Histone Acetyltransferases (HATs) in Chinese Cabbage: Insights from Histone H3 Acetylation and Expression Profiling of *HATs* in Response to Abiotic Stresses

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ABSTRACT. Histone acetyltransferase (HAT) is known as an epigenetic enzyme that acetylates specific lysine residues on the histone tail to promote chromatin dynamics and gene expression. In higher plants, HATs have been recognized as playing a fundamental role in plant development, growth, and the response to diverse environmental stresses. In this study, using comprehensive bioinformatic analyses, we identified 15 *HATs* in genome of chinese cabbage [*Brassica rapa* (*BraHATs*)], which are divided into four families. In addition, evolution analysis suggested that the *BraHAT* genes were duplicated mainly via a segmental duplication event originating 3.05–18.39 million years ago. To determine the effects of abiotic stresses, such as salt, wounding, and drought, on histone H3 acetylation in chinese cabbage, histone H3 acetylation was analyzed via immunoblot analysis, suggesting that the acetylation level of histone H3 increased in response to wounding and salt stresses. Furthermore, the analysis of *BraHAT* expression patterns using quantitative real-time polymerase chain reaction (qRT-PCR) suggested that the increased acetylation of histone H3 was related to *BraHAT* transcripts and/or the functional interplay between HAT and histone deacetylase (HDAC) activities. Taken together, our comparative genomic analysis of *HAT* genes in this important vegetable crop will provide a solid foundation to further our understanding of epigenetically regulated processes in response to environmental stresses.

In eukaryotes, histone modifications, including acetylation, methylation, phosphorylation, ubiquitination, and glycosylation, are major epigenetic mechanisms that regulate numerous biological processes. Among these modifications, the acetylation of histones by HAT neutralizes the positively charged tail region of histone, thereby decreasing their interaction with endogenous negatively charged DNA, leading to local chromatin opening, which facilitates the access of transcription factors to genomic sequences (Boycheva et al., 2014; Sun et al., 2015). By contrast, HDAC removes the acetyl functional groups from the lysine residues of histone tails and results in gene repression (Boycheva et al., 2014). This indicates that HATs and HDACs play a role as transcriptional activators or repressors involved in multiple biological processes.

Based on sequence homology with HATs in mammalian and yeast, plant HATs are divided into four types: the general control nonrepressible 5 (GCN5)-related N-terminal acetyltransferase (GNAT) family; the MYST (for MOZ, Ybf2/Sas3, Sas2, and Tip60) family; the p300/CREB (cAMP-responsive element-binding protein)-binding protein (CBP) family; and the TATA-binding protein-associated factor (TAF)_{II250} family (Boycheva et al., 2014; Wang et al., 2014a). Since genes encoding 12 arabidopsis (*Arabidopsis thaliana*) HATs were identified (Pandey et al., 2002), several HATs have been identified from grape [*Vitis vinifera* (Aquea et al., 2010)], rice

[*Oryza sativa* (Liu et al., 2012)], tomato [*Solanum lycopersicum* (Aiese Cigliano et al., 2013)], and litchi [*Litchi chinensis* (Peng et al., 2017)]. In yeast (*Saccharomyces cerevisiae*), the deletion of GCN5 HAT results in either up- or downregulation of a large number of genes, suggesting that GCN5 is linked to gene transcriptional regulation (Lee et al., 2000). The loss-of-function mutation of the GCN5 ortholog found in arabidopsis (*HAG1/AtGCN5*) leads to pleiotropic phenotypes, including dwarfism, aberrant meristem function, loss of apical dominance, short petals and stamens, and floral organ identity (Bertrand et al., 2003; Servet et al., 2010; Vlachonasios et al., 2003; Wang et al., 2014a). Furthermore, the mutation of other HATs causes the decreased rate of cell division in *HAG3* (encoding elongator complex protein 3) mutant (Nelissen et al., 2005), decreased chlorophyll content in arabidopsis HAF2 (encoding TAF_{II250}) mutant (Bertrand et al., 2005), and late flowering in arabidopsis HAC1 (encoding p300/CBP) mutant (Han et al., 2007). These phenotypes are conferred by alterations in gene expression, indicating the highly conserved function of HATs across animals, yeast, and plants. In addition, *AtGCN5*, which is one of the best characterized HATs in plants, regulates cold tolerance by interacting with C-repeat binding factor-1, and is required for stress-inducible gene expression by promoting the acetylation of the target gene promoter (Chinnusamy and Zhu, 2009; Stockinger et al., 2001; Yuan et al., 2013). Furthermore, the region-specific enrichment of the acetylation of histone H3K23 and H3K27 at the promoter and coding regions of the arabidopsis drought-responsive genes *RD29A*, *RD29B*, and *RD20* was observed in response to drought stress (Kim et al., 2008), suggesting that histone acetylation by HATs is important for the plant adaptation to environmental

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changes related to salinity, drought, and temperature (Yuan et al., 2013).

Despite the knowledge concerning HATs, evolutionary and functional information regarding HATs in vegetable crops are still relatively unknown, especially in the genus *Brassica*. *Brassica rapa* contains a relatively small genome (≈ 529 Mbp) compared with other *Brassica* species (Johnston et al., 2005) and has been used as a model crop for the genomic study of *Brassica* species (Alam et al., 2017). Here, we identified 15 putative *HAT* genes by means of genome-wide analysis of the *B. rapa* genome sequence. To investigate the evolutionary relationships of *B. rapa* *HATs*, we traced their expansion mechanisms and evolutionary history of this gene family by analyzing their duplication and distribution on the chromosomes. In addition, the expression profiles of *BraHATs* and alteration of histone H3 acetylation levels in response to salt, wounding, and drought were examined to assess functional divergence between the *BraHATs*.

Materials and Methods

DATABASE MINING AND IDENTIFICATION OF *BraHAT* GENES.

To enlist the complete family of HATs in *B. rapa*, *HAT* gene sequences were searched using BLASTp and tBLASTn, which are available in Phytozome v12.1 (Goodstein et al., 2012). The presence of the characteristic conserved domains in putative *BraHATs* was analyzed using the SMART tool (Schultz et al., 2000). In addition, the molecular weight (MW), the isoelectric point (pI), and subcellular localization of putative *BraHATs* were predicted using the Compute pI/Mw tool (Artimo et al., 2012) and Plant-mPLOC web services (Chou and Shen, 2010). A nomenclature system for HATs (generic name: *BraHAC1* to *BraHAC7* for CBP family, *BraHAG1* to *BraHAG7* for GNAT and MYST families, and *BraHAF1* for TAF_{II}250 family) was provisionally used to distinguish between each *HAT* gene as described by Pandey et al. (2002).

PHYLOGENETIC TREE AND GENE DUPLICATION ANALYSES OF *BraHAT* GENES. The protein sequence of putative *BraHATs* was aligned with HATs in arabidopsis and rice, and a phylogenetic tree using the condensed alignment in MEGA7.0 (Kumar et al., 2016) with the neighbor-joining algorithm (100 replications by default) was constructed.

To analyze the expansion of the *BraHAT* genes, the gene expansion mediated by segmental duplication was investigated, as described by Hyun et al. (2014). Syntenic gene pairs were retrieved from the Plant Genome Duplication Database (Lee et al., 2013) within a distance of 200 kb. The synonymous (Ks) and nonsynonymous (Ka) substitution rates were calculated between the duplicated *BraHAT* gene pairs using the PAL2NAL tool (Suyama et al., 2006). The values of the Ka/Ks ratio are depicted for positive selection (Ka/Ks > 1), negative selection (Ka/Ks < 1), and neutral selection (Ka/Ks = 1) as described by Yang and Nielsen (2000). The divergence time of gene pairs was calculated according to the formula $T = Ks/2\lambda$. The mean value of clock-like rate (λ) for *B. rapa* was 1.5×10^{-8} (Koch et al., 2000).

PLANT GROWTH CONDITION AND ABIOTIC STRESS TREATMENTS. Chinese cabbage (cv. Chunkwang) seeds were obtained from Sakata Korea Seed Co. (Seoul, South Korea). The seeds were germinated and grown in a growth chamber (16 h light/8 h dark) at 24 °C. To analyze the expression pattern of *BraHATs* in response to abiotic stresses, 4-week-old chinese cabbage plants

were treated with 250 mM NaCl or effectively wounded using a sterile syringe needle. For drought stress treatment, plants were subjected to water withholding for 2 or 4 d, which resulted in the soil water content reaching 20% or 5%, respectively. Samples were harvested at the times indicated in Fig. 2. Malondialdehyde content was determined using the thiobarbituric acid reaction as described by Eom et al. (2018).

ANALYSIS OF HISTONE H3 ACETYLATION USING WESTERN BLOT. A histone-enriched extract was prepared using the modified method of Aquea et al. (2017). Briefly, 100 mg of ground samples was mixed with an extraction buffer (10 mM Tris, pH 7.5, 0.4 M HCl, 2 mM EDTA, and 5 mM betamercaptoethanol) containing protease inhibitor cocktail (Thermo Fisher Scientific, Waltham, MA) and incubated overnight at 4 °C. After centrifugation at 12,000 g_n , the supernatants were incubated with the same volume of 50% trichloroacetic acid for 1 h on ice. The mixture was centrifuged at 17,000 g_n for 30 min at 4 °C. Then, the pellet was washed twice with ice-cold acetone, dried, and dissolved in 100 μ L of distilled water. The concentration of total protein was analyzed according to the Bradford method and bovine serum albumin was used as a standard.

For immunoblot analysis, 10 μ g protein was separated on a 15% SDS-polyacrylamide gel and transferred to a polyvinylidene difluoride membrane by electroblotting. The membranes were hybridized with specific antibodies, such as anti-histone H3 (ab18521; Abcam, Cambridge, MA), anti-H3K18Ac (ab1191; Abcam), anti-H3K27Ac (ab4729; Abcam), and anti-total H3Ac (06-599; Millipore, Bedford, MA). The signal was detected and visualized using a chemiluminescence system.

GENE EXPRESSION ANALYSIS USING qRT-PCR. Total RNA extracted from each sample were reverse-transcribed into cDNA, and qRT-PCR was performed as described by Eom et al. (2017). Chinese cabbage actin (Kim et al., 2014) was used as an internal reference gene, and specific primer pairs are listed in Supplemental Table 1.

Results and Discussion

IDENTIFICATION AND CHARACTERIZATION OF THE *HAT* FAMILY IN CHINESE CABBAGE. The availability of the chinese cabbage genome sequence (*B. rapa* FPsc v1.3) has made it possible to identify the putative *HAT* family in this plant species for the first time. Sequences of HATs from arabidopsis and rice were used as queries to obtain the amino sequences of HATs in chinese cabbage. According to the self-BLAST of sequences, the redundant sequences were removed, resulting in a total of 15 putative *HAT* genes from chinese cabbage (Table 1). In addition, all *BraHATs* had a calculated MW ranging from 50.9 to 199.9 kDa and a theoretical pI from 5.37 to 8.98. Based on subcellular localization, HATs are generally classified into two groups. Type A HATs are known as the nuclear proteins and acetylate nucleosome core histones, whereas Type B HATs are predominantly localized to the cytoplasm and acetylate-free histones to transfer their assembly into nucleosomes (Boycheva et al., 2014). Similarly, *BraHAG1* and *BraHAG6* are predicted to be cytoplasmic and nuclear proteins, whereas other *BraHATs* are predicted to localize to the nucleus (Table 1).

To analyze the phylogenetic relationship among the *HAT* family in plants, a phylogenetic tree was constructed and suggested that HATs were divided into four major classes (Fig. 1; Supplemental Fig. 1). The chinese cabbage genome encodes seven proteins (Fig. 1) clustered in the CBP family.

Name	Gene	Location	CDS (bp)	Amino acids (no.)	Introns (no.)	pI	MW (kDa)	Subcellular localization
BraHAC1	Brara.A03298	A01:27364854.27373116	4905	1634	15	8.83	184.3	Nucleus
BraHAC2	Brara.B01706	A02:9942192.9946098	2976	991	10	7.94	113.7	Nucleus
BraHAC3	Brara.C03444	A03:17432821.17441003	4812	1603	15	8.83	181.4	Nucleus
BraHAC4	Brara.F01165	A06:6506991.6514887	4308	1435	15	8.22	161.4	Nucleus
BraHAC5	Brara.G02584	A07:21359585.21363632	2784	927	9	6.2	106.6	Nucleus
BraHAC6	Brara.H02502	A08:20713162.20720728	4740	1579	15	8.74	176.7	Nucleus
BraHAC7	Brara.I04867	A09:41024531.41032046	4962	1653	15	8.67	183.6	Nucleus
BraHAG1	Brara.C01234	A03:5761380.5764158	1389	462	9	5.37	52.2	Cytoplasm and nucleus
BraHAG2	Brara.C02538	A03:12794703.12797812	1698	565	8	8.98	63.8	Nucleus
BraHAG3	Brara.D00450	A04:3341996.3346089	1578	525	12	6.18	58.9	Nucleus
BraHAG4	Brara.D00454	A04:3400250.3404506	1647	548	12	6.13	60.9	Nucleus
BraHAG5	Brara.I00786	A09:4383334.4386023	1317	438	8	7.26	51.0	Nucleus
BraHAG6	Brara.J01100	A10:10482109.10484932	1383	460	9	6.01	51.9	Cytoplasm and nucleus
BraHAG7	Brara.J02366	A10:17418999.17421438	1326	441	8	7.56	50.9	Nucleus
BraHAF1	Brara.A02897	A01:24652941.24661664	5289	1762	18	5.73	199.9	Nucleus

Phylogenetic tree and domain architecture of BraHAC proteins. The tree on the left shows relationships between BraHAC1-7, BraHAG1-7, BraHAG2, and BraHAF1, with bootstrap values at nodes. The domain architecture on the right shows the presence of various domains in each protein, including ZnF_TAZ, PHD, KAT11, ZnF_ZZ, CHROMO, MOZ_SAS, ZnF_C2H2, Acetyltransf_1, BROMO, Hat1_N, Elp3, UBQ, TBP-binding, and ZnF_C2HC. The proteins are grouped into three categories: CBP, MYST, and GCN5.

Most BraHACs contained a PHD domain (SM000249), a partially conserved plant homeodomain zinc finger, two types of zinc finger domains ZnF-TAZ (SM000551) and ZnF-ZZ (SM000291) that mediate protein–protein interactions in CBP/p300, and KAT11 (SM001250), required for H3K56 acetylation (Li et al., 2014; Tang et al., 2008). The MYST family consists of two BraHATs: BraHAG5 and BraHAG7. They contain three conserved domains, N-terminal Chromo (SM000298), ZnF_C2H2 (SM000355), and MOZ_SAS (PF01853), at the C terminal, which are typical of MYST acetyltransferases (Aiese Cigliano et al., 2013; Latrasse et al., 2008). The proteins belonging to the GNAT family are defined by the presence of Acetyltransf_1 domain (PF00583) (Sterner and Berger, 2000), suggesting that five BraHATs (BraHAG1, BraHAG2, BraHAG3, BraHAG4, and BraHAG6) are the GNAT family members (Fig. 1). The GNAT family can be further divided into four subfamilies designated GCN5, ELP3, HAT1, and HPA2. In the chinese cabbage genome, we identified homologs of GCN5 (BraHAG3 and BraHAG4),

EVOLUTION PATTERNS OF THE BraHAT FAMILY. Gene duplication occurring via unequal crossing over, retrotransposition, and whole-genome duplication is an important mechanism for acquiring new genes, which in turn facilitates the generation of

Fig. 2. Synteny analysis of *BraHAT* genes revealed five segmental duplicated pairs. Paralogous gene pairs were generated using the Plant Genome Duplication Database (Lee et al., 2013). Each query gene displays only ≈ 200 -kb regions. Blue lines indicate the other anchor gene in the region, and a red dot line represents the query locus.

new functions and creates genetic novelty in organisms (Flagel and Wendel, 2009; Magadum et al., 2013). Tandem and segmental duplications are known as the major sources of diversity for the evolution of large gene families in higher plants (Cannon et al., 2004). In the chinese cabbage genome ($n = 10$), 15 *HAT* genes are located on all chromosomes (Table 1). Chromosome 3 contained the highest density of *HAT* genes with three members (*BraHAC3*, *BraHAG1*, and *BraHAG2*), and two *HAT* genes were found on chromosome 1 (*BraHAC1* and *BraHAF1*), 4 (*BraHAG3* and *BraHAG4*), 9 (*BraHAC7* and *BraHAG5*), and 10 (*BraHAG6* and *BraHAG7*). Tandem duplicated genes are characterized as two or more adjacent homologous genes located on a single chromosome, whereas gene duplication on different chromosomes is defined as

segmental duplicated genes (Hyun et al., 2014; Ning et al., 2017), indicating that the GCN5 subfamily (*BraHAG3* and *BraHAG4*) in chinese cabbage seems to have evolved from tandem duplication. In addition, duplication analysis regarding the identification of chromosomal homologous segments within the genome exhibited five pairs of segmental duplicated paralogs (*BraHAC1/3*, *BraHAC2/5*, *BraHAC6/7*, *BraHAG1/6*, and *BraHAG5/7*) (Fig. 2), indicating that segmental duplication is an important factor leading to the expansion of the *HAT* family in chinese cabbage. Furthermore, the types and divergence time of the *BraHAT* paralogous gene pairs were analyzed by calculating K_s and K_a . All of the duplicated *BraHAT* gene pairs had K_a/K_s ratios less than one, suggesting that these segmental duplication pairs were subjected to

purifying selection (Table 2). The calculation of the divergence time of the duplicated *BraHAT* genes (Table 2) indicated that *BraHAC* gene divergence took place before or during the divergence of chinese cabbage and arabidopsis [9.6–16.1 million years ago (Mya)] (*Brassica rapa* Genome Sequencing Project Consortium, 2011), whereas *BraHAG* gene divergence occurred during or after the genome triplication events (5–9 Mya) in *B. rapa* (Wang et al., 2012).

COMPREHENSIVE ANALYSIS OF HISTONE H3 ACETYLATION AND *BraHAT* EXPRESSION PATTERNS IN RESPONSE TO ABIOTIC STRESSES. Abiotic stresses, such as salinity, heat, drought, cold, and ultraviolet irradiation, can affect histone methylation and/or acetylation patterns (Yuan et al., 2013). Therefore, it is widely accepted that histone modifications via the action of HATs, HDACs, histone methyltransferases, and histone demethylases epigenetically regulate the responses to various stresses (Chinnusamy and Zhu, 2009; Yuan et al., 2013). In arabidopsis, N-terminal lysine residues K9, K14, K18, K23, and K27 of histone H3 and K5, K8, K12, K16, and K20 of histone H4 are found to be acetylation/deacetylation targets (Zhang et al., 2007).

To determine whether abiotic stresses have an effect on histone H3 acetylation levels, initially, we analyzed the physiological responses of chinese cabbage subjected to salt, wounding, and drought stresses. The accumulation of reactive oxygen species in response to abiotic stresses is considered to be toxic by-products of aerobic metabolism (Miller et al., 2008). Therefore, oxidative stress markers including malondialdehyde (as a lipid peroxidation product) have been analyzed to identify variations in physiological response to abiotic stresses. As shown in Supplemental Fig. 2, malondialdehyde was accumulated in response to salt, wounding, and drought stresses, indicating that these treatments were effective. Then, histone-enriched extracts were prepared from each stress-treated sample, and the level of histone H3 acetylation was analyzed using a specific antibody. The increased level of histone H3 acetylation (anti-total H3Ac) was observed after exposure to wounding and salt stress, whereas drought stress did not affect histone H3 acetylation (Fig. 3). It has been shown that the lysine modifications on the histone H3 N-terminal tail are altered temporally and spatially in a gene- and region-specific manner (Kim et al., 2008). In chinese cabbage, the acetylation of histone H3K27 was significantly increased by exposure to wounding or salt stress for 2 d. The increased acetylation level of histone H3K18 was observed when the

Table 2. Divergence between paralogous *HAT* gene pairs in chinese cabbage.^z

Gene 1	Gene 2	S ^y	N ^y	Ks ^y	Ka ^y	Ka/Ks ^y	Mya ^x
<i>BraHAC6</i>	<i>BrHAC7</i>	1120.2	3589.8	0.3098	0.1135	0.3664	12.21
<i>BraHAC1</i>	<i>BrHAC3</i>	1061.8	3702.2	0.3897	0.1301	0.3340	11.13
<i>BraHAC2</i>	<i>BrHAC5</i>	615.5	1961.5	0.4606	0.2541	0.5518	18.39
<i>BraHAG5</i>	<i>BrHAG7</i>	304.7	1009.3	0.8688	0.0796	0.0916	3.05
<i>BraHAG1</i>	<i>BrHAG6</i>	369.5	1004.5	0.2734	0.0650	0.2377	7.92

^zThese gene pairs were identified at the terminal nodes of the gene tree shown in Fig. 2.

^yThe number of synonymous sites (S), number of nonsynonymous sites (N), synonymous substitution rate (Ks), and nonsynonymous substitution rate (Ka) are presented for each pair.

^xThe data of the duplication event were estimated according to $T = Ks/2\lambda$; Mya = million years ago.

Fig. 3. Alteration of histone H3 acetylation levels in response to wounding, salt, and drought stresses. Western blot analysis was performed with specific antibodies (anti-H3K18Ac for analyzing acetylation of H3K18, anti-H3K27Ac for analyzing acetylation of H3K27, and anti-total H3Ac for analyzing acetylation of H3). The relative intensity was determined by the ratio of histone H3 acetylation to total histone H3 using AzureSpot software (version 4.2; Azure Biosystems, Dublin, CA). The data are presented as mean $\pm$ SE. Unpaired *t* test compared with the nontreated group (NT) was used for significance analyses. * and ** indicate a significant difference at $P < 0.05$ and $P < 0.01$, respectively.

chinese cabbage plants were treated with NaCl for 2 d. Region-specific acetylation is associated with a region-specific change in the chromatin structure to increase the accessibility of stress-responsive transcription factor-binding sites (Roy et al., 2014), suggesting that differential acetylation of the H3K18 and H3K27 residues (Fig. 3) might function in transcriptional regulation under abiotic stresses. In fact, the enrichment of H3K18ac and H3K27ac with the expression of HATs is correlated with gene activation in response to abiotic stresses (Fang et al., 2014; Luo et al., 2012). In arabidopsis, it has been suggested that a large number of the sense/antisense transcripts have their expression changes correlated with H3K9ac and/or H3K27ac (Wang et al., 2014b). Taken together, these suggest

Fig. 4. Expression pattern of *BraHAT* genes in response to wounding, salt, and drought stresses. Transcript levels of the *BraHAT* genes were normalized to the constitutive expression level of chinese cabbage actin and were expressed relative to the values of the nontreated group (NT)***. Data are means $\pm$ SE. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ represent the significant differences in comparison with NT.

that modifications in histone acetylation patterns (Fig. 3) in response to abiotic stresses are associated with the expression of stress-response genes.

The expression patterns of *BraHAT* genes were analyzed in different tissues to investigate the spatial presence of transcripts for *BraHAT* proteins. As shown in Supplemental Fig. 3, all *BraHAT*s were expressed in all tissues tested. Most of all *BraHAT*s were primarily expressed in the cotyledon, except that *BraHAC1* and *BraHAG3* were highly expressed in the hypocotyl. In rice, the mRNA of the HAT genes was detected in all tissues but with differences in the transcript abundance (Liu et al., 2012). The constitutively expressed HATs in chinese cabbage suggested that these genes may play a role in plant growth and development. To investigate whether the differential acetylation of histone H3 under abiotic stress conditions was reflected by transcription of *BraHAT*s, the expression pattern of each *BraHAT* during response to stresses was analyzed by qRT-PCR. As shown in Fig. 4, the exposure of chinese cabbage to these stresses induced or repressed the expression of *BraHAT* genes. When chinese cabbage plants were treated with wounding, the slightly but significantly increased expression levels of *BraHAC2*, *BraHAC4*, *BraHAC7*, *BraHAG3*, and *BraHAG5* were observed. During response to salt stress, the transcript level of *BraHAC1*, *BraHAC2*, *BraHAC3*, *BraHAC4*, *BraHAC5*, *BraHAC6*, *BraHAC7*, *BraHAG1*, *BraHAG2*, *BraHAG3*, *BraHAG5*, and *BraHAG7* were slightly but significantly upregulated. These observations indicated that the increased acetylation of histone H3 (anti-total H3Ac) by wounding or salt treatment could be related to *BraHAT* transcription. The expression of *BraHAC1*, *BraHAC2*, *BraHAC3*, *BraHAC4*, *BraHAC7*, *BraHAG2*, *BraHAG5*, *BraHAG7*, and *BraHAF1* was slightly but significantly increased after 2 and/or 4 d of drought treatment (Fig. 4), whereas histone H3 acetylation was not changed by drought stress (Fig. 3). The dynamic equilibrium between histone acetylation and deacetylation is mostly maintained via two

opposing activities of HAT and HDAC enzymes. The functional interplay between HAT and HDAC regulates various physiological processes (Peserico and Simone, 2011). Although the observed increase in histone H3 acetylation might be reflected by transcription of *BraHAT*s under stress conditions, the variation in HDAC activities should be contributed to noncorrelation between transcription of *BraHAT*s and changes in acetylation levels in response to drought stress.

In conclusion, we have systematically identified and characterized the putative HAT proteins in *B. rapa* and proved new insights into how these genes have evolved in *B. rapa*. Comparison between the histone 3 acetylation and *BraHAT* transcripts suggests that the lysine modifications on the histone 3 N-terminal tail are affected by de novo transcription of *HAT*s in stress-treated chinese cabbage. Our results provide a reasonable starting point for future efforts to understand the epigenetic regulation of *BraHAT*s in response to environmental stresses.

Literature Cited

- Aiese Cigliano, R., W. Sanseverino, G. Cremona, M.R. Ercolano, C. Conicella, and F.M. Consiglio. 2013. Genome-wide analysis of histone modifiers in tomato: Gaining an insight into their developmental roles. *BMC Genomics* 14:57.
- Alam, I., Y.Q. Yang, Y. Wang, M.L. Zhu, H.B. Wang, B. Chalhoub, and Y.H. Lu. 2017. Genome-wide identification, evolution and expression analysis of RING finger protein genes in *Brassica rapa*. *Sci. Rpt.* 7:40690.
- Aquea, F., T. Timmermann, and P. Arce-Johnson. 2010. Analysis of histone acetyltransferase and deacetylase families of *Vitis vinifera*. *Plant Physiol. Biochem.* 48:194–199.
- Aquea, F., T. Timmermann, and A. Herrera-Vásquez. 2017. Chemical inhibition of the histone acetyltransferase activity in *Arabidopsis thaliana*. *Biochem. Biophys. Res. Commun.* 483:664–668.

- Artimo, P., M. Jonnalagedda, K. Arnold, D. Baratin, G. Csardi, E. de Castro, S. Duvaud, V. Flegel, A. Fortier, E. Gasteiger, A. Grosdidier, C. Hernandez, V. Ioannidis, D. Kuznetsov, R. Liechti, S. Moretti, K. Mostaguir, N. Redaschi, G. Rossier, I. Xenarios, and H. Stockinger. 2012. ExPASy: SIB bioinformatics resource portal. *Nucl. Acids Res.* 40:W597–W603.
- Bertrand, C., M. Benhamed, Y.F. Li, M. Ayadi, G. Lemonnier, J.P. Renou, M. Delarue, and D.X. Zhou. 2005. Arabidopsis HAF2 gene encoding TATA-binding protein (TBP)-associated factor TAF1, is required to integrate light signals to regulate gene expression and growth. *J. Biol. Chem.* 280:1465–1473.
- Bertrand, C., C. Bergounioux, S. Domenichini, M. Delarue, and D.X. Zhou. 2003. Arabidopsis histone acetyltransferase AtGCN5 regulates the floral meristem activity through the WUSCHEL/AGAMOUS pathway. *J. Biol. Chem.* 278:28246–28251.
- Boycheva, I., V. Vassileva, and A. Iantcheva. 2014. Histone acetyltransferases in plant development and plasticity. *Curr. Genomics* 15:28–37.
- Brassica rapa Genome Sequencing Project Consortium. 2011. The genome of the mesopolyploid crop species *Brassica rapa*. *Nature Genet.* 43:1035–1039.
- Cannon, S.B., A. Mitra, A. Baumgarten, N.D. Young, and G. May. 2004. The roles of segmental and tandem gene duplication in the evolution of large gene families in *Arabidopsis thaliana*. *BMC Plant Biol.* 4:10.
- Chinnusamy, V. and J.K. Zhu. 2009. Epigenetic regulation of stress responses in plants. *Curr. Opin. Plant Biol.* 12:133–139.
- Chou, K.C. and H.B. Shen. 2010. Plant-mPLOC: A top-down strategy to augment the power for predicting plant protein subcellular localization. *PLoS One* 5:e11335.
- Eom, S.H., S.-A. Baek, J.K. Kim, and T.K. Hyun. 2018. Transcriptome analysis in Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) provides the role of glucosinolate metabolism in response to drought stress. *Molecules* 23:E1186.
- Eom, S.H., J.K. Lee, H. Kim, and T.K. Hyun. 2017. De novo transcriptomic analysis to reveal functional genes involved in triterpenoid saponin biosynthesis in *Oplopanax elatus* NAKAI. *J. Appl. Bot. Food Qual.* 90:25–31.
- Fang, H., X. Liu, G. Thorn, J. Duan, and L. Tian. 2014. Expression analysis of histone acetyltransferases in rice under drought stress. *Biochem. Biophys. Res. Commun.* 443:400–405.
- Flagel, L.E. and J.F. Wendel. 2009. Gene duplication and evolutionary novelty in plants. *New Phytol.* 183:557–564.
- Goodstein, D.M., S. Shu, R. Howson, R. Neupane, R.D. Hayes, J. Fazo, T. Mitros, W. Dirks, U. Hellsten, N. Putnam, and D.S. Rokhsar. 2012. Phytozome: A comparative platform for green plant genomics. *Nucl. Acids Res.* 40:D1178–D1186.
- Han, S.K., J.D. Song, Y.S. Noh, and B. Noh. 2007. Role of plant CBP/p300-like genes in the regulation of flowering time. *Plant J.* 49:103–114.
- Hyun, T.K., S.H. Eom, X. Han, and J.S. Kim. 2014. Evolution and expression analysis of the soybean glutamate decarboxylase gene family. *J. Biosci.* 39:899–907.
- Johnston, J.S., A.E. Pepper, A.E. Hall, Z.J. Chen, G. Hodnett, J. Drabek, R. Lopez, and H.J. Price. 2005. Evolution of genome size in Brassicaceae. *Ann. Bot.* 95:229–235.
- Kim, J.M., T.K. To, J. Ishida, T. Morosawa, M. Kawashima, A. Matsui, T. Toyoda, H. Kimura, K. Shinozaki, and M. Seki. 2008. Alterations of lysine modifications on the histone H3 N-tail under drought stress conditions in *Arabidopsis thaliana*. *Plant Cell Physiol.* 49:1580–1588.
- Kim, R.-A., H. Lim, S.H. Park, H. Ji, U.-H. Yoon, T.-H. Kim, J.-I. Cho, C.-K. Kim, and G.-S. Lee. 2014. Molecular characterization of a putatively stress-related gene *BrTSR53* isolated from Chinese cabbage (*Brassica rapa*). *Korean J. Breeding Sci.* 46:143–151.
- Koch, M.A., B. Haubold, and T. Mitchell-Olds. 2000. Comparative evolutionary analysis of chalcone synthase and alcohol dehydrogenase loci in *Arabidopsis*, *Arabis*, and related genera (Brassicaceae). *Mol. Biol. Evol.* 17:1483–1498.
- Kumar, S., G. Stecher, and K. Tamura. 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* 33:1870–1874.
- Latrasse, D., M. Benhamed, Y. Henry, S. Domenichini, W. Kim, D.X. Zhou, and M. Delarue. 2008. The MYST histone acetyltransferases are essential for gametophyte development in arabidopsis. *BMC Plant Biol.* 8:12.
- Lee, T.I., H.C. Causton, F.C. Holstege, W.C. Shen, N. Hannett, E.G. Jennings, F. Winston, M.R. Green, and R.A. Young. 2000. Redundant roles for the TFIID and SAGA complexes in global transcription. *Nature* 405:701–704.
- Lee, T.H., H. Tang, X. Wang, and A.H. Paterson. 2013. PGDD: A database of gene and genome duplication in plants. *Nucl. Acids Res.* 41:D1152–D1158.
- Li, C., J. Xu, J. Li, Q. Li, and H. Yang. 2014. Involvement of arabidopsis histone acetyltransferase HAC family genes in the ethylene signaling pathway. *Plant Cell Physiol.* 55:426–435.
- Liu, X., M. Luo, W. Zhang, J. Zhao, J. Zhang, K. Wu, L. Tian, and J. Duan. 2012. Histone acetyltransferases in rice (*Oryza sativa* L.): Phylogenetic analysis, subcellular localization and expression. *BMC Plant Biol.* 12:145.
- Luo, M., X. Liu, P. Singh, Y. Cui, L. Zimmerli, and K. Wu. 2012. Chromatin modifications and remodeling in plant abiotic stress responses. *Biochim. Biophys. Acta* 1819:129–136.
- Magadum, S., U. Banerjee, P. Murugan, D. Gangapur, and R. Ravikesavan. 2013. Gene duplication as a major force in evolution. *J. Genet.* 92:155–161.
- Miller, G., V. Shulaev, and R. Mittler. 2008. Reactive oxygen signaling and abiotic stress. *Physiol. Plant.* 133:481–489.
- Nelissen, H., D. Fleury, L. Bruno, P. Robles, L. De Veylder, J. Traas, J. L. Micol, M. Van Montagu, D. Inzé, and M. Van Lijsebettens. 2005. The elongata mutants identify a functional Elongator complex in plants with a role in cell proliferation during organ growth. *Proc. Natl. Acad. Sci. USA* 102:7754–7759.
- Ning, P., C. Liu, J. Kang, and J. Lv. 2017. Genome-wide analysis of WRKY transcription factors in wheat (*Triticum aestivum* L.) and differential expression under water deficit condition. *PeerJ* 5:e3232.
- Pandey, R., A. Müller, C.A. Napoli, D.A. Selinger, C.S. Pikaard, E.J. Richards, J. Bender, D.W. Mount, and R.A. Jorgensen. 2002. Analysis of histone acetyltransferase and histone deacetylase families of *Arabidopsis thaliana* suggests functional diversification of chromatin modification among multicellular eukaryotes. *Nucl. Acids Res.* 30:5036–5055.
- Peng, M., P. Ying, X. Liu, C. Li, R. Xia, J. Li, and M. Zhao. 2017. Genome-wide identification of histone modifiers and their expression patterns during fruit abscission in litchi. *Front. Plant Sci.* 8:639.
- Peserico, A. and C. Simone. 2011. Physical and functional HAT/HDAC interplay regulates protein acetylation balance. *J. Biomed. Biotechnol.* 2011:371832.
- Roy, D., A. Paul, A. Roy, R. Ghosh, P. Ganguly, and S. Chaudhuri. 2014. Differential acetylation of histone H3 at the regulatory region of *OsDREB1b* promoter facilitates chromatin remodelling and transcription activation during cold stress. *PLoS One* 9:e100343.
- Schultz, J., R.R. Copley, T. Doerks, C.P. Ponting, and P. Bork. 2000. SMART: A web-based tool for the study of genetically mobile domains. *Nucl. Acids Res.* 28:231–234.
- Servet, C., N. Conde e Silva, and D.X. Zhou. 2010. Histone acetyltransferase AtGCN5/HAG1 is a versatile regulator of developmental and inducible gene expression in arabidopsis. *Mol. Plant* 3:670–677.
- Stern, D.E. and S.L. Berger. 2000. Acetylation of histones and transcription-related factors. *Microbiol. Mol. Biol. Rev.* 64:435–459.
- Stockinger, E.J., Y. Mao, M.K. Regier, S.J. Triezenberg, and M.F. Thomashow. 2001. Transcriptional adaptor and histone acetyltransferase proteins in arabidopsis and their interactions with CBF1, a transcriptional activator involved in cold-regulated gene expression. *Nucl. Acids Res.* 29:1524–1533.

- Sun, X.J., N. Man, Y. Tan, S.D. Nimer, and L. Wang. 2015. The role of histone acetyltransferases in normal and malignant hematopoiesis. *Front. Plant Sci.* 5:108.
- Suyama, M., D. Torrents, and P. Bork. 2006. PAL2NAL: Robust conversion of protein sequence alignments into the corresponding codon alignments. *Nucl. Acids Res.* 34:W609–W612.
- Tang, Y., M.A. Holbert, H. Wurtele, K. Meeth, W. Rocha, M. Gharib, E. Jiang, P. Thibault, A. Verreault, P.A. Cole, and R. Marmorstein. 2008. Fungal Rtt109 histone acetyltransferase is an unexpected structural homolog of metazoan p300/CBP. *Nat. Struct. Mol. Biol.* 15:738–745.
- Vlachonasios, K.E., M.F. Thomashow, and S.J. Triezenberg. 2003. Disruption mutations of ADA2b and GCN5 transcriptional adaptor genes dramatically affect arabidopsis growth, development, and gene expression. *Plant Cell* 15:626–638.
- Wang, Z., H. Cao, F. Chen, and Y. Liu. 2014a. The roles of histone acetylation in seed performance and plant development. *Plant Physiol. Biochem.* 84:125–133.
- Wang, H., P.J. Chung, J. Liu, I.C. Jang, M.J. Kean, J. Xu, and N.H. Chua. 2014b. Genome-wide identification of long noncoding natural antisense transcripts and their responses to light in arabidopsis. *Genome Res.* 24:444–453.
- Wang, Y., H. Tang, J.D. Debarry, X. Tan, J. Li, X. Wang, T.H. Lee, H. Jin, B. Marler, H. Guo, J.C. Kissinger, and A.H. Paterson. 2012. MCScanX: A toolkit for detection and evolutionary analysis of gene synteny and collinearity. *Nucl. Acids Res.* 40:e49.
- Yang, Z. and R. Nielsen. 2000. Estimating synonymous and non-synonymous substitution rates under realistic evolutionary models. *Mol. Biol. Evol.* 17:32–43.
- Yuan, L., X. Liu, M. Luo, S. Yang, and K. Wu. 2013. Involvement of histone modifications in plant abiotic stress responses. *J. Integr. Plant Biol.* 55:892–901.
- Zhang, K., V.V. Sridhar, J. Zhu, A. Kapoor, and J.K. Zhu. 2007. Distinctive core histone post-translational modification patterns in *Arabidopsis thaliana*. *PLoS One* 2:e1210.

Supplemental Fig. 1. Phylogenetic analysis of the HAT family in Chinese cabbage, Arabidopsis (Pandey et al., 2002), and rice (Liu et al., 2012).

Supplemental Fig. 2. Changes in malondialdehyde content in response to wounding, salt, and drought stresses. Data are means $\pm$ SE; * P < 0.05 and ** P < 0.01 represent the significant differences in comparison with the nontreated group (NT).

Supplemental Fig. 3. Transcript levels of *BraHATs* in different tissues. Data are means $\pm$ SE.

Supplemental Table 1. Primer sequences for quantitative real time polymerase chain reaction analysis.

Primer	Sequences (5'-3')
BraHAC1-F	CCCTGCTTGTGCATATCAGT
BraHAC1-Rev	CGCATTGACTCAAAGGCTTC
BraHAC2-F	TATATGGGCGTGTCCACCTC
BraHAC2-Rev	GTCCTACTACCTCTGCTTGGT
BraHAC3-F	CAGGTGAGTCCTCAACCAGT
BraHAC3-Rev	GCGAGGTTTCGTGCTTAACTT
BraHAC4-F	CACGCATTGCTGTACACTGA
BraHAC4-Rev	TCTCTATCCGTGGTGTCTGC
BraHAC5-F	TGACACAACAGTGCCTTTGG
BraHAC5-Rev	GTTGCAAAGCGAGCAGTAGA
BraHAC6-F	TGACGAACCATCCATCGCTA
BraHAC6-Rev	CCTAAACCATGCCGGAACAG
BraHAC7-F	CAAGCGGGATTTCAGCATCAA
BraHAC7-Rev	CTTCTTCCTTCGGCTCTTGC
BraHAG1-F	GATCTTGGTCTGCCTTCCT
BraHAG1-Rev	ATCGATGGCCGGTTTGATTG
BraHAG2-F	TGCTGGTTTCAAGGTGGTTG
BraHAG2-Rev	AAGGCCAGTTCCACGTATCA
BraHAG3-F	CGAGTCGCAACGCTAAGATT
BraHAG3-Rev	TCAAAGCCTCTTCTCTCGCA
BraHAG4-F	AGACTCGATTCCAGCTCAGG
BraHAG4-Rev	CCGCAGTTCTGTACGTCATC
BraHAG5-F	GACTCACACTCCCTCGGAAT
BraHAG5-Rev	CGATGACTTTGACGGGATGG
BraHAG6-F	CACGAACCATGTGAGAGCTG
BraHAG6-Rev	TGCTCCTCTTGATCCGGTTT
BraHAG7-F	CCGTCCGCGAAATCAGATAC
BraHAG7-Rev	GACGGATCCGACTCTGTGAT
BraHAF1-F	GCTCCCAGATGAAGCCATTG
BraHAF1-Rev	CTCGGACGTAGCTGAATCCT
Bra-Actin-F	TGGGCGTACTACTGGTATTGTG
Bra-Actin-Rev	TGTACCTCTCTCGGTGAGAAT