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) family (Boycheva et al., 2014; Wang et al., 2014). Since genes encoding 12 arabidopsis (Arabidopsis thaliana) HATs were identified (Pandey et al., 2002), several HATs have been identified from grape [Vitis vinifera (Aquela 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., 2014). 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 TAF9250) 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...
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 Mb) 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 Phytozone v12.1 (Goodstein et al., 2012). The presence of the characteristic conserved domains in putative BraHATs was analyzed using the SMART tool (Schulz 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 BraHAF12) was used as described by Pandey et al. (2002).

PHYLLOGENETIC 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 was constructed using the SMART tool (Schulz et al., 2000) with the neighbor-joining algorithm (100 replicates 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 $T = \frac{Ks}{2\lambda}$. The mean value of clock-like rate ($\lambda$) 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 ACETYLYATION USING WESTERN BLOT. A histone-enriched extract was prepared using the modified method of Aqueda 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, 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 for 30 min at 4 °C. Then, the pellet was washed twice with ice-cold acetone, dried, and dissolved in 100 μL 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 μg 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 pl 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 acetyl-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.
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), ELP3 (BraHAG2), and HAT1 (BraHAG1 and BraHAG6) and no homolog of the HPA2 subfamily. Similarly, no homolog of the HPA2 subgroup has been identified in the genome of yeast (Schizosaccharomyces pombe and S. cerevisiae), nematode (Caenorhabditis elegans), fruit fly (Drosophila melanogaster), human, arabidopsis, or rice (Liu et al., 2012; Pandey et al., 2002). Furthermore, the chinese cabbage genome has one TAFII250 protein (BraHAF1) that exhibited the same domain composition (TBP-binding, PF09247; UBQ, SM000213; ZnF_C2HC, SM000343; BROMO, SM000297) as that of arabidopsis, rice, and tomato HAFs (Aiese Cigliano et al., 2013; Liu et al., 2012; Pandey et al., 2002). Taken together, the presence of conserved domains described for each family suggests that BraHATs likely have the same function as their homologous proteins.

**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

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**Table 1. Histone acetyltransferase gene family in *Brassica rapa*.**

| Name      | Gene Location  | CDS (bp) | Amino acids (no.) | Introns (no.) | pI     | MW (kDa) | Subcellular localization |
|-----------|----------------|----------|-------------------|---------------|--------|----------|--------------------------|
| BraHAC1   | Brara.A03298   | A01:27374854.27377316 | 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   | A10:1741899.17421438 | 1326 | 441 | 8 | 7.56 | 50.9 | Nucleus |
| BraHAF1   | Brara.A02897   | A01:2259241.24661664 | 5289 | 1762 | 18 | 5.73 | 199.9 | Nucleus |

CDS = coding sequence; PI = isoelectric point; MW = molecular weight.
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 BraHAG1), 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 Ks and Ka. All of the duplicated BraHAT gene pairs had Ka/Ks ratios less than one, suggesting that these segmental duplication pairs were subjected to

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.
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 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). Taken together, these suggest

### Table 2. Divergence between paralogous HAT gene pairs in Chinese cabbage.

| Gene 1    | Gene 2    | $S$ | $N$ | $K_s$ | $K_a$ | $K_a/K_s$ | Mya |
|-----------|-----------|-----|-----|-------|-------|-----------|-----|
| BraHAC6   | BrHAC7    | 1120.2 | 3589.8 | 0.3098 | 0.1135 | 0.3664 | 12.21 |
| BraHAC1   | BrHAC3    | 1061.8 | 3702.2 | 0.3897 | 0.1301 | 0.3340 | 11.13 |
| BraHAC2   | BrHAC5    | 615.5  | 1961.5 | 0.4606 | 0.2541 | 0.5518 | 18.39 |
| BraHAG5   | BrHAG7    | 304.7  | 1009.3 | 0.8688 | 0.0796 | 0.0916 | 3.05  |
| BraHAG1   | BrHAG8    | 369.5  | 1004.5 | 0.2734 | 0.0650 | 0.2377 | 7.92  |

*These gene pairs were identified at the terminal nodes of the gene tree shown in Fig. 2.

**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 ± 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.
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 BraHATs were expressed in all tissues tested. Most of all BraHATs 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 BraHATs, 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 BraHATs under stress conditions, the variation in HDAC activities should be contributed to noncorrelation between transcription of BraHATs 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 HATs in stress-treated chinese cabbage. Our results provide a reasonable starting point for future efforts to understand the epigenetic regulation of BraHATs 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.
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 nonsynonymous 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 ± SE; * $P < 0.05$ and ** $P < 0.01$ represent the significant differences in comparison with the nontreated group (NT).
Supplemental Table 1. Primer sequences for quantitative real time polymerase chain reaction analysis.

| Primer       | Sequences (5′-3′)             |
|--------------|-------------------------------|
| BraHAC1-F    | CCCTGCTTGTCATATCAGT           |
| BraHAC1-Rev  | CGCATGACTCAAAGGCTTC           |
| BraHAC2-F    | TATATGGCGTGTCACCTC           |
| BraHAC2-Rev  | GTCACTACCTCCTCGTGGT           |
| BraHAC3-F    | CAGGTAGTCCTCAACCTG           |
| BraHAC3-Rev  | GCGAGGCTGTCCTAACTT           |
| BraHAC4-F    | CAGCATTGCTGTACCTGA           |
| BraHAC4-Rev  | TCTCTATCCGTTGGTCTGC          |
| BraHAC5-F    | TGACGAACATGCCTTTGG           |
| BraHAC5-Rev  | GGGCAAGCGCAGTGTAGA           |
| BraHAC6-F    | GGACGAAACATCCATGCA           |
| BraHAC6-Rev  | CCTAAACCATGCGGGAACAG         |
| BraHAC7-F    | CAAGCCGGATCACCATCAA          |
| BraHAC7-Rev  | CTCTCTCTCCTGGCTCTTG           |
| BraHAG1-F    | GATCTTGCTCAGCCTCTTCT         |
| BraHAG1-Rev  | ATCGATGCGGCTTTGGGATG         |
| BraHAG2-F    | TGCTGCTTTCAAGGGTCTGGG        |
| BraHAG2-Rev  | AAGGCGATGGCCTCACGATC          |
| BraHAG3-F    | CGAGGTCGGCAGCTAAGATT         |
| BraHAG3-Rev  | TCAAGGCTCTGCTCTCGCA          |
| BraHAG4-F    | AGACTGAGTCCAGCTAGGA          |
| BraHAG4-Rev  | CGGCAAGTTCTGTACGTCATC        |
| BraHAG5-F    | GACTCAACCTCCCGAAT            |
| BraHAG5-Rev  | GATGACTTTGACGGGATGG          |
| BraHAG6-F    | CAGGAAATGTGAGAGCCG           |
| BraHAG6-Rev  | TGCTCCTCTTGATCGGTGTT         |
| BraHAG7-F    | CGGTCGGAGAATACGATC           |
| BraHAG7-Rev  | GACGGAATCCGACTCTGTGAT        |
| BraHAF1-F    | GCCCTCAGGACTGAGCCG           |
| BraHAF1-Rev  | GCTGCCAGTGAGCCATG            |
| Bra-Actin-F  | TGGCCTCGTACTGTGATTG          |
| Bra-Actin-Rev| TGATACCTCTCGGTGAGAAT         |

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