Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Bioinformatics and Expression Analysis of Histone Modification Genes in Grapevine Predict Their Involvement in Seed Development, Powdery Mildew Resistance, and Hormonal Signaling

Li Wang
Hebei Agricultural University
Bilal Ahmad
Northwest Agriculture and Forestry University
Chen Liang
Hebei Agricultural University
Xiaoxin Shi
Hebei Agricultural University
Ruyi Sun
Hebei Agricultural University
Songlin Zhang
Northwest Agriculture and Forestry University
Guoqiang Du (gdu@hebau.edu.cn)
Hebei Agricultural University

Research article

Keywords: VvHMs, VvSDG, Seed development, Phytohormones, Evolutionary history

Posted Date: July 15th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-27843/v2

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Version of Record: A version of this preprint was published on September 4th, 2020. See the published version at https://doi.org/10.1186/s12870-020-02618-7.

Abstract

Background: Histone modification genes (HMs) play potential roles in plant growth and development via influencing gene expression and chromatin structure. However, limited information is available about HMs genes in grapes (*Vitis vinifera* L.).

Results: Here, we described detailed genome-wide identification of HMs gene families in grapevine. We identified 117 HMs genes in grapevine and classified these genes into 11 subfamilies based on conserved domains and phylogenetic relationships with Arabidopsis. We described the genes in terms of their chromosomal locations and exon-intron distribution. Further, we investigated the evolutionary history, gene ontology (GO) analysis, and syntenic relationships between grapes and Arabidopsis. According to results 21% HMs genes are the result of duplication (tandem and segmental) events and all the duplicated genes have negative mode of selection. GO analysis predicted the presence of HMs proteins in cytoplasm, nucleus, and intracellular organelles. According to seed development expression profiling, many HMs grapevine genes were differentially expressed in seeded and seedless cultivars, suggesting their roles in seed development. Moreover, we checked the response of HMs genes against powdery mildew infection at different time points. Results have suggested the involvement of some genes in disease resistance regulation mechanism. Furthermore, the expression profiles of HMs genes were analyzed in response to different plant hormones (Abscisic acid, Jasmonic acid, Salicylic acid, and Ethylene) at different time points. All of the genes showed differential expression against one or more hormones.

Conclusion: *VvHMs* genes might have potential roles in grapevine including seed development, disease resistance, and hormonal signaling pathways. Our study provides first detailed genome-wide identification and expression profiling of HMs genes in grapevine.

Background

There are several posttranscriptional modification systems and processes including methylation, acetylation, phosphorylation, ubiquitination, and sumoylation, which can influence gene expression [1]. Histone modification and DNA methylation are very important because they regulate the pattern of gene expression by altering DNA availability and chromatin structure. These mechanisms are necessary in plant life cycle for the normal development and disease resistance [2]. Acetylation, deacetylation, methylation, and demethylation are common histone modifications (HMs) processes. These HMs processes are mostly controlled by four different histone modification gene families, which include histone methyltransferases (HMTs), histone demethylases (HDMs), histone acetylases (HATs), and histone deacetylases (HDACs) [3, 4].

These HMs gene families HMTs, HDMs, HATs, and HDACs contain 2, 2, 4, and 3 subfamilies, respectively. Each subfamily has specific domain or structure, thereby, plays different role in plant growth and development. Set domain group (SDG) and protein arginine methyltransferases (PRMT) are subfamilies

of HMTs, and contain set domain and Prma domain, respectively. The HDMs have HDMA (SWIRN and C-terminal domain) and JMJ (JmjC domain-containing proteins) subfamily. In *A. thaliana*, SDG proteins have been divided into seven classes on the basis of sequence similarity with set domain. The classes are I), Enhancer of zesta, II) ASH1 homologs, III) Trx homologs and related proteins, IV) Proteins having SET and PHD domain, V), Su (var) homologs, VI) Proteins having interrupted SET domain (S-ET), and VII) Ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco) methyltransferase (RBCMT). Members of every class have specific structures and functions [5-7]. According to the sequence characterization and in silico analysis of data, HATs are divided into four subfamilies: (1) HAC have p300/CREB (cAMP responsive element-binding protein)-binding protein (CBP) family), (2) HAG contained the general control non repressible 5-related N-terminal acetyltransferase (GNAT) family, (3) HAF contained the TATA-binding protein-associated factor (TAFII250) family, and (4) HAM type had MOZ, Ybf2/Sas3, Sas2, and Tip60 (MYST) family. Furthermore, HDACs are divided into HDA (RPD3 (Reduced Potassium Dependence) /HDA1 (Histone Deacetylase)), SRT (Silent information regulator 2), and HDT (Histone Deacetylase 2) family [8, 9].

Methylation is an important gene regulation mechanism, which can affect different processes and have potential roles in plant growth and development. Histone lysine methylation modifications are mainly catalyzed by different groups of histone methyltransferases (HMTs). Mostly, SDG genes play role in histone lysine methylation modifications. CURLY LEAF (CLF) and MEDEA (MEA) are the first recognized members of SDG [10, 11]. HMTs play multiple roles in plant life cycle such as cell cycle regulation, floral organ development, flowering transition, transcriptional activation and silencing, regulation of dormancy, fungal pathogens resistance, and influence on hormone signaling pathway genes during different (biotic & abiotic) stresses [12, 13]. For example, *AtASHH2* methylated H3K4 was recognized as a positive regulator of gene expression. Some SDG genes play potential roles in heterochromatin formation and gene silencing [14]. *AtSDG5* has been reported for its involvement in embryo abortion and endosperm development. *AtSDG2* influences gametophyte and sporophyte development [11]. PRMT proteins also play different roles in plants such as AtPRMT4A and 4B collectively influenced flowering time in Arabidopsis. In Arabidopsis, mutations in PRMT5 resulted in dark green and curly leaves with stunted growth [15].

HATs and HDACs play critical roles in the regulation of gene expression in plant growth and plant response to different stresses. For example, *AtHAM1 and AtHAM2* have role in male and female gamete formation [16]. Silencing of *AtHD2A* expression resulted in aborted seed development [17]. Furthermore, *AtHDA7* and *AtHD2B* have role in embryo development and seed dormancy, respectively [18, 19]. Overexpression of *OsglHAT1* increased grain weight and plant biomass by increasing cell number and rate of grain filling [20]. Functional analysis of *AtHDA15* and *AtHDA19* have shown their involvement in phtomorphogenesis [21, 22]. In other studies, *AtHDA19* has shown involvement in leaf and root development. These results suggest that HDA genes have varying roles in plants. *AtHDA5* and *AtHDA9* can regulate flowering time. Moreover, some genes such as *OsHAC701*, *OsHAG703*, *AtHD2C*, and *OsHDT701* have been reported for temperature, salt, and abscisic acid (ABA) stress response [23-26]. *AtHDA19* is involved in plant defense against fungal and bacterial diseases via influencing the expression

of JA, SA, and ethylene signaling pathway genes. Finally, HATs and HDACs play potential roles in transcriptional regulation of many developmental processes via interacting with transcription factors and various chromatin-remodeling factors [27].

Different families of HMs genes have been identified in many plant species including, *Arabidopsis thaliana*, *Oryza sativa*, *Solanum lycopersicum*, *Malus domestica*, *Citrus sinensis*, *Fragaria vesca*, *Zea mays*, and *Brassica rapa* . However, all four HMs gene families have been described only in *Arabidopsis thaliana, Solanum lycopersicum, Malus domestica*, and *Citrus sinensis* [18, 28-33]. Moreover, most of the functional studies have been carried out only in model plants e.g. Arabidopsis and rice. There is little information about the HMs genes number and functions in grapevine. The potential roles of HMs genes in different plants during different stages of plant growth and involvement in defense mechanism, justifies the need of comprehensive bioinformatics and expression analysis of HMs genes in grapevine. To the best of our knowledge this is the first comprehensive bioinformatic analysis of all four HMs gene families in grapevine. In this study, we performed identification of HMs genes in grapevine and then divided into different families on the basis of phylogenetic analysis with Arabidopsis. Moreover, carried out their exon-intron, evolutionary, synteny, and Go analysis. We also analyzed the response of HMs genes against powdery mildew infection and under different hormone treatments, along with expression profiling during different stages of ovule development in seeded and seedless cultivars. This study will increase understandings about grapevine HMs genes and will provide assistance for functional characterization of genes.

Methods

Identification of HMs genes in grapevine

To identify a complete list of HMs genes in the grapevine, Hidden Markov Model (HMM) profile of each gene family was downloaded from Pfam database (http://pfam.xfam.org/search#tabview=tab1) [31]. These HMM profiles were used as query against three public databases: Grape Genome Database (http://www.genoscope.cns.fr), the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/), and the Grapevine Genome CRIBI Biotech website (http://genomes.cribi.unipd.it/) by HMMER software to find full list of HMs proteins in grapevine [34]. Moreover, for the identification of HDT family, Basic Local Alignment Search Tool (BLAST) was used because this family has no HMM profile in Pfam database [31]. Previously, identified Arabidopsis and apple proteins annotated as HDT were taken and used as query for BlastP. Initially 148 HMs genes were identified. All non-redundant putative HMs genes were checked for conserved domains using SMART (http://smart.embl-heidelberg.de), and genes having incomplete domain or DUF (Domain of unknown function) were removed. In this way, 117 *VvHMs* genes were selected for final consideration.

Phylogenetic and exon- intron distribution analysis of HMs genes

The HMs protein sequences of Arabidopsis and grapes were used for phylogenetic analysis. MEGA 6.0 software was used to construct phylogenetic trees with the following parameters: ‘W’ approach for

sequence alignments, the neighbor-joining (NJ) method, p-distance, complete deletion and 1000 bootstrap values [35]. Different phylogenetic trees were generated for different subfamilies. The full genomic sequences and coding sequences of grapes were downloaded from Grape Genome Database (http://www.genoscope.cns.fr) and online Gene Structure Display Server 2.071 (http://gsds.cbi.pku.edu.cn/index.php) was used for exon-intron analysis.

Synteny analysis of grapevine and Arabidopsis genes

Two or more genes on the same chromosome within 200 kb were designated as tandemly duplicated [36]. The list of synteny region pairs within grape genome and comparison of duplication events between grape and Arabidopsis were downloaded from Plant Genome Duplication Database (http://chibba.agtec.uga.edu/duplication/) [37]. Circos version 0.63 (http://circos.ca/) with default parameters was used for generation of diagrams.

Estimation of Ka/Ks ratio for duplicated genes and Go analysis

An online software (http://services.cbu.uib.no/tools/kaks) was used for calculation of Non-synonymous substitution rate (Ka) and synonymous substitution rate (Ks) of duplicated genes. The Ka/Ks ratio was used for the estimation of selection pressure. The Ka/Ks value denotes three different situations of selection pressure i.e. neutral (equal to 1), positive (more than 1), and negative (less than 1) [38]. GO enrichment analysis was performed using MetGenMAP with default parameters [39].

Plant materials

In this study, five grapevine cultivars were used including two seeded cultivars (Kyoho and Muscat kyoho), two seedless cultivars (Flame seedless and Crimson seedless), and one powdery mildew-resistant, Chinese wild cultivar ‘Shang-24’ (*V. quinquangularis*). All the cultivars were maintained under natural environmental conditions at the grapevine orchard of Agricultural University of Hebei, Baoding, Hebei, China (38°51'N, 115°29'E). Where the average annual temperature is 13.4 ℃, (Below - 4.3 ℃ in winter and above 26.4 ℃ in summer), the average annual sunshine hours are 2511, and the mean annual precipitation is 498.9 mm. Young leaves of ‘Shang-24’ were inoculated with *Erysiphe necator* (Schw.) and harvested at 12, 24, and 48 h post-inoculation. Control leaves were inoculated with sterile water. For the preparation of hormone solution, ABA, Methyl jasmonate (MeJA), and Salicylic acid (SA) were first dissolved in 95% ethanol and then diluted down with water, while ethylene was directly dissolved in water. Hormone treatments were performed as a foliar spray with 300 μM ABA, 0.5 g/L ethylene, 50 μM MeJA or 100 μM SA, and samples were collected at 1, 6, and 12 h post-treatment. Control leaves were sprayed with sterile water. Furthermore, for seed development studies ovules were dissected from seedless and seeded cultivars at 20, 30, 40, and 50 days after flowering (DAF). All leaves and ovule samples were immediately frozen in liquid nitrogen and stored at -80℃ for future use. All the plant materials used and collected in this work comply with China’s guidelines and legislation.

Transcriptome analysis of *VvHMs* genes during ovule development in seeded and seedless grape progenies

Expression profiles of *VvHMs* genes during ovule development from seeded and seedless progenies were analyzed using our previously published transcriptome data (BioProject Accession, PRJNA338255) [40]. Cross-progeny populations from the seeded maternal parent 'Red Globe' and the seedless paternal parent 'Centennial seedless' were used. A total of 65 progeny were obtained including 31 seedless and 34 seeded. Nine seedless progeny and 9 seeded ones were randomly selected from the populations and were used for RNA-sequencing. Reads per kilobase of exon model per million mapped reads (RPKM) of *VvHMs* were transformed into heat maps using Mev 4.8.1 [41]. RPKM value of each VvHM gene was normalized based on the mean RPKM value of genes in all samples analyzed. Further, processed data was log2 transformed and was used to generate heatmap with Mev 4.8.1. Red and green color scale indicate high and low expression levels, respectively.

RNA extraction and expression analysis by Quantitative RT-PCR

Total RNA was extracted from samples by using the EZNA Plant RNA Kit (R6827-01, OMEGA Biotek, Norcross, GA, USA) according to manufacturer's guidelines. Nano drop spectrophotometer (Thermo Fisher Scientific, Yokohama, Japan) was used to measure OD value of RNA, and each RNA sample was subjected to agarose gel electrophoresis to check its quality. The Prime Script RTase (Trans Gen Biotech, Beijing, China) was used to remove gDNA and then for reverse transcription. Genome DNA was removed by gDNA Eraser (contains DNase). A qPCR reaction on crude RNA was also performed in order to access the degree of gDNA contamination. After this cDNA was diluted six times with distilled water for a final concentration of 100ng/μl. The grape *ACTIN* gene (GenBank Accession number NC_012010) and *EF1-α* gene (GenBank Accession number NC_012012) were used as internal reference genes. All primers were designed using Primer 5 software and can be found in the Additional file 1: Table S1. Three biological replicates and three technical replicates were used. For each reaction, 100 ng cDNA was used and carried out in triplicate with a reaction volume of 20 μl containing 0.8 μl each primer (1.0 μM), 1.0 μl of cDNA, 10 μl of 2 X Fast Super EvaGreen ® qPCR Mastermix (US Everbright Inc., Suzhou, China), and 7.4 μl sterile distilled water. The amplification parameters were 95°C for 30 s, followed by 40 cycles of 95°C for 5 s and 60°C for 30 s. Melting-curve analyses were performed with initial incubation at 95°C for 15 s and then a constant increase from 60 to 95°C. Relative expression levels were analyzed using the IQ5 software and the geNorm software.

Statistical analysis

Data was analyzed using SPSS Statistics 22.0 software. ANOVA with a Tukey post-hoc analysis was used to check the overall significance of data. Sigma Plot 12 was used to draw the graphs.

Results

Genome-wide identification and annotation of HMs genes

A total of 117 putative HMs genes were identified in the grape genome having 48 HMTs, 22 HDMs, 33 HATs, and 14 HDACs genes. According to previous studies [31-33], all of the genes were divided into 11 subfamilies based on the conserved domains. HMTs have 43 SDG and 5 PRMT genes; HDMs contain 15 JMJ and 7 HDMA genes; HATs have 28 HAG, 2 HAM, 2 HAC, and 1 HAF gene; and HDACs have 10, 2, and 2 genes of HDA, SRT, and HDT, respectively. These genes were named according to their families and their position on chromosomes. The predicted length of proteins in the SDG, PRMT, JMJ, HDMA, HAG, and HDA subfamilies ranges from 184-2199, 337-651, 131-1905, 492-984, 157-667, and 349-698 amino acids, respectively. Detailed information about genes including accession number, gene ID, chromosomal position, length of coding sequence, and length of protein sequence are presented in Table 1.

Phylogenetic and exon-intron analysis

To illustrate the phylogenetic history and to classify the HMs gene into different subfamilies, phylogenetic trees were constructed between Arabidopsis and grapevine HMs protein sequences (Fig. 1). Moreover, to deeply study the phylogentic realtionships unrooted tress were generated among *VvHMS* genes of same group, and intron-exon analysis was performed (Figure 2).

VvHMTs:

All 43 SDG genes were divided into seven classes on the basis of phylogenetic tree constructed between Arabidopsis and grape (Fig. 1A). Class V was further subdivided into subclass I and subclass II. Class I was found more conserved with respect to gene numbers, as grape and Arabidopsis contained same; 3 number of genes. Further, different classes contained different number of exons but almost same number of exons with in the same class e.g. class I. However, all five members of PRMTs contained different number of exons, suggesting that PRMTs class is less conserved. Same number of HMTs genes were found in Arabidosis and grapevine. These results suggest that HMTs family is more conserved as compared to other families.

VvHDMs:

A phylogenetic tree was constructed between JMJ proteins of grapevine and Arabidopsis. According to the Fig. 1B JMJ grapevine and Arabidopsis proteins were clustered into 4 and 5 different clades, respectively. Class I (JMJ- domain only), has only one member which is from Arabidopsis. Class II (KDM3), class III (KDM4), and class V (JMJD6) all have equal number of proteins in both species 6, 3, and 2, respectively. However, class IV (KDM5) contained 5 and 3 proteins of Arabidopsis and grapes, respectively. These results suggest that class II, III, and V are more conserved with respect to protein number. Further, exon-intron distribution and size was not same in the same class. However, all duplicated gene pairs have similar exon-intron structure (*VvJMJ2*/ *VvJMJ13*, *VvJMJ4*/ *VvJMJ11*, and *VvJMJ12*/ *VvJMJ14*), with multiple exons and introns, but have difference in size and distribution of exons. Grapevine and Arabidopsis contained 7 and 4 HDMAs proteins, respectively.

VvHATs and *VvHDACs*:

In HATs family, HAG subgroup contained the highest (28) number of genes among all groups. In HATs group more difference in number of genes was noticed between grape and Arabidopsis as compared to other groups, suggesting that HATs group have underwent more changes during the process of evolution. Tandemly duplicated gene pairs (*VvHAG3*/ *VvHAG4* and *VvHAG7*/ *VvHAG8*) showed conserved exon-intron distribution. Moreover, the HAG proteins have high bootstrap values, suggesting high similarity with each other. According to the phylogenetic analysis (Fig. 1C), genes of *VvHDA* were further divided into three classes. Interestingly, class III contained only 2 members, one from each grape and Arabidopsis.

GO (Gene ontology) analysis of HMs

We performed in silico GO analysis of grapevine genes at biological, cellular, and molecular level (Additional file 2: Fig. S1). Prediction of biological process suggested the involvement of HMs genes in regulation of gene expression, cellular component organization, flower development, response to biotic and abiotic stimulus, protein modification processes, and transcription process. Most of the HMs proteins were found in cytoplasm, nucleus, and intracellular organelles. HMs genes might have a role in binding (chromatin, DNA, and protein) transcription and transferase regulation activities at molecular level.

Chromosomal distribution and expansion pattern of HMs genes in grapevine

All 117 HMs genes were unevenly distributed on 20 chromosomes (Fig. 3). Chromosome seven contained the highest number of genes (11) while chromosome nineteen contained the lowest only one gene. The 43 *VvSDG* genes were randomly distributed on fourteen chromosomes, because chromosomes 2, 3, 6, 9, 17, and 19 has no genes. Four chromosomes 1, 4, 16, and 18 contained equal number of genes; five. These four chromosomes contained almost 50% of genes. For all other gene families same trend of uneven distribution was noticed (Fig. 3). Tandem duplication was calculated according to the criteria described in materials and methods.

Only three events of tandem duplication (*VvHAG3*/ *VvHAG4*, *VvHAG7*/ *VvHAG8*, and *VvSDG39*/ *VvSDG40*) were observed among six genes on three chromosomes. Two duplication pairs belonged to HAG family, suggesting that this family is less conserved during the evolutionary process. We also calculated the segmental duplication among HMs grapevine genes. Total 15 genes underwent segmental duplication in the form of eight pairs; *VvSDG4*/ *VvSDG30*, *VvSDG11*/ *VvSDG35*, *VvSDG29*/ *VvSDG15*, *VvSDG31*/ *VvSDG15*, *VvJMJ2*/ *VvJMJ13*, *VvJMJ11*/ *VvJMJ4*, *VvJMJ12*/ *VvJMJ14*, and *VvHDT1*/ *VvHDT2*. Interestingly, *VvSDG15* paired with two genes, *VvSDG29* and *VvSDG31*. These results suggest that both tandem and segmental duplication (21%) events have played a role in the expansion of HMs genes. These results can provide clues for evolutionary history and functional analysis. The ratio between Ka and Ks provides help to describe the evolutionary process [42]. Furthermore, Ka/Ks ratios of all duplicated genes were less than 1, suggesting that all genes have purifying selection pressure (Additional file 3: Table S2).

Evolutionary relationships between grapes and Arabidopsis

The roles of HMs genes have been well studied in model plants e.g. Arabidopsis and rice. Therefore, to gain insights about the origin and potential functions of HMs genes in grapevine and to explore the evolutionary history, we performed synteny analysis of grapes and Arabidopsis.

As shown in Fig. 4, a total of 69 orthologous pairs were identified between HMs genes of Arabidopsis and grapes comprising of 24, 3, 7, 3, 18, 2, 1, 3, 2, and 6 pairs of SDGs, PRMTs, JMJs, HDMAs, HAGs, HAMs, HACs, HDAs, SRTs, and HDTs, respectively (Additional file 4: Table S3). However, HAFs genes have no syntenic block. There were 24 syntenic blocks of SDG genes having 21 Arabidopsis and 19 grapevine genes. Moreover, there were four pairs of SDG, two pairs of PRMT, one pair of JMJ, four pairs of HAG, and two pairs of HDT, where a single grapevine gene paired with more than one gene of Arabidopsis. There were also five HMs Arabidopsis (3 SDG and 2 HDT) genes, which formed pair with two grapevine genes. All of the pairs contained members from the same subfamily, suggesting that they have a common origin before speciation.

Transcriptomic analysis of HMs genes during seed development

To get some clues about the potential roles of HMs grapevine genes in seed development, transcriptomic analysis of HMs grapevine genes was performed (Fig. 5). The samples were taken at three different stages of seed development from seeded and seedless progenies. Most of the HMs genes were highly expressed during successive stages of seed formation in both seeded and seedless progenies. However, most of the HDA family members were expressed relatively very low. There were some exceptions as well, for example, *VvJMJ14* showed up-regulation during the later stages of ovule development in seeded progenies as compared to seedless. In contract, *VvSDG30*, *VvHDA1*, *VvHAG23*, and *VvHDT1* were highly expressed during all stages of seed development in seedless cultivars, suggesting their potential role in ovule abortion. Therefore, we speculate that HM_S genes have potential roles in seed development and ovule abortion. We observed that some of the grapevine HMs gene pairs generated from duplication events showed similar expression patterns in gene pairs. For example, most of the duplicated gene pairs of JMJ family were conserved with respect to exon-intron distribution and expression pattern. However, there were some exceptions as well, *VvSDG4*/ *VvSDG30* and *VvSDG15*-*VvSDG29*/ *VvSDG31* (Segmentally), i.e. *VvSDG4* and *VvSDG30* has significantly different expression during progressive stages of seed development. Further with regard to duplicated genes, JMJ subfamily is more conserved as compared to SDG subfamily.

Expression pattern of HMs genes during different stages of seed development in different grape cultivars

To verify the reliability of transcriptome data, and to explore whether the expression pattern of VvHMs genes is widespread in different grape varieties, we selected 15 HMs genes and analyzed the expression pattern in different seeded (Kyoho and Muscat kyoho) and seedless (Flame seedless and Crimson seedless) grape cultivars during successive stages of ovule development. The genes were selected according to the results of transcriptome data of seed development (Fig. 5). These genes were comprised of represntatives of all subfamilies including differently (*VvHAC1, VvHAG23*, *VvHAM2, VvHDA1, VvHDA8,*

VvHDT1, VvJMJ14, VvJMJ15, VvSDG20, VvSDG32, and *VvSDG38*) and ubiquitously (*VvHAG4, VvHDMA4, VvPRMT4*, , and *VvSRT2*) expressed genes during different stages of seed development in seeded and seedless cultivars. As shown in Fig. 6, *VvJMJ14, VvJMJ15,* and *VvSDG38*, were significantly highly expressed during later stages of seed development in seeded cultivars. *VvSDG20* showed significantly higher expression during all stages of seed development in seeded cultivars compared to seedless ones. Further, the change ratio between seeded and seedless cultivars was relatively higher in *VvSDG20* as compared to other differentially expressed genes. Based on the results, we speculate that these genes might have a role in seed development. In contract to these results, some genes were signficantly highly expressed in some stages of seed formation in seeedless cultivars, for example, *VvHAG23*, *VvHDA8, VvHDT1,* and *VvPRMT4*, , . Moreover, *VvHAG4* showed remarkable difference in the expression pattern during all stages of seed development in seedless cultivars than seeded ones. These results suggest that these genes might have a role in ovule abortion. In general, the results of qRT-PCR are consistent with the results of transcriptome.

Expression profiling of HMs genes in response to *E. necator* inoculation and hormone treatments

To investigate the potential role of HMs genes against powdery mildew infection, we performed qRT-PCR analysis of fifteen HMs genes (same genes as for seed study) after infecting 'Shang-24' with *Erysiphe necator*. At 12 hpi the expression of three genes (*VvHAC1*, *VvHAG4,* and *VvHAG23*) was significantly up-regulated but subsequently down-regulated at 24 hpi (Fig. 7). However, the expression of *VvHAM2, VvHDA1*, *VvPRMT4*, *VvHDT1*, *VvSDG38*, and *VvSRT2* was down-regulated at 12 hpi, and up-regulated at 24 hpi. Moreover, some of the genes (*VvJMJ14*, *VvJMJ15*, and *VvSDG32*) showed no significant difference in expression between treated and control plants. These results suggest that some of the HMs genes are responsive to powdery mildew and might have a role in resistance mechanism. We also checked the responses of 15 HMs grapevine genes to ABA, ethylene, JA, and SA applications. In plants treated with ABA, most of the genes were down regulated at 12 hpi (Fig. 8). But *VvHAC1*, *VvHAG23*, *SDG20*, and *VvSRT2* genes were significantly up-regulated at 12 hpi. *VvHAG4*, *VvJMJ14*, and *VvSDG20* showed significantly differential expression at all-time points. However, *VvSDG38* gene showed same expression in control and treated plants at all-time points, suggesting that, except this all other genes have link with ABA regulation in grapevine. For JA treated plants, *VvHAC1,VvHAG4*, *VvHDA1*, *VvHDA8*, *VvJM14*, and *VvSDG32* showed significant up-regulation at all-time points. However, *VvHAG23* and *VvHDMA4* showed significant up- regulation only at 1 hpi. Further, *VvHDT1* was significantly down-regulated at 6 and 12 hpi after JA treatment (Fig. 9). In SA treated plants, *VvHAC1*, *VvHAG23*, *VvHAM2*, *VvHDA1*, *VvHDA8*, *VvHDMA4*, *VvJMJ14*, *VvSDG20*, and *VvSDG32* gene were significantly up-regulated at 1 hpi and most of these genes were down-regulated at 12 hpi (Fig. 10). Furthermore, seven genes (*VvHAC1*, *VvHAG4*, *VvHAG23*, *VvHAM2*, *VvHDA1*, *VvSDG32*, and *VvSRT2*) showed significantly higher expression at 12 hpi in ethylene treated plants as compared to control (Fig. 11). Moreover, the expression of *VvHAG23*, *VvHAM2*, and *VvHDA1* was similar with each other at all-time points i.e. same expression at 1 hpi, down-regulation at 6 hpi and subsequent up-regulation at 12 hpi. However, *VvJMJ15* and *VvSDG20* showed significant down-regulation at 12 hpi. These results suggest that most of the HMs genes have role in

defense mechanism of grapevine in response to *E. necator* inoculation and have involvement in hormone pathways.

Discussion

HMs gene families have been well studied in model plants and affects many processes during plant growth and development including root growth, flower induction, floral organogenesis, embryo development, and plant responses to biotic and abiotic stresses [43-45]. In recent years HMs genes have been identified in fruit crops including citrus, apple, and strawberry. These studies have reported the putative functions of these genes in flower induction, fruit development, and response to blue mold infection, cold, and heat stress [31-33]. However, a limited information is available about the HMs gene numbers and functions in grapevine. In this study, we have described the detailed bioinformatics and expression analysis of HMs genes against *E. necator* inoculation and different hormonal treatments. We identified 117 *VvHMs* genes and divided them into 11 subfamilies on the basis of phylogenetic relationships with Arabidopsis and conserved domains. All the genes were named according to their family and position on the chromosomes [31, 32]. Previously, in grapevine Aquea et al. [46, 47] has reported 7, 13, and 33 genes in HAT, HDAC, and SDG subfamilies, respectively. However, we identified more number of genes as compared to previous studies, the difference in number of genes is due to advancements in gene searching websites and different searching methods. Among all families of VvHMs, the VvSDG family was more conserved and VvHAG was less conserved. Our results are also supported by exon-intron analysis (Fig. 2). Moreover, SDG family is also conserved with respect to gene number in different species i.e. grapevine, Arabidopsis, citrus, and tomato have 43, 41, 40, and 43 SDG genes, respectively [32]. According to predicted protein length HDA subfamily members are more conserved as compared to other subfamilies (Table1). Further, our findings are concordant with previous findings in other crops [31-33].

Gene duplication has played a vital role in the expansion of gene families [48]. According to studies segmental and tandem duplication both have significant contributions in the expansion of grapevine gene families [49]. We observed the previous trend with respect to genes distribution i.e. highly variable distribution of genes on chromosomes. Highest number of genes were present on chromosome seven. This suggests that most of the duplication events were occurred on this chromosome. These observations are supported by results as both type of duplication pairs (tandem and segmental) were found on chromosome seven, suggesting that this chromosome has played potential role in the expansion of HMs gene families. Moreover, more duplication events might be the reason of clustering of genes on chromosome but we have no evidences to support this hypothesis [50]. According to results, 21% of the HMs genes are the results of duplication events. We observed more segmental duplication (8 pairs) events as compared to tandem duplication (3 pairs) events. In case of duplication events we observed the previous trend as the segmental duplication has occurred more commonly than tandem duplication during the evolutionary process in plants [48]. Further, pairing of *VvSDG15* with two genes (*VvSDG29* and *VvSDG31*), suggests that it might have diverse roles in plant growth and development. Moreover, more tandem duplication events in VvHAG gene family further justify our results of gene

number and exon-intron analysis that HAG gene family is less conserved. Generally, duplicated gene pairs showed conservation with regard to exon-intron distribution and expression pattern. However, there were some differences as well, *VvSDG4*/ *VvSDG30* and *VvSDG15*-*VvSDG29*/ *VvSDG31* (Segmentally), i.e. *VvSDG15* showed ubiquitous expression during all stages of seed development in both seeded and seedless progenies, while expression of *VvSDG29* was low in seeds of both seeded and seedless progenies (Fig. 5). The duplicated gene pairs of JMJ subfamily were found more conserved in exon-intron distribution and expression pattern. The ratio between Ka and Ks provides help to describe the evolutionary process [43]. According to results all duplicated genes have negative mode of selection. Previously, similar results about HMs genes have been reported in other fruit crops i.e. Apple, citrus, and strawberry [31-33].

Synteny analysis is a very important bioinformatic tool as synteny blocks can provide inklings about gene evolution and function [51]. We found 68 synteny blocks (Segmental duplication pairs) between grapes and Arabidopsis HMs genes. Presence of more segmental duplication pairs between grapevine and Arabidopsis HM_S genes, suggest that they might have some common origin before speciation. However, it is difficult to explain evolutionary history only on the basis of syntenic relationships. Based on the potential roles of orthologous genes of grapevine in other crops we can predict functions of HMs genes in grapes.

Previously, some transcription factors and gene families have been reported for their roles in grapevine seed development, such as MADS-box, B3, and GASA gene family [34, 35, 42]. In Arabidopsis HMs genes, especially SDG family has been well studied for their potential roles in vegetative and reproductive growth of plant. Expression analysis of genes can provide clues about gene function. Based on presumed roles of HMs genes in other crops, we performed expression profiling of selected HMs grapevine genes at different stages of seed development, against infection of *Erysiphe necator*, and after application of multiple plant hormones. The results were obtained as anticipated, for example *VvSDG20* and *VvSDG32* showed different expression in seeds of seeded and seedless cultivars. According to previous study, *VvSDG12*, *VvSDG14*, *VvSDG16*, *VvSDG25*, and *VvSDG37* (Previously known as SDG6934, SDG66905, SDG66903, SDG66932, and SDG66911) showed high expression in flowers and fruits of grapevine [46]. In our study, these genes showed ubiquitous expression in both seeded and seedless ovules. Therefore, we speculate that these genes might have a general role in the reproductive process. Plant hormones, such as ABA, ethylene, JA, and SA, play important roles in regulating developmental processes and signaling networks involved in plant responses to biotic and abiotic stresses. In our study, *VvPRMT4* was up-regulated against *Erysiphe necator* and after SA treatments. Further, the expression of *VvSDG32* was also affected by JA and SA applications. Previously, the involvement of SDG genes in embryo development, fruit development, and response to biotic and abiotic stimulus in citrus [31], strawberry [33], and tomato [52] have been reported. In case of HDMs genes (*VvJMJ14*, *VvJMJ15*, and *VvHDMA4*) similar results like HMTs genes were noticed. Therefore, we speculated that HMTs and HDMs genes might have potential roles in seed development and disease resistance via affecting hormonal signaling pathways. In

general, our study provides some clues about gene functions based on bioinformatic and expression analysis. A future study is needed to get exact information about gene functions.

VvHAC1 gene showed differential expression in response to powdery infection, ABA, JA, SA, and ethylene application in treated and control plants. Li et al. [53] reported the involvement of *AtHAC1* (orthologous of *VvHAC1*) in ethylene signaling pathway. Further, mutants of *AtHAC1* showed resistance to high concentrations of glucose during early stages of root development. *VvHAM2* was up-regulated against JA and SA exogenous application and showed significant down-regulation to exogenous application of ethylene. In Arabidopsis, *HAM1/2* has been reported for their involvement in male and female gametogenesis [16]. These results suggest that HATs genes play vital role in plant development via regulating gene expression. HDACs genes also play potential roles in the regulation of gene expression and chromatin structure [47]. For example, *AtHDA19* played significant roles in plant defense via regulating multiple hormonal signaling pathways and networking with *WRKY38* and *WRKY62*. In our studies, *VvHDA1* (orthologous of *AtHDA19*) showed significant change in expression against disease attack and hormonal applications, suggesting its involvement in multiple biological processes. Moreover, *VvSRT2* showed significant down-regulation against powdery mildew attack and ethylene application. Similar type of results were obtained for SRT genes functions in Arabidopsis and rice [54, 55]. Finally, our study provides inklings about functions of HMs genes in grapevine.

Conclusion

In this study, we identified 117 *HMs* genes in grapes. We divided the genes into four subgroups and subsequently divided these groups into 11 subfamilies. Further, we analyzed the genes in terms of chromosomal locations, exon-intron distribution, and evolutionary history. According to evolutionary history, the segmental and tandem duplication both have played role in the expansion of *HMs* genes in grapevine. According to the results, *VvHMs* genes might have role in seed development, disease resistance, and multiple hormonal signaling pathways. Our study is useful for functional characterization of *VvHMs* genes.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The datasets supporting the conclusions of this article are included within the article and its additional files.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the Strat-up Funds of Hebei Agricultural University to Li Wang (YJ201806) and the Natural Science Foundation of Hebei, China (C2018204055). These funding bodies had no role in the design of this study, collection, analysis, and interpretation of data or in writing the manuscript.

Authors' contributions

LW designed the research; GD supervised the experiments; LW and CL performed most of the experiments; BA, RS and SZ provided technical assistance; LW and XS analyzed the data; BA and LW wrote the article with contributions of all the authors. All authors read and approved the final manuscript.

Acknowledgements

The authors thank all editors and reviewers for their comments on this manuscript.

Abbreviations

ABA: Abscisic acid; BLAST: Basic Local Alignment Search Tool; CBP: (cAMP responsive element-binding protein)-binding protein; CLF: CURLY LEAF; DAF: Days after flowering; Eth: ethylene; GNAT: General control non repressible 5-related N-terminal acetyltransferase; GO: Gene ontology; HATs: Histone acetylases; HDA1: Histone Deacetylase; HDACs: Histone deacetylases; HDMs: Histone demethylases; HDT: Histone Deacetylase 2; HMs: Histone modifications; HMTs: Histone methyltransferases; HMM: Hidden Markov Model; JMJ: JmjC domain-containing proteins; Ka: Non-synonymous substitution rate; Ks: Synonymous substitution rate; MEA: MEDEA; MeJA: Methyl jasmonate; NJ: neighbor-joining; PRMT: Protein arginine methyltransferases; RPD3: Reduced Potassium Dependence; SA: Salicylic acid; SDG: Set domain group; S-ET: SET domain; SRT: Silent information regulator; TAFII250: TATA-binding protein-associated factor;

References

1. Yuan L, Liu X, Luo M, Yang S, Wu K. Involvement of Histone Modifications in Plant Abiotic Stress Responses. J Integr Plant Biol. 2013;55(10):892-901.
2. Cedar H, Bergman Y. Linking DNA methylation and histone modification: patterns and paradigms. Nat Rev Genet. 2009;10(5):295-304.

3. Liu X, Yang S, Yu CW, Chen CY, Wu K. Histone Acetylation and Plant Development. The Enzymes. 2016;40:173-199.
4. Liu C, Lu F, Cui X, Cao X. Histone methylation in higher plants. Annu Rev Plant Biol. 2010;61:395-420.
5. Dong G, Ma D-P, Li J. The histone methyltransferase SDG8 regulates shoot branching in *Arabidopsis*. Biochem Biophys Res Commun. 2008;373(4):659-664.
6. Cazzonelli CI, Cuttriss AJ, Cossetto SB, Pye W, Crisp P, Whelan J, Finnegan EJ, Turnbull C, Pogson BJ. Regulation of Carotenoid Composition and Shoot Branching in *Arabidopsis* by a Chromatin Modifying Histone Methyltransferase, SDG8. Plant Cell. 2009;21(1):39-53.
7. Cazzonelli CI, Nisar N, Roberts AC, Murray KD, Borevitz JO, Pogson BJ. A chromatin modifying enzyme, SDG8, is involved in morphological, gene expression, and epigenetic responses to mechanical stimulation. Front Plant Sci. 2014;5.
8. Pandey R, Muller A, Napoli CA, Selinger DA, Pikaard CS, Richards EJ, Bender J, Mount DW, Jorgensen RA. Analysis of histone acetyltransferase and histone deacetylase families of *Arabidopsis thaliana* suggests functional diversification of chromatin modification among multicellular eukaryotes. Nucleic Acids Res. 2002;30(23):5036-5055.
9. Feng Q, Wang HB, Ng HH, Erdjument-Bromage H, Tempst P, Struhl K, Zhang Y. Methylation of H3-lysine 79 is mediated by a new family of HMTases without a SET domain. Curr Biol. 2002;12(12):1052-1058.
10. Goodrich J, Puangsomlee P, Martin M, Long D, Meyerowitz EM, Coupland G. A polycomb-group gene regulates homeotic gene expression in *Arabidopsis*. Nature. 1997; 386(6620):44-51.
11. Grossniklaus U, Vielle-Calzada JP, Hoeppner MA, Gagliano WB. Maternal control of embryogenesis by *MEDEA*, a *Polycomb* group gene in *Arabidopsis*. Science. 1998;280(5362):446-450.
12. Avramova Z. Transcriptional 'memory' of a stress: transient chromatin and memory (epigenetic) marks at stress-response genes. Plant J. 2015;83(1):149-159.
13. Thorstensen T, Grini PE, Aalen RB. SET domain proteins in plant development. BBA-Gene Regul Mech. 2011;1809(8):407-420.
14. Zhao Z, Yu Y, Meyer D, Wu C, Shen W-H. Prevention of early flowering by expression of *FLOWERING LOCUS C* requires methylation of histone H3 K36. Nat Cell Biol. 2005;7(12):1256-1260.
15. Pei Y, Niu L, Lu F, Liu C, Zhai J, Kong X, Cao X. Mutations in the type II protein arginine methyltransferase AtPRMT5 result in pleiotropic developmental defects in Arabidopsis. Plant Physiol. 2007;144(4):1913-1923.
16. Latrasse D, Benhamed M, Henry Y, Domenichini S, Kim W, Zhou D-X, Delarue M. The MYST histone acetyltransferases are essential for gametophyte development in Arabidopsis. BMC Plant Biol. 2008;8.
17. Wu KQ, Tian LN, Malik K, Brown D, Miki B. Functional analysis of HD2 histone deacetylase homologues in *Arabidopsis thaliana*. Plant J. 2000; 22(1):19-27.

18. Cigliano RA, Sanseverino W, Cremona G, Ercolano MR, Conicella C, Consiglio FM. Genome-wide analysis of histone modifiers in tomato: gaining an insight into their developmental roles. BMC Genomics. 2013;14:57.
19. Yano R, Takebayashi Y, Nambara E, Kamiya Y, Seo M. Combining association mapping and transcriptomics identify *HD2B* histone deacetylase as a genetic factor associated with seed dormancy in *Arabidopsis thaliana*. Plant J. 2013;74(5):815-828.
20. Song XJ, Kuroha T, Ayano M, Furuta T, Nagai K, Komeda N, Segami S, Miura K, Ogawa D, Kamura T et al. Rare allele of a previously unidentified histone H4 acetyltransferase enhances grain weight, yield, and plant biomass in rice. P Natl Acad Sci USA. 2015;112(1):76-81.
21. Liu XC, Chen CY, Wang KC, Luo M, Tai R, Yuan LY, Zhao ML, Yang SG, Tian G, Cui YH et al. PHYTOCHROME INTERACTING FACTOR3 Associates with the Histone Deacetylase HDA15 in Repression of Chlorophyll Biosynthesis and Photosynthesis in Etiolated *Arabidopsis* Seedlings. Plant Cell. 2013;25(4):1258-1273.
22. Benhamed M, Bertrand C, Servet C, Zhou D. *ArabidopsisGCN5,HD1,* and *TAF1/HAF2* Interact to Regulate Histone Acetylation Required for Light-Responsive Gene Expression. The Plant Cell. 2006;18(11):2893-2903.
23. Liu X, Luo M, Zhang W, Zhao JH, Zhang JX, Wu KQ, Tian LN, Duan J. Histone acetyltransferases in rice (*Oryza sativa* L.): phylogenetic analysis, subcellular localization and expression. BMC Plant Biol. 2012;12:17.
24. Long JA, Ohno C, Smith ZR, Meyerowitz EM. TOPLESS regulates apical embryonic fate in *Arabidopsis*. Science. 2006;312(5779):1520-1523.
25. To TK, Nakaminami K, Kim JM, Morosawa T, Ishida J, Tanaka M, Yokoyama S, Shinozaki K, Seki M. *Arabidopsis HDA6* is required for freezing tolerance. Biochem Biophys Res Commun. 2011;406(3):414-419.
26. Bertrand C, Bergounioux C, Domenichini S, Delarue M, Zhou DX. Arabidopsis histone acetyltransferase AtGCN5 regulates the floral meristem activity through the *WUSCHEL/AGAMOUS* pathway. J Biol Chem. 2003;278(30):28246-28251.
27. Liu X, Yang S, Yu CW, Chen CY, Wu K. Histone Acetylation and Plant Development. The Enzymes. 2016;40:173-199.
28. Pontvianne F, Blevins T, Pikaard CS. Arabidopsis Histone Lysine Methyltransferases. Adv Bot Res. 2010;53:1-22.
29. Lu ZH, Huang XL, Ouyang YD, Yao JL. Genome-Wide Identification, Phylogenetic and Co-Expression Analysis of *OsSET* Gene Family in Rice. Plos One. 2013;8(6):15.
30. Qian YX, Xi YL, Cheng BJ, Zhu SW. Genome-wide identification and expression profiling of DNA methyltransferase gene family in maize. Plant Cell Rep. 2014;33(10):1661-1672.
31. Xu J, Xu H, Liu Y, Wang X, Xu Q, Deng X. Genome-wide identification of sweet orange (*Citrus sinensis*) histone modification gene families and their expression analysis during the fruit development and fruit-blue mold infection process. Front Plant Sci. 2015;6:607.

32. Fan S, Wang J, Lei C, Gao C, Yang Y, Li Y, An N, Zhang D, Han M. Identification and characterization of histone modification gene family reveal their critical responses to flower induction in apple. BMC Plant Biology. 2018;18(1):173.
33. Gu T, Han Y, Huang R, Mcavoy RJ, Li Y. Identification and characterization of histone lysine methylation modifiers in *Fragaria vesca*. Sci Rep-UK. 2016;6(1):23581-23581.
34. Ahmad B, Zhang S, Yao J, Rahman MU, Hanif M, Zhu Y, Wang X. Genomic Organization of the B3-Domain Transcription Factor Family in Grapevine (*Vitis vinifera* L.) and Expression during Seed Development in Seedless and Seeded Cultivars. Int J Mol Sci. 2019;20(18).
35. Ahmad B, Yao J, Zhang S, Li X, Zhang X, Yadav V, Wang X: Genome-Wide Characterization and Expression Profiling of GASA Genes during Different Stages of Seed Development in Grapevine (*Vitis vinifera* L.) Predict Their Involvement in Seed Development. Int J Mol Sci. 2020;21(3).
36. Holub EB. The arms race is ancient history in *Arabidopsis*, the wildflower. Nat Rev Genet. 2001;2(7):516-527.
37. Lee TH, Tang HB, Wang XY, Paterson AH. PGDD: a database of gene and genome duplication in plants. Nucleic Acids Res. 2013;41(D1):D1152-D1158.
38. Siltberg J, Liberles DA. A simple covarion-based approach to analyse nucleotide substitution rates. J Evol Biol. 2002;15(4):588-594.
39. Joung JG, Corbett AM, Fellman SM, Tieman DM, Klee HJ, Giovannoni JJ, Fei ZJ. Plant MetGenMAP: An Integrative Analysis System for Plant Systems Biology. Plant Physiol. 2009;151(4):1758-1768.
40. Wang L, Hu X, Jiao C, Li Z, Fei Z, Yan X, Liu C, Wang Y, Wang X. Transcriptome analyses of seed development in grape hybrids reveals a possible mechanism influencing seed size. Bmc Genomics. 2016; 17(1):898.
41. Howe EA, Sinha R, Schlauch D, Quackenbush J. RNA-Seq analysis in MeV. Bioinformatics. 2011;27(22):3209-3210.
42. Wang L, Yin XJ, Cheng CX, Wang H, Guo RR, Xu XZ, Zhao J, Zheng Y, Wang XP. Evolutionary and expression analysis of a MADS-box gene superfamily involved in ovule development of seeded and seedless grapevines. Mol Genet Genomics. 2015;290(3):825-846.
43. Morgan CC, Loughran NB, Walsh TA, Harrison AJ, O'Connell MJ. Positive selection neighboring functionally essential sites and disease-implicated regions of mammalian reproductive proteins. BMC Evol Biol. 2010;10:17.
44. Nelissen H, Boccardi TM, Himanen K, Van Lijsebettens M. Impact of core histone modifications on transcriptional regulation and plant growth. Crit Rev Plant Sci. 2007;26(5-6):243-263.
45. Shen W-H, Xu L. Chromatin Remodeling in Stem Cell Maintenance in *Arabidopsis thaliana*. Mol Plant. 2009;2(4):600-609.
46. Aquea F, Vega A, Timmermann T, Josefina PM, Arce-Johnson P. Genome-wide analysis of the SET DOMAIN GROUP family in Grapevine. Plant Cell Rep. 2011;30(6):1087-1097.

47. Aquea F, Timmermann T, Arcejohnson P. Analysis of histone acetyltransferase and deacetylase families of *Vitis vinifera*. Plant Physiol Bioch. 2010;48(2):194-199.
48. Cannon S, Mitra A, Baumgarten A, Yong N, May G. The roles of segmental and tandem gene duplication in the evolution of large gene families in *Arabidopsis thaliana*. BMC Plant Biol. 2004;4:10.
49. Jaillon O, Aury J, Noel B, Policriti A, Clepet C, Casagrande A, Choisne N, Aubourg S, Vitulo N, Jubin C. The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. Nature. 2007;449(7161):463-467.
50. Xia F, Sun T, Yang S, Wang X, Chao J, Li X, Hu J, Cui M, Liu G, Wang D et al. Insight into the B3 Transcription Factor Superfamily and Expression Profiling of *B3* Genes in Axillary Buds after Topping in Tobacco (*Nicotiana tabacum* L.). Genes. 2019;10(2).
51. Lyons E, Pedersen B, Kane J, Alam M, Ming R, Tang HB, Wang XY, Bowers J, Paterson A, Lisch D et al. Finding and Comparing Syntenic Regions among Arabidopsis and the Outgroups Papaya, Poplar, and Grape: CoGe with Rosids. Plant Physiol. 2008;148(4):1772-1781.
52. Zhong SL, Fei ZJ, Chen YR, Zheng Y, Huang MY, Vrebalov J, McQuinn R, Gapper N, Liu B, Xiang J et al. Single-base resolution methylomes of tomato fruit development reveal epigenome modifications associated with ripening. Nat Biotechnol. 2013;31(2):154-159.
53. Li C, Xu J, Li J, Li Q, Yang H. Involvement of Arabidopsis Histone Acetyltransferase *HAC* Family Genes in the Ethylene Signaling Pathway. Plant Cell Physiol. 2014;55(2):426-435.
54. Huang L, Sun Q, Qin F, Li C, Zhao Y, Zhou D. Down-Regulation of a *SILENT INFORMATION REGULATOR2*-Related Histone Deacetylase Gene, *OsSRT1*, Induces DNA Fragmentation and Cell Death in Rice. Plant Physiol. 2007;144(3):1508-1519.
55. Wang C, Gao F, Wu J, Dai J, Wei C, Li Y. Arabidopsis Putative Deacetylase AtSRT2 Regulates Basal Defense by Suppressing *PAD4*, *EDS5* and *SID2* Expression. Plant Cell Physiol. 2010;51(8):1291-1299.

Table

Table 1. List of *VvHM* genes.

Gene Name	Accession No.	VCost.v3 ID	Chromosome	Start	End	CDS (bp)	ORF (aa)
SDG gene family							
VvSDG1	XP_019077603.1	Vitvi01g00086	1	949657	957893	1449	482
VvSDG2	XP_002281246.1	Vitvi01g00484	1	5390829	5401654	1452	484
VvSDG3	XP_002267249.1	Vitvi01g01058	1	14007416	14010547	1455	485
VvSDG4	XP_019078100.1	Vitvi01g01765	1	22469075	22482650	1458	486
VvSDG5	XP_010657698.1	Vitvi01g01812	1	22873387	22877304	984	328
VvSDG6	XP_010648716.1	Vitvi04g00550	4	5937871	5970124	3336	1111
VvSDG7	XP_019075140.1	Vitvi04g00878	4	11443796	11463685	1350	449
VvSDG8	XP_019075187.1	Vitvi04g01029	4	15011615	15039726	1698	565
VvSDG9	XP_002269759.2	Vitvi04g01208	4	17398366	17416188	3948	1316
VvSDG10	XP_002281774.1	Vitvi04g01653	4	22226309	22252207	1125	375
VvSDG11	XP_002282057.2	Vitvi05g00821	5	9227788	9230583	1554	518
VvSDG12	XP_002282386.1	Vitvi05g00855	5	9819374	9821392	1767	588
VvSDG13	XP_002268621.1	Vitvi05g02135	5	20741174	20798303	3207	1068
VvSDG14	XP_010652245.1	Vitvi07g00417	7	4010241	4015186	1149	382
VvSDG15	XP_019076867.1	Vitvi07g00675	7	7299138	7313963	2148	715
VvSDG16	XP_019076670.1	Vitvi07g01721	7	16552767	16572286	2805	935
VvSDG17	XP_002267602.2	Vitvi07g02035	7	20552339	20557870	1503	500
VvSDG18	XP_002277066.1	Vitvi08g00145	8	2654151	2659935	555	184
VvSDG19	XP_002278728.1	Vitvi08g00900	8	11294684	11297803	1434	478
VvSDG20	XP_002276611.1	Vitvi08g01786	8	20728208	20735141	1983	661
VvSDG21	XP_010655344.2	Vitvi10g00002	10	36174	37778	1512	504
VvSDG22	XP_002272781.1	Vitvi11g00800	11	9538580	9561355	1485	494
VvSDG23	XP_010656836.1	Vitvi11g01240	11	18553723	18566361	1752	584
VvSDG24	XP_002274324.1	Vitvi12g00537	12	7248181	7258220	1395	465
VvSDG25	XP_010657340.1	Vitvi12g00568	12	7477102	7493792	6600	2199
VvSDG26	XP_002267469.2	Vitvi12g00746	12	10000712	10005486	1494	497
VvSDG27	XP_010658301.1	Vitvi13g00002	13	12083	63791	2151	717
VvSDG28	XP_002275729.1	Vitvi13g00215	13	2012817	2037101	1464	487
VvSDG29	XP_010660173.1	Vitvi14g00738	14	12583680	12614451	582	193
VvSDG30	XP_010660678.1	Vitvi14g01462	14	24836624	24881775	2106	702
VvSDG31	XP_010661928.1	Vitvi15g00976	15	17677390	17689653	3012	1004
VvSDG32	XP_019081670.1	Vitvi16g00036	16	370309	399658	1317	438
VvSDG33	XP_002277774.1	Vitvi16g00290	16	5345035	5346423	1332	443
VvSDG34	XP_019081389.1	Vitvi16g00314	16	5891859	5894009	1671	557
VvSDG35	XP_010662976.1	Vitvi16g01288	16	20769710	20783861	3057	1019
VvSDG36	XP_002275757.1	Vitvi16g02079	16	21677420	21681935	1203	401
VvSDG37	XP_010664163.1	Vitvi18g00220	18	2259998	2287308	5877	1959
VvSDG38	XP_010664254.1	Vitvi18g00318	18	3446050	3490947	1433	481
VvSDG39	XP_019072296.1	Vitvi18g02964	18	19242564	19274978	2589	863
VvSDG40	XP_003634538.1	Vitvi18g02965	18	19280780	19290355	1113	370
VvSDG41	XP_010665141.1	Vitvi18g01629	18	19587111	19592826	1602	534
VvSDG42	XP_010646790.1	Vitvi02g01790	Un	22578033	22580210	1581	526
VvSDG43	XP_019074011.1	Vitvi10g00297	Un	28765455	28776585	3263	1127
PRMT gene family							
VvPRMT1	XP_019075123.1	Vitvi04g00275	4	2582427	2595604	1953	651
VvPRMT2	XP_002273923.1	Vitvi05g00322	5	3203018	3217695	1578	525
VvPRMT3	XP_002267286.2	Vitvi10g01540	10	17242398	17277211	1245	414
VvPRMT4	XP_002282760.1	Vitvi11g00208	11	2016637	2022471	1053	351
VvPRMT5	XP_002285026.1	Vitvi11g00426	11	4126320	4146088	1014	337
JMJ gene family							
VvJMJ1	XP_010660757.1	Vitvi02g00329	2	3074405	3119122	4488	1495
VvJMJ2	XP_010663122.1	Vitvi02g00759	2	8573734	8582220	2199	732

VvJMJ3	XP_010648867.1	Vitvi04g00769	4	9601201	9680561	2589	862
VvJMJ4	XP_010652382.1	Vitvi07g00618	7	6483761	6483761	2364	787
VvJMJ5	XP_002269129.1	Vitvi08g00216	8	4253675	4259819	393	131
VvJMJ6	XP_010655372.1	Vitvi10g00053	10	462316	466886	1941	646
VvJMJ7	XP_010655862.1	Vitvi10g01120	10	11527946	11538707	2856	952
VvJMJ8	XP_010655918.1	Vitvi10g01394	10	14142558	14157354	3060	1019
VvJMJ9	XP_010658392.1	Vitvi13g00630	13	5944228	5969863	4653	1551
VvJMJ10	XP_010659626.1	Vitvi14g00196	14	2053704	2057478	1473	490
VvJMJ11	XP_002266063.2	Vitvi14g00627	14	10528574	10546325	3537	1178
VvJMJ12	XP_019081261.1	Vitvi15g00950	15	17348763	17355815	2694	898
VvJMJ13	XP_002272599.2	Vitvi16g00120	16	1798809	1811815	3030	1009
VvJMJ14	XP_010662857.1	Vitvi16g01410	16	21915589	21926695	5987	1905
VvJMJ15	XP_010663055.1	Vitvi17g01001	17	11978867	11993275	2850	950
HDMA gene family							
VvHDMA1	XP_003631607.1	Vitvi03g00349	3	5641165	5655774	2952	984
VvHDMA2	XP_002265069.1	Vitvi03g01141	3	16377478	16379930	2010	669
VvHDMA3	XP_010658366.1	Vitvi13g00661	13	6501373	6505907	2805	935
VvHDMA4	XP_002280389.1	Vitvi17g00081	17	717752	722776	1692	563
VvHDMA5	XP_002275451.1	Vitvi18g00045	18	531976	535425	1479	492
VvHDMA6	XP_010664183.1	Vitvi18g00243	18	2543733	2568290	2028	675
VvHDMA7	XP_010664765.1	Vitvi18g01059	18	11641452	11647885	2316	772
HAG gene family							
VvHAG1	XP_002284766.1	Vitvi04g00414	4	4185433	4190152	495	165
VvHAG2	XP_002272537.1	Vitvi05g00123	5	788238	792303	1254	417
VvHAG3	XP_002278864.1	Vitvi05g00523	5	5399371	5399889	519	172
VvHAG4	XP_002282836.1	Vitvi05g00524	5	5401210	5402158	543	180
VvHAG5	XP_002278620.1	Vitvi06g00679	6	7667663	7669004	1155	384
VvHAG6	XP_010651587.1	Vitvi06g01092	6	14154983	14172657	867	289
VvHAG7	XP_002275146.2	Vitvi07g00052	7	77994	88805	1251	417
VvHAG8	XP_010651956.1	Vitvi07g00050	7	89628	110743	1317	439
VvHAG9	XP_002264391.3	Vitvi17g00723	7	948363	958053	1023	341
VvHAG10	XP_002278246.1	Vitvi07g00126	7	1190489	1221867	525	175
VvHAG11	XP_010652717.1	Vitvi07g01604	7	15521919	15523764	1266	422
VvHAG12	XP_010653125.1	Vitvi08g00051	8	875613	882448	666	222
VvHAG13	XP_010654086.1	Vitvi08g01460	8	17298621	17301373	474	157
VvHAG14	XP_019077012.1	Vitvi08g01691	8	19763293	19771775	2001	667
VvHAG15	XP_002264353.1	Vitvi09g01127	9	16169584	16171633	1203	400
VvHAG16	XP_002278105.2	Vitvi11g00083	11	928669	934564	1572	524
VvHAG17	XP_002270456.1	Vitvi11g00847	11	10597926	10613592	840	280
VvHAG18	XP_002267085.1	Vitvi12g00340	12	5074039	5075368	477	159
VvHAG19	XP_010657419.1	Vitvi13g01304	12	9037307	9044911	588	196
VvHAG20	XP_002270840.1	Vitvi13g01879	13	101740	106579	825	275
VvHAG21	XP_002273746.1	Vitvi13g00612	13	5707150	5718261	813	271
VvHAG22	XP_002273620.1	Vitvi13g00635	13	6006136	6007252	663	221
VvHAG23	XP_002273592.1	Vitvi13g01641	13	21451060	21451860	483	160
VvHAG24	XP_002276364.1	Vitvi14g01459	14	24820195	24822192	615	205
VvHAG25	XP_002273780.1	Vitvi16g00111	16	1730687	1738891	765	255
VvHAG26	XP_002262701.1	Vitvi17g01026	17	12379862	12388649	1368	455
VvHAG27	XP_019073388.1	Vitvi17g01073	17	390224	405802	930	310
VvHAG28	XP_019071767.1	Vitvi18g02063	18	25838001	25839742	864	288
HAM gene family							
VvHAM1	XP_002282931.1	Vitvi11g00219	11	2093896	2100497	1536	512
VvHAM2	XP_002285829.1	Vitvi18g00333	18	3700491	3718099	1140	379
HAC gene family							

VvHAC1	XP_019080308.1	Vitvi14g01230	9	22849657	22861791	3894	1297
VvHAC2	XP_019080308.1	Vitvi14g01230	14	21859980	21865827	1974	658
HAF gene family							
VvHAF1	XP_010656962.1	Vitvi12g00328	12	4766938	4820598	5397	1799
HDA gene family							
VvHDA1	XP_002283371.1	Vitvi03g00273	3	3061225	3067402	1185	394
VvHDA2	XP_002277742.1	Vitvi04g00077	4	781102	787349	1080	360
VvHDA3	XP_019075100.1	Vitvi04g01715	4	23072108	23108432	1314	437
VvHDA4	XP_010651716.1	Vitvi06g01425	6	19410537	19425212	1293	431
VvHDA5	XP_002277237.1	Vitvi06g01465	6	20001869	20006704	1047	349
VvHDA6	XP_019080589.1	Vitvi14g00998	14	18328471	18356529	1509	502
VvHDA7	XP_002266492.1	Vitvi15g00473	15	10328819	10349872	1293	431
VvHDA8	XP_002274270.2	Vitvi17g00365	17	4195887	4225096	2094	698
VvHDA9	XP_019082031.1	Vitvi17g00705	17	8144795	8155931	1941	646
VvHDA10	XP_010663108.1	Vitvi17g00894	17	10614427	10630758	1098	366
SRT gene family							
VvSRT1	XP_010652928.1	Vitvi07g01918	7	18710375	18723402	1266	422
VvSRT2	XP_010644574.1	Vitvi19g00685	19	8637608	8659887	1404	468
HDT gene family							
VvHDT1	XP_002270966.2	Vitvi06g01399	6	19041783	19047544	1062	354
VvHDT2	XP_010654035.1	Vitvi08g01518	8	17962761	17965626	342	114

Figures

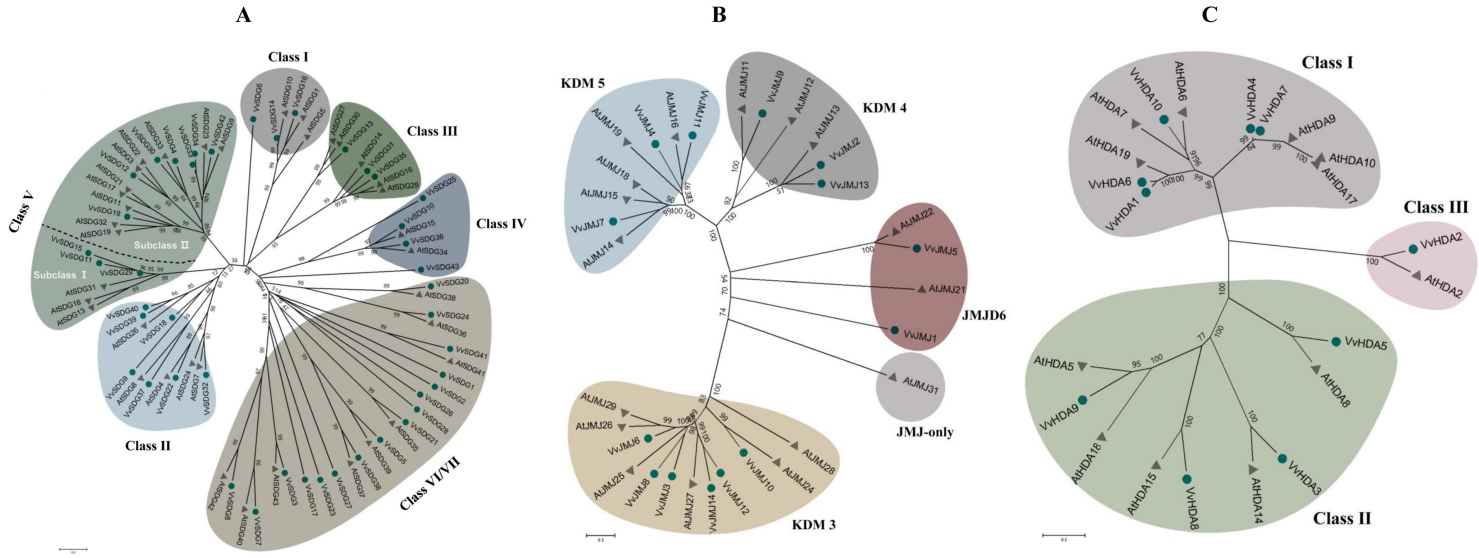

Figure 1

Phylogenetic trees of SDG (A), JMJ (B), and HAD (C) proteins of grapevine and Arabidopsis. Circles and pyramids denote grapevine and Arabidopsis proteins, respectively. Number near the tree branches

represent bootstrap values.

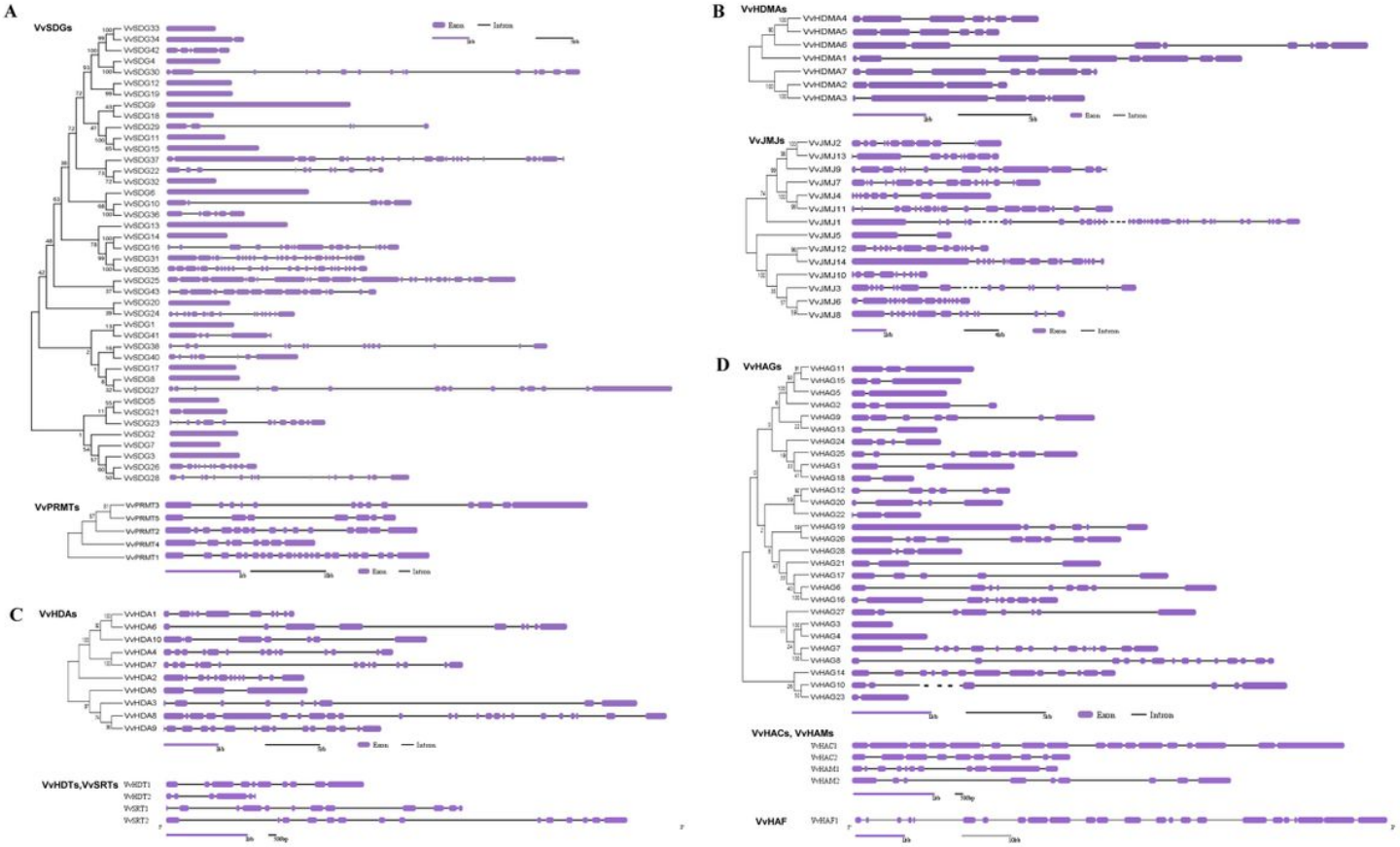

Figure 2

Phylogenetic tree and exon-intron distribution of HMs genes. (A). SDGs and PRMTs; (B). HDMAs and JMJs; (C). HDAs, SRTs, and HDTs; (D). HAGs, HAMs, HACs, and HAFs

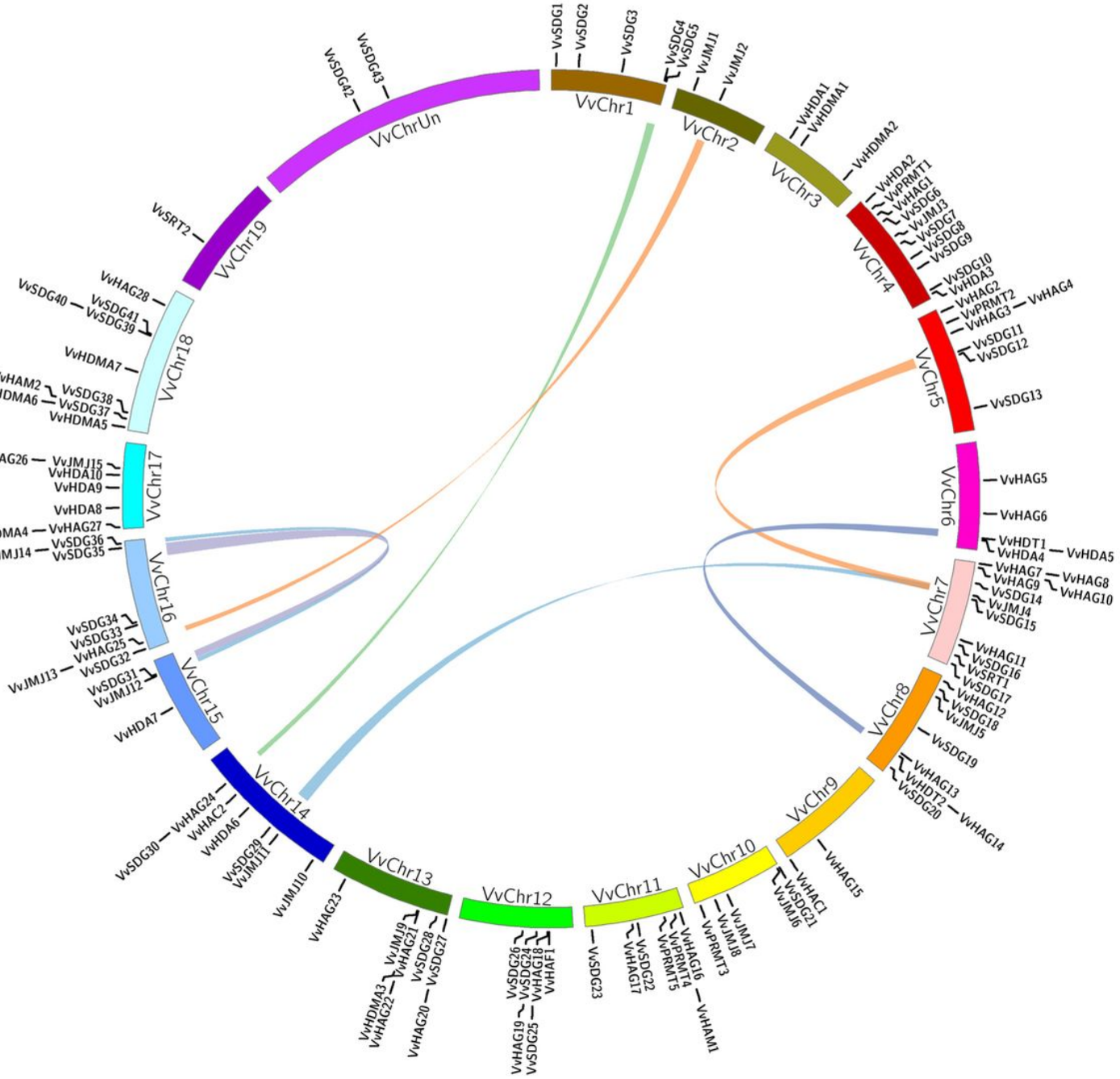

Figure 3

Synteny analysis and chromosomal distribution of grapevine HMs genes. Colored bars connecting two chromosomal regions denote syntenic regions; the corresponding genes on two chromosomes were regarded as segmental duplications. Chr: chromosomes.

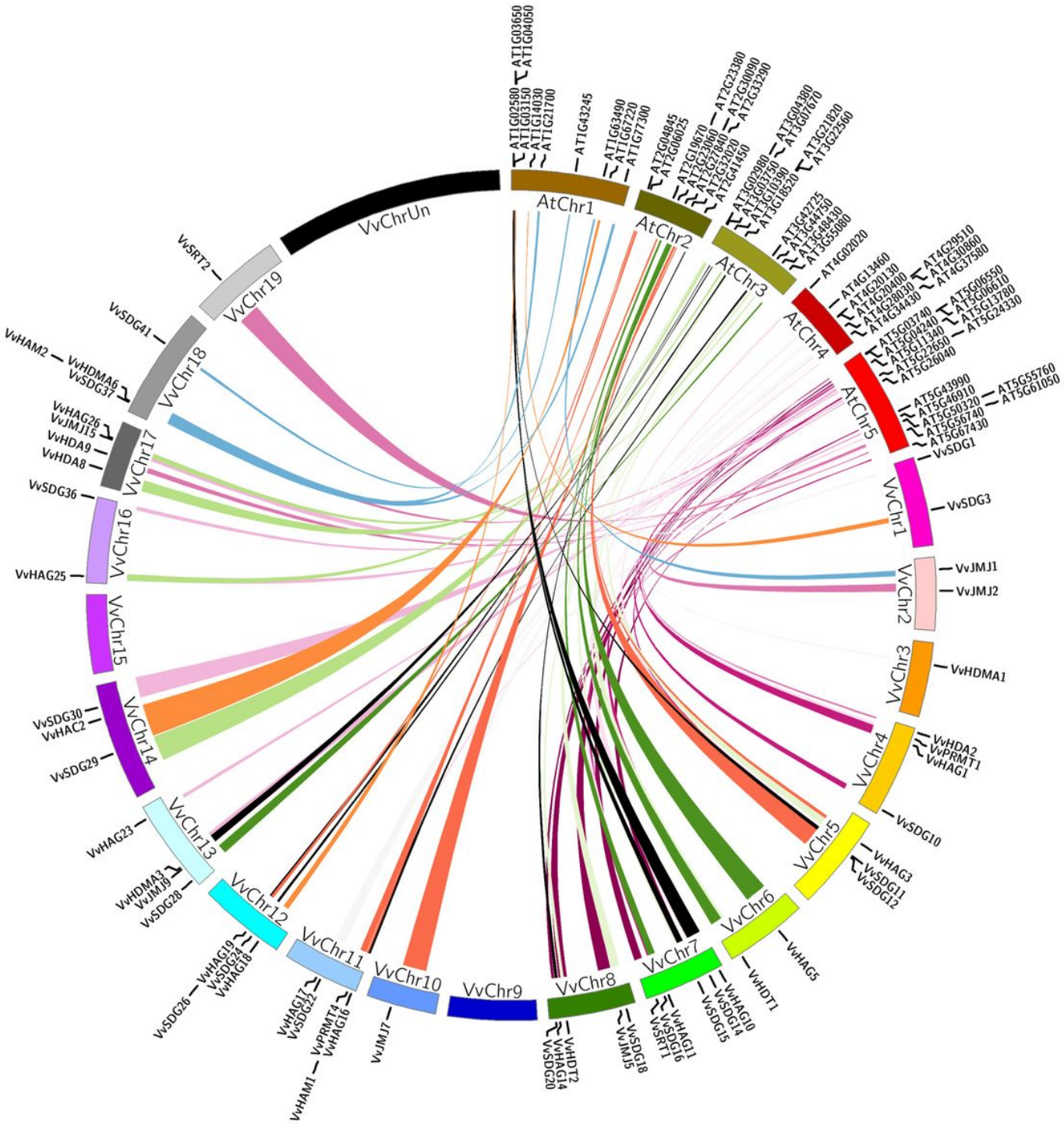

Figure 4

Synteny analysis of HMs genes between Arabidopsis and grapevine. Relative positions were depicted according to the grape and Arabidopsis chromosomes; colored lines represent syntenic regions

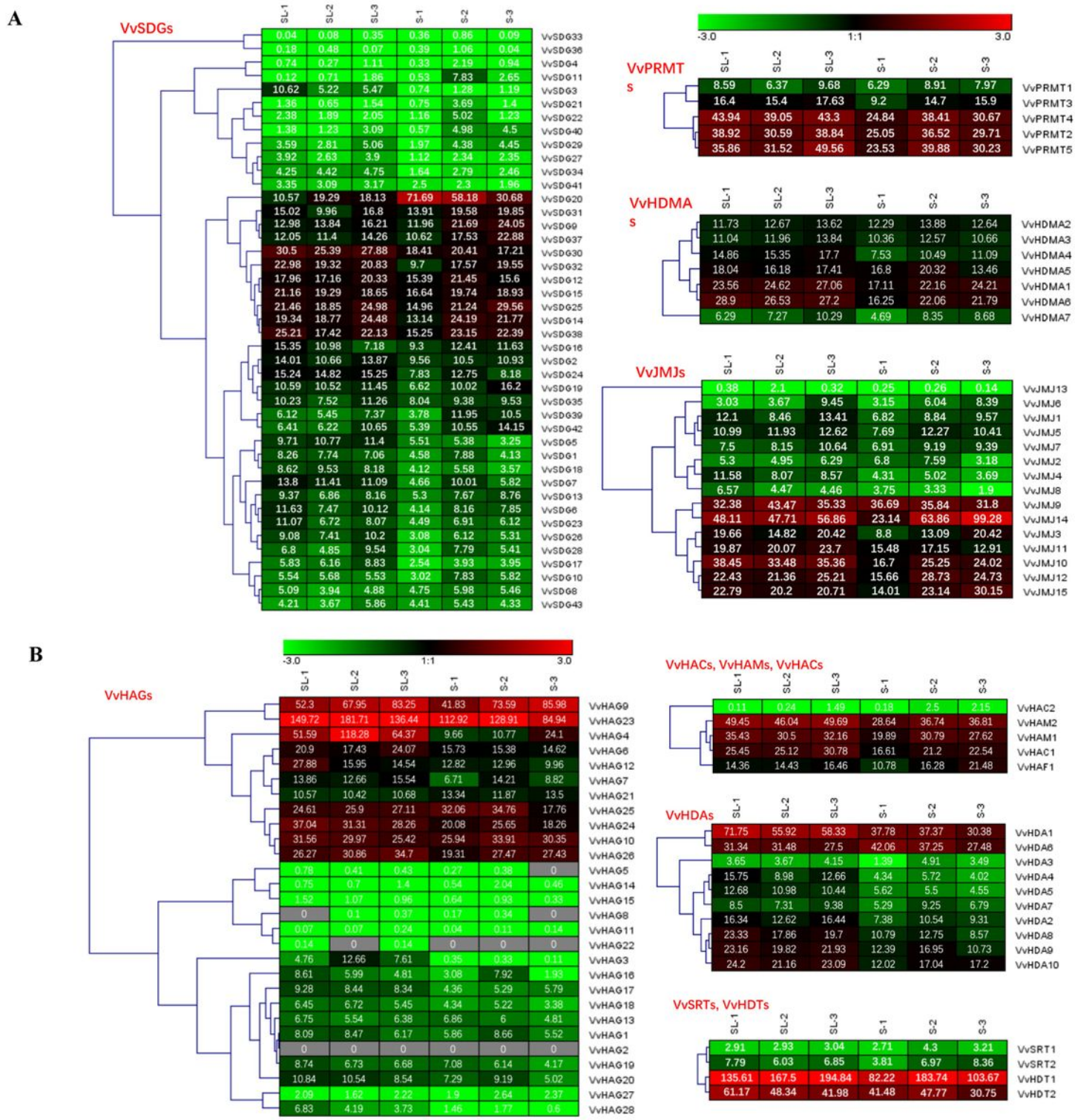

Figure 5

Heat map of VvHMs expression in transcriptome analysis during seed development in seeded and seedless progenies. Red and green color scale indicates high and low expression levels, respectively. Grey color indicates barely no gene expression detection. RPKM of VvHMs were indicated on the squares. SL denotes Seedless, S denotes Seeded

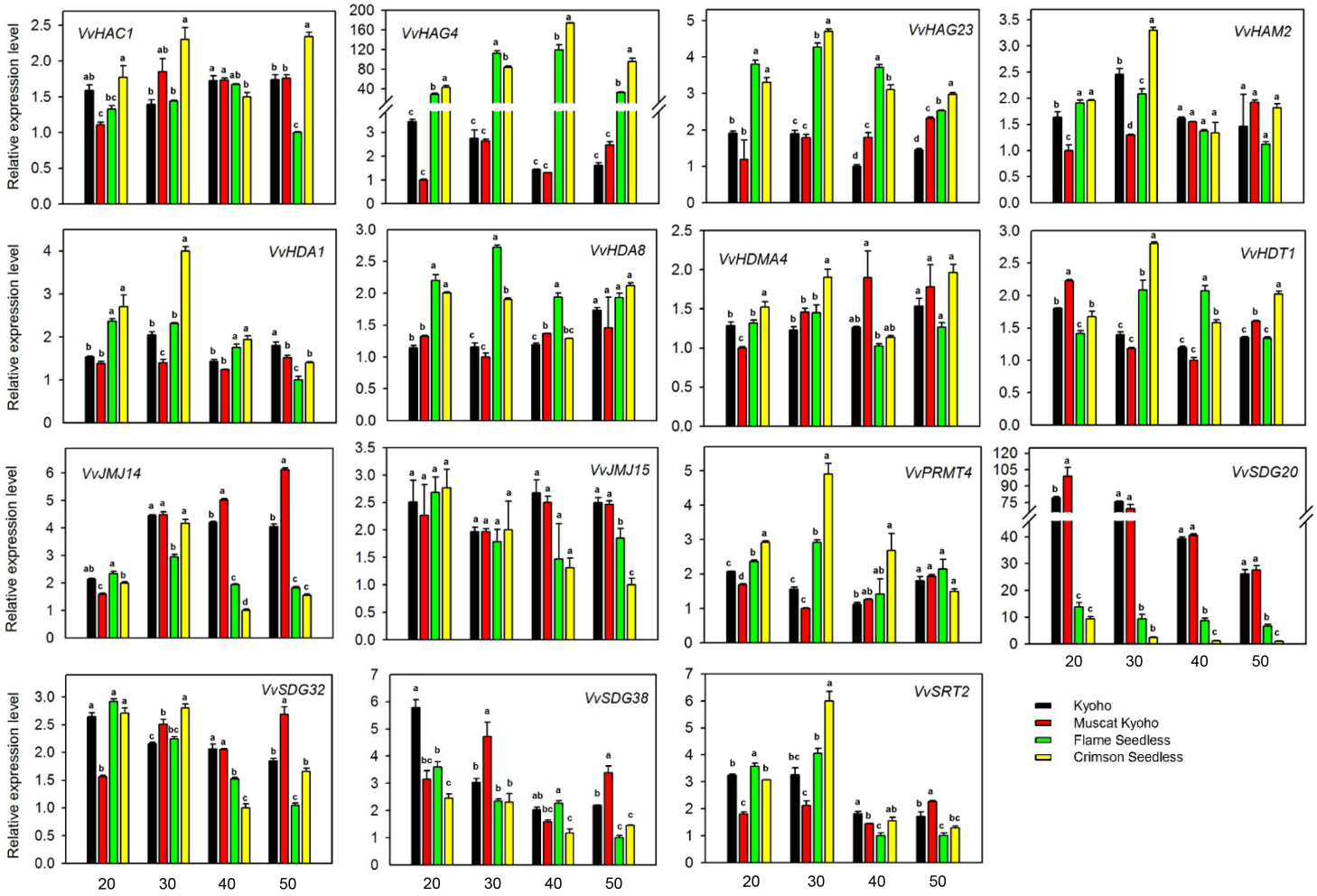

Figure 6

Real-time PCR analysis of VvHMs genes during ovule development in four cultivars. Different colors denote different cultivars and numbers on X-axis represent days after flowering (DAF). Different letters indicate statistically significant differences (ANOVA with a Tukey post-hoc analysis, 5% level). The mean ± S.D. of three biological replicates are presented.

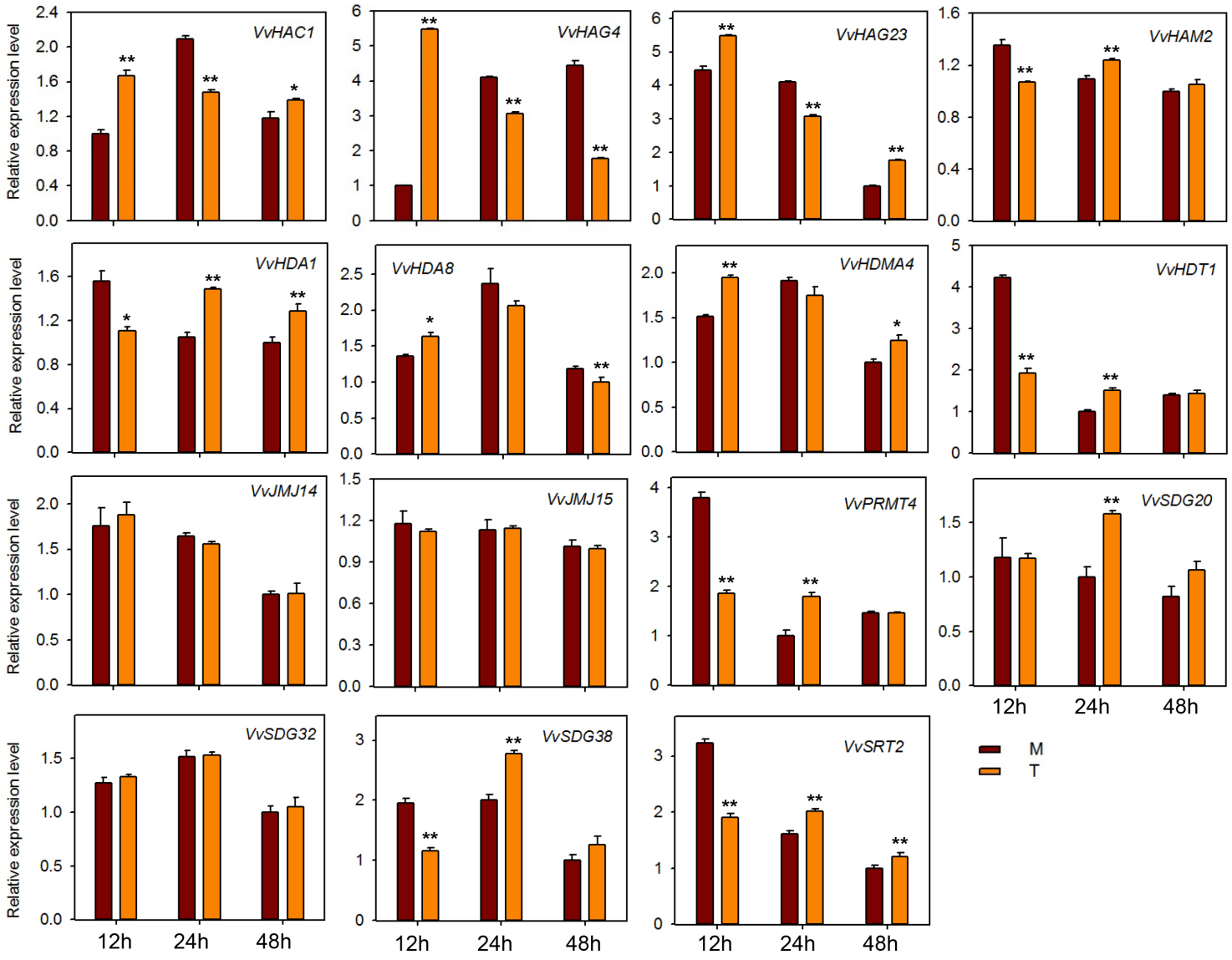

Figure 7

Real-time PCR expression analysis of VvHMs genes following Erysiphe necator inoculation at 12, 24, and 48 h post inoculation. M and T denotes mock and treatment, respectively. Asterisks indicate the corresponding statistically significance (ANOVA with a Tukey post-hoc analysis, *P < 0.05, **P < 0.01). The mean ± S.D. of three biological replicates are presented.

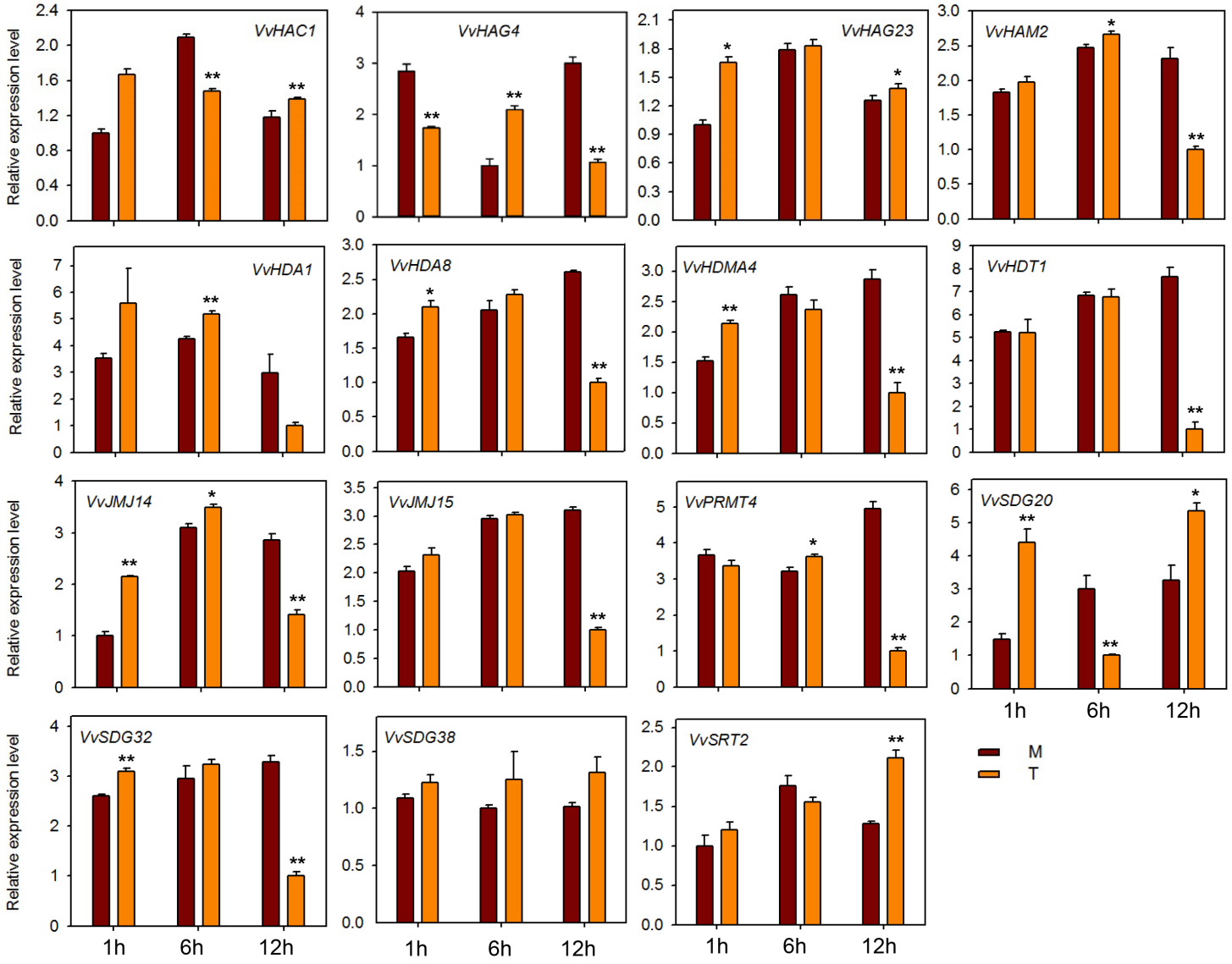

Figure 8

Real-time PCR expression analysis of VvHMs genes following ABA treatment. Leaves were collected at 1, 6, and 12 h after treatment. M and T denotes mock and treatment, respectively. Asterisks indicate the corresponding statistically significance (ANOVA with a Tukey post-hoc analysis, *P < 0.05, **P < 0.01). The mean ± S.D. of three biological replicates are presented.

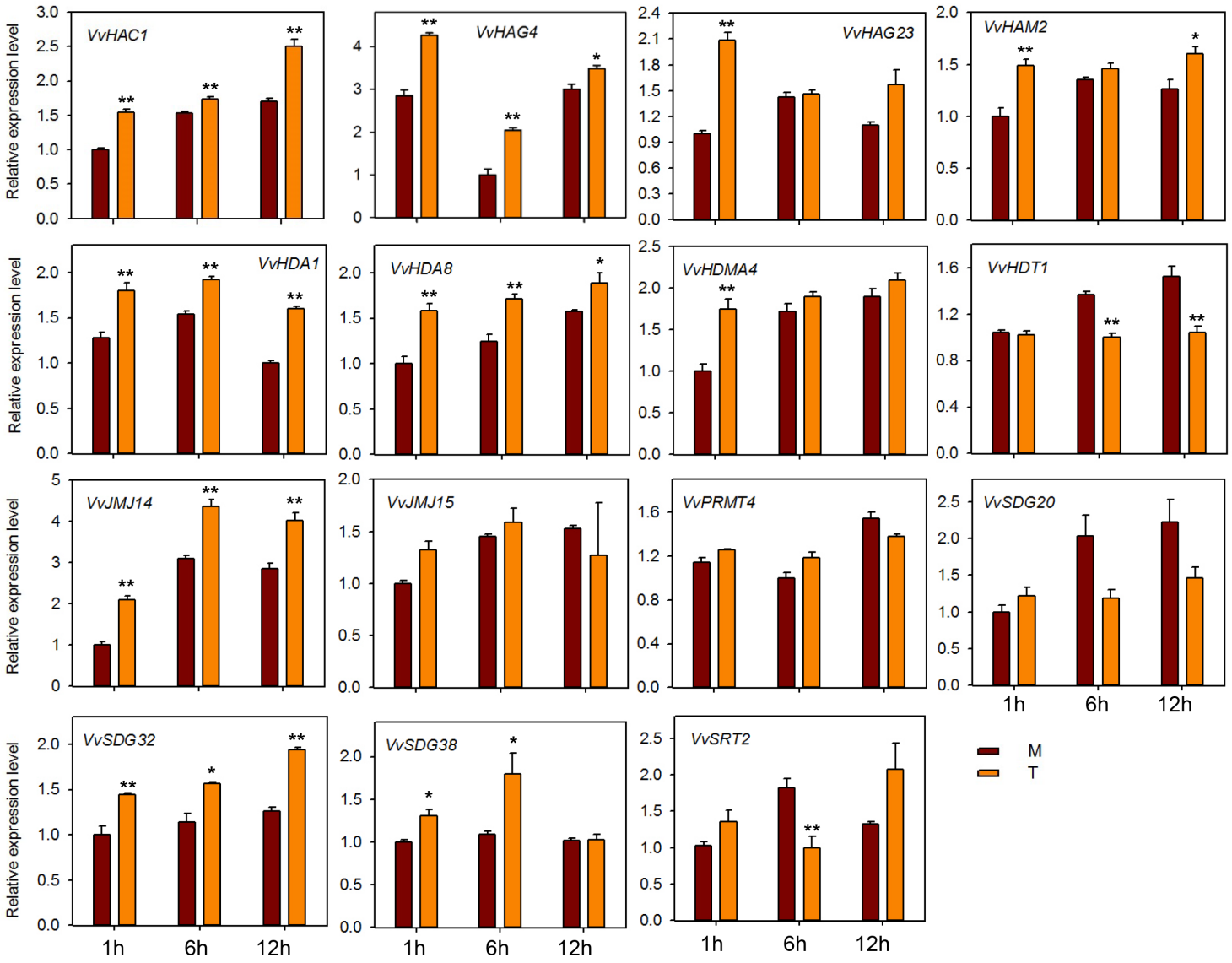

Figure 9

Real-time PCR expression analysis of VvHMs genes following JA treatment. Leaves were collected at 1, 6, and 12 h after treatment. M and T denotes mock and treatment, respectively. Asterisks indicate the corresponding statistically significance (ANOVA with a Tukey post-hoc analysis, *P < 0.05, **P < 0.01). The mean ± S.D. of three biological replicates are presented.

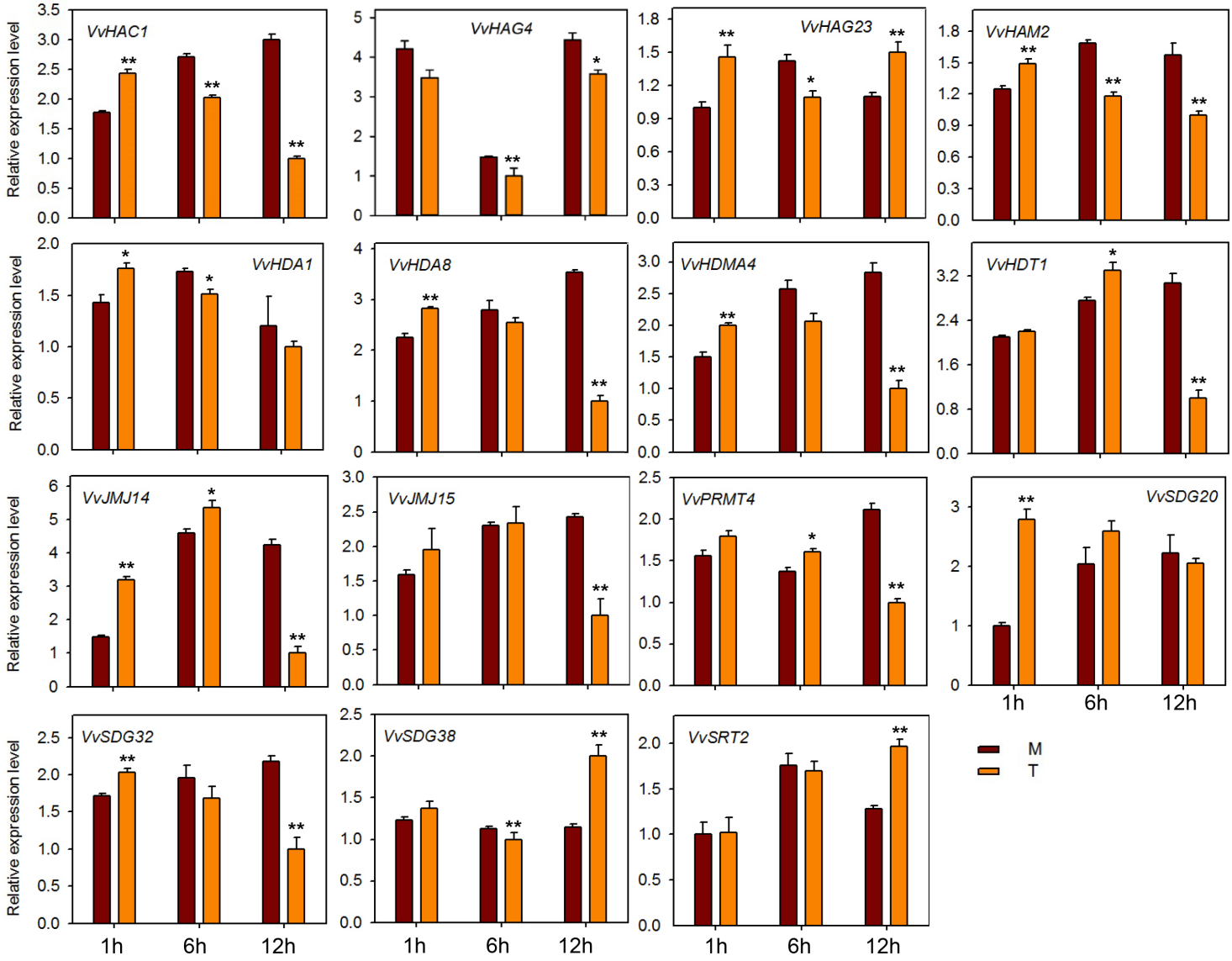

Figure 10

Real-time PCR expression analysis of VvHMs genes following SA treatment. Leaves were collected at 1, 6, and 12 h after treatment. M and T denotes mock and treatment, respectively. Asterisks indicate the corresponding statistically significance (ANOVA with a Tukey post-hoc analysis, *P < 0.05, **P < 0.01). The mean ± S.D. of three biological replicates are presented.

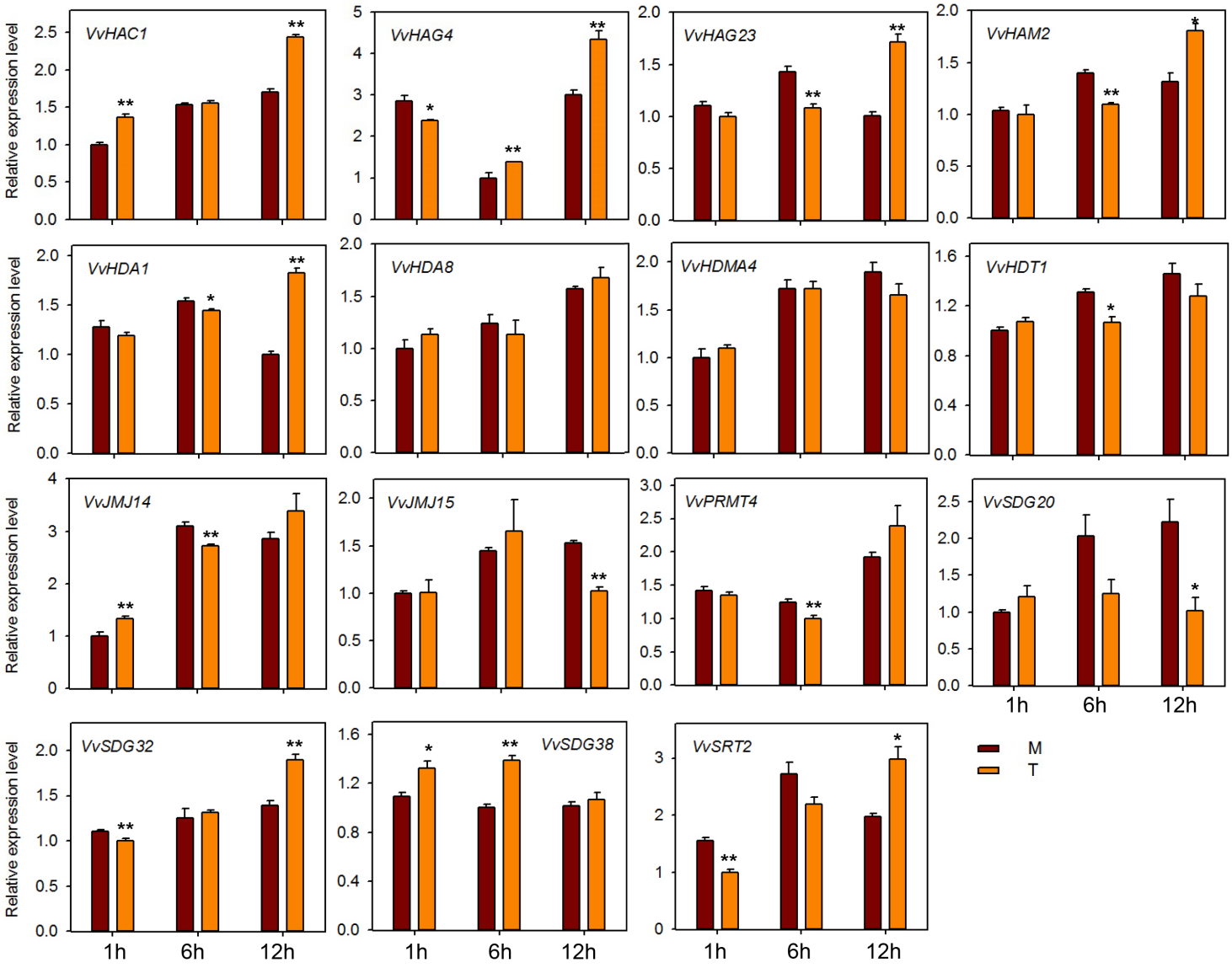

Figure 11

Real-time PCR expression analysis of VvHMs genes following Ethylene treatment. Leaves were collected at 1, 6, and 12 h after treatment. M and T denotes mock and treatment, respectively. Asterisks indicate the corresponding statistically significance (ANOVA with a Tukey post-hoc analysis, *P < 0.05, **P < 0.01). The mean ± S.D. of three biological replicates are presented.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1.xls
- Additionalfile3.xlsx
- Additionalfile2.tif
- Additionalfile4.xlsx