Identification and expression analysis of xyloglucan endotransglucosylase/hydrolase (XTH) family genes in grapevine (*Vitis vinifera* L.) (#69302)

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Identification and expression analysis of xyloglucan endotransglucosylase/hydrolase (XTH) family genes in grapevine (*Vitis vinifera* L.)

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Xyloglucan Endotransglucosylase/Hydrolase (XTH) ~~is a~~ vital enzyme during cell wall reformulation, ~~and it has~~ a major contribution ~~in helping plants to resist~~ abiotic stresses like drought, salinity, and freezing injury. However, the comprehensive ~~genomic~~ analyses of the *XTH* genes family and their functions in grape (*Vitis vinifera* L.) have not been completed yet. In the present study, 34 *XTH* genes were identified in the whole grape genome and then named according to their distribution on grape chromosomes. Based on the phylogenetic analysis with the *XTH* genes in *Arabidopsis*, *VvXTH* genes were classified into 3 groups. *Cis*-elements analysis indicated that various *cis*-elements related to stress were prevalent in the promoter sequence of most *VvXTHs*. Further, 14 *VvXTH* genes from different groups were randomly selected and their transcription level~~s~~ w~~ere~~ examined in 'Crismon' seedlings ~~under~~ drought and salt stresses. The results indicated that ~~most expressions of~~ the ~~above~~ *VvXTH* genes ~~are~~ up-regulated, suggesting that *VvXTH* genes are likely to take part in the responses to drought and salt stress in grape~~s~~. These results ~~will~~ provide useful information for developing further investigation ~~and validation~~ of the *VvXTH* gene in response to abiotic stresses in grapevine~~s~~.

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Abstract

Xyloglucan Endotransglucosylase/Hydrolase (XTH) is a vital enzyme during cell wall reformulation, and it has a major contribution in helping plants to resist abiotic stresses like drought, salinity, and freezing injury. However, the comprehensive genomic analyses of the *XTH* genes family and their functions in grape (*Vitis vinifera* L.) have not been completed yet. In the present study, 34 *XTH* genes were identified in the whole grape genome and then named according to their distribution on grape chromosomes. Based on the phylogenetic analysis with the *XTH* genes in *Arabidopsis*, *VvXTH* genes were classified into 3 groups. *Cis*-elements analysis indicated that various *cis*-elements related to stress were prevalent in the promoter sequence of most *VvXTHs*. Further, 14 *VvXTH* genes from different groups were randomly selected and their transcription levels were examined in 'Crismon' seedlings under drought and salt stresses. The results indicated that most expressions of the above *VvXTH* genes are up-regulated, suggesting that *VvXTH* genes are likely to take part in the responses to drought and salt stress in grapes. These results will provide useful information for developing further investigation and validation of the *VvXTH* gene in response to abiotic stresses in grapevines.

Introduction

Grapevine (*Vitis vinifera* L.) as one of the most economically fruit crops is globally widespread cultivation (*Zhu et al.*, *2019*). It has a wide range of applications in fresh food, winemaking, and fruit juice (*Feng et al.*, *2000*). The growth of grapes in natural environment inevitably suffers from a series of abiotic pressures from salinity, drought and extreme temperatures, which changes the morphology of the plant, disrupts the biofilm system and causes oxidative damage, and ultimately affects the quality and yield of the fruit. Hence, it is considered to be effective to discover novel genes involved in abiotic stress resistance and applications of genetic breeding for the improvement of stress resistance of grapes. The existence of a high-quality de novo assembled grape genome has made it possible to identify gene families in this species.

Xyloglucan Endotransglucosylase/Hydrolase (XTH), a cell wall that modifies enzyme, can complete cell wall structural modification and rearrangement by severing and repolymerizing cellulose mono-xyloglucan cross-linked structures (*Campbell et al.*, *2010*). It belongs to the GH16 (Glycoside Hydrolases 16) family which is a glycoside hydrolases subfamily containing a huge diversity of enzymes with different specificities like keratan sulfate, b-1,3-glucans, mixed linkage b-1,3(4)-glucans, xyloglucans, j-carrageenan, and agarose (*Mark et al.*, *2009*; *Stratilova et al.*, *2020*). All XTH proteins have a conserved structural domain, i.e., DEIDFEFLG (*Yokoyama et al.*, *2010*), which incorporates amino acid residues mediating catalytic activity (*Matsui et al.*, *2005*; *Liu et al.*, *2007*; *Miedes et al.*, *2009*; *Singh et al.*, *2011*). Members of XTH proteins may have one or two enzyme activities: one is xyloglucan endonuclease (XEH) activity and the other is xyloglucan endotransglucosylase (XET) activity. The former hydrolyses β-1, 4 glycosidic bonds of xyloglucan to form the cleavage and connection of xyloglucan chains, and the latter transfers and synthesizes xyloglucan fragments between xyloglucan chains (*Han et al.*, *2016*).

The *XTH* gene family was initially classified into three groups in *Arabidopsis thaliana*, named group I, II, and III (*Campbell et al.*, *1999*). However, the subsequent study in rice found that between groups I and II, there is no clear distinction, thereby the rice *XTH* genes were divided into 2 groups: group I/II and group III (*Eklof et al.*, *2010*). Further, it was found that the *XTH* members in group III could be divided into two subgroups (IIIA and IIIB) according to their three-dimensional structures (*Baumann et al.*, *2007*; *Fu et al.*, *2019*). What's more, a small outlier group was found to be close to the root of the tree and was named ancestral group. The *XTHs* of group I/II and group III-B showed primarily or entirely XET activity, whilst the XTH genes in group III-A mainly displayed XEH activity (*Eklof and Brumer 2010*; *Nomchit et al.*, *2010*; *Opazo et al.*, *2017*). Further studies found that each enzyme activity was determined by several structural characteristics. For example, in the protein structure of *TmNXG1*, loop2 is the key structure affecting hydrolysis and transglycosylase activity. *PttXET16-34* has an important N-glycan structure, which is present in all group I/II members and absent in almost all III-A groups (such as *TmNXG1*). Interestingly, the N-glycosylation site moves to the C-terminus to the other side of the active site cleft in group III-B.

Mounting researches revealed that XTH genes were instrumental for plants in coping with abiotic stresses through cell remodeling and enhanced cell wall biogenesis (*Eklof and Brumer*, *2010*), for instance, constitutive expression of *CaXTH3* is verified to enhance the resistance to salinity and drought pressures in tomato plants (*Choi et al.*, *2011*). *AtXTH11*, *AtXTH29* and *AtXTH33* were observed to be up-regulated through different secretory pathways in *Arabidopsis* seedlings treated with heat and drought stresses (*Caroli et al.*, *2021*). Study revealed that overexpression of persimmon *DkXTH1* promotes tolerance to salt and drought stress by improving photosynthesis and reducing lipid peroxidation (*Han et al.*, *2017*). It is also observed that

transgenic tobacco with estradiol-inducible expression of *SlXTH10* gene shows stronger growth under salinization and hypothermia conditions (*Norbert et al., 2020*). Besides, many *GmXTH* genes expression levels have been reported to be significantly associated with flooding stress. The transgenic soybeans overexpressing *AtXTH31* also exhibits higher tolerance to flooding stress (*Li et al., 2018*). In addition, *AtXTH19* mutant showed lower freezing tolerance during cold and sub-zero acclimation than the Col-0 wild type, related to differences in cell wall composition and structure (*Daisuke et al., 2020*). Taken together, these researches highlight the essential functions of *XTHs* in resisting abiotic pressure.

At present, *XTH* gene family has already been identified and analyzed in *Arabidopsis thaliana* (33), *Hordeum vulgare* (24), *Glycine max* (61), *Nicotiana tabacum* L. (54) and so on (*Nomchit, Harvey, Maria, Harry, Ines, Teeri and Fincher 2010,* (*Li et al., 2018*; *Wan and Henry, 2018*; *Meng et al., 2018*). However, the ~~grape *XTH* gene family has not yet been reported.~~ In the present study, a complete bioinformatics analysis of *XTH* gene family in grapevine ~~were~~ performed and ~~their~~ potential function~~s~~ in salt and drought stress responses were investigated. The findings of ~~current investigations~~ will ~~lay the foundation for~~ in-depth research on the potential functional ~~verification~~ of the~~se~~ *VvXTH* genes in grape~~s~~.

Materials & Methods

Identification of grape *XTH* ~~members~~ and analysis of ~~protein biochemical characteristics~~

The annotation sequences of the whole genome and the GFF3 file of the gene were downloaded by CRIBI (http://genomics.Cribi.unipd.it). We downloaded the hidden Markov models PF00722 and PF06958 of the *XTH* domain from the Pfam database (http://pfam.xfam.org) and get the

candidate gene sequence numbers of the grape *XTH* gene family with HMMer software, then the incomplete and redundant sequences were removed. The corresponding protein sequence was obtained by searching the sequence number in the grape protein database. The EMBL-EBI online tool (http://pfam.xfam.org/search/sequence) was used to further analyze the domains and confirm the gene family. Sequences without typical *XTH* domains were removed.

The relative molecular weight (MW), hydrophilicity (GRAVY), and isoelectric point (pI) of these VvXTH proteins were predicted and analyzed using ExPASy (https://www.expasy.org/). The subcellular localizations were predicted via ProtComp 9.0 (http://linux1.softberry.com). The single peptide (SP) was predicted by SignalP v4.1 server (http://www.cbs.dtu.dk/services/SignalP/).

Phylogenetic analysis of *VvXTH* gene family

To investigate the phylogenetic relationship of *VvXTH* genes, the 34 VvXTH protein sequences ~~of grapes~~ and the 33 *AtXTH* protein sequences ~~of~~ *Arabidopsis thaliana* were used for multiple sequence alignment by Clustal W program within MEGA 11.0 software. The phylogenetic tree was built using the neighbor-joining (NJ) method with 1 000 bootstrap replications and p-distance model and validated by the Maximum likehood method. For a better view of the phylogenetic tree, the final tree diagram file (*.nwk) was uploaded from MEGA to the Figtree and EVOLVIEW (https://evolgenius.info/evolview-v2/#login) online software.

Chromosomal distribution and synteny analysis of *VvXTH* genes

Grape Genome Browser (12X) (http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/) provided chromosomal locations data of all *VvXTH* genes. We used TBtools to locate and draw the distribution of genes on chromosomes. MCScanX was applied to identify gene replication events, using default parameters. The CIRCOS program (https://github.com/CJChen/TBtools) was

used to analysis syntenic relationships ~~of~~ *VvXTH* genes. *VvXTH* genes falling in the identified collinear blocks were regarded as segmental events while two genes whose adjacent distance were within 100 kb and whose similarity exceeds 75% are considered tandem duplications. In order to visualize the synteny relationship of orthologous *XTH* genes derived from grapes and *Arabidopsis thaliana*, Dual Systeny Plotter software (https://github.com/CJ-Chen/TBtools) was applied to construct syntenic analysis map (*Xie et al., 2018*). The ~~*Arabidopsis*~~ *thaliana* sequences were obtained from the Arabidopsis Information Resource (TAIR) database (*Han et al., 2013*). TBtools software was used ~~for~~ calculate nonsynonymous (Ka), synonymous (Ks), and Ka/Ks ratio of each gene pairs. The divergence time was calculated by $T = Ks/2\lambda$ ($\lambda = 6.5 \times 10^{-9}$ for Grapevine) (*Li et al., 2019*).

The gene structure, conservative motifs analysis and multiple sequence alignments of *VvXTH* gene family

Gene structures were performed with the Gene Structure Display Server (GSDS: http://gsds.cbi.pku.edu.cn/) software with default setting. Conserved motifs in *VvXTH* proteins were statistically identified by the online software of Multiple EM for Motif Elicitation (MEME) (https://meme-suite.org/meme/tools/meme) with default setting, then TBtools was used for clustering and visualization. Multiple sequence alignments were performed by Clustal X software and Espript 3.0 online program (https://espript.ibcp.fr/ESPript/ESPript/).

***Cis*-Elements Analysis of ~~*VvXTH* gene family~~**

The sequence within 1500 base pairs (bp) upstream of the ~~initiation~~ codon of the *VvXTH* genes were ~~gained~~ from Ensembl Plants (http://plants.ensembl.org/index.html) as the promoter region. The *cis*-elements were predicted with PlantCare Web Tools (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/).

Organ-specific expression analysis of *VvXTH* gene family

We Downloaded the microarray gene expression profile (GSE36128) of different organs and different growth stages of grapes from the Gene Expression Comprehensive (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). According to the gene ID, the information of the *VvXTH* genes were extracted from the GSE36128 data set, and then we normalized the average expression value of each gene in all organs. TBtools was used to draw heat maps and build clustering trees.

Plant growth and stress treatments

The tissue culture seedlings of grape "Crimson" seedless were grown on 1/2 Murashige and Skoog (MS) solid medium with 0.2 mM indole-3-butytric acid (IBA) under a 16-h-light /8-h-dark cycle at 24 ± 1°C for 4-week intervals. Then, six-week-old grape shoot seedlings were transferred to the liquid medium containing 200 mM NaCl or 200 mM Mannitol for salt and drought stress treatments, respectively. The treated seedlings were sampled at 0, 3, 6, 9, 12, and 24 h after treatment, then instantly freezing in liquid nitrogen and stored at -80°C for RNA extraction. For each sample, three biological replicates were collected.

Extraction of total RNA and expression analysis of *VvXTHs*

Total RNA was extracted from the samples treated with NaCl and Mannitol using HiPure HP Plant RNA Mini Kit (Magen, Guangzhou, China) based on the manufacturer's instructions. Subsequently, First-strand cDNA was synthesized from total RNA with the PrimeScript™ RT reagent kit with gDNA Eraser (Vazyme Biotech Co., Nanjing, China). qRT-PCR was performed according to the supplier's instructions of the SYBR® PrimeScript™ RT-PCR Kit (TaKaRa, Dalian, China) in the CFX96TM Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). The grape β-actin (XM_034827164.1) were used as the internal references. All the experiments were performed in three biological replicates. The relative levels of gene expression

were ascertained by the $2^{-\Delta\Delta Ct}$ methods. TBtools was used to draw a heat map for visualization. All the primers used in this study were listed in Table S1.

Results

Identification of *VvXTH* genes in grapes

Overall 36 candidate *VvXTH* genes were identified through searching two domains (Pfam: PF00722 and PF06955) by HMMer program. By removing genes that do not contain typical *XTH* domain, we finally identified 34 *VvXTH* genes named *VvXTH1-VvXTH34* according their distribution on the chromosomes.

The basic properties, including amino acid (AA), molecular weight (MW), signal peptide (SP), isoelectric point (pI), total average hydrophilicity (GRAVY) and subcellular localization, were analyzed to further characterize the *VvXTH* proteins (Table 1). The 34 VvXTH proteins consist of 251 to 369 amino acids in length. The MW ranged from 28.5 to 41.7 kDa, while the pI ranged from 4.61 to 9.45. All XTH protein members exhibited hydrophilicity. Subcellular location prediction results showed that most genes are localized in the Plasma membrane (29), and a few are localized in Extracellular (5), including *VvXTH10*, *VvXTH12* in group IIIA, *VvXTH2*, *VvXTH32* and *VvXTH33* in group IIIB. The majority of the proteins (80%) contain signal peptide sequences.

Phylogenetic ~~relationships~~ analysis and classification of *VvXTHs*

To investigate the evolutionary relationship and functional association of *VvXTHs* with *Arabidopsis XTH* genes, a phylogenetic tree was built utilizing the protein sequences of XTHs from *Vitis vinifera* and *Arabidopsis* (Figure 1). *VvXTHs* were grouped according to the previous grouping method of *AtXTH* and the evolutionary relationship between grapes and *Arabidopsis*.

The results of the phylogenetic analysis indicated that total of 34 *VvXTHs* were divided into three groups: 27 in group I/II, 2 in group IIIA, and 5 in group IIIB. In addition, one XTH protein (*VvXTH11*) was classified into the original ancestor group. The group I/II contains most of the members, and a substantial similarity can be observed between some members in the group. The termini of the phylogenetic tree branch showed a total of 22 sister pairs, of which 8 were orthologous pairs between *Arabidopsis thaliana* and grapes, and 6 were grape homologous gene pairs. It revealed that the number of *VvXTH* genes were expanded slightly in contrast to *Arabidopsis thaliana*.

Chromosomal localization and synteny analysis of *VvXTHs*

Thirty-four *VvXTH* genes were unevenly distributed on 13 chromosomes (Figure 2A). In particular, Chr.11 contains the largest number of *VvXTH* genes (15), whereas other chromosomes contain much fewer genes, for example, a total of 4, 3, 2genes were located on Chr.5, Chr.10 and Chr.1, respectively. In addition, Chr.2 , Chr.3, Chr.6, Chr.7, Chr.8, Chr.12, Chr.15, Chr.16 and Chr.17 contain only 1 gene each. Therefore, it can be inferred that no association should be seen between the number of *XTH* genes and the length of chromosomes. Furthermore, the genes located on Chr.11 and Chr.5 are closely clustered together. According to the chromosome location and genome annotation information, a total of 81 tandem duplicate gene pairs were obtained (Figure 2A). As shown in the Figure 2B, there is no *VvXTHs* falling in the identified collinear blocks, which indicated that segmental duplication is not involved in *VvXTHs* expansion. The above results indicate that the expansion of *VvXTHs* especially group I/II gene members were driven by tandem duplication. We trace the duplication time of *VvXTH* genes by analysing their Ka (non-synonymous rate), Ks (synonymous rate) and Ka/Ks ratio. The Ka/Ks ratios of all *VvXTHs* were less than 1, ranging from 0.07 to 0.28. In addition, the time to duplication for all *VvXTHs* was also

calculated. The duplication times ranged from 2.91-78.39 Mya (million years ago) (Table S2). To further determine the evolution and development of the *VvXTH* gene family, we constructed a comparison diagram of grapes and *Arabidopsis thaliana*. As shown in Figure 3, there are 8 *VvXTH* genes that are synonymous with the *XTH* genes in Arabidopsis. Among them, *VvXTH10* is collinear with *AtXTH31* and *AtXTH32* in *Arabidopsis* while *VvXTH1* is collinear with *AtXTH27* and *AtXTH28* in *Arabidopsis*. It is speculated that there may be functional redundancy between them, which implies their important part in the evolutionary progress

Gene structure, conserved motifs pattern, and multiple sequence alignment analysis of *VvXTHs*

For the 34 *VvXTH* genes that have been identified, their intron-exon structure is drawn together with the order of the subfamily in the phylogenetic tree (Figure 4A). The more closely related genes among this subfamily are characterized by a similar structure, which is agreeable with the results of phylogenetic analysis. Except for *VvXTH32* which does not contain introns, all other *VvXTH* gene members contain different introns, and the number is in the range of 2-4. The group I/II contains a large number of members, and most members have 2 introns. The sister pair genes, including *VvXTH23/26*, *VvXTH25/28*, *VvXTH14/15*, *VvXTH8/9* at the terminal branch of the evolutionary tree, have highly similar exon/intron structures. In addition, compared with the adjacent gene *VvXTH27*, *VvXTH24* loses an exon and exhibits different intron and exon lengths. The members of group IIIA have 3 introns and 4 exons, with high structural similarity. The members of group IIIB showed different numbers and lengths of intron/exon structures in the long evolutionary process (Figure 4C). In general, most *VvXTHs* have the same intron/exon structure pattern, and they remain conserved during evolution, which is consistent with the results in other plants.

Based on the results of MEME motif analysis, motif 3 and motif 4 are highly conserved in all *VvXTHs*. Motif3 (Figure 4D) is a characteristic domain DEIDFEFLG that catalyzes enzymatic contact reaction. In addition, all members except *VvXTH30* contain motif 1. As shown in Figure 4B, members of the same group share a similar motif composition. For instance, motif 2 only exists in group I/II; motif 8 only exists in all members of group IIIA and IIIB. As for the genes in the same clade, especially those closely related, such as 1) *VvXTH23*, *VvXTH26*, *VvXTH25*, and *VvXTH28*, 2) *VvXTH6*, *VvXTH7*, *VvXTH8* and *VvXTH9* or 3) *VvXTH10* and *VvXTH12*, they can have much more similar motif structures. In addition, motif 7, 9, and 10 only exist in group I/II, and most of the group members contain the above motifs. The members of group IIIA contain 5 motifs with the same distribution. Group IIIB contains 4-6 motifs while these members share 4 same motifs, only *VvXTH4* and *VvXTH32* have motif 6, and only *VvXTH33* does not have motif 5. At the same time, the result of multiple sequence alignment also confirmed that the conserved domain DEIDFEFLG is present in all *VvXTH* genes. Moreover, except for *VvXTH2* (IIIB), *VvXTH10* (IIIA)and *VvXTH12* (IIIA), potential N-glycosylation residues in 31 *VvXTHs* are located near the active site (Figure 4E).

The organ-specific expression patterns analysis of *VvXTHs*

Through the expression profile (GSE36128) analysis of GEO data set, we obtained the specific expression patterns of *VvXTH* genes in different organs and developmental periods of grapes, so as to predict the function of *VvXTH* in grape growth and development (Figure 5). According to the results of cluster analysis, the *VvXTH* families were classified into 4 groups: A, B, C, and D. Group A contains 7 genes with high expression levels in berry peels, berry skins, shafts and tendrils; Group B includes 4 members, with high expression levels only in stems and tendrils, and almost have low expression in other organs; Group C includes 8 genes, which expression levels are very

low in all organs; Group D has the largest number, with 15 members, and shows higher expression in berries, shafts and tendrils. In addition, during the V, MR, and R periods, the *VvXTH* genes have a higher expression level in the pulp, peel, and stem, it is inferred that *VvXTH* genes may related to fruit ripening. In short, *VvXTHs* are universally present in all organs of grapes, and they are probably engaged in the growth and development of plants.

Cis-Elements analysis of *VvXTHs*

PlantCARE database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) was utilized to assay *cis*-elements of the 1.5kb upstream DNA sequence of the VvXTH protein coding genes, the results showed that all 34 *VvXTH* genes contained a variety of abiotic and biotic stress response elements, phytohormone response elements and response elements related to growth and development (Figure 6). Among them, the two drought stress response elements MYB and MYC exist in almost all members, and 80% of the gene members contain defense and stress response elements STRE, which indicates that the *VvXTH* gene family probably have an important function when subjected to abiotic or biotic stress; Ethylene response elements ERE, abscisic acid response element ABRE, and salicylic acid response element TCA-element are abundantly present in the members of *VvXTH*, which indicates that *VvXTH* gene families may be involved in hormone regulation and interact with hormones in the process of plant growth and responding to stress; The light response elements G-box, Box-4 and the meristem development control element CAT-box are present in most *VvXTH* members, indicating that the *VvXTH* gene families may have a significant effect in the regulatory control of plant growth and development.

qRT-PCR verification of *VvXTHs* under drought and saline stresses

Promoter analysis demonstrated the widespread presence of *cis*-elements associated with abiotic stress in the promoter region of *VvXTHs*, revealing a possible induction of *VvXTHs* expression by

298 abiotic stress. To further investigate the potential role of *VvXTH* genes in response to abiotic stress,
299 the grape seedlings were exposed to 200 mM NaCl or 200 mM Mannitol, and the expression of 14
300 *VvXTH* genes that were randomly selected members were examined. In roots, the expression levels
301 of 11 genes were up-regulated under salt stress, among which 4 members were significantly up-
302 regulated. *VvXTH5*, *VvXTH20* and *VvXTH34* were increased more than two-fold, and *VvXTH4* was
303 increased more than four-fold. Interestingly, the expression of *VvXTH4* reached peak at 9 h, which
304 is obviously different from the other three genes, which reached peak at 3 h. This shows that they
305 may respond to salt stress in different ways. Under drought stress, most of the genes whose
306 expression was up-regulated reached peak at 3 h, and some genes were up-regulated by more than
307 four-fold (*VvXTH3*, *VvXTH20*); *VvXTH10* reached the peak at 12 h, indicating that it may come
308 into play at a later time. Compared with roots, the number of up-regulated *VvXTH* genes in leaves
309 decreased after stress, but they expressed a higher fold of up-regulation. Among them, *VvXTH3*,
310 *VvXTH10*, and *VvXTH31* were up-regulated about ten-fold. Especially under salt stress, the above
311 genes might take crucial roles in leaves response to salinity stress. Taken together, the expression
312 of *VvXTHs* could be induced by salt and drought stress, suggesting *VvXTHs* may be participating
313 in reactions to abiotic damages in grapes.

314 ## Discussion

315 XTH is a cell wall modification enzyme that rebuilds cell walls through the construction and
316 composition of xyloglucan cross-links. According to previous literatures, various members are
317 aware to take critical positions in development and stress reaction of plant. To date, genome-wide
318 identification and characterization has been performed in *Arabidopsis thaliana*, *Oryza sativa*,
319 *Medicago truncatula*, *Nicotiana tabacum*, *Solanum lycopersicum*, and *Ananas comosus*, but has
320 not been performed in grapevine (*Meng et al.*, *2018*; *Li et al.*, *2019*; *Yokoyama*, *2004*; *Kurasawa*

321 *et al.*, *2009*; *Xuan et al.*, *2016*). The release of the latest grape genome database makes it possible
322 to identify the grape *XTH* genes family. In this study, 34 grapevine *XTH* genes were systematically
323 identified and characterized using bioinformatic approaches. Experimental results show that the
324 number of identified *VvXTH* (34) was slightly higher than *Arabidopsis thaliana* (33) and *Oryza*
325 *sativa*. (29) (*Yokoyama*, *2004*; *Kurasawa et al.*, *2009*), which may be related to pedigree-specific
326 gains and losses as well as the gene duplication events. Gene duplication as a primary driver for
327 the expansion of gene families, tandem duplications and segmental duplications have been
328 considered as a primary duplication modes. In previous studies of the *XTH* gene family, there are
329 gene tandem duplication or segment duplication in barley, soybean, and tobacco (*Fu et al.*, *2019*;
330 *Li et al.*, *2018*; *Meng et al.*, *2018*). Thirty-four *VvXTH* genes are located on 13 chromosomes,
331 where Chr.11 and Chr.5 each have a gene cluster. Based on the definition of gene tandem
332 duplication, *VvXTH17- VvXTH30* and *VvXTH6-VvXTH9* form gene events. According to the
333 results of Ka/Ks ratio, all genes are less than 1, which denotes that they are under an intense
334 purifying selection (*Hurst*, *2002*). Hence, the role played by gene tandem duplication for *VvXTH*
335 gene family expansion is irreplaceable for the increase of *VvXTH* gene members and functional
336 diversification. According to protein function and amino acid sequence as other plants, Thirty-four
337 *VvXTHs* are divided into I/II, IIIA, IIIB and ancestor group: 26 genes were classified into group
338 I/II whilst 7 genes formed group III, IIIA contains 2 members, IIIB contains 5 members and 1 was
339 divided into the ancestral group. Previous studies showed that because there was no clear
340 distinction between groups I and II, they were combined into one group and named group I/II. In
341 this study, the group that also clustered group I and group II together constituted the group
342 containing the largest number of members. It was known that the *XTH* genes in group III-A mainly
343 displayed XEH activity, while group III-B showed obvious XET activity, suggesting there were

functional distinction between group IIIA and IIIB. According to previous researches, Serine or Threonine near the catalytic center of XET has a typical N-glycosylation residues. The result of multiple sequence alignment showed the members of group IIIA do not contain N-glycosylated residues while group I/II and IIIB (except for *VvXTH2*) contain N-glycosylated, therefore, we speculated that *VvXTH10* and *VvXTH12* in group IIIA might possess XEH activity, and *VvXTH4*, *VvXTH32* and *VvXTH33* in group IIIB might have XET activities in grape, which is in agreement with previous research findings (*Mark et al.*, *2009*; *Miedes and Lorences*, *2009*).

Analyzing gene structure including the number and distribution of introns/exons is of great significance to further clarify the origin, evolution, or genetic relationship of species. The structure of *XTH* gene family members is complex, and the family members contain more introns than many other genes. To be specific, most members of the grape *XTH* gene family contain 3 or 4 introns, and some genes have shorter intron regions, which may be related to gene splicing (*Mount et al.*, *2012*). It is precisely because of the existence of multiple introns that gene splicing is more complicated, and to a certain extent, the expression products of *XTH* gene increase. According to previous comparison of the amino acid sequence of *Arabidopsis thaliana*, *Populus tomentosa*, *Hordeum vulgare*, *Brassicarapa*, and *Brassica oleracea*, even if the difference of protein size is obvious, the domain DEIDFEFLG is conserved in the XTH proteins reported so far (*Ya-Ru et al.*, *2017*). Motif 3 is considered to be a conserved domain contained in the XTH protein with specific functions. In this study, all 34 *VvXTH* genes are identified as DEIDFEFLG (motif3) (*Opazo et al.*, *2017*), which suggests that XTH proteins may play similar role in the plant kingdom. It is reported that DEIDFEFLG contains amino acid residues that mediate catalytic activity, which can catalyze hydrolase activity and complete cell wall structural modification and rearrangement by cutting and repolymerizing cellulose single chains (*Li et al.*, *2018*; *Behar et al.*, *2018*). The resulting

xyloglucan cross-linked structure has critical functions in maturation and resistance to abiotic stress (*Bulone et al.*, *2019*).

Previous studies have shown that *XTH* genes are of vital importance in the process of plant resistance to abiotic stress (*Chen et al.*, *2019*; *Dong et al.*, *2019*; *Li et al.*, *2019*). The expression of *CaXTH3* is induced by a variety of abiotic stresses such as drought, high salt, and low temperature, whilst the tolerance of *CaXTH3* transgenic tomato plants to salt and drought stress is significantly improved (*Choi et al.*, *2011*). Meanwhile, the heterologous expression of *PeXTH* in tobacco improves plant osmotic tolerance by reducing water loss and reducing the speed of stomata opening (*Han et al.*, *2014*). In order to study the potential function of *VvXTH* gene against abiotic stress, this study carried out promoter analysis and tissue expression analysis. To respond to drought stress, almost all members of the upstream promoter region of grape *XTH* gene family contain MBS, MYB, MYC, ARE *cis*-elements. Furthermore, 47% of the members contain ABRE, aiming to respond to ABA supervision. Under drought conditions, ABA promotes root growth and development, inhibits seed germination, promotes the shedding of senescent tissues and organs, and improves the survival rate of plants. In addition, a few members of the grape *XTH* gene family also contain DRE action elements, which means that *XTH* genes can potentially deal with salt stress apart from drought stress. Besides, it is also interesting to observe that the expression of *VvXTH* varies in different organs. Genes from the same gene cluster of gene tandem repeat events, such as *VvXTH6* and *VvXTH7*, *VvXTH26* and *VvXTH30*, are expressed differently in organs at different stages. This finding implies that, in the process of evolution, genes that are closely related have undergone sub-functional evolution, new functionalization or non-functionalization, helping grapes adapt to a variety of growth environments. According to the above results, the expression profile of *VvXTH* gene under different stresses was further analyzed. To our best knowledge, this

study is the first to explore the *VvXTH* under drought and salt stress. Consistent with the results of previous studies, qRT-PCR results revealed the complexity of *VvXTH* expression under different stresses.

We speculate that the above genes may enhance cell wall participation in stress by combining xyloglucan, and further molecular and genetic identification are still needed to verify their functions.

Conclusions

In this study, 34 grape *XTH* genes were identified. *VvXTHs* were divided into group I/II, IIIA and IIIB according to the *Arabidopsis* grouping method. Experimental results indicate that the *VvXTH* genes are unevenly distributed on 13 chromosomes, and that there are possibly tandem duplication of genes on Chr.5 and Chr.11. Furthermore, all *VvXTHs* contain conserved domains. The expression analysis showed that some *VvXTHs* can effectively response to salt and drought stress. In this sense, the present investigation results will lay a foundation for future investigations of the function of the *VvXTH* genes.

Acknowledgements

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Table

Table 1 Molecular characteristics of thirty-four *VvXTHs* identified in the grape genome

AA: amino acid; MW: molecular weight; SP: signal position; pI: isoelectric point; GRAVY: total average hydrophilicity

Figure Legends

Figure 1 Phylogenetic tree of full-length *VvXTH* and *AtXTH* proteins

The tree was constructed using the identified 34 *VvXTHs* in grape and 33 *AtXTHs* from *Arabidopsis*. The different colored branches and arcs show Group I/II, IIIA, IIIB, and Ancestral Group, respectively. Bootstrap values are shown on the branches. The blue five-pointed star represents *AtXTH* family members. The red triangle represents *VvXTH* family members.

Figure 2 Chromosomal distribution and synteny analysis of *VvXTH* genes

(A) The 34 *VvXTHs* were mapped onto grape chromosomes based on their physical positions. 81 tandemly duplicated gene-pairs are labeled by red lines. The scale on the left is in megabases (Mb). (B) Schematic representations for the chromosomal distribution and interchromosomal relationships of *VvXTHs*. Gray lines indicate all synteny blocks in the grape genome. Gene IDs on the chromosomes indicate their physical positions.

Figure 3 Synteny analysis of *XTH* between grape and *Arabidopsis*

Gray lines in the background indicate the collinear blocks within grape and *Arabidopsis* while the different color lines highlight the syntenic *XTH* gene pairs.

Figure 4 Phylogenetic relationships, structures and conserved protein motifs in 34 *VvXTHs*

(A) A Phylogenetic tree inferred from full length sequence of *VvXTHs*. Color of branches represents different groups. (B) The motif composition of the *VvXTHs* protein identified using MEME. The different colored boxes represent different motifs and their positions in each *VvXTHs*

574 sequence. Each motif is indicated by a colored box in the legend at the bottom. (C) Exon-intron
575 structure of *VvXTHs*. The boxes represent exons or UTRs (untranslated regions), and lines
576 represent introns. (D) Schematic representation of the conserved domain in grape. (E) Multiple
577 sequence alignments of the conserved domains of the *VvXTHs*. The black lines indicate the
578 conserved domain. N-glycosylation residues are indicated as asterisks.

579 **Figure 5 Heatmap of *VvXTHs* expression in different organs and stages of development**

580 Rows represent *VvXTH* members, while columns show different developmental stages and organs.
581 The expression level of *VvXTHs* is shown by the intensity of color. The phylogenetic tree on the
582 left side of the heat map is a hierarchical clustering of the expression profiles of *VvXTHs* in 54
583 samples.

584 **Figure 6 *Cis*-element analysis of 1.5 kb upstream region of *VvXTHs* start codon**

585 The bars on the top represent the total number of *cis*-elements in each gene promoter region.
586 Different colors represent different type of *cis*-elements. The color intensity and number in the
587 cells indicate the numbers of *cis*-element in these *VvXTHs*.

588 **Figure 7 Expression profiles of the *VvXTHs* under abiotic stress**

589 Heatmap shows the relative expression of 14 *VvXTHs*, detected by qRT-PCR, in roots and leaves
590 of 6-week-old 'Crimson' grape seedlings after treatment with 200 mM NaCl and 200 mM
591 Mannitol for 3, 6, 9, 12, and 24 h (0 h treatment as the control), respectively. Experiments were
592 performed in biological triplicate.

593

594 Supplementary materials

595 **Table S1 Ka/Ks analysis and duplication date estimated for grape duplicated *XTH* paralogs**

596 **Table S2. Primer sequences for qRT-PCR**

Figure 1

Figure 1 Phylogenetic tree of full-length *VvXTH* and *AtXTH* proteins

The tree was constructed using the identified 34 *VvXTHs* in grape and 33 *AtXTHs* from *Arabidopsis*. The different colored branches and arcs show Group I/II, IIIA, IIIB, and Ancestral Group, respectively. Bootstrap values are shown on the branches. The blue five-pointed star represents *AtXTH* family members. The red triangle represents *VvXTH* family members.

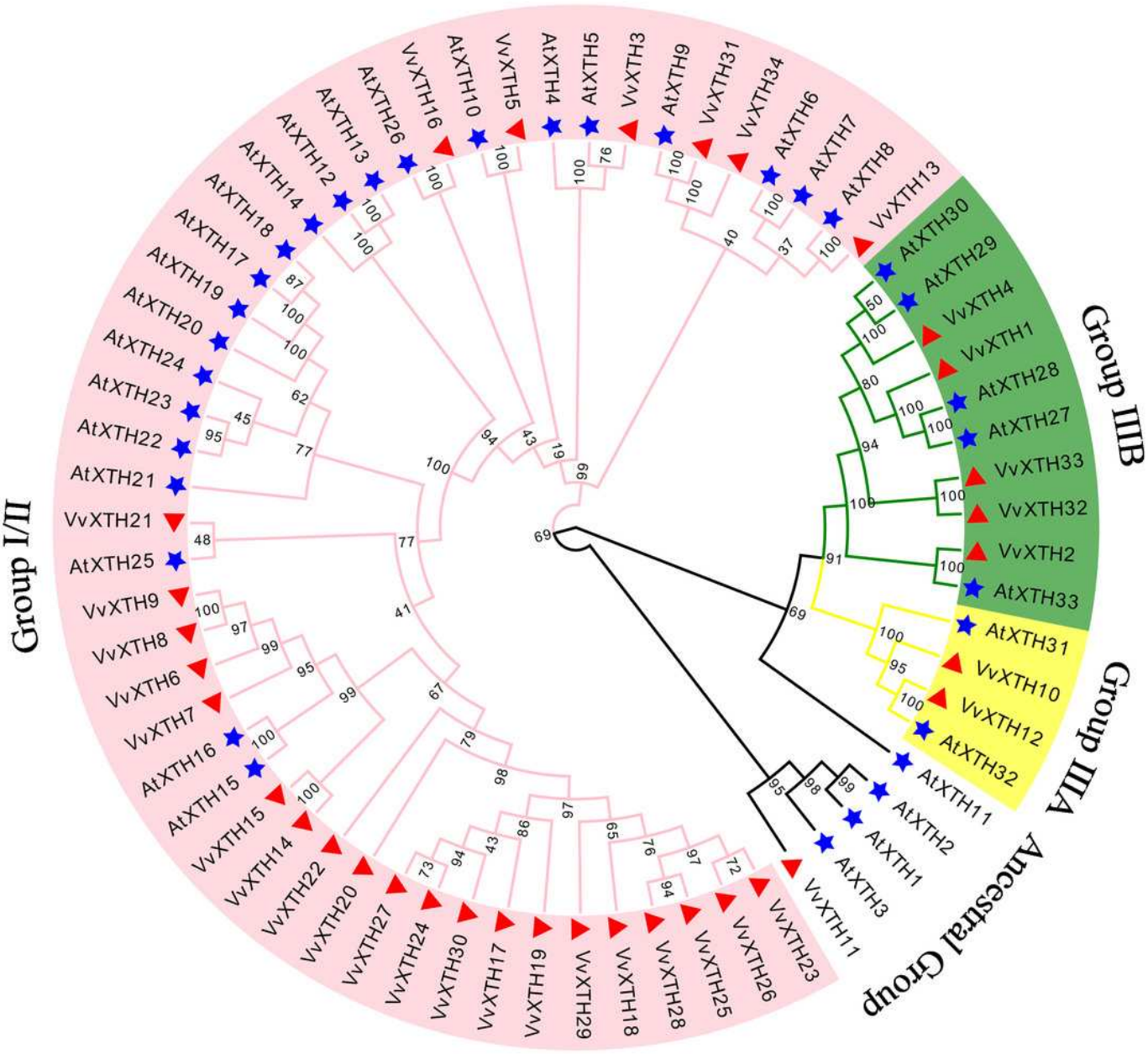
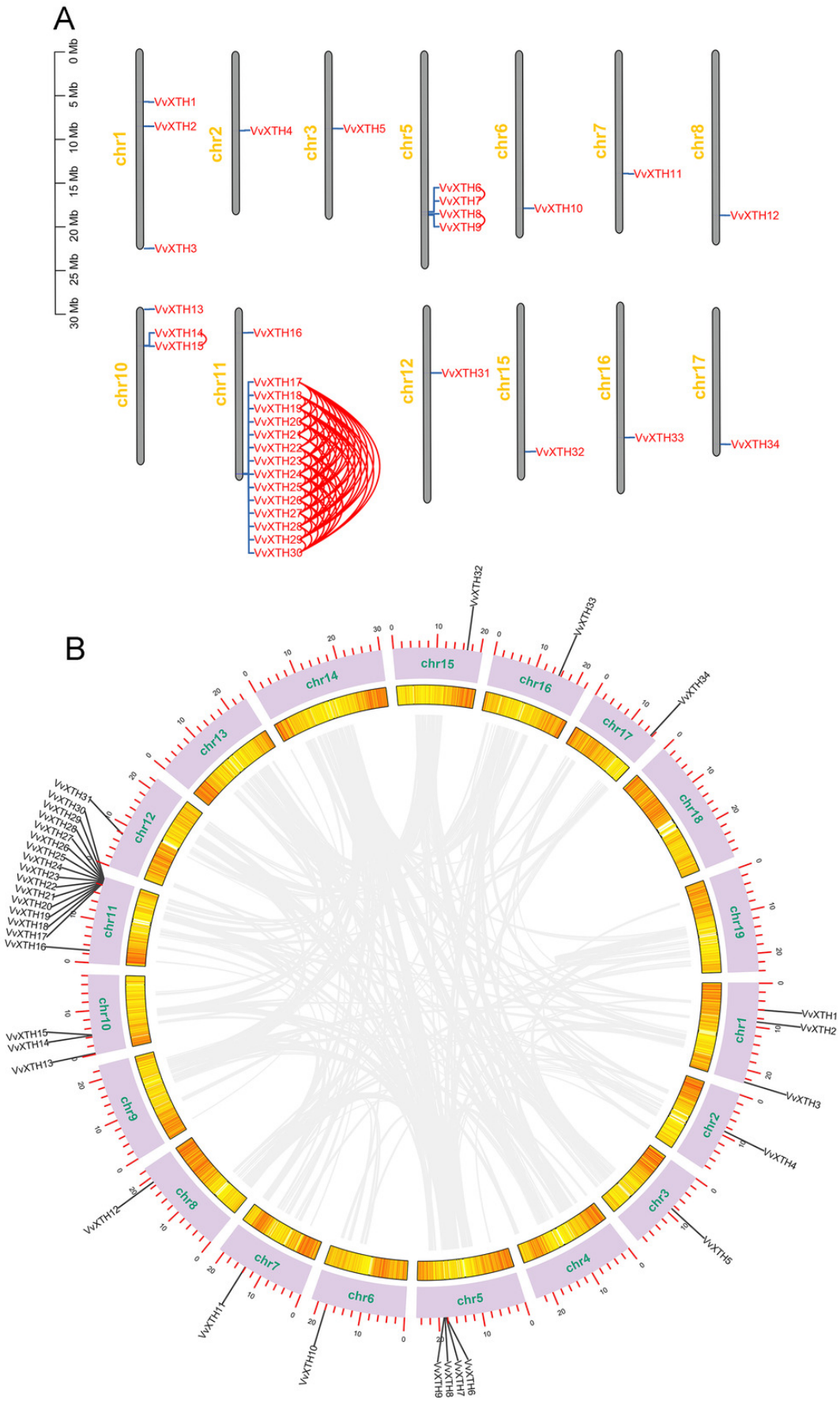

Group I/II
Group IIIB
Group IIIA
Ancestral Group
AtXTH30
AtXTH29
VvXTH4
VvXTH1
AtXTH28
AtXTH27
VvXTH33
VvXTH32
VvXTH2
AtXTH33
AtXTH31
VvXTH10
VvXTH12
AtXTH32
AtXTH11
AtXTH2
AtXTH1
AtXTH3
VvXTH11
VvXTH23
VvXTH26
VvXTH25
VvXTH28
VvXTH18
VvXTH29
VvXTH19
AtXTH17
AtXTH30
AtXTH24
VvXTH27
VvXTH20
VvXTH22
VvXTH14
VvXTH15
AtXTH15
AtXTH16
VvXTH7
VvXTH6
VvXTH8
VvXTH9
AtXTH25
VvXTH21
AtXTH21
AtXTH22
AtXTH23
AtXTH24
AtXTH20
AtXTH19
AtXTH17
AtXTH18
AtXTH14
AtXTH12
AtXTH13
AtXTH26
VvXTH16
AtXTH10
VvXTH5
AtXTH4
AtXTH5
VvXTH3
AtXTH9
VvXTH31
VvXTH34
AtXTH6
AtXTH7
AtXTH8
VvXTH13

Figure 2

Figure 2 Chromosomal distribution and synteny analysis of *VvXTH* genes

(A) The 34 *VvXTHs* were mapped onto grape chromosomes based on their physical positions. 81 tandemly duplicated gene-pairs are labeled by red lines. The scale on the left is in megabases (Mb). (B) Schematic representations for the chromosomal distribution and interchromosomal relationships of *VvXTHs*. Gray lines indicate all synteny blocks in the grape genome. Gene IDs on the chromosomes indicate their physical positions.


A
0 Mb
5 Mb
10 Mb
15 Mb
20 Mb
25 Mb
30 Mb
chr1
VvXTH1
VvXTH2
VvXTH3
chr2
VvXTH4
chr3
VvXTH5
chr5
VvXTH6
VvXTH7
VvXTH8
VvXTH9
chr6
VvXTH10
chr7
VvXTH11
chr8
VvXTH12
chr10
VvXTH13
VvXTH14
VvXTH15
chr11
VvXTH16
VvXTH17
VvXTH18
VvXTH19
VvXTH20
VvXTH21
VvXTH22
VvXTH23
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VvXTH25
VvXTH26
VvXTH27
VvXTH28
VvXTH29
VvXTH30
chr12
VvXTH31
chr15
VvXTH32
chr16
VvXTH33
chr17
VvXTH34
B
chr14
chr13
chr9
chr4

Figure 3

Figure 3 Synteny analysis of *XTH* between grape and *Arabidopsis*

Gray lines in the background indicate the collinear blocks within grape and *Arabidopsis* while the different color lines highlight the syntenic *XTH* gene pairs.

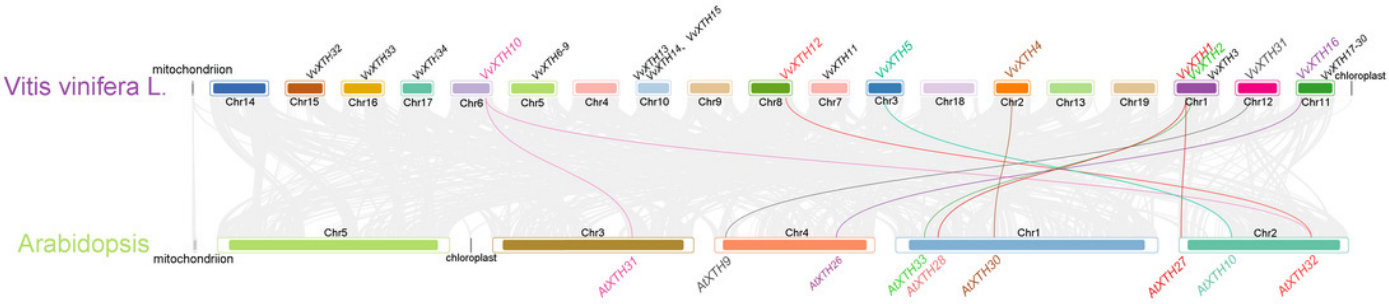
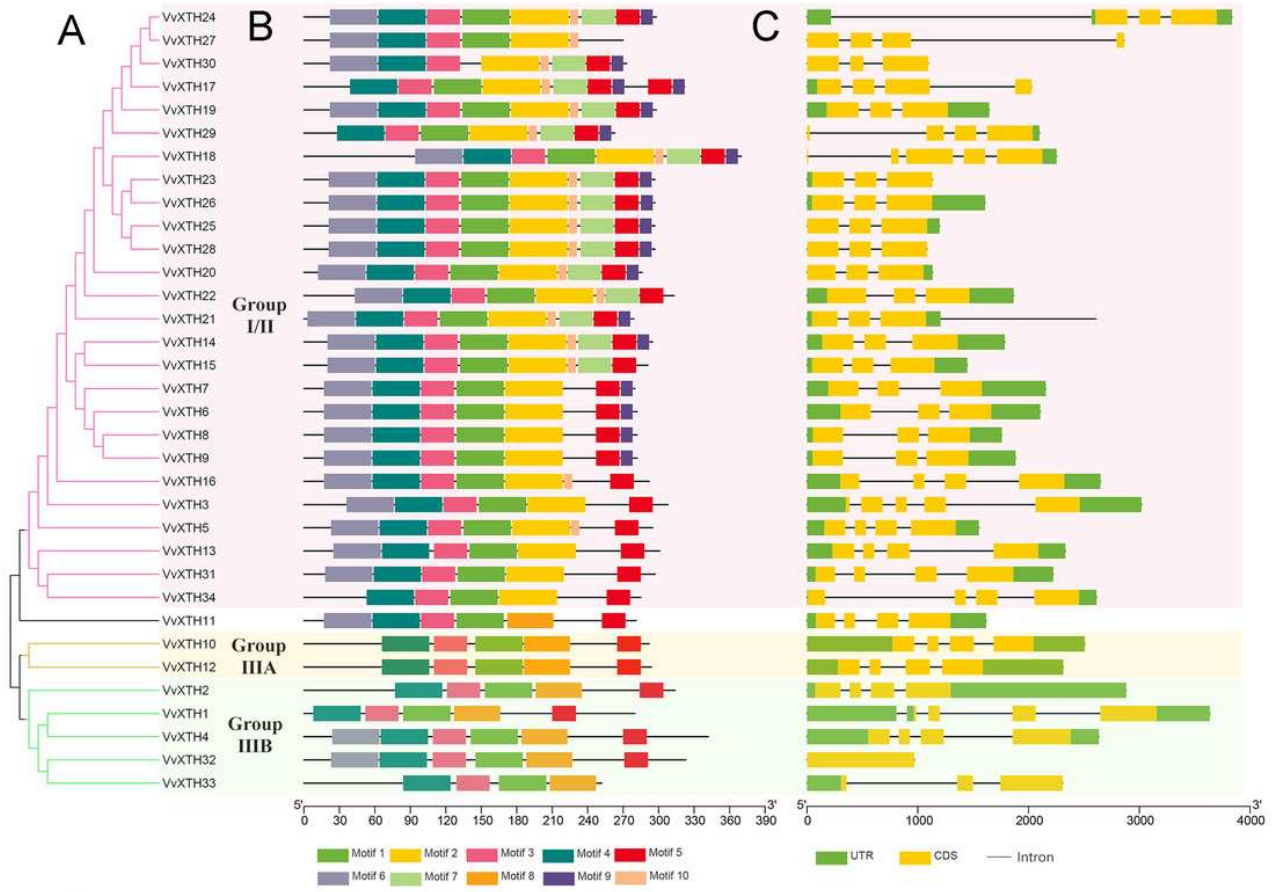


Figure 4

Figure 4 Phylogenetic relationships, structures and conserved protein motifs in 34 *VvXTHs*

(A) A Phylogenetic tree inferred from full length sequence of *VvXTHs*. Color of branches represents different groups. (B) The motif composition of the *VvXTHs* protein identified using MEME. The different colored boxes represent different motifs and their positions in each *VvXTHs* sequence. Each motif is indicated by a colored box in the legend at the bottom. (C) Exon-intron structure of *VvXTHs*. The boxes represent exons or UTRs (untranslated regions), and lines represent introns. (D) Schematic representation of the conserved domain in grape. (E) Multiple sequence alignments of the conserved domains of the *VvXTHs*. The black lines indicate the conserved domain. N-glycosylation residues are indicated as asterisks.



E

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                                    40        50        60        70        80        90       100       110
VvXTH1  YTAGVVVAFYMSNADMFEANHDELDFEFLGNIRGKDWRIQTNVYGNG....STNVGREERYHLWFDPSEDFHQYSILWSDNLIIFYVDNVP
VvXTH2  YTSGVVVAFYMSNADLFPHNHDEIDIELLGHEKRKDWVLQTNMYGNG....SVSTGREEKFRLWFDPTEQYHHYSFIWNRHHIVFLVDNVP
VvXTH3  DSAGTVTAFYLSS...QNSEHDEIDFEFLGNRSGQPYILQTNVFTGGKG.....DREQRIYLWFDPTKAFHSYSVLWNTFLIIFLVDDVP
VvXTH4  YTAGVVVAFYTSNGDVFEKTHDELDFEFLGNVKGKPWRFQTNVYGNG....STSRGREERYRLWFDPSKEFHRYSILWTAKNIIFYVDEVP
VvXTH5  HSAGTVVAYYLTS...DQPNRDEVDFEFLGNVSGQPYILQTNVFADGFD.....NREERIYLWFDPTEDFHTYSILWNLHQIVFMVDWVP
VvXTH6  NSAGTVTAYYLSS...QGSTHDEIDFEFLGNLSGDPYILHTNVFTQGKG.....NREQQFYLWFDPTRNFHTYSIIWTARHIIFLVDNVP
VvXTH7  NSAGTVTAYYLSS...QGPTHDEIDFEFLGNLSGDPYILHTNMFTQGKG.....NREQQFYLWFDPTRNFHTYSVIWNPQQIIFLVDNTP
VvXTH8  NSAGTVTAYYLSS...QGPTHDEIDFEFLGNLSGDPYILHTNVFTQGKG.....NREQQFYLWFDPTRNFHTYSIAWSAQHIIFLVDNVP
VvXTH9  NSAGTVTAYYLSS...QGPTHDEIDFEFLGNLSGDPYILHTNVFTQGKG.....NREQQFYLWFDPTRNFHTYSIAWSAQHIIFLVDNVP
VvXTH10 YTAGVITSFYLSNNEDYPGNHDEIDIEFLGTTLDKPYTLQTNVYMKGSG.DGRLIGREVKIHLWFDPTEDFHNYAILWTPDEIIFLVDDVP
VvXTH11 DSAGVVTAYYLTS...HTKNHDELDFEFLGNKEGKPYTLQTNVFSNGQG.....DREQRILLWFDPTADFHSYKILWNQHQIVFYVDDIP
VvXTH12 YTAGVITAFYLSNNEAHPGFHDEVDIEFLGTTFGKPYTLQTNVYIRGSG.DGRIIGREMKFHLWFDPTKDFHHYAILWSPKDIIFFVDDVP
VvXTH13 DSAGVVTAYYMCSENGAGPERDELDIEFLGNRSGEPYLIQTNVYKNGTG.....GREMRHMLWFDPTEEFHSYSILWNNEQILFVVDRVP
VvXTH14 NSAGTVTAYYLSS...QGQTHDEIDFEFLGNLSGDPYILHTNVFTQGKG.....NREQQFYLWFDPTKNFHTYSIIWNPQRIIFLVDNFP
VvXTH15 NSAGTVTAYYLSS...QGPTHDEIDFEFLGNLSGDPYILHTNVFTQGKG.....NREQQFYLWFDPTENFHTYSIIWNLQNIIFLVDNIP
VvXTH16 NSAGTVTAYYLSS...TGSKHDEIDFEFLGNVSGQPYTIHTNIYTQGNG.....SREQQFYLWFDPTADFHNYTIHWNPTEVVWYIDSIP
VvXTH17 NSAGTVTAYYLSS...QGPTHDEIDFEFLGNLSGDPYILHTNVFSQGKG.....NREQQFYLWFDPTADFHTYSILWNPQRIIFSVDGTP
VvXTH18 NSAGTVTAYYLSS...QGPTHDEIDFEFLGNLSGDPYILHTNVFSQGKG.....NREQQFYLWFDPTADFHTYSILWNPQRIIFSVDGTP
VvXTH19 NSAGTVTAYYLSS...QGPTHDEIDFEFLGNLSGDPYILHTNVFSQGKG.....NREQQFYLWFDPTADFHTYSILWNPQRIIFSVDGTP
VvXTH20 NSAGTVTAYYLSS...QGSAHDEIDFEFLGNLSGDPYILHTNVFSQGKG.....NREQQFYLWFDPTADFHTYSILWNPQLIIFSVDGTP
VvXTH21 NSAGTVTAFYLSS...PGPTRDEIDFEFLGNLSGDPYVHTNVYTQGKG.....EREQQFYLWFDPTADFHTYSFTWNPKHIIFSVDDTP
VvXTH22 NSAGTVTAYYFSS...LGSTHDEIDFEFLGNLSGDPYILHTNVFSQGKG.....NREQQFYLWFDPTANFHTYSVLWNPHNILFSVDGTP
VvXTH23 NSAGTVTAYYLSS...QGPTHDEIDFEFLGNLSGDPYILHTNVFSQGKG.....NREQQFYLWFDPTADFHTYSVLWNPQRIIFSVDGTP
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VvXTH25 NSAGTVTAYYLSS...QGPTHDEIDFEFLGNLSGDPYILHTNVFSQGKG.....NREQQFYLWFDPTADFHTYSILWNPQRIIFSVDGTP
VvXTH26 NSAGTVTAYYLSS...QGPTHDEIDFEFLGNLSGDPYILHTNVFSQGKG.....NREQQFYLWFDPTADFHTYSVLWNPQRIIFSVDGTP
VvXTH27 NSAGTVTAYYLSS...QGPTHDEIDFEFLGNLSGDPYILHTNVFSQGKG.....NREQQFYLWFDPTADFHTYSILWNPQRIIFSVDGTP
VvXTH28 NSAGTVTAYYLSS...QGPTHDEIDFEFLGNLSGDPYILHTNVFSQGKG.....NREQQFYLWFDPTADFHTYSILWNPQRIIFSVDGTP
VvXTH29 NSAGTVTAYYLSS...QGPTHDEIDFEFLGNLSGDPYILHTNVFSQGKG.....NREQQFYLWFDPTANFHSYSILWNPQRIIFSVDGTP
VvXTH30 NSAGTVTAYYLSS...QGPTHDEIDFEFLGNLSGDPYILHTNVFGQGKG.....NRE.....................HFSVDGTP
VvXTH31 DSAGTVTAFYMSS...DGPNHNEFDFEFLGNTTGEPYLVQTNIYVNGVG.....NREQRLNLWFDPTKDFHSYTFMWNQRQVVFLVDETP
VvXTH32 DTAGVVVAFFTMNQDIYSRNQDEATFEFLGNVKGKEWVVQTNIHGNTTIRNSTSKGREERYTLWFDPSADFHKYSILWTENHIIFYVDDVP
VvXTH33 GTVGVVVAFDSMNQDRYSKNQDEVTFEILGNVKGKEWAVQTNVHGNTTIRNSTSKGREERYTLRFDPSADFHKYSIFWTENHIIFYVDDVP
VvXTH34 DSAGTVTALYMAS...EGLNHDELDFEFLGNVSGEPYLVQTNVYVNGTG.....NREQRHTLWFDPTAAFHTYSFLWNRYSILFLVDEVP
                                                  Active site  *   *
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Figure 5

Figure 5 Heatmap of *VvXTHs* expression in different organs and stages of development

Rows represent *VvXTH* members, while columns show different developmental stages and organs. The expression level of *VvXTHs* is shown by the intensity of color. The phylogenetic tree on the left side of the heat map is a hierarchical clustering of the expression profiles of *VvXTHs* in 54 samples.

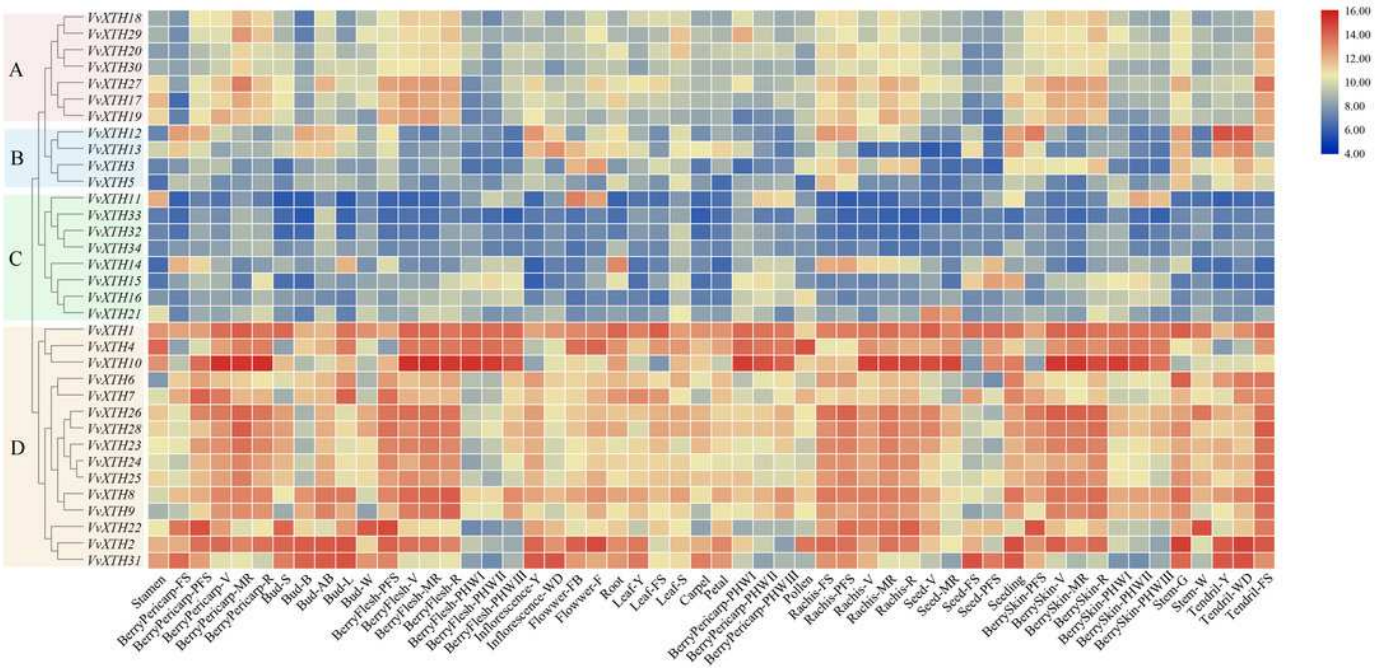

Figure 6

Figure 6 *Cis*-element analysis of 1.5 kb upstream region of *VvXTHs* start codon

The bars on the top represent the total number of *cis*-elements in each gene promoter region. Different colors represent different type of *cis*-elements. The color intensity and number in the cells indicate the numbers of *cis*-element in these *VvXTHs*.

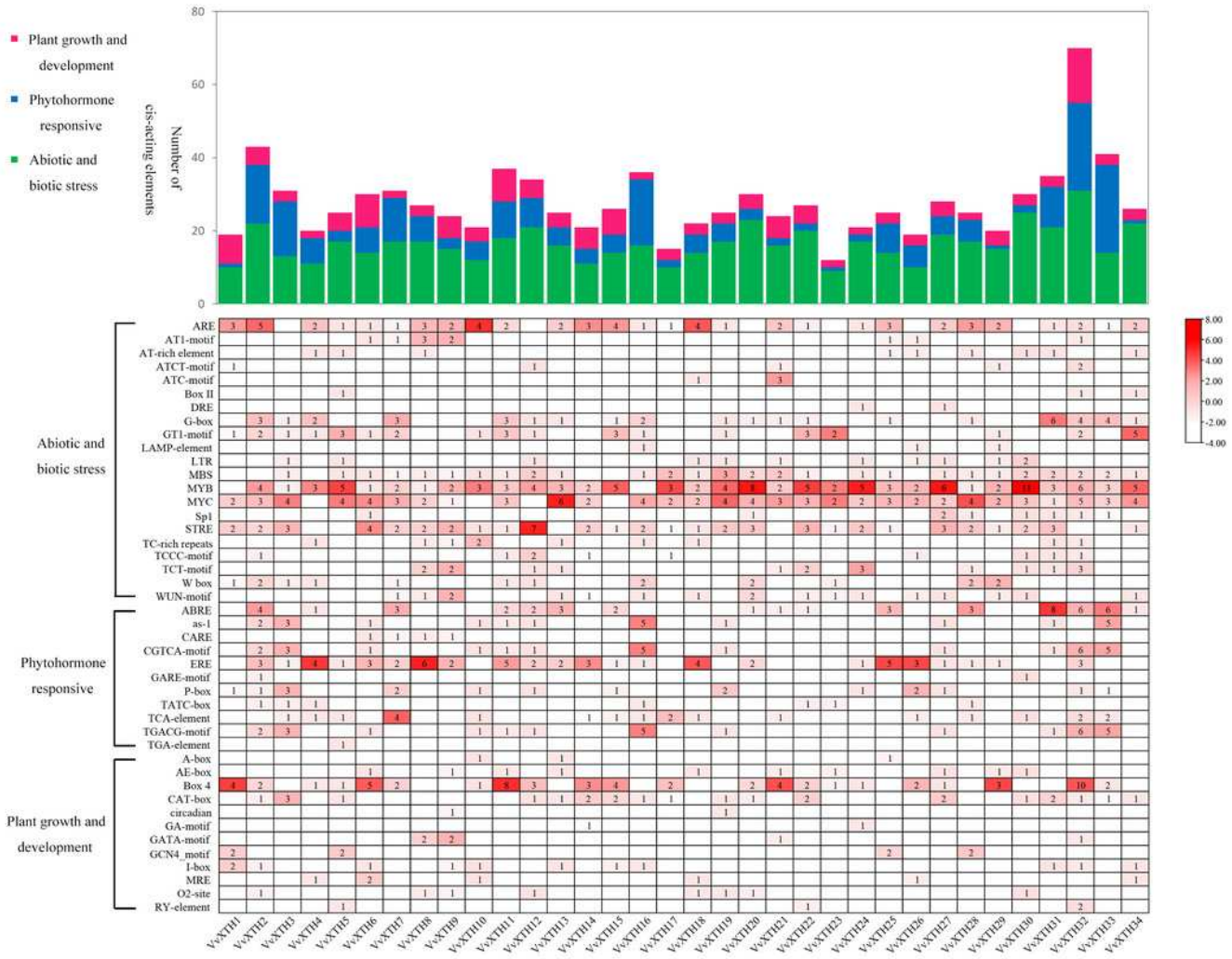

Figure 7

Figure 7 Expression profiles of the *VvXTHs* under abiotic stress

Heatmap shows the relative expression of 14 *VvXTHs*, detected by qRT-PCR, in roots and leaves of 6-week-old ‘Crimson’ grape seedlings after treatment with 200 mM NaCl and 200 mM Mannitol for 3, 6, 9, 12, and 24 h (0 h treatment as the control), respectively. Experiments were performed in biological triplicate.

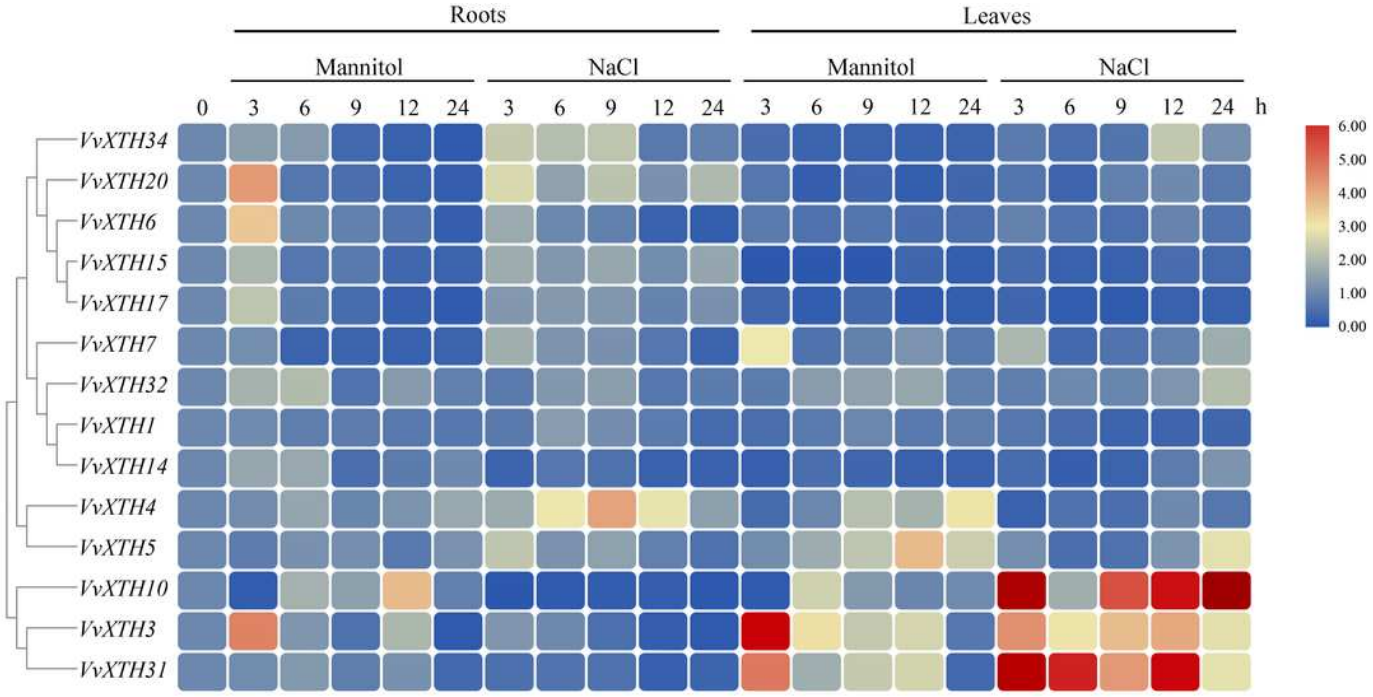

Table 1(on next page)

Table 1. Molecular characteristics of thirty-four *VvXTHs* identified in the grape genome

AA: amino acid; MW: molecular weight; SP: signal position; pI: isoelectric point; GRAVY: total average hydrophilicity

Table 1. Molecular characteristics of thirty-four *VvXTHs* identified in the grape genome

Name	Gene Identifier	AA	MW(Da)	SP	pI	GRAVY	Subcellular Localization
VvXTH1	VIT_201s0011g06250	279	32099.88	–	6.60	-0.649	Plasma membrane
VvXTH2	VIT_201s0026g00200	313	35198.85	24	6.83	-0.296	Extracellular
VvXTH3	VIT_201s0150g00460	307	35270.14	35	8.65	-0.366	Plasma membrane
VvXTH4	VIT_202s0012g02220	341	38867.80	–	8.99	-0.374	Plasma membrane
VvXTH5	VIT_203s0088g00650	295	34401.83	25	7.12	-0.372	Plasma membrane
VvXTH6	VIT_205s0062g00240	281	32143.11	24	9.22	-0.389	Plasma membrane
VvXTH7	VIT_205s0062g00250	279	32239.31	24	9.07	-0.449	Plasma membrane
VvXTH8	VIT_205s0062g00480	281	32088.01	24	9.08	-0.406	Plasma membrane
VvXTH9	VIT_205s0062g00610	281	32173.18	24	9.14	-0.408	Plasma membrane
VvXTH10	VIT_206s0061g00550	291	32696.72	18	5.74	-0.438	Extracellular
VvXTH11	VIT_207s0185g00050	280	32102.92	19	7.11	-0.555	Plasma membrane
VvXTH12	VIT_208s0007g04950	293	33761.16	18	9.45	-0.457	Extracellular
VvXTH13	VIT_210s0116g00520	300	34816.90	27	4.61	-0.584	Plasma membrane
VvXTH14	VIT_210s0003g02440	294	33673.17	27	9.44	-0.375	Plasma membrane
VvXTH15	VIT_210s0003g02480	290	32860.23	27	8.18	-0.278	Plasma membrane
VvXTH16	VIT_211s0016g03480	291	33246.42	17	8.24	-0.338	Plasma membrane
VvXTH17	VIT_211s0052g01180	321	36502.43	–	4.81	-0.596	Plasma membrane
VvXTH18	VIT_211s0052g01190	369	41704.56	–	6.36	-0.454	Plasma membrane
VvXTH19	VIT_211s0052g01200	297	32951.68	29	5.22	-0.392	Plasma membrane
VvXTH20	VIT_211s0052g01220	285	31821.45	19	5.92	-0.404	Plasma membrane
VvXTH21	VIT_211s0052g01230	278	31187.75	–	5.13	-0.405	Plasma membrane
VvXTH22	VIT_211s0052g01250	312	35059.55	–	8.42	-0.338	Plasma membrane
VvXTH23	VIT_211s0052g01260	296	32919.61	26	5.07	-0.393	Plasma membrane
VvXTH24	VIT_211s0052g01270	297	33155.89	29	4.97	-0.361	Plasma membrane
VvXTH25	VIT_211s0052g01280	296	32939.66	26	5.63	-0.420	Plasma membrane
VvXTH26	VIT_211s0052g01300	280	31322.79	26	5.37	-0.419	Plasma membrane
VvXTH27	VIT_211s0052g01310	269	30176.71	29	5.69	-0.275	Plasma membrane
VvXTH28	VIT_211s0052g01320	296	33018.79	26	5.93	-0.425	Plasma membrane
VvXTH29	VIT_211s0052g01330	262	29508.94	18	5.62	-0.459	Plasma membrane
VvXTH30	VIT_211s0052g01340	272	29935.10	29	4.96	-0.457	Plasma membrane
VvXTH31	VIT_212s0134g00160	296	33657.80	27	5.60	-0.320	Plasma membrane
VvXTH32	VIT_215s0048g02850	322	37077.07	23	6.11	-0.346	Extracellular
VvXTH33	VIT_216s0100g00170	251	28460.18	–	6.65	-0.232	Extracellular
VvXTH34	VIT_217s0053g00610	284	32329.43	21	5.50	-0.361	Plasma membrane

AA: amino acid; MW: molecular weight; SP: signal position; pI: isoelectric point; GRAVY: total average hydrophilicity