Identification, characterization and functional analysis of grape (*Vitis vinifera* L.) Mitochondrial Transcription Termination Factor (mTERF) genes in responding to biotic stress and exogenous phytohormone

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Abstract

Mitochondrial transcription termination factor (mTERF) is a large gene family which plays a significant role during plant growth against various environmental stresses. However, knowledge of mTERF genes in grapevine (*Vitis* L.) is limited. In this research, a comprehensive analysis of grape mTERF genes, including chromosome locations, phylogeny, protein motifs, gene structures, gene duplications, synteny analysis and expression profiles, was conducted. As a result, a total of 25 mTERF genes were identified from the grape genome, which are distributed on 13 chromosomes with diverse densities and segmental duplication events. The grape mTERF gene family is classified into nine clades based on phylogenetic analysis and structural characteristics. These *VvmTERF* genes showed differential expression patterns in response to multiple phytohormone treatments and biotic stresses, including treatments with abscisic acid and methyl jasmonate,and inoculation of *Plasmopara viticola* and *Erysiphe necator* These research findings, as the first of its kind in grapevine, will provide useful information for future development of new stress tolerant grape cultivars through genetic manipulation of *VvmTERF* genes.

Introduction

In eukaryotes, genetic information is not only stored in the nucleus, but also in organelle genomes such as mitochondria and chloroplasts. Due to the loss of organelle genes and the continuous transfer of organelle-nuclear genes in the process of organelle evolution, the number of organelle genes have been drastically reduced [1–3]. In living organisms, the organelle expression system largely depends on nuclear-coding proteins, which include RNA polymerase, sigma factor, and specific RNA maturation factors [4–8]. Meanwhile, partial organelle protein families which have similar modular structures consisting of repetitive helical motifs also play an important role in organelle gene expression. These functional proteins include PPRs, HAT, OPRs and mTERFs [4, 9].

Commonly located in organelles and cytoplasm, mitochondrial transcription termination factor (mTERF) genes comprise a large family which is vital in regulation of mitochondrial gene transcription [10]. mTERF proteins are able to bind to specific sites in mitochondria, leading to the cessation of mitochondrial gene transcription. The mTERF protein structure based on repetition of 30 amino acids form the typical mTERF motif [11]. The crucial characteristic of human mTERF motif is that there is a proline at position 8, 11, 18 and 25. Therefore, the motif is conservative of leucine or hydrophobic amino acids, indicating that there are at least three leucine motifs in the mTERFs [12]. Previous researches indicated that mTERF proteins could have multiple biological functions of intracellular regulation. For example, human mTERF1, with 342 amino acids in length, can promote the termination of mitochondrial gene transcription. It has been proved *in vitro* that mTERF1 arrests on mitochondrial RNA polymerase(mtRNAP)by binding to 28 nucleotide sequences downstream of the 3'end of 16SrRNA [13, 14]. The mTERF1 protein possess the function of regulating the transcriptional initiation of mitochondrial rDNA and mitochondrial DNA replication [15]. In addition, mTERF2 protein showed a significant downregulation of mitochondrial transcription level *in vitro*, suggesting that mTERF2 protein may affect mitochondrial transcription by binding with regulatory activators of mtDNA transcription initiation [16].

In recent years, plant mTERF genes and their roles in regulating expression of mitochondrial genes have received a good deal of attention. Bioinformatic analysis shows that the mTERF genes are a large and complex protein family existing in metazoans and plants[17]. There are at least 35 mTERFs in *Arabidopsis thaliana*, mainly located in mitochondria or chloroplasts [18, 19]. By far, several mTERF genes from *A. thaliana* were obtained and their functions in abiotic stresses were studied. For example, the seed germination rate of *mterf1* (*soldat 10*) mutant was considerably lower than wild type under the same condition[20]. Over expression of the *AtmTERF5* (*mad1*) gene affects the germination rate of transgenic lines under simulated drought stress as higher germination rate was observed under mannitol treatment [21]. Besides, *A. thaliana mterf9* mutant was insensitive to ABA treatment. Under the treatment of NaCl and ABA, the root growth retardation of *mTERF9* mutant plants displayed the phenotype of short root and light fresh weight compared to the wild type [22]. Furthermore, past studies in maize showed that ZmTERF4 protein can coimmunoprecipitate with multiple chloroplast introns and the splicing of some of these introns is disrupted even in *Zm-mterf4* mutants, indicating that it might play significant roles in meditating communication between organelle and the nucleus[23]. These conclusions expand the functional knowledge of the mTERF family.

As a large economic worth fruit crop [24], grapevine is an important resource for identifying stress resistance genes to enhance grape quality. At present, the basic structure and preliminary functions of mTERF family proteins has been continuously explored, but their detailed functions and regulation mechanisms under different stresses still need further study. The current investigation is therefore to study members of the grape mTERF gene family (VvmTERF) for their potential functions to stress resistance.

Materials And Methods

Identification and annotation of grape mTERF genes

Conserved mTERF domains were first used to detect grape genes in mTERF HMM (Hidden Markov Model) file (PF02536) from the Pfam database [25] using the HMMER 3.0 package [26]. The domains were then used as a query to search the GenBank nonredundant protein database and the Grape Genome Database (http://www.rosaceae.org/projects/grape_genome) using the BLAST program. All mTERF proteins with an E value <0.01 were collected and the domains were manually checked in each identified *VvmTERF* gene.

Multiple sequence alignment, phylogenetic analysis and classification

A total of 25 *VvmTERF* genes containing mTERF core domains were identified. The CLUSTALX software was then used for multiple sequence alignment analysis [27]. Furthermore, a multiple sequence alignment including grape mTERF genes and those from Arabidopsis (*AtmTERF*) and maize (*ZmTERF*) were conducted using CLUSTALW version. Based on the neighbor-joining method and with the bootstrap test replicated 1000 times, a phylogenetic tree was constructed using MEGA 5.0 software [28]. The

VvmTERF genes were classified into clades ground on multiple sequence alignments with those *AtmTERF* and *ZmTERF* genes.

MEME motifs and conserved sequences analysis of grape mTERF proteins

The identification of known conserved motifs in grape mTERF proteins was conducted by SMART [29] and Pfam [25] database searching. The potential motifs in the putative mTERF family gene sequences were predicted by Multiple Em for Motif Elicitation (MEME) program [30] with the parameters as follow: the optimum width of every single motif distributed between 6 to 50, and the maximum number of motifs to find was 15. After that, the collection and cluster of mTERF motifs from grape mTERF proteins were conducted using the ClustalW 2.0 program software [27], and graphical representation of amino acid residues was arranged by TBtools [31].

Exon–intron structure analysis, synteny analysis and gene duplication

According to alignments of grape mTERF gene coding sequences and their respective full-length sequences, the exon-intron structure was determined on Grape Genome Browser: http://www.genoscope.cns.fr/externe/GenomeBrowser/*Vitis*/. And the online program Gene Structure Display Server (GSDS: http://gsds.cbi.pku.edu.ch)[32] was carried out to obtain relative diagrams. Besides, the definition of mTERF genes with tandem duplication events was adjacent homologous genes on a single chromosome, while gene duplication events among diverse chromosomes were defined as segmental duplications [33]. The specific physical location of each *VvmTERF* gene on its individual chromosome determines whether it was considered a tandem duplication event. Therefore a synteny analysis map of grape mTERF genes was constructed via the syntenic blocks, and a further synteny analysis between grape and *AtmTERF* genes was acquired from the Plant Genome Duplication Database [34]. The generation of related diagrams was obtained by the Circos version website (http://circos.ca/).

Cis-element analysis of grape mTERF genes promoter

The 2000 bps upstream promoter sequence of each grape mTERF gene coding regions were obtained from the grape genome database (https://wwwdev.genoscope.cns.fr/vitis). PlantCARE online analysis program (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) was used to find the predicted cis-element.

Expression profiles of grape mTERF gene family in different tissues and organs

The expression profile of grape mTERF gene family were confirmed in a *V. vinifera* cv 'Corvina' (clone48) gene expression atlas of various organs at different developmental stages. Microarray data were collected from the NCBI gene expression omnibus (GEO) datasets under the series entry GSE36128 (http://www.ncbi.nlm.nih.gov/geo/) [35]. The mean expression value of grape mTERF genes in all tissues and organs were analyzed and detailed displayed by Multiple Experiment Viewer software (MeV) [36]. Measured using RNA-Seq data, the expression patterns of *VvmTERF* genes in various berry

developmental stages were gained from gene expression omnibus (GEO) database of NCBI (GSE77218) [37].

Plant materials and stress treatments

To notarize the expression regulation of grape mTERF genes under abiotic and biotic stresses, grape leaves and organs were obtained from *V. vinifera* 'Thompson Seedless' grape grown in greenhouse. When the shoots of the grapevines reach 30 centimeters long and young leaves were fully expanded, the plants were applied for hormone treatment. Hormone treatments were carried out on similar growth condition grape leaves and sprayed with 100 μM salicylic acid (SA), 300 μM abscisic acid (ABA), 50 μM methyl jasmonate (MeJA), and 0.5 g/l ethylene (Eth), respectively. Leaves from the treated vines were collected at 0.5, 1, 3, 6, 12, 24, and 48 h post treatment [38]. Grape leaves sprayed with sterile water were harvested as the negative control.

Samples of the powdery mildew (*Erysiphe necator*) and downy mildew (*Plasmopara viticola*) pathogens were used to inoculate young leaves of *V. vinifera* 'Thompson Seedless'. Leaves were sampled at 6, 12, 24, 48, 72, 96, and 120 h post inoculation and untreated leaves collected as the negative control. At each time point of all treatments, nine leaves from three separate plants were homogenized, and the treatments were conducted three times independently. These grape leaves were frozen in liquid nitrogen and stored at −80°C.

Semiquantitative PCR and real-time quantitative PCR analysis

The method of total RNA extraction referred to Zhang et al [39]. Then RNA was digested by RNase-free DNase I (OMEGA Bio Inc., USA) to remove genomic DNA. The grape *Actin1* gene (GenBank Accession number AY680701) and *EF1-α* gene were chosen as housekeeping genes and amplified with the primers showing in Table S1, which also includes Gene-specific primers for the 25 *VvmTERF* genes. For the semi-quantitative reverse transcription-PCR experiment, the volume of reaction system was 20 μL which includes 1 μL cDNA template, 1 μL gene-specific primers, 10 μL PCR Master Mix (Qingke Biotech Co. Ltd., Shanghai, China) and 8 μL sterile water, the specific proportion and program were set according to the PCR Master Mix instruction book. Each PCR reaction was conducted twice. the Gene Tools software was used for quality control of the results of semi-quantitative PCR, after that log-transformed values of the relative expression patterns of *VvmTERF* genes under various phytohormone and biotic stresses treatment were used to perform hierarchical cluster via Genesis software.

Quantitative real-time PCR analysis was performed with an IQ5 real-time PCR instrument (Bio-Rad, Hercules, CA, USA). All reactions were performed in triplicate with a reaction system of 20 μL including 1 μL specific primers, 1 μL cDNA, 10 μL SYBR green (Yeasen Biotech Co Ltd, Shanghai, China), and 8 μL sterile water, the specific proportion was on the instruction book as well as the PCR parameters. The expression levels of grape mTERF genes were analyzed using IQ5 software with the normalized expression method. The t-test was conducted using the SPSS software (SPSS 17.0, Chicago, IL, USA).

Results

Identification of mTERF genes in grape genome

MTERF genes in the grape genome were identified by BLASTP with HMMER 3.0 [26] searching key domain mTERF PFAM file (PF02636). A total of 25 grape mTERF genes were identified, which were named as *VvmTERF1-VvmTERF25* according to sequence of their chromosomal locations (Table 1). A high conserved mTERF domain was found in all the VvmTERF proteins.

Phylogenetic analysis and classification of grape mTERF genes

In order to evaluate the evolution relationship of *VvmTERF* gene family, a total of 95 mTERF genes from Arabidopsis (35), maize (35) and grape (25) genomes were collected for a phylogenetic tree construction using MEGAX software. Detailed sequence information of Arabidopsis and maize mTERF genes were obtained from a previous study [40]. The tree topology result demonstrated that nine groups (CladeI–IX) were classified based on their conversed domains (Figure 1). Of the 25 *VvmTERF* genes, Clade contained 7 genes, the most among all the clades, while other clades had 1 to 5 members, respectively. Only one grape mTERF gene, *VvmTERF24*, belonged to Clade I where 4 members were identified in Arabidopsis and in maize, respectively [19, 40]. It is worth noting that the well functional characterized mTERF genes from Arabidopsis, such as *SOLDAT10* (*AtmTERF1*, AT2G03050), *BSM/RUG2* (*AtmTERF4*, AT4G02990), and *SHOT1* (*AtmTERF18*, AT3G60400) were distributed in group II, IV and VI, respectively. Meanwhile, a certain of grape mTERF genes belong to these groups, indicating their close evolutionary relationships with Arabidopsis mTERF genes from the same group.

Exon–intron structure analysis of VvmTERF genes

Structures analysis on the exon and intron boundaries of the *VvmTERF* genes will provide important clues as they played significant roles in evolution of various gene families. The number of exons per grape mTERF gene ranged from 1 to 22 (Figure 2). Among them, *VvmTERF20* had the most exons of 22, followed by *VvmTERF14* (10), *VvmTERF16* (7), *VvmTERF18* (6), *VvmTERF*9 (6), *VvmTERF24* (6) and *VvmTERF4* (6)while *VvmTERF3*, *VvmTERF8*, *VvmTERF11-13* and *VvmTERF21* had only one exon each. These results indicated that during the long evolution of *VvmTERF* gene family, both exon loss and gain have occurred, which might lead to diversified function among the otherwise closely related mTERF genes. In clade , for example, the number of exons was quite large, ranging from three to ten, while the genes in clade I and had a relatively smaller number, ranging from one to six exons. It is worth noted that *VvmTERF* genes from same clades had similar exon/intro structures while genes from different groups normally showed distinct structures. This difference in exon/intro patterns might be resulted from a series of gene replication events.

VvmTERF conserved motifs analysis

Searching for known conserved motifs in grape mTERF proteins was conducted via Pfam [25] and SMART [29] databases. In order to predict the potential motifs in the putative grape mTERF family gene

sequences, the MEME (Multiple Em for Motif Elicitation) program [30] was used and 15 mTERF motifs in grape were identified and clustered (Figure 2 and Table 2) using the ClustalW 2.0 program [27]. Grape mTERF sequences had 1–15 mTERF motifs. For example, all class sequences had more than 10 mTERF motifs, and clade IX mTERF sequences had 5–8 mTERF motifs. Identified in human mTERF proteins previously[12], conversed mTERF motifs containing repeats of leucine zipper-like heptad X_3LX_3 structure was also found in grape mTERF motifs (Table 2), suggesting that fundamental structures and functions of mTERF proteins in *Vitis* might be similar to human mTERF proteins.

Aiming to find predicted motifs shared among related proteins within the grape mTERF gene family, the MEME database program [30] was performed. As shown in Figure 2, a total of 15 motifs, designated as motifs 1–15, were discovered among these 25 proteins. Among them, motifs 2 and 8 were found in most grape mTERF proteins. Motif sequences comparison with PFAM mTERF domain alignment revealed that motifs 1, 4 and 5 partly covered the PFAM mTERF domain (PF02536), and motif 5 belonged to specific organelle-targeting mTERF proteins, such as the group grape mTERF proteins (Figure 2). It is probably that group-specific motifs lead to characteristic functions in various life activities.

Synteny analysis of VvmTERF and AtmTERF genes

Arabidopsis is a well-studied model species which can provide available genomic information to a less-studied species through genomic comparison method [38, 41]. As showed in Figure 3, a large-scale syntenies study containing 6 pairs of grape and Arabidopsis mTERF genes were recognized. Grape orthologues including *VvmTERF2*, *VvmTERF6*, *VvmTERF13*, *VvmTERF15*, *VvmTERF24* and *VvmTERF25* displayed synteny location with Arabidopsis mTERF genes *AtmTERF6*, *AtmTERF4*, *AtmTERF19*, *AtmTERF10*, *AtmTERF9* and *AtmTERF17*, respectively (Table S2). The number of synteny results indicated that several mTERF genes might arise before the divergence of Arabidopsis and grape lineages, and also suggested that partial deletion of the grape genes might occur in specific syntenic locations during genome evolution.

Cis-element analysis of grape mTERF gene promoters

To understand the possible regulatory mechanism of *VvmTERF* genes in multiple stress responses and functions in chloroplast and mitochondrion, a 2-kb sequence upstream of the translational initiation site of each *VvmTERF* gene was analyzed by the PlantCARE database. Meanwhile, *Actin1* was chosen in grape genome as the housekeeping gene (Figure 4). The sequences of *VvmTERF* gene promoters were found to contain various hormone regulation-related cis-elements such as those responsive to auxin, MeJA (Methyl Jasmonate), gibberellin, abscisic acid and salicylic acid. In addition, various defense and stress-related elements were also observed. These elements included light and wound responsive elements, osmotic stress-related elements, and low temperature and drought responsive elements.

Analysis of expression profiles among the grape mTERF genes in different tissues and organs

To discover the potential function of VvmTERF proteins during different stages of grape development, the tissue/organ-specific expression profiles of *VvmTERF* genes were analyzed in the *V. vinifera* cv. Corvina global gene expression atlas from the GEO DataSet (GSE36128). This dataset contained expression information of 54 sample tissues and organs in different developmental phases acquired by microarray database (Figure 5). The results showed that some *VvmTERF* genes such as *VvmTERF6*, *9*, *11* and *23* displayed similar expression patterns in different tissues and organs, while other *VvmTERF* genes like *VvmTERF1*, *3*, *10* and *16* demonstrated tissue/organ-specific expression profiles, suggesting multiple roles played by these *VvmTERF* genes in grapevine.

Expression patterns of VvmTERF genes under different exogenous hormone treatments

To explore potential stress-related genes characterized in this research, plant hormones ABA, MeJA, SA and ethylene were chosen because of their well-known functions in regulating plant signaling networks [42]. Interestingly, almost all these *VvmTERF* gene expressions were influenced by exogenous hormone treatments (Figure 6). For example, after the ABA treatment, a total of 13 *VvmTERF* genes displayed multiple degrees of up regulation while 8 genes were down regulated. MeJA treatment led to the expression increase of 17 *VvmTERF* genes and decrease on 7 genes. However, the expression patterns under SA and Eth treatments were different from those regulated by ABA and MeJA as more down regulated genes were observed. A total of 5 *Vv*mTERF genes were up regulated and 12 were down regulated by SA, while 7 were up regulated and 14 were down regulated by exogenous Eth hormone treatment. According to the semi-quantitative RT-PCR result, *VvmTERF2*, *VvmTERF6*, *VvmTERF16*, *VvmTERF23* and *VvmTERF26*, which were downregulated by the ETH treatment, displayed obvious upregulation by the MeJA treatment, indicating an existence of different regulatory networks among these phytohormones.

Expression profiles of VvmTERF genes in response to biotic infections

In order to adapt to changing environments, the ability of tolerating to diverse stresses becomes a significant trait in plant. Identification and functional analysis of genes involved in biological signal transduction pathways is of great significance in providing a fundamental information for plant development and stress responses. To investigate their role in responding to biotic stress, express analysis of the 25 *VvmTERF* genes were conducted in potted ‘Thompson Seedless’ grapevines in greenhouse after inoculating with powdery mildew (PM) and downy mildew (DM) pathogens. As shown in Figure 6, most *VvmTERF* genes demonstrated a tendency of downward expression after the inoculation. For example, the expression of clade genes-*VvmTERF5*, *7-12*-decreased in both *E. necator* and *P. viticola* treatments, *while VvmTERF7* and *VvmTERF10* genes have slightly decreased after *P. viticola* inoculation (Fig 6). Besides, the expression level of *VvmTERF6, VvmTERF14* and *VvmTERF19* held steady in both biotic treatments. On the other hand, *VvmTERF11*, *VvmTERF17* and *VvmTERF21* displayed an increasing trend in both PM and DM treatments in comparison with the control. Based on semi RT-PCR analysis, three grape mTERF genes (*VvmTERF2*, *VvmTERF4* and *VvmTERF20*) were chosen

for further detailed analysis using real-time qPCR. The qPCR results were consistent with the those obtained by semi RT-PCR.

Discussions

Widely identified in metazoans and plants, mitochondrial transcription termination factors (mTERFs) can regulate the expression of organelle genes at different levels [43]. Previous researches showed that mTERF plays a significant regulatory role in the function of mitochondrial genes, which have important meanings for mitochondrial function, biological evolution, gene diagnosis and treatment [44]. In plants, the expression of mitochondrial genes is fundamental for various plant biological functions. To fully explore their functions of grape mTERF genes, it is essential to identify and characterize mTERF genes in grape genome. In the current study, 25 grape mTERF genes were identified. Their structures, evolutionary history and expression patterns in responding to biotic stresses and hormone treatments were also analyzed.

Identification, annotation and evolution of VvmTERF genes

In this study we searched mTERF genes in grapevine and found 25 mTERF genes that all can be mapped onto the sequenced grape genome. The number of grape mTERF genes was less than that of Arabidopsis although grape has a much larger genome. Therefore, the underrated number of *VvmTERF* genes would be probably due to unsequenced genomic gaps or mis-annotated genes of grape genome.

According to phylogenetic relationships, mTERF genes of Arabidopsis have been classified into eight groups [18]. In this study, a constructed phylogenetic tree which gathered the mTERF proteins from Arabidopsis, maize and grape had close topological framework to the tree constructed. Based on phylogenetic classification, grape mTERF genes were classified into nine groups. The number of clades and of *VvmTERF* genes are small, which may be caused by a different pattern of duplication events. Furthermore, most of the *VvmTERF* genes were closely related to *AtmTERF* genes, which is in step with the fact that both grape and Arabidopsis are eudicots and exiting an appearance of close evolutional distance. As a result of highly conserved features, those mTERF genes which contain the same subclass displayed similar functions. Multiple Arabidopsis mTERF genes functions have been tested, for instance, *AtmTERF5*, *9*, *10* and *11* have functions on the resistance to salt and osmotic stress, and *AtmTERF5*, *9* and *10* also play roles in responding to ABA regulation [45]. Although the Arabidopsis mTERF genes can provide predicted characterization of *VvmTERF* genes, the functional analysis of *VvmTERF* genes homologs still need more detailed experimental demonstration.

Expansion and synteny analysis of grape mTERF gene family

During evolution, tandem, segmental and whole genome duplications have been commonly found in many organisms [38, 46]. In our study based on the chromosome locations, motifs and sequences, we concluded that some of the *VvmTERF* genes such as *VvmTERF3-4* and *VvmTERF8-11* might arise by tandem duplications. Genome duplications leading to rearrangement and extension of the mTERF gene

family have been reported in other plant species [19, 40, 47], and these duplication events helped expansion of the Arabidopsis genome genes [48]. Some of the Arabidopsis mTERF genes were considered to be generated by tandem duplications and one block duplication [18, 19]. On the other hand, it has been demonstrated that grape has gone through whole-genome duplication events distinctly [49]. Therefore, tandem and segmental duplications could probably contribute for most gene extensions, although there are different opinions on the exact nature and timing of these events [49, 50]. Similarly, tandem and segmental duplications have probably played a key role for grape mTERF gene expansions and their structural and functional diversity. Therefore, according to the comparison with respective orthologs of the most common model plant Arabidopsis, the putative functions of grape mTERF genes can be speculated. This current work analyzed the tandem duplication events of the 25 grape mTERF genes on the 13 grape chromosomes based on the research techniques of Holub [51], within 200 kb length on all chromosomes containing more than two genes that will be deemed to regard as a tandem duplication event.

In order to research a less-studied species, we often use genomic comparison method which could effectively transfer genomic knowledge obtained from a well-studied model species (e.g. Arabidopsis) to a less studied organism [39, 41]. In this research, as seen in Fig 4, synteny analysis of the grape and Arabidopsis genomes demonstrated that six pairs of *mTERF* genes (*VvmTERF2-AtmTERF6*, *VvmTERF6-AtmTERF4*, *VvmTERF13-AtmTERF19*, *VvmTERF15-AtmTERF10*, *VvmTERF24-AtmTERF9* and *VvmTERF25-AtmTERF17*) are located in syntenic genomic regions (Figure 4). Accompanied by selected genes loss, Arabidopsis and grape genomes have also went through multiple and crucial chromosomal rearrangements and fusion processes during their evolution, which results in the identification of genes mismatches on chromosomes. In this case, we can deduce that the mTERF genes of grape and Arabidopsis in the same linear region may have a common ancestor. The first identified mTERF gene in Arabidopsis is *AtmTERF1* (*SOLDAT10*), which is mainly involved in fluorescent phenotype and O^{2-} signaling cell death [20]. Furthermore, the *AtmTERF4* (*BSM/RUG2*) gene is crucial for plant development. The *rug2-1* and *bsm* mutant are unable to grow up compared with the wild type plant. Sequence analysis revealed that *VvmTERF6* was homologous to *AtmTERF4* which might imply that the *VvmTERF6* may have similar function in regulating plant development.

Functional character of grape mTERF genes in hormone treatments and biotic stresses

In the previous researches, Linder [10] firstly described the mTERF gene family in plants. A number of studies on identification of plant mTERFs followed. For example, identification and functional analysis of mTERF gene family were reported in maize and capsicum [40, 47]. However, information about mTERF functions in plants is still rather limited and thus the plant mTERF genes should be further studied. In this report, we analyzed the expression patterns of *VvmTERF* after challenge with various pathogens and phytohormones. Under these different treatments, the *VvmTERF* genes showed various expression patterns, which implied that they might play a wide range of roles in plant growth and responding to various stress.

Among the phytohormones, It has been reported that ABA widely involved in various biological function, biotic and abiotic stress [52-54], while plant hormone Eth, SA and MeJA have synergistic effects on biological stress signals after pathogen infection [55]. Our results revealed that the grape mTERF genes responded to the expression patterns of different plant hormones, and these results were consistent with previous studies in other species such as maize and capsicum mTERF genes [40, 47].

A total of 35 mTERF genes were identified in *A. thaliana*, among which 6 mTERF genes were discovered specifically functional. For example, gene *SOLDAT10* and *SHOT1* can respond abiotic stresses [20, 56], as well as gene *TWIRT1* has meristem function [57] and gene *RUG2* associated with leaf morphology [58]. *VvmTERF6* is a homolog of *AtmTERF4* that has functions of organelles development and photoautotrophic growth. In Fig 5, expression alteration of *VvmTERF6* gene in the development stages of grape showed distinct upregulated pattern, indicating potential function during growth periods. What's more, *AtmTERF5* which also named *MDA1* rooting in model plant Arabidopsis showed responses to abiotic treatment. On account of reducing sensitivity to hormone abscisic acid, mutant *mda1* seedlings are exhibited insensitive to osmotic and salt stresses, while grown Arabidopsis *mda1* plants demonstrate decreased tolerance of cold, salt stress or ABA treatment [21]. Hence, since this gene family has expanded in plants, scientists found that these genes were still powerful enough to cause many mutant plants to exhibit potent phenotypes, some even showed embryo-lethal feature. In addition to the results acquired from the mutant analysis, previously published mTERF genes expression data which had also clearly showed the potential role for mTERFs through plant stress response. In the report, *ZmTERF12* and *ZmTERF28* were down- and up-regulated by ABA hormone treatment, respectively [40]. Taken together, we analyzed the response of *VvmTERF* genes to various plant hormones and found potential key targets for enhancing grape stress resistance, which provided basic information for future studies on function of *VvmTERF* genes and its related signal transduction network.

Reactive active oxygen (ROS) refers to the general term for substances composed of oxygen, containing oxygen and active properties in the body or in the natural environment [59, 60]. The bactericidal effect of ROS in plant cells has been known for a long time. When exogenous O^{2-} and H_2O_2 are used in plants, they have a direct toxic effect on fungi and bacteria, which can inhibit the spore germination and growth of fungi [61]. The burst of reactive active oxygen can be induced by pathogenic bacteria, and ROS burst has been considered as one of the host defense responses, which plays an important role in plant disease resistance [62]. Previous researches demonstrated that a few plant mTERF genes are related with ROS burst process, such as *SOLDAT10* and *SHOT1* [56]. Given this, the role of mTERF genes in response to biotic stresses needs more information. Then we conducted the related experiment here and results confirmed our hypothesis before. In this study, we found that some grape mTERF genes responded to powdery or downy mildew treatments. For example, *VvmTERF22* was up regulated by PM at 12h, and *VvmTERF21* positively responded to downy mildew infection. We detected several *VvmTERF* genes expression levels which are actively responsive to PM or DM treatments, indicating these genes might display significant characters in the grape protection, but further research is needful to demonstrate that they participate in biotic stress responses in grapevine.

Conclusions

The family of transcriptional activators encoded by the mTERF genes is widely found in plants and animals. Although significant progresses have been made in identifying mTERF genes in model plant species, little fruit crop mTERF genes has been known in fruit crops. In this study, we identified a total of 25 *VvmTERF* genes in the grape genome, and also investigated their structural, phylogenetical and syntenic features. Through comparative analysis of homology between grape and Arabidopsis, it is found that several mTERF genes of grape and Arabidopsis are located in the homologous regions, indicating that they may present close evolutionary relationship. Expression analysis of *VvmTERF* genes showed that multiple genes could respond to different biological stresses and hormone treatments. Results from this study pave the way for future researches to investigate roles of *VvmTERF* genes on disease resistance of grapevines.

Abbreviations

mTERFs
mitochondrial transcription termination factor
VvmTERFs
Vitis vinifera mitochondrial transcription termination factor
Eth
Ethylene
SA
Salicylic acid
MeJA
Methyl Jasmonate
ABA
Abscisic acid
RT-qPCR
Reverse transcription quantitative PCR

Declarations

Conflicts of Interests

The authors declare no conflict of interests.

Authors' contributions

JL and XY: conceived and designed the experiments. XY, SS and YG: performed the experiments, contributed reagents/materials/analysis tools and analyzed the data; XY and JL wrote the manuscript.

YG: provided guidance for the entire study. All authors approved the final manuscript.

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Tables

Due to technical limitations, table 1 and 2 is only available as a download in the Supplemental Files section.

Figures

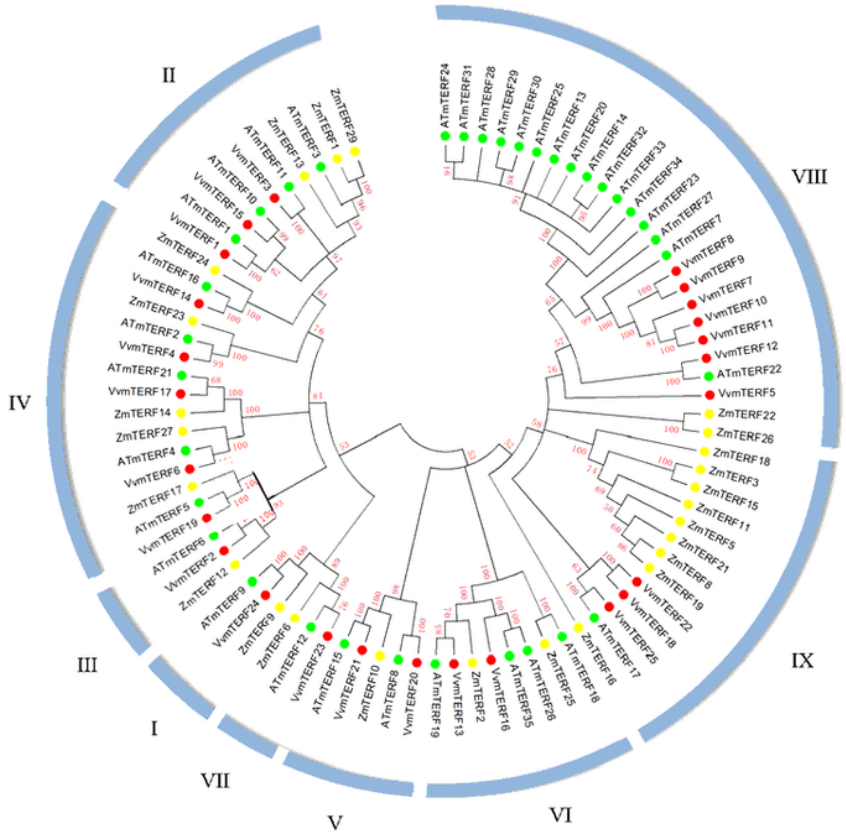

Figure 1

Phylogenetic analysis among the grape, Arabidopsis and maize mTERF proteins. The unrooted tree was constructed using MEGAX software by Neighbor-joining method. The numbers represent the bootstrap values (%) for 500 bootstrap replicates and only bootstrap values > 60% are shown. Nine groups designated I–IX are shown outside.

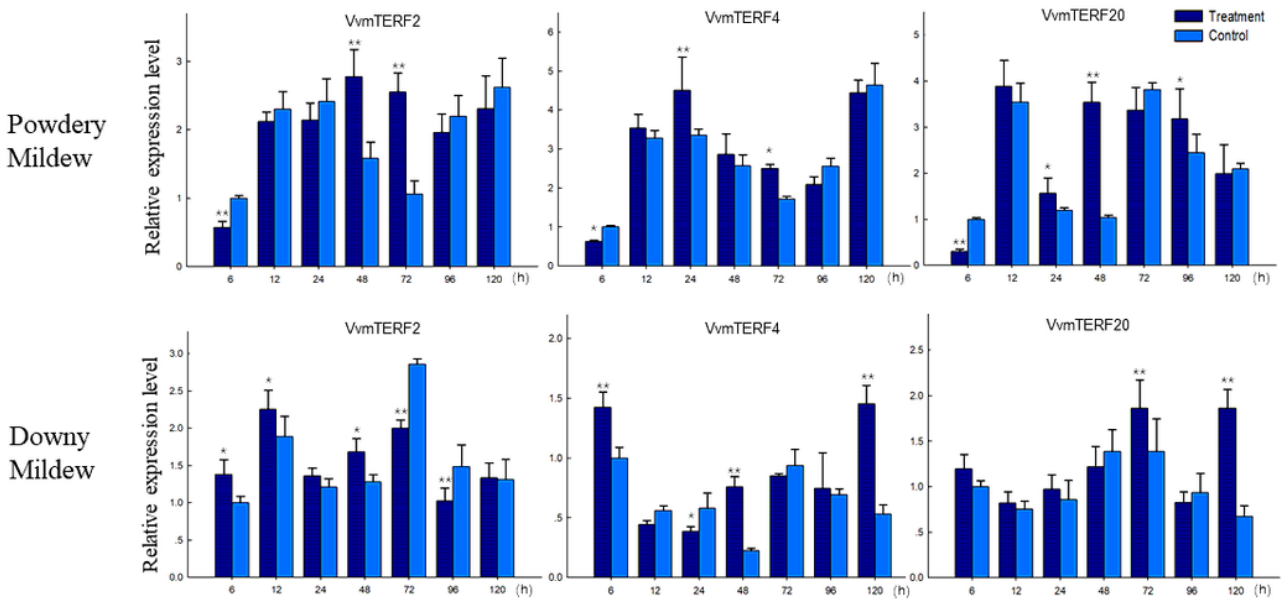

Figure 2

A) Sequence analysis of introns and exons in grape mTERF genes. The yellow boxes and dark lines represent exons and introns sequences, respectively. B) Schematic diagram of predicted recognized conservative modules in grape mTERF protein. The MEME program was used to mine the presumptive conservative motif of grape mTERF protein. Different colored boxes were used to show putative fifteen motifs and the sequences of regular motifs were displayed in the Table 2.

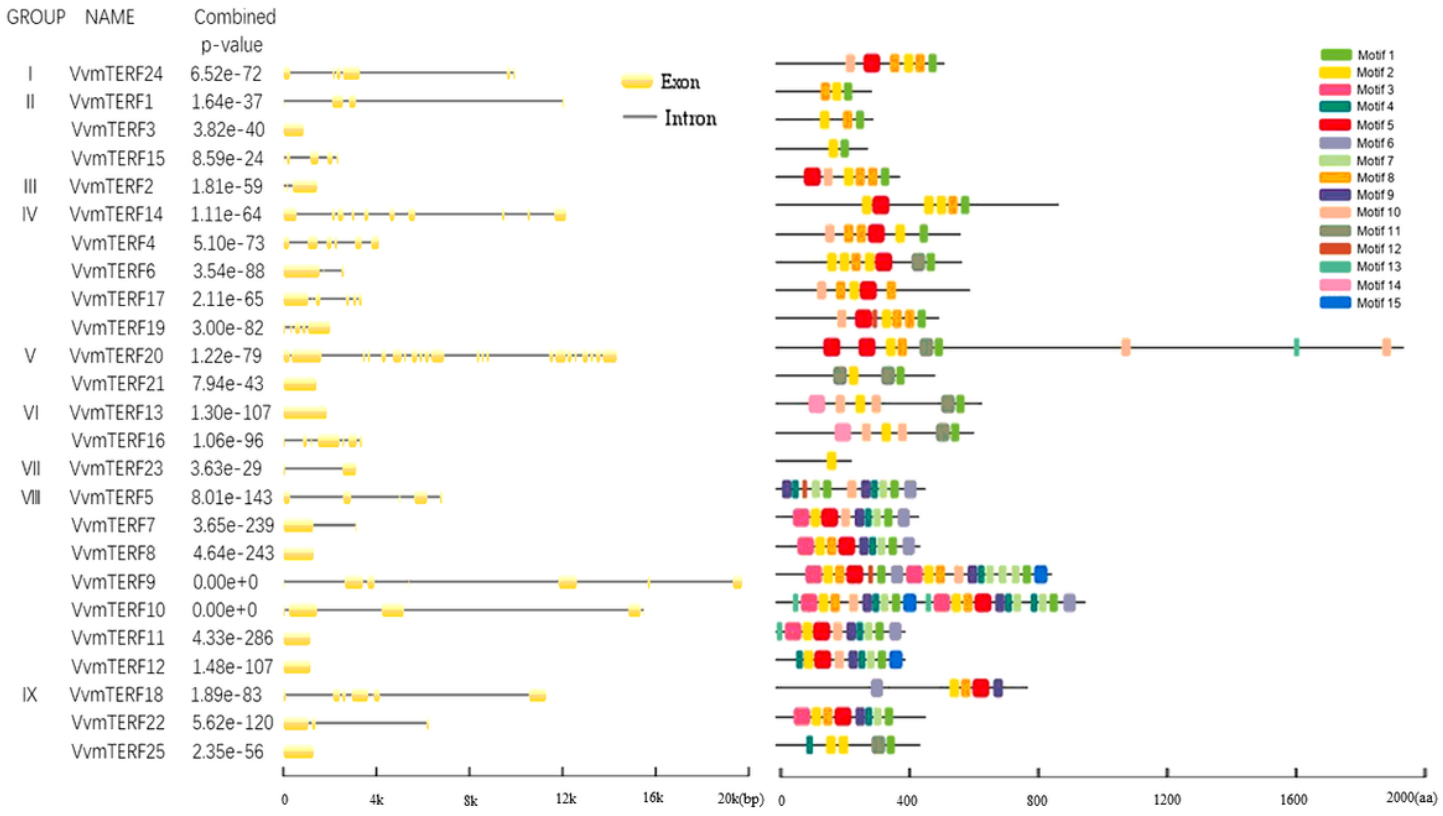

Figure 3

Localization, duplication and synteny analysis of grape mTERF genes. Chromosomes 1-19 are marked using different colors and labeled with their names in a circular form. Syntenic regions are demonstrated by coloured curves between grape and Arabidopsis mTERF genes.

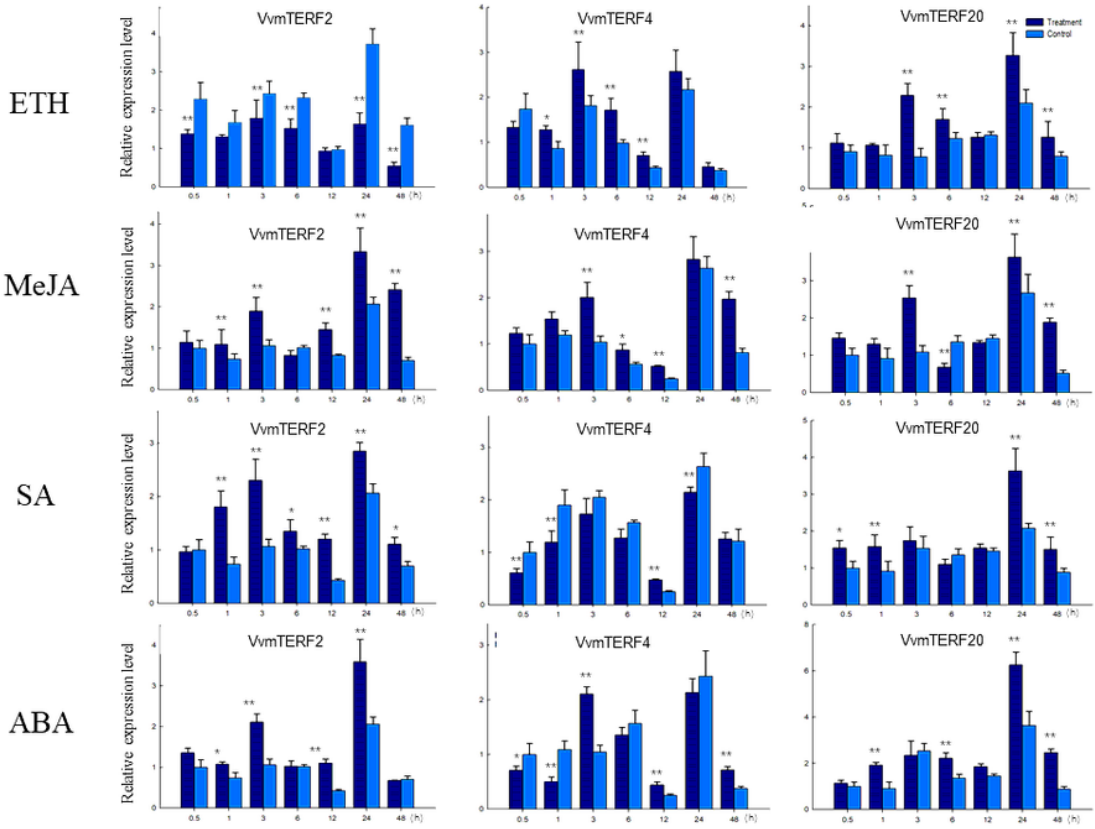

Figure 4

Predicted cis elements of VvmTERF gene promoters. Promoter sequences of each VvmTERF genes and the housekeeping gene were analyzed by PlantCARE website (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/).

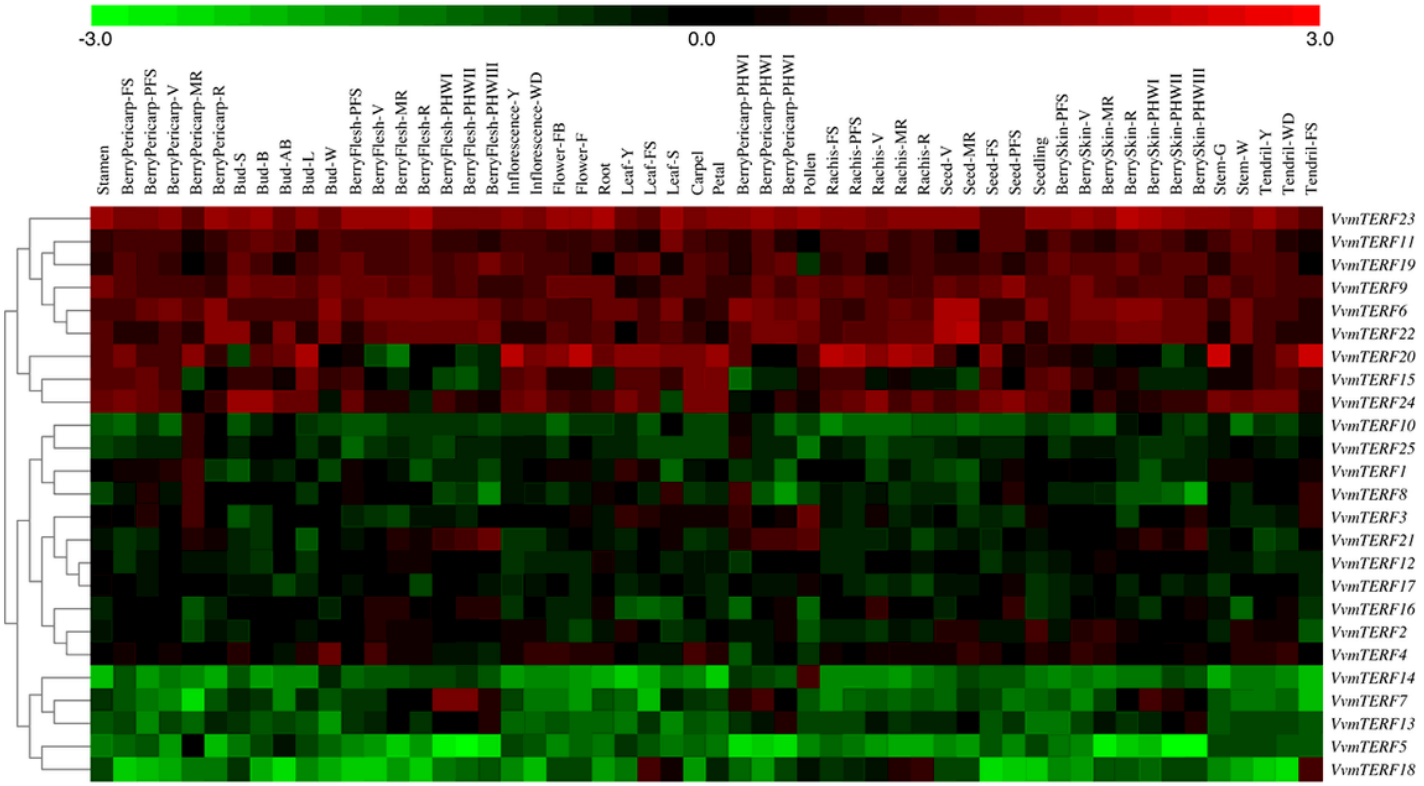

Figure 5

Expression profiles of VvmTERF genes in different tissues/organs under various developmental stages. Red and green boxes represent high and low expression levels, respectively. Bud-AB, bud after burst; Bud-B, Bud burst; Bud-W, winter bud; Bud-L, latent bud; Bud-S, bud swell; Flower-F, flowering; Flower-FB, flowering begins; FS, fruit set; Inflorescence-Y, young inflorescence with single flowers separated; Inflorescence-WD, well-developed inflorescence; Leaf-FS, mature leaf; Leaf-S, senescing leaf; Leaf-Y, young leaf; MR, mid-ripening; R, ripening; PFS, post fruit set; Stem-G, green stem; Stem-W, woody stem; V, véraison.

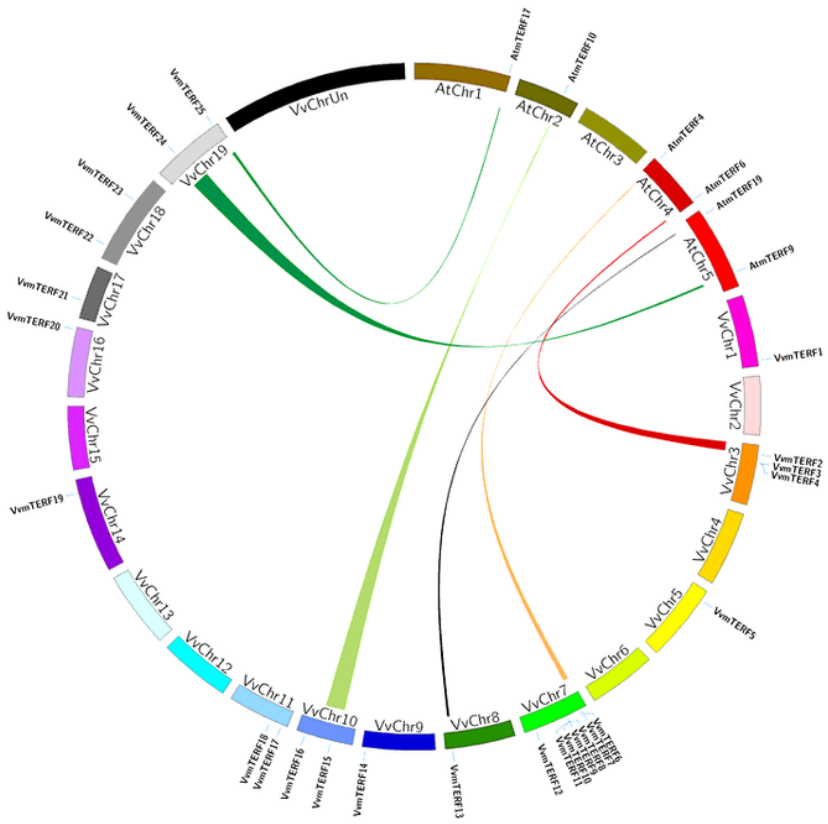

Figure 6

Expression patterns of 25 mTERF genes in grape under treatments of phytohormones and biotic stresses. The Gene Tools software was used to quantify the brightness of semi-quantitative RT-PCR bands. MeV software was used for hierarchical cluster analysis to compare the grape mTERE genes under different hormone treatment and stresses. Red/green were used to indicate increased or decreased expression levels□respectively.

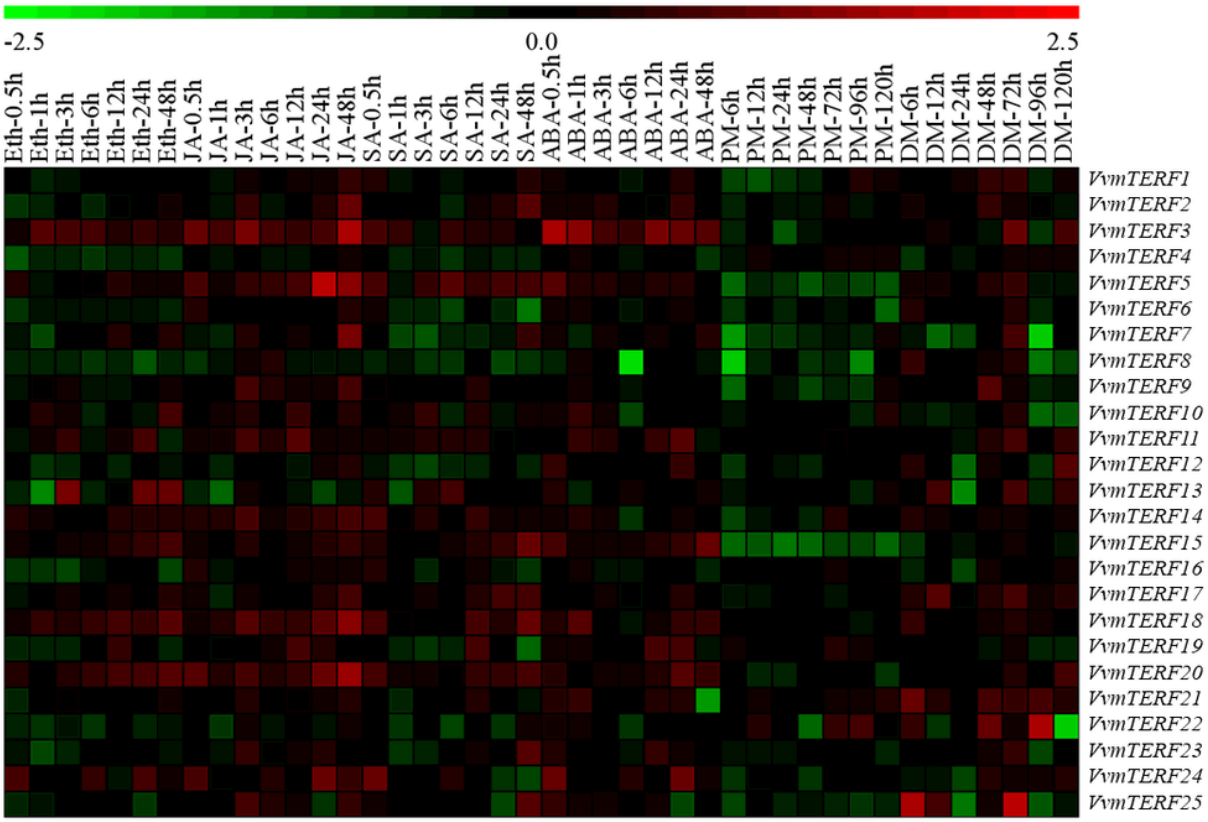

Figure 7

Real-time quantitative PCR expression patterns of 3 selected grape mTERF genes regulated by different hormone treatments including abscisic acid (ABA), salicylic acid (SA), methyl jasmonic acid (MeJA), and ethanol (Eth). Asterisks indicate that the corresponding genes were distinctly up- or down-regulated following various treatments by t-test (*P<0.05, **P<0.01). The mean and SD values were derived from three biological and three technical repetitions.

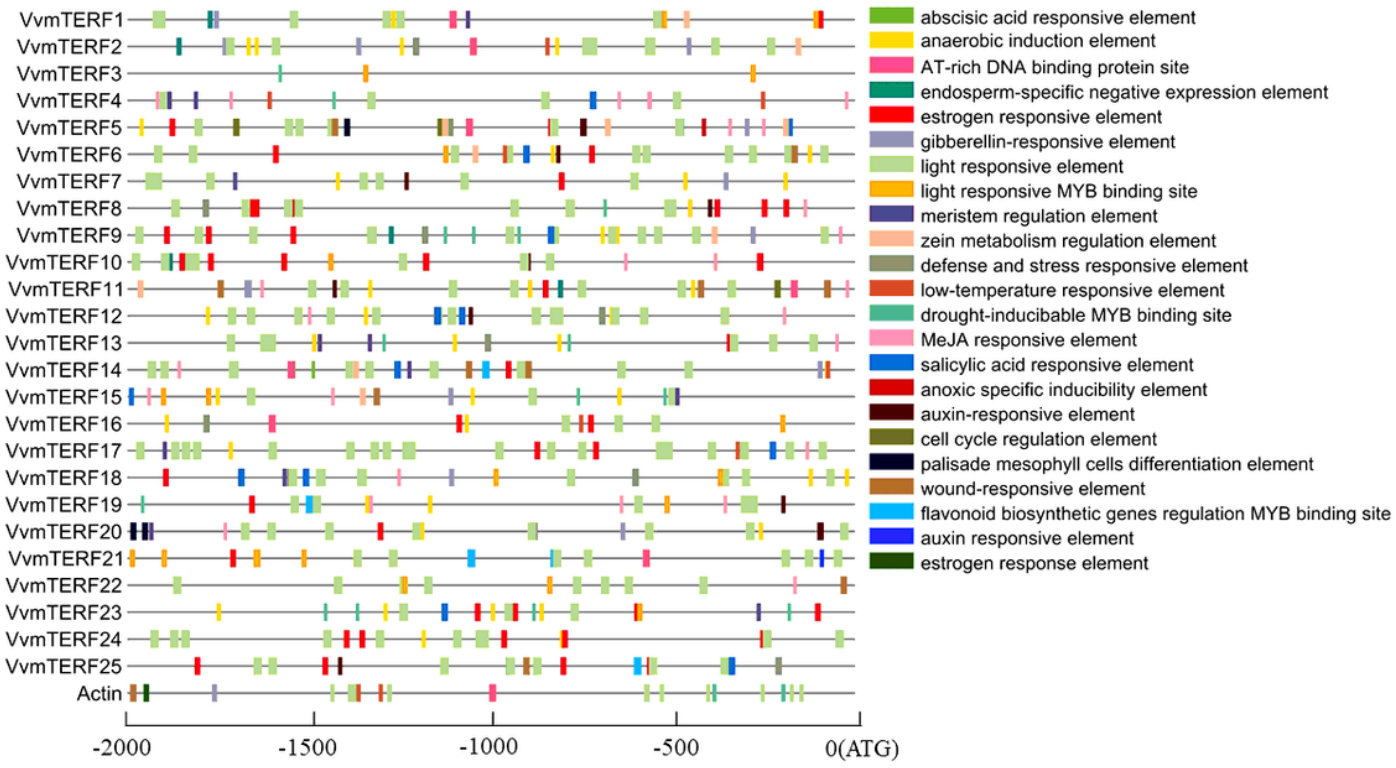

Figure 8

Real-time quantitative PCR expression patterns of 3 selected grape mTERF genes regulated by inoculating powdery mildew and downy mildew pathogens. Asterisks indicate that the corresponding genes were distinctly up- or down-regulated following different treatments by t-test (*P<0.05, **P<0.01). The mean and SD values were derived from three biological and three technical repetitions.

Supplementary Files

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