A comprehensive analysis of cotton VQ gene superfamily reveals their potential and extensive roles in regulating cotton abiotic stress

Shuxun Yu (ysx195311@163.com)
Chinese Academy of Agricultural Sciences Cotton Research Institute https://orcid.org/0000-0002-9715-3462

Pengyun Chen
Chinese Academy of Agricultural Sciences Cotton Research Institute

Fei wei
Zhengzhou University

Shuaishuai Cheng
Northwest Agriculture and Forestry University

Liang Ma
Chinese Academy of Agricultural Sciences Cotton Research Institute

Hantao Wang
Chinese Academy of Agricultural Sciences Cotton Research Institute

Meng Zhang
Chinese Academy of Agricultural Sciences Cotton Research Institute

Guangzhi Mao
Chinese Academy of Agricultural Sciences Cotton Research Institute

Jianhua Lu
Chinese Academy of Agricultural Sciences Cotton Research Institute

Pengbo Hao
Northwest Agriculture and Forestry University

Adeel Ahmad
Chinese Academy of Agricultural Sciences Cotton Research Institute

Lijiao Gu
Chinese Academy of Agricultural Sciences Cotton Research Institute

Qiang Ma
Chinese Academy of Agricultural Sciences Cotton Research Institute

Aimin Wu
Chinese Academy of Agricultural Sciences Cotton Research Institute

Hengling Wei
Chinese Academy of Agricultural Sciences Cotton Research Institute

Research article

Keywords: Gossypium, valine glutamine (VQ), phylogenetic, expression analysis

Posted Date: July 1st, 2020

DOI: https://doi.org/10.21203/rs.3.rs-38226/v1

Version of Record: A version of this preprint was published on November 16th, 2020. See the published version at https://doi.org/10.1186/s12864-020-07171-z.

Abstract

Background

Valine-glutamine (VQ) motif-containing proteins play important roles in plant growth, development and abiotic stress response. For many plant species, the *VQ* genes have been identified and their functions have been described. However, little is known about the origin, evolution, and functions (and underlying mechanisms) of the *VQ* family genes in cotton.

Results

In this study, we comprehensively analyzed the characteristics of 268 *VQ* genes from four *Gossypium* genomes and found that the *VQ* proteins evolved into ten clades, and each clade had a similar structural and conservative motif. The expansion of the *VQ* gene was mainly through segmental duplication, followed by dispersal. Expression analysis revealed that the *VQ* genes play important roles in response to salt and drought stress, especially *GhVQ18* and *GhVQ84* were significantly high expression in PEG stress and salt stress. Further analysis showed that *GhVQ* genes were co-expressed with *GhWRKY* transcription factors (TFs), and microRNAs (miRNAs) could hybridize to their cis-regulatory elements.

Conclusions

The results in this study broaden our understanding of the *VQ* gene family in plants, and the analysis of the structure, conserved elements, and expression patterns of the *VQ* genes provide a solid foundation for exploring their specific functions in the abiotic stress responses in cotton. Our study provides significant insight into the potential functions of *VQ* genes in cotton.

Background

The *VQ* genes form a large gene family with important roles in growth, development and abiotic stress tolerance in plants [1–3]. The VQ proteins have a conserved VQ motif [F**hVQ*hTG (F, phenylalanine; *, any amino acid; h, hydrophobic residue; V, valine; Q, glutamine; T, tryptophan; G, glycine)] [4, 5] and interact with WRKY TFs via the conserved residues V and Q. In Arabidopsis, many *VQ* genes have been reported to function in plant development and responses to abiotic stress, such as sigma factor-binding protein 1 (*SIB1*, also known as *AtVQ23*) and *SIB2* (*AtVQ16*), which have been reported to interact with *AtWRKY33* to increase the resistance of plants to *Botrytis cinerea* [6]. In another research, SIB1 and SIB2 have also been proven could interact with AtWRKY57, and SIB1 and SIB2 can enhance the competitions on *AtWRKY57* to *AtWRKKY33* in regulating *JASMONATE ZIM-DOMAIN1* (*JAZ1*) and *JAZ5* [7]. *JASMONATE-ASSOCIATED VQ MOTIF GENE1* (*JAV1/AtVQ22*) was reported to serve as a key negative regulation gene in the jasmonate pathway [8]. For instance, the AtVQ09 protein could act as a repressor

of the *AtWRKY08* factor to establish tolerance to salinity stress [9]. Recently, *MdVQ10* and *MdVQ15* were reported to interact with *MdWRKY52* to regulate apple defense mechanisms and development [10].

Beyond stress responses, *VQ* genes have also been reported to perform other functions, such as HAIKU (*IKU1*, *AtVQ14*) that was reported to interact with MINISEED3 (*MINI3*, *AtWRKY10*) to reduce the expression of *IKU2* to affect the seed size [1], and *AtVQ20* that plays an important role in regulating the male gametogenesis in plants [11]. In addition, *VQ* TFs were also reported to interact with ETHYLENE RESPONSE FACTOR (*ERF*), Mitogen-activated protein kinase (*MAPK* or *MPK*), and miRNA in response to environmental stresses in plants [12, 13]. On the other hand, abiotic stress-related genes have been isolated from Arabidopsis and other plants [18–21], including VQ TFs, WRKY TFs, and other TFs.

Cotton is an important, widely cultivated fiber and oil crop that is essential for the textile industry and provides a nutrient-rich edible oil [14]. Various biotic and abiotic stresses, including pathogen infection, drought and salinity stresses, have consistently and severely affected the formation of cotton production [15–17], although some cultivars with broad-spectrum resistance to biotic and abiotic stresses have been developed. Recently, *VQ* family genes have been identified at genome-wide levels in several plants, including Arabidopsis (*Arabidopsis thanliana*) (34 *VQs*) [22], soybean (*Glycine max*) (74 *VQs*) [23], maize (*Zea mays*) (61 *VQs*) [24], Chinese cabbage (*Brassica rapa spp. pekinensis*) (57 *VQs*) [25], apple (*Malus Domestica Borkh*) (49 *VQs*) [10], tea (*Camellia sinensis*) (25 *VQs*) [26] and tomato (*Solanum lycopersicum*) (26 *VQs*) [27]. However, the genomic information and genetic evolution relationships of *VQ* genes are not clear in *Gossypium spp.*, and their expression patterns in in different tissues and responses to abiotic stresses remain unknown.

With the released four cultivated *Gossypium* spp. (*G. hirsutum* Linn., *G. barbadense* L., *G. raimondii* Ulbr and *G. arboretum* L.) genome sequences and their annotation [28–30], several important achievements and progress have been achieved in the study of cotton's genomics. In this study, using the annotations of four cotton genomes, we identified the cotton *VQ* genes, perform phylogenetic, conserved structural motif, whole-genome duplication (WGD) analysis, functional interaction network analyses, and predict microRNA target profiles. The comprehensive analysis of the *VQ* gene family in cotton will contribute to identifying new key candidate genes for diverse stress resistance in cotton breeding.

Results

Identification and comparative analysis of *VQ* genes in plants

To identify the *VQ* gene family in the four kinds of cotton, the AtVQ proteins and the VQ-domain Pfam (PF05678) were used as query sequences to search against the four cotton protein databases. In total, 89 *GhVQ*, 89 *GbVQ*, 45 *GrVQ*, and 45 *GaVQ* genes were identified and named (Additional File 1, Supplemental Table S1). In addition, the physiological and biochemical properties of the 268 *VQ* genes were determined, including CDS length, GC count, isoelectric point (pI) and molecular weight (MW) (Additional File 1, Supplemental Table S1). The CDS lengths of these *VQ* genes ranged from 279 bp (*GhVQ89* and *GbVQ89*) to 1443 bp (*GbVQ15*), the average GC content of the transcript was 46.01, their exon numbers

varied from 1 to 9, and only a small percentage of VQ genes contained introns (3.37% *GhVQ*, 3.37% *GbVQ*, 6.67% *GaVQ*, and 31.11% *GrVQ* genes). The pI values varied from 4.159 (*GbVQ33* and *GbVQ78*) to 11.496 (*GhVQ07*) and the MW values ranged from 10.346 kDa (*GbVQ89*) to 52.058 kDa (*GbVQ15*) (Additional File 2, Supplemental Fig. S1).

To perform comparative genomic analyses, we also searched another 11 plant species for VQ proteins. The evolutionary relationships of the species and the number of their *VQ* genes are shown in Fig. 1. The data showed that the number of *VQ* genes in *A. trichopoda*, *P. dactylifera*, *V. vinifera*, *P. trichocarpa*, and *T. cacao* were less than that in the four cotton species (Additional File 3, Supplemental Table S2). The comparative structure analysis of *VQ* genes showed that almost all the *VQ* genes have a few introns and encode relatively small proteins, and only 3 *GhVQ*, 3 *GbVQ*, 3 *GaVQ* and 14 *GrVQ* genes have more than one intron. We speculate that the WGD events that occurred during the evolution of angiosperms increased the numbers of the cotton *VQ* genes, and these events have helped the *VQ* genes to gain new functions through neofunctionalization. However, the evolutionary forces that shape the current intron/exon gene structures remain unknown.

Phylogenetic analysis of VQ proteins

To explore the relationships among *VQ* genes in cotton, we conducted a phylogenetic analysis of the VQ proteins from the 15 plant species (Fig. 2), and a phylogenetic tree between *Gossypium* spp. and Arabidopsis was also constructed (Additional File 4, Supplemental Fig. S2). The tree contained 656 VQ proteins and was divided into ten clades based on the nomenclature of the *VQ* genes in Arabidopsis. The largest group (Group Ⅰ) contains 20 *GhVQs*, 20 *GbVQs*, 10 *GaVQs*, and 10 *GrVQs*. Group Ⅱ was the smallest group, which including 4 *GhVQs*, 5 *GbVQs*, 3 *GaVQs* and 3 *GrVQs*. Previous research has verified that VQ proteins contain a conserved motif composed of F**hVQ*hTG. In our study, among the 656 VQ proteins, 212 proteins (in Group Ⅰ and Group Ⅳ) had the amino acid “M” next to “VQ” (simple M-VQ model); 159 proteins (in Group Ⅲ, Group Ⅴ, and Group Ⅵ) had the amino acid “V” next to “VQ” (simple V–VQ model); and 285 proteins (in Group Ⅶ, Group Ⅷ, Group Ⅸ, Group Ⅹ and Group Ⅺ) had the amino acid “L” next to “VQ” (simple L-VQ model) (Additional File 5, Supplemental Fig. S3). The *VQ* genes with rarer amino acids of the *Gossypium* spp. were also scattered in Group Ⅰ to Group Ⅺ, and the clusters of VQ genes were similar to those in angiosperms [3].

Cis-regulation elements and structural composition of the *VQ* genes

The cis-regulation elements in promoters (from 2000 bp to -1 bp) of the four cotton *VQ* genes were analyzed using the PlantCARE tool. We identified 715, 701, 386 and 399 cis-regulation elements from the promoters of the *GhVQ*, *GbVQ*, *GaVQ* and *GrVQ* genes, respectively. Among these were: seven kinds of hormone-responsive cis-regulation elements, ABRE, P-box, TGA-box, TGA-element, TCA-element, CGTCA-motif, and GARE-motif, which were associated with ABA, ethylene, salicylic acid (SA), methyl jasmonate (MeJA), auxin (IAA), and gibberellin (GA), respectively; and six types of stress-related regulatory elements, MBS, TC-rich repeats, LTR, DRE-motif, W-box, and CCAAT-box, responding to drought inducibility, cold stress, and defense stress (Fig. 3). Moreover, promoters of 66 *GhVQs*, 69 *GbVQs*, 36 *GrVQs* and 34 *GaVQs*

possessed WRKY-binding sites (W-box) (Fig. 3). The diversity of the cis-regulation elements in the *VQ* genes' promoter indicated that *VQ* genes might participate in regulating the cotton response to endogenous hormones and diverse environmental stimuli.

Motif compositions and exon-intron structures of the *VQs* are shown in Fig. 4. Combining the phylogenetic groups of the *VQs* in the four cotton species, we found that there were more motif types in Group Ⅱ, including Motif 1, Motif 4, Motif 7, Motif 9, and Motif 10; and followed by Group Ⅰ, containing Motif 1, Motif 2, Motif 3, Motif 6, and Motif 8. Not surprisingly, Motif 1 existed in almost all of the *VQ* genes, suggesting that it is the most conservative motif. The differences in motif composition among the four cotton *VQs* indicated that they might perform different functions in each cotton species. Most of the *VQ* genes were identified as having no intron: 96.63% (86/89) in *GhVQ* genes, 96.63% (86/89) in *GbVQ* genes, 68.89% (31/45) in *GrVQ* genes, and 93.33% (42/45) in *GaVQ* genes. The remaining *VQ* genes, which were widely distributed in Group Ⅰ and Group Ⅱ, contained from one to eight introns. In general, VQ proteins in the same clades would share similar motif elements and structural compositions, indicating that the members in the same subgroup could have similar functions.

Chromosomal distribution, synteny analysis and duplication type identification

In this study, the *VQ* genes were detected located in most chromosomes with a few exceptions, i.e., Gh_A09 and Gh_D02 in *G. hirsutum*, Gb_A09, Gb_A13 and Gb_D02 in *G. barbadense*, Ga_Chr03 and Ga_Chr09 in *G. arboretum*, and Gr_Chr05 *G. raimondii* (Fig. 5). For the two allotetraploid species of cotton, Gh_D05 (eight genes/~9%), Gb_A05 (eight genes/~9%), and Gb_D05 (eight genes/~9%) contained more *VQ* genes than other chromosomes, while Gh_A02, Gh_A03, Gh_A13, Gh_D08, Gh_D13, Gb_A02, Gb_A03, Gb_D08, Gb_D09, and Gb_D13 only contained one gene. For the two diploid cotton species, Ga_Chr05 (eight genes/~17.8%), Gr_Chr02 (seven genes/ ~17.8%), Gr_Chr07 (seven genes/ ~17.8%) and Gr_Chr09 (seven genes/ ~17.8%) contained more VQ genes, and Ga_Chr08, Ga_Chr13, Gr_Chr04, Gr_Chr12, and Gr_Chr13 only contained one gene. Most *VQ* genes in the four *Gossypium* species were distributed at both ends of the chromosomes, which corresponded to the position of the telomere.

The orthologous genes were first identified between *G. hirsutum* and *G. arboreum* with *G. raimondii*. A total of 83 *GhVQs* were orthologous genes in the two-diploid cotton, of which 40 gene pairs showed A genome origin, while 43 gene pairs showed D genome origin (Additional Files 6 and 7, Supplemental Fig. S4 and S7, Table S3). Subsequently, orthologous gene identification was also conducted between *G. barbadense* and *G. arboreum* with *G. raimondii*, and there were 84 orthologous *GbVQs*, of which 40 gene pairs showed A genome origin, while 44 gene pairs showed D genome origin (Additional Files 7 and 8, Supplemental Fig. S5 and S3). Orthologous genes between G*. hirsutum* and G. *barbadense* were also identified with 39 gene pairs in Gh_At and Gb_At subgenomes and 42 gene pairs in Gh_Dt and Gb_Dt subgenomes (Fig. 6 and Additional File 7, Supplemental Table S3). In addition, *GhVQ27*, *GhVQ61*, *GhVQ68*, *GbVQ28*, *GbVQ60* and *GbVQ67* had no orthologous genes in the diploid species of cotton.

As previously described, duplication contributed to the expansion of genes in the polyploid events in plants [31]. The tetraploid species of cotton have undergone a genome duplication since their divergence

from the two diploid species of cotton. In our study, we have identified the *VQ* duplication event, and the WGD/segmental event likely contributed to the expression of this gene family. The percentages of *VQs* derived from WGD were 60.47% in the At-subgenome of *G. hirsutum*, 63.04% in the Dt-subgenome of *G. hirsutum*, 69.04% in the At-subgenome of *G. barbadense*, 59.57% in the Dt-subgenome of *G. barbadense*, 62.22% in *G. raimondii*, and 57.78% in *G. arboretum* (Additional File 9, Supplemental Table S4). Gene duplication events after the divergence of *Gossypium* spp. resulted in a high number of paralogous genes in both allotetraploid cotton species.

Prediction of miRNA target sites

miRNA had been predicted to target the VQ genes in Arabidopsis [32] and tea [33]. To determine the miRNA-mediated post-transcriptional regulation of *VQ* genes in two allotetraploid species of cotton, we predicted the target sites of *G.hirsutum* miRNAs in the coding (CDS) regions of the *GhVQs* and *GbVQs*. In *G.hirsutum*, 46 sites of 34 *GhVQs* were detected that could be targeted by 22 miRNAs, while 46 sites of 32 *GbVQs* could be targeted by 21 miRNAs (Fig. 6 and Additional File 10, Supplemental Table S5). Of these, six *VQ* genes (*GhVQ02*, *GhVQ40*, *GhVQ86*, *GbVQ02*, *GbVQ40* and *GbVQ86*) were predicted to be targeted by Ghr-miR172 in the CDS regions; and six *VQ* genes (*GhVQ39*, *GhVQ52*, *GhVQ85*, *GbVQ39*, *GbVQ51* and *GbVQ85*) were targeted by Ghr-miR156 (Ghr-miR156a, Ghr-miR156b, Ghr-miR156c and Ghr-miR156d) at ten prediction sites. Ghr-miR172 and Ghr-miR156 were reported to be involved in some biological processes in plants, including the responses to development and abiotic stress [34-36]. However, to verify the regulation mechanism and function of those predicted miRNAs and their targets in cotton will require further experiments.

Expression pattern analysis and function verification

Expression profiles of the *VQ* genes in the two allotetraploid kinds of cotton were analyzed with available transcriptome data (Additional Files 11 and 12, Supplemental Fig. S6 and S7 and Supplemental Table S6). In this study, the *GhVQ* and *GbVQ* genes with average FPKM values > 5 and present in at least two samples were identified as potentially expressed transcripts. Fifty-seven *GhVQ* and 39 *GbVQ* were selected, and their expression profiles tested in ten tissues, including anther, bract, filament, leaf, petal, pistil, root, sepal, stem and torus (Fig. 7). For 57 *GhVQ* genes, 22 genes were highly expressed in root and *GhVQ82* had the highest expression level; 14 genes were highly expressed in leaf, while there were only a few genes expressed in the anther, bract, filament, petal, pistil, sepal, stem and torus (Fig. 7A). For 39 *GbVQ* genes, 17 *GbVQ* genes were highly expressed in root, and 12 genes were highly expressed in leaf (Fig. 7B). The different expression profiles of *VQ* genes suggest that they have different functions in different tissues and developmental stages.

VQ genes were widely identified in the abiotic stress responses in angiosperms [3]. In this study, using the published data, the expression patterns of *VQ* genes in the allotetraploid cotton types under salt, drought, cold and heat stresses were analyzed. In total, 43 *GhVQ* and 37 *GbVQ* genes had different expressions under the four abiotic stress treatments (Fig. 8). Under salt stress, 29 *GhVQ* genes were significantly up-regulated at 12 h, and 21 *GbVQ* genes were up-regulated at 6 h (Fig. 8A and C). In the PEG treatment,

most of the *GhVQ* and *GbVQ* genes were highly expressed at 12 h (Fig. 8B and D). During cold stress, 33 *GhVQ* and 19 *GbVQ* genes were up-regulated in response to low-temperature stress at 24 h (Fig. 8E and G). Most of the *GhVQ* and *GbVQ* genes under the hot treatment were highly expressed at 1 h (Fig. 8F and H). To achieve validation of the *GhVQ* genes responsive to salt and drought stresses, we conducted a qRT-PCR analysis of 12 *GhVQ* genes with PEG and salt treatment for 72 h. In the presence of PEG stress, *GhVQ08*, *GhVQ18*, *GhVQ62*, *GhVQ64*, *GhVQ80*, and *GhVQ84* had high expression levels at 48 h, while the selected *GhVQ* genes, except *GhVQ18* and *GhVQ84*, were highly expressed during 24–48 h under salt treatment (Fig. 9). The qRT-PCR result was different from the RNA-seq, but these findings suggested that some *GhVQ* genes were involved in response to drought and salt stresses.

Co-expression and interaction network of *GhVQ* genes

To understand the *VQ* genes function in the drought and salt stresses, we conducted a co-expression analysis. Ten *GhVQ* genes were co-expressed with another 227 functional genes (Fig. 10 and Additional File 13, Supplemental Table S7). Among these, six and seven genes were identified in different modules of drought stress and salt stress, respectively, while *GhVQ37*, *GhVQ59* and *GhVQ83* were detected coexisting in the two stress treatments. Moreover, these 227 genes co-expressing with ten *GhVQ* genes, contained multiple TFs, including domain AP2, bHLH, F-box, GRAS, p450, PLB03212, WD40 and WRKY (Fig. 10 A–E and Additional File 13, Supplemental Table S7). The functional regulation networks of the GhVQ proteins were constructed using the website of STRING11.0 with the module reference of Arabidopsis association, and the results revealed that the GhVQ proteins participated in the following plant defense gene interaction networks, including WRKYs, MYB15, MPK4, AR781, CSN5B and SIGA proteins (Fig.10 F). Indeed, VQ proteins could interact with WRKY and other TFs to defense against abiotic stresses in cotton.

Discussion

In previous studies, the *VQ* family genes have been systematically analyzed in Arabidopsis [22], soybean [23], rice [37], banana [38], maize [39] and bamboo [40], and shown to play significant roles in regulating growth, development processes, and responding to biotic and abiotic stresses [2]. It is now important to complete a comprehensive analysis of the *VQ* gene family and to explore their evolution interactions in *Gossypium spp*.

The expansion, duplication and structural characteristics of VQ motif-containing genes in cotton

In this study, we analyzed the *VQ* genes of four cotton (*G. hirsutum*, *G. barbadense*., *G. raimondii*, and *G. arboretum*) and another 11 plant species, and found that the numbers of *VQ* genes in 15 species genomes are inconsistently related to the size of the genome. We found that there are 89 *GhVQs*, 89 *GbVQs*, 45 *GaVQs*, and 45 *GrVQs* in the four analyzed kinds of cotton, respectively, and that these numbers are higher than in *cacao* (27 *TcVQs*) or in Arabidopsis (34 *AtVQs*), but the numbers of *GrVQs* and *GaVQs* are fewer than the *DzVQs* (Fig. 1 and Additional File 2, Supplemental Table S2). Previous studies have shown that diverse WGD events lead to the different sizes of plant genomes [41–43]. Our

results show that *VQ* genes in these four cottons were more likely to be proximal, tandem, and segmental genes, while the majority of *VQ* genes in rice [37] and Arabidopsis [22] are singleton genes. Through the analysis of the phylogenetic and structural features of the 15 plants *VQ* domains, the *VQ* genes could be divided into ten clades. This result shows that Group III could be expanded in the eudicots, particularly in the Mallow species (Fig. 2). While Groups □, □, □, □, □, and □ have no *VvVQ*, suggesting that these might have been lost in ancient genome duplication events.

Furthermore, ten conserved motifs were identified in the four cotton VQ proteins, and Motif 1 was found to correspond to the VQ-containing motif, which is widely found in angiosperms. Similar previous studies have suggested that *VQ* genes have few introns in higher plants, and there were only 3 *GhVQ*, 3 *GbVQ*, 14 *GrVQ*, and 3 *GaVQ* genes with multiple introns. Additionally, the motif composition and intron content of the cotton VQ proteins/genes in our study were consistent with the results of the phylogenetic analysis. Collectively, our data suggested that *Gossypium* spp. *VQ* genes might be affected by intronic evolution.

VQ genes play important roles in abiotic stress signaling pathways

Previous reports have shown that *VQ* genes are involved in various endogenous and environmental signals, which is consistent with their diverse roles in plant development and the responses to abiotic stress [2, 5, 22, 37]. For example, the *AtVQ08*, *AtVQ14*, *AtVQ17*, *AtVQ18* and *AtVQ22* were identified as being involved in seed development, chloroplast development, and plant growth. On the other hand, a proportion of the *GmVQ* [23], *PeVQ* [40], *VvVQ* [44] and *CsVQ* [26] genes were also confirmed as being involved in regulating the growth at different tissues and different developmental stages. In this study, we also found that most *GhVQ* and *GbVQ* genes were differentially expressed in the different tissues, including ovule, fiber, anther, leaf, root, sepal and stem, suggesting that the *VQ* genes also play an important role in cotton growth and development (Fig. 7, Additional Files 6 and 7, Supplemental Fig. S6 and S7). Most *VQ* genes also play important roles in the plant responses to various abiotic stresses [2, 3, 27, 39]. In our work, we assessed the expression levels of the *GhVQ* and *GbVQ* under salt, drought, cold, and heat stresses, and found that the majority of *VQ* genes were up-regulated under drought, salt, and cold stress, or down-regulated under heat stress. These findings are similar to those of previous reports in Arabidopsis [5], rice [37], maize [39] and cabbage [25]. Also, in the promoters of the four cotton *VQ* genes, we identified many cis-elements that were reported to exist in other abiotic stresses responsive genes (Fig. 3), implying that the *VQ* genes in cotton are also actively involved in the cotton response to various abiotic stresses and that the response mechanism is complex and diverse.

In previous studies, *VQ* genes were reported to interact with WRKY TFs and regulate a variety of physiological and biochemical processes and abiotic stresses. For instance, *AtVQ09* can act as a repressor of *WRKY8* to establish salinity stress tolerance [9]; *AtVQ15* interacts with *WRKY25/51* in osmotic stress responses [45, 46], *MKS1* (*VQ21*) and *SIB1/2* are known to bind WRKY33 [6], and *VQ08* interacts with *WRKY14/17* in response to abiotic stresses [45]. Here, by constructing co-expression and an Arabidopsis associated model, multiple cotton VQ proteins, such as *GhVQ37*, *GhVQ59*, and *GhVQ83*, were presumed to closely interact with different WRKY TFs, implying that cotton VQ genes could interact

with WRKY TFs in the process of cotton stress resistance. Moreover, we also predicted some putative target sites of cotton microRNAs in the cotton *VQ* genes, including miRNA156s and miR172, which were identified as having important roles in various life processes of plants. These results indicate that the *VQ* genes in cotton are extensively involved in growth, development, and response to stress, and their regulation together with WRKY TFs and microRNAs could account for their physical interactions during the responses.

Conclusions

In this study, using bioinformatics plus expression profiles, we identified and presented the structure, phylogenetic relationships, and tissue specificity of *VQ* family genes in four kinds of cotton. Our data show that the gene structure and motif coding regions are conserved across plants, and a segmental, dispersed, and tandem duplication is the main reason for the expansion of the *VQ* gene family in cotton. Our cis-element and expression analyses indeed indicated that the majority of *VQ* genes are activated in response to abiotic stress, and some of *VQ* genes were also co-expressed with WRKY TFs and hybridize with the miRNAs involved in cotton growth, development, and abiotic stress. Our study could serve as a foundation for future exploration of the specific function of cotton *VQ* genes in the abiotic stress responses and the interactions with *WRKY* genes or microRNAs.

Materials And Methods

Identification and classification of VQ motif-containing genes in plants

The latest versions of predicted proteomes of *Gossypium raimondii* (https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Graimondii) [30], *Gossypium arboreum* (ftp://bioinfo.ayit.edu.cn/downloads/) [29], *Gossypium hirsutum*, and *Gossypium barbadense* (http://ibi.zju.edu.cn/cotton/) [28] were used in this study. The genome data of other plants were obtained from the JGI database (http://www.phytozome.net) and National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/): *A. thanliana*, *V. vinifera*, *T. cacao*, *P. trichocarpa*, *P. dactylifera*, *O. sative*, *M. acuminate*, *D. zibethinus*, *C. papaya*, *B. rapacious*, and *A. trichopoda*. The pre-classified groups of these species were based on their phylogenetic relationships (http://www.timetree.org/) [47]. The VQ conserved domain (PF05678) was used as a query to scan the *Gossypium spp.* protein databases, and the *A. thaliana* VQ proteins were used as the queries to search against the above proteomes through the basic local alignment search tool (BLAST, v 2.10.0)[48] (score value $\geq$ 0.0001 and E-value = 1×10^{-3}) for each newly identified gene. The obtained putative VQ motif-containing sequences were confirmed in the NCBI Conserved Domain Database (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) [49] and SMART database (http://smart.embl-heidelberg.de/) [50]. Then, the physical and chemical properties of the *VQ* family members, including amino acid length, mRNA length, MW, and pI, were analyzed using the online tools of the web site of

softberry website (http://linux1.softberry.com/berry.phtml) and the ExPASy website (https://web.expasy.org/translate/) [51], and the relative results were plotted by the ggstatsplot (v 0.4.0) [52].

Phylogenetic analysis and synteny analysis of the VQ genes in plants

All the VQ motif-containing proteins from the four kinds of cotton and the other 11 plant species were aligned using MAFFT (Multiple Alignment using Fast Fourier Transform, v 7.4.0.7) (L-INS-algorithms) [53] with default parameters, and conserved site sequences were selected by the Gblock (v 0.91b) software (http://molevol.cmima.csic.es/castresana/Gblocks_server.html) [54]. Furthermore, a phylogenetic tree was constructed using the IQ-TREE software (v 1.6.9) (http://www.iqtree.org/)[55] with the maximum likelihood method, and the substitution model was calculated with ModelFinder (intergraded in IQ-TREE; best-fit model: JTT + R5 chosen according to BIC). The obtained treefile was visualized using ggtree (v 2.0.2) [56] and AI (Adobe Illustrator CS6).

The synteny and collinearity of duplication genes in *Gossypium* species were analyzed using the modified MCScan algorithm of the MCScanX package (default parameters) (http://chibba.pgml.uga.edu/mcscan2/) [57], including *G. hirsutum* and *G. arboreum*, *G. hirsutum*, and *G. raimondii*; *G. barbadense* and *G. arboreum*; *G. barbadense* and *G. raimondii*; and *G. hirsutum* and *G. barbadense*. All results were drawn using Circos (http://circos.ca/) [58].

Declarations

Ethics approval and consent to participate:

Not applicable

Consent for publication:

Not applicable

Availability of data and material:

All data supporting the conclusions of this article are included in the article and its additional files.

Competing interests:

The authors declare that they have no competing interests.

Funding:

This project is based on research that was supported by Central Public-interest Scientific Institution Basal Research Fund (No. Y2018YJ05).

Author Contributions:

SXY and HLW designed the research program. FW, SSC, LM and HTW analyzed the data. MZ, GZM, JHL, PBH, AA, LJG, QM and AMW revised the language and collected the data. PYC performed the experiment and wrote the manuscript. All authors have read and approved the final manuscript.

Acknowledgements

We are grateful to all the colleagues in our laboratory.

References

1. Wang A, Garcia D, Zhang H, Feng K, Chaudhury A, Berger F, Peacock WJ, Dennis ES, Luo M. The VQ motif protein IKU1 regulates endosperm growth and seed size in Arabidopsis. Plant J. 2010;63(4):670–9.
2. Jiang SY, Sevugan M, Ramachandran S. Valine-glutamine (VQ) motif coding genes are ancient and non-plant-specific with comprehensive expression regulation by various biotic and abiotic stresses. BMC Genomics. 2018;19(1):342.
3. Cai HY, Zhang M, Liu YH, He Q, Chai MN, Liu LP, Chen FQ, Huang YM, Yan MK, Zhao HM, et al. Genome-Wide Classification and Evolutionary and Functional Analyses of the VQ Family. Tropical Plant Biology. 2019;12(2):117–31.
4. Xie YD, Li W, Guo D, Dong J, Zhang Q, Fu Y, Ren D, Peng M, Xia Y. The Arabidopsis gene SIGMA FACTOR-BINDING PROTEIN 1 plays a role in the salicylate- and jasmonate-mediated defence responses. Plant Cell Environ. 2010;33(5):828–39.
5. Jing Y, Lin R. The VQ Motif-Containing Protein Family of Plant-Specific Transcriptional Regulators. Plant Physiol. 2015;169(1):371–8.
6. Lai Z, Li Y, Wang F, Cheng Y, Fan B, Yu JQ, Chen Z. Arabidopsis sigma factor binding proteins are activators of the WRKY33 transcription factor in plant defense. Plant Cell. 2011;23(10):3824–41.
7. Jiang Y, Yu D. The WRKY57 Transcription Factor Affects the Expression of Jasmonate ZIM-Domain Genes Transcriptionally to Compromise Botrytis cinerea Resistance. Plant Physiol. 2016;171(4):2771–82.
8. Hu P, Zhou W, Cheng Z, Fan M, Wang L, Xie D. JAV1 controls jasmonate-regulated plant defense. Mol Cell. 2013;50(4):504–15.
9. Hu Y, Chen L, Wang H, Zhang L, Wang F, Yu D. Arabidopsis transcription factor WRKY8 functions antagonistically with its interacting partner VQ9 to modulate salinity stress tolerance. Plant J. 2013;74(5):730–45.
10. Dong Q, Zhao S, Duan D, Tian Y, Wang Y, Mao K, Zhou Z, Ma F. Structural and functional analyses of genes encoding VQ proteins in apple. Plant Sci. 2018;272:208–19.
11. Lei R, Li X, Ma Z, Lv Y, Hu Y, Yu D. Arabidopsis WRKY 2 and WRKY 34 transcription factors interact with VQ 20 protein to modulate pollen development and function. Plant J. 2017;91(6):962–76.

12. Pecher P, Eschen-Lippold L, Herklotz S, Kuhle K, Naumann K, Bethke G, Uhrig J, Weyhe M, Scheel D, Lee J. The Arabidopsis thaliana mitogen-activated protein kinases MPK3 and MPK6 target a subclass of 'VQ-motif'-containing proteins to regulate immune responses. New Phytol. 2014;203(2):592–606.
13. Xie F, Wang Q, Sun R, Zhang B. Deep sequencing reveals important roles of microRNAs in response to drought and salinity stress in cotton. J Exp Bot. 2015;66(3):789–804.
14. Roberts EM, Rao NR, Huang JY, Trolinder NL, Haigler CH. Effects of cycling temperatures on fiber metabolism in cultured cotton ovules. Plant Physiol. 1992;100(2):979–86.
15. Khan A, Pan X, Najeeb U, Tan DKY, Fahad S, Zahoor R, Luo H. Coping with drought: stress and adaptive mechanisms, and management through cultural and molecular alternatives in cotton as vital constituents for plant stress resilience and fitness. Biol Res. 2018;51(1):47.
16. Wang W, Vinocur B, Altman A. Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta. 2003;218(1):1–14.
17. Cramer GR, Urano K, Delrot S, Pezzotti M, Shinozaki K. Effects of abiotic stress on plants: a systems biology perspective. BMC Plant Biol. 2011;11(1):163.
18. Eulgem T, Rushton PJ, Robatzek S, Somssich IE. The WRKY superfamily of plant transcription factors. Trends Plant Sci. 2000;5(5):199–206.
19. Dou L, Zhang X, Pang C, Song M, Wei H, Fan S, Yu S. Genome-wide analysis of the WRKY gene family in cotton. Mol Genet Genomics. 2014;289(6):1103–21.
20. Grandbastien MA. LTR retrotransposons, handy hitchhikers of plant regulation and stress response. Biochim Biophys Acta. 2015;1849(4):403–16.
21. Zhu JK. Plant salt tolerance. Trends Plant Sci. 2001;6(2):66–71.
22. Li Y, Jing Y, Li J, Xu G, Lin R. Arabidopsis VQ MOTIF-CONTAINING PROTEIN29 represses seedling deetiolation by interacting with PHYTOCHROME-INTERACTING FACTOR1. Plant Physiol. 2014;164(4):2068–80.
23. Wang Y, Jiang Z, Li Z, Zhao Y, Tan W, Liu Z, Cui S, Yu X, Ma J, Wang G, et al. Genome-wide identification and expression analysis of the VQ gene family in soybean (Glycine max). PeerJ. 2019;7:e7509.
24. Song Wb Z, Hm Z, Xb, Lei L, Lai J. Genome-wide identification of VQ motif-containing proteins and their expression profiles under abiotic stresses in maize. Frontiers in plant science. 2016;6:1177.
25. Zhang G, Wang F, Li J, Ding Q, Zhang Y, Li H, Zhang J, Gao J. Genome-Wide Identification and Analysis of the VQ Motif-Containing Protein Family in Chinese Cabbage (Brassica rapa L. ssp. Pekinensis). Int J Mol Sci. 2015;16(12):28683–704.
26. Guo J, Chen J, Yang J, Yu Y, Yang Y, Wang W. Identification, characterization and expression analysis of the VQ motif-containing gene family in tea plant (Camellia sinensis). BMC Genomics. 2018;19(1):710.

27. Ding H, Yuan G, Mo S, Qian Y, Wu Y, Chen Q, Xu X, Wu X, Ge C. Genome-wide analysis of the plant-specific VQ motif-containing proteins in tomato (Solanum lycopersicum) and characterization of SlVQ6 in thermotolerance. Plant Physiol Biochem. 2019;143:29–39.
28. Hu Y, Chen J, Fang L, Zhang Z, Ma W, Niu Y, Ju L, Deng J, Zhao T, Lian J, et al. Gossypium barbadense and Gossypium hirsutum genomes provide insights into the origin and evolution of allotetraploid cotton. Nat Genet. 2019;51(4):739–48.
29. Du X, Huang G, He S, Yang Z, Sun G, Ma X, Li N, Zhang X, Sun J, Liu M, et al. Resequencing of 243 diploid cotton accessions based on an updated A genome identifies the genetic basis of key agronomic traits. Nat Genet. 2018;50(6):796–802.
30. Paterson AH, Wendel JF, Gundlach H, Guo H, Jenkins J, Jin D, Llewellyn D, Showmaker KC, Shu S, Udall J, et al. Repeated polyploidization of Gossypium genomes and the evolution of spinnable cotton fibres. Nature. 2012;492(7429):423–7.
31. Lee TH, Tang H, Wang X, Paterson AH. PGDD: a database of gene and genome duplication in plants. Nucleic Acids Res. 2013;41(Database issue):D1152–8.
32. Liang G, He H, Li Y, Yu D. A new strategy for construction of artificial miRNA vectors in Arabidopsis. Planta. 2012;235(6):1421–9.
33. Zhang Y, Zhu X, Chen X, Song C, Zou Z, Wang Y, Wang M, Fang W, Li X. Identification and characterization of cold-responsive microRNAs in tea plant (Camellia sinensis) and their targets using high-throughput sequencing and degradome analysis. BMC Plant Biol. 2014;14(1):271.
34. Wu G, Park MY, Conway SR, Wang JW, Weigel D, Poethig RS. The sequential action of miR156 and miR172 regulates developmental timing in Arabidopsis. Cell. 2009;138(4):750–9.
35. Naqvi AR, Haq QM, Mukherjee SK. MicroRNA profiling of tomato leaf curl New Delhi virus (tolcndv) infected tomato leaves indicates that deregulation of mir159/319 and mir172 might be linked with leaf curl disease. Virol J. 2010;7(1):281.
36. Stief A, Altmann S, Hoffmann K, Pant BD, Scheible WR, Baurle I. Arabidopsis miR156 Regulates Tolerance to Recurring Environmental Stress through SPL Transcription Factors. Plant Cell. 2014;26(4):1792–807.
37. Kim DY, Kwon SI, Choi C, Lee H, Ahn I, Park SR, Bae SC, Lee SC, Hwang DJ. Expression analysis of rice VQ genes in response to biotic and abiotic stresses. Gene. 2013;529(2):208–14.
38. Ye YJ, Xiao YY, Han YC, Shan W, Fan ZQ, Xu QG, Kuang JF, Lu WJ, Lakshmanan P, Chen JY. Banana fruit VQ motif-containing protein5 represses cold-responsive transcription factor MaWRKY26 involved in the regulation of JA biosynthetic genes. Sci Rep. 2016;6(1):23632.
39. Song W, Zhao H, Zhang X, Lei L, Lai J. Genome-Wide Identification of VQ Motif-Containing Proteins and their Expression Profiles Under Abiotic Stresses in Maize. Front Plant Sci. 2015;6:1177.
40. Wang Y, Liu H, Zhu D, Gao Y, Yan H, Xiang Y. Genome-wide analysis of VQ motif-containing proteins in Moso bamboo (Phyllostachys edulis). Planta. 2017;246(1):165–81.
41. Adams KL, Wendel JF. Polyploidy and genome evolution in plants. Curr Opin Plant Biol. 2005;8(2):135–41.

42. Fawcett JA, Maere S, Van de Peer Y. Plants with double genomes might have had a better chance to survive the Cretaceous-Tertiary extinction event. Proc Natl Acad Sci USA. 2009;106(14):5737–42.
43. Pellicer J, Hidalgo O, Dodsworth S, Leitch IJ. Genome Size Diversity and Its Impact on the Evolution of Land Plants. Genes (Basel). 2018;9(2):88.
44. Wang M, Vannozzi A, Wang G, Zhong Y, Corso M, Cavallini E, Cheng ZM. A comprehensive survey of the grapevine VQ gene family and its transcriptional correlation with WRKY proteins. Front Plant Sci. 2015;6:417.
45. Cheng Y, Zhou Y, Yang Y, Chi YJ, Zhou J, Chen JY, Wang F, Fan B, Shi K, Zhou YH, et al. Structural and functional analysis of VQ motif-containing proteins in Arabidopsis as interacting proteins of WRKY transcription factors. Plant Physiol. 2012;159(2):810–25.
46. Perruc E, Charpenteau M, Ramirez BC, Jauneau A, Galaud JP, Ranjeva R, Ranty B. A novel calmodulin-binding protein functions as a negative regulator of osmotic stress tolerance in Arabidopsis thaliana seedlings. Plant J. 2004;38(3):410–20.
47. Kumar S, Stecher G, Suleski M, Hedges SB. TimeTree: A Resource for Timelines, Timetrees, and Divergence Times. Molecular biology evolution. 2017;34(7):1812–9.
48. Altschul SF. Basic local alignment search tool (BLAST). Journal of molecular biology. 1990;215(3):403–10.
49. Marchler Bauer A, Lu S, Anderson JB, Chitsaz F, Derbyshire MK, Carol D-S, Fong JH, Geer LY, Geer RC, Gonzales NR. **CDD: a Conserved Domain Database for the functional annotation of proteins**. *Nucleic Acids Research* 2010:225–229.
50. Ivica L, Tobias D, Peer B. SMART 6: recent updates and new developments. Nucleic Acids Res. 2009;37:229–32.
51. Panu A, Manohar J, Konstantin A, Delphine B, Gabor C, Edouard DC, Séverine D, Volker F, Arnaud F, Elisabeth G. ExPASy: SIB bioinformatics resource portal. Nucleic Acids Res. 2012;40:597–603.
52. Patil I: '**ggplot2**' **Based Plots with Statistical Details [R package ggstatsplot version 0.0.1]**. 2018.
53. Katoh K, Standley DM. MAFFT: iterative refinement and additional methods. Methods Mol Biol. 2014;1079(1079):131–46.
54. Talavera G, Castresana J. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Syst Biol. 2007;56(4):564–77.
55. Lam-Tung N, Schmidt HA, Arndt VH, Quang MB. **IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies**. *Molecular Biology & Evolution* 2014(1):1.
56. Yu Gc, Smith DK, Zhu Hc, Guan Y, Lam TT, Mclnerny G. GGtree: an r package for visualization and annotation of phylogenetic trees with their covariates and other associated data. Methods Ecol Evol. 2016;8(1):28–36.
57. Wang Y, Tang H, Debarry JD, Tan X, Li J, Wang X, Lee TH, Jin H, Marler B, Guo H, et al. MCScanX: a toolkit for detection and evolutionary analysis of gene synteny and collinearity. Nucleic Acids Res. 2012;40(7):e49.

58. Krzywinski M, Schein J, Birol I, Connors J, Gascoyne R, Horsman D, Jones SJ, Marra MA. Circos: an information aesthetic for comparative genomics. Genome Res. 2009;19(9):1639–45.
59. Hu B, Jin J, Guo AY, Zhang H, Luo J, Gao G. GSDS 2.0: an upgraded gene feature visualization server. Bioinformatics. 2015;31(8):1296–7.
60. Bailey TL, Boden M, Buske FA, Frith M, Grant CE, Clementi L, Ren J, Li WW, Noble WS. **MEME SUITE: tools for motif discovery and searching**. *Nucleic Acids Res* 2009, **37**(Web Server issue):W202-208.
61. Chen C, Xia R, Chen H, He Y. **TBtools, a Toolkit for Biologists integrating various HTS-data handling tools with a user-friendly interface**. *bioRxiv* 2018:289660.
62. Lescot M, Dehais P, Thijs G, Marchal K, Moreau Y, Van de Peer Y, Rouze P, Rombauts S. PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. Nucleic Acids Res. 2002;30(1):325–7.
63. Dai X, Zhao PX. psRNATarget: a plant small RNA target analysis server. Nucleic Acids Res. 2011;39:W155–9. (Web Server issue).
64. Leinonen R, Sugawara H, Shumway M, International Nucleotide Sequence Database C. The sequence read archive. Nucleic Acids Res. 2011;39(Database issue):D19–21.
65. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014;30(15):2114–20.
66. Kim D, Langmead B, Salzberg SL. HISAT: a fast spliced aligner with low memory requirements. Nat Methods. 2015;12(4):357–60.
67. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R. Genome Project Data Processing S: **The Sequence Alignment/Map format and SAMtools**. Bioinformatics. 2009;25(16):2078–9.
68. Pertea M, Pertea GM, Antonescu CM, Chang TC, Mendell JT, Salzberg SL. StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. Nat Biotechnol. 2015;33(3):290–5.
69. Kolde R, Kolde MR. **R: Pheatmap: Pretty Heatmaps**. *R Package* 2015, 1(7).
70. Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. BMC Bioinform. 2008;9(1):559.
71. Wagner R, Fischer M. The string-to-string correction problem. J ACM. 1974;21(1):168–73.
72. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13(11):2498–504.

Figures

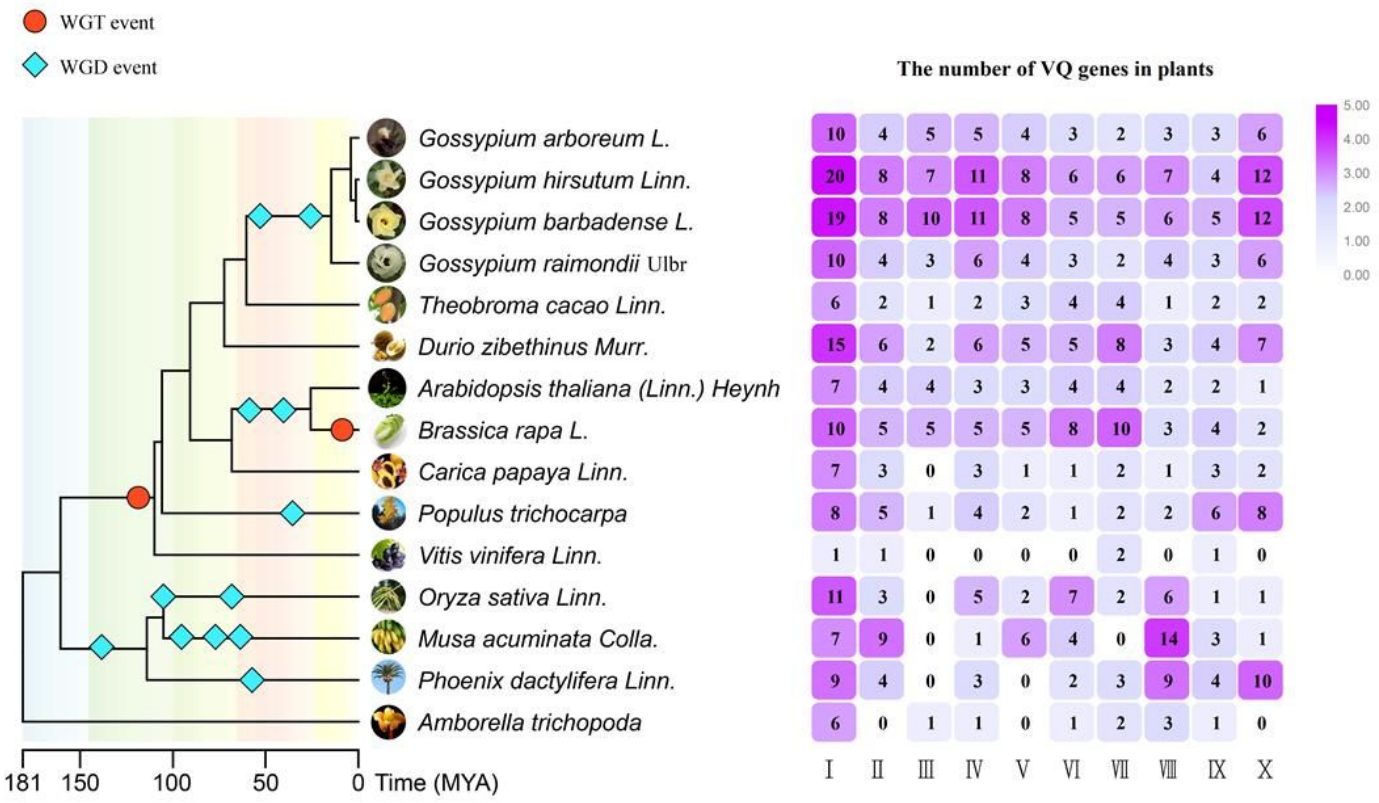

Figure 1

The VQ gene family evolutionary relationship and the number details of the 15 plant species. The left of this figure shows the evolutionary relationships of the species; the right of this figure shows the number details of the VQ family of each group.

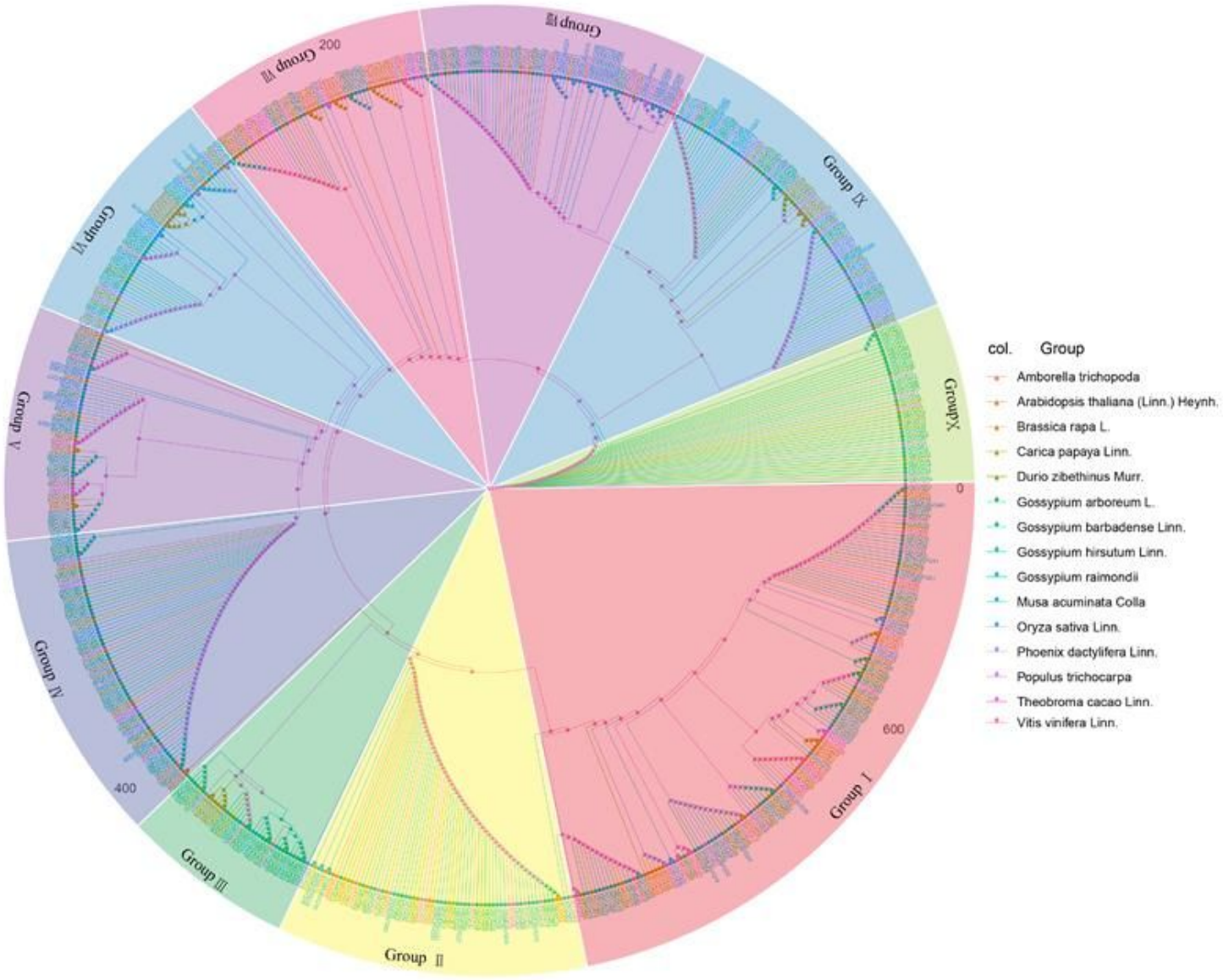

Figure 2

The VQ gene family phylogenetic relationship of the 15 plant species. The Phylogenetic tree includes 268 VQ genes from cotton and 388 VQ genes from other 11 plants. Maximum likelihood (ML) bootstrap values are shown in the major nodes.

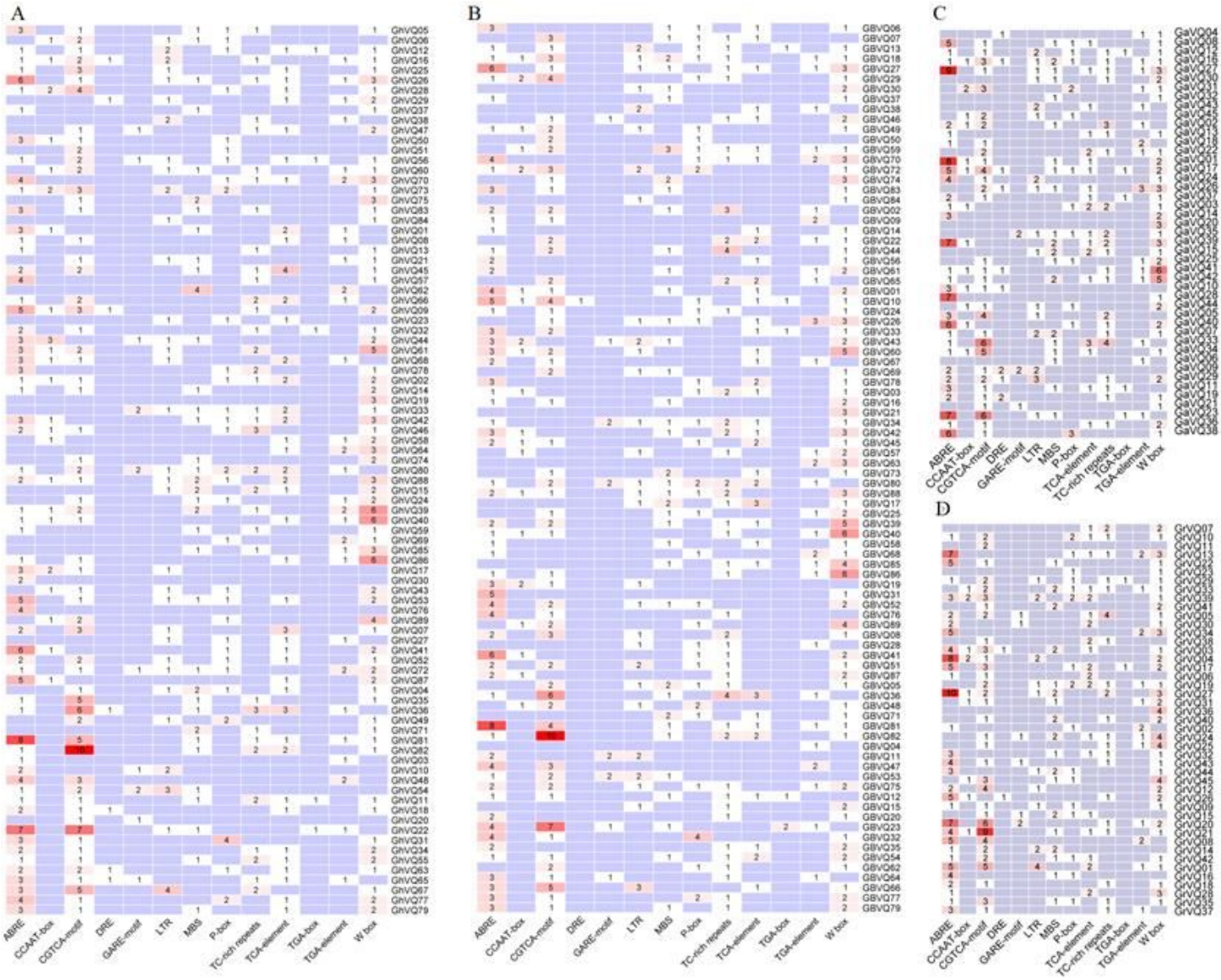

Figure 3

Potential cis-elements in promoters of VQ genes in cotton. a, b, c, d are the identified cis-elements of GhVQ, GbVQ, GaVQ and GrVQ genes, respectively.

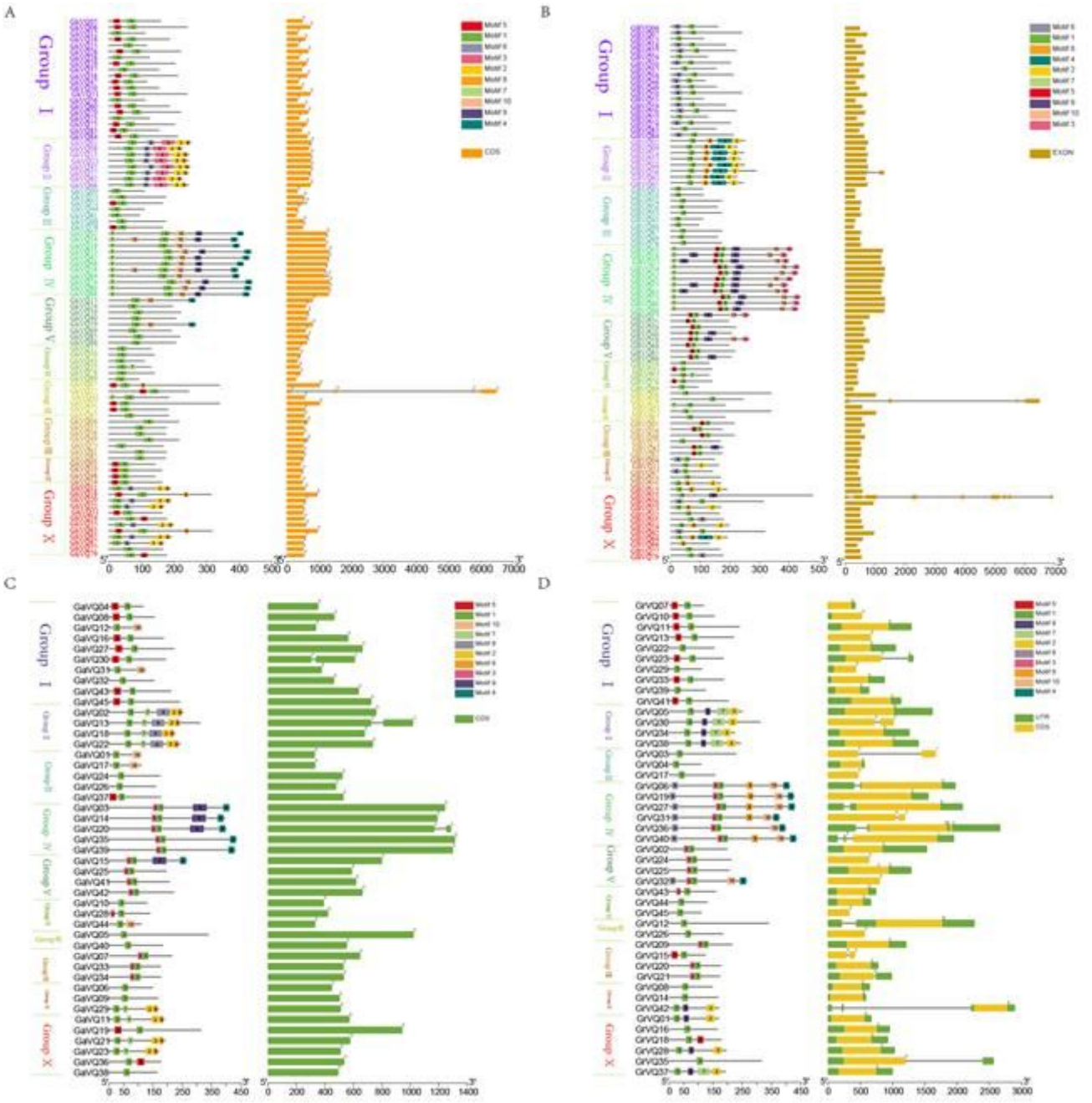

Figure 4

The converted motif and gene structure of VQ genes in cotton. a, b, c, d are the converted motif and gene structure of GhVQ, GbVQ, GaVQ and GrVQ genes, respectively.

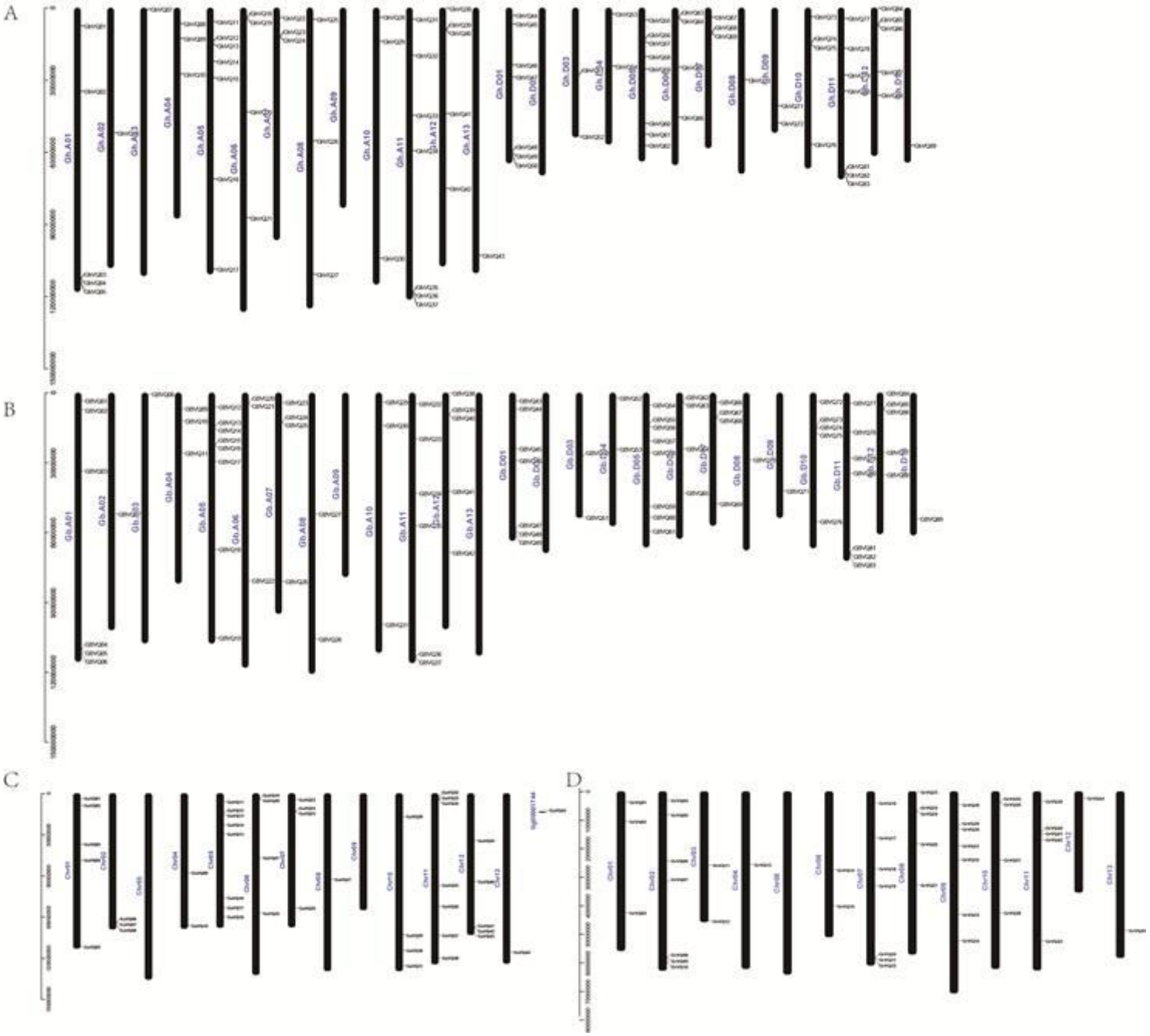

Figure 5

Distribution of the VQ genes on chromosomes. a The 89 GhVQ genes distribution in G. hirsutum. b The 89 GbVQ genes distribution in G. barbadense. c The 45 GaVQ genes distribution in G. arboreum. d The 45 GrVQ genes distribution in G. raimondii.

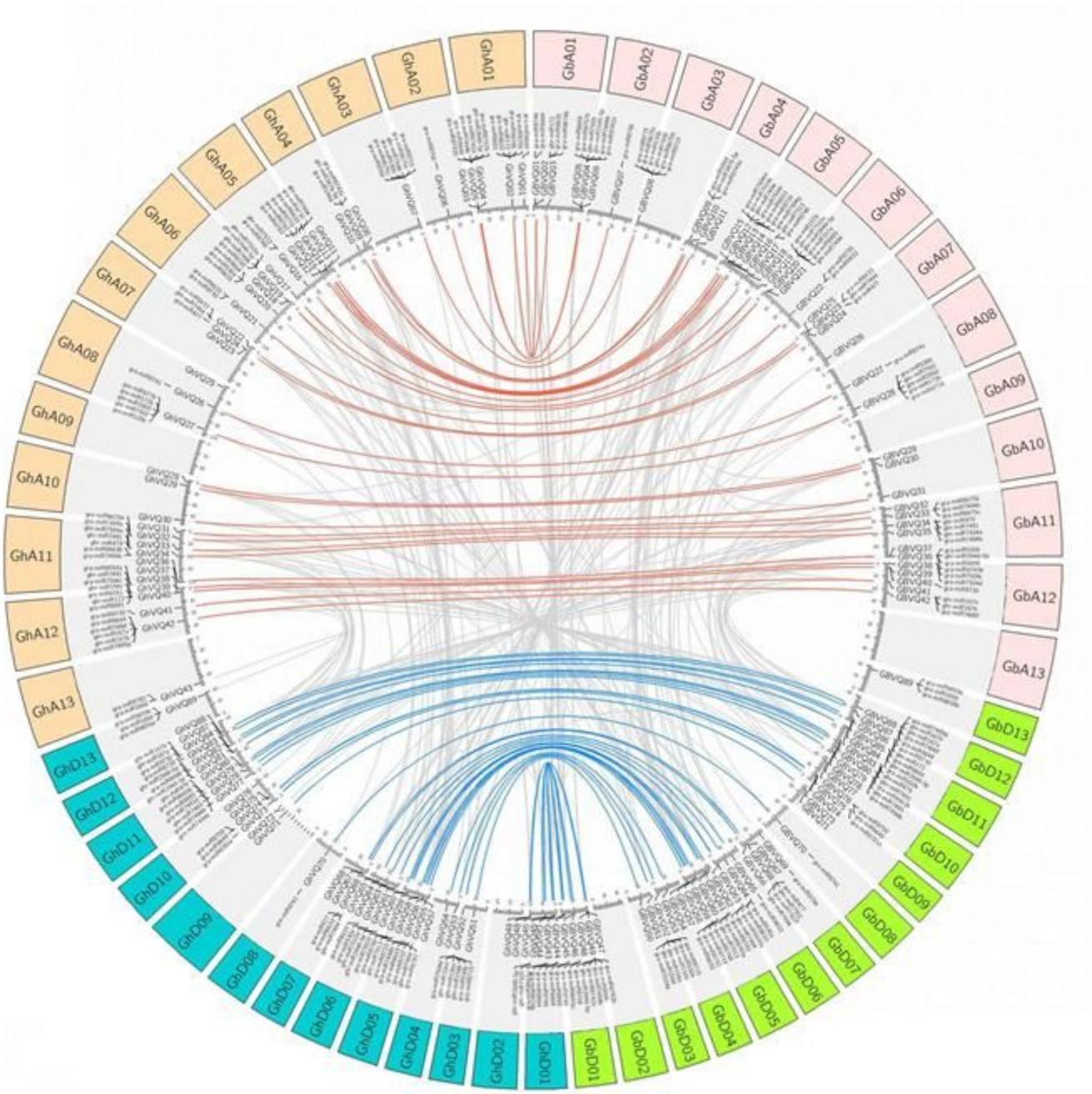

Figure 6

Ortholog and paralog pairs of VQ genes in G. hirsutum and G. barbadense. The lines regarding orthologous gene pairs are colored by blue and orange, and the paralogous gene pairs are colored by grey lines. The central links are predicted miRNA target genes.

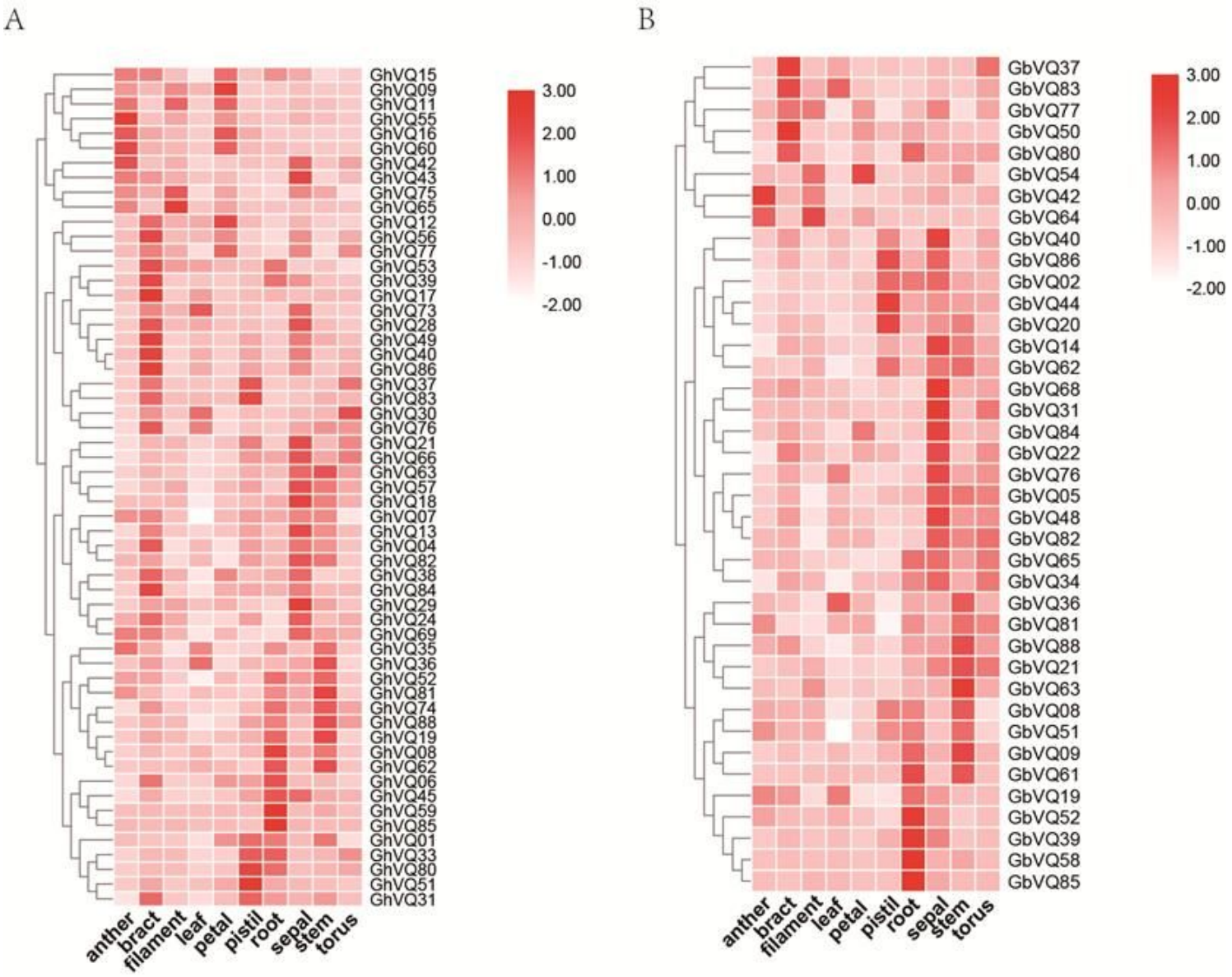

Figure 7

The expression of cotton VQ genes in different cotton tissues. a The expression of the selected VQ genes in G. hirsutum; b The expression of the selected VQ genes in G. barbadense.

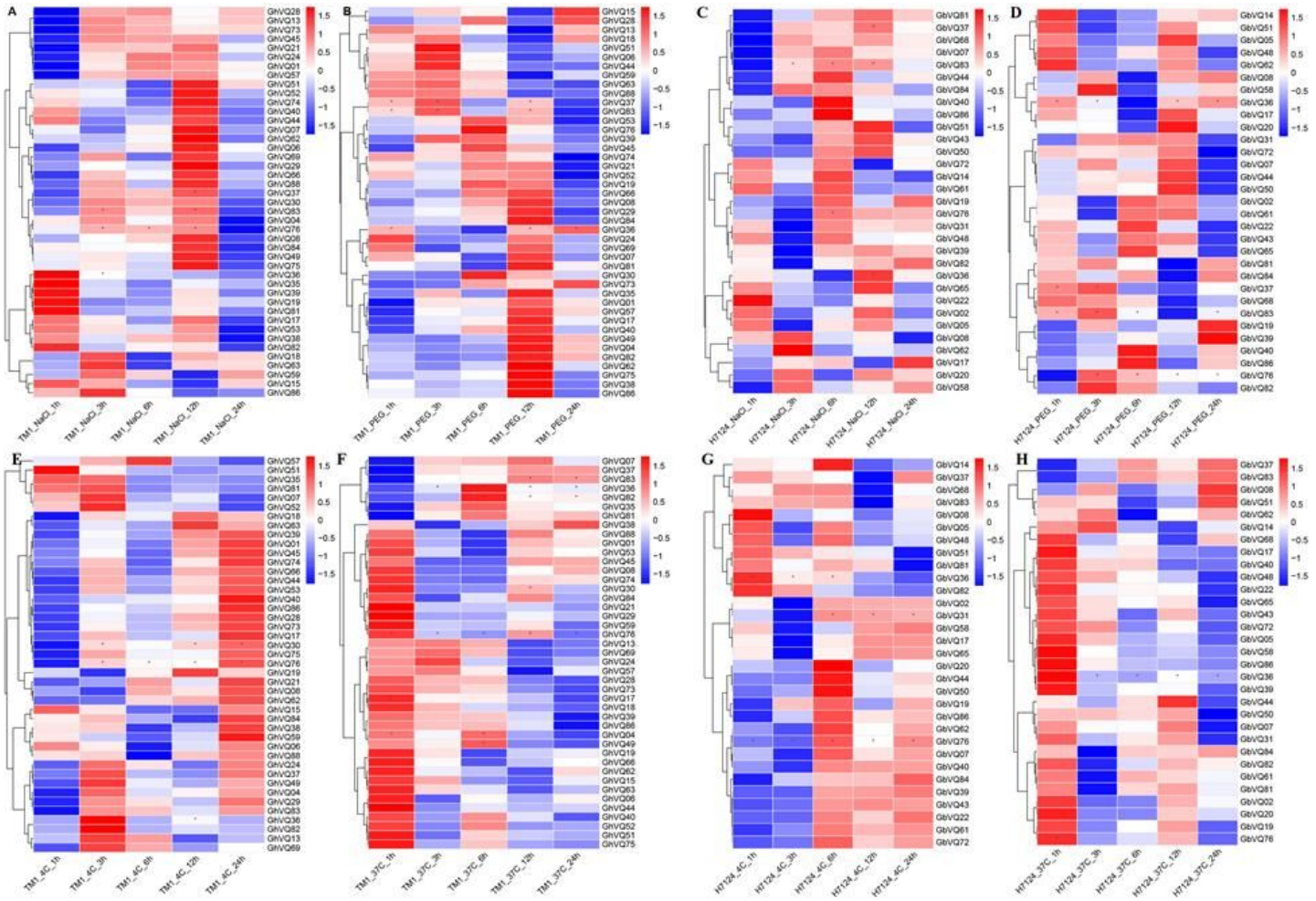

Figure 8

Stress-induced expression profiles of GhVQ and GbVQ genes. a, b, c, d, e, f, and h are the expressions of 43 GhVQ and 31 GbVQ genes under salt (a and c), drought (b and d), cold (e and g) and heat (f and h) stresses. The colors vary from blue to red representing the scale of the relative expression levels.

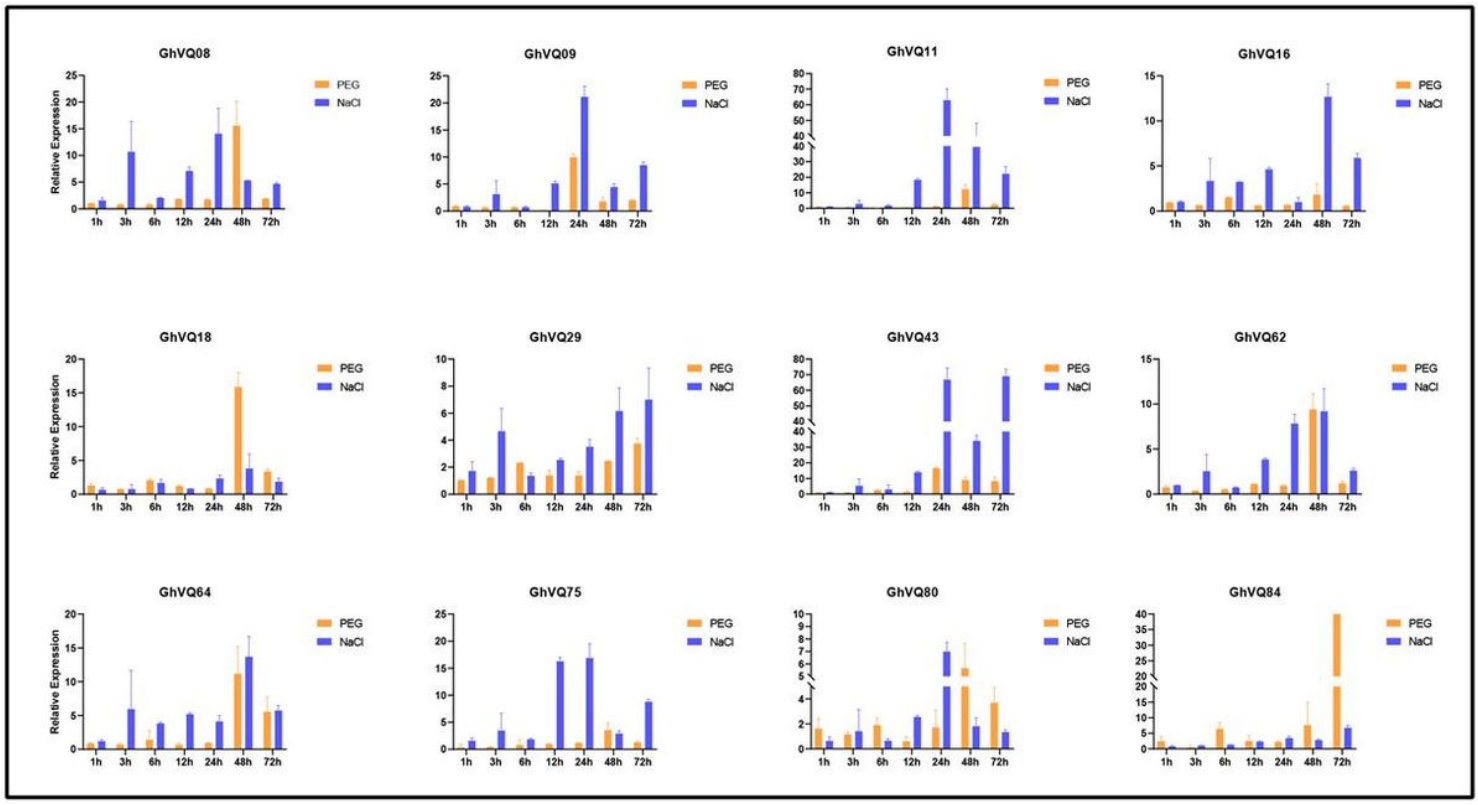

Figure 9

The qPCR relative transcriptional expression levels of GhVQs in different stresses. The time of posts of different treatments are shown on the x-axis, and the relative expression levels are shown on the y-axis.

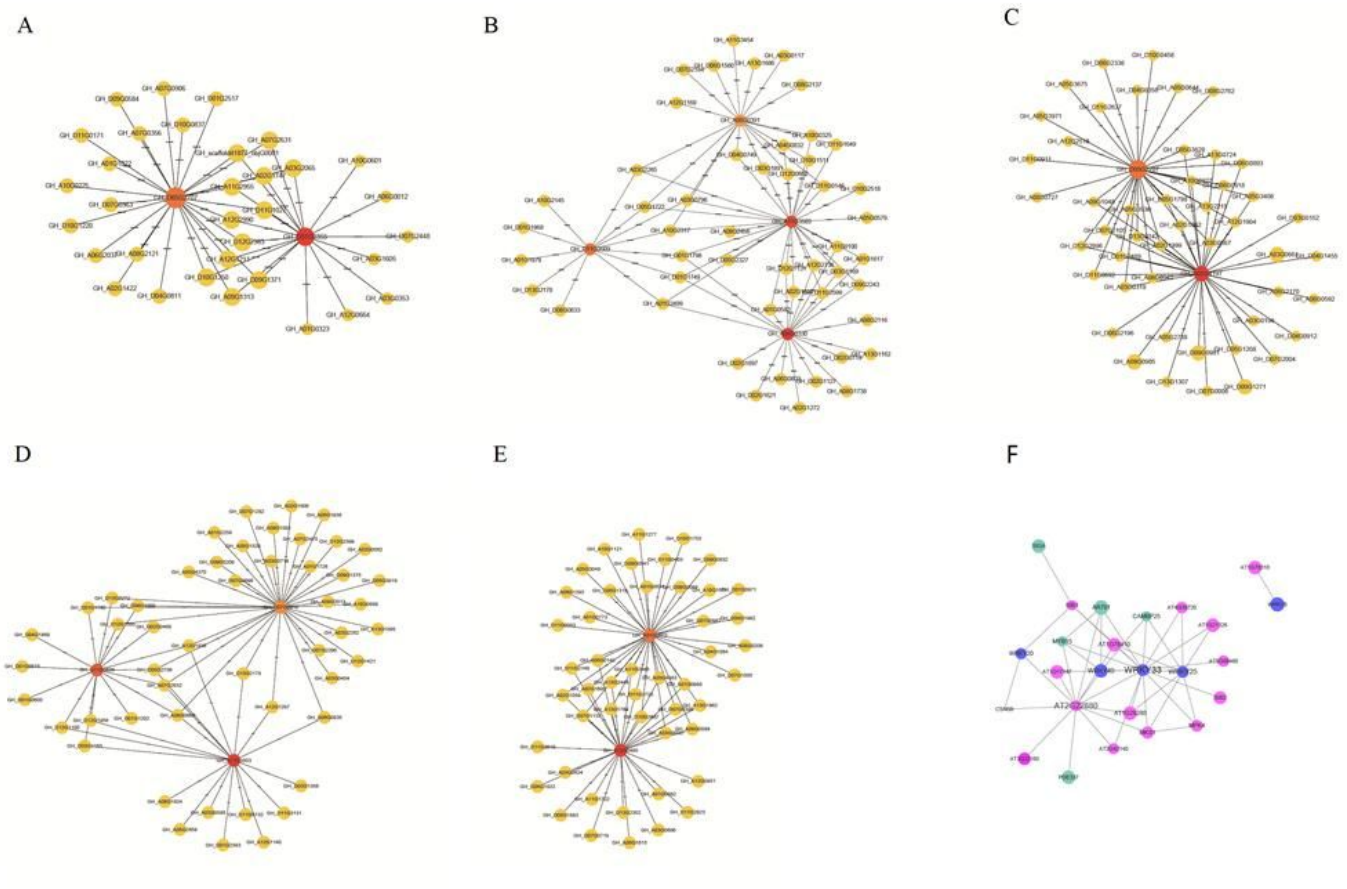

Figure 10

Co-expression and functional interaction network of GhVQ genes. Orange circular ones are the GhVQ co-expression genes (a, b, c, d and e), while yellow ones are other co-expression genes. f The functional interacting network models of GhVQ genes. Homologous genes in cotton and Arabidopsis are shown in pink and blue, respectively.

Supplementary Files

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

- TableS8.xlsx
- TableS7.xlsx
- TableS6.xlsx
- TableS5.xlsx
- TableS4.xlsx
- TableS3.xlsx
- TableS2.xlsx
- TableS1.xlsx

- Fig.S1.jpg
- Fig.S2.jpg
- Fig.S3.jpg
- Fig.S4.jpg
- Fig.S5.jpg
- Fig.S6.jpg
- Fig.S7.jpg