

Genome-wide Analysis and Expression Profiling Suggest Diverse Roles of TCP Genes During Development and Stress Responses in Grapevine (*Vitis vinifera* L)

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Submitted for publication: December 2017

Accepted for publication: April 2018

Key words: Genome-wide analysis, TCP transcription factor, grapevine, stress, real-time PCR

Teosinte branched 1/cycloidea/proliferating cell factor 1 (TCP) proteins are plant-specific transcription factors playing crucial roles in various biological processes, such as leaf development, flower symmetry, shoot branching and senescence. However, no comprehensive analysis of the TCP gene family has been reported in grapevine (*Vitis vinifera* L). Herein, a total of 15 TCP family members were identified in the genome of grapevine, located on eight of the 19 chromosomes. Phylogenetic and structural analyses showed that the *Vv*TCPs were classified into two groups, designated as Class I and Class II. The Class II genes were further divided into two subclasses, the CIN subclass and the CYC/TB1 subclass. Genes belonging to the same subclass shared similar gene structures, conserved domains and motifs. Real-time PCR showed that almost all members of Class II exhibited organ-specific expression patterns, while members of Class I and the CIN Class were ubiquitously expressed in all the tissues examined, indicating multiple roles in the development of different grapevine organs. In addition, many members were strongly modulated by abiotic (cold, heat, drought) and biotic (downy mildew and powdery mildew infection) stresses, suggesting important and diverse regulatory roles in adverse conditions and plant immunity. The comprehensive in silico analysis of the grapevine TCP transcription factor family gives us some references to potential functions in grapevine development and stress responses.

INTRODUCTION

The teosinte branched 1/cycloidea/proliferating cell factor 1 (TCP) gene family is a plant-specific transcription factor, which is of great importance in the evolution and developmental control of plants (Navaud *et al.*, 2007). It was first described in 1999 and named after the first four characterised family members: TEOSINTE BRANCHED 1 (TB1) in maize (*Zea mays*), CYCLOIDEA (CYC) in snapdragon (*Antirrhinum majus*), and two PROLIFERATING CELL FACTORS (PCF1 and PCF2) in rice (*Oryza sativa*) (Doebley *et al.*, 1995; Luo *et al.*, 1996; Kosugi & Ohashi, 1997; Cubas *et al.*, 1999). All the TCP family members share a conserved region consisted of 59 residue-long amino acids, designated as the TCP domain (Cubas *et al.*, 1999), which form a basic helix-loop-helix (bHLH) structure and are essential for DNA binding and protein interactions (Cubas *et al.*, 1999). Based on differences within the TCP domains, TCP family members were classified into two subfamilies, known as Class I (or PCF or TCP-P) and Class II (or TCP-C) (Navaud *et al.*, 2007), which have been suggested to play contrasting roles in plant proliferation and growth (Hubbard

et al., 2002; Takeda *et al.*, 2003; Aguilar-Martínez *et al.*, 2007; Hervé *et al.*, 2009; Martin-Trillo & Cubas, 2010). TCP Class II was further subdivided into the CIN and CYC/TB1 clades (Martin-Trillo & Cubas, 2010). Compared to that of Class I, the TCP domain within the Class II members possessed a four amino acid insertion, and some of the members in TCP Class II possessed an R domain (an 18- to 20-residue arginine-rich motif) and an ECE motif (a glutamic acid–cysteine–glutamic acid stretch), although both were of unknown biological function (Navaud *et al.*, 2007; Martin-Trillo & Cubas, 2010).

TCP transcription factors were reported to be implicated in a series of developmental processes, including flower organ morphogenesis (Damerval *et al.*, 2007; Broholm *et al.*, 2008; Tanaka *et al.*, 2011), the circadian clock (Giraud *et al.*, 2010), leaf shape (Kieffer *et al.*, 2011), internode length (Kieffer *et al.*, 2011), root hair elongation (Hao *et al.*, 2012), cotton fibre elongation (Hao *et al.*, 2012) and tendril development (Wang *et al.*, 2015b). Also, an increasing number of TB1 homologs were identified as negative regulator of axillary

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Acknowledgments: This work was financially supported by the National Natural Science Foundation of China (Grant No. 31872049), the Key Research and Development Program of Ningxia (2016BZ0602), and the China Agriculture Research System for Grape (CARS-29-zp-6)

bud outgrowth and tillering (Takeda *et al.*, 2003; Aguilar-Martínez *et al.*, 2007; Martín-Trillo *et al.*, 2011; Xu *et al.*, 2015). In addition, *TCP15* in *Arabidopsis* was recently found to play roles in modulating redox development and regulating anthocyanin biosynthesis (Viola *et al.*, 2016). TCP factors not only act as mediators of hormone-induced changes in cell proliferation, but also as modulators, or even key players, of hormone synthesis, transport and signal transduction (reviewed in Nicolas & Cubas, 2016).

To date, many TCP genes have been identified in various plants, such as *Arabidopsis thaliana* (Yao *et al.*, 2007), *Oryza sativa* (Yao *et al.*, 2007), *Malus domestica* (Xu *et al.*, 2014), *Solanum lycopersicum* (Parapunova *et al.*, 2014), *Citrullus lanatus* (Shi *et al.*, 2016), *Gossypium raimondii* (Ma *et al.*, 2014) and *Gossypium arboreum* (Ma *et al.*, 2016), etc. The sequenced genome of grapevine (Jaillon *et al.*, 2007), and the rise of next-generation sequencing, have made it easier for genome-wide analysis of all genes belonging to specific gene families. The genome-wide analysis of the NAC (Wang *et al.*, 2013), bZIP (Liu *et al.*, 2014), WRKY (Wang *et al.*, 2014), MADS-box (Grimplet *et al.*, 2016) and HSF (Hu *et al.*, 2016) gene families in grapevine have been reported successively. However, no report on the genome-wide analysis of the grapevine TCP gene family is currently available.

In this study, we performed for the first time a comprehensive analysis of the TCP gene family members in grapevine (*Vitis vinifera* L.) genome. Next, the phylogenetic analysis, chromosomal location, gene structure and conserved motif were carefully analysed. Finally, the expression profiles in different grapevine tissues and the expression patterns in response to abiotic (heat, cold and drought treatment) and biotic (downy mildew and powdery mildew infection) stimuli were detected via real-time PCR. This study provides valuable information for understanding the classification and molecular and biological functions of *VvTCPs*, which can be of great value in *Vitis* breeding.

MATERIALS AND METHODS

Identification of TCP genes in grapevine (*Vitis vinifera* L.)

The protein sequences of the 15 putative grapevine TCP members available in the Plant Transcription Factor Database (<http://plantfdb.cbi.pku.edu.cn/>) (Jin *et al.*, 2017) were used as queries to perform BLAST-P (Protein Basic Local Alignment Search Tool) searches in NCBI (National Center for Biotechnology Information, <http://blast.ncbi.nlm.nih.gov/Blast.cgi>), setting the threshold of the E-value as 1e-10 to ensure the discovery of all potential TCP-type DNA-binding domain-encoding sequences in the grape genome (*V. vinifera* cv. Pinot Noir, PN40024, 12X) (<http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/>). Then, the candidate TCP proteins were manually inspected with the InterProScan program (<http://www.ebi.ac.uk/Tools/pfa/iprscan5/>) (Quevillon *et al.*, 2005) to validate the presence and completeness of the TCP domain (PF03634). The multiple alignment of identified gene sequences was also performed by MEGA 5.0 (Tamura *et al.*, 2011) to remove redundant sequences.

Chromosomal location and gene structure analysis of *VvTCP* genes

Molecular weight and isoelectric point were predicted using the ProtParam tool (<http://web.expasy.org/protparam/>) (Gasteiger *et al.*, 2005); chromosomal locations of the *TCP* genes were retrieved from the Grape Genome Browser (12X) (<http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/>), and the distribution of genes in chromosomes were depicted based on the above results. Gene structures were analysed and drawn with the GSDS (Gene Structure Display Server: <http://gsds.cbi.pku.edu.cn>) (Hu *et al.*, 2015) by aligning the genome sequences with their corresponding coding sequences.

Sequence alignment and phylogenetic analysis

DNAMAN 6.0 software (Lynnon Biosoft, Quebec, Canada) was employed to perform multiple sequence alignments, with the parameters setting as default. The TCP amino acid sequences of *Arabidopsis* were downloaded from the Plant Transcription Factor Database, and a phylogenetic tree containing the TCP amino acid sequences of *Arabidopsis* and grapevine was constructed based on MUSCLE (Edgar, 2004) alignment, as well as the unrooted neighbour-joining method using MEGA 5.0 (Tamura *et al.*, 2011). Neighbour-joining analysis with pairwise deletion was performed using the Jones Taylor Thornton (JTT) model (Jones *et al.*, 1992). Bootstrap analysis was performed with 1 000 replicates to assess the level of statistical support for each tree node (Tamura *et al.*, 2011). The protein domains of the putative *VvTCPs* were identified using MEME online tools (<http://meme.nbcr.net/meme/>) (Bailey *et al.*, 2009). Default settings were retained for the parameters.

Plant materials and treatments

One-year-old 'Cabernet Sauvignon' (*Vitis vinifera* L.) cuttings were planted in plastic pots (diameter: 14 cm, height: 10 cm) filled with soil mix (peat:perlite, 4:1, v:v), and grown in the greenhouse of the Enology College, Northwest A&F University, Yangling, China (34.29°N, 108.35°E), with the temperature ranging from 22°C to 27°C and the relative humidity from 70% to 90%. The roots, stems, leaves (old and young leaves), tendrils, buds (prompt and latent buds), and inflorescences were collected to analyse the tissue-specific expression of the *VvTCP* genes.

The extreme temperature treatments were performed by transferring the cuttings to two growth chambers in which the temperature setting was 45°C and 4°C respectively; another set of cuttings was maintained under the control temperature of 23°C. The drought treatment was simulated by adding Hoagland solution (Hoagland & Arnon, 1950) with 10% PEG; another set of cuttings was cultivated similarly with Hoagland solution (Hoagland & Arnon, 1950). All the leaves were collected 12 h after the treatments. All of the experiments were repeated independently in triplicate. The samples were frozen immediately in liquid nitrogen and then stored at -80°C for further experiments.

RNA extraction and real-time PCR

Total RNA was extracted from the materials described above using Plant RNAout (TIANDZ, China) according to the

manufacturer's instructions. Genomic DNA contamination was removed from the total RNA using RNase-free DNase I (Thermo Fisher Scientific Inc., USA). RNA concentration and quality were assessed using the NanoDrop ND-2000 Spectrophotometer (Thermo Fisher Scientific Inc., USA). The first strand of cDNA was synthesised from RNA using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific Inc., USA). A total of 20 µl of qPCR mixture was employed, containing 2 µl first-strand cDNAs, 0.6 µl of the forward and reverse primers for each gene, 6.8 µl of ddH₂O and 10 µl of 2 × FastStart Universal SYBR Green Master (Roche, Germany). The relative expression of each TCP gene was normalised using the method of $2^{-\Delta\Delta Ct}$ against the reference gene, *VvGAPDH*. The primers were designed via the Primer Premier 5.0 (Lalitha, 2000) and are listed in Table 1.

RESULTS

Identification of TCP genes in the grapevine genome

Fifteen TCP proteins were identified in the transcription factor database. A total of 16 homologous TCP proteins were obtained using the BLAST-P in NCBI, 15 of which were detected to contain TCP domain, while others were the repetitive sequences or the alternative splicing transcripts of the TCP genes identified in the transcription factor database. Therefore, as was predicted in the plant transcription factor database, there are 15 members of TCP transcription factors in the grapevine genome (Table 2). According to the chromosomal locations, these putative grapevine (*Vitis vinifera* L) TCP genes were provisionally named *VvTCP1* to *VvTCP15*. As described in Table 2, these grapevine TCPs had a nucleotide length ranging from 654 (*VvTCP13*) to

1 398 bp (*VvTCP9*), a peptide length ranging from 217 to 465 amino acids, a molecular weight ranging from 24.7374 to 52.1817 kDa, and a theoretical isoelectric point that varied from 6.9057 (*VvTCP4*) to 10.7992 (*VvTCP5*).

According to the genomic data, we depicted the chromosome location of all the *VvTCPs*, finding that these genes were not evenly distributed over the genome of *Vitis vinifera* L. They were located on Chr 1, Chr 2, Chr 10, Chr 12, Chr 14, Chr 15, Chr 17 and Chr 19. Chr 14 and Chr 17 both had three TCP genes, and Chr 1, Chr 10 and Chr 12 all had two genes (Fig. 1).

Phylogenetic analysis of TCP proteins

To shed light on the evolutionary relationships of the TCP transcription factor family in grapevine, the full-length protein sequences of 15 *VvTCPs* and 24 TCPs from *Arabidopsis* (Yao *et al.*, 2007) were subjected to a multiple sequence alignment using the MEGA 5.0 program. The multiple sequence alignment file was then used to construct an unrooted phylogenetic tree. As illustrated in the neighbour-joining phylogenetic tree (Fig. 2), the *VvTCPs* were also classified into two groups, designated Class I (TCP-P or PCF class) and Class II (TCP-C). The Class II genes were further classified into two subgroups, CYC/TB1 and CIN. Class I and Class II included six and nine genes respectively, and the group of CYC/TB1 was the smallest one and was consistent with that of *Arabidopsis*, rice (*Oryza sativa*) (Yao *et al.*, 2007) and apple (*Malus domestica*) (Xu *et al.*, 2014). It was also suggested that grapevine TCP members in each group all had their counterparts in *Arabidopsis*, and they were also not evenly distributed in each group. In addition, only two sister pairs of paralogous *VvTCP* genes (*VvTCP2* and

TABLE 1
Primers used in real-time PCR

Gene name	Forward primer (5'-3')	Reverse primer (5'-3')
VvTCP1	AAGAACAGAAAACCTCGTAGTAAAT	TCAATGATGCCCACAGTAGCCA
VvTCP2	AGAAAGAGGCAGCCAGAGCAGGA	GGCAAGAGAGCCATTGGAGTAGG
VvTCP3	CCATCCATCGCTTGACTCTTTTG	TTATCGTTGGCGTTGTTCTCCTC
VvTCP4	AGTTTGGAGCACGCATCATCG	GAATAGCAGTGTTAGCAGACAATCGC
VvTCP5	CTACCAACAAAACAAAACACCAAACA	GCTGCCTCTGGGAGTCAACAATA
VvTCP6	TCACAGAACTACTACTGGGGGT	GGGATAAGATGGTCGGGAACAAA
VvTCP7	TGGCTAATCAAGAAGGCAAAAT	GTGTCAGCAATGGAATCAGAGT
VvTCP8	CCTCCCTCGCCATCGCTA	GACTTGTGCCCCAACTCTCTCG
VvTCP9	CTGAGGGCTAAGTCAAAGGAAGT	CAAATGATAGTAAGGACCATAAGGC
VvTCP10	AGGGCAAGGGAAAGGACAAG	GAGGTGGGTGGGGAAAATAGC
VvTCP11	GGCACACGAAGGTAGAGGGA	CGGAGGAGACTGAAACGGCA
VvTCP12	ATCCCAACTTCTCGGTCCCTCAA	GCCTCTTCCTCCCTATGCTCTCC
VvTCP13	AAGACAGTGGTCAGCATTAGAA	CTGGAGTGGTGGAAGTTTATCAA
VvTCP14	TCTTCCTTCTTACTTTCCGCTC	ATCGGCTCGGTCACACTGTTACTT
VvTCP15	CCCAACCCCAATCCTAACCCCTAA	GTGTCCGCAATGGTATCAGAGTC
VvGAPDH	ACTGCCTTGCTCCTCTTGCGAAG	CCAGTGCTGCTAGGAATGATGTTGAATG

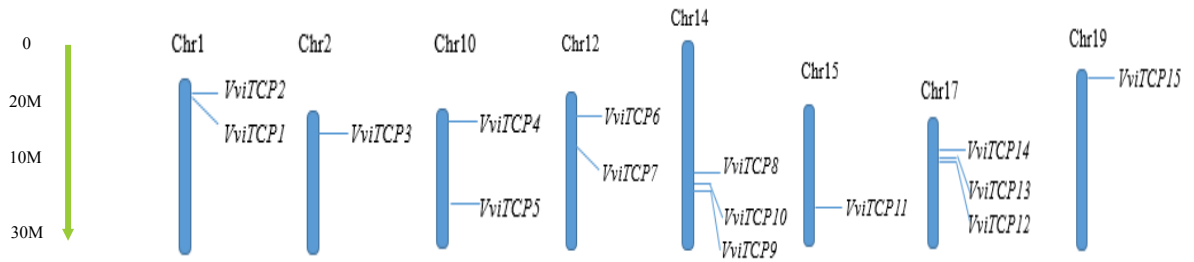


FIGURE 1

The distribution of grapevine TCP gene families in the chromosomes. The location of each TCP gene is indicated by a line.

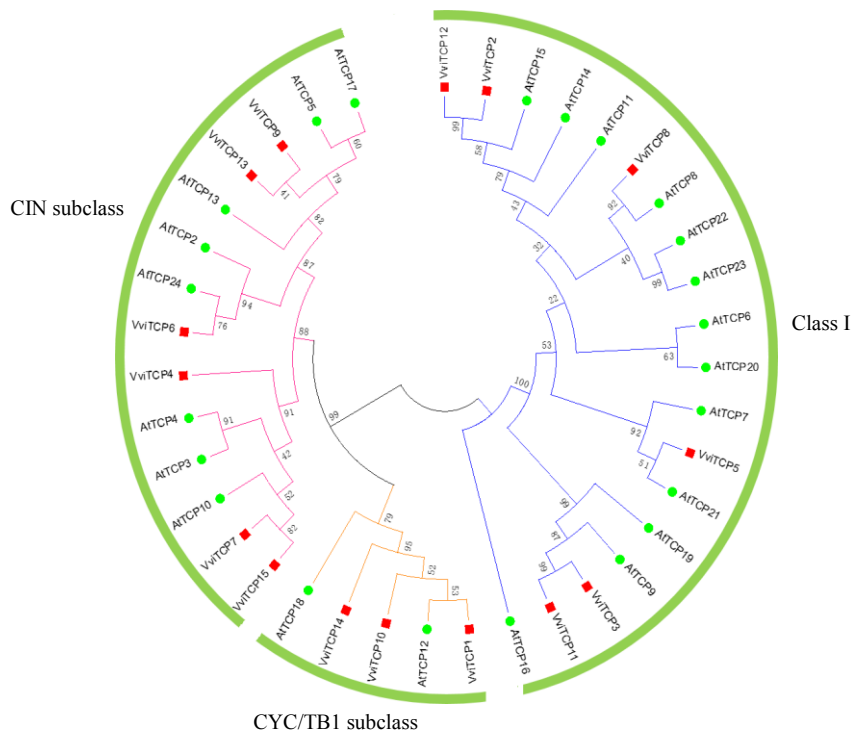


FIGURE 2

Phylogenetic relationships of *Arabidopsis* and grapevine TCP proteins. The phylogenetic tree was constructed using MEGA 5.0 with the neighbour-joining (NJ) method with 1 000 bootstrap replicates.

VvTCP2, *VvTCP3* and *VvTCP13*) were identified (Fig. 3), with strong bootstrap support (> 90%). This may account for the fewer number of TCP members in grapevine than in other species.

Conserved domains and gene structure analysis of TCP gene family in grapevine

The sequence alignment analysis of these TCP proteins showed that the specific TCP domain of bHLH was highly conserved in all the members. However, the R domain, an 18- to 20-residue arginine-rich motif, was only present in the Class II members. A four amino acid deletion was observed in Class II (Fig. 3). In addition, the sequence conservation outside the TCP domain was low, and sequence lengths on both sides of the TCP domain varied greatly among the

15 members. This result was mainly consistent with the phylogenetic analysis described above.

Gene structure was analysed and drawn with the GSDS (<http://gsds.cbi.pku.edu.cn>) by aligning the genome sequences with their corresponding CDS sequences. As was shown in Fig. 4, most TCP members (14 members, 93.3%) contained both exons and introns, except for one member, *VvTCP5*, which had no intron. Niu and Yang (2011) reported that highly expressed genes had higher intron densities (number of introns per kilobase of coding sequence) than weakly expressed genes, and introns could mediate the enhancement of transcription by splicing. As in other species, members of Class II had more introns than those of Class I, indicating a higher level of regulation by introns. Six genes (40%) had two introns, three genes (20%) had three introns,

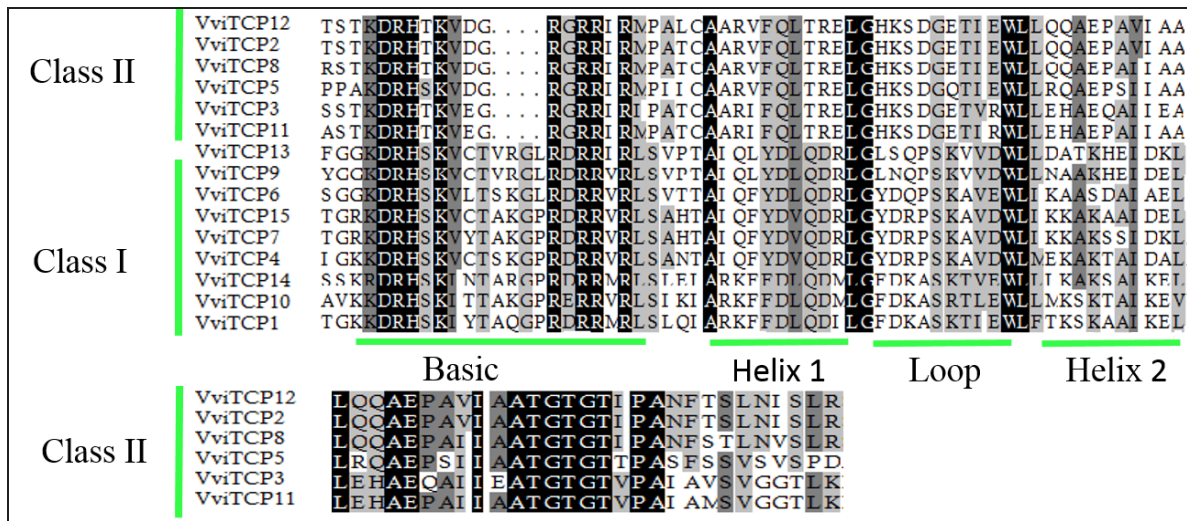


FIGURE 3
Alignment of amino acid sequences among grapevine TCP members.

TABLE 2
Characteristics of the TCP transcription factor family in *Vitis vinifera* L

Name	TF ID	Chromosome location	Nucleotide length (bp)	Amino acid length	Molecular weight (kDa)	Isoelectric point
VvTCP1	GSVIVT01011962001	chr1:2574244..2575738	1215	404	44.8869	10.3531
VvTCP2	GSVIVT01020011001	chr1:11610081..11611375	954	317	35.1046	9.8435
VvTCP3	GSVIVT01019876001	chr2:4139671..4141512	1296	431	46.746	7.9585
VvTCP4	GSVIVT01021167001	chr10:2112286..2113434	1149	382	42.3984	6.9057
VvTCP5	GSVIVT01026145001	chr10:12936187..12943830	867	288	30.5565	10.7992
VvTCP6	GSVIVT01021528001	chr10:6666048..6669048	960	319	34.8686	9.7124
VvTCP7	GSVIVT01020666001	chr12:3280522..3283144	924	307	33.6998	6.9128
VvTCP8	GSVIVT01023283001	chr12:20149670..20152476	1164	387	42.3781	8.0579
VvTCP9	GSVIVT01032911001	chr14:24045544..24048408	1398	465	52.1817	9.0525
VvTCP10	GSVIVT01036449001	chr14:22124290..22125983	822	273	31.2935	10.0656
VvTCP11	GSVIVT01027588001	chr15:15268031..15269562	1023	340	36.3764	9.5841
VvTCP12	GSVIVT01008023001	chr17:6588505..6590555	1038	345	38.089	8.3349
VvTCP13	GSVIVT01008109001	chr17:5711518..5719116	654	217	24.7374	9.719
VvTCP14	GSVIVT01008234001	chr17:4343550..4345620	1101	366	41.5708	8.9711
VvTCP15	GSVIVT01014236001	chr19:1804594..1806793	903	300	32.9047	7.5709

only one (6.7%) had four introns, two (13%) contained six introns and only one (6.7%) contained seven introns. Among all the members, *VvTCP12* was the longest gene, with an intron region of nearly 6 Kb. Generally speaking, genes belonging to the same subclades had a similar number of exons (Fig. 4).

Conserved motifs analysis of TCP gene family in grapevine

We analysed the motif compositions of the 15 proteins in *Vitis vinifera* L using the MEME online tool and found that the number of motifs in grapevine TCP families varied from two to seven (Fig. 4). All the VvTCPs had motif 1 and motif 2, which were specific to the TCP domain, and only the members of Class I had motif 3. Some motifs, such as motif

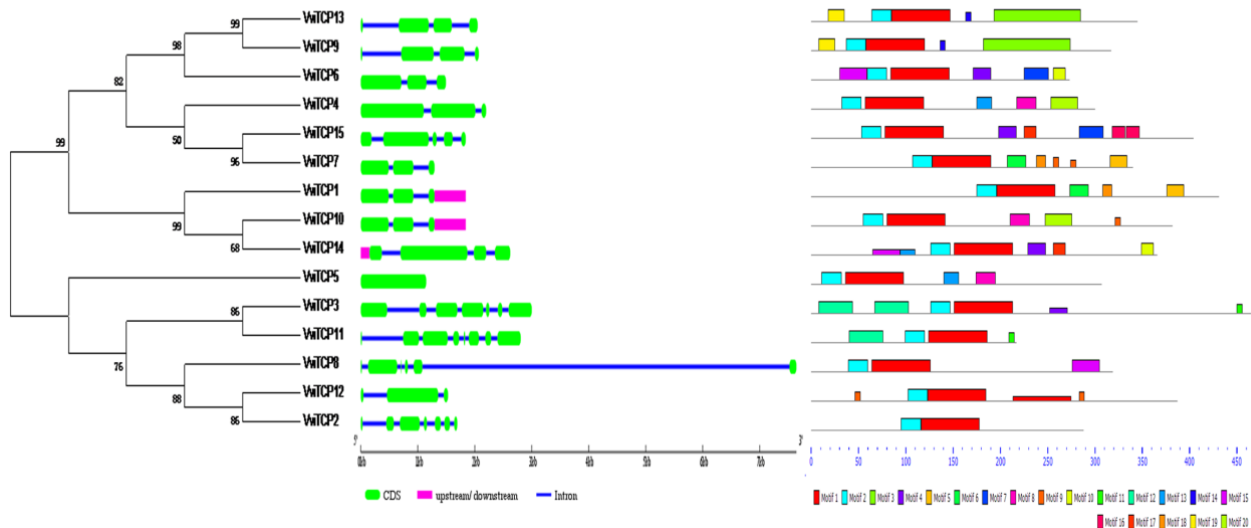


FIGURE 4

Phylogenetic analysis and gene structure of TCP genes in grapevine. The phylogenetic tree was constructed using MEGA 5.0 with the neighbour-joining (NJ) method with 1 000 bootstrap replicates. The green and red boxes represent exons and upstream/downstream sequences respectively, and the blue lines represent introns.

4, motif 5 and motif 7, existed exclusively in the group of Class I, while others, like motif 12, were present only in the group of Class II. In summary, members in the same subgroup showed the same motif composition and distribution.

Expression profiles of VvTCP genes in different organs and under different treatments

Real-time PCR was used to detect the expression patterns in different tissues in grapevine, and under different treatments. As shown in Fig. 5, all the members of *VvTCP* were shown to be expressed in the examined tissues. However, tissue-specific expression was also observed, such as *VvTCP1* in the inflorescence and latent buds, and *VvTCP2* in the stems (Fig. 5). We also found that most of the genes were apparently suppressed or induced when exposed to abiotic and biotic stress (Fig. 6). Among the 15 genes, six (*VvTCP1*, *VvTCP4*, *VvTCP8*, *VvTCP9*, *VvTCP13*, *VvTCP14*) were up-regulated by heat (45°C), and cold (4°C) up-regulated six genes (*VvTCP4*, *VvTCP5*, *VvTCP8*, *VvTCP12*, *VvTCP13*, *VvTCP15*). Intriguingly, most *VvTCP* gene expression was strongly modulated under the infection of two common but harmful pathogens, namely downy and powdery mildew, implying an additional role in disease responses except for the functions previously reported. Overall, the expression profile analysis indicated that *VvTCP* genes might play multiple roles in grapevine tissue development and abiotic and biotic stress responses.

DISCUSSION

The TCP transcription factors were versatile and of great importance to the process of plant growth and development. Family members of *TCP* genes in *Arabidopsis* (*Arabidopsis thaliana*) (Yao *et al.*, 2007), rice (*Oryza sativa*) (Yao *et al.*, 2007), cotton (*Gossypium arboreum* and *Gossypium raimondii*) (Ma *et al.*, 2014, 2016), apple (*Malus domestica*) (Xu *et al.*, 2014), tomato (*Solanum lycopersicum*)

(Parapunova *et al.*, 2014), watermelon (*Citrullus lanatus*) (Shi *et al.*, 2016) and *Prunus mume* (Zhou *et al.*, 2016) have been identified, and some of the genes have been well characterised, while no comprehensive analysis of grapevine has been reported until now. In our study, 15 *TCP* members in the grapevine genome were identified. Compared to the other species mentioned above, the number in grapevine is fewer, probably because fewer gene-duplication events occurred.

The phylogenetic tree constructed on the basis of the full length of *VvTCP* proteins in grapevine and *Arabidopsis* further validates the previous classification of *AtTCPs* (Yao *et al.*, 2007). The *TCP* members in grapevine were divided into two groups, namely Class I and Class II (Fig. 2). Class II was further classified into two subgroups, the *CYC/TB1* and the *CIN* subgroup, in which *VvTCP* members were unevenly distributed (Fig. 2). The multiple alignment of protein sequences in the *VvTCPs* was consistent with the results of the phylogenetic tree (Figs 2 and 3). Most members of the same subgroup shared relatively similar gene structure, conserved domain and motifs between grapevine and *Arabidopsis* (Xu *et al.*, 2014), providing a solid reference for us to explore the functions of the *VvTCPs*.

The phylogenetic tree indicated that *VvTCP12* and *VvTCP2* showed high similarity with *AtTCP14* and *AtTCP15*, and real-time PCR showed that these genes were highly expressed in old and young leaves and in inflorescences, suggesting that they may also modulate cell proliferation in the developing leaf blade and in specific floral tissues (Hiratsu *et al.*, 2003; Kieffer *et al.*, 2011). Aguilar-Martínez and Sinha (2013) reported that *TCP* genes in Class I, such as *AtTCP7*, *AtTCP8*, *AtTCP22* and *AtTCP23*, could interact with *SRDX* to control cell proliferation in leaf development. *VvTCP8* was shown to be 92% homologous with *AtTCP8*, suggesting possible functions for *VvTCP8* in the regulation of leaf development. *TCP24* in *Arabidopsis*

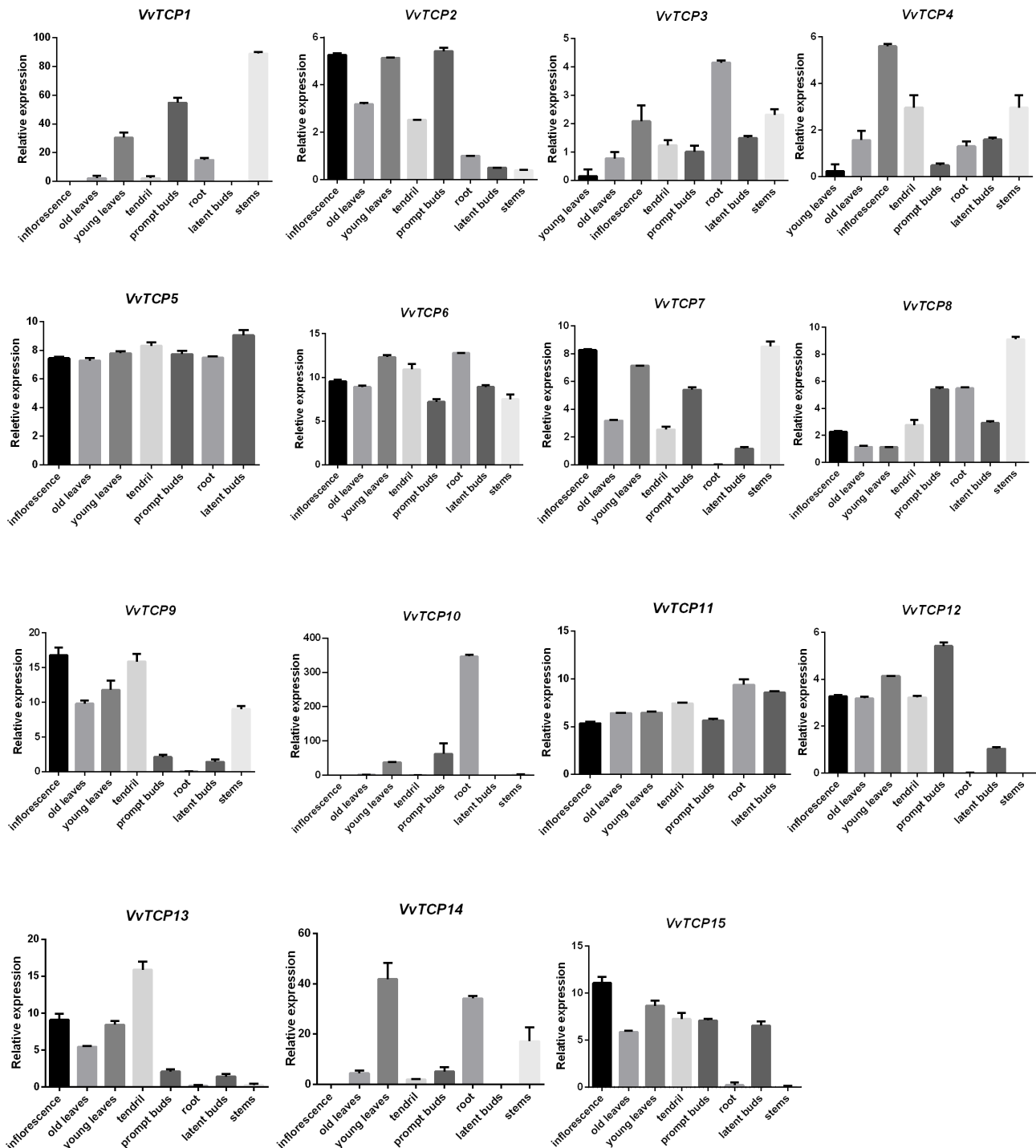


FIGURE 5

Expression patterns of *VvTCPs* in different grapevine tissues. The Y-axis indicates the relative expression level; the X-axis (inflorescence, old leaves, young leaves, tendrils, prompt buds, root, latent buds, stems) indicates different organs. Standard errors are plotted using vertical lines.

was reported to act as an important regulator of secondary cell wall thickening and to modulate anther endothecium development (Wang *et al.*, 2015a), and *VvTCP6* showed 76% similarity with it, indicating the same functions. In addition, *VvTCP1*, *VvTCP10* and *VvTCP14* were highly homologous to the *AtTCP18*, which is known as *BRANCHED1(BRC1)* and *TEOSINTE BRANCHED1-LIKE(TBL1)*, and could act downstream of cytokinins and strigolactones to coordinate

the outgrowth of axillary buds (Aguilar-Martínez *et al.*, 2007; Finlayson, 2007). Although they did not present bud-specific expression (Fig. 5) as in *Arabidopsis* (Aguilar-Martínez *et al.*, 2007), they were differentially expressed between latent bud and prompt buds (Fig. 5), which are two kinds of grapevine buds exhibiting different characteristics in terms of dormancy. Therefore, they were identified to be candidates of *BRANCHED 1*, thus to regulate grapevine bud

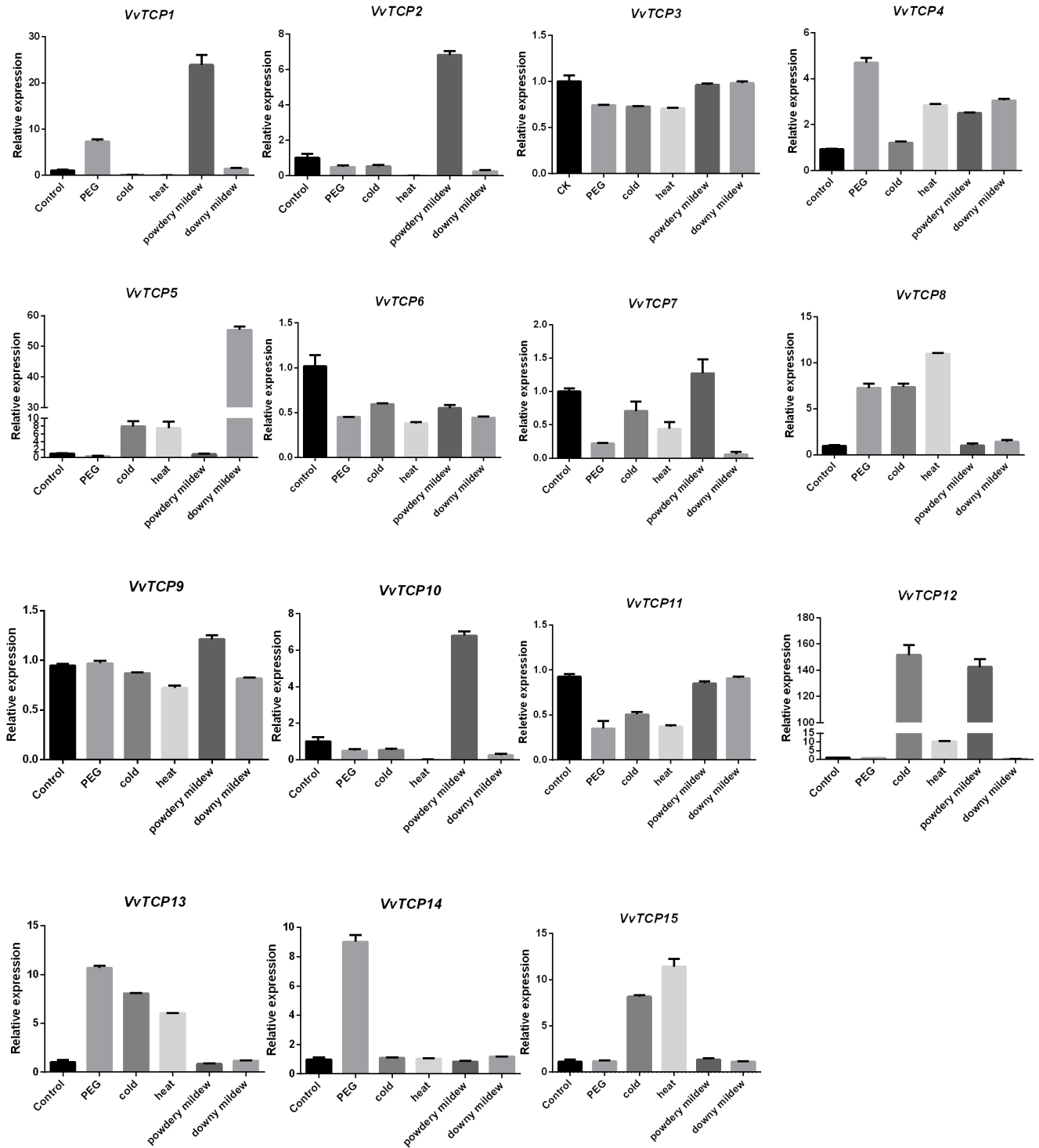


FIGURE 6

Expression patterns of *VvTCPs* under different abiotic and biotic stresses. The Y-axis indicates the relative expression level; the X-axis (control, PEG, cold, heat, powdery mildew, downy mildew) indicates different treatments. Standard errors are plotted using vertical lines.

break and shoot branching. Interestingly, recent research on grapevine bud burst also identified TCP transcription factors as one of the hallmarks of the orderly and functional resumption of growth following quiescence in buds (Meitha *et al.*, 2018). Hence, the detailed biological functions of TCP transcription factors in grapevine bud break would be an issue worthy of further investigation.

The expression patterns of 15 *VvTCPs* in eight organs were detected via real-time PCR. The results show that almost all the members in Class II had organ-specific expression (Fig. 5), while all members in Class I and the CIN members were ubiquitously expressed in all the tissues examined, further validating their diverse functions in the development of different organs. The divergence between

the two groups was also consistent with that in other species (Xu *et al.*, 2014; Zhou *et al.*, 2016). Most members, such as *VvTCP2*, *VvTCP5*, *VvTCP6*, *VvTCP7* and *VvTCP14*, had higher expression in young leaves (Fig. 5), implying that they play a role in modulating leaf development.

Plants commonly encounter various abiotic and biotic stresses during their lifecycle. Some studies have reported that TCP factors could act as targets of pathogenic effectors and are likely to play an important role in plant immunity (Lopez *et al.*, 2015). Many TCP members, such as *TCP13*, *TCP14*, *TCP15*, *TCP19* and *TCP21*, are reported to be targeted by effectors from three pathogens that span the eukaryote–eubacteria divergence, represented by the bacterium *Pseudomonas syringae* (*Psy*), the oomycete *Hyaloperonospora arabidopsidis* (*Hpa*), and the true fungus *Golovinomyces orontii* (*Gor*) (Mukhtar *et al.*, 2011; Weßling *et al.*, 2014). Deletion mutants of *TCP13*, *TCP14* and *TCP19* exhibited enhanced disease susceptibility phenotypes (Mukhtar *et al.*, 2011). In our study, several *VvTCPs*, including *VvTCP1*, *VvTCP2*, *VvTCP10* and *VvTCP12*, were highly induced after infection with powdery mildew, and *VvTCP5* expression was up-regulated under downy mildew infection, indicating an important regulatory role in plant disease response, providing an important reference in the study of grapevine disease response (Fig. 6).

So far, there have been limited reports on the response of TCP members under abiotic stress. The response of TCPs to abiotic stress in monocots was found to be mediated mainly by abscisic acid (ABA) (Zhou *et al.*, 2013). When exposed to salt, drought and cold stress, *OsTCP19* expression in rice was greatly up-regulated, and it could interact with the *ABA INSENSITIVE₄* (*ABI₄*), a transcription factor involved in ABA signal transduction (Zhou *et al.*, 2013; Mukhopadhyay *et al.*, 2015). In our study, many TCP genes were also apparently regulated by cold, heat and drought stresses, for example the transcript level of *VvTCP1*, *VvTCP4*, *VvTCP8*, *VvTCP13* and *VvTCP14* was greatly induced by drought treatment (10% PEG), while six genes (*VvTCP1*, *VvTCP4*, *VvTCP8*, *VvTCP9*, *VvTCP13*, *VvTCP14*) were up-regulated by heat (45°C) and six (*VvTCP4*, *VvTCP5*, *VvTCP8*, *VvTCP12*, *VvTCP13*, *VvTCP15*) were up-regulated by cold (4°C), suggesting that all these genes had potential roles to play in response to abiotic stress (Fig. 6). We speculate that, when exposed to adverse conditions, these genes may form homo- or hetero-dimers, and also may interact with other transcription factors to form functional protein complexes, as previously reported in other plant species (Parapunova *et al.*, 2014), to enhance their adaptability to adversity. Further research is needed to validate its role in the response to different stresses.

Overall, the *in silico* analysis of grapevine TCP transcription factors could provide insight into the potential functions of TCP genes and help to identify some genes associated with different biological processes in important agronomical traits of grapevine, such as bud break, shoot branching, and stress tolerance. This knowledge will support the future improvement of grapevine cultivars via genetic engineering.

CONCLUSIONS

In this study, 15 unevenly distributed TCP genes were identified on eight chromosomes in the genome of *Vitis vinifera* L. The *VvTCP* genes were classified into two groups, as in other species. Real-time PCR indicated that most members of *VvTCPs* were expressed differentially in different grapevine tissues, and several genes' expression was induced or suppressed under abiotic (heat, cold and PEG) and biotic (downy mildew and powdery mildew infection) stresses, indicating multiple roles in different organs and in various biological processes, especially roles in regulating the process of bud dormancy and modulating plant immunity. To the best of our knowledge, this is the first report on genome-wide analysis of the grapevine TCP gene family, and the first report of its relationship with abiotic and biotic responses. This study provides abundant information for understanding the classification and potential functions of TCP genes in grapevine, which would be of great help to provide guidance in *Vitis* breeding using genetic engineering tools, and thus be of great significance to the development of the grape industry.

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