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Identification, classification, and transcriptional analysis of *TCP* gene family from *Scutellaria baicalensis* and *SbTCP* genes response under MeJA and SA treatments

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Abstract: TCP transcription factor is a plant-specific gene family which plays important roles in many developmental control pathways, regulating secondary metabolites and plant responses to abiotic and biotic stresses. Nevertheless, this gene family remain unknown in *Scutellaria baicalensis*. Here, by identifying and analysing all the TCP transcription factor family members based on the transcriptome of *S. baicalensis*, a total of 19 *SbTCP* genes were obtained following gene classification, the phylogenetic relationship, conserved domain structure, functional differentiation, and an expression activity analysis. Phylogenetic analysis grouped the *SbTCP* genes into two subfamilies; we also found that *SbTCP* with the same motif structure clustered together in the evolutionary tree, and these results suggest that SbTCP proteins with the same gene structure have similar functions. Gene Ontology (GO) categorised the *SbTCP* genes into 17 functional subcategories, suggesting that they have diversified in functionality, even though their putative proteins share a number of conserved motifs. After the MeJA and SA treatments, the expression of *SbTCP* candidate genes containing MeJA and SA promoter elements was significantly higher or lower compared with the control, indicating that these candidate *SbTCP* genes could respond to different concentrations of MeJA and SA treatments. These comprehensive data provide a reference for elucidating the functions of TCP transcription factor family in the growth, development, and MeJA and SA stress response of *S. baicalensis*, this study can create a new avenue for understanding the role of *TCP* gene family in *S. baicalensis*.

Keywords: *Scutellaria baicalensis*; TCP transcription factor; evolutionary and phylogenetic analysis; gene expression; expression pattern analysis

Scutellaria baicalensis, an important herbal plant in China, belongs to the *Lamiaceae* family, also known as Chinese skullcap in traditional Chinese medicine (Zhao et al. 2019). It was widely cultivated in China, East Asia and some European countries and was found to contain a diverse array of bioactive compounds, including flavonoids, alkaloids, and terpenoids (Zhao et al. 2016; Chanchal et al. 2023). These compounds have demonstrated a wide range of pharmacological activities, including rheumatology and immunology, anti-inflammatory, antioxidant, anti-cancer, neuroprotective, and hepatoprotective effects (Zhang et al. 2003; Fox et al. 2012; Yang et al. 2012; Chen et al. 2013; Xu et al. 2020; Tan et al. 2022; Chanchal et al. 2023).

TCP transcription factor is a plant-specific protein in green algae to dicotyledonous plants. They are involved in multiple developmental control pathways

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and the regulation of secondary metabolites, such as brassinosteroid (BR), jasmonic acid (JA) flavonoids and so on, which were first reported in 1999 with an important family membership (Cubas et al. 1999). Its nomenclature is derived from the initial letters of the four first identified genes, TB1 (Teosinte Branched 1) in maise, CYC (Cycloidea) gene in goldenrod, and PCF1 (Proliferating Cell Factors 1) and PCF2 (Proliferating Cell Factors 2) in rice (Doebley et al. 1995; Luo et al. 1996). The TCP transcription factor encodes proteins with an atypical basic-Helix-Loop-Helix (bHLH) structure of 59 amino acids, a structural domain that is important for DNA binding and is involved in protein-protein interactions and localisation and dimerisation, with a nuclear localisation signal in the basic region. The two-helix structure consists of hydrophilic amino acids, also known as the TCP structural domain (Li et al. 2021). Based on the amino acid sequence similarity of the TCP structural domains, TCP proteins can be divided into two groups: group I of PCF subfamily and group II of CIN and CYC/TB1 subfamily (Navaud et al. 2007). Meanwhile, TCP transcription factor gene family has been reported in a variety of plants, such as *Arabidopsis thaliana* (Danisman et al. 2013), *Oryza sativa* (Yao et al. 2007), *Gossypium hirsutum* (Li et al. 2017), *Dendrobium chrysotoxum* (Huang et al. 2023), *Solanum melongena* (Li et al. 2022a), *Solanum lycopersicum* (Parapunova et al. 2014), *Cymbidium goeringii* (Liu et al. 2022a), *Panax ginseng* (Liu et al. 2024) and so on. However, no one has yet reported that this gene family is in *S. baicalensis*, so we conducted a systematic study of *SbTCP* gene family.

Salicylic acid (SA) and Methyl Jasmonate (MeJA), as the signaling molecules of plant defence response, can induce the accumulation of a wide range of secondary metabolites related to stress tolerance in plants and have been widely used in inducer studies in different plants. SA facilitated the enhancement of flavonoids content in the indeterminate roots of *Epimedium* (Hayat et al. 2010). Mohd Zaheer (Zaheer et al. 2016) showed that different concentrations of jasmonic acid, salicylic acid, acetylsalicylic acid and methyl salicylic acid all resulted in an increase in the content of andrographolide in the adventitious roots of *Andrographis paniculata*. The addition of 200 μM MeJA to the ginseng adventitious roots could directionally increase the content of ginseng mono saponins (Hu et al. 2023); MeJA also could effectively induce the accumulation of PPD-type saponins in *Panax notoginseng* leaves (Li et al. 2022b). Therefore, screening SbTCP candidate genes in *S. baicalensis* that can respond to MeJA and SA has become more important for studying the regulation of various secondary metabolites biosynthesis in herbal plants by MeJA and SA treatments.

In this study, the *TCP* gene family was screened and identified based on the *S. baicalensis* transcriptome database, and the structure, expression, phylogeny, and functional prediction of the *TCP* gene family were analysed. On this basis, candidate *SbTCP* genes with the ability to respond to MeJA and SA, were screened to provide genetic resources for the further analysis of their biological functions in response MeJA and SA treatments. On the one hand, this study can enrich the study of *TCP* gene family in plants; on the other hand, it can provide a theoretical basis for further analysis of the regulatory mechanisms of MeJA and SA.

MATERIALS AND METHODS

Collection of leaf and root samples from *S. baicalensis*. In this study, the leaf and root samples of *S. baicalensis* were grown from a cooperative base. The samples were taken from leaf and root with three biological replicates, and the samples were frozen in liquid nitrogen and stored in a –80 °C deep freezer for the subsequent experiments at the School of Biological and Pharmaceutical Engineering, Jilin Agricultural Science and Technology University.

Identification *TCP* gene family from transcriptome database. *TCP* gene family is a family of plant transcription factors; we used the Pfam (Finn et al. 2016) (http://pfam-legacy,xfam.org, accessed on September 2, 2023) downloads the TCP protein structural domains (Pfam ID: PF03634) for comparison with the transcriptome database to obtain preliminary TCP transcription factor sequence information, and *SbTCP* genes were identified from the transcriptome database of *S. baicalensis* using HMMER (Finn et al. 2011) (http://hmmer.janelia.org, accessed on September 3, 2023) with the *e-value* is set to $1.0E^{-06}$. NCBI CDD domain pattern (http://www.ncbi.nlm.nih.gov/Stru-cture/cdd/wrpsb.cgi, accessed on December 5, 2023) and SMART (https://smart.embl.de/, accessed on September 6, 2023) were used to confirm the presence in *TCP* genes conserved structural domains, and genes containing TCP conserved structural domains were

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selected and defined as *SbTCP* genes. We named the obtained *SbTCP* gene sequences with sequence names ranging from *SbTCP*01 to *SbTCP*19. Multiple sequence alignment was performed using TBtools (version 2.045) (Chen et al. 2020) and plotted using Simple MSA Viewer in this software for multiple sequence comparison analysis. The ExPASy-Prot Param software (https://web.expasy.org/protparam/, accessed on September 9, 2023) was used to predict the basic information of the SbTCP proteins, including the *SbTCP* gene of mRNA sequences length (bp), SbTCP proteins number of amino acids, subcellular localisation, molecular weights (Da), theoretical isoelectric points (pI) and conserved structural domains group.

Evolutionary and phylogenetic relationship analysis. Multiple sequence alignments of SbTCP protein sequences were compared using MEGA X (Kumar et al. 2018), and an evolutionary and phylogenetic tree was constructed using the neighbor-joining (NJ) method using MEGA X (Kumar et al. 2018); the bootstrap replicates were set to 2 000, model plants were selected as outgroup species from two species *Arabidopsis thaliana* (*At*) and *Oryza sativa* (*Os*). The final phylogenetic tree was edited using Evolview (version 3.0) (https://www.evolgenius.info/evolview, accessed on September 15, 2023) (Subramanian et al. 2019).

Conserved motifs and gene structure. MEME (http://meme.nbcr.net/meme/, accessed on September 12, 2023) was used to analyse the conserved motif analysis using. The maximum and minimum conserved motif lengths were from 5 and 10 motifs amino acids, respectively. Then, NCBI Batch CDD (http://www.ncbi.nlm.nih.gov/Stru-cture/cdd/wrpsb.cgi, accessed on September 14, 2023) was used to analyse the genes' structure. The conserved motifs and gene's structure characterisation were aligned and plotted using TBtools (version 2.045) (Chen et al. 2020).

Gene ontology (GO) functional annotation and enrichment analysis. Blast2GO (version 6.0.3) (Conesa et al. 2005) was used to perform functional gene annotation. The results of annotation and GO classification were used to assess the functional differentiation of *SbTCP* genes. Omicshare online tools (https://www.omicshare.com/, accessed on September 20, 2023) were used to show GO enrichment circle graph.

The cis-acting element analysis. PlantCARE (https://bioinformatics.psb.ugent.be/webtools/plantcare/html/, accessed on September 17, 2023) was used to find plant cis-acting elements, which can also be used to find important regulatory elements such as transcription factor binding sites in promoter sequences. The information on cis-acting element analysis on promotors in SbTCP was aligned and plotted using TBtools (version 2.045) (Chen et al. 2020) of PlantCARE Result Classify.

Expression of *SbTCP* genes in leaf and root tissues. We screened *SbTCP* genes that were differentially expressed in the leaf and root of *S. baicalensis*, and analysed the differential expression patterns of *SbTCP* genes in these two tissues. Ultimately, candidate *SbTCP* genes responsive to MeJA and SA were identified by cis-acting element screening. Omicshare online tools (https://www.omicshare.com/, accessed on October 7, 2023) was used to draw a dotrod heatmap and dynamic radar map.

Candidate *SbTCP* gene expression analysis under MeJA and SA treatments. The healing tissue material of *S. baicalensis* 2.0 g was inoculated into a 100 mL flask containing 40 mLB5 liquid medium, placed in a shaker of 22 °C, and shaken at 110 rpm. On day 20, methyl jasmonate (MeJA) and salicylic acid (SA) treatments was added to the culture-healing tissue material of *S. baicalensis*. The concentration of MeJA was 100 μM, 200 μM, and SA was 50 mg/mL, 100 mg/mL. Each experimental group used three replicates and one control. The candidate *SbTCP* genes expression analysis of MeJA and SA treatments 48 h using qRT-PCR, the genes expression results of qRT-PCR were analysed by the $2^{-\Delta\Delta CT}$ method (Wang et al. 2019) and Excel version 2019 was used to draw a bar chart. All the primers required for qRT-PCR are in Electronic supplementary material (ESM) Table S1.

RESULTS

Identification and classification *TCP* sequence analysis of *S. baicalensis* from transcriptome. A total of 19 TCP transcription factor (TF) sequences from *S. baicalensis* were identified, which had complete CDS (coding sequence) and ORF (open reading frame), and they named from *SbTCP01* to *SbTCP19* (ESM Table S2); they all present the conserved the TCP domain and a basic bHLH structure (Figure 1). In our study, 19 TCP mRNA nucleotide sequences are between 577–2 705 bp, and code amino acid numbers between 80 and 411,

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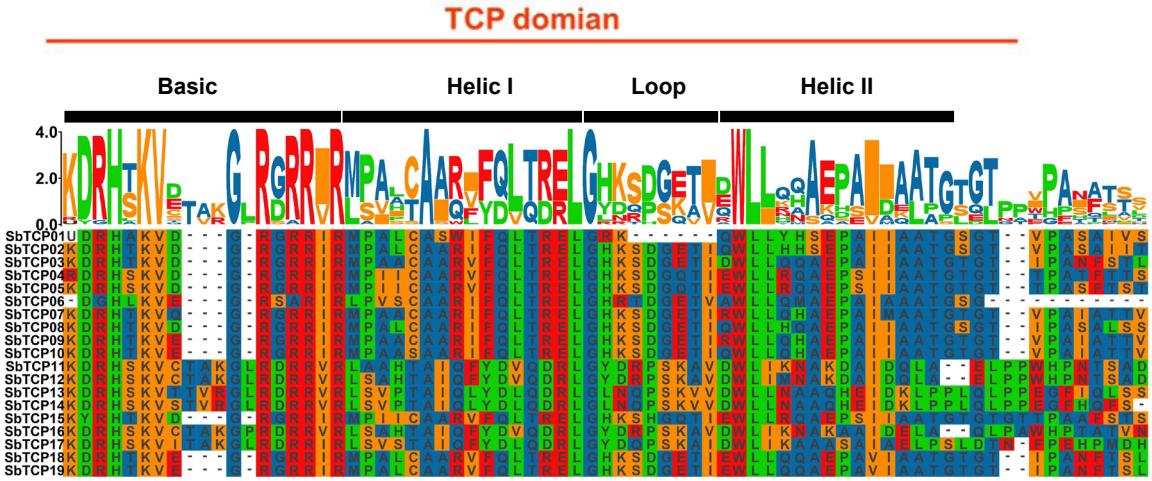

Figure 1. Multiplex sequence alignment analysis of 19 SbTCP protein characteristics in *Scutellaria baicalensis*
Each letter and colour represent one amino acid, the left column corresponds to the *SbTCP* gene name, and the top black region indicates the highly conserved residues of the *SbTCP* gene family members

while the *SbTCP05* gene has the longest sequence and the *SbTCP06* gene has the shortest sequence, of which the molecular weight of these SbTCP proteins ranged from 8 533.7 to 44 515.87 Da. In molecular characterisation, we found that the theoretical isoelectric point (pI) of *SbTCP* genes was between 6.08 (*SbTCP06*) to 10.84 (*SbTCP09*), of which 15 members had an alkaline pKa range, and 4 members had an acidic pKa range, suggesting SbTCP family members of protein functions only in different acidic and basic environments. Meanwhile, the subcellular localisation of all *SbTCP* gene family members was on the nucleus, further validating that we screened the *TCP* gene sequences were transcription factors (ESM Table S2).

Evolutionary and phylogenetic analysis of the *TCP* gene family from *S. baicalensis*. In the evolutionary and phylogenetic analysis of the *SbTCP* gene family, 28 *TCP* genes were identified from *S. baicalensis* (Sb), *A. thaliana* (At), and *O. sativa* (Os). The *TCP* genes from dicotyledonous model plants of *A. thaliana* (six *TCP* genes from three subfamilies) and monocotyledonous model plants of *O. sativa* (three *TCP* genes from three subfamilies) were used as outgroups in our study. The 19 *SbTCP* genes were clustered into two groups, including group I (PCF) of thirteen *TCP* genes and the II group of six *TCP* genes (CIN and CYC/TB1) in Figure 2. In evolutionary and phylogenetic studies, the *TCP* gene family is unique and ancient in plants, which plays crucial roles in multiple growth and development processes. We found the *TCP* gene family in *S. baicalensis*, an ancient gene family that originated before splitting be-

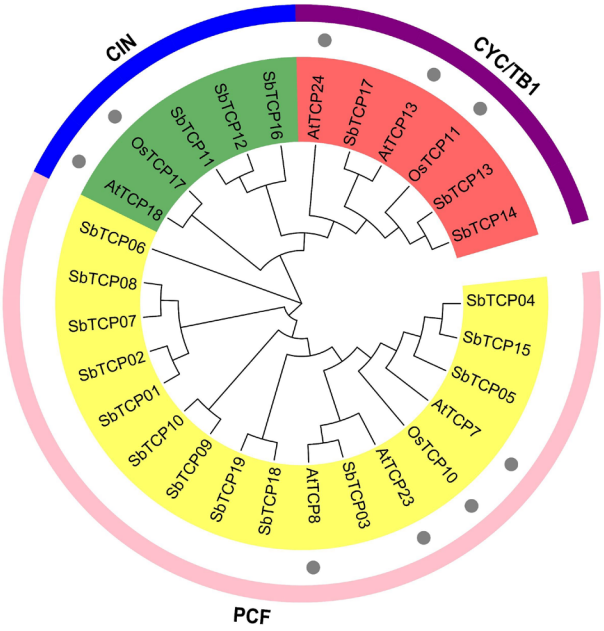

Figure 2. Evolutionary and phylogenetic analysis of TCP proteins in *Scutellaria baicalensis* (Sb), *Arabidopsis thaliana* (At), and *Oryza sativa* (Os)
The circles mark the *TCP* genes from *A. thaliana* and *O. sativa*, no mark the *TCP* genes from *S. baicalensis*. PCF, CIN, and CYC/TB1 indicated different subfamilies of *TCP* gene family. The phylogenetic tree was constructed from the TCP proteins *A. thaliana* and *O. sativa* as the outgroups with 2 000 bootstrap replicates using the neighbor-joining (NJ) method by MEGA X, respectively

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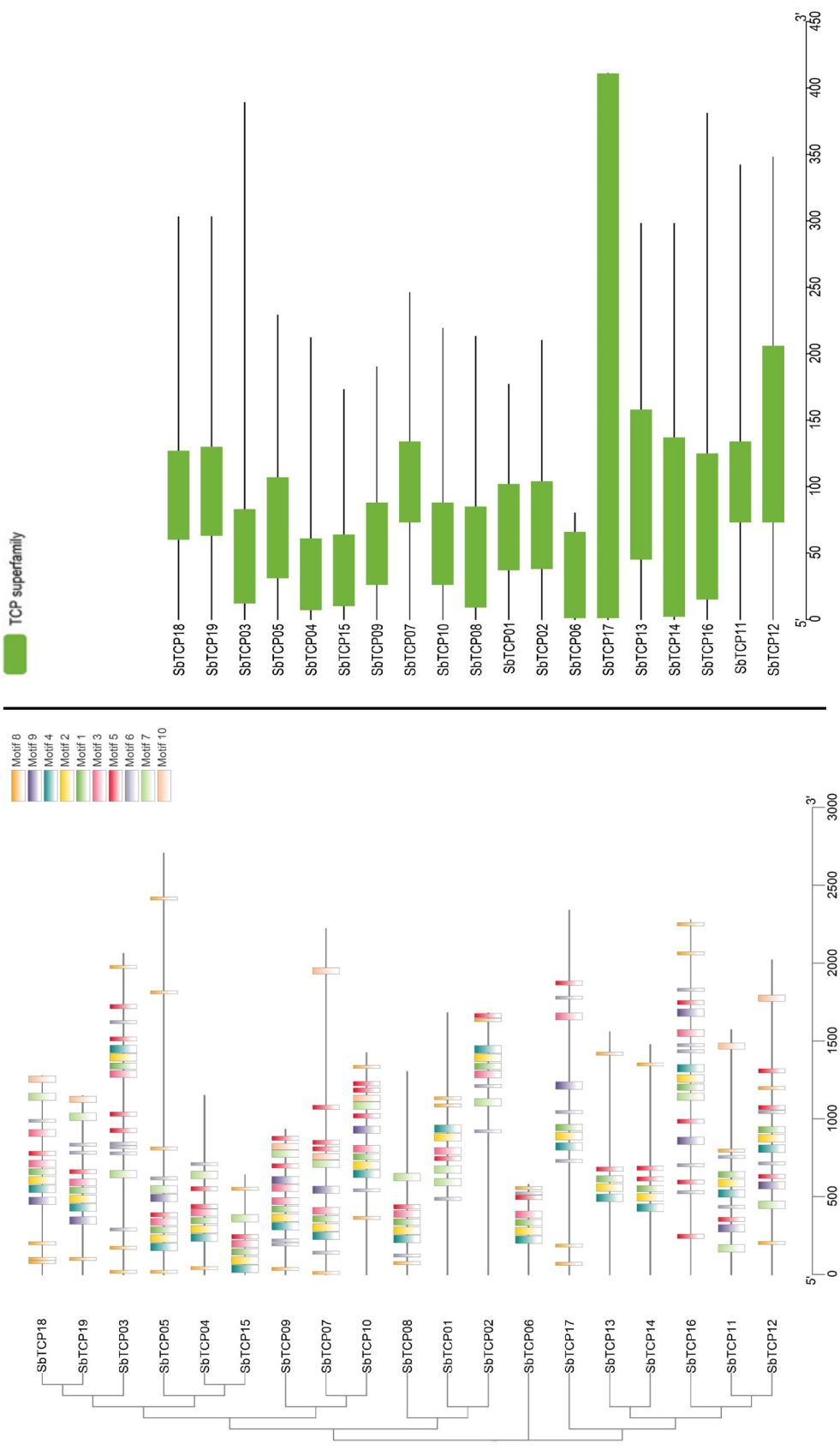

Figure 3. Characterisation conserved motifs and gene structures of 19 TCP proteins in *Scutellaria baicalensis*; Exon/intron distribution in the *SbTCP* gene family members in *S. baicalensis*

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tween the monocot (*O. sativa*) and dicot (*A. thaliana*) plants. In the *SbTCP* gene family, group I had one subfamily (PCF), the largest number of the *SbTCP* gene family members (thirteen genes) were identified, And group II had two subfamilies (CIN and CYC/TB1), each subfamily had three *SbTCP* gene family members. Similarly, 19 *TCP* gene families were delineated in *S. baicalensis*. The number of TCP gene family members was similar to *A. thaliana* (23) (Danisman et al. 2013). *O. sativa* (22) (Yao et al. 2007), but the number of gene family members was less compared with *G. hirsutum* (74) (Li et al. 2017), *D. chrysotoxum* (50) (Huang et al. 2023), *S. melongena* (30) (Li et al. 2022a), *S. lycopersicum* (30) (Parapunova et al. 2014), *P. ginseng* (28) (Liu et al. 2024) *S. tuberosum* (46) (Wang et al. 2019), *Zea mays* (36) (Ding et al. 2019) and others plants. In this study, the *TCP* gene family members differ in number in *S. baicalensis* with other species because we used the transcriptome data for the identification of *TCP* gene family, we may not capture all *SbTCP* genes, and no altered species genomes have been reported.

Conserved motifs and gene structure analysis of *SbTCP* genes. The conserved motifs and gene structure of *SbTCP* genes results are shown in Figure 3. A total of ten motifs and one TCP superfamily conserved structural domains were found in SbTCP proteins. The results showed that the motif number ranged from five to ten in 19 SbTCP protein members and was classified into ten types. In Figure 3, motif 2, motif 4, motif 5, and motif 8 were present in all SbTCP protein sequences and identified these four motifs as the core conserved structural domains of SbTCP TCP proteins. In different subfamilies, we also found the core conserved domain numbers to be different (Figure 4). All the motifs from 1 to 10 are distributed in subfamily PCF (group I); for example, SbTCP07, SbTCP09, SbTCP10, SbTCP18, and SbTCP19 have all ten motifs, but subfamily CIN and CYC/TB1 (group II) are no all motif, for example in subfamily CIN, SbTCP13 and SbTCP14 have five motifs (motif 1, motif 2, motif 4, motif 5, and motif 8); in subfamily CYC/TB1, SbTCP11 and SbTCP12 have nine motifs (no motif 3), and SbTCP16 also has nine motifs (no motif 10). Meanwhile, we also found that SbTCP with the same motif structure clustered together in the evolutionary tree, and these results suggest that SbTCP proteins with the same gene structure have similar functions.

GO functional annotation and enrichment analysis of *SbTCP* genes. Of the 19 *SbTCP* genes GO functional annotation, all the *SbTCP* genes (100%) were categorised into three categories (Figure 5A). There are no genes without GO function annotations. In GO enrichments analysis (Figure 5B), six terms in biological processes (BP), include biological regulation, regulation of the biological process, cellular process, metabolic process, localisation, and single-organism process. This indicates *SbTCP* gene's putative role in defence re-

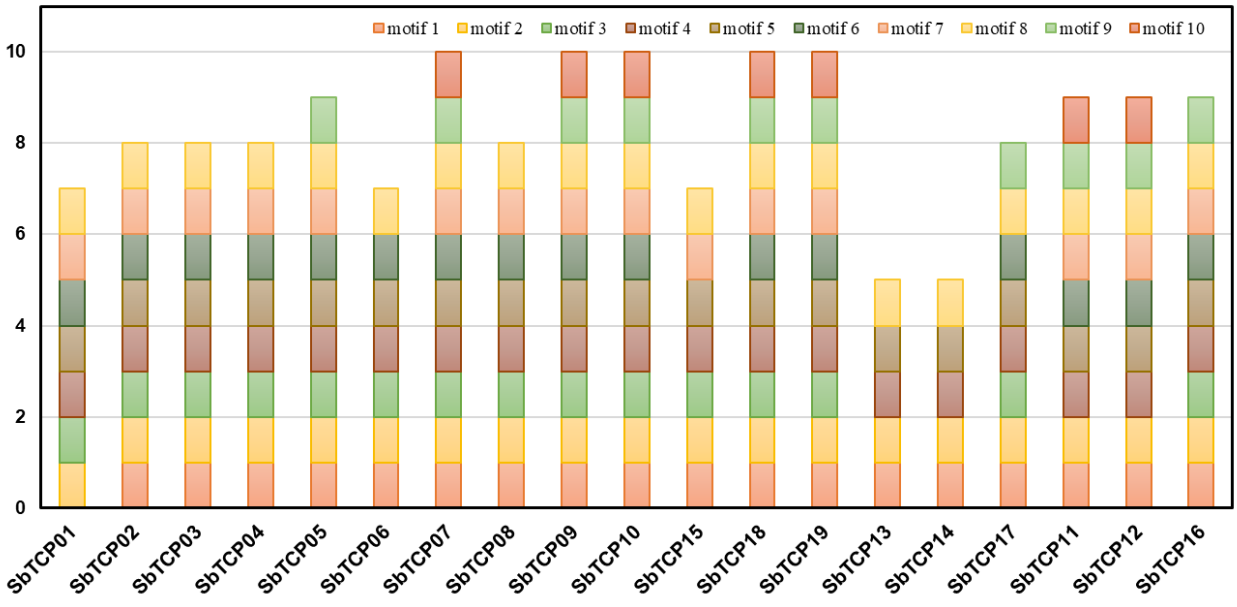

Figure 4. Species distribution conserved motifs of all SbTCP proteins

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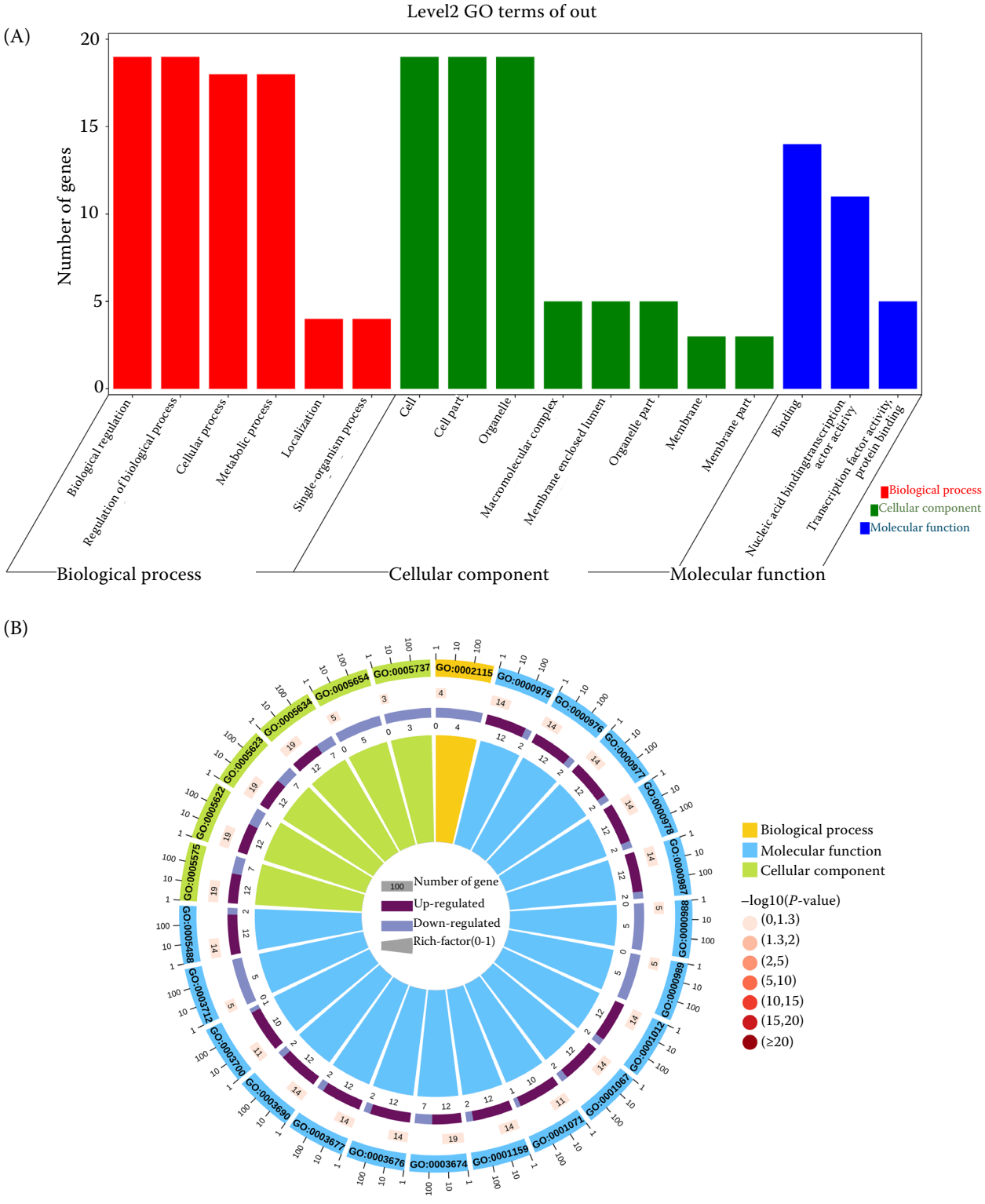

Figure 5. GO functional annotation and enrichments of the SbTCP gene family

(A) The *SbTCP* genes are classified into 28 functional categories at GO level 2, including seventeen biological process (BP) functional categories (red column), eight cellular component (CC) functional categories (green column), and three molecular functions (MF) functional category (blue column). (B) GO enrichment circle graph. The first circle: the top 25 GO terms enriched; the second circle: the number of GO terms in total genes as background; the third circle: the number *SbTCP* genes; the fourth circle: The rich factor value from 0 to 1

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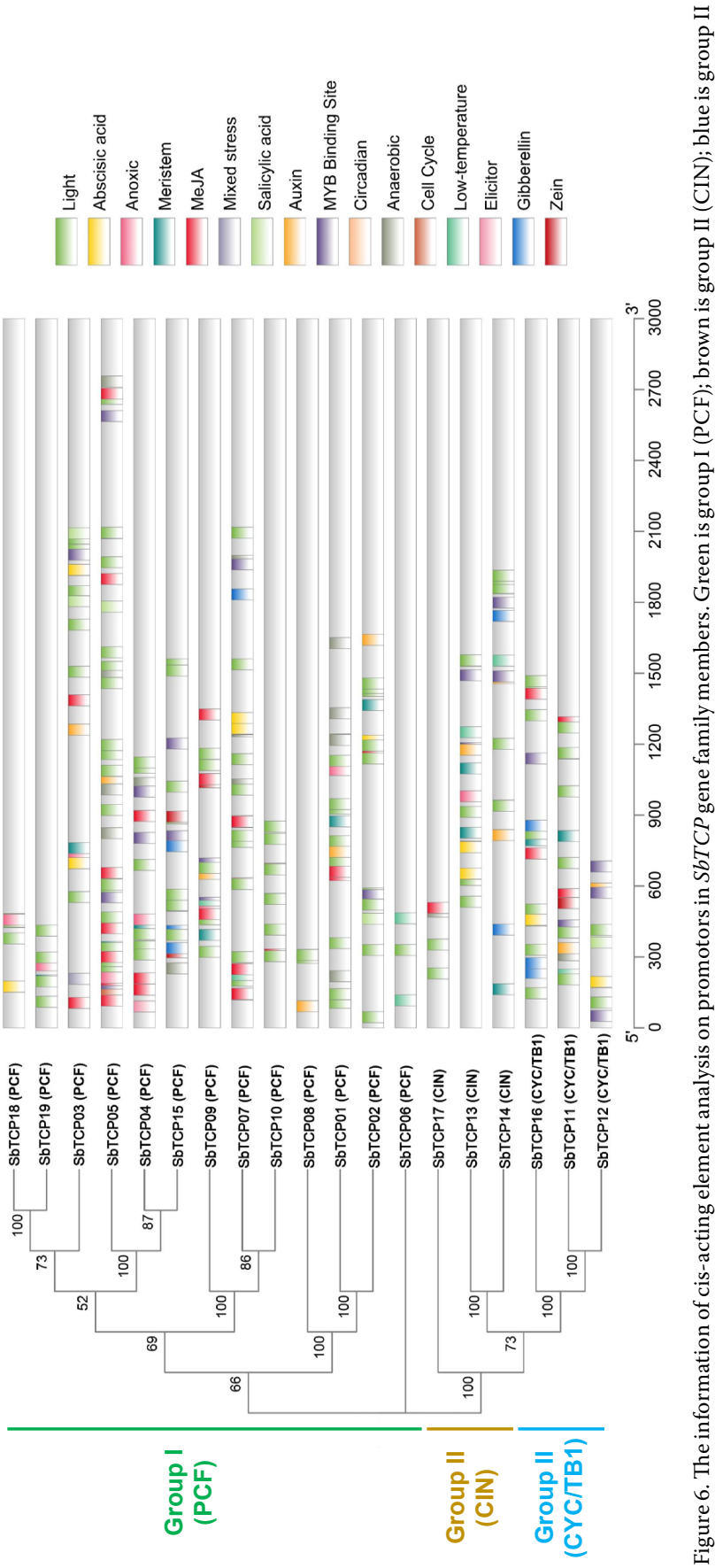

Figure 6. The information of cis-acting element analysis on promotors in *SbTCP* gene family members. Green is group I (PCF); brown is group II (CIN); blue is group II (CYC/TB1)

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sponse, regulation of secondary metabolism, and stress-coping mechanisms. Eight terms in cellular components (CC), including cell and cell part, organelle, macromolecular complex, membrane-enclosed lumen, organelle part, membrane, and membrane part, suggest their role in the regulation of transcription machinery. Three terms in molecular function (MF), including binding, nucleic acid binding, transcription factor activity and transcription factor activity protein binding, were significantly highlighted. This indicates *SbTCP* gene family members in the binding function and thus exercise the gene function of transcription factors. GO functional annotation and enrichment results can illustrate the diversity of *SbTCP* gene functions, which can be involved in growth and development, as well as secondary metabolism regulation, and these genes can provide rich gene resources for the in-depth study of functional genes in *S. baicalensis*.

The cis-acting element analysis on promotors of *SbTCP* genes. To further analyse the potential function of *SbTCP* gene family members, we classified and identified cis-acting elements in the promoter regions. All 19 *SbTCP* genes were analysed for cis-acting elements; we found *SbTCP* genes were divided into 16 categories, and the number of cis-acting elements number was ranged from 2 to 11 in *SbTCP* genes; no *SbTCP* gene had all the 16 categories of cis-acting elements; however, all *SbTCP* genes were also found to contain one category of light. Meanwhile, we concerned attention to the high number of *SbTCP* genes capable of responding to MeJA (*SbTCP01*, *SbTCP02*, *SbTCP03*, *SbTCP04*, *SbTCP05*, *SbTCP07*, *SbTCP08*, *SbTCP09*, *SbTCP10*, *SbTCP11*, *SbTCP14*, *SbTCP16*, *SbTCP17*, *SbTCP18*, and *SbTCP19*), and salicylic acid (*SbTCP01*, *SbTCP02*, *SbTCP03*, *SbTCP04*, *SbTCP17*, and *SbTCP19*) elements, which provided a data source and a theoretical basis for us to screen the function of *TCP* genes for important functions in leaf and root from *S. baicalensis* (Figure 6).

Expression pattern analysis of *SbTCP* genes in leaf and root tissues. To study the expression pattern of the *SbTCP* genes in leaf and root tissues from *S. baicalensis*, we found 14 *SbTCP* genes differentially expressed in leaf and root, of which seven up-regulated and seven down-regulated *SbTCP* genes were expressed (Figure 7).

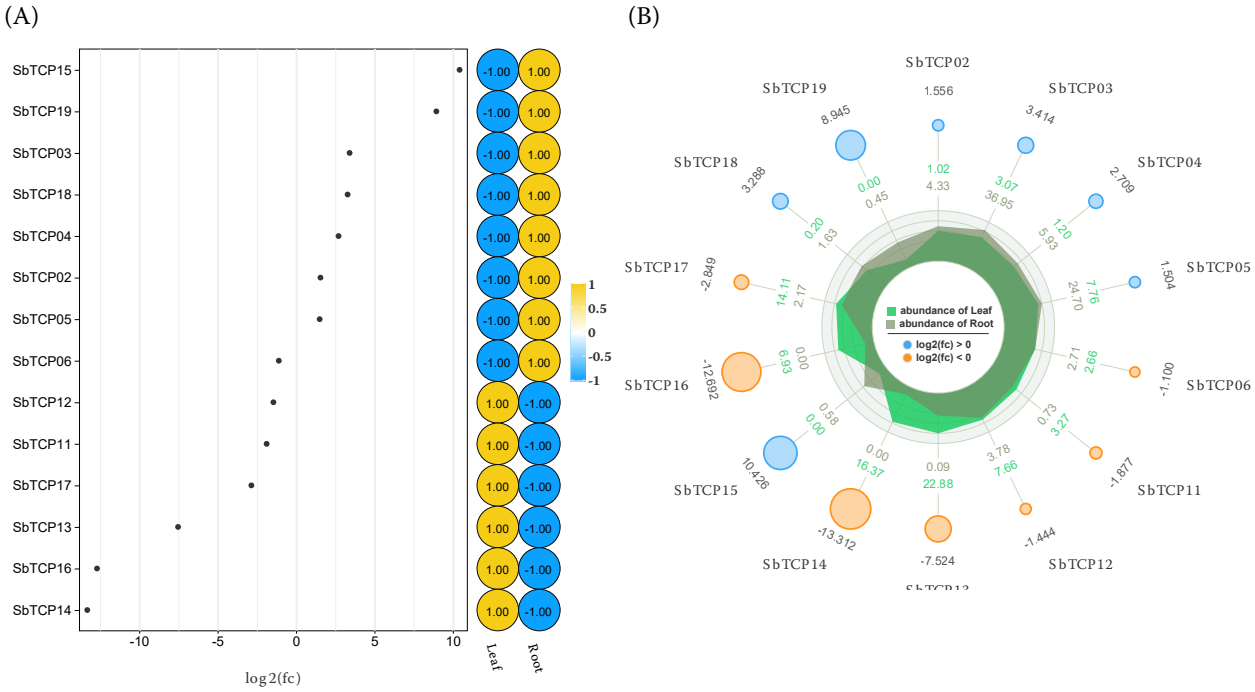

Figure 7. Dotrod heatmap and dynamic radar map of the *SbTCP* genes at leaf and root tissues in *Scutellaria baicalensis*
(A) Dotrod heatmap of the *SbTCP* genes at leaf and root; (B) dynamic radar map of the *SbTCP* genes at leaf and root; the blue circles indicate up-regulated genes, the brown circles indicate down-regulated genes, and the circle size varies according to the log2(fc) value; the green circles represent the average expression in the leaf, the grey circles represent the average expression in the root, and the irregular shapes in the circles represent the expression abundance in different tissues on each axis

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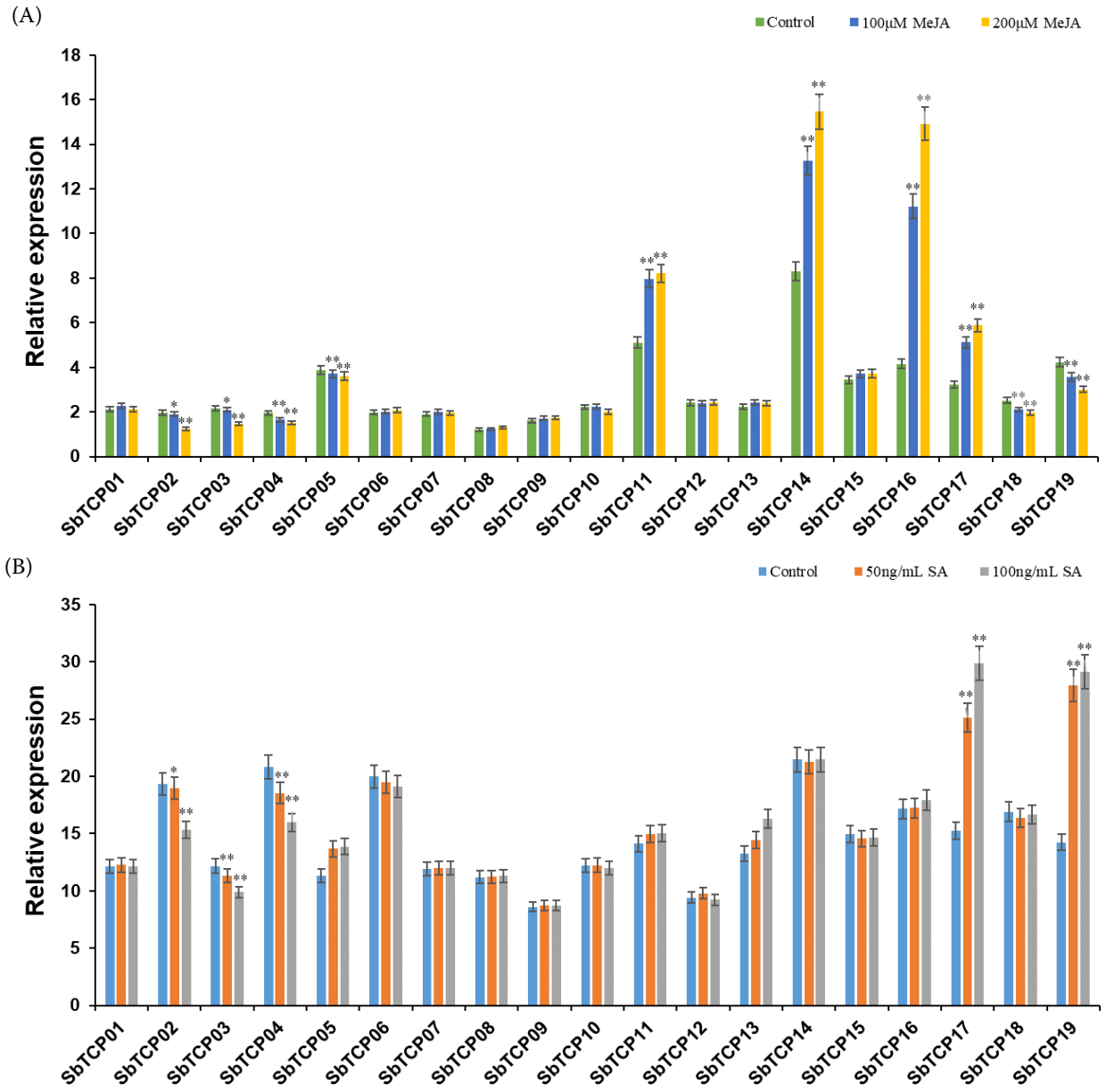

Figure 8. The expressions analysis of candidate *SbTCP* genes under MeJA and SA treatments by the qRT-PCR
(A) Expression profiles of *SbTCP* in different MeJA concentrations; (B) expression profiles of *SbTCP* in different SA concentration. The *Y*-axis indicates *SbTCP* genes relative expression levels; the *X*-axis indicates different S*bTCP* genes, no treatment group as the control; * significant difference at $P \leq 0.05$, ** significant difference at $P \leq 0.01$

In Figure 7B, we found 14 *SbTCP* genes differentially expressed from 3 different subfamilies, including some group I (8 of PCF) and all group II (3 of CIN and 3 of CYC/TB1). Meanwhile, all seven *SbTCP* genes were up-regulated genes from group I (*SbTCP02*, *SbTCP03*, *SbTCP04*, *SbTCP05*, *SbTCP15*, *SbTCP18*, and *SbTCP19*); of other seven *SbTCP* genes were down-regulated genes, including one gene was from group I (*SbTCP06*) and six genes were from group II (*SbTCP13*, *SbTCP14*, *SbTCP17*, *SbTCP11*, *SbTCP12*, and *SbTCP16*). Combined with the results of cis-acting element analysis, we obtained ten *SbTCP* genes (*SbTCP02*, *SbTCP03*, *SbTCP04*, *SbTCP05*, *SbTCP11*, *SbTCP14*, *SbTCP16*, *SbTCP17*, *SbTCP18*, and *SbTCP19*) involved in MeJA regulation and five *SbTCP* genes (*SbTCP02*, *SbTCP03*, *SbTCP04*, *SbTCP17*, and *SbTCP19*) involved in SA regulation in leaf and root from *S. baicalensis*. These *SbTCP* candidate genes will continue to be inves-

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tigated in studying gene expression patterns under MeJA and SA treatments.

Candidate *SbTCP* gene expression analysis under MeJA and SA treatments. To further investigate the expression of *SbTCP* candidate genes under MeJA and SA treatments, 48 h was used to analyse their gene expression by the qRT-PCR. Among 19 *SbTCP* genes, ten *SbTCP* genes (*SbTCP02*, *SbTCP03*, *SbTCP04*, *SbTCP05*, *SbTCP11*, *SbTCP14*, *SbTCP16*, *SbTCP17*, *SbTCP18*, and *SbTCP19*) were able to respond to MeJA treatment, of which six *SbTCP* genes (*SbTCP02*, *SbTCP03*, *SbTCP04*, *SbTCP05*, *SbTCP18*, and *SbTCP19*) were significantly downregulated, and four *SbTCP* genes (*SbTCP11*, *SbTCP14*, *SbTCP16*, and *SbTCP17*) were significantly up-regulated under MeJA treatment, and the other nine *SbTCP* genes no cis-acting element of MeJA showed no significant changes (Figure 8A). Among 19 *SbTCP* genes, also five *SbTCP* genes (*SbTCP02*, *SbTCP03*, *SbTCP04*, *SbTCP17*, and *SbTCP19*) were able to respond to SA treatment, of which three *SbTCP* genes (*SbTCP02*, *SbTCP03*, and *SbTCP04*) were significantly downregulated, and two *SbTCP* genes (*SbTCP17* and *SbTCP19*) were significantly up-regulated under SA treatment, and the other fourteen *SbTCP* genes no cis-acting element of SA showed no significant changes (Figure 8B). These qRT-PCR results are consistent with the results obtained from our analyses, which will provide candidate genes and a theoretical basis for further studies of *TCP* gene's function under the regulation of MeJA and SA.

DISCUSSION

TCP transcription factors involved in plant development. TCP transcription factors gene family play important roles in leaf development. For example, plants with multiple mutations in *A. thaliana* group I four *TCP* genes (*AtTCP7*, *AtTCP8*, *AtTCP22*, and *AtTCP23*) can exhibit a reduced rosette leaf number phenotype (Viola et al. 2011). The AtTCP11-EAR mutant of *A. thaliana* exhibits a leaf curl phenotype and is impaired in apical dominance (Aguilar-Martínez & Sinha 2013). After double mutation of *AtTCP14* and *AtTCP15* genes, plants exhibit widened leaf bases, shorter petioles, and upward curling of leaf margins. The phenotypes are stronger after expression of AtTCP14-EAR or AtTCP15-EAR in *A. thaliana* (Kieffer et al. 2011). *AtTCP20* and *OsPCF1*/*OsPCF2* genes play crucial roles in cell proliferation and growth (Li et al. 2005; Martin-Trillo & Cubas 2010). *AtTCP9* and *AtTCP20* genes regulate leaf senescence in the jasmonate signaling pathway (Martin-Trillo & Cubas 2010). These results show that group I *TCP* genes can regulate leaf development by controlling cell proliferation.

The CIN sub-group genes regulate leaf shape by inhibiting the proliferation of leaf margin cells. In the CIN sub-group of multiple mutant plants, loss of TCP gene function leads to overexpression of leaf margin genes, which results in sustained division of leaf cells and an increase in leaf area to produce folded and curled Leaf blades. By analysing the expression pattern of CIN-like genes, it was demonstrated that CIN-like genes control leaf shape and area size by controlling cell differentiation (Efroni et al. 2008). The *LA* gene in tomato, a homologue of *AtTCP4*, was found to function in obtaining a composite leaf to a single leaf to the mutant in tomato (Ori et al. 2007). In contrast to group I *TCP* genes, group II *TCP* genes regulate leaf development mainly by inhibiting cell proliferation and differentiation.

TB1 in maise is the first *TCP* gene family member identified, and studies have shown that TB1 is involved in branch development in maise. TB1 is mainly expressed in the axillary meristem and stamens of floral primordia, inhibiting the development of lateral buds and male flowers, and its expression level gradually decreases as the plant grows (Oebley et al. 1997). In rice, *OsTB1* gene also negatively regulates lateral branch development, and studies have found that overexpression of *OsTB1* plants shows a significantly reduced number of tillers compared with the wild type. BRC1 (*AtTCP18*) and BRC2 (*AtTCP12*) in *Arabidopsis* are homologous genes of *TB1* and are transcribed at high levels in tissues mainly containing axillary buds. The number of branches in the BRC mutant is increased, and the phenotype of the BRC 2 mutant is between that of the wild type and the brc1 mutant, indicating that BRC1 plays a major role in controlling branch development in *Arabidopsis* (Aguilar-Martínez et al. 2007). Loss of function of the *PsBRC1* gene in peas shows a phenotype of increased plant side branches (Braun et al. 2012). Studies have shown that *BRC1* gene from group II of *TCP* gene family in cucumbers can inhibit plant branching, and RNAi plants show increased branching (Shen et al. 2019). In our study, the *SbTCP* genes obtained during screening were also annotated to the

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GO terms of growth and development. This provides a reference for subsequent research directions on the growth and development function of *SbTCP* genes from *S. baicalensis*.

TCP transcription factors involved in the hormone signaling. The TCP transcription factors gene family members of groups I and II are involved in the hormone pathway. They can play a role downstream of the hormone pathway or in hormone synthesis, promoting or inhibiting the synthesis of hormones, thereby affecting the growth and development of plants. Auxin is a natural hormone commonly found in plants. In *A. thaliana*, the *TCP15* gene regulates the development of pistils and siliques by regulating auxin biosynthesis (Koyama et al. 2010). It can also affect the development of plant leaves and floral organs by inducing the expression of the auxin response gene *SAUR65* (Lucero et al. 2015). *TCP4* gene can promote the expression of early leaf auxin response genes *SAURAC1* and *SAUR23* to regulate leaf growth (Sarvepalli et al. 2011). Auxin influx carrier (AUX1) and efflux carrier (PIN) determine the polar transport of auxin, and the TCP gene also regulates the polar transport of auxin. In addition, *TCP2* gene affects hypocotyl elongation by mediating the jasmonate signaling pathway (He et al. 2021). After SA treatments, TCP proteins are involved in the orchestrated regulation of *ICS1* gene expression in *A. thaliana* (Wang et al. 2015). Under MeJA treatments, *StTCP4.1* and *StTCP11* may be involved in jasmonic acid in *Senna tora* (Liu et al. 2022b). In *Panax ginseng*, the *PgTCP26-02* gene belongs to the PCF subfamily. The expression level of the *PgTCP26-02* gene showed a downward trend, and in different farm cultivars, it was also negatively correlated with ginsenoside synthesis key enzyme genes (Liu et al. 2024).

The above research shows that TCP transcription factors regulate the biosynthesis of anthocyanins, chlorophyll, and carotenoids. However, the functions of TCP transcription factors have not been reported in *S. baicalensis*. They play a role in the MeJA and SA-mediated secondary metabolism biosynthesis of *S. baicalensis*. Its function and mechanism of action need to be further studied.

CONCLUSION

In this study, the *TCP* gene family was screened and identified based on the *S. baicalensis* transcriptome database, and the structure, expression, phylogeny, and functional prediction of the *TCP* gene family were analysed. On this basis, candidate *SbTCP* genes with the ability to respond to MeJA and SA. This study further complements the functions possessed by TCP transcription factors in *S. baicalensis* and lays the foundation for the study of the mechanism of the molecular role of members of this gene family in the regulation of secondary metabolite synthesis.

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