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Genomic characterization and expression analysis of *TCP* transcription factors in *Setaria italica* and *Setaria viridis*

Wangdan Xiong[#], Yiran Zhao[#], Hanchi Gao, Yinghui Li, Wei Tang, Lichao Ma, Guofeng Yang, and Juan Sun

Grassland Agri-Husbandry Research Center, College of Grassland Science, Qingdao Agricultural University, Qingdao, Shandong, China

ABSTRACT

The plant-specific *TCP* transcription factor plays important roles in plant development and environment adaptation. *Setaria italica* and *Setaria viridis*, the C4 model plants, can grow on drought or arid soils. However, there is no systematic information about the genomic dissection and the expression of *Setaria TCP* genes. A total of 22 *TCP* genes were both identified from *S. italica* and *S. viridis* genomes. They all contained bHLH domain and were grouped into three main clades (PCF, CIN, and CYC/TB1). The *TCP* genes in the same clades shared similar gene structures. Cis-element in the *TCP* promoter regions were analyzed and associated with hormones and stress responsiveness. Ten *TCP* genes were predicted to be targets of miRNA319. Moreover, gene ontology analysis indicated three Si*TCP* and three *SvTCP* genes were involved in the regulation of shoot development, and *SiTCP16/SvTCP16* were clustered together with tillering controlling gene *TB1*. The *TCP* genes were differentially expressed in the organs, but *SiTCP/SvTCP* orthologs shared similar expression patterns. Ten *SiTCP* members were downregulated under drought or salinity stresses, indicating they may play regulatory roles in abiotic stresses. The study provides detailed information regarding *Setaria TCP* genes, providing the theoretical basis for agricultural applications.

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Introduction

Setaria italica and its wild ancestor *S. viridis* are C4 graminaceous diploid grasses with a short life cycle and small genomes, which have been designated as models for C4 panicoid plants.[1–3] Both are relatively drought tolerant and can be grown on drought or arid soils.[2] Besides, *S. italica* is designated as a dual-purpose grain and forage grass, which is cultivated globally including in Northern China.[2] Recently, the available genomic and transcriptional sequences of these two grasses make it to be models for studying the developmental adaption of forage grasses.[4,5]

Transcription factors (TF), such as *NAC, MYB, bHLH, bZIP, WRKY*, and *AP2/ERF*, play important roles in plant growth, development, and responses to stresses.[6–8] In addition to the mentioned TF gene families, Teosinte branched 1/Cycloidea/Proliferating cell factors 1 (*TCP*) gene family also play important roles in plant biological processes.[9,10] The first identified *TCP* genes, TEOSINTE BRANCHED 1/CYCLOIDEA/PROLIFERATING CELL FACTORS 1 AND 2, are involved in apical dominance regulation, floral asymmetry, and proliferation.[11–14] The non-canonical bHLH motif is located at the N-terminal of TCP domain, and they can be divided into two main classes, Class I (PCF) and Class II (CIN and CYC/TB1 clades) according to the differences of the TCP domain.[10] Studies have elucidated that many *TCP* genes play important roles in plant growth and development, such as seed germination, apical dominance, leaf and flower development, and bud outgrowth.[15–19] AtTCP15 can directly activate the expression levels of *GA20ox1* in *Arabidopsis* during thermomorphogenesis.[20] The *Arabidopsis tcp2 tcp4* mutant has enlarged flat leaves, and the *tcp2 tcp3 tcp4 tcp10* mutant with strongly crinkled leaves in *Arabidopsis*.[21] Tomato *SlTCP9* and *SlTCP7* are involved in axillary bud initiation and outgrowth.[22]

With the development of sequencing and bioinformatic technology, *TCP* gene family has been systematically analyzed across the plant genomes, such as *Arabidopsis*, rice, cucumber, maize, switchgrass, etc.[23–28] A total of 24 *TCP* members were identified in *Arabidopsis*, of which two members, *AtTCP19* and *AtTCP20*, showed similar functions in controlling leaf senescence.[23] Eleven *TCP* genes were identified in the grapevine genome, and most of their expression levels were inhibited by drought and waterlogging stresses.[27] In switchgrass, 42 *TCP* genes are identified and 29 members were regulated under salinity conditions.[25] Moreover, *TCP* genes are targets of miR319, which is involved in the response to drought and salinity stress.[29] Therefore, *TCP* gene members can also be involved in plant development and abiotic stress.[21,29] Abiotic stresses, like salinity, heat, and drought, dramatically affect plant growth and decrease its biomass.[30] However, little is known about the genes of *Setaria TCPs* and their function in plant developmental processes, as well as under abiotic stresses.

CONTACT Juan Sun sunjuan@qau.edu.cn Grassland Agri-Husbandry Research Center, College of Grassland Science, Qingdao Agricultural University, Qingdao, Shandong 266109, China
[#]Co-first author

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Here, we analyzed the genome-wide *TCP* genes of two closely related species, *S. italica* and *S. viridis*. A total of 44 *TCP* members (22 *SiTCPs* and 22 *SvTCPs*) were identified. The gene structure, chromosome location, promoter cis-element analysis, gene annotation, tissue-specific expression pattern, and their expression changes under drought and salinity were analyzed. These results will be helpful for further analyzing the detailed function of *Setaria TCP* genes and utilizing them in agricultural application.

Materials and methods

Identification of TCP *genes from* S. Italica *and* S. viridis

Genes encoding *TCP* proteins were retrieved from *S. Italica* and *S. viridis* genomes, which were searched from the Phytozome database (https://phytozome.jgi.doe.gov). The *Arabidopsis* TCP proteins were searched as query sequences with an E-value lower than 0.00001 and the identified TCP proteins were confirmed for the presence of PFAM domain PF03634 using HMMSCAN (http://www.ebi.ac.uk/Tools/hmmer/search/hmmscan). The corresponding detailed information, genomic DNA, and coding sequences along with their chromosomal positions were also downloaded from the Phytozome database verified by comparison to cDNA sequences in the TSA and EST databases at GenBank. The information of *TCP* genes in *Arabidopsis thaliana, Oryza sativa, Panicum virgatum, Sorghum bicolor*, and *Zea mays* was referred to previously published studies.[23–26]

Protein properties and phylogenetic analysis

Protein properties including molecular weight and isoelectric point (pI) were predicted using the online tool of ExPASy (http://web.expasy.org/compute_pi/). Multiple sequence alignments were performed using Clustal X. TCP proteins from *Arabidopsis*, rice, *S. Italica*, and *S. viridis* were used to construct a phylogenetic tree with MEGA by Neighbor-Joining method, and the bootstrap test was performed with 1000 iterations.

Gene structure and chromosomal locations

The genomic and coding sequences of TCP proteins were used to generate their exon/intron structures using the GSDS website (http://gsds.gao-lab.org/). The MEME online tool was used to analyze TCP protein conserved motifs (https://meme-suite.org/meme/tools/meme). The chromosomal position of *TCP* genes was imported into MapChart and a physical map was constructed based on the physical map in Phytozome.[31]

The miR319 target site prediction and GO annotation analysis

The full-length *TCP* nucleotide sequences in the psRNA online website (http://www.mirbase.org/) were used to predict the miR319 target sites. All TCP protein sequences were submitted to the eggNOG website (http://eggnog-mapper.embl.de/) for gene ontology (GO) annotation analysis.

Gene promoter analysis

The 2 kb upstream sequences of *TCP* gene sequences were retrieved from the Phytozome database, and they were screened for cis-regulatory elements using the PlantCARE web server (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/).

Expression profiles of *SiTCP* and *SvTCP* genes in different tissues or organs

The transcriptome data of *S. italica* and *S. viridis* were downloaded from the phytozome database. The expression profiles of five tissues (germ shoot 6 days in dark mesh water, root 10 days, shoot 1 week, leaf 2 weeks, and mature panicle) were retrieved and the normalized counts in transcripts per million (TPM) were used to make the expression heat map.

Expression pattern of *SiTCP* genes under drought and salinity treatment

Plants of *S. italic* (Jigu 20) were used for drought treatments. Plants were hydroponically grown in a chamber under a 28°C/16 h and 24°C/8 h cycle. Thirty-day-old seedlings were treated with 20% PEG 6000 and 200 mM NaCl, respectively. Seedlings grown in Hoagland nutrient solution were used as the control. Samples were collected at 6 h and 12 h intervals, immediately frozen in liquid nitrogen, and stored at −80°C for further experiments.

Total RNA of the shoot samples was extracted using the TRIzol method (Invitrogen Life Technologies, USA) and treated with RNase-free DNase I (Roche). The total RNAs were reverse transcribed into cDNA using a PrimeScript™ RT Kit (TransGen Biotech, Beijing, China). Three independent biological replicates were maintained for transcriptome analysis and the reads per kilobase per million (RPKM) values were used to normalize the mapped reads. A heat map was generated based on the RPKM values for each gene using RNA-Seq data. The differential expressed genes were verified using quantitative RT-PCR (qRT-PCR). The SYBR Premix ExTaq™ (Takara, Dalian, China) was used for qRT-PCR, and the cycle thresholds were determined using a Roche LightCycler® 480 II sequence detection system (Roche, Shanghai, China). The expression data were analyzed using $2^{-\Delta\Delta CT}$ method. The primers for target and internal control genes are listed in Table S1. The *β*-actin gene, *Seita.7G294000*, was used as an internal control.[32]

Results

Characterization of TCP *genes in* S. italica *and* S. viridis

To identify *TCP* genes in *Setaria*, TCP proteins in *Arabidopsis* were used to search their whole genome from Phytozome database. The identified proteins were confirmed with the PF03634 domain and all of them contained the conserved bHLH domain (Figure 1). A total of 22 TCP proteins were both identified in *S. italica* and *S. viridis*, and

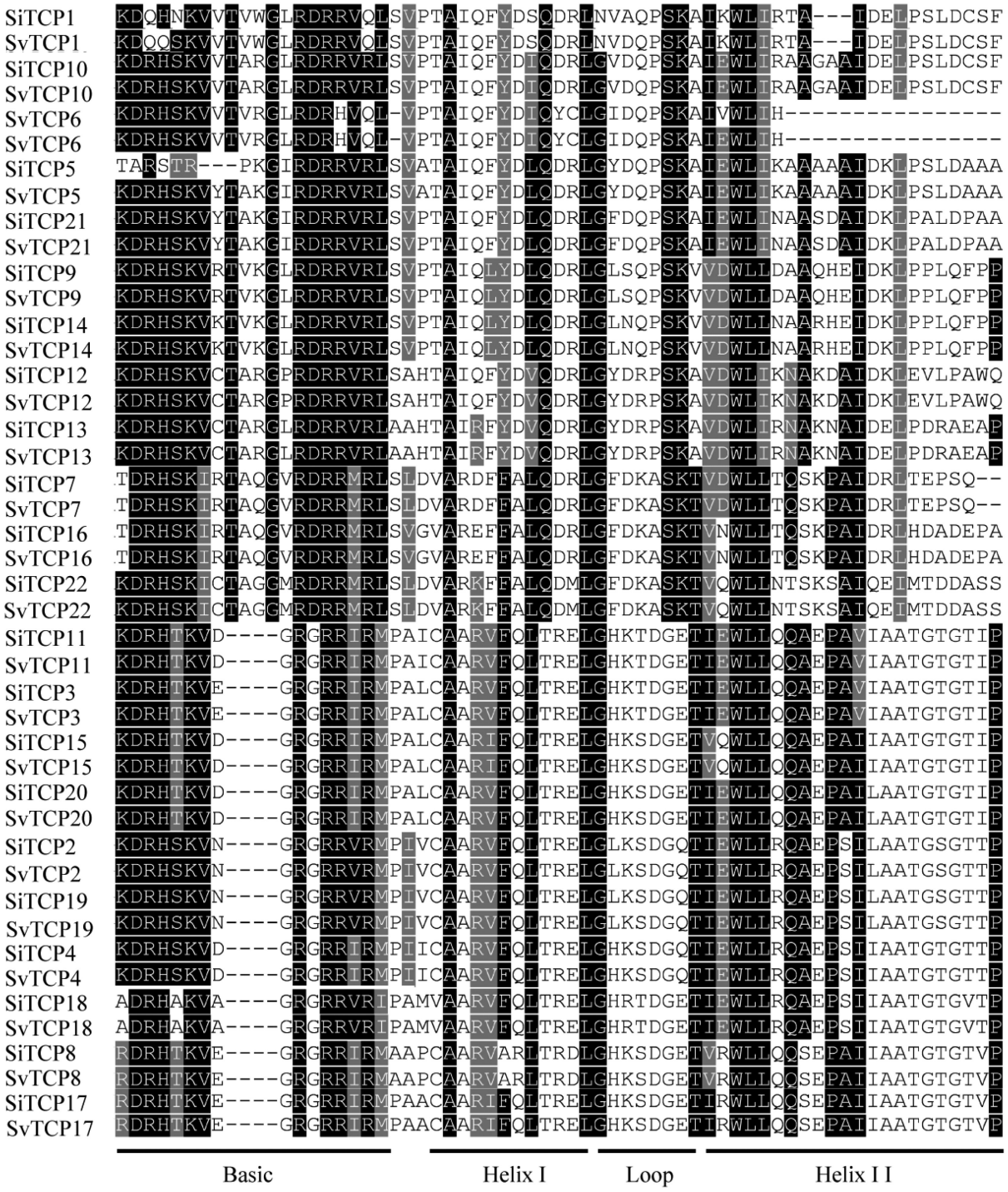

Figure 1. Alignment of the conserved basic helix-loop-helix sequence of *Setaria* TCP proteins was generated by GenDoc. The black box and gray color box indicate highly conserved and less conserved amino acids.

they were named as *SiTCP1-22* and *SvTCP1-22* (Table 1). Among the identified *TCP* proteins, their sequences exhibit variations in length [144 to 454 amino acids (aa)] and their molecular weight ranged from 9.07 to 47.64 kDa. The isoelectric point varied from 4.67 (SiTCP8 and SvTCP8) to 11.35 (SiTCP22). In *S. italica*, SiTCP6 was identified to be the smallest protein with 144 aa, whereas the largest one was SiTCP12 (450 aa). For *SvTCP* proteins, their lengths were ranging from 144 (SvTCP6) to 454 aa (SvTCP5).

Chromosomal location analysis of *TCP* genes

A total of 44 TCP proteins were identified in *S. italic* and *S. viridis*, and they were named as *SiTCP1-22* and *SvTCP1-22* based on their physical locations (Figure 2). *Setaria TCP* genes were unevenly distributed on chromosomes but *TCP* genes show similar distribution in two model species. *SiTCPs* were located on seven chromosomes except for chromosome 8 (Figure 2). The phenomenon was the same for *SvTPS* genes

Table 1. Overview of the *TCP* genes detected in *S. italica* and *S. viridis*.

Gene	ID	CDS	Exons	aa	PI	MW (kD)	Gene Location
SiTCP1	Seita.1G230500	738	3	245	10.1	26.93	scaffold_1:30672521.30674126 (+)
SiTCP2	Seita.1G246200	663	1	220	9.69	22.59	scaffold_1:32300730.32302263 (+)
SiTCP3	Seita.1G319500	1212	1	403	9.42	40.58	scaffold_1:38005478.38007811 (+)
SiTCP4	Seita.1G376200	687	1	228	10.09	23.22	scaffold_1:41873145.41873831 (-)
SiTCP5	Seita.2G032900	1329	2	442	9.51	46.39	scaffold_2:2693522.2694888 (+)
SiTCP6	Seita.2G101000	435	1	144	11.35	15.83	scaffold_2:9156462.9156896 (-)
SiTCP7	Seita.2G202000	801	1	266	5.75	28.25	scaffold_2:30250292.30251791 (+)
SiTCP8	Seita.2G279300	999	1	332	4.67	33.94	scaffold_2:37688669.37689667 (-)
SiTCP9	Seita.3G181900	885	1	294	8.58	30.81	scaffold_3:13728299.13730944 (-)
SiTCP10	Seita.3G391900	894	1	297	6.59	31.03	scaffold_3:49321162.49325142 (-)
SiTCP11	Seita.4G097900	1170	1	389	7.88	39.48	scaffold_4:8347568.8355680 (+)
SiTCP12	Seita.5G149100	1353	1	450	6.59	46.47	scaffold_5:13316304.13319606 (+)
SiTCP13	Seita.5G322500	1218	2	405	6.31	42.9	scaffold_5:37185471.37186845 (+)
SiTCP14	Seita.5G327600	846	1	281	8.11	29.91	scaffold_5:37626213.37628883 (-)
SiTCP15	Seita.5G433900	999	1	332	6.27	35.02	scaffold_5:45178220.45184296 (+)
SiTCP16	Seita.6G157000	735	1	244	6.6	25.89	scaffold_6:27794357.27795091 (+)
SiTCP17	scaffold_6_312	1107	1	368	5.76	37.92	scaffold_6:34677727.34678833 (-)
SiTCP18	Seita.7G035500	519	1	172	8.26	17.87	scaffold_7:11228252.11228771 (-)
SiTCP19	Seita.7G174000	606	1	201	10.01	20.81	scaffold_7:25468158.25469242 (+)
SiTCP20	Seita.7G288200	927	2	308	5.74	32.36	scaffold_7:33312907.33314931 (-)
SiTCP21	Seita.9G064600	1176	1	391	9.14	40.05	scaffold_9:3743634.3744848 (-)
SiTCP22	Seita.9G123400	1110	1	369	8.06	39.45	scaffold_9:7678020.7680228 (-)
SvTCP1	Sevir.1G234700	939	2	312	10.58	33.45	Chr_01:30131241.30132844 (+)
SvTCP2	Sevir.1G250500	663	1	220	9.69	22.61	Chr_01:31588253.31589516 (+)
SvTCP3	Sevir.1G325700	1212	1	403	9.42	40.61	Chr_01:37178214.37180567 (+)
SvTCP4	Sevir.1G382800	687	1	228	10.09	23.24	Chr_01:40985803.40989499 (-)
SvTCP5	Sevir.2G037600	1365	1	454	9.49	47.64	Chr_02:3041684.3048953 (+)
SvTCP6	Sevir.2G103700	435	1	144	11.33	15.97	Chr_02:9275090.9275524 (-)
SvTCP7	Sevir.2G210100	801	1	266	5.75	28.25	Chr_02:29207436.29209064 (+)
SvTCP8	Sevir.2G289200	999	1	332	4.67	33.94	Chr_02:36556914.36558513 (-)
SvTCP9	Sevir.3G186300	885	1	294	8.58	30.83	Chr_03:13554151.13556691 (-)
SvTCP10	Sevir.3G409000	894	1	297	6.59	31.15	Chr_03:48360918.48367616 (-)
SvTCP11	Sevir.4G097000	1179	1	392	7.88	39.88	Chr_04:8302245.8305033 (+)
SvTCP12	Sevir.5G147800	1344	1	447	6.57	46.22	Chr_05:12779170.12781956 (+)
SvTCP13	Sevir.5G326100	1218	2	405	6.31	42.93	Chr_05:36130432.36131806 (+)
SvTCP14	Sevir.5G331400	846	1	281	8.11	29.91	Chr_05:36554888.36557559 (-)
SvTCP15	Sevir.5G440100	1002	1	333	6.27	35.12	Chr_05:43962101.43968310 (+)
SvTCP16	Sevir.6G163100	735	1	244	6.53	25.88	Chr_06:27380179.27380913 (+)
SvTCP17	Sevir.6G241000	1110	1	369	5.76	37.97	Chr_06:34054313.34055897 (-)
SvTCP18	Sevir.7G022600	519	1	172	8.26	17.86	Chr_07:7690715.7694570 (-)
SvTCP19	Sevir.7G183600	606	1	201	10.01	20.82	Chr_07:24414807.24415897 (+)
SvTCP20	Sevir.7G298200	1248	2	415	5.91	42.12	Chr_07:32316298.32318899 (+)
SvTCP21	Sevir.9G064100	1176	1	391	9.14	40.76	Chr_09:3739283.3743093 (-)
SvTCP22	Sevir.9G122200	1110	1	369	8.06	39.47	Chr_09:7597867.7599660 (-)

location and no gene was located on chromosome 8 (Figure 2). For both genomes, chromosome 1, 2, and 5 possess four *TCP* genes, chromosome 7 contains three genes, chromosome 3, 6, and 9 contain two genes, and chromosome 4 contains one gene. Interestingly, orthologs of *SiTCP* genes in *S. viridis* were located in the similar site on the chromosome.

Phylogenetic analysis of *TCP* genes

To evaluate the relationship among *Setaria* TCP proteins, full-length amino acid sequences of 22 SiTCPs and 22 SvTCPs, together with AtTCPs and OsTCPs, were used to construct an unrooted phylogenetic tree (Figure 3). All TCP proteins were divided into two main classes, Class I (PCF) and Class II (CIN and CYC/TB1). There were 20 Class I members and 24 Class II members for *Setaria* TCP proteins. Specifically, both species contain 9 PCF type members, 3 CYC/TB1 type members and 10 CIN type members (Figure 3). The number of *TCP* genes was 42 and 46 in switchgrass and maize (Table S2). There was a gap of the number of TCP genes in PCF and CYC/TB1 and the gene number of PCF and CYC/TB1 clade was 17 and 19 in maize, respectively. This result suggested that the *SiTCP* and *SvTCP* genes did not undergo a segmental duplication event.[24]

The number of CYC/TB1 type members in *Setaria* was the same as that in *Arabidopsis* (3) and rice (3) but the number was doubled in switchgrass (6) and maize (6) (Figure S1). Moreover, SiTCP22 and SvTCP22 were clustered closely together with the functional analyzed *TB1* genes in rice, maize, and switchgrass. Interestingly, these two protein sequences were the same according to their information from the phytozome database, and only two nucleotides are different in their coding sequences of *SiTCP22* and *SvTCP22*.

According to the phylogenic tree (Figure 3), 10 *SiTCP/SvTCP* genes (5 each) are closely clustered with the mentioned miR319 targeted *OsTCPs* (*OsPCF5, OsPCF6, OsPCF7, OsPCF8*, and *OsTCP21*).[33] Indeed, these homologs in *Setaria* all contained the putative recognition site of miR319 and no other genes were recognized as targets of miR319 (Figure S2). The alignment of

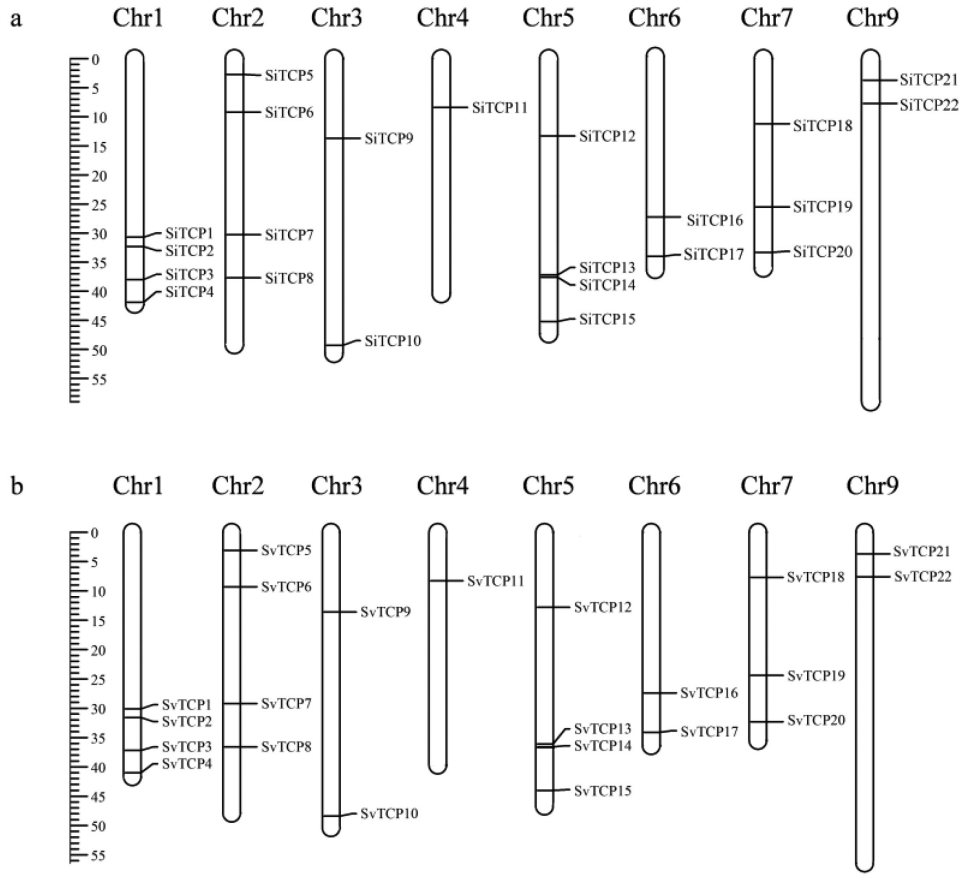

Figure 2. Chromosomal location of *SiTCP* (a) and *SvTCP* (b) genes based on the physical map. The scale on the left represents the physical length of the chromosomes; Mb = million base pairs.

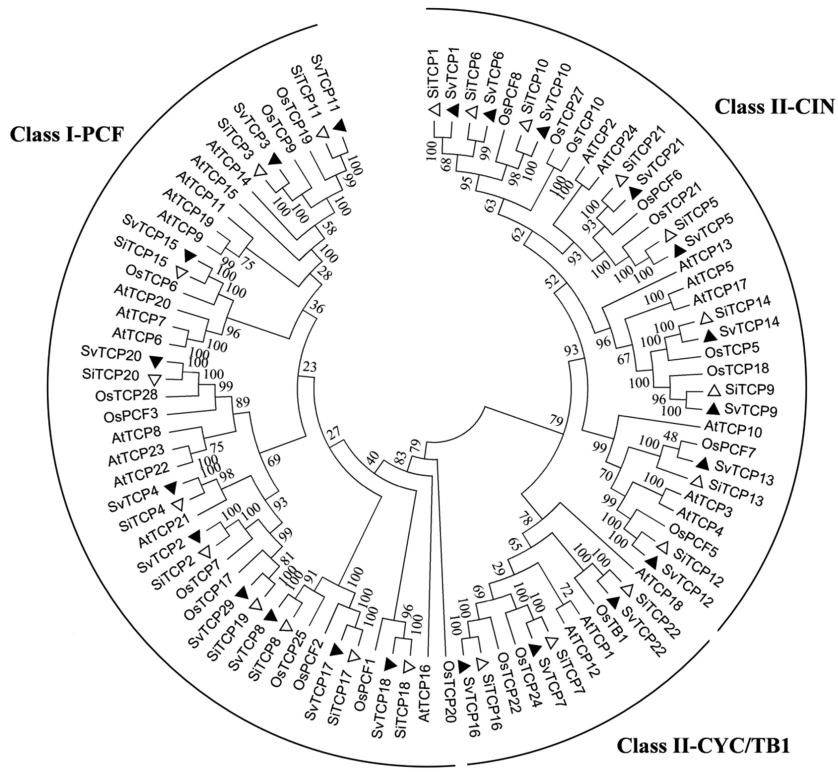

Figure 3. Phylogenetic analysis of *Setaria* TCP proteins together with TCP proteins in *Arabidopsis* and rice. An unrooted neighbor-joining (NJ) tree was constructed using MEGA5.0 after the multiple alignment of peptide sequences retrieved from the Phytozome database, and the bootstrap test was performed with 1000 iterations. The hollow and solid triangles represent TCP proteins in *S. italic* and *S. viridis*, respectively.

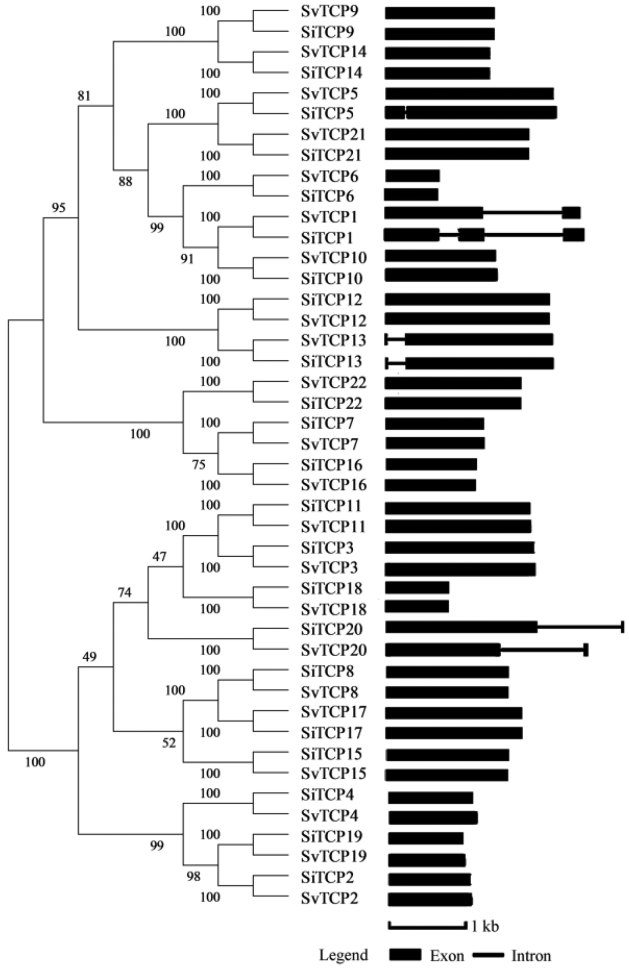

Figure 4. Structural analysis of *Setaria TCP* genes. The gene structure of *Setaria TCP* genes. Black boxes and lines indicate coding sequences and introns, respectively.

miR319 recognition sequences showed that the miR319-TCP regulation module was highly conserved among species (Figure S2).

Gene structure of *TCP* genes and motif analysis of their encoding proteins

Gene structure analysis indicated that 37 of them contain only one exon in their coding sequence region. Still, six genes contain two exons, and one gene contains three exons (Figure 4). Moreover, genes in the same subclade contain similar gene structure. For example, *SiTCP20* and *SvTCP20* contain one intron at the C terminal end. This was the same for gene *SiTCP13* and gene *SvTCP13*. Still, there was a variation in clustered *TCP* genes. *SiTCP1* contains three exons, while *SvTCP1* gene only contains two exons.

Furthermore, the conserved motifs of *Setaria* TCP proteins were analyzed using the MEME suite (Figure 5). Ten conserved motifs (named as motif 1 to motif 10) were identified in *Setaria* TCPs and proteins clustered together contain the same motif. Motif 1 was widely distributed in *Setaria* TCP family. Moreover, TCP proteins clustered together possess similar motifs and the orthologs of SiTCP and SvTCP proteins also mostly share the same motifs. Specifically, motif 2 was only present in all Class II TCP members (24). Motif 3 and motif 4 were only present in all Class I TCP (PCF) members (20). Motif 9 was observed in 18 members of class II TCP subfamily, except for SiTCP6, SiTCP7, SiTCP14, SvTCP6, SvTCP7, and SvTCP14. Motif 8 was observed in 10 members of the CIN clade and two members of the PCF clade. Eighteen TCP proteins contain motif 5 and 6 of them contain two motif 5.

Functional annotation of *Setaria TCP* genes

Furthermore, GO annotation and enrichment analysis was performed to recognize the contribution of *Setaria TCP* genes (Table S3). The results showed that all identified *Setaria TCP* genes were enriched in DNA binding transcription factor activity (GO:0003700) and sequence-specific DNA binding (GO:0043565) based on molecular function. The enrichment term based on cellular component was nucleus (GO:0005634) and the enriched terms based on biological process were regulation of shoot system development (GO:0048831) and regulation of secondary shoot formation (GO:2000032).

Promoter analysis of *Setaria TCP* genes

To elucidate the transcriptional regulation of *TCP* genes, the cis-elements of their promoter region were analyzed. A 2 kb sequence upstream of the open reading frame of the *SiTCPs* was subjected to PlantCARE analysis (Table 2; Table S4). A number of cis-acting DNA elements were commonly identified in the promoters, which would be correlated with phytohormone and stress response. Notably, cis-acting DNA elements such as TGA-element, ABRE, CGTCA-motif, TGACG-motif, GARE-motif, P-box, TATC-box, and TCA-element are relevant to auxin, abscisic acid, MeJA, gibberellin, and salicylic acid responsiveness. The DNA element MBS was correlated with drought stress response. These findings suggested that the expression level of *Setaria TCP* genes would change in different tissues or under stress conditions.

Expression profiling of *Setaria TCP* genes

Expression pattern of *Setaria TCP* genes was analyzed in five tissues, namely, root 10 days, germ shoot 6 days dark, shoot 1 week, leaf 2 weeks, and mature panicle. They revealed a differential expression pattern (Figure 6, Table S3). Some genes showed tissue-specific higher expression in the root, such as *SvTCP3, SiTCP3*, and *SvTCP11*. Some gene pairs such as *SiTCP9* and *SvTCP9, SiTCP12* and *SvTCP12, SiTCP13*, and *SvTCP13*, were expressed relatively higher in shoot and mature panicles. A total of 15 genes (*SiTCP1, SiTCP6, SiTCP7, SiTCP8, SiTCP16, SiTCP17, SiTCP22, SvTCP1, SvTCP6, SvTCP7, SvTCP8, SvTCP16*, and *SvTCP22*) showed no or negligible expression in all five tested tissues (Figure 6). However, *TCP* genes with a similar expression pattern in *Setaria* were clustered together or located in the same clade. All *Setaria TCP* genes in CYC-TB1 clade expressed relatively very lower in the tested tissues. Gene pairs, like *SiTCP8*/*SvTCP8*, were found to be expressed at low levels in all tested tissues, while *SiTCP4*/*SvTCP4* were found to be highly expressed in all tested tissues.

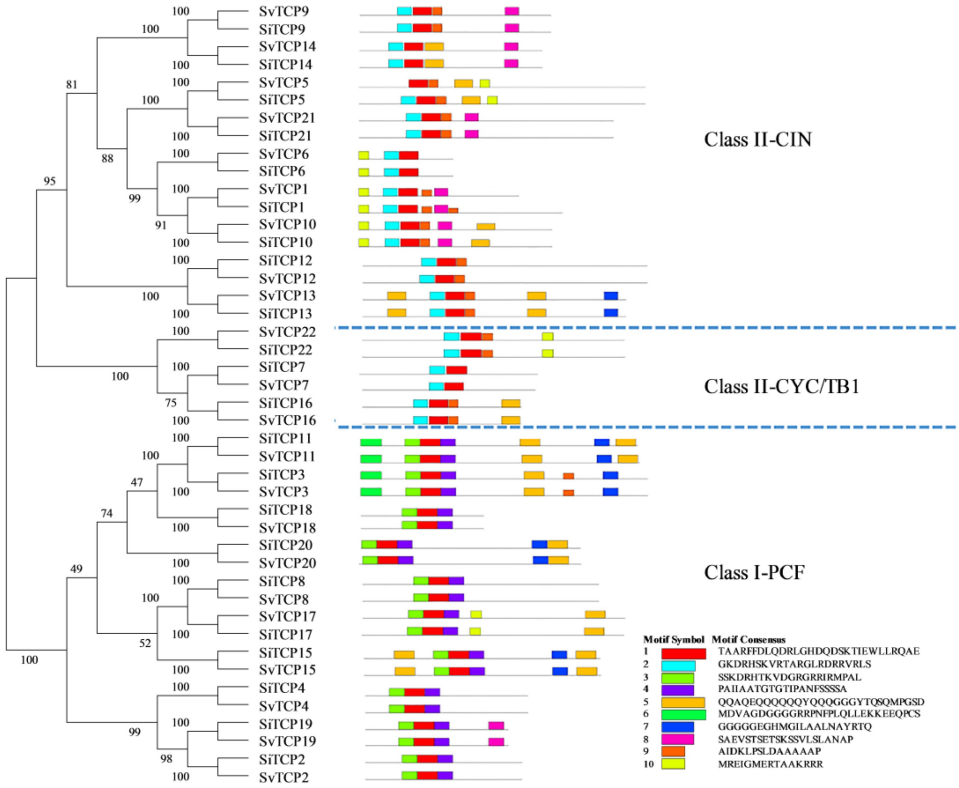

Figure 5. The motif analysis of *Setaria* TCP proteins. Motifs were represented in different colors using MEME suite.

Expression profiles of *SiTCP* genes in response to drought and salinity treatments

To investigate the expression of *SiTCP* genes in response to abiotic stress, the expression profiles were examined during 6 h and 12 h after drought or salinity treatments. The relative transcript abundance showed a differential expression pattern for 10 *SiTCP* genes under drought and salinity (Figure 7). The differential expression genes of the CIN clade were selected and verified by qRT-PCR and the results were consistent with the RNA-seq data (Figure 8). Specifically, the expression levels of *SiTCP2, SiTCP3, SiTCP4, SiTCP5*, and *SiTCP12* was reduced in drought and salinity compared with the control. For *SiTCP9, SiTCP13, SiTCP14*, and *SiTCP19*, they were only downregulated significantly under drought stress.

Table 2. The conserved DNA sequence motifs analysis of *Setaria TCP* gene promoters. The number indicated the total number of cis-elements in the promoters of *Setaria TCP* genes.

Function	Site Name	SiTCP	SvTCP
Meristem expression	CAT-box	16	15
Zein metabolism regulation	O2-site	16	14
Endosperm expression	GCN4_motif	4	4
Seed-specific regulation	RY-element	4	4
Auxin responsiveness	TGA-element	11	9
Abscisic acid responsiveness	ABRE	60	79
MeJA-responsiveness	CGTCA-motif	40	46
	TGACG-motif	34	46
Gibberellin-responsiveness	GARE-motif	4	5
	P-box	13	14
	TATC-box	4	8
Salicylic acid responsiveness	TCA-element	10	13
Low-temperature responsiveness	LTR	16	15
Drought-inducibility	MBS	13	18
Anaerobic responsiveness	ARE	31	37
	GC-motif	25	19
Defense and stress responsiveness	TC-rich repeats	5	7

Discussion

S. italica is an important dual-purpose grain and forage grass, which can be grown in drought and barren area.[1–3] *S. italica* and its ancestor *S. viridis* have been designated as the model C4 plant for studying plant development and environment adaptation.[4,5] The *TCP* transcription factors are conserved and plant-specific, playing important roles in plant growth and development processes.[15,16,19] Studies also supported the idea that TCP transcription factors can respond to abiotic stresses.[16] To our knowledge, there is no systematic analysis of *TCP* genes in *S. italica* and *S. viridis*. Here, 22 *TCP* genes were identified in *S. italica* and the number was also 22 in *S. viridis*. A systematic analysis of *Setaria TCP* genes was performed including their chromosome location, gene structure, phylogenetic relationship, promoter analysis of cis elements function annotation and expression pattern, providing information for further exploration the function of *Setaria TCP* genes in plant growth and environment adaptation.

In *S. italica* and *S. viridis* genomes, the *TCP* gene number was similar to that in *Arabidopsis* (24) and rice (28).[27] All *TCP* genes contained a TCP domain and they shared similar gene structures. No introns existed in most of *Setaria TCP* genes (37 of 44 members). A total of 22 *SiTCP/SvTCP* orthologous were identified, which were clustered together and contained the same gene structure. *TCP* genes were unevenly distributed on

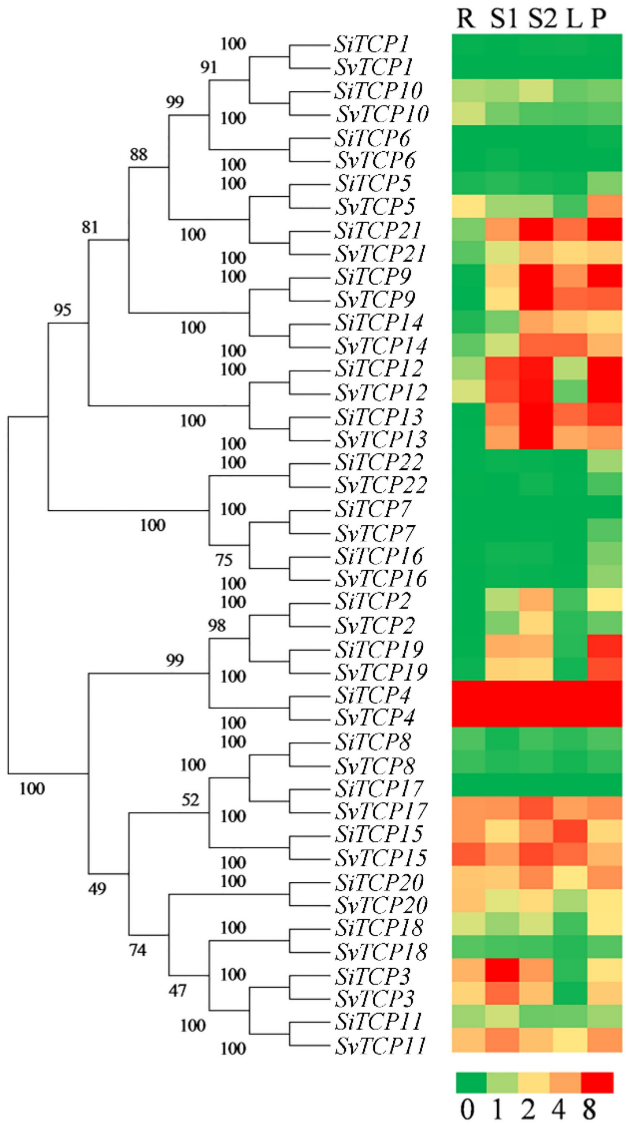

Figure 6. Expression pattern of *Setaria TCP* genes. The heatmap was created by taking the transcripts per million values of *Setaria TCP* genes in five tissues. The green, yellow, and red colors display low to high expression levels. R, root 10 days; S1, germ shoot 6 days; S2, shoot 1 week; L, leaf 2 weeks; P, mature panicle.

the chromosome and no *TCP* genes were located on chromosome 8 for both *S. italica* and *S. viridis*. No tandem duplication events were found in *the Setaria TCP* gene family. This was different from *TCP* gene number in maize and switchgrass, in which the number was almost doubled (46 in maize and 42 in switchgrass).[24,25] The *TCP* gene number was different in PCF clade and CYC/TB1 clade (Table S2).

The CYC/TB1 clade was relatively conserved and there are three *TCP* genes belonging to CYC/TB1 clade in *Arabidopsis*, rice, *S. italica* and *S. viridis*. The number was doubled in switchgrass (six) and 19 *TCP* genes belong to CYC/TB1 clade in maize. The characterized *TB1* genes of rice (*OsTB1*), maize (*ZmTB1*) and *Arabidopsis* (*AtTCP18*) were all clustered together with *SiTCP22* and *SvTCP22*.[11,34,35] The biomass yield of forage is concerned in production, which was closely related with the plant tillering. Here, the coding sequences of *SiTCP22* and *SvTCP22* were the same. Moreover, the expression pattern of *SiTCP22* and *SvTCP22* in shoots, roots, shoots, leaves, and mature panicles was similar. *TB1*, one of the TCP members, was the identified major gene controlling plant outgrowth in maize, rice and other plants.[36] Recently, OsTB2 was identified to be positively regulating tillering by interacting with the homologous OsTB1 protein.[37] Here, *SiTCP7* and *SvTCP7* were clustered with OsTB2. It was speculated that there TCP7 and TCP22 would modulate the differentiation of the tillering number in *S. viridis* and *S. italica*.

The expression pattern of *Setaria TCPs* varied in shoots, roots, shoots, leaves, and mature panicles, but those clustered together in the phylogenetic tree showed similar expression patterns. Gene pairs, such as *SiTCP9/SvTCP9*, *SiTCP12/SvTCP12*, and *SiTCP13/SvTCP13*, showed a relatively higher expression in shoot and mature panicles. *SiTCP9* and *SvTCP9* were clustered together with *AtTCP5/13/17*, which can act directly at the *APETALA1*

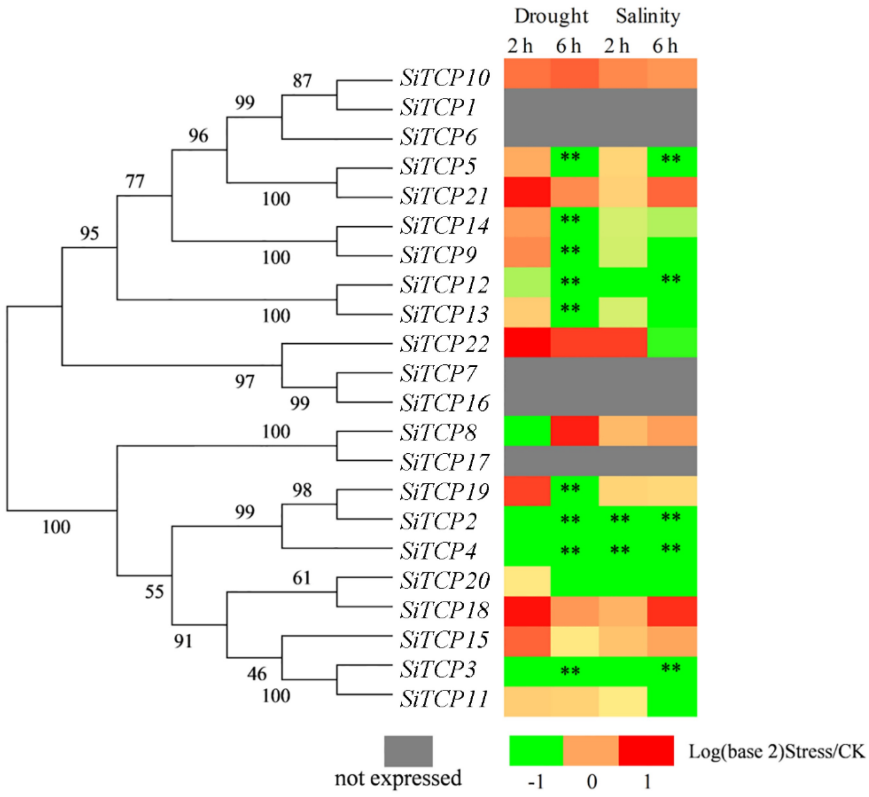

Figure 7. The expression level of *SiTCP* genes in *S. italica* shoot under drought and salinity condition. The heatmap was created by taking the log(base2) stress/control values of *Setaria TCP* genes in shoot. The *p*-values were obtained using student's t-test for each comparison. Error bar represented the SD (n = 3). *, *P*< .05; **, *P*< .001.

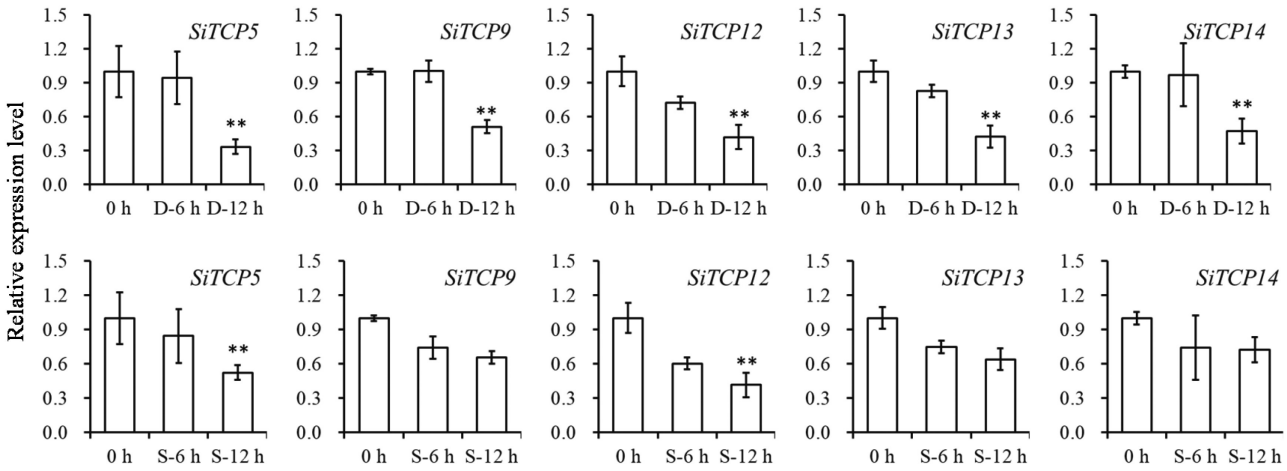

Figure 8. qPCR analysis of *SiTCP* genes from the shoots under drought and salinity treatment. The *p*-values were obtained using Student's t-test for each comparison. Error bar represented the SD (n = 3). *, *P*< .05; **, *P*< .001.

(*AP1*) promoter to control flowering in *Arabidopsis*.[38] *SiTCP4* and *SvTCP4*, the homologous genes of AtTCP*21* in *Arabidopsis*, were expressed highly in all tested tissues. Mutation of AtTCP*21* would cause leaf curling upward in *Arabidopsis*, exhibiting smaller leaf cells and shorter hypocotyls than the wild type. The results indicated that part of the mentioned *Setaria TCP* genes may be involved in plant development, such as leaf development and flowering.

Research has also suggested that *TCP* transcription factors play important roles in abiotic stresses.[20,24,25,29,39,40] Since *S. italica* is drought and salinity tolerant, whether *SiTCP* genes respond to abiotic stresses was still obscure.[41] In the study, the expression level of *SiTCP* genes under short-term drought and salinity treatment was analyzed. Nine *SiTCP* genes (*SiTCP2/3/4/5/9/12/*13/14/*19*) were downregulated under stress conditions. Cis elements of the *SiTCP* gene promoters

were analyzed and demonstrated that there were hormone or stress associated recognition sites (Table 2, Table S4). For instance, the MBS element, which was found to be associated with MYB binding site involved in drought stress, was identified in the promoters of eight *SiTCP* genes (*SiTCP2/3/4/7/8/14/15/19*).[42] Indeed, five of the eight mentioned *SiTCP* genes (*SiTCP2/3/4/14/19*) exhibited lower expression to drought stress. Moreover, *TCP* genes are targets of miR319, which is involved in the response to drought and salinity stress. Overexpressing rice miR319 in creeping bentgrass exhibited enhanced drought and salt tolerance.[29] It was also reported that miR319 was upregulated in sugarcane under cold stress.[43] Repression of a miR319 target, *PvPCF5*, also improves the salt tolerance of transgenic switchgrass plants.[44] Meanwhile, three of the predicted miR319 target *SiTCP* genes (*SiTCP5/12/13*) were significantly downregulated in short-term drought or salinity. It was suggested that overexpressing miR319 in *Setaria* plants may enhance their drought and salt tolerance.

Conclusion

A systematic analysis of *TCP* gene family was conducted in the model C4 plants of *S. italica* and *S. viridis*. A total of 22 *SiTCP* and 22 *SvTCP* genes were identified and they were distributed on 8 chromosomes each. They were phylogenetically divided into three clades. Their gene structure, motifs, promoter cis-element analysis, gene annotation, miRNA319 prediction, tissue expression pattern and expression profiles under abiotic treatments were analyzed to gain a systematic information of *Setaria TCP* genes. After all, comprehensive analyses of *Setaria TCP* genes would provide an indication of TCP regulatory function in plant development and abiotic stress regulation in the future.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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