Indian J. Genet., 78(1): 104-110 (2018)
DOI: 10.5958/0975-6906.2018.00012.3

Identification, characterization, and comparative transcriptome analysis of TCP family genes in foxtail millet

Mingxin Guo[+], Yanwei Cheng[+], Jinxin Qiao and Xusheng Zhao*

College of Life Science, Luoyang Normal University, Luoyang, 471934, Henan, China

(Received: November 2017; Revised: January 2018; Accepted: February 2018)

Abstract

Teosinte Branched 1, Cycloidea, and Proliferating Cell Factors (TCP), belonging to a plant-specific transcription factors family, play versatile roles in multiple processes of plant growth and development. In the present study, 18 *SiTCP* genes were identified and phylogenetic analysis suggested that the *SiTCP* genes were divided into two main classes, which was supported by multiple sequence alignment of the core TCP domain. To study the evolution of foxtail millet and rice, *Sorghum bicolor*, *Arabidopsis*, we compared the domain distribution and exon/intron structure of TCP proteins of four species. The results showed that R domain had expansion in *Arabidopsis*, while the other three monocots had relative conserved domains distribution. Furthermore, all four plant species had a conserved exon/intron structure during evolutionary process. Comparative transcript profiles of *TCP* genes in similar tissues among foxtail millet, rice and *Arabidopsis* indicated that the *TCP* genes of monocots (foxtail millet and rice) exhibited similar expression patterns among the same clade, which were different from eudicot *Arabidopsis*. The present study is the first analyses of the TCP gene family in foxtail millet, will provide valuable information for functional analyses of this important gene family.

Key words: TCP protein, foxtail millet (*Setaria italica*), phylogeny, TCP genes expression profile

Introduction

The TCP proteins are a plant-specific transcription factors (TFs) family involved in the regulation of plant growth, development and abiotic stress tolerance (Cubas et al. 1999; Martin-Trillo et al. 2010; Mukhopadhyay and Tyagi, 2015). All TCP TFs share the TCP domain, which is named after the first characterized members: TB1 (TEOSINTE BRANCHED 1) in maize, CYC (CYCLOIDEA) in *Antirrhinum majus*, and the PCFs (PROLIFERATING CELL FACTORS) in rice (Cubas et al. 1999). The TCP domain is an approximately 59-amino-acid-long, non-canonical basic helix–loop–helix domain that is responsible for nuclear targeting, DNA binding and mediating protein-protein interactions (Cubas et al. 1999; Kosugi and Ohashi 2002). In addition to the TCP domain, several TCP members have a second conserved region known as the R domain, which is rich in polar residues (arginine, lysine and glutamic acid) and predicted to form a hydrophilic α-helix (Cubas et al. 1999).

The *ZmTB1* gene affects the fate of maize axillary meristems by preventing the outgrowth of buds at lower nodes and promoting the development of female inflorescences (ears) at upper nodes. Knockdown or knockout *ZmTB1* gene cause enhanced lateral branching, suggesting that ZmTB1 functions as a negative regulator for the growth of axillary buds (Doebley et al. 1997). The homologs of the maize *ZmTB1* gene from rice, *OsTB1* (Minakuchi et al. 2010; Takeda et al. 2003), Sorghum (*Sorghum bicolor*), *SbTB1* (Kebrom and Burson, 2006), *Arabidopsis*, *AtBRC1*(*AtTCP18*) (Aguilar-Martínez et al. 2007), and pea (*Pisum sativum*), *PsBRC1* (Braun et al. 2012), all negatively regulate lateral branching. The *CYC* together with a related gene, *DICHOTOMA* (*DICH*), establish the dorsoventral asymmetry of the *Antirrhinum* flower (Carpenter and Coen 1990; Luo et al. 1996). PCF1 and PCF2 specifically bind to *cis* elements, which are essential for meristematic tissue-specific expression of the rice proliferating cell nuclear antigen (*PCNA*) gene (Kosugi and Ohashi, 1997). However, no systematic studies have been carried out in foxtail millet. In this study, we identified 18 *TCP* genes in

*Corresponding author's e-mail: 15736750861@163.com; [+]These authors contributed equally to this work.
Published by the Indian Society of Genetics & Plant Breeding, F2, First Floor, NASC Complex, PB#11312, IARI, New Delhi 110 012
Online management by indianjournals.com; www.isgpb.com

foxtail millet and then conducted comprehensive bioinformatics analyses of its phylogeny, conserved domains and motifs, gene structure and comparative transcriptome analysis of SiTCPs with OsTCPs and AtTCPs.

Materials and methods

Sequence retrieval and identification of TCP proteins from foxtail millet

The amino acid sequences of the TCP domain (IPR017887) were obtained from Interpro EMBL-EBI database (http://www.ebi.ac.uk/interpro/) and searched against the Gramene (www.gramene.org/) and Phytozome (www.phytozome.net/) databases of foxtail millet. All hits with expected (E) values less than 0.1 were retrieved and the non-redundant sequences were examined for the presence of conserved TCP domains by searching the Interpro database. The TCP protein sequences from *Arabidopsis thaliana*, *Sorghum bicolor* and rice were retrieved from the Plant Transcription Factor Database (PlantTFDB) (http://planttfdb.cbi.pku.edu.cn/).

Phylogenetic analysis, multiple sequence alignment

To generate the phylogenetic trees of TCP TFs family genes, the full-length amino acid sequences of the putative TCP proteins were imported into MEGA6.0 and an unrooted phylogenetic tree based on the Maximum-likelihood (ML) method was generated with the following parameters: pairwise deletion of gaps/missing data; Poisson correlation model; bootstrap 1,000 replicates (Tamura et al. 2013). Multiple sequence alignment was performed using the ClustalX (1.83) software (Thompson et al. 1997), and the alignment result was then imported into the GeneDoc software for further visualization (Nicholas et al. 1997).

Protein motif and exon/intron structure analysis

Conserved motif structures of TCP proteins were analyzed by MEME4.11.2 (Multiple Expectation Maximization for Motif Elicitation) (http://meme-suite.org/tools/meme) with the following parameters; distribution of motif occurrences: any number of repetitions; number of different motifs: 12; minimum motif width: 6; and maximum motif width: 100. The *TCP* gene CDS (coding sequence) and genomic DNA sequences were used to derive exon/intron structures with the online tool Gene Structure Display Server (GSDS, http://gsds.cbi.pku.edu.cn/) (Guo et al. 2007).

Digital expression pattern analysis

For foxtail millet, the RNA-seq data of four tissues; namely, root (SRX128223), stem (SRX128225), leaf (SRX128224) and spica (SRX128226) were retrieved from the European Nucleotide Archive (http://www.ebi.ac.uk/ena). The reads were processed to generate RPKM as previously described (Mishra et al. 2014). For rice, the expression profiling data was acquired from the GEO database. GSE19024 (Wang et al. 2010) was selected for tissue-specific expression in rice. Briefly, the expression values of five tissues from rice *indica* var. Minghui63, namely, root, stem, leaf blade, leaf sheath and young panicle were retrieved from GSE19024. For *Arabidopsis*, the transcript level of four tissue including root, stem, leaf and silique were chosen from high quality AtGenExpress ATH1-22k microarray data. A heat map for the tissue-specific expression profiles was then generated using the Multi Experiment Viewer (MeV4) software package (Saeed et al. 2003).

Results and discussion

Identification and domain analysis of TCP proteins in foxtail millet

The TCP domain (IPR017887) was used to conduct BLASTP searches through the Gramene (www.gramene.org/) and Phytozome (www.phytozome.net/) database. Subsequently, the redundant sequences of candidate TCP genes were removed according to their chromosome locations, resulting in 18 TCP members in foxtail millet (Table 1). To verify the reliability of the initial results, a survey was conducted to confirm the presence of the conserved TCP domain using the InterPro database. The results showed that all 18 candidate TCP proteins harbored the TCP domain. The 18 nonredundant *SiTCP* genes were annotated as *SiTCP1* to *SiTCP18* according to their locations in foxtail millet chromosomes 1-9 (Table 1). Detailed information regarding the TCP proteins including locus IDs, length of proteins, molecular weight, isoelectric point, chromosome location, number of exons, and NCBI accession number are provided in Table 1.

In addition to the TCP domain, two TCP proteins (SiTCP3 and SiTCP17) were found to possess the R domain. The locations of the TCP and R domain of each SiTCP proteins are listed in Supplementary Table S1.

Table 1. Characteristic features of TCP gene family members identified in foxtail millet

Locus ID	Gene name	Length (aa)	MW (kDa)	PI	Chr. Location	Exons	Accession No.
Si019114m	SiTCP1	245	26.95	10.10	Chr1:30672521..30674126	3	XP_004955073
Si017396m	SiTCP2	402	40.61	9.81	Chr1:38005478..38007811	1	XP_004953859
Si029871m	SiTCP3	442	46.42	9.51	Chr2:2693522..2694888	2	XP_014660159
Si033183m	SiTCP4	266	28.27	6.12	Chr2:30250292..30251791	1	XP_004956790
Si030591m	SiTCP5	332	33.96	4.67	Chr2:37688669..37689667	1	XP_004957394
Si022833m	SiTCP6	294	30.83	8.58	Chr3:13728299..13730944	1	XP_004961521
Si022811m	SiTCP7	297	31.05	6.59	Chr3:49321162..49325142	1	XP_004963209
Si006631m	SiTCP8	389	39.51	8.27	Chr4:8347568..8355680	1	XP_004965036
Si004564m	SiTCP9	450	46.50	7.12	Chr5:13316304..13319606	1	XP_004968615
Si004293m	SiTCP10	405	42.93	6.31	Chr5:37185471..37186845	2	XP_004972181
Si002496m	SiTCP11	281	29.93	8.86	Chr5:37626213..37628883	1	XP_004970055
Si015230m	SiTCP12	244	25.90	7.13	Chr6:27794357..27795091	1	XP_004973503
Si011076m	SiTCP13	201	20.82	10.60	Chr7:25468158..25469242	1	XP_004976294
Si010765m	SiTCP14	308	32.38	5.74	Chr7:33312907..33314931	1	XP_004977266
Si026501m	SiTCP15	325	39.49	8.77	Chr8:4662196..4663989	1	XP_004978832
Si036075m	SiTCP16	391	40.08	9.61	Chr9:3743634..3744848	1	XP_004986203
Si038692m	SiTCP17	369	39.47	7.99	Chr9:7678020..7680228	1	XP_004982035
Si039258m	SiTCP18	135	14.23	11.35	Chr9:48105907..48106411	3	

MW = Molecular weight; PI = Isoelectric point; Chr = Chromosome

Phylogenetic analysis and classification of foxtail millet TCP genes

To assess the classification of the *SiTCP* genes and gain some insight into the potential function of *SiTCP* genes from well-studied TCPs in model plants, a total of 83 TCP proteins comprising 24 proteins from *Arabidopsis*, 21 from rice, 20 from *Sorghum bicolor* and 18 from foxtail millet were used to construct a phylogenetic tree (Fig. 1A). Phylogenetic analysis showed that foxtail millet TCP proteins can be divided into two classes: class I (or PCF) and class II (Fig. 1A). The TCP proteins of *Arabidopsis*, *Sorghum bicolor* and rice were further classified into two major classes (Fig. 1A), which is consistent with the results of previous reports (Yao et al. 2007; Francis et al. 2016). Additional analysis showed the class II subfamily of four species could be further divided into two subclades, class IIa or CYC/TB1 and class IIb or CIN (Fig. 1A), which indicated that TCP genes are relatively conserved across different species. Comparison of the TCP genes of four species showed that there was the same gene number in CYC/TB1 and CIN but different number of genes in PCF (Fig. 1B). It indicated that CYC/TB1 and CIN were more conserved than PCF during evolutionary process and more duplication events were involved for PCF gene family expansion, which is consistent with previous reports that AtTCP PCF subfamily experienced more duplication events than OsTCP (Yao et al. 2007). To gain a better understanding of the classification of SiTCP proteins, multiple sequence alignment of the core TCP domain was performed. Based on previous reports (Yao et al. 2007), the class II TCP proteins contained a 4-amino-acid insertion in the basic region. The compositions of the loop and helixes I and II differed greatly between the class I and II proteins (Fig. 2A).

There were two SiTCPs containing an R domain (Fig. 2B) that were classified as class II proteins. Among them, SiTCP3 was a CIN type member, and SiTCP17 was a CYC/TB1 type member. Similarly, two TCP proteins (CIN and CYC/TB1) harboring R domain were existed in the other two monocots (rice and *Sorghum bicolor*). While, eudicot *Arabidopsis* contained four TCP proteins harboring R domain (Supplementary Fig. S1). Among them, two TCPs were CIN member and the other two TCPs were CYC/TB1 member. This twice number of TCP proteins harboring

R domain in *Arabidopsis* implied that these genes had expansion in eudicot evolution after the monocot-eudicot divergence.

In the CYC/TB1 subclades, SiTCP17 with SbTB1 was clustered into one small clade (Fig. 1A). Furthermore, alignment of the predicted protein sequences of SiTCP17 with ZmTB1, OsTB1, and SbTB1, which were verified to function in shoot branching (Doebley et al. 1997; Doebley et al. 2007; Minakuchi et al. 2010; Takeda et al. 2003) showed that these CYC/TB1 proteins all having TCP and R domains and high similarity (Supplementary Fig. S2). These results indicated that SiTCP17 may play a role in shoot branching in foxtail millet.

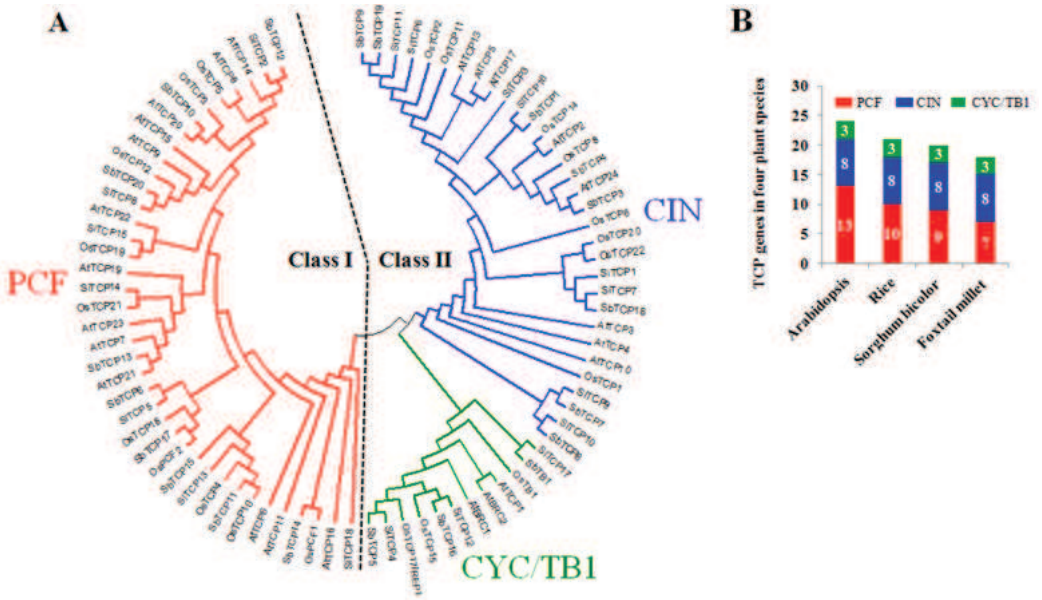


Fig. 1. Phylogenetic analysis of TCP proteins from Arabidopsis, rice, *Sorghum bicolor* and foxtail millet. (A) The phylogenetic tree of four plant species. (B) TCP family members of four plant species

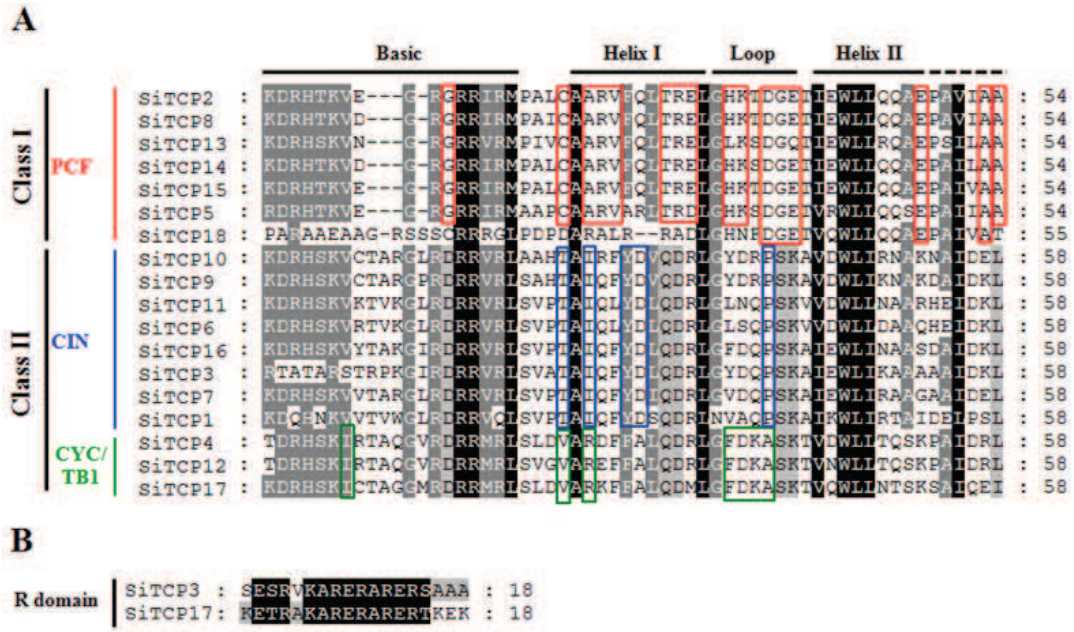


Fig. 2. Multiple sequence alignment of foxtail millet TCP transcription factors. (A) Alignment of the TCP domain for the predicted foxtail millet TCP proteins. Black and gray boxes showed the conserved amino acids in two TCP subfamilies; red, residues conserved in the PCF subclade; blue, conserved in the CIN subclade; green, conserved in the CYC/TB1 proteins. The basic, helix I, loop, and helix II regions are indicated. (B) Multiple sequence alignment of the R domain

Conserved motif identification and gene structure analysis

To evaluate the structural divergences and evolutionary relationships of TCP proteins in foxtail millet, the conserved motifs and exon/intron organization of SiTCPs were analyzed. A new phylogenetic tree was then constructed using the full-length amino acid sequences of SiTCPs (Fig. 3). We used the MEME4.11.2 tool to predict the conserved motifs. About 12 different motifs of between 6 and 100 residues were detected in foxtail millet (Supplementary Table S2). As expected, all 18 SiTCPs harbored the highly conserved TCP domain (motif 1). Members of the same subclade usually had a similar motif composition (Fig. 3). Thus, the motif distribution confirmed that the TCP proteins were conserved during evolution. The differences in motif distribution among classes and subclades of TCP proteins were the structural basis for the diversity in gene functions.

Overall, the *SiTCP* genes exhibited a relative conserved exon/intron organization: 14 of 18 SiTCP genes had no intron, while *SiTCP3* and *SiTCP10*

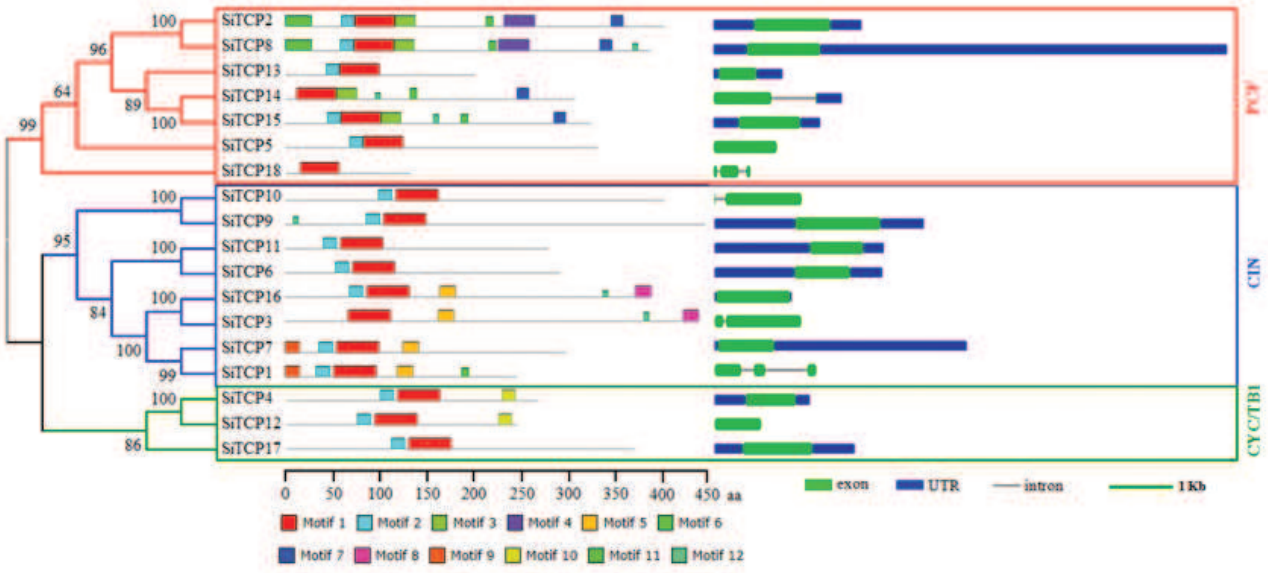


Fig. 3. Motif distribution and exon/intron structures of foxtail millet TCP genes. The protein motifs of the foxtail millet TCP genes are shown on the left. The exon/intron organization is shown on the right

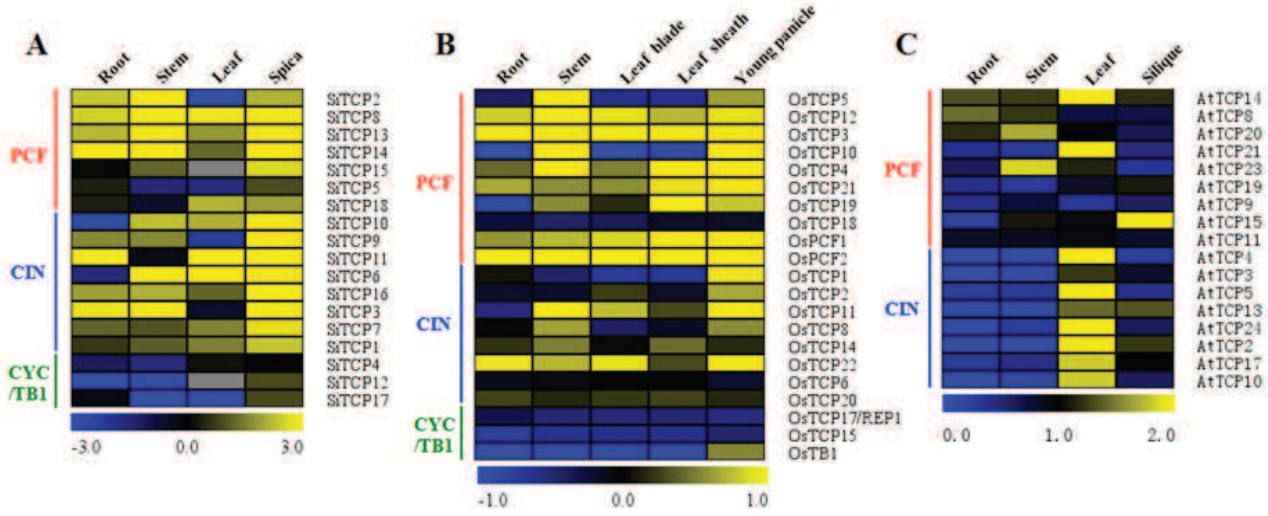


Fig. 4. Comparative tissue-specific transcript profiles of TCP genes in foxtail millet, rice and *Arabidopsis*. (A) Expression profiles of the 18 *SiTCP* genes. Color scale represents RPKM normalized $\log_2$ transformed counts, (B) Expression profiles of the 21 *OsTCP* genes. The color scale indicates the relative gene expression levels (Z-scores) and (C) Expression profiles of the 17 *AtTCP* genes

possessed one intron; and *SiTCP1* and *SiTCP18* had two introns (Fig. 3). In addition, the *SiTCPs* in the same subclade had very similar exon/intron distribution patterns in terms of exon length. To study the evolution of foxtail millet and other plant species TCPs, we also diagramed the TCPs exon/intron structures of rice and *Arabidopsis*. The results showed that most of OsTCPs and AtTCPs had one exon, several TCP members had two or three exons (Supplementary Fig. S3). It has been reported that most of SbTCPs also had only one exon (Francis et al. 2016). This suggested that these conserved exon/intron structures might have originated in the emergence of land plants and experienced less variation during evolutionary process.

Comparative transcriptome analysis of TCP family genes in foxtail millet

To investigate the tissue-specific transcript profiles of TCP genes in foxtail millet, publicly available RNA-seq. expression data was used to analyze TCP gene expression. As shown in Fig. 4A, most of the *SiTCP* genes exhibited tissue-specific transcript accumulation patterns, while other *SiTCP* genes were constitutively expressed in all tissues at relatively high transcript levels. These findings indicated the functional divergence of *SiTCP* genes during foxtail millet growth and development. In general, the PCF and CIN type *SiTCP* genes had wider expression domains and higher expression levels than the CYC/TB1 type genes.

To further investigate the conservation and/or diversification of the expression patterns of TCP genes in grasses, we compared the transcript levels of the physiologically similar tissues of foxtail millet, rice and *Arabidopsis* (Fig. 4). There were no available public publishing transcript data of seven TCP members including four PCF and three CYC/TB1 members in *Arabidopsis*. Therefore, we investigated 17 TCP genes expression levels in four tissues. In the PCF subclade, the expression pattern of *OsTCP* genes was found to be similar to that of *SiTCP* genes. However, PCF members in *Arabidopsis* showed different expression pattern compared to foxtail millet and rice. Most of PCF members showed strong expression levels in only one tissue, for example, AtTCP14 and AtTCP21 in leaf, AtTCP23 in stem and AtTCP15 in silique. The other three PCF members including AtTCP9, AtTCP11, AtTCP19 all showed relative low transcript levels in all four tissues.

In the CIN subclade, all *SiTCP* genes showed high transcript levels in spica. Similarly, most of the *OsTCP* genes exhibited high transcript levels in young panicles, except *OsTCP6*. However, all CIN members in *Arabidopsis* all exhibited high transcript levels in leaf, but low expression levels in the other three tissues, implying their important role in leaf development. This expression pattern were in accord with the functions of CIN members in *Arabidopsis*. *AtTCP2*, *AtTCP3*, *AtTCP4*, *AtTCP10* and *AtTCP24* play an important role in leaf development (Palatnik and Allen 2003; Palatnik et al. 2007).

In the CYC/TB1 subclade, the members of foxtail millet and rice all showed relative low transcript levels in detected tissues. These findings indicated that these members might play roles in specific organs or sites. The different expression pattern of TCP genes of three plant species indicated the diversification roles of TCP protein during evolutionary process.

Authors' contribution

Conceptualization of research (MXG, XSZ); Designing of the experiments (MXG, YWC); Contribution of experimental materials (YWC, JXQ); Execution of field/lab experiments and data collection (MXG, JXQ); Analysis of data and interpretation (MXG, XSZ); Preparation of manuscript (MXG, YWC, XSZ).

Declaration

The authors declare no conflict of interest.

Acknowledgements

This work was supported by the Key Science and Technology Program of Henan Province (Grant No. 162102110160, No. 152102110098, No. 152102210334) and Key Scientific Research Project for Higher Education of Henan Province (Grant No. 16B220003, No. 17A180030).

References

Aguilar-Martínez J. A., Poza-Carrión C. and Cubas P. 2007. Arabidopsis *BRANCHED1* acts as an integrator of branching signals within axillary buds. Plant Cell, **19**: 458-472.

Carpenter R. and Coen E. S. 1990. Floral homeotic mutations produced by transposon-mutagenesis in *Antirrhinum majus*. Genes Dev., **4**: 1483-1493.

Braun N., de Saint Germain A., Pillot J.P. *et al*. 2012. The pea TCP transcription factor *PsBRC1* acts downstream of strigolactones to control shoot branching. Plant Physiol., **158**: 225-238.

Cubas P., Lauter N., Doebley J. et al. 1999. The TCP domain: a motif found in proteins regulating plant

growth and development. Plant J., **18**: 215-222.

Doebley J., Stec A. and Hubbard L. 1997. The evolution of apical dominance in maize. Nature, **386**: 485-488.

Francis A., Dhaka N., Bakshi M. et al. 2016. Comparative phylogenomic analysis provides insights into TCP gene functions in Sorghum. Sci. Rep., **6**: 38488.

Guo A. Y., Zhu Q. H., Chen X. et al. 2007. GSDS: a gene structure display server. Yi Chuan = Hereditas/ Zhongguo Yi Chuan Xue Hui Bian Ji, **29**: 1023-1026.

Kebrom T. H., Burson B. L. and Finlayson S. A. 2006. Phytochrome B represses *teosinte branched1* expression and induces sorghum axillary bud outgrowth in response to light signals. Plant Physiol., **140**: 1109-1117.

Kosugi S. and Ohashi Y. 1997. PCF1 and PCF2 specifically bind to *cis* elements in the rice proliferating cell nuclear antigen gene. Plant Cell, **9**: 1607-1619.

Kosugi S. and Ohashi Y. 2002. DNA binding and dimerization speciûcity and potential targets for the TCP protein family. Plant J., **30**: 337-348.

Koyama T., Sato F. and Ohme-Takagi M. 2010. A role of TCP1 in the longitudinal elongation of leaves in Arabidopsis. Biosci. Biotech. Bioch., **74**: 2145-2147.

Luo D., Carpenter R., Vincent C. *et al.* 1996. Origin of ûoral asymmetry in *Antirrhinum*. Nature, **383**: 794-799.

Martin-Trillo M. and Cubas P. 2010. TCP genes: a family snapshot ten years later. Trends Plant Sci., **15**: 31-39.

Minakuchi K., Kameoka H., Yasuno N. *et al*. 2010. *FINE CULM1*(*FC1*) works downstream of strigolactones to inhibit the outgrowth of axillary buds in rice. Plant Cell Physiol., **51**: 1127-1135.

Mishra A. K., Muthamilarasan M., Khan Y. et al. 2014. Genome-wide investigation and expression analyses of WD40 protein family in the model plant foxtail millet (*Setaria italica* L.). PLoS One, **9**: e86852.

Mukhopadhyay P. and Tyagi A. K. 2015. *OsTCP19* influences developmental and abiotic stress signaling by modulating ABI4-mediated pathways. Sci. Rep., **5**: 9998.

Palatnik J., Allen E., Wu X. *et al*. 2003. Control of leaf morphogenesis by microRNAs. Nature, **425**: 257-263.

Nicholas K. B., Nicholas H. B. and Deerfield W. I. 1997. GeneDoc: Analysis and visualization of genetic variation. Embnet News, **4**: 28-30.

Palatnik J. F., Wollmann H., Schommer C. et al. 2007. Sequence and expression differences underlie functional specialization of Arabidopsis microRNAs miR159 and miR319. Dev. Cell, **13**: 115-125.

Saeed A. I, Sharov V., White J. et al. 2003. TM4: a free, open-source system for microarray data management and analysis. Biotechniques, **34**: 374-378.

Takeda T., Suwa Y., Suzuki M. et al. 2003. The *OsTB1* gene negatively regulates lateral branching in rice. Plant J., **33**: 513-520.

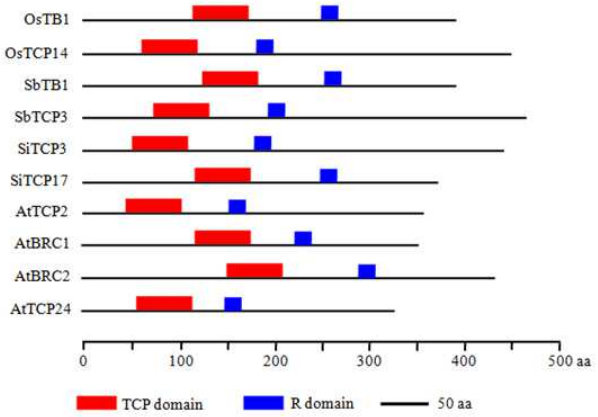
Tamura K., Stecher G., Peterson D. et al. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol. Biol. Evol., **30**: 2725-2729.

Thompson J. D., Gibson T. J., Plewniak F. et al. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res., **25**: 4876-4882.

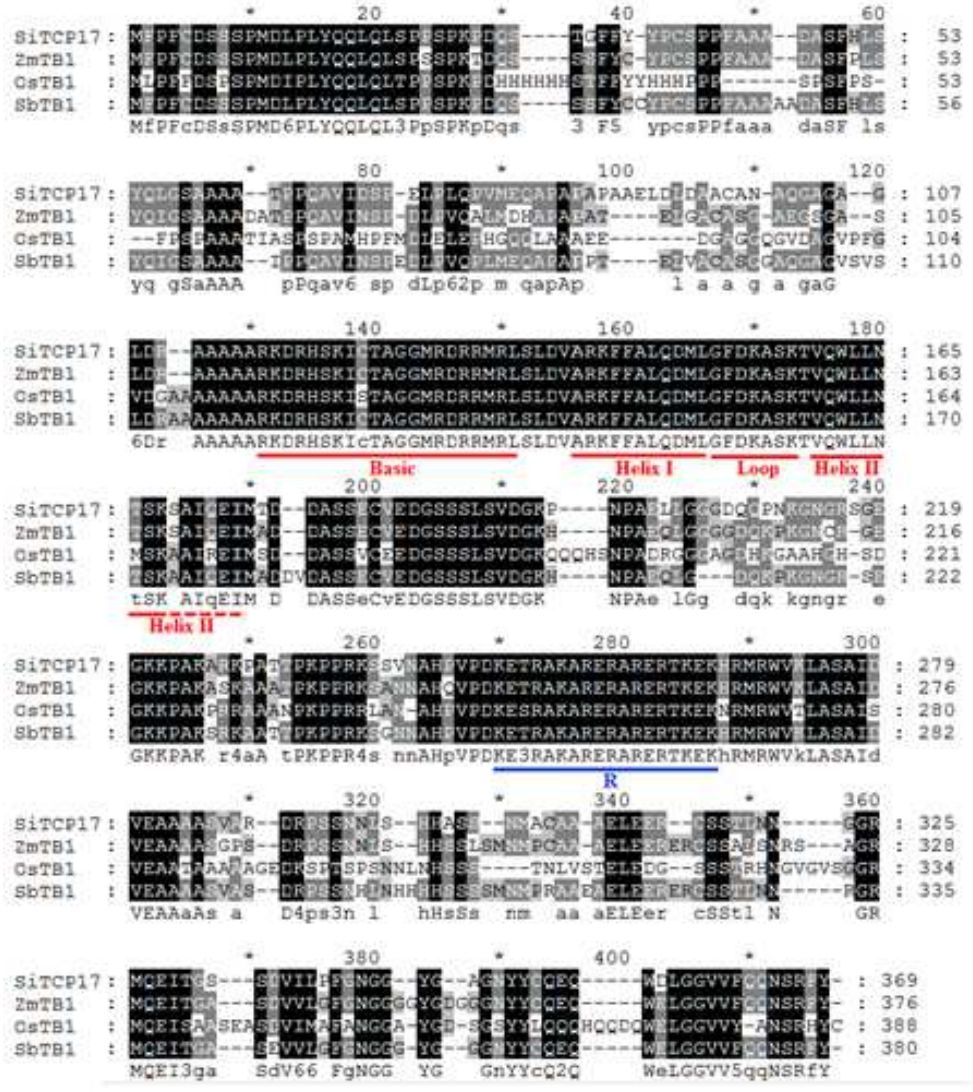
Wang L., Xie W., Chen Y. et al. 2010. A dynamic gene expression atlas covering the entire life cycle of rice. Plant J., **61**: 752-766.

Yao X., Ma H., Wang J. et al. 2007. Genome-wide comparative analysis and expression pattern of TCP gene families in *Arabidopsis thaliana* and *Oryza sativa*. J. Integr. Plant Biol., **49**: 885-897.

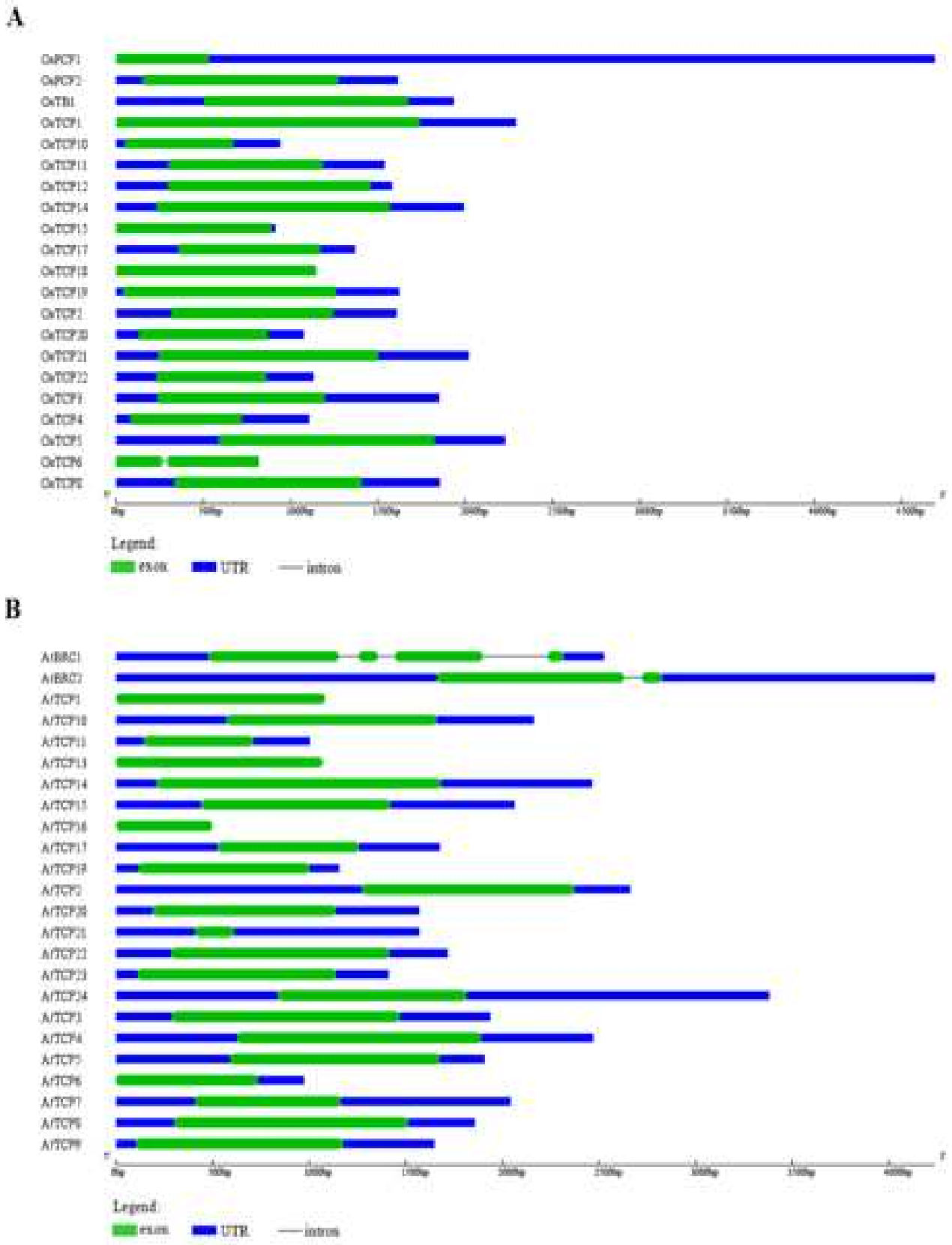
Supplimentary Figure S1. The distribution of TCP and R domains of partial TCP proteins



Supplimentary Figure S2. Multiple sequence alignment of SiTCP17, ZmTB1, OsTB1, SbTB1. Two conserved moifs speciûc to TCP proteins are indicated (bHLH and R motifs). Conserved amino acids are highlighted in shades of black and gray. White letter with black background (100% identity), white letter with gray background (80%), black letter with gray background (60%)



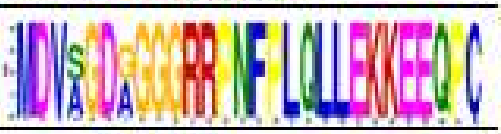
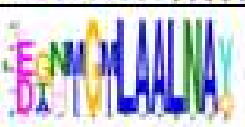
Supplimentary Figure S3. Exon/intron structures of rice and Arabidopsis TCP genes. (A) Rice. (B) Arabidopsis



Supplimentary Table S1. Summary of functional domains of TCP proteins in foxtail millet

Gramene locus ID	Gene name	Subfamily	Subclade	TCP (IPR017887)		R (IPR017888)	
				Start	End	Start	End
Si019114m	SiTCP1	II	CIN	39	96	-	-
Si017396m	SiTCP2	I	PCF	66	119	-	-
Si029871m	SiTCP3	II	CIN	55	112	181	198
Si033183m	SiTCP4	II	CYC/TB1	105	162	-	-
Si030591m	SiTCP5	I	CYC/TB1	75	128	-	-
Si022833m	SiTCP6	II	CIN	60	117	-	-
Si022811m	SiTCP7	II	CIN	42	106	-	-
Si006631m	SiTCP8	I	PCF	65	118	-	-
Si004564m	SiTCP9	II	CIN	93	150	-	-
Si004293m	SiTCP10	II	CIN	106	163	-	-
Si002496m	SiTCP11	II	CIN	47	104	-	-
Si015230m	SiTCP12	II	CYC/TB1	81	138	-	-
Si011076m	SiTCP13	I	PCF	50	103	-	-
Si010765m	SiTCP14	I	PCF	4	57	-	-
Si026501m	SiTCP15	I	PCF	51	104	-	-
Si036075m	SiTCP16	II	CIN	75	132	-	-
Si038692m	SiTCP17	II	CYC/TB1	117	174	249	266
Si039258m	SiTCP18	I	CYC/TB1	6	60	-	-

Supplimentary Table S2. Conserved motiss found in TCP proteins in foxtail millet

Motif	E-value	Sites	Width	Motif logo
Motif 1	2.4e-358	17	45	
Motif 2	1.4e-070	15	15	
Motif 3	3.2e-020	4	19	
Motif 4	1.1e-008	2	33	
Motif 5	3.1e-008	4	17	
Motif 6	1.1e-005	2	28	
Motif 7	3.1e-005	4	13	
Motif 8	9.2e-004	2	17	
Motif 9	2.3e-003	2	15	
Motif 10	1.5e-003	2	14	
Motif 11	1.4e-001	5	8	
Motif 12	7.3e-001	6	6