Biochemical Genetics (2022) 60:204–222
https://doi.org/10.1007/s10528-021-10100-8

ORIGINAL ARTICLE

Genome-Wide Identification and Transcript Analysis of TCP Gene Family in Banana (*Musa acuminata* L.)

Jingyi Wang[1] · Zhuo Wang[1,2] · Caihong Jia[1] · Hongxia Miao[1,2] · Jianbin Zhang[1] · Juhua Liu[1,2] · Biyu Xu[1] · Zhiqiang Jin[1,2]

Received: 28 February 2020 / Accepted: 9 June 2021 / Published online: 22 June 2021

Abstract

Plant-specific TEOSINTE-BRANCHED1/CYCLOIDEA/PROLIFERATING CELL FACTOR1 (TCP) gene family has versatile functions in diverse aspects of plants. However, less research on banana TCPs was done comprehensively. Accordingly, 48 banana TCP genes were characterized on aspects of gene structure, conserved motifs, phylogenetic relationship, and expression patterns. Members of *the MaTCP* gene family were unevenly distributed among 11 chromosomes and purification selection was the driving force of *the MaTCP* gene family. Gene duplication analysis indicated that segmental duplication is the major contributor to family expansion. Promoter analysis showed that *MaTCPs* might be involved in banana growth, development, and abiotic stress responses. Further, the expression of 12 *MaTCPs* was analyzed by real-time quantitative RT-PCR, and the protein interaction analysis showed that *MaPCF10* and *MaPCF13* may have an important function in banana fruit development and ripening. These results lay the foundation for further study of the functions of TCP genes in banana.

Keywords Banana · Co-expression network · Expression characteristics · *MaTCPs* · Phylogenetic analysis

Jingyi Wang and Zhuo Wang are equally Contributed.

✉ Juhua Liu
liujuhua@itbb.org.cn

✉ Biyu Xu
xubiyu@itbb.org.cn

✉ Zhiqiang Jin
jinzhiqiang@itbb.org.cn

Extended author information available on the last page of the article

Introduction

The TCP gene family, a kind of plant-specific transcription factors (TFs), has versatile functions in diverse aspects of plant growth and development. The name of the TCP gene family originated from the first four identified genes: TEOSINTE BRANCHED1 (TB1) from maize, CYCLOIDEA (CYC) from snapdragon, and PCFs (PCF1 and PCF2 proteins from rice) (Doebley et al. 1997; Cubas et al. 1999; Luo et al. 1999). Members of this family have about 60-amino-acids encoded basic Helix–Loop–Helix (bHLH) conserved regions, which performs the functions of DNA binding, protein–protein interaction, and protein nuclear localization (Cubas et al. 1999). According to the structure of the bHLH conserved region, the TCP gene family can be divided into two subfamilies, namely Class I (PCF or TCP-P) and Class II (TCP-C) (Manassero et al. 2013). Class II is further subdivided into the CINCINNATA (CIN) and CYC/TB1 subgroups. Class I TCP proteins contain only one typical TCP conserved domain and have 4 amino acid deletions, while Class II TCP members usually have unique R domains (arginine motifs with 18–20 residues) and ECE motif (a glutamate-cysteine-glutamate peptide chain), which is speculated that they may have the function of mediating protein interactions (Cubas et al. 1999; Li 2015). Moreover, the DNA binding sequence for the two classes differs slightly but partly overlaps (GGNCCCAC for class I and GTGGNCCC for class II) (Kosugi and Ohashi 2002; Masuda et al. 2008).

The TCP gene family is widely involved in the biological processes of the plant life cycle, including branching (Takeda et al. 2003; Aguilar-Martínez et al. 2007), leaf development (Kieffer et al. 2011), flower development (Kieffer et al. 2011), hormone pathways (Aguilar-Martínez et al. 2007), seed germination (Tatematsu et al. 2008), gametophyte development (Pagnussat et al. 2005), mitochondrial biogenesis (Hammani et al. 2011), regulation of the circadian clock (Giraud et al. 2010), and fruit development and ripening (Parapunova et al. 2014) in various plants. To date, genome-wide identification of the TCP proteins has been performed in a variety of plants, such as Arabidopsis (Riechmann et al. 2000), rice (Yao et al. 2007), tomato (Parapunova et al. 2014), cotton (Ma et al. 2014), apple (Xu et al. 2014), strawberry (Wei et al. 2016) and grapevine (Leng et al. 2019). However, very little is known about the TCP family in banana, which was the most consumed fruit growing around the world. Based on transcriptomic data, Song et al. (2018) initially analyzed 25 banana TCP family genes and explored the function in banana fruit ripening. However, based on RNA-seq alone, it was difficult to achieve more complete and detailed annotations of the TCP genes in banana. In 2012 and 2016, the *M. acuminata* doubled-haploid genotype (A genome) sequencing and annotations were completed successfully (D'Hont et al. 2012, Martin et al. 2016), which laid the foundation for the genomic-identification of the TCP gene family in banana.

Despite the possible role of TCP family genes in banana fruit ripening has been studied based on RNA-seq data, our understanding of this family still lags behind compared with other plants like Arabidopsis, rice, and maize. Due to the

critical role of TCP transcription factors in the control of plant development, fruit development and ripening, and abiotic stress responses, we performed the comprehensive analysis of the *MaTCP* family in banana. In this study, 48 *MaTCP* genes were characterized in *M. acuminata* using the newly genome version 2 (Martin et al. 2016). Comprehensive analyses of their phylogenetic relationship, conserved motifs, gene structure, location and duplication pattern, and cis-acting elements were conducted. Then, the dynamic expression patterns of these genes were investigated in various organs, in different stages of fruit development and ripening, and response to different abiotic stresses in banana. Further, the expression of 12 *MaTCP* genes was analyzed by real-time quantitative RT-PCR, and the protein–protein interaction network of *MaTCP*s was predicted. The results provide valuable information for further functional analyses of TCP genes in *M. acuminata* and other Musa species.

Materials and Methods

TCP Genes Identification and Phylogenetic Analysis

Arabidopsis and *Oryza sativa* TCP proteins were obtained from the TAIR (http://www.arabidopsis.org) and RGAP (http://rice.plantbiology.msu.edu) databases, respectively. Banana TCP proteins were retrieved from the DH-Pahang genome database (http://banana-genome-hub.southgreen.fr/). BLAST analysis was used to identify the predicted banana TCPs with all the TCPs from *A. thaliana* and *O. sativa* queries. After removing the repeat sequences, all the candidate TCPs were confirmed to encode the conserved TCP domain with SMART (http://smart.embl-heidelberg.de/) (Schultz et al. 1998).

A neighbor-joining (NJ) phylogenetic tree was constructed for the identified *M. acuminata*, 24 *A. thaliana* and 22 *O. sativa* TCPs using MEGA5.0 (Tamura et al. 2011) with 1000 bootstraps. The exon/intron structures were analyzed using the online Gene Structure Display Server (GSDS) with coding sequences and genomic sequences (Hu et al. 2015a) (http://gsds.cbi.pku.edu.cn/). The MEME (http://meme-suite.org/tools/meme) and SMART were used to identify and annotate the conserved motifs of *MaTCP*s. To predict miR319 target sites, full-length *MaTCP* nucleotide sequences were analyzed using the online software psRNATarget (Dai and Zhao, 2011) (http://plantgrn.noble.org/psRNATarget).The upstream 1-kb fragments from *MaTCP* genes were used to predict the *cis*-acting elements by PlantCARE (Lescot et al. 2002).

Chromosomal Location and Gene Duplication

The chromosomal location of each TCP gene was retrieved from the DH-Pahang genome database. Paralogous TCP genes were determined by the genomic sequence identity and the following two criteria were used: > 70% alignment coverage of the longer gene and > 70% identity in the aligned region (Yang et al. 2008). In addition,

to further analyze gene duplication events, the synonymous substitution rate (Ks) and nonsynonymous substitution rate (Ka) were calculated using the software DnaSp (Librado and Rozas, 2009). The divergence times of the gene duplication events were also calculated based on the formula $T = Ks/2\lambda$ ($\lambda = 4.6 \times 10^{-9}$) (Lescot et al. 2008).

Plant Material and Transcriptome Analysis

BaXi Jiao (*M. acuminata*, Cavendish cultivar group, AAA) was used as the main plant material. Roots, leaves, flowers and fruits at 80 days after emergence from pseudostem (DAF) were sampled for the tissue-specific expression analysis. Fruits at 0, 20 and 80 DAF (0 days after harvest (DPH)), 8 DPH, and 14 DPH were collected for fruit development and ripening analysis. For studying the expression profile of the *MaTCP*s under abiotic stresses, the five-leaf stage banana plantlets were subjected to 200 mM mannitol or 300 mM NaCl for 7 days and 4 °C for 22 h, respectively. Transcriptome sequencing (RNA-seq) was performed as described previously (Hu et al. 2015b). Gene expression levels were calculated as FPKM. The gene expression profiles of *MaTCP*s were visualized by the heatmap via Multi Experiment Viewer (MeV) software (http://mev.tm4.org/).

Real-Time Quantitative RT-PCR

The qRT-PCR was performed using SYBR® Premix Ex Taq™ (TaKaRa, Shiga, Japan) on a Mx3005P™ Real-Time PCR System (Stratagene, CA, USA). The banana *MaActin* (EF672732) was used as a reference gene. The relative expression levels of the target genes were assessed based on the $2^{-\Delta\Delta Ct}$ method (Livark and Schmittgen 2001). For each gene, three biological replicates were carried out.

Construction of the Regulatory Networks

To further explore the function of MaTCPs, the potential protein interaction and co-expression networks were identified based on the banana genome database and transcriptome analysis. The co-expression network analysis was performed as described previously (Wang et al. 2020). We extracted the co-expression network of all MaTCPs and the network connections were visualized using Cytoscape software v.3.4.0 (Shannon et al. 2003).

Results

Identification and Evolutionary Analysis of *MaTCPs* in Banana

A total of 48 TCP genes were characterized by the *M. acuminata* genome. These TCP proteins have a peptide length ranging from 248 (*MaPCF4*) to 607 (*MaCIN9*) amino acids, with an average of 362.8 amino acids. The molecular weight (Mw) and

isoelectric point (pI) varied from 25.23 (*MaPCF4*) to 65.18 (kDa) (*MaCIN9*) and from 5.25 (*MaPCF7*) to 10.12 (*MaTB2*), respectively. The other detailed information was shown in Supplementary Table S1. The alignment analysis showed that *MaTCP*s shared high homology in the TCP domain (Fig. 1A). Banana TCP proteins could be divided into two subfamilies. There was a four amino acid deletion in the

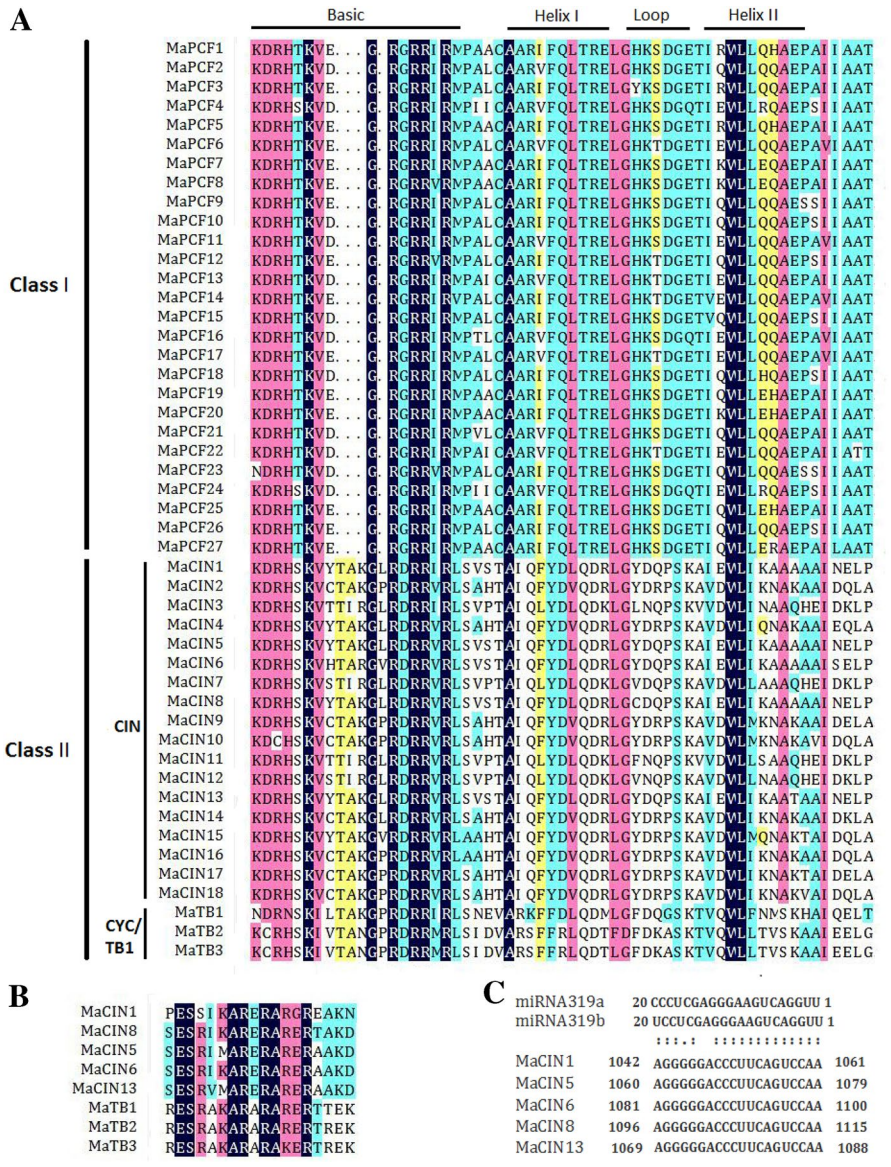

Fig. 1 Multiple alignments of TCP protein sequences for *M. acuminata*. **a** Alignment of the TCP domain for the predicted banana TCP proteins. **b** Alignment of the R-domain of class Π subfamily members. **c** Alignment of miR319 complementary sequences with *MaTCP* genes

basic helix–loop–helix-type motif of Class I compared with that of class II proteins. The two subfamilies, class I and class II, contained 27 and 21 genes, respectively. Moreover, the class II subfamily is differentiated into two subclades, CYC/TB1 and CIN (Figs. 1A and 2). The CYC/TB1 subclade contained three banana genes, *MaTB1*, *MaTB2*, and *MaTB3* and CIN included 18 members, *MaCIN1–MaCIN18* (Figs. 1A and 2). In *M. acuminata*, all three genes, *MaTB1*, *MaTB2*, and *MaTB3,* in CYC/TB1 and 5 genes, *MaCIN1*, *MaCIN5*, *MaCIN6*, *MaCIN8*, and *MaCIN13*, in the CIN subclade encoded proteins with the R-domain (Fig. 1B). This result was consistent with the previous research in Arabidopsis (Yao et al. 2007). Meanwhile, 5 genes in the CIN subclade (*MaCIN1*, *MaCIN5*, *MaCIN6*, *MaCIN8* and *MaCIN*13) harbored putative target site of miR319 (Fig. 1C). Similarly, there were five CIN family members (*AtTCP2*, *AtTCP3*, *AtTCP4*, *AtTCP10* and *AtTCP24*) contained miR319 binding sites in Arabidopsis (Palatnik et al. 2003). Phylogenetic tree showed that *MaCIN1*, *MaCIN5*, *MaCIN6*, *MaCIN8* and *MaCIN*13 are grouped with *AtTCP2*

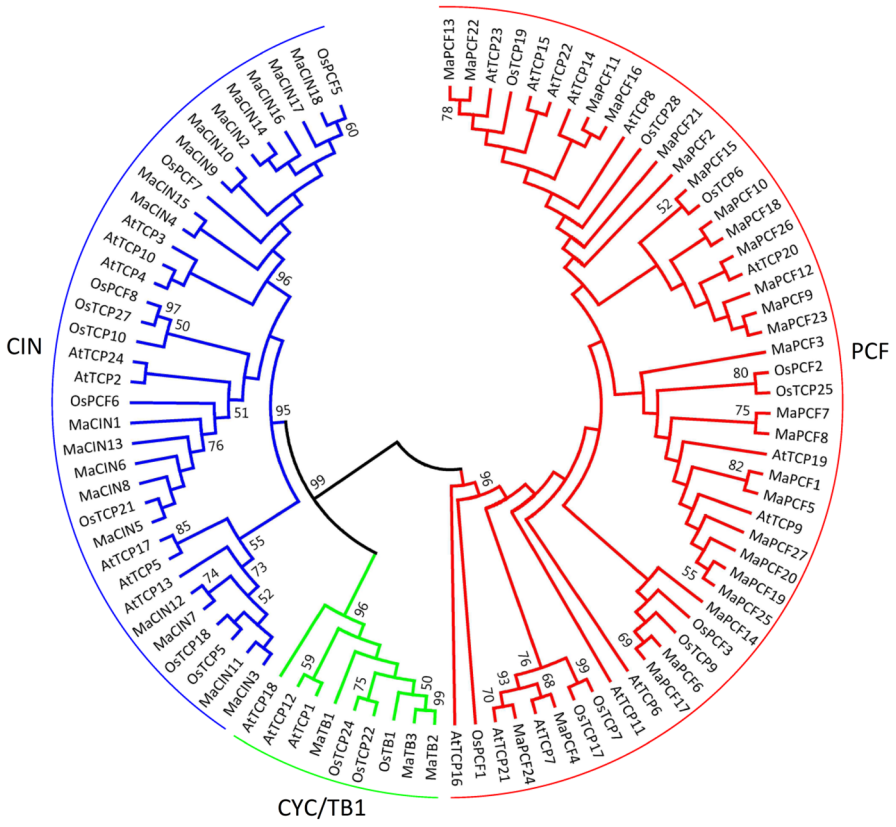

Fig. 2 Phylogenetic relationships of TCP transcription factors from banana, Arabidopsis and rice. The unrooted phylogenetic tree was constructed using MEGA5.0 by Neighbor-Joining method and the bootstrap test was performed with 1000 iterations. Red, blue, and green lines indicate the PCF, CIN and CYC/TB1 clades, respectively (Color figure online)

and *AtTCP24* of *A. thaliana* (Fig. 2). This result was also found in cassava (Lei et al. 2017), *P. mume* (Zhou et al. 2016) and cotton (Ma et al. 2014). It is revealed that the miR319 target sites were retained during the evolution and diversification of plants.

To study the evolutionary relationships of TCP family proteins, a phylogenetic tree was constructed by aligning 48, 24 and 22 TCP proteins from banana, Arabidopsis and rice. Two individual subfamilies (Classes I and II) were generated in Fig. 2. Proteins in Class II were further divided into 2 subclades: CYC/TB1 and CIN. All of the Arabidopsis and rice TCPs fell in the same Class or clade as previously reported (Manassero et al. 2013), confirming the reliability of our phylogenetic tree. Comparing the family gene number in banana, Arabidopsis and rice, the gene number in banana was the 2.0-fold size of that in Arabidopsis and rice. In addition, the biased expansion in *M. acuminata* was found. It occurred mainly in Class I and in CIN subclade, and the CYC/TB1 clade remained the same size as in Arabidopsis and rice (Table 1). Moreover, in comparison with other polyploidy crops, the gene member of *MaTCPs* was much higher than that of potato (23) and shortly higher than that of Chinese cabbage (39) and turnip (39), but it was significantly lower than that of wheat (66) (Table 1). It showed that species that experienced whole genome duplication or polyploidization seemed to have more duplication genes than species that did not experience such events (Liu et al. 2019).

Gene Structure and Conserved Motif Analysis of *MaTCP* Genes

A new phylogenetic tree of banana TCP genes was constructed, together with their exon/intron structure and conserved motif analysis (Fig. 3). Two genes (*MaPCF14* and *MaCIN9*) had 1–2 introns, while the others had no introns (Fig. 3B). A total of 20 conserved motifs were identified in banana TCP proteins (Fig. 3C and Supplementary Table S2). In short, their distribution was found to be almost consistent with the phylogenetic tree. Motif 1, which was identified as the TCP domain, was found in all the banana TCP proteins. Motif 2 was only found in all class I (PCF class). Motif 11 was only found in all class II. Motif 4 was only found in all CIN subclade. Eight proteins from class II (3 genes in CYC/TB1 and 5 genes in CIN subclade) were found to have the R domain (motif 16). Moreover, some proteins from

Table 1 TCP gene in *Arabidopsis*, rice, potato, Chinese cabbage, turnip, wheat and banana

Species	Class I TCPs	Class II TCPs		Total	References
	PCF	CIN	CYC/TB1		
Arabidopsis thaliana	13	8	3	24	Yao et al. 2007
Oryza sativa	10	9	3	22	Yao et al. 2007
Solanum tuberosum (Potato)	13	7	3	23	Bao et al. 2019
Brassica rapa (Chinese cabbage)	19	14	6	39	Liu et al. 2018
Brassica rapa (turnip)	20	13	6	39	Liu et al. 2018
Triticum aestivum (wheat)	33	21	12	66	Zhao et al. 2018
Musa acuminata	27	18	3	48	

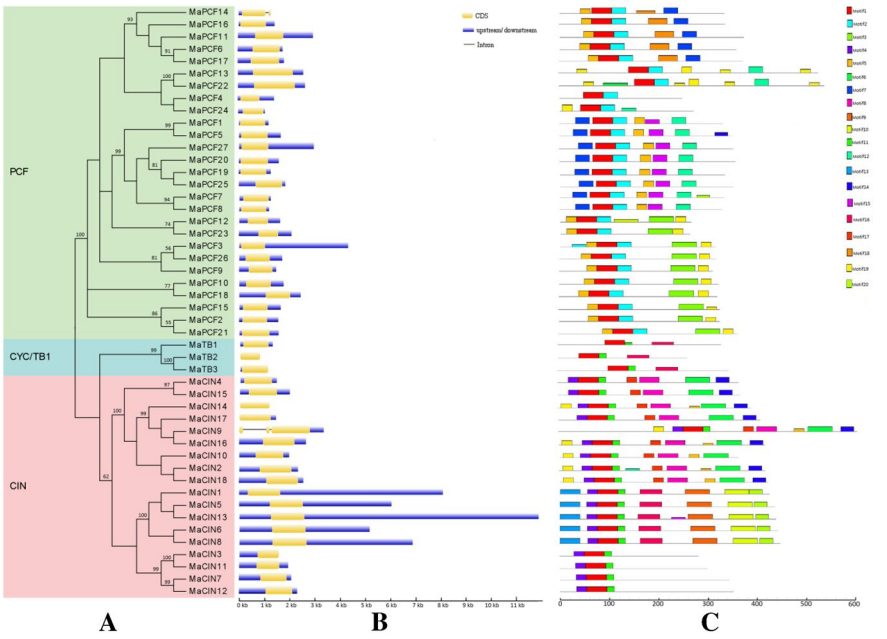

Fig. 3 Genomic structure and motif composition of banana TCPs. **a** Phylogenetic tree of *M. acuminata* TCP proteins. **b** Genomic structure of banana TCP genes. Exons, introns, and UTRs are indicated with yellow boxes, black lines and blue boxes, respectively. **c** Motif composition of banana TCP proteins. Conserved motifs in the banana TCP proteins are indicated by colored boxes (Color figure online)

the two classes had several special motifs, respectively. For example, 5 genes in CIN subclade (*MaCIN1*, *MaCIN5*, *MaCIN6*, *MaCIN8* and *MaCIN13*) harbored motifs 9, 10 and 13, while 8 genes in PCF class (*MaPCF1*, *MaPCF5*, *MaPCF7*, *MaPCF8*, *MaPCF25*, and *MaPCF27*) had motifs 7 and 15 (Fig. 3C).

Chromosomal Location and Gene Duplication Analysis

Based on the genomic data, 48 TCP genes were unevenly distributed on 10 of 11 banana chromosomes. The number of genes on each chromosome ranged from 0 (Chr 9) to 8 (Chr 7). The distribution of *MaTCP* genes on the 10 banana chromosomes was shown in Fig. 4 and the exact position of each gene on the banana chromosome was given in Supplementary Table S1. Thirty-four duplication events, including 20 gene pairs from PCF class and 14 pairs from Class II, were identified, accounting for 70.83% of the total TCP genes (Fig. 4 and Supplementary Table S3). In addition, 22 genes involved in two or more segmental duplication events (*MaPCF2/MaPCF15/MaPCF21, MaPCF3/MaPCF9/MaPCF26, MaPCF6/MaPCF16/MaPCF17, MaPCF7/MaPCF8/MaPCF19/MaPCF20/MaPCF25/MaPCF27* and *MaCIN2/MaCIN10/MaCIN14/ MaCIN9/MaCIN16/ MaCIN17/MaCIN18*).

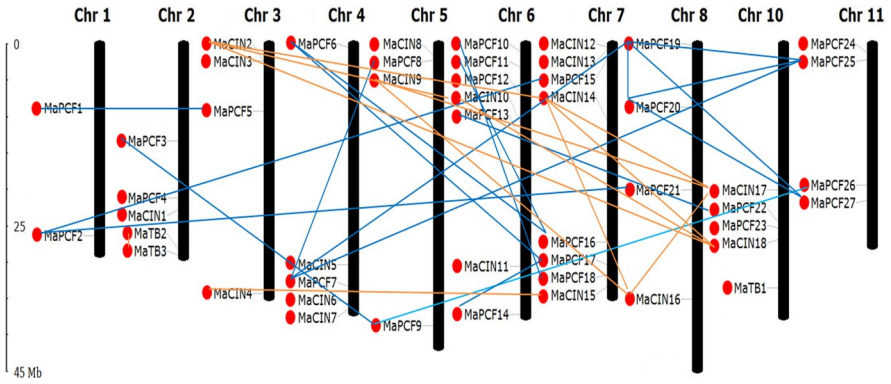

Fig. 4 Chromosomal location and gene duplication of TCP genes in *M. acuminata*. The scale is in megabases (Mb). The chromosome numbers are indicated at the top of each chromosome. The paralogous TCP genes from Class I and Class Π are connected with a blue and orange line respectively

Two or more adjacent homologous genes within 200 kb on a single chromosome were defined as tandem duplications (Guo et al. 2013). According to the definition, no tandem duplication event was identified. Thirty-four pairs of TCP genes associated with segmental duplication, indicating that segmental duplications had a vital role in *MaTCPs* expansion in the banana genome. Furthermore, the *Ka/Ks* values of paralogous genes were calculated. Interestingly, three pairs (*MaPCF1/MaPCF5*, *MaPCF13/MaPCF22* and *MaTB2/MaTB3*) exhibited Ka/Ks > 1.00 values, signifying positive selection, whereas the remaining underwent purifying selection with values less than 1.00. Finally, we also calculated the divergence time for these duplicated genes. The results showed that these gene pairs were estimated to diverge at approximately 5.80 ~ 100.58 million year ago (MYA) (Supplementary Table S3).

Expression Profiles of *MaTCPs* in Different Organs

The relative transcript abundance of *MaTCPs* in roots, leaves, flowers and fruits is shown in Fig. 5A and Supplementary Table S4. Generally, 39 out of 48 *MaTCPs* were detected in at least one tissue. A total of 10, 10, 11 and 8 genes had high expression (value > 10) in roots, leaves, flowers, and fruits, respectively. Notably, three *MaTCPs* (*MaPCF2*, *MaPCF4* and *MaPCF24*) exhibited high transcript levels (value > 10) in four organs. However, the representatives of class II TCPs were expressed mostly in leaves. Most of them were not expressed in roots and fruits. *MaCIN2* and *MaCIN11* expressed highly (value > 10) in leaves. *MaCIN1* and *MaTB1* expressed highly (value > 10) in flowers and fruits, respectively.

Expression Profiles of *MaTCPs* in the Fruit Development and Ripening

The expression profiles of *MaTCPs* in fruit development and ripening were also studied. Generally, 38 out of the 48 genes showed expression in different stages

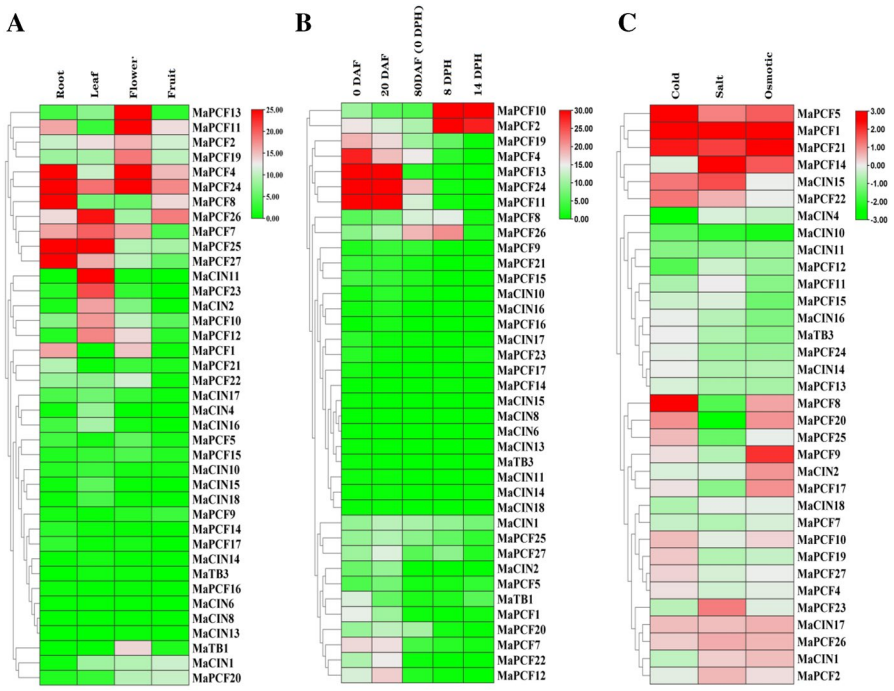

Fig. 5 Expression patterns of *MaTCPs*: **a** in the roots, leaves, flowers and fruits, **b** in different stages of fruit development and ripening, and **c** in response to cold, salt, and osmotic stresses in banana. The heat map **a** and **b** were created based on the FPKM value of MaTCPs. The heat map **c** was created based on log2-based values of *MaTCP*s. Differences in gene expression are shown in color as the scale

of fruit development and ripening (Fig. 5B, Supplementary Table S5). Five genes (*MaPCF2*, *MaPCF4*, *MaPCF11*, *MaPCF19* and *MaPCF24*) expressed highly (values > 10) at 0, 20 and 80 DAF. Notably, *MaPCF2* and *MaPCF10* expressed highly (value > 10) at 8 and 14 DPH of the fruits, suggesting that they might have an extensive and vital role in the fruit development and ripening.

Moreover, banana TCP genes with close phylogenetic relationships showed both similar and divergent expression patterns. For example, *MaPCF13* and *MaPCF22* were both expressed at a high level at 0 and 20 DAF and at a low level at 80 DAF, 8 and 14 DPH and the expression level of *MaPCF13* was higher than that of *MaPCF22* in each tissue we tested. With respect to *MaPCF7* and *MaPCF8*, however, divergent expression patterns were found at 0, 20, 80 DAF and 8 DPH, respectively (Supplementary Table S5).

Expression Profiles of *MaTCPs* Under Cold, Salt and Osmotic Stresses

Under the three stress conditions, the expression levels of most genes (34 out of the 48 *MaTCPs*) were modulated (Fig. 5C, Supplementary Table S6). Among them, 16, 12 and 14 genes showed increased expression while 18, 22 and 20 genes were

downregulated by cold, salt and osmotic stress, respectively. Intriguingly, there were 6 (*MaCIN15, MaPCF1*, *MaPCF5*, *MaPCF8*, *MaPCF21* and *MaPCF22*), 5 (*MaCIN15, MaPCF1, MaPCF14, MaPCF21* and *MaPCF23*) and 5 (*MaPCF1*, *MaPCF5*, *MaPCF9*, *MaPCF14*, and *MaPCF21*) *MaTCP* genes that were notably induced (value > 1) in response to cold, salt or osmotic stress, respectively. In addition, 3 *MaTCP* genes (*MaPCF1, MaPCF5* and *MaPCF21*) were upregulated in all of the stress conditions, suggesting they have major functions in response to various stress conditions.

Validation of the *MaTCP* Genes by qRT-PCR

Based on transcriptome sequencing data analysis, 12 differentially expressed *MaTCP*s were selected for qRT-PCR analysis. Primers for the quantification assay were shown in Supplementary Table S7. Four genes (*MaPCF4*, *MaPCF12*, *MaPCF13* and *MaPCF19*) were tested in various organs and 4 genes (*MaPCF2*, *MaPCF8*, *MaPCF10* and *MaPCF26*) were tested at different stages of fruit development and ripening. Four *MaTCP* genes (*MaPCF1, MaPCF5, MaPCF21* and *MaCIN15*) that were upregulated in response to each of the stress treatments were tested. The results showed that the expression patterns of the selected *MaTCP*s had the same trend and consistent results between RNA-seq data and qRT-PCR data (Fig. 6).

Putative Cis-Elements Analysis in the *MaTCP* Promoters

From promoter analysis, three groups of cis-elements, including plant growth and development, phytohormone responses and abiotic and biotic stress responses, were detected (Fig. 7 and Supplementary Table S8). For plant growth and development, three types of cis-elements (Skn-1_motif and GCN4_motif, CCGTCC box and O2-site) are involved in endosperm expression, meristem specific activation and zein metabolism regulation respectively were found in the most *MaTCP* promoters. The as-2-box and CAT-box, involved in light-responsive and meristem expression respectively, were detected in the promoter of six diverse *MaTCP*s. In addition, the HD-Zip1 and HD-Zip2 motifs, involved in leaf development were only presented in the *MaPCF1* promoter. In hormone responses, 2 MeJA-responsive elements (CGTCA-motif and TGACG-motif), salicylic acid-responsive element (TCA-element) and 2 ABA-responsive elements (ABRE, CE3) were found in 27, 25 and 19 *MaTCP*s respectively. Moreover, ethylene-responsive element (ERE), 2 auxin-responsive elements (AuxRR-core and TGA-element) and 3 gibberellin-responsive elements (TATC-box, P-box, and GRAE-motif) were also detected in 3, 7 and 9 *MaTCP*s, respectively. In stress responses, 10 stress-relative elements were detected. In details, 50.0% (24/48) and 52.1% (25/48) *MaTCP* promoter regions had drought-inducible element (MBS) and anaerobic induction element (ARE), respectively. Additionally, 29.2% (14/48), 25% (12/48) and 20.8% (10/48) *MaTCP* promoter regions had TC-rich repeats involved in defense and stress responses, fungal elicitor responsive element (Box-W1) and heat stress-responsive element (HSE),

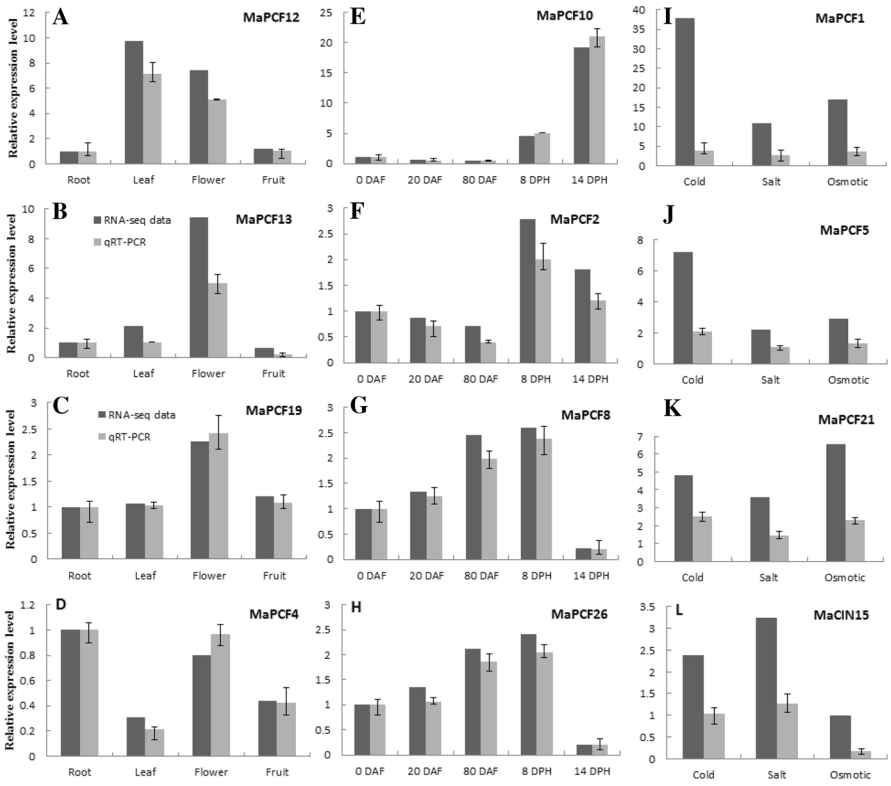

Fig. 6 Relative expression of *MaTCP*s in BX by qRT-PCR. **a**–**d** expression patterns of *MaPCF4*, *MaPCF12*, *MaPCF13* and *MPCF19* in different organs. The mRNA fold difference was relative to that of BX root samples used as calibrator. **e**–**h** expression patterns of *MaPCF2*, *MaPCF8*, *MaPCF10* and *MaPCF26* in different stages of fruit development and ripening. The mRNA fold difference was relative to that of BX 0DAF samples used as calibrator. **i**–**l** expression patterns of *MaPCF1*, *MaPCF5*, *MaPCF21* and *MaCIN15* in response to cold, salt and osmotic stresses. The mRNA fold difference was relative to that of untreated samples used as calibrator. Data are means ± SD of $n = 3$ biological replicates

respectively. Low temperature-responsive element (LTR) and GC-motif involved in anoxic specific inducibility were only found in 7 and 6 *MaTCP*s, respectively.

The Interaction Network of *MaTCP* Genes

To further explore the function of *MaTCP*s, the potential protein interaction and co-expression networks were identified using Cytoscape. Two MaTCP-mediated networks were constructed, and 23 and 47 interactive proteins for MaPCF10 and 13, respectively, were detected (Fig. 8, Supplementary Table S9 and S10).

The 47 key proteins that interacted with MaPCF13 were divided into 46 classes including the most abundant TF family bHLH (20), followed by the MYB (17), HB (12), C2H2 (11), SBP (8) and MIKC (8) with single-member for NAC, C3H, ARF, C2C2.CO.like, GNAT, etc. The 23 key proteins that interacted with MaPCF10 were

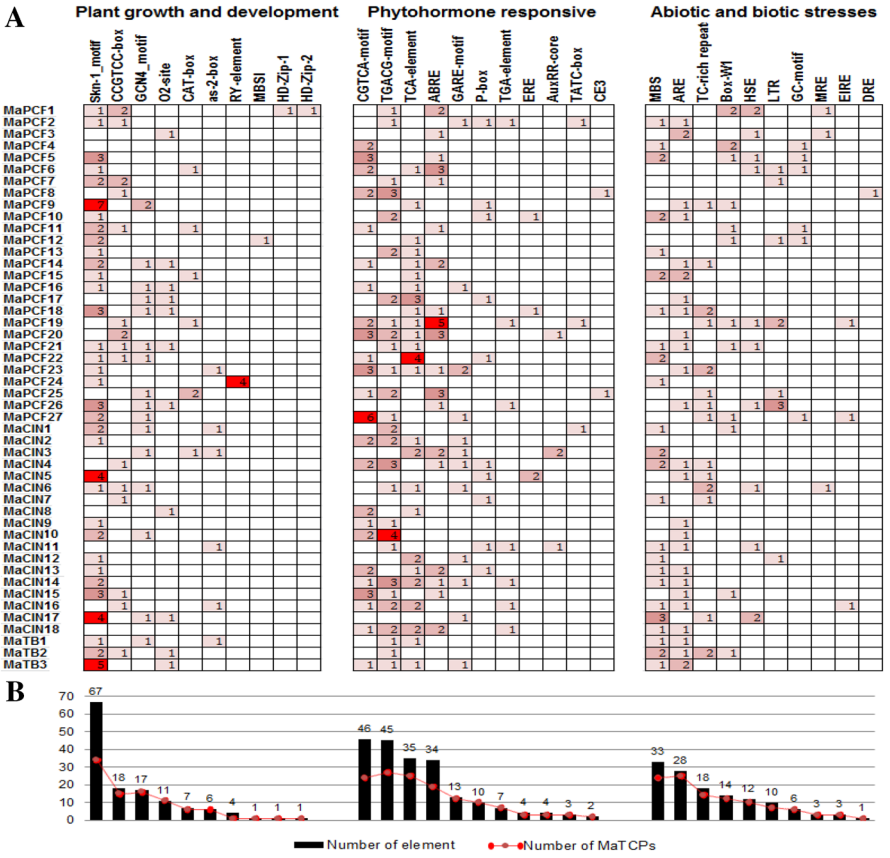

Fig. 7 Kinds and numbers of the cis-acting element analysis found in the promoter regions of banana TCP genes. **a** A number of each cis-acting element in the promoter region of *MaTCP*s. **b** Statistics for the total number of *MaTCP*s, including the corresponding cis-acting elements (red dot) and the total number of cis-acting elements in *MaTCP*s (black box) (Color figure online)

divided into 21 classes, including bHLH (3), HB (3) and C2H2 (3), followed by NAC (2), ERF (2), PHD (2), MYB (2), GRAS (2) and bZIP (2), with single members for HSF, G2-like, NF-YC, OFP, WRKY, C2C2-GATA, etc. Upon comparison of the proteins interacting with MaPCF10 and 13, 16 kinds of proteins (bHLHs, bZIP, MADS-box, MYB, HB, C2H2, OFP, ERF, WRKY, PHD, C2C2-GATA, G2-like, NAC, LOB, HSF and Orphans) were found to be very same, showing that these proteins may be fundamental for banana fruit development and ripening. Another 6 kinds of proteins (NF.YC, LIM, GRAS, Jumonji, NF.X1 and Trihelix) were found to specifically interact with MaPCF10, suggesting that these proteins may interact with MaPCF10 to participate in banana fruit ripening. In addition, 30 kinds of proteins (SET, PLATZ, AUX.IAA, C3H, AP2, C2C2-Dof, VOZ, SWI.SNF.BAF60b, HMG, ARF, IWS1, SBP, zf.HD, C2C2.CO.like, GRF, BBR.BPC, GANT, BES1, C2C2. YABBY, E2F.DP, SNF2, CPP, NF.YB, ARID, TRAF, ARR.B, GRAS, B3, TUB and

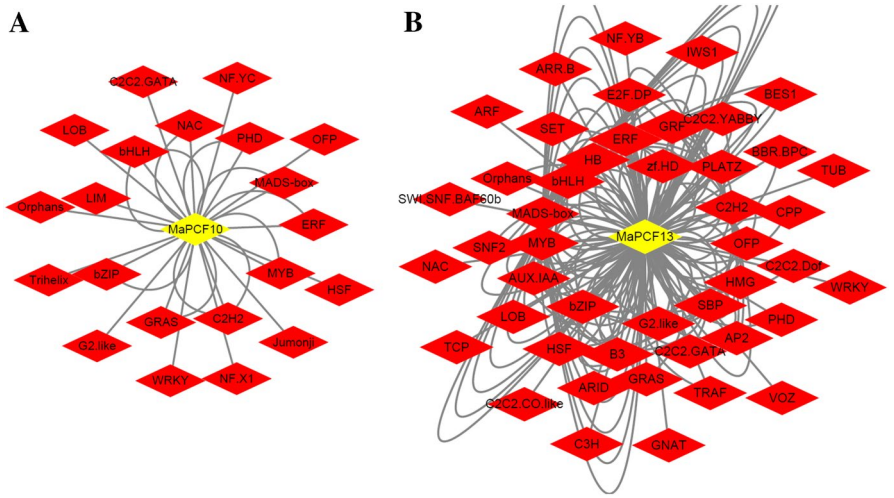

Fig. 8 The interactive networks of *MaPCF10* and 13 using Cytoscape. **a** Interaction network of *MaPCF10*. **b** Interaction network of *MaPCF13*

TCP) were found to specifically interact with MaPCF13, indicating that these proteins together with MaPCF13 may play a role in the banana fruit development.

Discussion

The TCP proteins are a kind of plant-specific TFs that are involved in regulating plant biological processes such as branch, leaf and flower development, hormone signaling and stress response. It is widely distributed in plant and the memmber of this gene family varies greatly among various species. To date, genome-wide identification of the TCP proteins has been performed in a variety of plants. Song et al. (2018) initially identified 25 banana TCP sequences based on banana transcriptome data related to fruit ripening. In our study, a total of 48 TCP genes were detected in *M. acuminata* genome based on the latest released banana genome database version 2 (Martin et al. 2016). 48 TCPs identified in our study included the 25 TCPs reported by Song et al. (2018) and the corresponding information was listed in Supplementary Table S1. It is significantly higher than that reported by Song et al. (2018). It is more comprehensive and accurate for gene family identification using the whole-genome information. Compared to other plants, the number of TCP genes in *M. acuminata* in our study is similar to that in *Malus domestica* (52) (Xu et al. 2014) and much higher than that in grape (18) (Leng et al. 2019), strawberry (19) (Wei et al. 2016), Arabidopsis (24) and rice (22) (Martín-Trillo and Cubas 2010). The number of TCP genes in *M. acuminata* is 2.0 times that in Arabidopsis, which is consistent with the fact that the protein-coding genes in the *M. acuminata* genome (36,542 genes) is about 1.4 times that in Arabidopsis (25,498 genes) (Arabidopsis Genome Initiative 2000; D'Hont et al. 2012).

Banana TCP gene encodes a protein sequence length ranging from 248 to 607 amino acids. Phylogenetic analysis shows that the family can be divided into two major classes, Class I and Class II, namely PCFs and CYC/TB1, which are consistent with the results of Arabidopsis and rice TCP proteins (Fig. 2). These TCPs in the same subclass had similar gene structures and protein motifs (Fig. 3), which implied that they may play similar molecular roles. Here, we found that most of the genes (95.83%) had no intron. It is in concert with the research that plants tend to retain the genes with no intron or a short intron (Mattick and Gagen 2001). Furthermore, all the proteins carried a highly conserved TCP domain (motif 1). Proteins in Class I and Class II also have different specific motifs respectively. The R domain (motif 16), with the function to mediate protein interactions, was also found in 8 proteins from class II (3 genes in CYC/TB1 and 5 genes in CIN subclade). It is speculated that these members may play a key role in protein interaction.

Gene duplication events are of great significance in gene family expansion and evolution. Segmental, tandem, and whole-genome duplication (WGD) are the main modes for the gene family expansion (Leister 2004). In this study, we found that most of the banana TCP genes were involved in segmental duplication in the banana genome, indicating that segmental duplication may be the significant driving force for the banana TCP gene family expansion. It is similar to the results of Arabidopsis, rice and maize (Yao et al. 2007; Chai et al. 2017). Based on synonymous substitution rate (Ks) analysis, the banana genome has undergone three WGDs during evolution, namely the α+ß WGD events (Ks≈0 ~ 0.45) and the γ WGD event (Ks≈0.45 ~ 0.85) that occurred 64.8 million years ago (Mya) and 96Mya respectively (D'Hont et al. 2012). The Ks value of 22 paralogous gene pairs was ranged from 0.0534 to 0.8180, showing the divergence time for these duplicated genes was ranged from 5.80 to 88.91 MYA. It suggested that WGD is also responsible for the expansion of the *MaTCP* gene family. This result was similar to *MaERF* and *MaWRKY* gene family (Lakhwani et al. 2016; Goel et al. 2016). In addition, we identified 2 TCP gene paralogs (*MaPCF7/MaPCF19* and *MaCIN4/MaCIN15* with Ks > 0.85,) attributing probably to an older duplication event. The Ka/Ks value of most of the paralogous gene pairs ranged from 0.15 to 0.67, showing the purifying selection of these genes (Supplementary Table S3). In summary, the banana TCP gene family may originate from more ancient duplication events and has undergone multiple WGDs. The combined effects of segmental duplication and WGDs may be the direct reason for the expansion of the TCP gene family in *M. acuminata*.

The expression pattern of TCP TFs in different tissues has been reported in plants, such as Arabidopsis (Martin-Trillo and Cubas, 2010), maize (Chai et al. 2017), apple (Xu et al. 2014) and strawberry (Wei et al. 2016). There are few studies on the dynamic and spatially expression profile of *MaTCP* genes in banana. Based on RNA-seq data, the tissue-specific expression of MaTCP was analyzed (Fig. 5A and Supplementary Table S4). It was found that the genes from two subclasses had a divergence tissue-specific expression. A previous study had reported that 8 CIN-type genes (*AtTCP2*, *AtTCP3*, *AtTCP4*, *AtTCP5*, *AtTCP10*, *AtTCP13*, *AtTCP17* and *AtTCP24*) may regulate leaf growth in Arabidopsis (Palatnik et al. 2003; Ori et al. 2007). In this study, 55% of the CIN-type (Class II) were highly expressed in the leaf. However, about 60% of the PCF genes

(Class I) were detected in all of the organs and were predominantly expressed in the root, leaf and flower. We speculate that Class I *MaTCP* genes might be involved in housekeeping functions during banana growth and development.

Previous research showed that Class I TCP proteins were involved in fruit development and ripening (Parapunova et al. 2014; Pillet et al. 2015; Wei et al. 2016). For example, *FaTCP11* and *FvTCP9* were found to play a role in fruit development respectively in strawberry (Pillet et al. 2015; Wei et al. 2016). Three tomato TCPs (*SlTCP12*, *15* and *18*) were reported to be preferentially expressed in tomato fruit and their promoter elements could be bound by the ripening–related proteins such as RIN, CNR and SlAP2a, respectively (Parapunova et al. 2014). Our results also showed that *MaTCPs* are likely to play a role in fruit development and ripening. Five genes (*MaPCF2*, *MaPCF4*, *MaPCF11*, *MaPCF19* and *MaPCF24*) showed highly expression during the stage of fruit development (0, 20 and 80 DAF). Eight genes (*MaPCF1, MaPCF7, MaPCF12, MaPCF13, MaPCF20, MaPCF22, MaPCF25* and *MaPCF27*) high expressed during the early stage of fruit development (0 and 20 DAF) (Fig. 5B and Supplementary Table S4). It is indicated that these genes may have a function in banana fruit development. Particularly, *MaPCF2* and *MaPCF10* were significantly expressed at 8 and 14 DPH, which was verified by qRT-PCR (Fig. 5). As for CIN-type, only MaCIN1 showed high expression in banana fruit development and ripening. Song et al. (2018) reported that *MaTCP5* and *MaTCP20* promoted the transcription of MaXTH10/11 that may play a role in fruit softening during banana ripening, whereas *MaTCP19* repressed their transcription, by directly binding to their promoters. Combined with the research, *MaTCP5, MaTCP19* and *MaTCP20* are equivalent to *MaCIN1, MaPCF10* and *MaPCF18* respectively in our study. These results indicated that *MaPCF10* might have a vital role in banana fruit ripening.

Moreover, most *MaTCP* genes showed apparent responses to different kinds of abiotic stresses (Fig. 5C). Numerous cis-acting elements involved in plant growth, development, hormone responses as well as stress responses were also found in the promoter region of the TCP genes (Fig. 6), implying that *MaTCPs* are also involved in abiotic stress signaling or tolerance, which is worthy of further study.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10528-021-10100-8.

Acknowledgements This research was supported by the Natural Science Foundation of Hainan Province (318MS090), the National Natural Science Foundation of China (31501043, 31872161), the National Natural Science Foundation of Hainan Province (2019CXTD412) and the Earmarked Fund for Modern Agro-industry Technology Research System (CARS-31).

Author Contributions JHL, ZQJ and BYX conceived the study. JYW, ZW and CHJ performed the experiments and carried out the analysis. XHM and JBZ carried out the analysis. JYW designed the experiments and wrote the manuscript. All authors read and approved the final manuscript.

Declarations

Conflict of interest The authors claim no conflict of interest.

References

Aguilar-Martínez JA, Poza-Carrión C, Cubas P (2007) *Arabidopsis* BRANCHED1 acts as an integrator of branching signals within axillary buds. Plant Cell 19(2):458–472. https://doi.org/10.1105/tpc.106.048934

Arabidopsis Genome Initiative (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature 408(6814):796–815. https://doi.org/10.1038/35048692

Bao S, Zhang Z, Lian Q, Sun Q, Zhang R (2019) Evolution and expression of genes encoding TCP transcription factors in *Solanum tuberosum* reveal the involvement of *StTCP23* in plant defence. BMC Genet 20:91. https://doi.org/10.1186/s12863-019-0793-1

Chai W, Jiang P, Huang G, Jiang H, Li X (2017) Identification and expression profiling analysis of TCP family genes involved in growth and development in maize. Physiol Mol Biol Plants 23(4):779–791. https://doi.org/10.1007/s12298-017-0476-1

Cubas P, Lauter N, Doebley J, Coen E (1999) The TCP domain: a motif found in proteins regulating plant growth and development. Plant J 18(2):215–222. https://doi.org/10.1046/j.1365-313x.1999.00444.x

Dai X, Zhao P (2011) psRNATarget: A plant small RNA target analysis server. Nucleic Acids Res. 39:W155–W159. https://doi.org/10.1093/nar/gkr319.P

D'Hont A, Denoeud F, Aury JM, Baurens FC, Carreel F, Garsmeur O et al (2012) The banana (*Musa acuminata*) genome and the evolution of monocotyledonous plants. Nature 488(7410):213–217. https://doi.org/10.1038/nature11241

Doebley J, Stec A, Hubbard L (1997) The evolution of apical dominance in maize. Nature 386(6624):485–488. https://doi.org/10.1038/386485a0

Giraud E, Ng S, Carrie C, Duncan O, Low J, Lee CP, Van Aken O, Millar AH, Murcha M, Whelan J (2010) TCP transcription factors link the regulation of genes encoding mitochondrial proteins with the circadian clock in *Arabidopsis thaliana*. Plant Cell 22(12):3921–3934. https://doi.org/10.1105/tpc.110.074518

Goel R, Pandey A, Trivedi PK, Asif MH (2016) Genome-wide analysis of the Musa WRKY gene family: evolution and differential expression during development and stress. Front Plant Sci 7:299. https://doi.org/10.3389/fpls.2016.00299

Guo R, Xu X, Carole B, Li X, Gao M, Zheng Y, Wang X (2013) Genome-wide identification, evolutionary and expression analysis of the aspartic protease gene superfamily in grape. BMC Genom 14:554. https://doi.org/10.1186/1471-2164-14-554

Hammani K, Gobert A, Hleibieh K, Choulier L, Small I, Giegé P (2011) An *Arabidopsis* dual-localized pentatricopeptide repeat protein interacts with nuclear proteins involved in gene expression regulation. Plant Cell 23(2):730–740. https://doi.org/10.1105/tpc.110.081638

Hu B, Jin J, Guo AY, Zhang H, Luo J, Gao G (2015a) GSDS 2.0: an upgraded gene feature visualization server. Bioinformatics 31(8):1296–1297. https://doi.org/10.1093/bioinformatics/btu817

Hu W, Zuo J, Hou X, Yan Y, Wei Y, Liu J, Li M, Xu B, Jin Z (2015b) The auxin response factor gene family in banana: genome-wide identification and expression analyses during development, ripening, and abiotic stress. Front Plant Sci 6:742. https://doi.org/10.3389/fpls.2015.00742

Kieffer M, Master V, Waites R, Davies B (2011) TCP14 and TCP15 affect internode length and leaf shape in *Arabidopsis*. Plant J 68(1):147–158. https://doi.org/10.1111/j.1365-313X.2011.04674.x

Kosugi S, Ohashi Y (2002) DNA binding and dimerization specificity and potential targets for the TCP protein family. Plant J 30(3):337–348. https://doi.org/10.1046/j.1365-313x.2002.01294.x

Lakhwani D, Pandey A, Dhar YV, Bag SK, Trivedi PK, Asif MH (2016) Genome-wide analysis of the AP2/ERF family in *Musa* species reveals divergence and neofunctionalisation during evolution. Sci Rep 6:18878. https://doi.org/10.1038/srep18878

Leister D (2004) Tandem and segmental gene duplication and recombination in the evolution of plant disease resistance genes. Trends Genet 20(3):116–122. https://doi.org/10.1016/j.tig.2004.01.007

Lei N, Yu X, Li S, Zeng C, Zou L, Liao W, Peng M (2017) Phylogeny and expression pattern analysis of TCP transcription factors in cassava seedlings exposed to cold and/or drought stress. Sci Rep 7:10016. https://doi.org/10.1038/s41598-017-09398-5

Leng X, Wei H, Xu X, Ghuge SA, Jia D, Liu G, Wang Y, Yuan Y (2019) Genome-wide identification and transcript analysis of TCP transcription factors in grapevine. BMC Genom 20(1):786. https://doi.org/10.1186/s12864-019-6159-2

Lescot M, Déhais P, Thijs G, Marchal K, Moreau Y, Van de Peer Y, Rouzé P, Rombauts S (2002) PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. Nucleic Acids Res 30(1):325–327. https://doi.org/10.1093/nar/30.1.325

Lescot M, Piffanelli P, Ciampi AY, Ruiz M, Blanc G, Leebens-Mack J, da Silva FR, Santos CM, D'Hont A, Garsmeur O, Vilarinhos AD, Kanamori H, Matsumoto T, Ronning CM, Cheung F, Haas BJ, Althoff R, Arbogast T, Hine E, Pappas GJ Jr, Sasaki T, Souza MT Jr, Miller RN, Glaszmann JC, Town CD (2008) Insights into the *Musa* genome: syntenic relationships to rice and between *Musa* species. BMC Genomics 9:58. https://doi.org/10.1186/1471-2164-9-58

Li S (2015) The *Arabidopsis thaliana* TCP transcription factors: a broadening horizon beyond development. Plant Signal Behav 10(7):e1044192. https://doi.org/10.1080/15592324.2015.1044192

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25(11):1451–1452. https://doi.org/10.1093/bioinformatics/btp187

Liu Y, Guan X, Liu S, Yang M, Ren J, Guo M, Huang Z, Zhang Y (2018) Genome-wide identification and analysis of TCP transcription factors involved in the formation of leafy head in Chinese cabbage. Int J Mol Sci 19(3):847. https://doi.org/10.3390/ijms19030847

Liu MM, Wang MM, Yang J, Wen J, Guo PC, Wu YW, Ke YZ, Li PF, Li JN, Du H (2019) Evolutionary and comparative expression analyses of TCP transcription factor gene family in land plants. Int J Mol Sci 20(14):3591. https://doi.org/10.3390/ijms20143591

Livark KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25(4):402–408. https://doi.org/10.1006/meth.2001.1262

Luo D, Carpenter R, Copsey L, Vincent C, Clark J, Coen E (1999) Control of organ asymmetry in flowers of *Antirrhinum*. Cell 99(4):367–376. https://doi.org/10.1016/s0092-8674(00)81523-8

Ma J, Wang Q, Sun R, Xie F, Jones DC, Zhang B (2014) Genome-wide identification and expression analysis of TCP transcription factors in *Gossypium raimondii*. Sci Rep 4:6645. https://doi.org/10.1038/srep06645

Manassero NG, Viola IL, Welchen E, Gonzalez DH (2013) TCP transcription factors: architectures of plant form. Biomol Concepts 4(2):111–127. https://doi.org/10.1515/bmc-2012-0051

Martín-Trillo M, Cubas P (2010) TCP genes: a family snapshot ten years later. Trends Plant Sci 15(1):31–39. https://doi.org/10.1016/j.tplants.2009.11.003

Martin G, Baurens FC, Droc G, Rouard M, Cenci A, Kilian A, Hastie A, Dolezel J, Aury JM, Alberti A, Carreel F, D'Hont A (2016) Improvement of the banana "*Musa acuminata*" reference sequence using NGS data and semi-automated bioinformatic methods. BMC Genom 17:243. https://doi.org/10.1186/s12864-016-2579-4

Masuda HP, Cabral LM, De Veylder L, Tanurdzic M, de Almeida EJ, Geelen D, Inzé D, Martienssen RA, Ferreira PC, Hemerly AS (2008) ABAP1 is a novel plant Armadillo BTB protein involved in DNA replication and transcription. EMBO J 27(20):2746–2756. https://doi.org/10.1038/emboj.2008.191

Mattick JS, Gagen MJ (2001) The evolution of controlled multitasked gene networks: the introns and other noncoding RNAs in the development of complex organisms. Mol Biol Evol 18(9):1611–1630. https://doi.org/10.1093/oxfordjournals.molbev.a003951

Ori N, Cohen AR, Etzioni A, Brand A, Yanai O, Shleizer S, Menda N, Amsellem Z, Efroni I, Pekker I, Alvarez JP, Blum E, Zamir D, Eshed Y (2007) Regulation of LANCEOLATE by miR319 is required for compound- leaf development in tomato. Nat Genet 39(6):787–791. https://doi.org/10.1038/ng2036

Pagnussat GC, Yu HJ, Ngo QA, Rajani S, Mayalagu S, Johnson CS, Capron A, Xie LF, Ye D, Sundaresan V (2005) Genetic and molecular identification of genes required for female gametophyte development and function in *Arabidopsis*. Development 132(3):603–614. https://doi.org/10.1242/dev.01595

Palatnik JF, Allen E, Wu X, Schommer C, Schwab R, Carrington JC, Weigel D (2003) Control of leaf morphogenesis by microRNAs. Nature 425(6955):257–263. https://doi.org/10.1038/nature01958

Parapunova V, Busscher M, Busscher-Lange J, Lammers M, Karlova R, Bovy AG, Angenent GC, de Maagd RA (2014) Identification, cloning and characterization of the tomato TCP transcription factor family. BMC Plant Biol 14:157. https://doi.org/10.1186/1471-2229-14-157

Pillet J, Yu HW, Chambers AH, Whitaker VM, Folta KM (2015) Identification of candidate flavonoid pathway genes using transcriptome correlation network analysis in ripe strawberry (*Fragaria* × *ananassa*) fruits. J Exp Bot 66(15):4455–4467. https://doi.org/10.1093/jxb/erv205

Riechmann JL, Heard J, Martin G, Reuber L, Jiang C, Keddie J, Adam L, Pineda O, Ratcliffe OJ, Samaha RR, Creelman R, Pilgrim M, Broun P, Zhang JZ, Ghandehari D, Sherman BK, Yu G (2000) *Arabidopsis* transcription factors: genome-wide comparative analysis among eukaryotes. Science 290(5499):2105–2110. https://doi.org/10.1126/science.290.5499.2105

Schultz J, Milpetz F, Bork P, Ponting CP (1998) SMART, a simple modular architecture research tool: identification of signaling domains. Proc Natl Acad Sci 95(11):5857–5864. https://doi.org/10.1073/pnas.95.11.5857

Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 13(11):2498–2504. https://doi.org/10.1101/gr.1239303

Song CB, Shan W, Yang YY, Tan XL, Fan ZQ, Chen JY, Lu WJ, Kuang JF (2018) Heterodimerization of MaTCP proteins modulates the transcription of *MaXTH10/11* genes during banana fruit ripening. Biochim Biophys Acta Gene Regul Mech 1861(7):613–622. https://doi.org/10.1016/j.bbagrm.2018.06.005

Takeda T, Suwa Y, Suzuki M, Kitano H, Ueguchi-Tanaka M, Ashikari M, Matsuoka M, Ueguchi C (2003) The OsTB1 gene negatively regulates lateral branching in rice. Plant J 33(3):513–520. https://doi.org/10.1046/j.1365-313x.2003.01648.x

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28(10):2731–2739. https://doi.org/10.1093/molbev/msr121

Tatematsu K, Nakabayashi K, Kamiya Y, Nambara E (2008) Transcription factor *AtTCP14* regulates embryonic growth potential during seed germination in *Arabidopsis thaliana*. Plant J 53(1):42–52. https://doi.org/10.1111/j.1365-313X.2007.03308.x

Wang Z, Jia C, Wang JY, Miao HX, Liu JH, Chen C, Yang HX, Xu B, Jin Z (2020) Genome-Wide Analysis of Basic Helix-Loop-Helix Transcription Factors to Elucidate Candidate Genes Related to Fruit Ripening and Stress in Banana (*Musa acuminata* L. AAA Group, cv. Cavendish). Front Plant Sci 11:650. https://doi.org/10.3389/fpls.2020.00650

Wei W, Hu Y, Cui MY, Han YT, Gao K, Feng JY (2016) Identification and transcript analysis of the TCP transcription factors in the diploid woodland strawberry *Fragaria vesca*. Front Plant Sci 7:1937. https://doi.org/10.3389/fpls.2016.01937

Xu R, Sun P, Jia F, Lu L, Li Y, Zhang S, Huang J (2014) Genome wide analysis of TCP transcription factor gene family in *Malus domestica*. J Genet 93(3):733–746. https://doi.org/10.1007/s12041-014-0446-0

Yang S, Zhang X, Yue JX, Tian D, Chen JQ (2008) Recent duplications dominate NBS-encoding gene expansion in two woody species. Mol Genet Genom 280(3):187–198. https://doi.org/10.1007/s00438-008-0355-0

Yao X, Ma H, Wang J, Zhang D (2007) Genome-wide comparative analysis and expression pattern of TCP gene families in *Arabidopsis thaliana* and *Oryza sativa*. J Integr Plant Biol 49(6):885–897. https://doi.org/10.1111/j.1744-7909.2007.00509.x

Zhao J, Zhai Z, Li Y, Geng S, Song G, Guan J, Jia M, Wang F, Sun G, Feng N, Kong X, Chen L, Mao L, Li A (2018) Genome-wide identification and expression profiling of the TCP family genes in spike and grain development of wheat (*Triticum aestivum* L.). Front in Plant Sci 9:1282. https://doi.org/10.3389/fpls.2018.01282

Zhou Y, Xu Z, Zhao K, Yang W, Cheng T, Wang J, Zhang Q (2016) Genome-wide identification, characterization and expression analysis of the TCP gene family in *Prunus mume*. Front Plant Sci 7:1301. https://doi.org/10.3389/fpls.2016.01301

Authors and Affiliations

Jingyi Wang[1] · Zhuo Wang[1,2] · Caihong Jia[1] · Hongxia Miao[1,2] · Jianbin Zhang[1] · Juhua Liu[1,2] · Biyu Xu[1] · Zhiqiang Jin[1,2]

[1] Key Laboratory of Tropical Crop Biotechnology, Ministry of Agriculture, Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, 4 Xueyuan Road, Haikou 571101, China

[2] Hainan Key Laboratory for Protection and Utilization of Tropical Bioresource, Hainan Academy of Tropical Agricultural Resource, Chinese Academy of Tropical Agricultural Sciences, 4 Xueyuan Road, Haikou 571101, China