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Research paper

Genome-wide identification of members of the *TCP* gene family in switchgrass (*Panicum virgatum* L.) and analysis of their expression

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ABSTRACT

Teosinte branched 1/Cycloidea/Proliferating cell factor 1 (TCP) proteins belongs to a plant-specific transcription factor family that plays important roles in plant development. TCP gene-regulated plant branching occurs downstream in the strigolactone pathway. In this study, 41 *TCP* genes were identified in the genome of *Panicum virgatum* L. (switchgrass). These genes all contained the TCP conserved domain, and they belonged to two subfamilies distributed across 18 chromosomes. Analysis of gene expression using RNA-Seq data showed that 16 TCP genes were highly expressed in the inflorescence and shoot. The expression patterns of 13 selected *PvTCP* genes were analyzed in different tissues, and their responses to strigolactones (SLs) were examined. The selected genes were expressed differentially in a range of tissues and to application of SLs, indicating that *PvTCPs* were involved in a range of developmental and physiological processes. This genome-wide analysis and determination of *PvTCP* gene-expression patterns yielded valuable information on switchgrass development that will inform studies into improving switchgrass and other species for crop production.

1. Introduction

Transcription factors (TFs) play vital roles in many developmental processes in plants, notably regulation of transcriptional networks. Currently, 58 families of transcription factors are listed in the Plant Transcription Factor Database (http://planttfdb.cbi.pku.edu.cn/index.php).

The Teosinte branched 1/Cycloidea/Proliferating cell factor 1 (TCP) family includes plant-specific transcription factors that have a non-canonical basic helix–loop–helix motif (the TCP domain) (Cubas et al., 1999). TCP proteins are divided into two classes, namely TCP-C and TCP-P (sometimes called the PCF class), on the basis of differences in their TCP domains. The TCP-C class has two clades, termed the CIN clade and the CYC/TB1 clade (Navaud et al., 2007). CIN clade genes participate in lateral organ development (Palatnik et al., 2003; Koyama et al., 2007; Ori et al., 2007; Schommer et al., 2008), while CYC/TB1 clade genes are involved in flower development or lateral shoot development (Hubbard et al., 2002; Takeda et al., 2003; Yuan et al., 2009). However, mutants of *TCP-P* class genes have mild or no phenotypic defects (Takeda et al., 2006; Herve et al., 2009). *TCP* genes have been analyzed in various plant species, such as *Arabidopsis thaliana*, *Oryza sativa* (rice), *Solanum lycopersicum* (tomato), *Malus domestica* (apple), *Prunus mume* (Chinese plum), and *Orchis italica* (Italian orchid) (Martin-Trillo and Cubas, 2010; Parapunova et al., 2014; Xu et al., 2014; De Paolo et al., 2015; Zhou et al., 2016).

TCP genes are involved in many biological processes, such as flower development, flower symmetry, shoot branching, leaf development, leaf morphogenesis, leaf senescence, male and female gametophyte development, and the circadian clock (Palatnik et al., 2003; Li et al., 2005; Aguilar-Martínez et al., 2007; Busch and Zachgo, 2007; Koyama et al., 2007; Pozacarrión et al., 2007; Efroni et al., 2008; Schommer et al., 2008; Nag et al., 2009; Giraud et al., 2010; Koyama and Ohme-Takagi, 2010; Sarvepalli and Nath, 2011; Yang et al., 2012). The genes involved in these processes generally act through plant hormone-mediated signaling. Some *TCP* genes act downstream of hormonally-mediated pathways as transcriptional modulators of cell division. Other *TCP* genes act upstream of plant hormones and influence levels of hormone synthesis, transport, and signal transduction (Nicolas and

Abbreviations list: TCP, Teosinte branched 1/Cycloidea/Proliferating cell factor 1; TFs, Transcription factors; SLs, Strigolactones; SMART, Switching Mechanism At 5′ end of the RNA Transcript

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Table 1
TCP gene family in switchgrass.

Gene	Gene locus ID	Genomic location	CDS length	Protein length
PvTCP1	Pavir.1KG397100	Chr. 1K: 63645349..63646113	663	220
PvTCP2	Pavir.1KG510200	Chr. 1K: 75765762..75768115	1188	395
PvTCP3	Pavir.1KG552700	Chr. 1K: 79521775..79523415	681	226
PvTCP4	Pavir.1NG030900	Chr. 1N: 3370022..3371927	426	141
PvTCP5	Pavir.1NG539700	Chr. 1N: 95301934..95303989	1206	401
PvTCP6	Pavir.1NG547900	Chr. 1N: 96465389..96467986	663	220
PvTCP7	Pavir.2KG296300	Chr. 2K: 59186963..59189445	801	266
PvTCP8	Pavir.2NG040500	Chr. 2N: 5718258..5719550	1293	430
PvTCP9	Pavir.2NG168500	Chr. 2N: 32012380..32013416	933	310
PvTCP10	Pavir.2NG320400	Chr. 2N: 66635714..66637139	795	264
PvTCP11	Pavir.2NG441900	Chr. 2N: 81045305..81046294	990	329
PvTCP12	Pavir.3KG357500	Chr. 3K: 39629282..39631990	870	289
PvTCP13	Pavir.3KG547300	Chr. 3K: 69673046..69678099	870	289
PvTCP14	Pavir.3NG031100	Chr. 3N: 2368139..2370112	1200	399
PvTCP15	Pavir.3NG279000	Chr. 3N: 50705121..50709007	864	287
PvTCP16	Pavir.4KG172900	Chr. 4K: 29298318..29299788	1197	398
PvTCP17	Pavir.4NG098900	Chr. 4N: 13149150..13151818	1173	390
PvTCP18	Pavir.4NG231900	Chr. 4N: 42483446..42485664	978	325
PvTCP19	Pavir.5KG544700	Chr. 5K: 94391370..94392775	1251	416
PvTCP20	Pavir.5KG556600	Chr. 5K: 95364773..95369286	837	278
PvTCP21	Pavir.5KG742600	Chr. 5K: 113279411..113281724	978	325
PvTCP22	Pavir.5NG501800	Chr. 5N: 86933569..86934999	1233	410
PvTCP23	Pavir.5NG508900	Chr. 5N: 87491419..87493406	531	176
PvTCP24	Pavir.6KG270000	Chr. 6K: 55905938..55907215	849	282
PvTCP25	Pavir.6KG395100	Chr. 6K: 70566109..70567758	1065	354
PvTCP26	Pavir.6NG051800	Chr. 6N: 10745904..10747230	735	244
PvTCP27	Pavir.6NG140000	Chr. 6N: 27715493..27716203	711	236
PvTCP28	Pavir.6NG344700	Chr. 6N: 77613527..77615377	1329	442
PvTCP29	Pavir.7KG023900	Chr. 7K: 4785956..4803221	525	174
PvTCP30	Pavir.7KG255700	Chr. 7K: 56383609..56384723	606	201
PvTCP31	Pavir.7NG066100	Chr. 7N: 15663289..15674390	285	94
PvTCP32	Pavir.7NG333200	Chr. 7N: 60097820..60098422	549	182
PvTCP33	Pavir.8KG079400	Chr. 8K: 9383778..9386025	1209	402
PvTCP34	Pavir.8NG062800	Chr. 8N: 8490834..8493093	1191	396
PvTCP35	Pavir.9KG031700	Chr. 9K: 2411064..2412737	1110	369
PvTCP36	Pavir.9NG079800	Chr. 9N: 7699993..7703805	1158	385
PvTCP37	Pavir.9NG142700	Chr. 9N: 13486659..13488379	1116	371
PvTCP38	Pavir.J125500	scaffold_14983: 1395..1995	582	193
PvTCP39	Pavir.J227000	scaffold_20: 54419..57032	882	293
PvTCP40	Pavir.J362100	scaffold_275: 1..2111	1335	444
PvTCP41	Pavir.J675700	scaffold_7087: 83..2442	1353	450

Cubas, 2016).

Strigolactones (SLs) are one of the most important groups of phytohormones, influential in plant development and function in shoot branching, root development, seed germination, and leaf senescence. The *TCP* genes, *BRANCHED1* (*BRC1*) of dicots and *TEOSINTE BRANCHED 1* (*TB1*) of monocots, mediate shoot and branching suppression by SLs (Rameau et al., 2014; Chevalier et al., 2014; Guan et al., 2012). *BRC1* is expressed at very low levels in axillary buds and cauline leaves of SL-related mutants (Aguilar-Martínez et al., 2007; Finlayson, 2007; Braun et al., 2012; Chevalier et al., 2014; Drummond et al., 2015). The level of expression of *BRC1* is increased in *SMAX1-LIKE/D53* mutants, which are repressors of the SL pathway (Soundappan et al., 2015; Wang et al., 2015). SLs are reported to induce expression of *PsBRC1* in *Pisum sativum* (pea) (Braun et al., 2012; Dun et al., 2012).

Switchgrass is an important plant in the biofuel industry because of its high bioproduction. It is a perennial C4 plant that can produce hundreds of tillers during its lifetime, and it produces large amounts of dry matter. The stalks of switchgrass are used to produce ethanol. As a consequence of these attributes, studies on the mechanisms that regulate biological yield in this species are underway. Recently, the switchgrass *TCP* family gene, *PvTB1*, was cloned and shown to be involved in regulation of tillering. Thus, *PvTB1* is a potentially valuable gene for improving the yield of switchgrass (Xu et al., 2016). The miRNA *miR319* and its target gene *PvPCF5*, involved in leaf development, were also recently identified from switchgrass (Xie et al., 2017).

The aim of this study was to investigate the responses of the switchgrass *TCP* gene family to application of SLs. This information will form the basis for cloning and further functional analyses of *TCP* genes. Moreover, this approach should identify further valuable *TCP*-family genes for improving agronomic traits in switchgrass.

2. Material and methods

2.1. PvTCP gene identification

Sequences were retrieved from the switchgrass genome sequence database (Department of Energy Joint Genome Institute, USA, JGI, *Panicum virgatum* v4.1) with a Hidden Markov Model (HMM) method using the conserved structural domain of *TCP* (PF03634) as the probe. A search of the retrieved results with the same *TCP* conserved domain structure sequence was done using SMART, and we identified all sequences with an E-value of < 0.01 as *PvTCP* genes.

2.2. Phylogenetic analysis of TCP genes

Multiple sequence alignment of all the *PvTCP* genes that were identified was performed using Clustal X software (version 2.0). All the *TCP* genes from switchgrass, rice (a monocotyledonous model plant) and *A. thaliana* (a dicotyledonous model plant) were used to construct a distance tree using the Neighbor-Joining method within MEGA5.3 and the bootstrap test was performed with 1000 iterations.

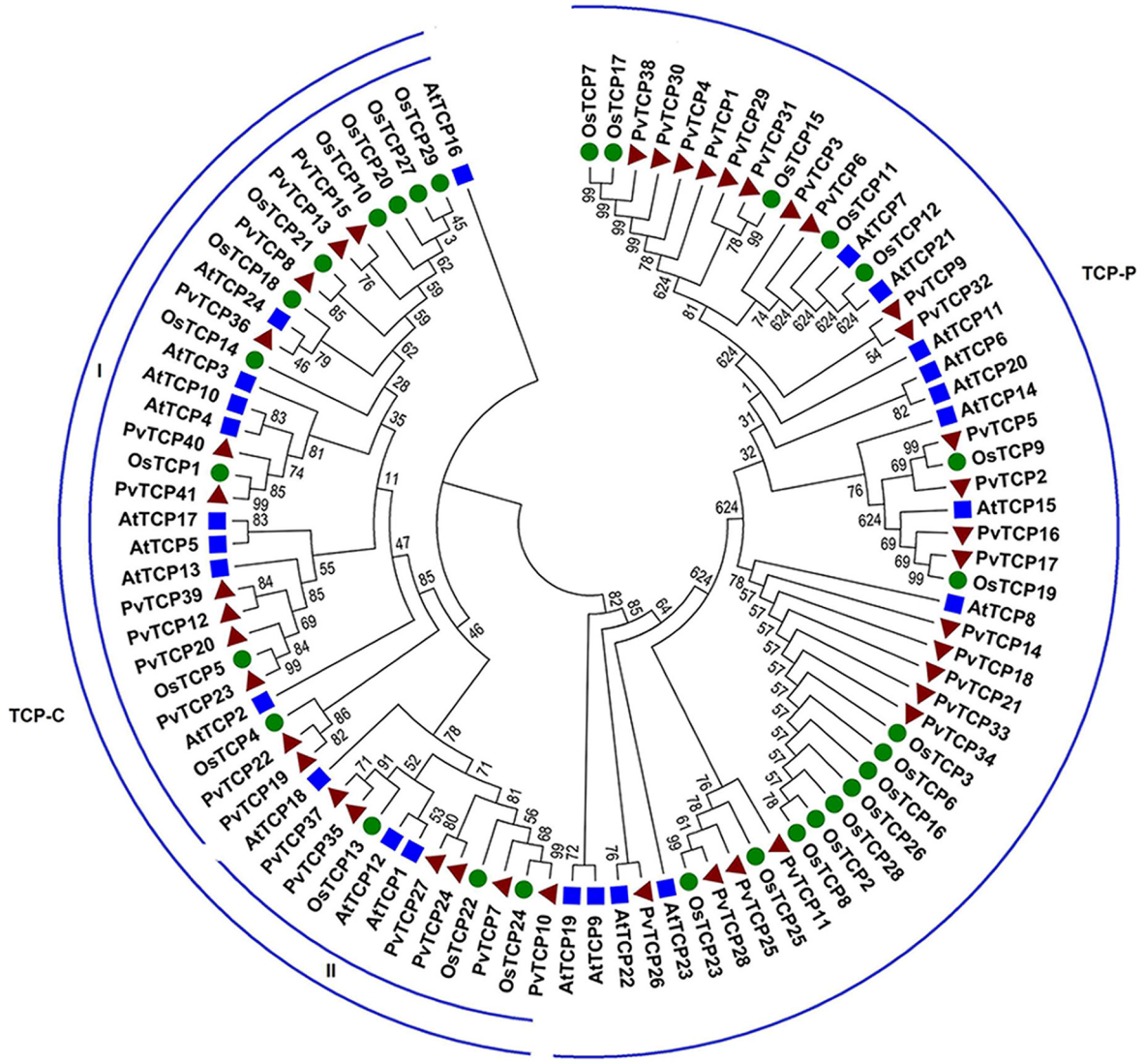

Fig. 1. Predicted phylogeny of *TCP* genes in switchgrass, rice and *Arabidopsis thaliana*. Branch lengths indicate distance. The triangles, circles and tetragons indicate *TCP* genes originating from switchgrass, rice and *A. thaliana*, respectively. The *TCP* gene family included two subfamilies: TCP-P and TCP-C. The TCP-C subfamily was subdivided into Clade I and Clade II. The unrooted distance tree was constructed by pairwise alignment of sequences within MEGA5.3 using the Neighbor-Joining method, and the bootstrap test was performed with 1000 iterations.

2.3. Structure analysis of PvTCP genes

We used the whole genome and coding sequences of *PvTCP* genes from the switchgrass genome database to generate their exon/intron structures using the Gene Structure Display Server (GSDS2.0) website (http://gsds.cbi.pku.edu.cn/).

MEME 4.11.2 online tools (http://meme-suite.org/tools/meme) were used to analyze switchgrass *TCP* gene structures and to classify conserved protein motifs.

2.4. Chromosomal location and miR319 target site prediction

Analysis of the switchgrass genome using CIRCOS software indicated that the 41 *TCP* genes were located on 18 chromosomes. The *miR319* target sites were predicted using full-length *PvTCP* nucleotide sequences within the psRNATarget online application.

2.5. Plant materials and GR24 treatment

Plants of the lowland ecotype switchgrass 'Alamo'(2n = 4x = 36) was used in this study. Plants were grown in a greenhouse under a 26 °C/16 h (day) and 23 °C/8 h (night) cycle. For GR24 treatment, one-month-old 'Alamo' seedlings were grown in Yoshida nutrient solution containing 1 μM or 10 μM GR24 (DAQIN technology co. LTD, Beijing), a synthetic analog of strigolactone, for 24 h. Seedlings grown in Yoshida nutrient solution without GR24 served as controls.

2.6. RNA extraction, cDNA synthesis, and real-time PCR

Total RNA was extracted from seedlings using TRIzol® reagent (Thermo Fisher Scientific, Waltham, Massachusetts, USA), according to the supplied protocol. First-strand cDNA was synthesized using a PrimeScript™ II 1st Strand cDNA Synthesis Kit (Takara Bio Inc., Kusatsu, Shiga, Japan) according to the manufacturer's instructions. Quantitative real-time PCR analysis was performed using a StepOnePlus™ real-time PCR system (Thermo Fisher Scientific). Each reaction was carried out in triplicate within a reaction volume of 20 μl containing 1.6 μl of gene-specific primers (1.0 μM), 1.0 μl of cDNA, 10 μl of SYBR green (TaKaRa Bio Inc., Kusatsu, Shiga, Japan), and 7.4 μl sterile distilled water. *ACTIN1* was used as the reference gene. The primers for the real-time PCR are listed in Table S1.

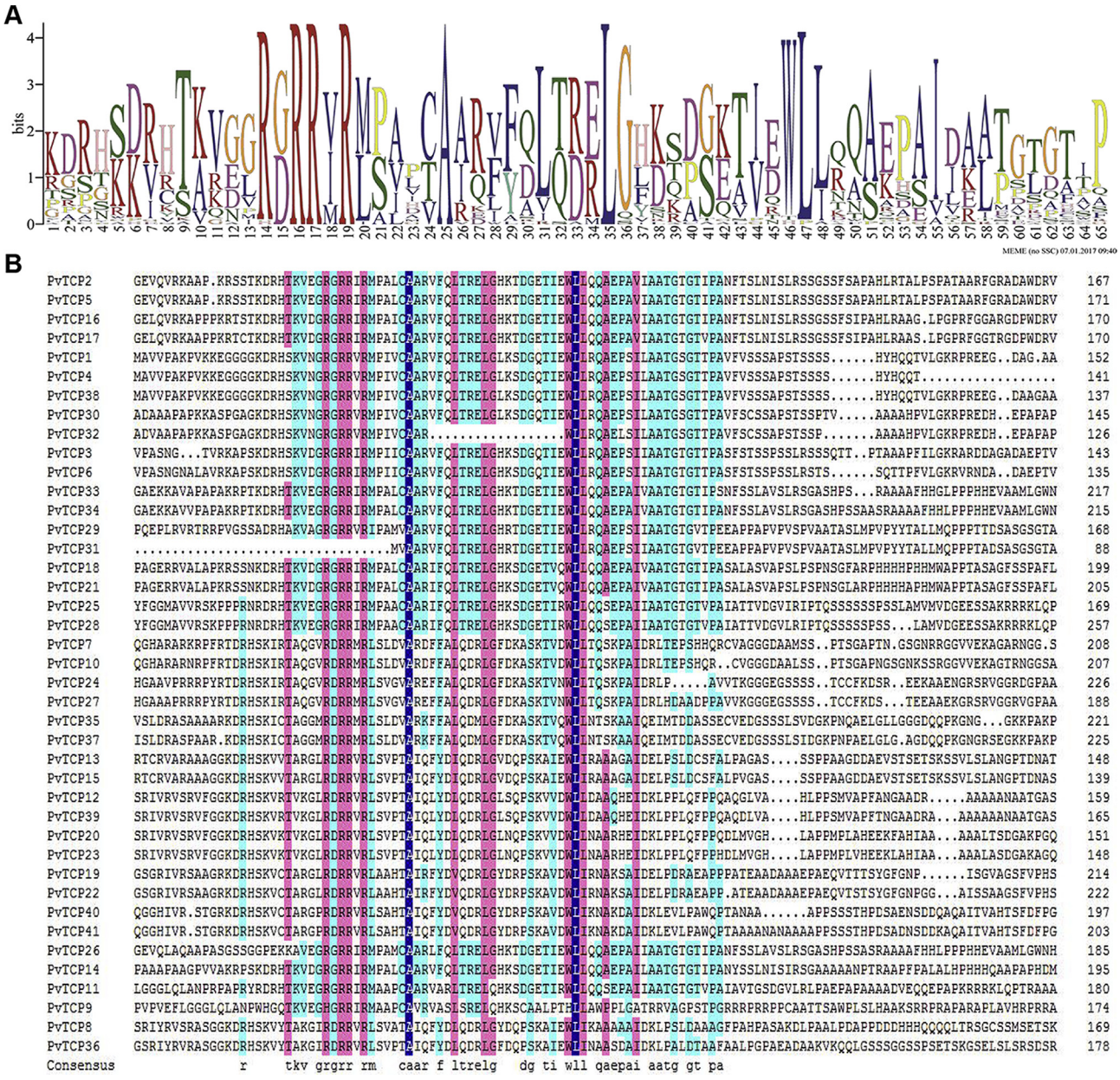

Fig. 2. Alignment of TCP domains from 41 putative *PvTCP* genes. A) The conserved motif of *PvTCP* genes predicted by MEME 4.11.2 online tools (http://meme-suite.org/tools/meme). Amino acids are expressed in the standard single letter code. The size of the letters at each position represents their frequency. B) An alignment of 41 putative PvTCP proteins. The colored box indicates conserved amino acids. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.7. Statistics analysis

Data were examined by analysis of variance (ANOVA), followed by Tukey's HSD test using the statistical software SPSS version 21.0 (SPSS, Chicago, Illinois, USA). Differences between means were compared with Tukey's HSD test at the 0.05 significance level.

3. Results

3.1. Identification of PvTCP genes

In order to identify *PvTCP* genes, all annotated protein sequences were downloaded from the latest version of the switchgrass genome database (https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Pvirgatum_er). Hidden Markov Model (HMM) profiling of the TCP domain (PF03634) (http://pfam.xfam.org/) was used to query the switchgrass protein database using the HMMER 3.0 program with the default E-value. With the online program SMART, we identified all proteins that contained a conserved TCP domain. In total, 41 *TCP* genes were identified in the tetraploid 'Alamo' ecotype of switchgrass. The latest available switchgrass genomic data identified two genomes, referred to as K and N, each with nine chromosomes. *TCP* genes were distributed across all 18 chromosomes and were named *PvTCP1* to *PvTCP41* according to their locus on the chromosomes (Table 1).

3.2. Pairwise distance, conserved motifs, and structural analysis

We used the sequences of the 41 *PvTCPs*, with 24 *A. thaliana AtTCPs* and 29 rice *OsTCPs*, to construct a Neighbor-Joining pairwise distance tree (Fig. 1). There were two obvious subfamilies in this tree, named TCP-C class and TCP-P class (PCF class). The TCP-C subfamily could be divided into Clade I and II; Clade I, the smaller one, was also named

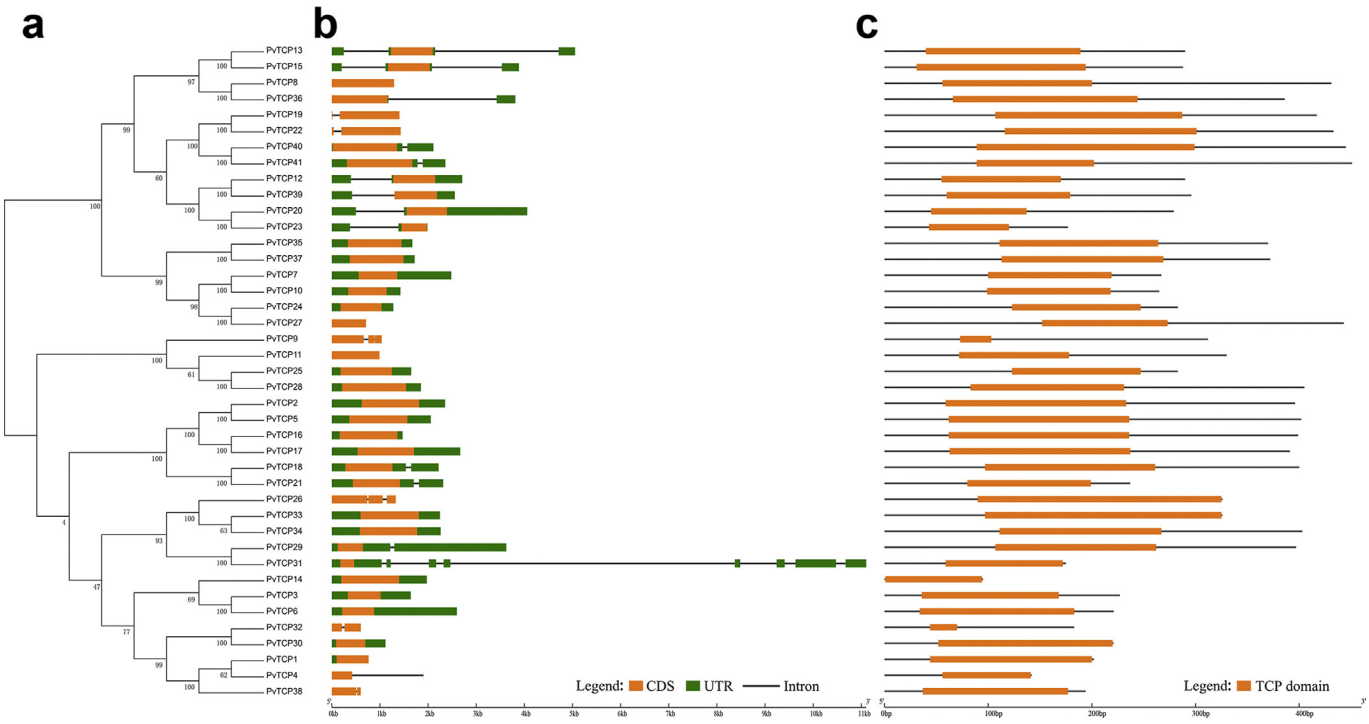

Fig. 3. Conserved motifs and structural analysis of *PvTCP* genes. A) Estimated phylogeny of *PvTCP* genes. B. The gene structure of *PvTCP* genes. Orange box, green box and line indicate coding sequences, untranslated regions, and introns, respectively. C. The TCP domain of *PvTCP* genes is represented by orange boxes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

CYC/TB1; Clade II, the larger one, was also named CIN. There were 18 *PvTCPs* in the TCP-C subfamily and 23 *PvTCPs* in the TCP-P subfamily. There were 12 CIN-type *PvTCP* genes and 6 CYC/TB1-type genes in TCP-C subfamily. The reported gene *PvTB1*(*PvTCP3* and *PvTCP4*) (Xu et al., 2016) and *PvPCF5*(*PvTCP40* and *PvTCP41*) (Xie et al., 2017) belonged separately to the CYC/TB1-type and CIN-type, respectively. The *PvTCPs* were closer to homologous genes of rice than of *A. thaliana*. Comparative analysis revealed that the pattern of distribution of the *TCP* gene family in each of the three species was similar, indicating that most *TCPs* appear to have evolved before the divergence of dicotyledons and monocotyledons 140 to 150 Myr ago (Chaw et al., 2004). Some *TCP* genes, such as *PvTCP29*, *PvTCP31* and *OsTCP15*, were present on branches lacking an *AtTCP* gene. No orthologues of *AtTCP16* were identified from switchgrass or rice. These results also confirm that the two subfamilies of *TCP* genes appeared before the divergence of dicotyledons and monocotyledons. Genes that showed different distributions in monocot and dicot plants mainly belonged to the TCP–P subfamily. The TCP–C subfamily genes were more highly conserved than those of the TCP–P subfamily.

A MEME analysis showed that all 41 predicted protein sequences had a highly conserved bHLH domain (TCP domain) in switchgrass (Fig. 2). Analyses of gene structures indicated that most did not contain introns in coding regions, with the exceptions of *PvTCP9*, *PvTCP19*, *PvTCP22*, *PvTCP28*, *PvTCP32* and *PvTCP38* (Fig. 3b). However, most genes in the CYC/TB1 clade had introns in 5′-UTRs (untranslated regions), which may influence expression of *TCP* genes (Chung et al., 2006). Genes belonging to the same clade shared similar TCP domains and structures, notably *PvTCP2/PvTCP5/PvTCP16/PvTCP17* and *PvTCP7/PvTCP10/PvTCP24/PvTCP27* (Fig. 3C).

3.3. Chromosomal location and synteny analysis

PvTCP genes were located on all 18 chromosomes according to the annotation of the switchgrass genome (Fig. 4). The largest number of *PvTCP* genes on one chromosome was four, located on chromosome 2N. Four chromosomes (1K 1N, 5K and 6N) carried three *PvTCP* genes, and eight chromosomes (3K, 3N, 4N, 5N, 6K, 7K, 7N and 9N) carried two *PvTCP* genes. Chromosomes 2K, 4K, 8K, 8N and 9K each carried one *PvTCP* gene. Four genes could not currently be assigned to chromosomes because of gaps in the annotated switchgrass genome.

Synteny analysis of the switchgrass *TCP* genes showed that two duplication events had occurred during evolution: *PvTCP1/PvTCP4/PvTCP38* and *PvTCP26/PvTCP33/PvTCP34*. The genes involved in the duplication events belonged to the TCP-P class. No tandem repeats of *PvTCP* genes were found.

3.4. Expression analysis of PvTCP genes

Gene expression patterns can be informative on whether genes play a role at particular developmental stages. According to the gene expression data from the JGI Plant Gene Atlas (https://phytozome.jgi.doe.gov/phytomine/begin.do), *PvTCP18* and *PvTCP21* are expressed highly in all tissues. Genes *PvTCP12*, *PvTCP18*, *PvTCP19*, *PvTCP21*, *PvTCP22*, *PvTCP30*, *PvTCP36*, and *PvTCP39* are highly expressed in the developing stem and inflorescence, therefore, these genes may play important roles in tillering and panicle branch development. *PvTCP16* is mainly expressed in the root system, indicating that the gene may play an important role in root development. Most switchgrass *TCP* genes were induced by nitrogen fertilizer, suggesting they may be involved in plant morphogenesis (Fig. 5).

In order to confirm expression patterned of *PvTCP* genes, quantitative PCR was performed to compare expression levels of *PvTCP* genes in different tissues. This analysis showed that *PvTCP1*, *PvTCP12*, *PvTCP19* and *PvTCP20* were expressed highly in panicles, while *PvTCP24*, *PvTCP25*, *PvTCP35* and *PvTCP36* were highly expressed mainly at the shoot node, and *PvTCP14* and *PvTCP16* were expressed highly in roots (Fig. 6).

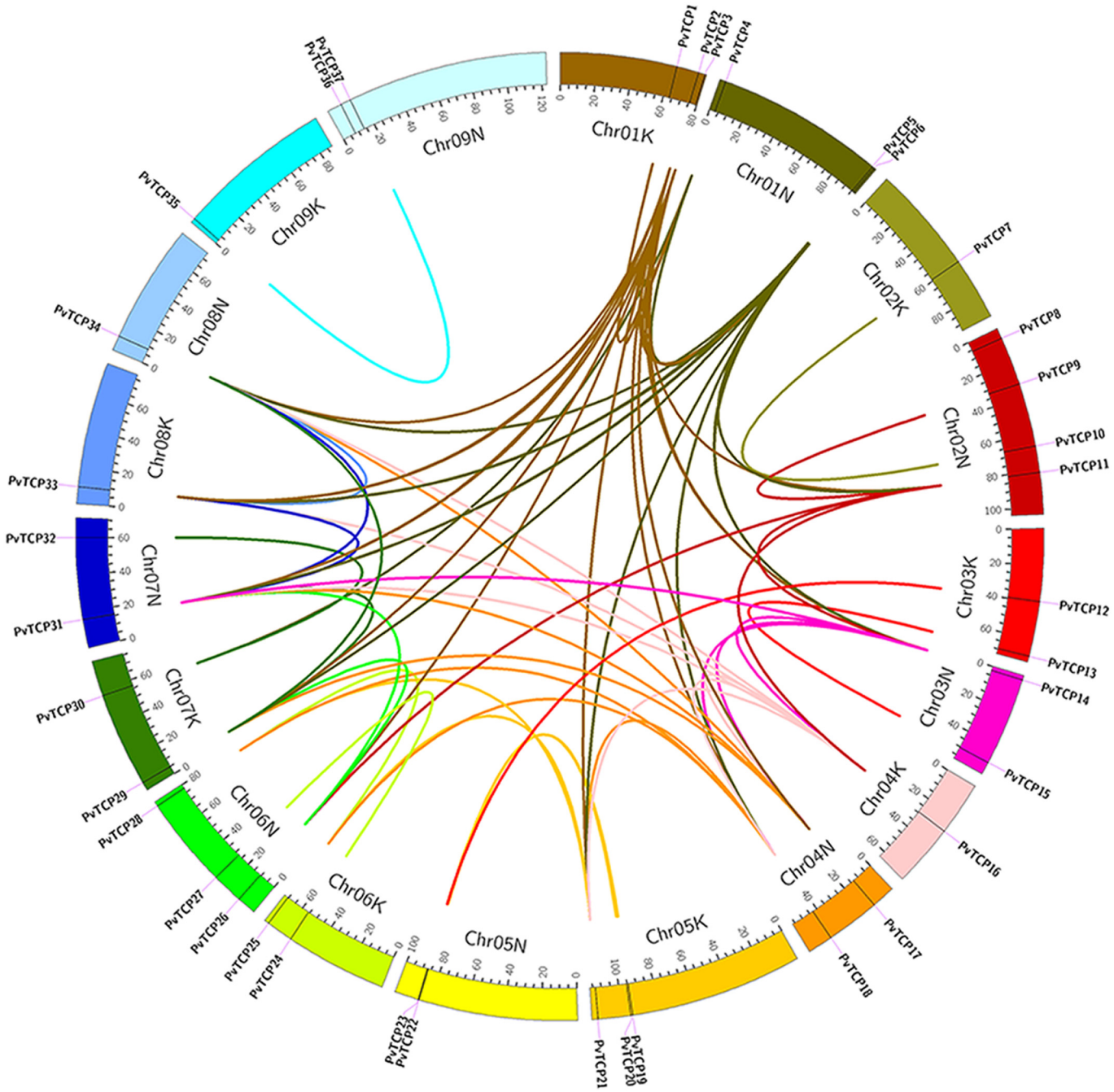

Fig. 4. The chromosomal location and synteny analysis of *PvTCP* genes. A Circos diagram illustrates the chromosomal locations of *PvTCP* genes and their synteny. The colored lines display similarity of different genes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.5. Response to strigolactones

Strigolactones (SLs) are plant hormones that play important roles in plant development. *TB1*, belonging to Class II of the TCP-C subfamily, has been shown to function downstream of SL signaling (Drummond et al., 2015; Chevalier et al., 2014; Braun et al., 2012; Finlayson, 2007). The relationship between the *TCP* family genes and SLs was investigated by quantitative PCR to determine gene expression levels after treatment with the synthetic SL analog, GR24. The results showed that *PvTCP12*, *PvTCP19* and *PvTCP33* expression increased after GR24 treatment, whereas *PvTCP1*, *PvTCP13*, *PvTCP14*, *PvTCP16*, *PvTCP20*, *PvTCP25*, *PvTCP36* and *PvTCP40* expression was inhibited by GR24 treatment. *PvTCP18* expression was induced by a low concentration of GR24(1 μM) but inhibited by a high concentration (10 μM) of GR24. In contrast, *PvTCP8* expression was inhibited by a low concentration of GR24 and induced by a high concentration (Fig. 7). The response of these genes to SLs were inconsistent, which may mean that the role of *TCPs* in the SLs pathway were different.

4. Discussion

In this study, we identified 41 genes in switchgrass belonging to the *TCP* family, genes of which were distributed across 18 chromosomes. Expression analysis showed that *TCP* genes showed different expression patterns, reflecting their different roles in a range of development processes. Although the 'Alamo' ecotype of switchgrass used here is a

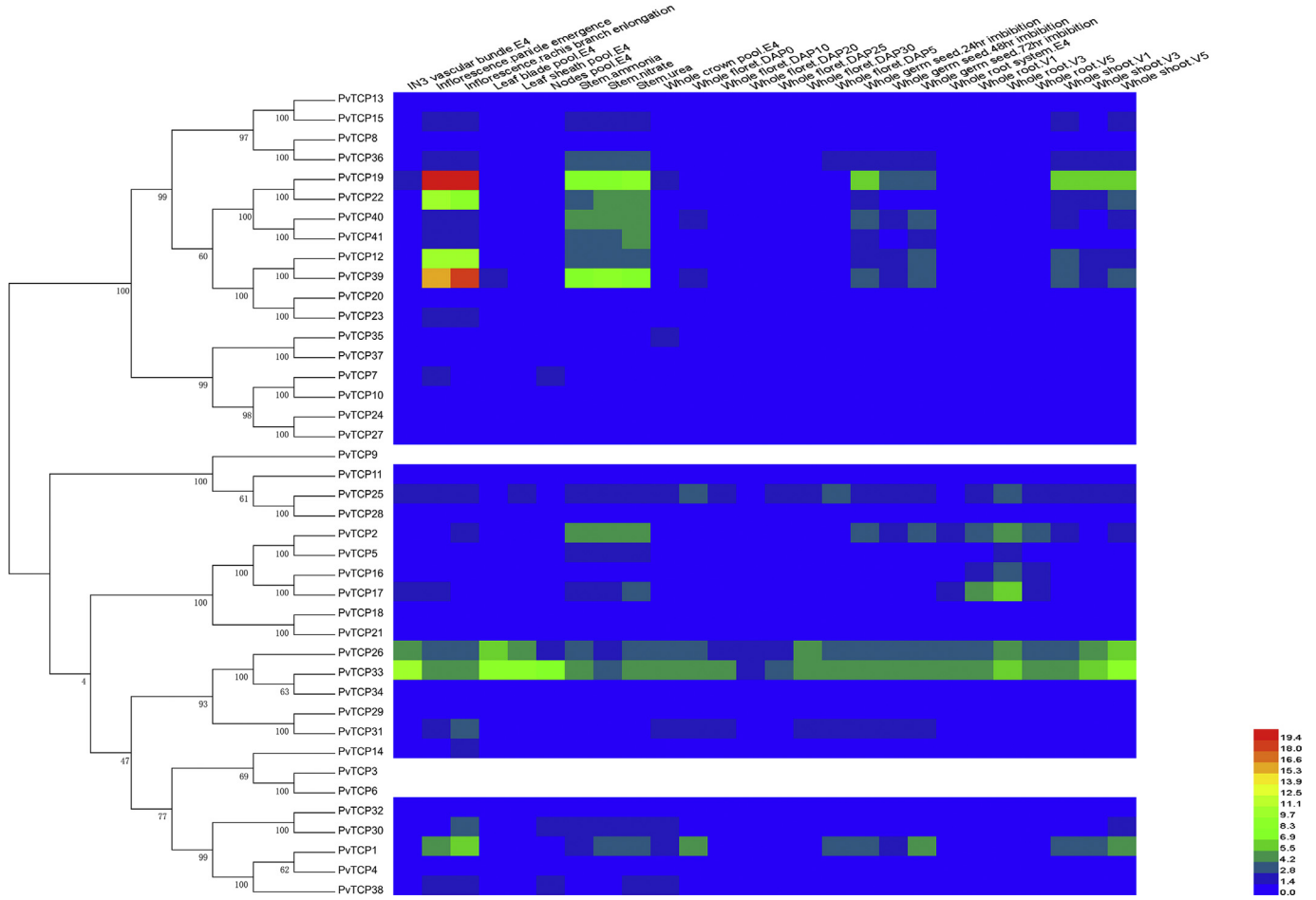

Fig. 5. Gene expression profiling of *PvTCP* genes. The gene expression data of every gene from the JGI Plant Gene Atlas (https://phytozome.jgi.doe.gov/phytomine/begin.do) was analyzed by HemI 1.0.

tetraploid, and contains two similar genomes, not all *TCP* genes existed in pairs. This may be due to the incomplete nature of the current switchgrass genome, and this may be why some *TCP* genes were not identified.

TCP transcription factors play important roles in plant growth and development. Previous studies showed that *TCP* genes are involved in gametogenesis, plant hormone signal transduction, mitochondrial development, biological rhythms, lateral development, and seed germination. Genes of the TCP-C subfamily inhibit plant growth and cell differentiation in similar ways (Nicolas and Cubas, 2016), while TCP-P family genes may promote plant growth and cell differentiation (Takeda et al., 2006; Herve et al., 2009). In this study, we found that the expression pattern of *PvTCPs* in different tissues varied (Fig. 6). These results indicated that different *TCP* genes may be involved in different organ development.

TCP genes play important roles in plant development. For example, *TB1* regulated branching in maize, *A. thaliana* and rice (Hubbard et al., 2002; Takeda et al., 2003; Pozacarrión et al., 2007). We found that *PvTB1* (*PvTCP3* and *PvTCP4*) regulated tillering in switchgrass (Xu et al., 2016). *PvPCF5* (*PvTCP40* and *PvTCP41*) was involved in leaf development like the orthologue *OsPCF5* in rice (Xie et al., 2017; Yang et al., 2013).

SLs regulate plant development at different stages and in different tissues and organs (Morffy et al., 2016). The levels of *TCP* gene expression were affected by SLs. The expression levels of *PvTCP12*, *PvTCP19*, *PvTCP33* and *PvTCP35* were induced by application of SLs, whereas *PvTCP1*, *PvTCP13*, *PvTCP14*, *PvTCP16*, *PvTCP20*, *PvTCP25*, *PvTCP36* and *PvTCP40* were inhibited by SLs. Expression of *PvTCP18* was regulated differently by low or high contention of SLs (Fig. 7). These results indicated *TCP* genes may be involved in SL regulatory networks in plant development, and are involved in different processes regulated by SLs. *PvTCP18* could be a prime candidate for further investigation in the SLs regulation network of plant development.

The microRNA319 (*miR319*) family, an ancient miRNA group, is conserved among diverse plant species. *miR319* targets members of the CIN clade (Class II) of the *TCP* transcription factor gene family (Palatnik et al., 2003; Ori et al., 2007; Nag et al., 2009; Martin-Trillo and Cubas, 2010). miR319 in switchgrass (Pv-miR319) has been identified and studied in recent years (Matts et al., 2010; Xie et al., 2010; Li et al., 2011). We used the *Pv-miR319* sequence and psRNATarget online software to analyze full-length *PvTCPs* nucleotide sequences and identified four TCP genes (*PvTCP13*, *PvTCP15*, *PvTCP8* and *PvTCP36*) with *miR319* target sites (Fig. S). It was found that there were relatively negative correlation between *miR319* and its predicted target genes, compared the expression pattern of miR319 (Xie et al., 2017) and *PvTCPs* (*PvTCP13*, *PvTCP 36* in Fig. 6). *PvTCP13* and *PvTCP36* had significantly negative correlations with miR319 in shoot, leaf and sheath. These results suggested that miR319 may involve in the development of different tissues of switchgrass through targeting to some *PvTCP* genes.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gene.2019.03.059.

Conflict of interest

The authors declare that they have no competing interest.

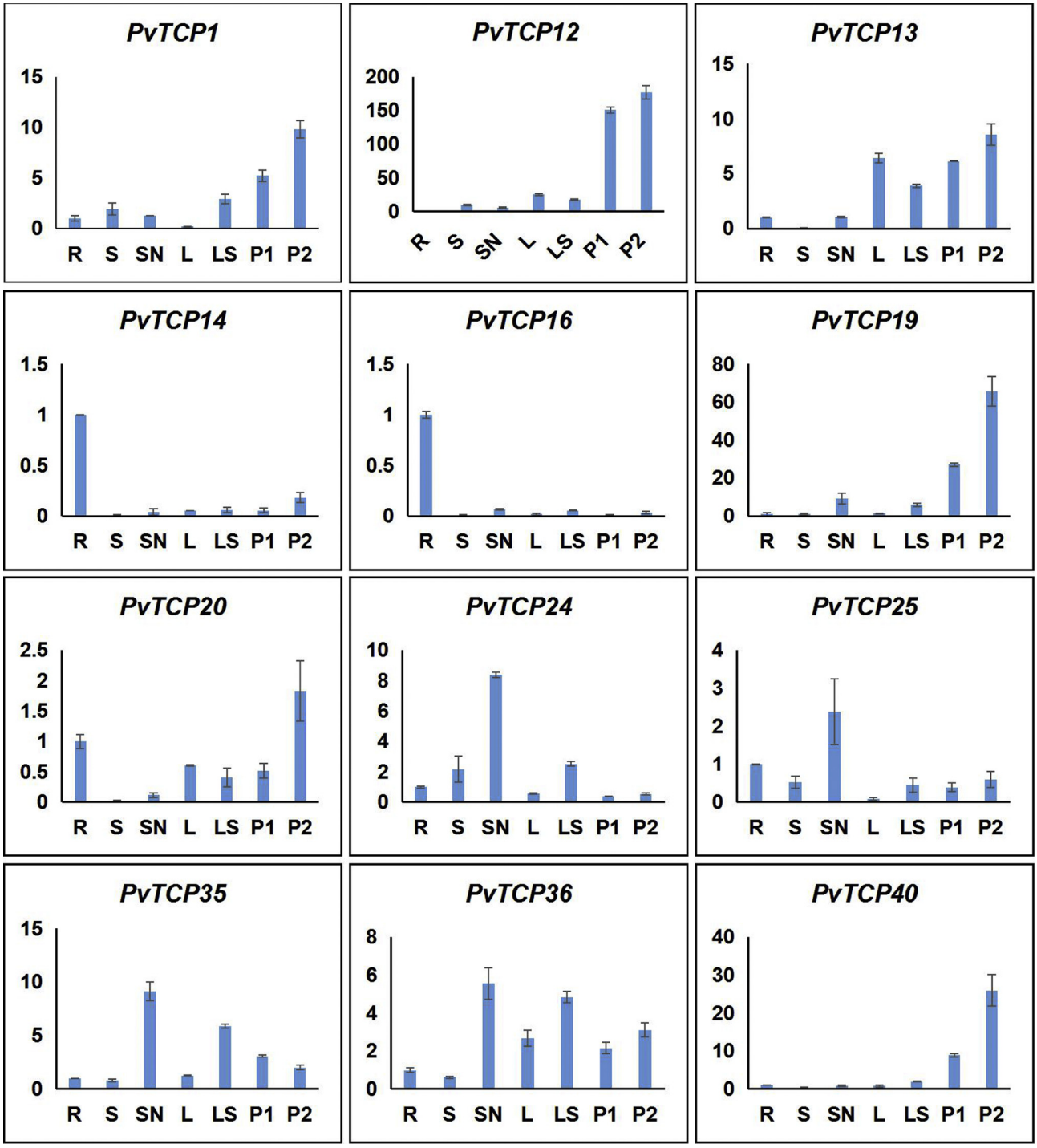

Fig. 6. Expression patterns of some *PvTCP* genes determined as by quantitative RT-PCR. Abbreviations are: R, root; S, shoot base; SN, shoot node; L, leaf; LS, leaf sheath; P1, panicle (early); P2, panicle (late); reference gene, *PvActin1*. The relative expression of *PvTCP* in roots was set up as 1. Leaf, leaf sheath, stem node, and P1 panicle were collected at the early heading stage, and the P2 ear was collected in the later heading stage. Roots were collected from developing seedlings grown hydroponically. Error bars indicate standard deviation (SD). Error bars represent means ± SDs; $n = 3$.

Authors' contribution

Fengli Sun and Aiquan Zheng preformed most of the experiments and data analysis. Fengli Sun designed the work and wrote the paper. Tingting Cheng performed some Q-PCR experiments. Yongfeng Wang performed part of bioinformation analysis. Kunliang Xie performed part of bioinformation analysis. Chao Zhang provided suggestion about the work and revised the paper. Yajun Xi designed the work and wrote the paper.

Declaration of interest statement

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

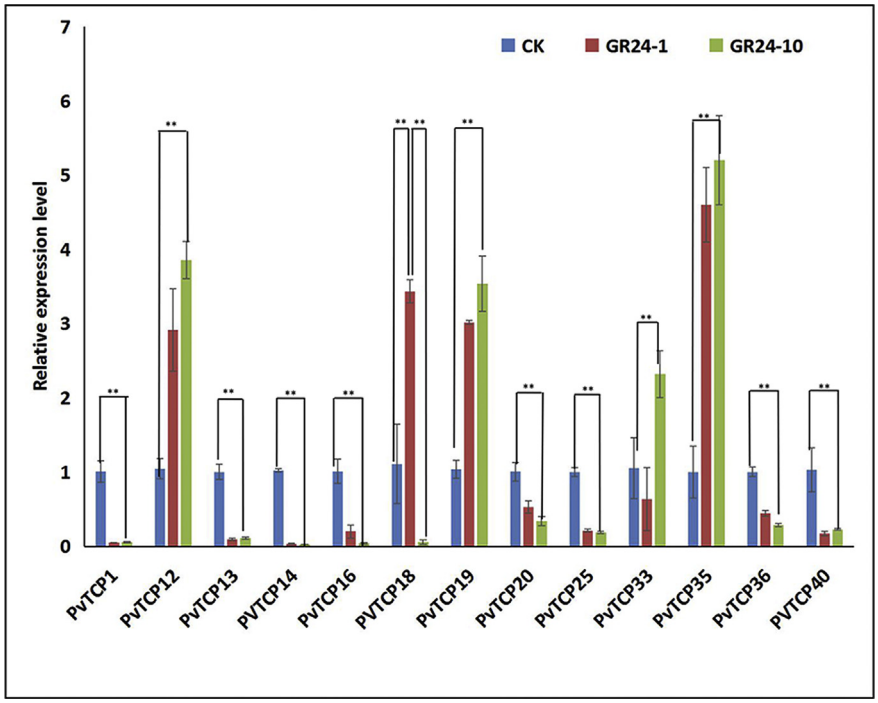

Fig. 7. Expression levels of *PvTCP* genes influenced by application of striglactones. Expression level was relative to the reference gene, *PvActin1*. Relative expression of root was set up as 1. Error bars represent means ± SDs; $n = 3$. Two stars indicate a highly significant difference ($P < 0.01$).

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