

GENOME-WIDE IDENTIFICATION OF TCP TRANSCRIPTION FACTORS FAMILY IN *POPULUS* SECT. *TURANGA* (*POPULUS PRUINOSA* SCHRENK AND *POPULUS EUPHRATICA* OLIVE) REVEALED THE ROLES OF TCPS IN LEAF MORPHOLOGY

WANG, J. N.^{1,2,3#} – XU, J. D.^{4#} – SUN, J. H.^{1,2,3} – QIU, C.^{1,2,3} – WU, Z. H.^{2,5*} – LI, Z. J.^{1,2,3*}

¹College of Plant Sciences, Tarim University, Alar 843300, China

²Key Laboratory of Biological Resource Protection and Utilization of Tarim Basin, Xinjiang Production and Construction Group, Alar 843300, China

³Desert Poplar Research Center of Tarim University, Alar 843300, China

⁴Hubei Provincial Key Laboratory for Protection and Application of Special Plant Germplasm in Wuling Area of China, College of Life Sciences, South Central University for Nationalities, Wuhan 430040, China

⁵College of Life Sciences, Zhejiang normal University, Jinhua, Zhejiang, 321004, China

[#]Contributed equally

^{*}Corresponding authors

e-mail: zhww@scuec.edu.cn, lizhijun0202@126.com; phone: +86-997-468-1202

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Abstract. The TCP (TEOSINTE BRANCHED1/CYCLOIDEA/PROLIFERATING CELL FACTORS) gene family is a plant-specific transcription factor family and plays an important regulatory role in plant growth and development. However, the identification, characterization, and expression levels of TCPS during leaf development in *Populus pruinosa* and *Populus euphratica* remain unclear. In this study, 33 and 34 non-redundant TCPS in *P. pruinosa* and *P. euphratica*, (PpTCPS/PeTCPS) are identified, respectively, which contain TCP-conserved domains and are unevenly distributed on 19 chromosomes. Among them, PpTCP19, PpTCP27, PeTCP19, and PeTCP28 undergo positive selection in the *Populus* sect. *Turanga*. Furthermore, transcriptome data on different leaf morphologies of *P. pruinosa*/*P. euphratica* and available functional data of TCPS in *Arabidopsis thaliana* (AtTCP) support the involvement of the TCP gene family in the leaf development of the *Populus* sect. *Turanga*. PpTCP7, PpTCP19, PeTCP7, and PeTCP19 may regulate leaf morphology by restricting cell division at the boundaries of leaves and sepals in *P. pruinosa* and *P. euphratica*, respectively. These results elucidate a foundation for an in-depth analysis of the correlation between the expression pattern of the TCP gene and leaf morphology changes in the *Populus* sect. *Turanga*, and provide valuable information for the functional study of TCP transcription factors in the *Populus* sect. *Turanga*.

Keywords: desert poplar species, phylogenetic analysis, TCP gene family, heteromorphic leaves

Introduction

Plant transcription factors (TFs) respond to various biotic and abiotic stresses by regulating the expression of target genes during plant growth and development (Huo et al., 2019; Jin et al., 2017). TCPS are one of the largest families that constitute plant-specific TF (Martín-Trillo et al., 2010). The TCP gene family is named after the earliest identified members of the TCP domain, including TB1 (teosinte branched1) from *Zea mays* L., CYC (cycloidea) in *Antirrhinum majus* L., and PCF1 and PCF 2

(PROLIFERATING CELL FACTORS 1 and 2) in *Oryza sativa* L. (Luo et al., 1996; Doebley et al., 1997; Kosugi and Ohashi, 1997). In addition, it contains approximately 59 amino acids and has an atypical basic helix (bHLH) structure (Cubas et al., 1999), and is located at the N-terminus, thereby allowing it to interact with DNA or proteins (Kosugi and Ohashi, 2002). Plant-specific TCPs play regulatory roles in different processes of plant growth and development, including the establishment of leaf morphology (Yu et al., 2022; Palatnik et al., 2003; Qi et al., 2019; Zhang et al., 2021; Li et al., 2022; Lin et al., 2016), the formation of trichomes (Vadde et al., 2018), and the floral organ morphogenesis as well as leaf growth (Nag et al., 2009; Koyama et al., 2011). Functional analysis of the CINCINNATA (CIN) subclass genes in *Arabidopsis* demonstrate that class II TCPs participate in plant leaf morphogenesis by inhibiting the proliferation of leaf margin cells (Palatnik et al., 2003). *TCP15* in *Arabidopsis* is involved in ROS-mediated signal transduction during exposure to high-light-intensity conditions (Viola et al., 2016). Moreover, *AtTCP14* and *AtTCP15* control the SPINDLY (SPY) sensitivity to cytokine (CK) and regulate the expressions of CK-responsive genes (Steiner et al., 2012). Furthermore, miR156 interacts with TCP through its target SPL9, the complex that promotes the complexity of leaves under the control of cup-shaped cotyledon (CUC), thereby suggesting that TCP may play an important role in the leaf morphology regulatory cascade center on the miR156 module (Wang et al., 2011; Yang et al., 2011).

Herein, *P. pruinosa* and *P. euphratica* have been studied, both belong to the sister species of the *Populus* sect. *Turanga* and are dominant tree species that occur commonly in the arid deserts of Central Asia (Gai et al., 2021). The natural distribution of *P. pruinosa* is limited to Western China and adjacent areas, while *P. euphratica* extends from Western China to Southern Morocco. They become natural protective barriers for desert forest ecosystems because of their high tolerance to salinity and drought stress (Sun et al., 2023; Wu et al., 2022; Han et al., 2013). A leaf is an organ with the largest area exposed to the atmosphere, and the change of its shape will reflect plants' adaptation to the environment. The morphological features of *P. pruinosa* and *P. euphratica* differentiate well, and they both have heteromorphic leaves. The leaf morphology of an adult *P. pruinosa* is oblong, round, and broad ovate from the bottom to the top of the canopy (Liu et al., 2016), meanwhile, adult *P. euphratica* is strip, lanceolate, ovate, and broad ovate from bottom to top (Wang et al., 1998; Hao, 2017; Zheng et al., 2007). Liu et al. (2016) studied the ontogeny process of the heteromorphic leaves of *P. pruinosa* and found that their ontogeny process appeared in sequence with oblong, round, and broad ovate leaves. Different photosynthetic areas and the accumulation of photosynthetic products respond to the constant demand for photosynthetic products and mineral nutrient metabolites in individual growth and development. Meanwhile, *P. euphratica* appears linear, lanceolate, ovate, and broad ovate leaves in turn with the process of individual development. Zeng et al. (Zeng, 2020) also studied the physiological and biochemical characteristics of typical heteromorphic leaves of *P. euphratica* and found that the specific leaf weight, dark respiration rate, and light saturation point of *P. euphratica* in linear, lanceolate, ovate, and broad ovate leaves gradually increased, which made it to have a different accumulation of photosynthetic products at different stages of development. Although various studies have reported the physiological and biochemical characteristics of heteromorphic leaves of *P. pruinosa* and *P. euphratica*, the role of TCPs in the leaf morphology of the *Populus* sect. *Turanga* species remains unknown.

This study conducted a systematic analysis of the TCP gene family of the *Populus* sect. *Turanga* including the identification of TCPs, physicochemical properties, phylogeny, and expression patterns during leaf morphology changes in the *Populus* sect. *Turanga*. Consequently, our data offer detailed information on TCPs in *P. pruinosa* and *P. euphratica* (*PpTCPs*/*PeTCPs*) character, which deeply improves our understanding of TCPs in the *Populus* sect. *Turanga* and elucidates the function of *PpTCPs*/*PeTCPs* in leaf morphology.

Materials and methods

Plant materials and experimental design

All samples of this experiment were sourced from the *P. pruinosa* and *P. euphratica* forest (40°32'36.90"N, 81°17'56.52"E) in Alar City, Xinjiang Province, China. *P. pruinosa* and *P. euphratica* leaves matured from the end of July to August. When there were 7–13 leaves on a bud, the first three fastest growing leaves in the branch of oblong, round, and broad ovate leaves of *P. pruinosa*, the linear, lanceolate, ovate, and broad ovate leaves of *P. euphratica* were selected as materials based on the variation law of *P.h*l (Zhao and Qin., 2017). All samples were quickly frozen in liquid nitrogen and stored at –80 °C for transcriptome sequencing. This experiment was performed in three biological replicates (three biological replicates here indicated three different plants).

Identification of TCPs in Populus sect. Turanga

PpTCPs/*PeTCPs* were analyzed based on *P. pruinosa* (<https://www.ncbi.nlm.nih.gov/PRJCA006811/>)/*P. euphratica* genome numbers (<https://ngdc.cnbc.ac.cn/PRJCA005959/>). The BLAST algorithm was applied to identify all potential *PpTCPs*/*PeTCPs* with TCP or TCP-like domains. BLASTP searches were performed to identify chromosome-level genomes in *P. pruinosa* and *P. euphratica* using amino acid sequences containing the TCP or TCP-like domains of *Arabidopsis* proteins (the genetic information of *P. pruinosa* can be obtained from the genome of *P. pruinosa* at the chromosome level. https://figshare.com/articles/online_resource/Pprgenome_fa/20705107/2). In addition, TCP proteins were identified using HMMER (<http://hmmer.org/Download.html>). Hidden Markov Model (HMM) profiles corresponding to the TCP-conserved domain (PF03634) downloaded from the Pfam protein family database (<http://pfam.xfam.org/search>) were scanned to identify the TCP proteins (Potter and Finn, 2018). Then, the same TCP-like domain sequences were verified using SMART (Simple Modular Architecture Research Tool, <http://smart.embl-heidelberg.de/>) and NCBI-CDD (<https://www.ncbi.nlm.nih.gov/cdd>). At E values < 1e⁻⁵, the protein was identified as a member of the *PpTCPs*/*PeTCPs* transcription factor family. Finally, the physicochemical properties of *PpTCP*/*PeTCP* proteins, including amino acid length (aa), protein molecular weight (mw), and isoelectric points (pI) were obtained using ProtParam in the ExPASy website (<https://web.expasy.org/protparam/>) (Duvaud et al., 2021). Wolf PAORT (<https://wolfsort.hgc.jp/>) was used to predict the subcellular localization of *PpTCP*/*PeTCP* proteins (Horton et al., 2007).

Gene duplications and repeats sequence of *Populus* sect. *Turanga*

Genome-wide replication modes were identified using MCScanX (<https://megasoftware.net/>), and replication patterns of *PpTCPs*/*PeTCPs* were counted. After being manually inspected, SMART was used to analyze the repeat amino acid sites in *PpTCP*/*PeTCP* proteins. Then, the alignments of full-length amino acid sequences of *PpTCPs*/*PeTCPs* were performed using the MUSCLE method of MEGA-X with default settings. Subsequently, after amino acid sequence alignment, gap trimming was performed using the multiple alignment trimming tool of TBtools software with a site coverage cutoff parameter of 0.95.

Phylogenetic relationship, gene structure and conserved motif composition of *PpTCPs*/*PeTCPs*

The gap trimming was conducted with MUSCLE method of MEGA-X with default settings and a parameter of 0.95 (Wang et al., 2010). The neighbor-joining method was used to build a phylogenetic tree (neighbor-joining), the bootstrapping repetition algorithm was set to 1000, and other parameters were set at default. The structure of *PpTCPs*/*PeTCPs* encoded proteins and the conserved motifs were screened and identified using the online website GSDS (<http://gsds.cbi.pku.edu.cn/>) and MEME (<http://meme-suite.org/tools/meme>). Based on this, the results of domain analysis were combined, and the structure and conserved motifs were drawn with TBtools (Chen et al., 2020).

Collinearity and phylogenetic analysis of TCPs in multispecies

Orthologous pairs of *P. pruinosa* and *Arabidopsis*, *Salix brachista*, and *P. euphratica* were aligned using the BLASTP. Then, collinear regions between *P. pruinosa* and *Arabidopsis*, *P. pruinosa* and *Salix brachista*, as well as *P. pruinosa* and *P. euphratica* were screened by MCscan and visualized using JCVI (<https://zenodo.org/record/31631/>). The SMART website was used to retrieve the domain coordinates in the TCP protein sequences of *P. pruinosa*/*P. euphratica* and *Arabidopsis*. Subsequently, the combined protein sequences were used to construct a phylogenetic tree between the two species using the EvolView (<http://www.evolgenius.info/evolview/>) and TBtools software was used to display the phylogenetic tree. Identical (Ka) and non-identical mutation frequency (Ks) values of the *PpTCPs* sequence were calculated using the TBtools software. $Ka/Ks < 1$, $Ka/Ks = 1$, and $Ka/Ks > 1$ indicated purification selection pressure, neutral evolution, and positive selection pressure, respectively.

Prediction of cis-acting elements in the promoters of TCPs of *Populus* sect. *Turanga*

The 2000 bp upstream sequence of the CDS transcription start site of *PpTCPs*/*PeTCPs* was extracted using TBtools, and Plant CARE (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>) was used as a cis-acting element to predict the promoter region.

Transcriptome sequencing and data analysis of TCPs at different leaf morphology

Different leaf morphologies and their control samples of *P. pruinosa* and *P. pruinosa* leaves were selected for transcriptome sequencing. Moreover, RNA extraction, cDNA

library construction, RNA-seq, and raw data analysis were performed by Illumina novaseq 6000 (Frasergen, Wuhan, China). After the library was qualified, DNB (DNA Nano Ball) was prepared, then loaded onto the sequencing chip, and sequenced using a high-throughput sequencer made by MGI; the SOAPnuke (v2.1.0) software was used (Chen et al., 2018) to filter the original reads to obtain high-quality clean reads to process off-machine data. Afterward, the obtained high-quality Illumina clean reads were compared with *P. pruinosa* and *P. euphratica* reference genomes using the Hisat2 (v2.1.0) software (Kim et al., 2019). Meanwhile, the StringTie (v1.3.4d) (Pertea et al., 2016) software was used to carry out quantitative expression analysis of genes in each sample. Gene expression patterns were quantified using FPKM (fragments per kilobase per million), which essentially referred to fragments per kilobase as compared to exons of the reference genome per million reads. Among them, $|\log_2 \text{Fold Change}| > 1.5$ and P value < 0.05 were considered differentially expressed genes. The obtained data were subjected to cluster analysis and expression heat map drawing using the TBtools software.

Results

Identification and chromosomal classification of *PpTCPs* and *PeTCPs*

Herein, a total of 33 *PpTCPs* were identified in the *P. pruinosa* genome by HMMER and BLASTP, and they were named *PpTCP1–PpTCP33* based on the chromosomal arrangement of genes in the genome. A total of 34 *PeTCPs* were identified in the *P. euphratica* genome using the same methods, and they were named *PeTCP1–PeTCP34*. Chromosome mapping found that *TCPs* were unevenly distributed on the 19 chromosomes of the two species of the poplar. Moreover, *TCPs* were distributed on 17 out of the 19 *P. pruinosa* chromosomes in an uneven manner, with the number of *PpTCPs* per chromosome ranging from 0 to 5. Chromosomes 4 contained five genes, while no *TCP* was found on Chromosomes 17 and 18 (Fig. 1A). Meanwhile, a total of 34 *PeTCPs* were distributed across 19 chromosomes, except for Chr08 with different densities (Fig. 1B), these results suggested that each chromosome contributed differently to the evolution of *PpTCPs/PeTCPs*. The amino acid number, protein molecular weight (MW), and isoelectric point values of *PpTCP* proteins ranged from 176 (*PpTCP22*) to 572 (*PpTCP16*), from 19.32 kDa (*PpTCP22*) to 60.15 (*PpTCP16*), and from 5.44 (*PpTCP22*) to 9.96 (*PpTCP17*), respectively. Meanwhile, the amino acid number, MV, and the isoelectric point value of *PeTCP* proteins ranged from 120 (*PeTCP5*) to 661 (*PeTCP20*), from 12.96 kDa (*PeTCP5*) to 73.09 kDa (*PeTCP20*), and from 6.51 (*PeTCP29*) to 10.95 (*PeTCP5*), respectively (Tables 1a, b, c and Electronic Appendix 1). In addition, subcellular localization found that *PpTCP/PeTCP* proteins were primarily located in the nucleus.

Table 1a. The identification and character analysis of *PpTCPs*

TCP gene	Subcellular localization
PpTCP1	nucl: 14
PpTCP2	nucl: 13, golg: 1 PpTCP3 details nucl: 12, chlo: 1, extr: 1
PpTCP3	nucl: 12, chlo: 1, extr: 1
PpTCP4	nucl: 12.5, cyto_nucl: 7.5, cyto: 1.5
PpTCP5	nucl: 13, golg: 1
PpTCP6	nucl: 12.5, cyto_nucl: 7, plas: 1

PpTCP7	nucl: 13, cyto: 1
PpTCP8	nucl: 13, chlo: 1
PpTCP9	nucl: 7, cyto: 5, extr: 1, cysk: 1
PpTCP10	nucl: 9, chlo: 1, cyto: 1, extr: 1, vacu: 1, pero: 1
PpTCP11	nucl: 14
PpTCP12	nucl: 10.5, nucl_plas: 6, mito: 2, cyto: 1
PpTCP13	chlo: 6, mito: 5, nucl: 3
PpTCP15	nucl: 10, mito: 2, plas: 1.5, golg_plas: 1.5
PpTCP16	nucl: 14
PpTCP17	nucl: 14
PpTCP18	nucl: 14
PpTCP19	nucl: 13, chlo: 1
PpTCP20	nucl: 12, chlo: 1, cyto: 1
PpTCP21	nucl: 13.5, cyto_nucl: 7.5
PpTCP22	nucl: 11, cyto: 2, cysk: 1
PpTCP23	nucl: 14
PpTCP24	nucl: 14 PpTCP14 details nucl: 12, chlo: 1, cyto: 1
PpTCP25	nucl: 13, plas: 1
PpTCP26	nucl: 14
PpTCP26	nucl: 14
PpTCP27	nucl: 10.5, cyto_nucl: 6, chlo: 1, extr: 1, vacu: 1
PpTCP28	nucl: 14
PpTCP29	chlo: 7, nucl: 3.5, mito: 3, cyto_nucl: 2.5
PpTCP30	nucl: 14
PpTCP31	nucl: 14
PpTCP32	nucl: 14
PpTCP33	nucl: 13.5, cyto_nucl: 7.5

Table 1b. The identification and character analysis of PpTCPS

PpTCP gene	PpTCP ID in genome	Length of amino acid	Relative molecular mass (Da)	Isoelectric point
PpTCP1	PprTF01G0485	200	21543.21	9.38
PpTCP2	PprTF01G0945	366	40732.16	9.57
PpTCP3	PprTF01G2752	365	40459.25	7.79
PpTCP4	PprTF01G3161	396	41467.58	5.71
PpTCP5	PprTF02G1396	320	33914.62	8.99
PpTCP6	PprTF03G1426	334	35402.45	6.5
PpTCP7	PprTF04G0329	478	51676.41	7.75
PpTCP8	PprTF04G0501	346	38055.71	5.99
PpTCP9	PprTF04G0806	276	28086.34	9.51
PpTCP10	PprTF04G0910	344	36797.01	9.3
PpTCP11	PprTF04G1758	395	42714.85	6.9
PpTCP12	PprTF05G0677	269	27542.84	9.72
PpTCP13	PprTF06G1062	346	38078.61	6.13
PpTCP14	PprTF07G0637	407	44236.78	7.31
PpTCP15	PprTF08G1035	489	54904.14	8.61
PpTCP16	PprTF09G0110	572	60149.01	6.73
PpTCP17	PprTF10G1116	332	35489.49	9.96
PpTCP18	PprTF11G0334	377	41441.03	6.71
PpTCP19	PprTF11G0540	389	44464.9	8.23
PpTCP20	PprTF11G0622	346	38078.61	6.13
PpTCP21	PprTF12G0077	302	32655.41	6.24
PpTCP22	PprTF12G0730	176	19321.53	5.44
PpTCP23	PprTF13G0866	389	44369.86	8.2
PpTCP24	PprTF13G0942	419	45686.41	6.46

PpTCP25	PprTF14G1024	417	44215.9	7.17
PpTCP26	PprTF15G0431	318	35593.78	8.23
PpTCP27	PprTF15G0488	357	37949.04	6.09
PpTCP28	PprTF15G1133	197	21251.72	6.59
PpTCP29	PprTF16G0759	302	32790.68	9.19
PpTCP30	PprTF19G0500	417	44537.21	6.52
PpTCP31	PprTF19G0575	472	51782.41	7.41
PpTCP32	PprTF19G0791	395	42682.97	7.01
PpTCP33	PprTF001Sca0109	389	44677.01	8.03

Table 1c. The identification and character analysis of *PpTCPS*

TCP gene	Subcellular localization
PpTCP1	nucl: 14
PpTCP2	nucl: 13, golg: 1 PpTCP3 details nucl: 12, chlo: 1, extr: 1
PpTCP3	nucl: 12, chlo: 1, extr: 1
PpTCP4	nucl: 12.5, cyto_nucl: 7.5, cyto: 1.5
PpTCP5	nucl: 13, golg: 1
PpTCP6	nucl: 12.5, cyto_nucl: 7, plas: 1
PpTCP7	nucl: 13, cyto: 1
PpTCP8	nucl: 13, chlo: 1
PpTCP9	nucl: 7, cyto: 5, extr: 1, cysk: 1
PpTCP10	nucl: 9, chlo: 1, cyto: 1, extr: 1, vacu: 1, pero: 1
PpTCP11	nucl: 14
PpTCP12	nucl: 10.5, nucl_plas: 6, mito: 2, cyto: 1
PpTCP13	chlo: 6, mito: 5, nucl: 3
PpTCP15	nucl: 10, mito: 2, plas: 1.5, golg_plas: 1.5
PpTCP16	nucl: 14
PpTCP17	nucl: 14
PpTCP18	nucl: 14
PpTCP19	nucl: 13, chlo: 1
PpTCP20	nucl: 12, chlo: 1, cyto: 1
PpTCP21	nucl: 13.5, cyto_nucl: 7.5
PpTCP22	nucl: 11, cyto: 2, cysk: 1
PpTCP23	nucl: 14
PpTCP24	nucl: 14 PpTCP14 details nucl: 12, chlo: 1, cyto: 1
PpTCP25	nucl: 13, plas: 1
PpTCP26	nucl: 14
PpTCP26	nucl: 14
PpTCP27	nucl: 10.5, cyto_nucl: 6, chlo: 1, extr: 1, vacu: 1
PpTCP28	nucl: 14
PpTCP29	chlo: 7, nucl: 3.5, mito: 3, cyto_nucl: 2.5
PpTCP30	nucl: 14
PpTCP31	nucl: 14
PpTCP32	nucl: 14
PpTCP33	nucl: 13.5, cyto_nucl: 7.5

Sequences and protein structure analysis of *TCPS* in *Populus* sect. *Turanga*

The amino acid sites of TCP domains in *PpTCP* and *PeTCP* proteins were analyzed using SMART to study the domain sequence characteristics of TCP proteins (Fig. 2A, B). The results showed that all TCP protein sequences have a highly conserved domain (bHLH) consisting of 63–65 amino acids. Analysis of the conserved domains of the two

poplars found that the basic region was the most conserved, consisting of 20 amino acids. Among them, *P. pruinosa* contained seven conserved amino acid residues (2D, 3R, 6K, 10R, 12R, 13R, and 15R), while *P. euphratica* contained five (6K, 10R, 12R, 13R, and 15R) (Fig. A1A, B). In addition, the helix regions of TCP proteins were relatively conserved in the two poplars, and the hydrophobic amino acids were widely distributed.

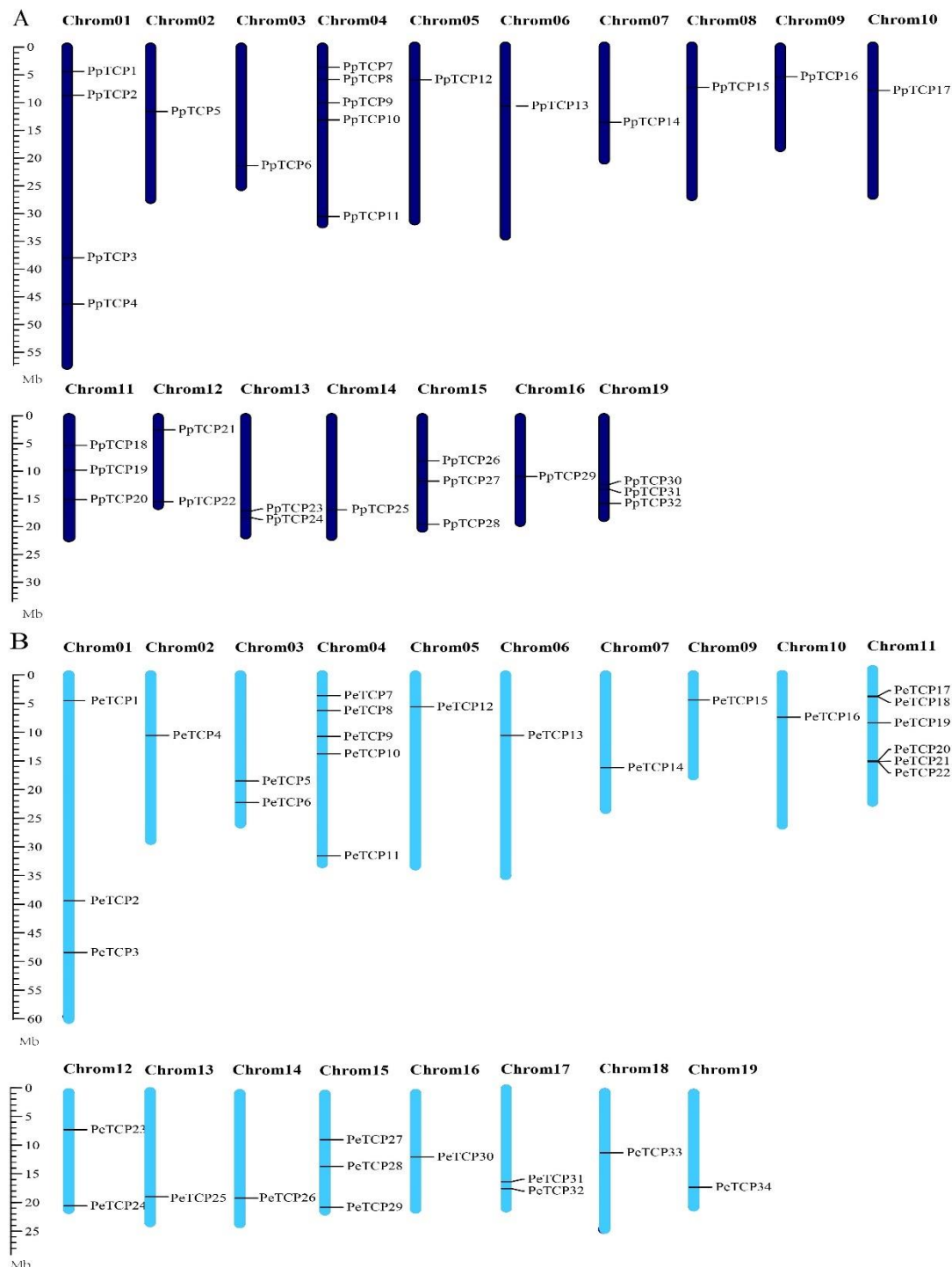


Figure 1. The chromosomal locations of *Populus* sect. *Turanga*. (A) The chromosomal locations of TCPS in *P. pruinosa*. (B) The chromosomal locations of TCPS in *P. euphratica*. Graphical representation of locations for TCPS on each chromosome

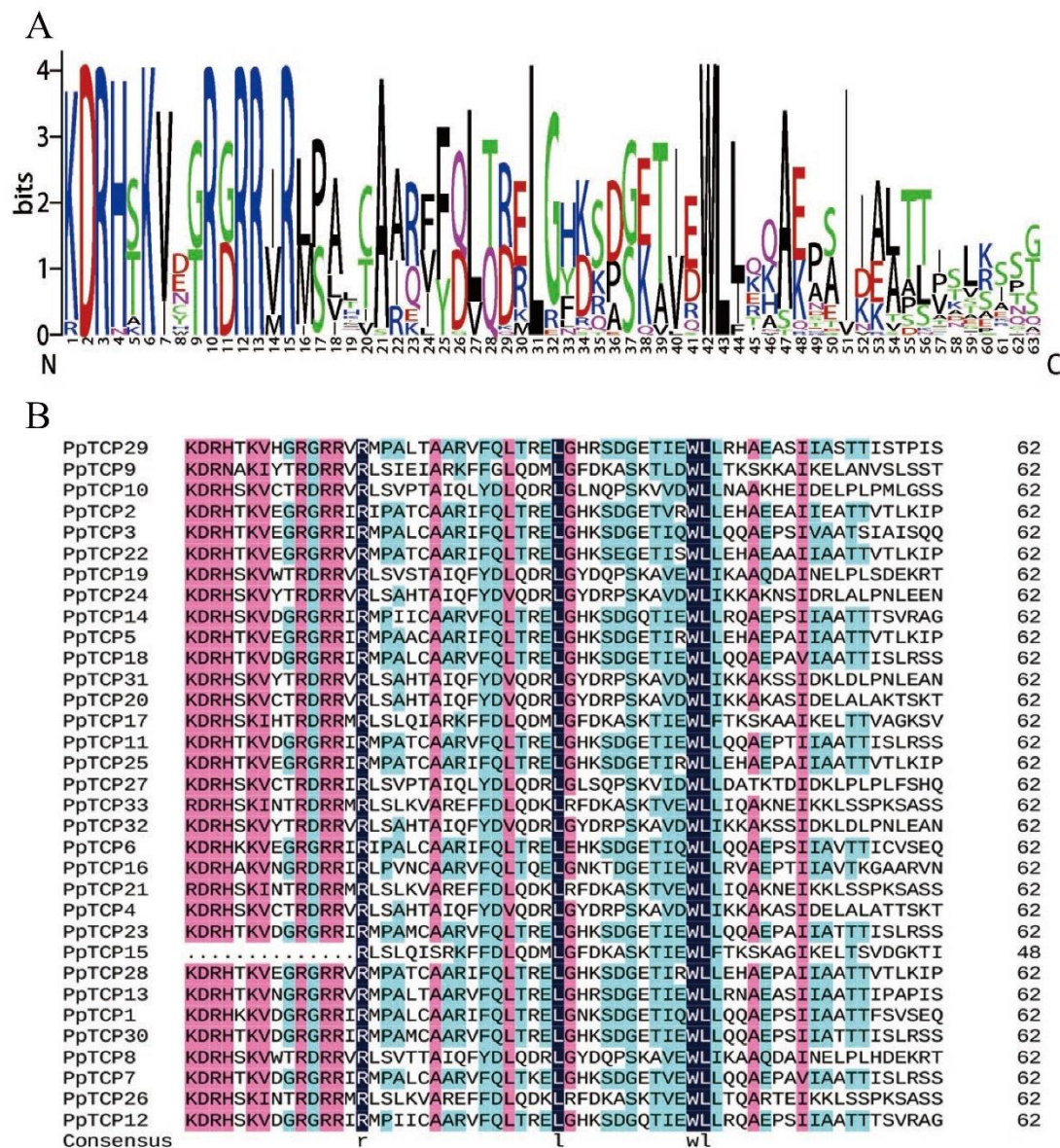


Figure 2. Alignment of TCP domains from 33 PpTCPs. (A) Amino acids are expressed in the standard single letter code. The size of the letters at each position represents their frequency. Numbers in the horizontal axis indicate the position of amino acids. (B) The conserved motif of PpTCPs predicted by MEME.5.4.1 online tools (<http://meme-suite.org/tools/meme>). The conserved amino acids are indicated with colored box. Numbers in the vertical axis indicate the total number of amino acids in this TCP domains

The secondary structure prediction results showed that 33 PpTCP proteins were primarily random coil in *P. pruinosa*, accounting for 51.26% (PpTCP15)–81.92% (PpTCP10); followed by α helix and extend strand, accounting for 9.04% (PpTCP10)–39.34% (PpTCP9) and 6.17% (PpTCP21)–17.10% (PpTCP12); β turn accounted for the lowest proportion, ranging from 0.55% (PpTCP9)–6.25% (PpTCP16). Except for PpTCP2 and PpTCP28, in which the secondary structure was random coil > extend strand > α helix > β turn, the secondary structure of the remaining 31 PpTCP proteins was random coil > α helix > extend strand > β turn (Table 2a). The secondary structure

prediction results showed that 34 PeTCP proteins were primarily random coil in *P. euphratica*, accounting for 50.80% (PeTCP32)–77.69% (PeTCP31); followed by α helix and extend strand, accounting for 10.74% (PeTCP31)–37.50% (PeTCP27), and 5.96% (PeTCP1)–18.33% (PpTCP29); and β turn accounted for the lowest proportion, ranging from 0.53% (PeTCP9)–8.33% (PeTCP5) (Table 2b).

Table 2a. Secondary structure of PpTCPS

Protein	Alpha helix/%	Extended strand/%	Beta turn/%	Random coil/%
PpTCP1	17.55%	9.27%	4.64%	68.54%
PpTCP2	11.62%	12.88%	5.30%	70.20%
PpTCP3	21.56%	8.12%	5.62%	64.69%
PpTCP4	13.60%	10.26%	1.43%	74.70%
PpTCP5	16.28%	10.47%	2.91%	70.35%
PpTCP6	20.20%	9.27%	4.30%	66.23%
PpTCP7	18.53%	11.17%	3.81%	66.50%
PpTCP8	15.04%	12.08%	2.12%	70.76%
PpTCP9	39.34%	8.20%	0.55%	51.91%
PpTCP10	9.04%	6.85%	2.19%	81.92%
PpTCP11	15.38%	11.36%	3.15%	70.10%
PpTCP12	20.82%	17.10%	5.95%	56.13%
PpTCP13	26.90%	8.12%	4.06%	60.91%
PpTCP14	21.38%	13.77%	5.43%	59.42%
PpTCP15	36.48%	10.69%	1.57%	51.26%
PpTCP16	22.73%	12.50%	6.25%	58.52%
PpTCP17	29.55%	14.21%	4.91%	51.33%
PpTCP18	19.24%	10.38%	4.81%	65.57%
PpTCP19	14.85%	13.18%	2.72%	69.25%
PpTCP20	14.00%	10.57%	3.19%	72.24%
PpTCP21	36.76%	6.17%	1.03%	56.04%
PpTCP22	17.66%	13.77%	3.89%	64.67%
PpTCP23	15.83%	10.07%	2.64%	71.46%
PpTCP24	13.01%	10.69%	2.89%	73.41%
PpTCP25	14.16%	12.35%	3.92%	69.58%
PpTCP26	35.99%	6.17%	1.80%	56.04%
PpTCP27	13.53%	10.88%	4.24%	71.35%
PpTCP28	13.45%	14.85%	4.76%	66.95%
PpTCP29	24.50%	17.00%	5.00%	53.50%
PpTCP30	19.90%	12.23%	3.84%	64.03%
PpTCP31	14.74%	11.85%	3.76%	69.65%
PpTCP32	14.74%	11.85%	3.76%	69.65%
PpTCP33	32.65%	9.25%	1.03%	57.07%

Table 2b. Secondary structure of PeTCPS

Protein	Alpha helix/%	Extended strand/%	Beta turn/%	Random coil/%
PeTCP1	25.17%	5.96%	4.30%	64.57%
PeTCP2	21.25%	8.44%	5.62%	64.69%
PeTCP3	15.35%	11.03%	2.16%	71.46%
PeTCP4	17.37%	12.87%	3.89%	65.87%
PeTCP5	25.00%	15.83%	8.33%	50.83%
PeTCP6	20.20%	9.27%	4.30%	66.23%
PeTCP7	21.27%	11.90%	4.30%	62.53%
PeTCP8	15.34%	11.97%	2.10%	70.59%

PeTCP9	32.01%	9.26%	0.53%	58.20%
PeTCP10	12.88%	7.67%	1.92%	77.53%
PeTCP11	19.07%	11.05%	3.57%	66.31%
PeTCP12	22.14%	15.13%	6.27%	56.46%
PeTCP13	26.63%	13.07%	5.03%	55.28%
PeTCP14	20.29%	11.59%	5.07%	63.04%
PeTCP15	18.75%	10.80%	2.84%	67.61%
PeTCP16	25.86%	12.28%	1.51%	60.34%
PeTCP17	19.24%	10.38%	4.81%	65.57%
PeTCP18	19.24%	10.38%	4.81%	65.57%
PeTCP19	17.57%	11.51%	2.72%	68.20%
PeTCP20	24.51%	13.92%	3.18%	58.40%
PeTCP21	24.41%	13.15%	2.82%	59.62%
PeTCP22	25.60%	14.15%	3.18%	57.07%
PeTCP23	36.76%	6.17%	1.03%	56.04%
PeTCP24	19.05%	17.46%	6.67%	56.83%
PeTCP25	15.07%	10.77%	2.63%	71.53%
PeTCP26	15.36%	10.24%	4.52%	69.88%
PeTCP27	37.50%	6.25%	2.50%	53.75%
PeTCP28	13.26%	9.28%	2.92%	74.54%
PeTCP29	15.83%	18.33%	5.83%	60.00%
PeTCP30	24.50%	17.00%	5.00%	53.50%
PeTCP31	10.74%	9.37%	2.20%	77.69%
PeTCP32	36.90%	11.23%	1.07%	50.80%
PeTCP33	24.50%	17.00%	5.00%	53.50%
PeTCP34	19.90%	12.23%	3.84%	64.03%

Replication modes and positive selection analysis of *Pp*TCPs and *Pe*TCPs

Gene duplication events play important roles not only in genomic rearrangement and expansion but also in the diversification of gene functions, thereby implicating them as the primary driving forces throughout the evolutionary process of genomes, including the WGD (whole genome duplication)/segmental duplication, tandem and dispersed (Wu and Poethig, 2006). The replication events between *P. pruinosa* and *P. euphratica* were analyzed. We found that the replication patterns of *Pp*TCPs genes include 24 WGD/segmental duplication and 9 dispersed (Fig. 3A; Table 3a). The *Pe*TCPs were mainly generated by WGD/segmental duplication (24), followed by dispersed (8) and tandem duplication (2) (Fig. 3B; Table 3b).

Table 3a. The genes duplication modes of *Pp*TCPs

Gene_ID	Dup_type	PpTCP gene
PprTF01G0485	WGD or segmental	PpTCP1
PprTF01G0945	WGD or segmental	PpTCP2
PprTF01G2752	WGD or segmental	PpTCP3
PprTF01G3161	WGD or segmental	PpTCP4
PprTF02G1396	WGD or segmental	PpTCP5
PprTF03G1426	WGD or segmental	PpTCP6
PprTF04G0329	WGD or segmental	PpTCP7
PprTF04G0501	Dispersed	PpTCP8
PprTF04G0806	WGD or segmental	PpTCP9
PprTF04G0910	Dispersed	PpTCP10
PprTF04G1758	Dispersed	PpTCP11

PprTF05G0677	WGD or segmental	PpTCP12
PprTF06G1062	Dispersed	PpTCP13
PprTF07G0637	WGD or segmental	PpTCP14
PprTF08G1035	WGD or segmental	PpTCP15
PprTF09G0110	Dispersed	PpTCP16
PprTF10G1116	WGD or segmental	PpTCP17
PprTF11G0334	WGD or segmental	PpTCP18
PprTF11G0540	Dispersed	PpTCP19
PprTF11G0622	Dispersed	PpTCP20
PprTF12G0077	WGD or segmental	PpTCP21
PprTF12G0730	WGD or segmental	PpTCP22
PprTF13G0866	WGD or segmental	PpTCP23
PprTF13G0942	WGD or segmental	PpTCP24
PprTF14G1024	WGD or segmental	PpTCP25
PprTF15G0431	WGD or segmental	PpTCP26
PprTF15G0488	Dispersed	PpTCP27
PprTF15G1133	WGD or segmental	PpTCP28
PprTF16G0759	Dispersed	PpTCP29
PprTF19G0500	WGD or segmental	PpTCP30
PprTF19G0575	WGD or segmental	PpTCP31
PprTF19G0791	WGD or segmental	PpTCP32
PprTF001Sca0109	WGD or segmental	PpTCP33

Table 3b. Secondary structure of *PeTCPS*

Gene_ID	Dup_type	PeTCP gene
PeuTF01G00582.1	WGD or segmental	PeTCP1
PeuTF01G03221.1	WGD or segmental	PeTCP2
PeuTF01G03710.1	WGD or segmental	PeTCP3
PeuTF02G01346.1	WGD or segmental	PeTCP4
PeuTF03G01171.1	WGD or segmental	PeTCP5
PeuTF03G01663.1	WGD or segmental	PeTCP6
PeuTF04G00384.1	WGD or segmental	PeTCP7
PeuTF04G00594.1	Dispersed	PeTCP8
PeuTF04G00957.1	WGD or segmental	PeTCP9
PeuTF04G01093.1	WGD or segmental	PeTCP10
PeuTF04G02091.1	Dispersed	PeTCP11
PeuTF05G00694.1	WGD or segmental	PeTCP12
PeuTF06G01253.1	Dispersed	PeTCP13
PeuTF07G00787.1	WGD or segmental	PeTCP14
PeuTF09G00105.1	Dispersed	PeTCP15
PeuTF10G01164.1	WGD or segmental	PeTCP16
PeuTF11G00417.1	WGD or segmental	PeTCP17
PeuTF11G00427.1	Dispersed	PeTCP18
PeuTF11G00676.1	Dispersed	PeTCP19
PeuTF11G00791.1	WGD or segmental	PeTCP20
PeuTF11G00795.1	Tandem	PeTCP21
PeuTF11G00796.1	Tandem	PeTCP22
PeuTF12G00485.1	WGD or segmental	PeTCP23
PeuTF12G01222.1	WGD or segmental	PeTCP24
PeuTF13G01061.1	WGD or segmental	PeTCP25
PeuTF14G01204.1	WGD or segmental	PeTCP26
PeuTF15G00494.1	WGD or segmental	PeTCP27
PeuTF15G00571.1	WGD or segmental	PeTCP28
PeuTF15G01225.1	WGD or segmental	PeTCP29

PeuTF16G00852.1	Dispersed	PeTCP30
PeuTF17G00828.1	WGD or segmental	PeTCP31
PeuTF17G00950.1	WGD or segmental	PeTCP32
PeuTF18G00522.1	Dispersed	PeTCP33
PeuTF19G00734.1	WGD or segmental	PeTCP34

Ka/Ks ratio was an important indicator for the evaluation of selection pressure during sequence evolution (Hurst, 2002). We determined the Ka, Ks, and Ka/Ks ratios of *PpTCPs* and *PeTCPs* to explore the evolutionary history of *TCPs*. The results showed that among the 26 pairs of homologous genes, only *PpTCP19* (homologous to *PeTCP19*) and *PpTCP27* (homologous to *PeTCP28*) had a Ka/Ks ratio greater than 1, and the Ka/Ks ratios of the remaining 24 pairs of homologous genes were less than 1. This result indicated that most *PpTCPs* have undergone purification selection, except *PpTCP19* and *PpTCP27*; *PeTCP19* and *PeTCP28*, which have undergone positive selection.

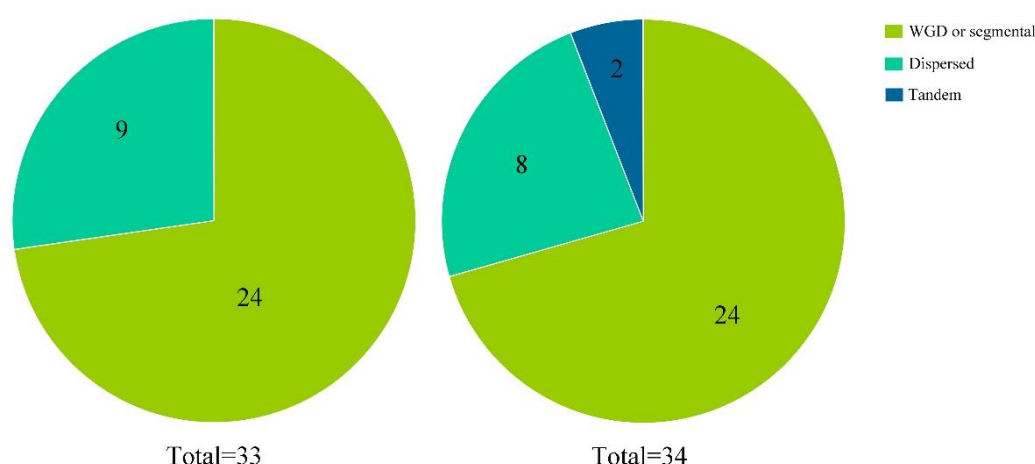


Figure 3. Replication modes of TCP gene family in *Populus* sect. *Turanga*. (A) Replication modes of TCP gene family in *P. pruinosa*. (B) Replication modes of TCP gene family in *P. euphratica*

Analysis of cis-acting elements in the promoter region of *PpTCPs* and *PeTCPs*

Plant CARE was used to analyze *cis*-acting elements in the upstream 2 kb promoter regions of *PpTCPs/PeTCPs* to explore the potential functions of *PpTCPs/PeTCPs*. The results showed that the promoter regions of *PpTCPs/PeTCPs* contained a large number of DNA sequence elements such as CAT-box, TC-rich repeats, ABRE, and TGA-element (Fig. 4A, B). These promoter elements play important roles in meristem expression, defense and stress responses, abscisic acid responses, as well as auxin responses. *PpTCP4*, *PpTCP18*, *PeTCP3*, *PeTCP17*, and *PeTCP18* contain TGA-element *cis*-acting elements. *PpTCP1*, *PpTCP3*, *PpTCP4*, *PpTCP8*, *PpTCP13*, *PpTCP14*, *PpTCP19*, *PpTCP23*, *PpTCP24*, *PpTCP26*, *PpTCP30*; *PeTCP1*, *PeTCP2*, *PeTCP3*, *PeTCP5*, *PeTCP19*, *PeTCP21*, *PeTCP25*, *PeTCP27*, and *PeTCP34* contain CAT-box *cis*-acting elements, thereby indicating that these *TCPs* may play important roles in leaf morphology.

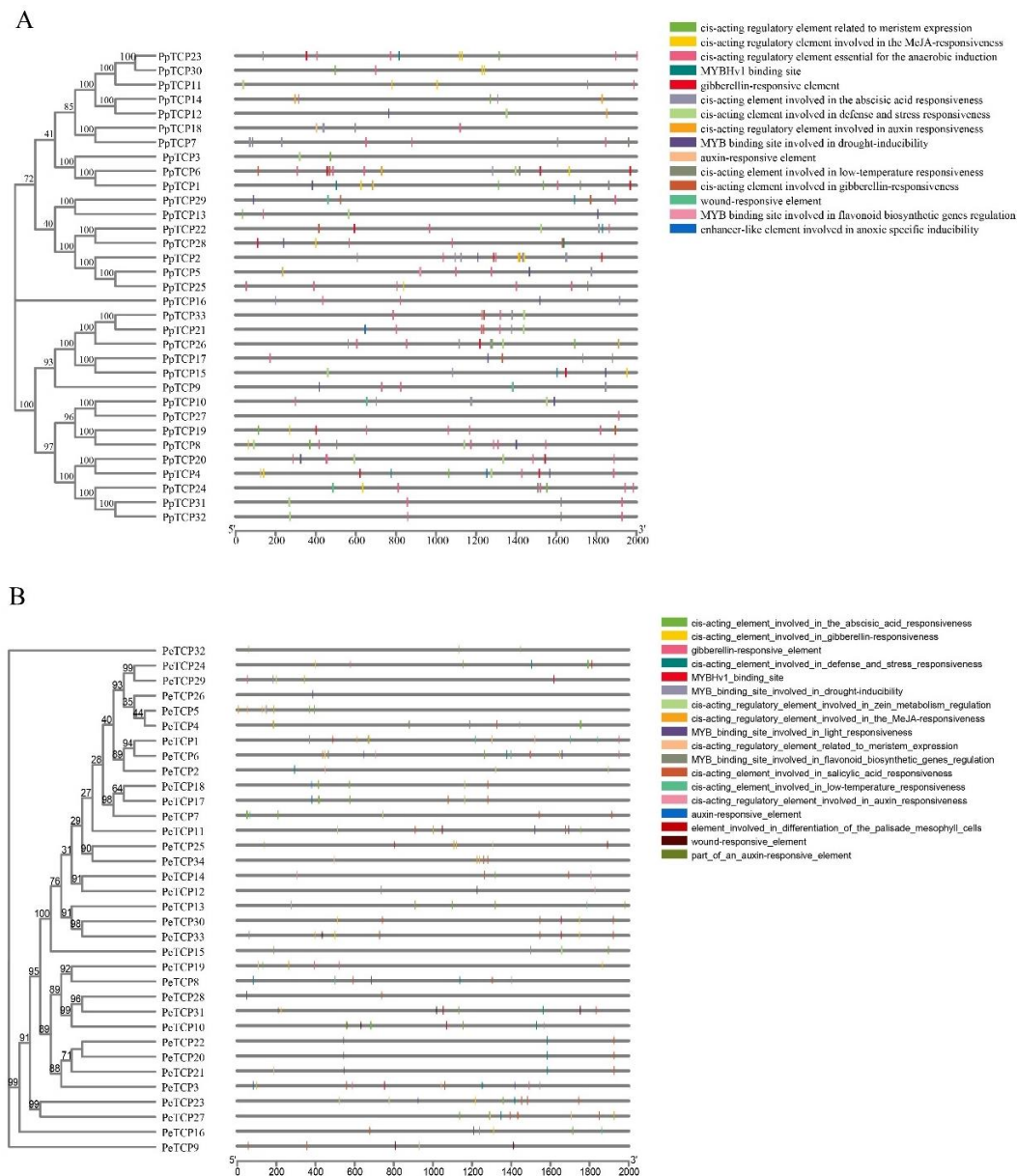


Figure 4. Cis-acting elements identified in the promoters of TCPs in *Populus* sect. *Turanga*. (A) Cis-acting elements identified in the promoters of TCPs in *P. pruinosa*. (B) Cis-acting elements identified in the promoters of TCPs in *P. euphratica*. The micro-segments with different function annotations are represented by different colors. The scale bar represents 100 bp

Collinearity and phylogenetic analysis of interspecific TCP gene family

Herein, we performed a collinear analysis of TCPs between *P. pruinosa* and other species, such as *P. euphratica*, *Salix brachista*, and *Arabidopsis* to explore the potential evolutionary process of *PpTCPs* (Fig. 5). First, a total of 34, 30, and 24 TCPs members were identified in *P. euphratica*, *Salix brachista*, and *Arabidopsis*, respectively (Table 4a, b). Next, the results of collinearity analysis showed that there were 32, 30, and 24 *PpTCPs* have collinearity between *P. euphratica*, *Salix brachista*, and *Arabidopsis* TCPs, respectively (Electronic Appendix 2). Among them, 22 *PpTCPs* (*PpTCP3*, *PpTCP4*, *PpTCP5*, *PpTCP8*, *PpTCP9*, *PpTCP10*, *PpTCP11*, *PpTCP12*,

PpTCP13, *PpTCP14*, *PpTCP17*, *PpTCP19*, *PpTCP20*, *PpTCP21*, *PpTCP23*, *PpTCP25*, *PpTCP26*, *PpTCP27*, *PpTCP28*, and *PpTCP30*) were collinear with three other species, thereby suggesting that these genes may have existed before their ancestors diverged. There were only three *PpTCPs* have a collinear relationship with *PeTCP*, including *PpTCP24*, *PpTCP31*, and *PpTCP32*, thereby suggesting that these *PpTCPs* may play important roles in the long-term adaptive evolution of *P. pruinosa*.

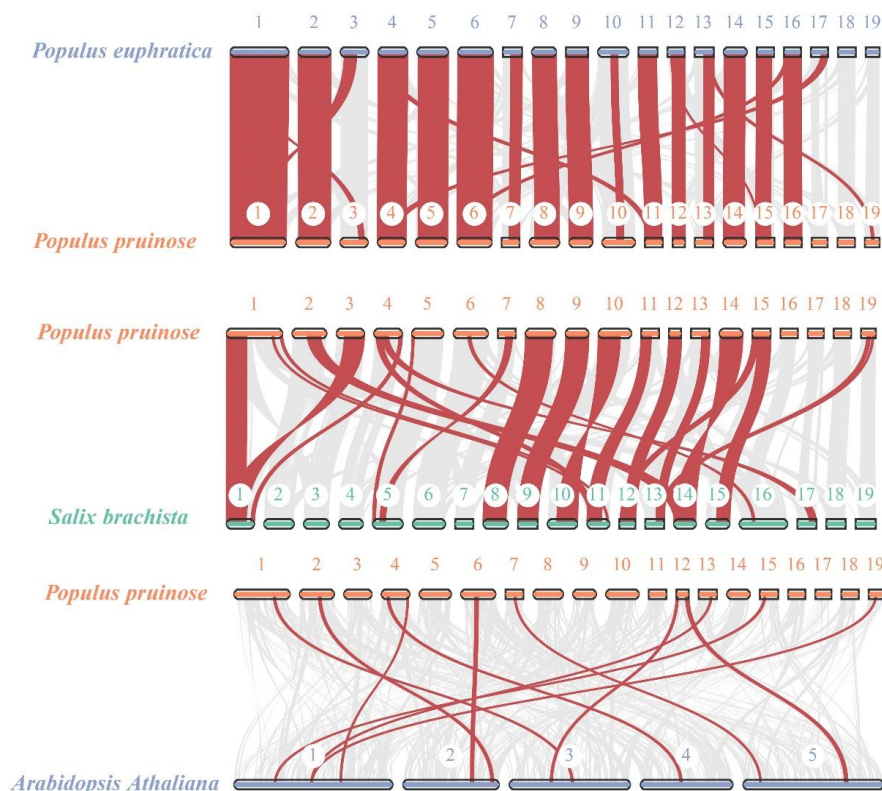


Figure 5. Collinear analysis of TCPs between *P. pruinosa* and three representative plant species (*P. euphratica*, *S. brachista* and *A. thaliana*). The gray line in the background indicates collinear blocks within the *P. euphratica*, *S. brachista* and *A. thaliana* genomes, while the red lines highlight the syntenic TCP gene pairs

A phylogenetic tree of TCPs in *P. pruinosa* and *Arabidopsis*, *P. euphratica* and *Arabidopsis* was constructed to study the evolutionary relationship between *PpTCPs*/*PeTCPs* (Fig. 6A, B), referring to TCPs in *Arabidopsis*. The interspecific phylogenetic tree of *P. pruinosa* was divided into two subgroups based on the topological structure of the interspecific tree (classes I and II). TCPs from these two species showed dispersed distribution in both classes I and II, with *PpTCPs* containing 18 and 15 members, and *PeTCPs* containing 20, 14, and 2 members. Previous studies showed that the TCP of *Arabidopsis* classes I and II CIN branches were involved in the regulation of leaf morphology, including *AtTCP3*, *AtTCP4*, *AtTCP5*, *AtTCP7*, *AtTCP9*, *AtTCP10*, *AtTCP11*, *AtTCP13*, *AtTCP14*, *AtTCP15*, *AtTCP20*, *AtTCP21*, *AtTCP23*, and *AtTCP24* (Li et al., 2005; Schommer et al., 2008; Herve et al., 2009; Koyama and Tomotsugu, 2010; Viola et al., 2011; Kieffer et al., 2011). The number of *PpTCPs* in class II was relatively small, which was consistent with the classification of

Arabidopsis. *PpTCP1*, *PpTCP2*, *PpTCP3*, *PpTCP5*, *PpTCP6*, *PpTCP7*, *PpTCP11*, *PpTCP12*, *PpTCP13*, *PpTCP14*, *PpTCP16*, *PpTCP18*, *PpTCP22*, *PpTCP23*, *PpTCP25*, *PpTCP28*, *PpTCP29*, and *PpTCP30* were clustered with class I subgroup in *Arabidopsis*. Meanwhile, *PpTCP9*, *PpTCP15*, *PpTCP17*, *PpTCP21*, *PpTCP26*, and *PpTCP33* were clustered with class II CYC/TB1 branches in *Arabidopsis*. *PeTCP1*, *PeTCP2*, and other 20 *PeTCPs* were clustered with class I in *Arabidopsis*, *PeTCP9*, *PeTCP32*, and other five *PeTCPs* were clustered with the class II CYC/TB1 branch in *Arabidopsis*. Given that *AtTCPs* with similar functions often show a preference to belong to a subgroup, 21 *PpTCPs* such as *PpTCP6* and 25 *PeTCPs* such as *PeTCP1* may be involved in the regulation of leaf morphology in *P. pruinosa* and *P. euphratica*.

Table 4a. The identification of TCPs in *S. brachis*

TCP gene	Gene ID in genome	Amino acid sequence
SbTCP1	KAB5520921.1	MPALCAARIFQLTRELGHKSDGETIQWLLQQAEPSSIAATGTGTIPASALAAAGGAISQQGASL SAGLHQKIDDLGGSSSRASWAMLGGNLGRPHVTTAGLWPSVGGYGFQSSSDTTGPSTTNI GTEAAAAAAGSSYLQKLGFPGFDLPSNNMGPMSTFSGGGTQQLPGLGLGLSQDGHIGVLSL QALNHIYQQMGHARVQQQQQHFHQNPSSKDDSQSGQWDCAPQNSLVLGLTSYLIQRGEAI NFSHLAVCYMHLTCCVSV
SbTCP2	KAB5540656.1	MILSSFTTKEKSKLSAMNKAASKTKVADPKPRKDRHVKNVNGRDRRIRLPFNCAARVFQLTR ELGNRTDGETIEWLLRVAEPTIVAVTGNGIGPTGANLGYSQAAIVNTAPPVSYSSDLHPLSSA GLSSPLMDPSVEPIQTQGSISRNCCVVGDEPLSFTSEFDNLETDDFMDFPVSDMFPSVPGNDHA
SbTCP3	KAB5544726.1	MFSSSSSFPDQYANHTMVASMENPSSIESCSQTLRHFPDPFLDDNDLLVGELLSSQQQQQQ QQQQEVLSNTALAAEAHDPTTPSASNEEAKINNKKEKSNVDTSKQRIHQRRGTGKDRHSKI HTAQGPDRRRMRLSLQIARKFFDLQDMLGFDKASKTIEWLLTKSKAGIKELTDSAPRVRRRC SISADGKSVPTSEREVVSGIKPPETDGDKRGAEAKKDSLVSNPKEKRSKKVHKPVFNLVDRD SREKARARERERTREMKNGTDTSSDRSSQANPNLEKFESSSPLEYGENLASVNREMKSP VKVSEVKESNHLQTDQMDYVSIAGNFFGITNSPRSFSCMDFSESVEFLPGGTNCKDEFPGF PVNWDMTNDRIRYKYNALTNMKLPPLNAHEESPNSFFMNPNDQNSISILMTTATNANQ SSSNSFFVEDHSDCLYRSA
SbTCP4	KAB5534273.1	MEAEIEQRQPRKFSRVGGNGIKDSSRIAQKNGTDHQPDPDEEDGVLRKRTNSNNGACGGINA AGVDGGTFTGLRAWHHSSRIIRVSRASGGKDRHSKVWTSKGSRRDRVRLSVTTAIQFYDLQD RLGYDQPSKAVEWLIKASQDAINELPSLNHCFFDTPKQLSDEKSSSDGTEQGFDSDADVELED NFRQNQNLSSKSAACSTSTSTSGKSGLSLRSRDIRYSREKARERARERTAKEKEKEYDSRMAH HSNINPISQNSTFTTELLTGIGGASNNNNSTGSPGTSHEANNLFQKVAAARQWSPIMPMDYF GSGLLGPSSSRSTHHSSGFPQIQLGNSIPQAMTMSIPPFCLSGESHQEHQHPFVSDHLIPVA ATAQPPASSGDYNLNFITSSGLAAGFNRGTLQSNSSSPSLLPHLQRFSTSTIDGSTTNLPFFIGA ATPQAMESHHHHHQFPPLQLCYGDGTRHSDQKKGKGN
SbTCP5	KAB5548029.1	MSDMSGAVNNGSLIDPQRNPQPGGAGGGGGGGGGGGGGTNGALVAKPPSKDRHSKVDGR GRRIRMPICAAARVFQLTRELGHKSDGQTIWLLRQAEPSSIAATGTGTPASFSTVSVVRAG GNSNSISLSSNNIHSAGSLEHKPLLGPAFFILGKMRPEEDGNGGGKNDGDVSVGPTIGSLM GPTATAGPGGFWALPARTDFWGFAAAATPEMVVQPTAVQQSSLFMHQHAAAAMGEASAA RVGNLYLPGHLNLLASLSGGTASSGRREDDHR
SbTCP6	KAB5534021.1	MEGGDDQFHHQHHRPTFPFQLLEKKEDDGPCCSSSPYPSPISPSTTEPNNSNTNTNHSTSNL QIEVAEPSKKTTPPKRTSPKDRHTKVDGRGRRIRMPALCAARVFQLTRELGHKSDGETIEWLLQ QAEPAVIAATGTGTIPANFTSLNISLRSSGSSMSLPSQLRSGNFNPNFSLQQRGRSLFPGIGLETS QAPTFLSFQSSNNLNAVLAQKQELRDNSSELSTETESLSRKQRSEQDLSPSQHQMGNYLL QSSTGAIPASHGQIPANFWMLANSNNNQVMMSGDPIWTFPSANNSSVYRGTMSSGLHFMNFFA PVTLLPSQQLGSTSIGGASGGNSGISEGHFNMLAGLNPRYPSGLSESQASGSHSHGGGGGGS DDRHDTSHHHS
SbTCP7	KAB5516571.1	MFSYTNSTISLFPNLSSPAFNPPPYITDHETNDIFHHHRHRHHIDPLAVPLVSTDQLPVTHEIV MNMAASNNSMISKQDVPALDGELYNRSHFLAGKKS VKKDRHSKIFTAQLRDRRVRLSIEIA RKFFDLQDMLGFDKASKTLDWLLTRSKNAIEELAKNGDGKCLSSPSSNCEALSESGYLEDRV LSEIQEQRMKKMQTIQVLDLLSKESRAKARERARERTRVKMCARRLHETVKCPDFSSSCLKQA SSLNQHQSCKESSSYNATCSIKVVSHGQAEPPSSHHSLANQSAKGDIEESVVMPRKLPKSPAP MGYHQNLRLMKDASCNSSSSSYLPNLAQNWDISSAVAHSSFRPITSSNQPTGLL
SbTCP8	KAB5574444.1	MPATCAARVFQLTRELGHKSDGETIEWLLQQAETTHAATGTGTIPANFSTLNVLSRSSGSLT APPSKSAFHSFHSALGLSHHPHYEEGFHPAFLGFHHQQQHLITADQIAQALPIGADVGGSGG ESRGGGEENYMRKRFREDLFKEDNPQEQGESGGGGGGGGEGPPIKAFKSDLQPKTQQQETGS SGLLRSSNILPTTAMWAMAPAPSSGAGNTFWMLPMSAGAGGGPPLATATSVSCPSEPQIW PFATTPASGNPLQAPLHFVPRFNLPTTNLEFQGRGRGSPQLGSMSPQLGSMMLMQQQQHHL HHQQQPSQHLGLMPESNLGMLAALNNAYSRGDLNMNSQQNNPLDHHHHHRHQQQQQQQT QGTTDSGDEDPNSSQ

SbTCP9	KAB5524729.1	MFPLSSNGYDPMYSYNHQAYNMPFFSDIISNSKQYVPPPISEFCHLPSPFFAYDQPELEDHSILFQ QSYDLLLHQQLRATATRTAPSQSTVDNNMVDSSKNDVIDEITEICNKQSHSSTEQIPRKRSSK KDRHRSKINTAQGPRDRRMRLSLKVAREFFDLQDKLRFDKASKTVEWLLTQARTEIKKISGGF PVMNYSCTTVGAKSASSTSECEVLSEINIDSTLKVSRVSKGKSSLCKKERRTSRASSSRKAP LNPFakesREKARARERERTKQKLRSRRLDESKPCELEAVNQEFNQFAGCWSPFETGDQE SGTHNINPSLEVQLVEAEVPFYQEKEQLDTREGMIDETLVSIGKWSPSFPNHLHKNIPQENQF TDFQYPLYKTWDHEAYNIDGLQQLERLNIIEEAPSAAAGEEENSCNLEISSVPEDGFEKELMGL TGGFPGGEKGLEKFIENPPPKQSVAKLTITNKPKPELPLLLPGNSHDNIAWVITVAQAGRH PFSLPFGPFLVRLTEAYVQTHVAA
SbTCP10	KAB5531871.1	MFPLSDNGNDAISCSDDQAYNMPLFSDIGSYSKQDVPPPFSCFLAPSPFIPCDQQEFEDHLIFLQ QNYDLFFQEQLRATVSTNLSQSTVANMVDNSKSDVIELTEICDKKHSSTDQILRKRSSK RDRHRSKINTAQGPRDRRMRLSLKVAREFFDLQDKLSFDKASKTVEWLLVQAKSEIKKLSSGS PHMSYSGSAGTKNASSTSECEMLSEMNAETTTKGRNVSNKGRSSPCAKKERRARRPSSRKTP LNPFakesREKARARERERTKQKLRSRRLDESKPCELEAVNQEFNQFAGCWDTPLETGDQHSIGI QNFNPSLEVQLAAEVDAAIYHEQEKLGTTEGLIDEELVIMSKYWSPSPFNHLYNTGTQPQEVIIIS DDIHFSIINSQTYNTKHGIRHAPLAVCASMDTHLSHHP
SbTCP11	KAB5525432.1	MDSQKHAHHTIEIDQEQSITVASLSDQTDPAALQLKEETLTDPEESRELEEHDNSNIRINSGG SNSCLVAHQVQRQVVTAPKRNTKDRHTKVEGRGRRVRMPATCAARIFQLTRELGHKSDGE TIRWLEHAEPAAIATGTGTVPAAIVSVNGTLKIPTESSAAAAADSDADGLEHKKRKRPCN SDFVDITEAARQNSVSSGLAPIATTSPQGLVPLWPMGTFMFPQGSVGVGGTNQAQVFAFPA TSTAPFFNMAARPISSEFVSAMQPGVQLAGNVSVGFGGESLGGSGSPNTIGSASSSSSGSSAAG ASIGGGGGGTQLLRDFSLEIYDKKELQLGHPVNHHDHQAPCSES
SbTCP12	KAB5573634.1	MASFQEHERGPEDEGGTSDLSTSAGDQEDNRNSGSFSSNECLKISTIDEAEFQVPLKEEPID SDPSSDPDQRTNSMGVPLAMQRQTSMSIMPVVTTTATIRRPSTKDRHTKVEGRGRRIRIP ATCAARIFQLTRELRLHKSDDGETVRWLEHAEQAIIEATGTGTVPAAIVSVGGTLKIPTTSSNNS NSLTETPKKRKRPSRSEFCIDSEAAAVSISQTSGLAPVTPTAPIAAAAATPQGLVPVYAVGNTGM MVPANAFWMIPQAATTASPAQIWLALSPSLNPVFNVAARPISIVASANSENTEGVNTAA AASVVNIPSEVEFRAPSPVAASTSVGAKVAKKSTMAPSLSSDSGGGKGGAQMLRDFSLEIY DKQELQLMGRPGNNQPR
SbTCP13	KAB5569131.1	MASIQRQEEDDMRTVDLRINSRELPNIRASPTNKKNCNDMSVALKEEPCTEERSPTPPLGDM PLAAVPMPLTTVAPPKRASTKDRHTKVEGRGRRIRMPAACAAARIFQLTRELGHKSDGETIRW LLERAEPAVIAATGTGTVPAAIVSVNGTLKIPTTSSSPEGELDATKKRKRPANSEYVDAND TVSVSSSLALMTTQTPQPKRPLPPQPPQTVAEVPSLVPMWAIPSNNAVVPGAFFMLPSLAGPS NQPPQIFTFAAATPLINISARPISSFVSSMQSNIAVAMPVSGSTASGSMATKAGISMMAPSSSSAC TLTSTSTTNRTPQMLRDFSLEIYDKQELQFMPRS
SbTCP14	KAB5538251.1	MFSSSSSFNPFQYANPTMIASTYADPENPSSIEYYSPTSFLQHFPDLFLDDDDLQVGELLSQQQ QQPEVFGSNTHFLAVETPEINPEASNEESKRKINNSKKRGNVNTSKQPIRQRTGKKDRHSKI NTAQGPRDRRMRLSLQIARKFFDLQDMLGFDKASKTIEWLFTKSKAAIKELTDTVPKVRKWS RTSAGGKSVSSTSESEVVSIGIKLTLDRNGDRRVMEAKSDSLVSKPKEKRSKVKHVPFNPVD RESREKARARARDRTREKMKNGIDKLSQSSQANPDSLEKFGYSSSLEYGENFASGRQEINS VKVVDEGEESNNHHLLPHQMDHVSIIIDKFFGITNSPRSSSIFDFSESVEVPTLKDEFSGFPLK WDMTDGRVQYIYNALPNMKLPSGNVQAQNPANFMTTPHALEQNSTTMIMARLNAHVEES PNSAFMNMSNPNEQNPSILKTIDNGNQFSSSRIFARDYDRL
SbTCP15	KAB5519866.1	MASETVLNFSSTNTQHQQPKLNNSVVPSTPATGGSQSKMAHPRKSTTSQSKDRHTKV HGRGRRVRMPALTAARVFQLTRELGHRSDDGETIEWLLRHAEASIIASTGTGTPIPISTTVGST PISSSSPPASCKVHPVNCIGPEMFSLTAPSSRVLDYRHMPFTALLQPMATVAVDAAEEGR QQEHKSAIFFLVPLVGLGLRNWKGSSCKLLCSPFSTWLEANNMRADCTRLGRTIVLLTVSGI WTHHLSLFPCYIVNPKRFSSSGFPGRGLLQVSFCNMILICRRVENNRNVLCQPGESTGLAGAR LFVDGGHDEIDEK
SbTCP16	KAB5557667.1	MLPFIDPQLPFPLSSPRSLAFQLARSSPSNGYFYGFDCWLTELTVVPLVQLDHMASSKKPPPK RTSTKDRHTKVVDGRGRRIRMPALCAARVYKMGISFLTGLVMVFGYAYPAYECYKTVELNK PEIEHLRFWCQYWILVAVLTVGERIGDAFISWVPMYSEAKLAFYIYLWYPKTKGTSYVYDSFF RPYVAKHENDIDRSLLELRTRAGDMAVYVWQRAASYGQTRVFDVLQYIASQSTPRPRPAQF KGVVSLVYKMQSCTRVSWHDHLYFYWRTLHVGDGKLQLGVPQPQGARARQPPAPSRQPST NRQAIPAQAETEEPPSPSTSSSSQNQMEVAEVEAGPSKVLETAAPKAVPSKAVPSKAVPAT ASNAQKENAAASEVFSQPKPTEDGAAVETEEAPSSTANENENPAPKETVMEQTVRVTRGLR KTRSEKNR
SbTCP17	KAB5516483.1	MITSSKEVDCPPKKEEDTKDGKISKASSSAPWLRKDPRIVRVSRAFGGKDRHSKVCTIRGL RDRRVRLSVATAIQLYDLQDRLGLNQPSKVVDWLLNAAKQDIDELPLPVMPPGTFLNHQA MLGSSHELGAQSQSGKEVFKMNSINWEDPNAPFRPNNSWNADAFWRAKSKEVMDQLNEKE YWKKRNEEDTQDSNIEGNSAAQVSSNFFHIATSHSSLPSLINNGMPYGSFFHLEPPNPLSHM GNHGFSTHTGDIHNLNSLPLSPALSLSSGSQFFVCQPGTTQSYFSPSHVTASMENDRPQINHFQV LTPSTQNLFPNSLTPSPYHTSQSMKPSQYFSATPSLLRSDQNSSEPPDHKVDPGFPV

SbTCP18	KAB5534493.1	MGWSSHGGRRLSGLVFSFRYKYVPCPDVGFDSGSGNIEWQSLLEAAEAASGIRVVAAVAQ PPRRASKQSPKFTREPTFVSKASKYARKMGESHHAETSSRLGISDVTGEIVEVQGGHIVRST GRKDRHSKVCATAGPRDRRVRLSAHTAIQFYDVQDRLGYDRPSKAFDWLIKARASIDELAE LPPWDPATAGFTPKTSKTATRSTQQQSISDEKEHGLSVENVATSKTAAVAEVQNMQQMAE NLNSSSGFLPPSLDSDVIADTIKTFPMDASTETSSPTIRFQNYPPDLLSRSSQSQDLRLSLLSF QEPILLQHQAHHHGHQAQSEHQVLFSGTTAHLGFCISGGWSEQQHPPEITRFQRNPVAVN AEDVGGGGGRAGLIFNASLPPTQTMPPSPVQPLFGQNQFFSQRGTLQSSNTPSFRAWTPAIN LDHLQQQQTASIGIGFTASDGGFSGFRVPARVEGEEEECNKPSSVSSDSRRLVHCLRQSTMGL RRCFQDWLEGWDSGRLEMNQSQIPWEKREIRMEFQDGRVTMSQFSIDNRSCFAME
SbTCP19	KAB5521290.1	MGESHHQAATSSRLGIRNTVGEIVEVQGGHIVRSTGRKDRHSKVCATAGPRDRRVRLSAHTA IQFYDVQDRLGYDRPSKAVDWLIKAKASIDELAELPAWDPTTAGFTPTTSKTTTRRSTQRLNI SDESEYQLSIENAAASKTAAAEIAQNMQQQMDENPNCSGFFPPLSDSDAIADTIKTFPMD ASTETSTPTIQFQNYPPDLLSRSSQSQDLRLSLQSFQEPILLQHQAHHQAQNEHQVLLSGATA HYVGLDGSWGSEQHHTPDITRFQRNLVAVNTAGGGGGGLFNTPTVPPLQTMSPPLVQ PFFGQNQFFSQRGTLQSSNTPSVLAWIDPSITPDHHHHHHHQQQIPQIHQQTAISIGIGFTASG GEFYGFRVPARIQEEEEHDGIHNKPSSASDTRH
SbTCP20	KAB5527026.1	MSAAPKEEPCTEERSPTPLAVMPFAAIPMPLTTVTTPKRASTKDRHTKVEGRGRIRIMPATC AARIFQLTRELGHKSDGETIRWLEHAEPAAIAATGTGTVPAAIAMSNGTLKIPTTTNSSGEPEE PSAKKKRKRANSEYVDVNDVSISASFAPITTRPPQPAPQTVAAVPQGLVPMWAIPSNVTV PGAFFMVPTMPGPSNKPQIFTPAAATPLINISPRISSFVSSMQSNAVALPVSGSKPGKGISM MAPSSSAHTIRTASTTTNSTPQMLRDFSLEIYDKQELQLMSRSSKQ
SbTCP21	KAB5529934.1	MELMESQRNSANQHNGSGGAKDHRHGQKQESVAASLQLVPLESRSSQLQQHESTATTPTS QGPAMGSISSQIGIHPSTSTNSALTKSTTKRPSKDRHTKVDGRGRIRIMPAMCAARVFQLTR ELGHKSDGETIEWLLQAEPAIATTGTGTIPANFSTLNVSLRSSGATISSKSAPLSFHSGLAFY DANNASDARRAMASNPMLGFHHQLYPQNLVSDDNYMRKIFREDLFKETTQQQGTETIEAS NSAKSRAGVQDQETAGSIRPTTNMLTTPMWAVAPAATTNGGNTFWMLPVGGGATPTASVQ EPQMWTFPAAAAGVPSMQRVNFGGGGRVSSPVQLGSMIVQQQLGANQQLGLGISESNMGM TLGGLNPYSSSRVGLGMNLEQHNQDNQPPGSDSGDENPNDSQ
SbTCP22	KAB5556618.1	MSDNSGAVNNGALIDPQRNPPGSGGGGATNGALVVKKPPSKDRHSKVDGRGRIRIMPICA ARVFQLTRELGHKSDGTIEWLLRQAEPPIAATGTGTPASFSTVSVSVRAGGNSNISLSSN NVHSAASLDHKLPLGPAPFILGKMRPEEDGNGGKDDGCVIVGPTIGSLMGPTSTAAAGSGG FWALPARPDFWGFATAPPEMVVQPTAVQQSSLFMHQHAAAAAAAAAAMGEASAARLGNLY PGHLNLLASLSGGTGSSGRREEDQR
SbTCP23	KAB5529995.1	MSCGVLGAWSLRSAMAAVFGISNKEHSLGIHSLSKNPQLLQNNQQHGEAINSNQTEGVPNK QSLHQTTEYAKYRRRPEMKSTGGEIIVQGGHIVRSTGRKDRHSKVCATAGPRDRRVRLSAH TAIQFYDVQDRLGYDRPSKAVDWLIKAKNSIDMLAELPPWQPPANNANLEENQNAAGSSE MGIAEEPSSSGYSFQLHGQLTDHNPNGNDSSFLEPTIDPAIDPTMRSFFPTCSTNSSMSFQSYPT VIARTANHTEDLGLSLHSFQDQVLLHGQSQAQVTAQTPSTDQNLFEQSAPVGFDFANFQRMMA WSNDTNADNRVAGGFTSSPPLTPHQAMLAQGSFAFSQRGTLQSSFPISIRAWNDLHMASIEH HRAQELHQSILFSSRFASEGLTGFSIPARIHGENEQNVVSDRRPPSSPNSSQS
SbTCP24	KAB5561009.1	MIPSSKQVGSAPAKKEENSNDKISKASSSETWLRLKDPRIVRVSRAGGKDRHSKVCITIRGLR DRRVRLSVPTAIQLYDLQDRLGLNQPSKVVDWLLNAAKHEIDELPPLPIPPGIFSLNRQAMP SSHEVGSSQSSKEVFKMSNSINWEDTSGFTRPGFWNADAILRAKSKEVAIDPVNEKENWKKR DEEDKQDGNIEGNSAQVLSSSFFQRASHSSPLSLINNAMPYGSFFHLETPSFPLSHMGNHGFA AQTDGIHNLNVLPFSSALSLSSGSQILVCPPTVTTSYFPSHATASMEIDPRQINHFQWLTPSTQN LLPNSLTPSPYPINQSMRPSQYFNVTPRVLHSHNSESPPDKDLFPCK
SbTCP25	KAB5561725.1	MNSKSTSKIKVDDPKSRKDRRLKVNGRDRRIRLPINCAARIFQLTRELVTGNGAPATSVNTTM VPQPPGLHPSAGSVGLS SSNIYACEPRSTETIAQQLNIKEKNSEVRENDPLVFPPGFDNLGTNFDMEFLK
SbTCP26	KAB5573228.1	MKPNILTDGIERTGKYMFPFRGHLLQSKRLLFSSVPMPEKGPNNHQLQVPSFLNPPQKASVPES NINNHNHRHPAEIKDFQI MIANRDDNKKQLAPKRSSNKDRHKKVDGRGRIRIMPALCAARIFQLTRELGNKSDGETIQW LLQQAEPPIAATGTGTIPASALAVAGASVSEQNSVSAGLHAKTEGLPADTGSRDRTNWT MMNTNLGRSNLASGVWPSAAGIGSGFVPNSGQASNFGNENSNLTPKYGFHGVFEPNINMG LMSFYSMFSGTNQFPGLGLSLQDGMFNPQASSPFYQQMVGGRVNLNNEEQQEQPDP KDDSQGSRQ
SbTCP27	KAB5524813.1	MISNPRDKGLRGKQAGGSSHEAGKISEATPSTSSRQWSAFRNPRIVRVSRTEFGGKDRHSKVC TVRGLRDRRIRLSVPTAIQLYDLQHKLGLSQPSKVVDWLLDATKNIDKLPPLQIPLPGYGQF HQPVLFSHQSNIASPFFDPNSTFIKDVGFHSLGMKIDTSAADLDGRVQNCSSGTGFSRDIEASTR AKDKQAETITEKGKWRANEGGGIGSYSAAEQVSAQNFFPLATHSSMPTLLSNTNMPFNSYH WEASNLSTEFSGHGFQSQAESNLNSPSSPLSSGSQLFCCPPPPMPLFSPYPPCVNESRDMN HFQLSSSSSQHILPSTRAMSSRTKPFSLNLNPGEDK

SbTCP28	KAB5512700.1	MNMIERLQRVEKDVKKSVLVGRIKVLILLERNSPHSSLFFLWDLTLTRDRDRWGGNLHIRKLG QIKLFWEHQFCDLWKQEATATILGEKNLKRNLDFQRNQHPHNPIHQIPRPEAEAEQSLGT QSLSQHPQLHLQNQQYGEAINPNQPEVVPSTESLEYAKHSQRMGMKSTAGEIIVQGGHI VRSTGRKDRHSKVYTAKGPRDRRVRLSAHTAIQFYDVQDRLGYDRPSKAVDWLIKAKASSI DKLAELPPWQPTANNANENLEANQAGSSEMAIAEEPSSGYTFQLHRQLADHNPSNDSSFL APTIDPDAMKSFFPTSSANSSMNFQSYPTVISRSTNHTEDLGLSLHSFQDQGLLHGQSQADTT HTPSTDHNLFEQSAPVGFDFHANFQRMVAVSNDTNAENRVAGGFTFNPPPLTPHQAMLAQAQ AFSQRGPLQSSFPHSIRSWNDVHMASTDHHRQAQEIHQSLIFGSRFVSDGLSGFSIPSRHGEDEQ NVVSDRPSSSSPNSQN
SbTCP29	KAB5512590.1	MELMESQRNNRNQQRNGGADEDDHGGKEESAASLQLVAPLESQSSQLQHHGSTASPTSQ GPFMGSIASQAGVHPSTSTSNSTITKSTVKRPSKDRHTKVDGRGRIRMPAMCAARVFLTRE LGHKSDGETIEWLLQQAEPHIIATGTGTIPANFSTLNVSLRSSGATISPSVSKSAPLSFHSGLAF YDANDASDTRRAMASNPMLGFHHQLYPQNLVSDDNYLKRTFREDLFKETTQQRSTETIEA SNSGKSRTGVQDQETAGSFRPATNLPTPMWAVAPAATTNGGNTFWMLPVGGGAAPAAAAP EPQMWTFPTAGAAAAGVTSMQRVNFGGSGRVSSPVQLGSMIVQQPVGANQQLGLGISESSM GMIFGGASAYNSSRVGLGMNLDQNNQENQPQGSDSGDEPNDSQ
SbTCP30	KAB5560754.1	MEAEIQRQPCFSGRSGSDTSRIAQNGCGDHQYPDEEEDGELKRSNPSSGGGAINRLRE WHHSSRIIRVSRASGGKDRHSKVWTSQGLRDRRVRLSVTAIQFYDLQDRLGYDQPSKAVE WLKAAQDSINELPPLSHPPDTPKQLSDEKRTSDGTEQGFDSDADVELNDPNFNQNNQNNQ QNQHLSLRSACSSTSETSKGSLSLARSGLRVNRVKARDRARERTAKERENETRNAHHHPIS QNSTFTELLTGIGNVSNNNNSNSSNNNNAASPSEANLFQKAAASRQWPLTPMDYIGTGL LGPSSSRATHHSSGFPQQLGNSIPQPMTMSVPPFNVSGESHQELQHFPFVSDHLIPVAATT QPVGDNLTFTISSSLAAGFNRGTLQSNSSSPSFFSHLQRFSTSSPIDGSTTNVPFLLAGAQPQSM ENHHHHQFPHGLQLCYGDGTRHSDQKKGKGN

Table 4b. The identification of TCPs in *A. thaliana*

TCP gene	Gene ID in genome	Amino acid sequence
AtTCP1	AT1G67260.1	MSSSTNDYNDGNNGVYPLSLYSSLSGHQDIIHNPYNHQLKASPGHMSAVPESLIDYMAF KSNNVVNQGFEPFVSKEIKKVVKKDRHSKIQTAAQGIRDRRVRLSIGIARQFFDLQDMLGFD KASKTLDWLLKKSRAKIEVVQAKNLNDDDEDFGNIGGDVQEEEEKEEDNDGDKSFVYGLS PGYGEVVEATKAGIRKKKSELNRISSKGLGAKARGKAKERTKEMMAYDNPETASDITQS EIMDPFKRSIVNEGDMTHLFYKEIEFDNQESILTNTMLPTKMGQSYNQNNGLMLVDQS SSSNYNTFLPQNLDSYDQNPFDQTLVYVTDKNFPKGKVIQDSFVN
AtTCP2	AT4G18390.1	MIGDLMKNNNGDVVDNEVNNRLSRWHHNSRIIRVSRASGGKDRHSKVLTSKGPRDRRV LSVSTALQFYDLQDRLGYDQPSKAVEWLKAAEDSISELPSLNTHFPTDDENHNQTLTTVA ANSLSKSACSSNSDTSKNSSGLSLRSELDRKARERARERTAKETKERDHNHTSFTDLLNSGS DPVNSNRQWMASAPSSSPMEYFSSGLILGSGQQTHFPISTNSHPFSSISDHHHHHPHHQHQS FVPDHLISPAESNGGAFNLDFNMSTPSGAGAAVSAASGGGFGFNRGTLQSNSTNQHQSF NLQRFTSESGGPQFLGALPAENHHHHNQFQLYYENGCRNSSEHKKGKGN
AtTCP3	AT1G53230.1	MAPDNDHFLDSPPLLEMRHHSATENGCGEIVEVQGGHIVRSTGRKDRHSKVCTAKGP RDRRVRLSAPTAIQFYDVQDRLGFDRPSKAVDWLITKAKSAIDDLAQLPPWNPADTLRQHAA AAANAKPRKTKTLISPPPPQPEETEHHRIGEEEDNESSFLPASMSDSIADTIKSFVASTQQS YHHQPPSRGNTQNQDLLRLSLQSFQNGPPFPNQTPEALFSGQSNQLAFFDSSTASWEQSHQSP EFGKIQLVSWNNVGAESAGSTGPFVAFSPSLHPVYSQSLLSQSGPLQSIPTMIRAWFD PHHHHHHHQSMTTDDLHHHHHPYHIPPGIHSAPGIAFASSGEFSGFRIPARFQGEQEEHGGD NKPSSASSDSRH
AtTCP4	AT3G15030.1	MSDDQFHPPPPSSMRHRSTDAADGGCGEIVEVQGGHIVRSTGRKDRHSKVCTAKGPRDRR VRLSAHTAIQFYDVQDRLGFDRPSKAVDWLIKAKTSIDELAEPLPPWNPADAIRLAAANAKP RRTTAKTQISPPPPQQQQQQQLQFGVFGNGGGAEPHSPNNESSFLPPMSDSIADTIKSF PVIGSSTEAPSNHNLHMHYHHQHPDLSRTNSQNQDLRLSLQSFDPGPPSLHHQHHTSA SASEPTLFYQSNPLGFDTSWEQSSSEFGRIQLVAVNSGGGGGATDTGNGGGFLFAPPTPS TTSFQPVLGQSQQLYSQRGPLQSSSPMIRAWFDPHHHHQSISTDDLNNHHHLLPPPVHQAIP GIGFASGEFSSGFRIPARFQGEQEEHHDGLTHKPSSASSISRH
AtTCP5	AT5G60970.1	MRSGECEDEEIQAKQERDQNNHQNVLNHLMLQQQPPSSVSSSRQWTSAFRNPRIVRVSRF GGKDRHSKVCTVRGLRDRIRLSVPTAIQLYDLQDRLGLSQPSKVIDWLLEAAKDDVDKLPP LQFPHGFNQMYPNLIFGNSGFGESPSTSTTFPGTNLGFLENWDLGSSRTRARLDTTTTQR ESFDLDKKGWIKNDENSNDHQGFNTNHQQQFPLTNPNNTSAYYNLGHLLQSLDQSGNN VTVAISNVAANNNNNLNLHPPSSSAGDGSQLEFGPTPPAMSSLFPTYPSFLGASHHHHVVDGA GHLQLFSSNSNTASQQHMMPGNTSLIRPFHMLMSSNHDTDHSSDNESDS
AtTCP6	AT5G41030.1	MVMEPKKNQNLPSFLNPSRQNDNDKKRKQTEVKGFDIVVGEKRRKKKENEEDQEIQILYE KEKKKPNKDRHLKVEGRGRVRVPLCAARIYQLTKELGHKSDGETLEWLLQHAEPSILSAT VNGIKPTESVVSQPLTADLMCHSVVEEASRTQMEANGLWRNETGGQTIGGFDLNYGIGFDN GVPEIGFDNQTPGLELRLSQVGVLPNPQVFQMGKEQFRVLHHHSHEDQQQSAEENG

AtTCP7	AT5G23280.1	MSINNNNNNNNNNDGLMISSNGALIEQQPSVVVKKPPAKDRHSKVDGRGRRIRMPICAAR VFQLTRELGHKSDGQTIEWLLRQAEPSSIAATGTGTTPASFSTASVSIRGATNSTSLDHKPTSLL GGTSPFILGKRVRADEDSSNNSHNHSSVVGKDEFTTTTTPAGFWAVPARPDFGQVWSFAGAPQE MFLQQQHHHQPLFVHQQQQQQAAMGEASAARVGNLPGHLNLLASLGGSPGSDRREED PR
AtTCP8	AT1G58100.1	MDLSDIRNNNDTAAVATGGGARQLVDASLSIVPRSTPPEDSTLATTSSSTATATTTKRSTKDR HTKVDGRGRRIRMPALCAARVFQLTRELGHKSDGETIEWLLQQAEPPIVAATGTGTIPANFST LSVSLRSSGSTLSAPPSKSVPLYGALGLTHHQYDEQGGGVFAAHTSPLLGFHHQLQHHQNNQ NQNQDPVETIPEGNFSSRKRYSVDLSKENDDRKQENKSLKESETSGPTAAPMWAVAPPSR SGAGNTFWMLPVPTTAGNQMESSNNNTAAGHRAPPMWPFVNSAGGGAGGGGAATHFM AGTGFSFPMQYRGSPLQLGSFLAQPPQPTQNLGLSMPDSNLGMLAALNSAYSRRGGNANANA EQANNAVEHQEKQQQSDHDDDSREENSNSSE
AtTCP9	AT2G45680.1	MATIQKLEEVAGKDQTLRAVDLTIINGVRNVETSRPFQVNPTVSLEPKAEPVMPFSMSLAPP SSTGPPLKRASTKDRHTKVEGRGRRIRMPATCAARIFQLTRELGHKSDGETIRWLLNAEPAII AATGTGTVPALAMSVNGTLKIPTTTNADSDMGENLMKKKRKRPSSEYIDISDAYASSGLAP IATTTTIQPPQALASSTVAQQLLPQGMYPMWAIPSNAMIPTVGAFFLIPQIAGPSNQPLAFAP AAAASPSYVAAVQASTMARPPPLQVVPSSGFVSVDVSGSNLSRATSVMAPSSSGVTTGS SSSIATTTTHTLRDFSLEIYEKQELHQFMSTTTARSSNH
AtTCP10	AT2G31070.1	MGLKGYSVGEGGGEIVEVQGGHIIRATGRKDRHSKVFTSKGPRDRRVRLSAHTAIQFYDVQD RLGYDRPSKAVDWLIKKAKTAIDKLEGETTTTTTTRQEPVNTKPESPTLVFQRENNDQTQFV AANLDPEDAMKTFFPATTTTNGGGGTNINFQNYPHQDDNNMVSRTTTPPNLSQDLGLSLHP FQGNNTTVVVPETNNFTTTHFDTFGRISGWNHDLTMTSSSSSEHQEQEERSNGGFMVN HHPHHHHHQPSMMTLNSQQQVFLGGQQQQQQRGTQLQSLFHSFRSWDHHQTTSDDHHH HQNQASSMFASSQYGSHGMMMMQGLSFPNTTRLLHGEEATQPNSSSPPNSHL
AtTCP11	AT2G37000.1	MIFQNVCRNESNFNAIASERSQTQFGVSKSSSSGGGCISARTKDRHTKVNGRSRRVTMPALA AARIFQLTRELGHKTEGETIEWLLSQAEPSSIAATGYGTLISNWVDVAADDSSSSSSMTSPQT QTQTPQSPSCRLDLCQPIGQYPPVNGYSHMPFTAMLEPMTTAESEVEIAEEEEERRRRHH
AtTCP12	AT1G68800.1	MFPSLDTNGYDLFDPFIPHQTTMFPSFITHIQSPNSHHYSSPFPSSDFLESFDESFLINQFLLQ QQDVAANVVESPWKFCCKLELKKKNEKCVDGSTSEVQWRRTVKKRDRHSKICTAQGPRD RRMRLSLQIARKFFDLQDMLGFDKASKTIEWLFSKSKTSIKQLKERRVAASEGGGKDEHLQVD EKEKDETLKLRVSKRRTKTMESSFKTKESRERARKRARERTMAKMKMRLFETSETISDPHQE TREIKITNGVQLEKENKEQEWSTNDVHMVEYQMDSVSIEKFLGLTSDSSSSSIFGDSEECY TSLSSVRGMSTPREHNTTSIATVDEEKSPISFSLYDYLCY
AtTCP13	AT3G02150.2	MNIVSWKDANDEVAGGATRREREVKEDQEETEVRATSGKTVIKKQPTSISSSSSSSWMKSKD PRIVRVSRAFGGKDRHSKVCTLRGLRDRRVRLSVPTAIQLYDLQERLGVDPQSKAVDWLLDA AKEEIDELPLPISPENFSIFNHHSFLNLGQRPGQDPTQLGFKINGCVQKSTTTSREENDREKG ENDVVYTNHHVGSYGTYNLEHHHHHHHQLSLQADYHSHQLHSLVPFSPQILVCPMTTSP TTTTIQSLFPSSSSAGSGTMETLDPQMVSHFQMPLMGNSSSSSSQNISTLYSLLHGSSSNNGG RDIDNRMSSVQFNRTNSTTTANMSRHLGSECTSRGSDHHM
AtTCP14	AT3G47620.1	MQKPTSSILNVIMDGGDSVGGGGGDDHHRHLHHHRPTFPFQLLGKHPDDNHQQQSPSSS SSLFLSHQHQLSQSQPSQSQSQSQPQTQKELLQTQESAVVAACKPPLKRASTKDRHTKV DGRGRRIRMPALCAARVFQLTRELGHKSDGETIEWLLQQAEPSSIAATGTGTIPANFTSLNISL RSSGSSMSLP SHFRSAASTFSPNNIFSPAMLQQQQQQQRGGGVGFHHPHLQGRAPTSSLFPGID NFTPTTSFLNFHNPTKQEGDQDSEELNSEKKRRIQTTSDLHQQQQQHHDQIGGYTLQSSNSG STATAAAQIPGNFWMVAAAAAAGGGGGNNNQTTGGLMTASIGTGGGGGEPVWTFPSINT AAAALYRSGVSGVPAGVSSGLHFMNFAAPMAFLTQQQQLATTSNHEINEDSNNEGGRSD GGGDHHTNQRHHHHQQQHHHNILSGLNQYGRQVSGDSQASGSLGGGDEEDQQD
AtTCP15	AT1G69690.1	MDPDPDHNHRPNFPLQLLDSSTSSSTSLAIISTSEPNSPKPPPKRTSTKDRHTKVEGRGRR IRMPAMCAARVFQLTRELGHKSDGETIEWLLQQAEPPIVAATGTGTIPANFTSLNISLSSRS LSAAHLRTTPSSSYFHSHPHSMTHHLQHQQHVRPKNESHSSSSSSQLLDHNQMGNYLVQST AGSLPTSQSPATAPFWSSGDNTQNLWAFNINPHHSGVAVGDVYNPNSSGSGGGSGVHLMNF AAPIALFSGQLASGYGGGGGGGGEHSHYGVLAALNAAYRPVAETGNHNNNNQNRDGDHH HHNQEDGSTSHHS
AtTCP16	AT3G45150.1	MDSKNGINNSQKARRTPKDRHLKIGGRDRRIRIPPSVAPQLFRLTKELGFKTDGETVSWLLQN AEPPIFAATGHGVTTTNSNEDIQPNRNFPSYTFNGDNISNNVFPCTVNTGHRQMVFVPSTMTD HAPSTNYSTISDNYNSTFNNGNATASDTTSAATTTATTTV
AtTCP17	AT5G08070.1	MGIKKEDQKSSLSLLTQRWNNPRIVRVSRAFGGKDRHSKVCTVRGLRDRRIRLSVMTAIQVY DLQERLGLSQPSKVIDWLLVAKNDVDLLPPLQFPFGHQLNPNLTGLGESFPGVFDLGRTRQ EALDLEKRWVNLVDHVFDDHHHNSNSISQSNKLYFPTTSSSSSYHYNLGHLQQSLLDQSG NVTVAFSNNYNNNNLNPAAETMSSLFPTRYPSFLGGGQLQLFSSTSSQPDHIE

AtTCP18	AT3G18550.1	MNNNIFSTTTTINDDYMLFPYNDHYSSQPLLFPSPSSSINDILIHSTNTSNNHLDHHHQFQQPS PFSHFEPAPDCALLTSFHPENNGHDDNQITPNDNHHPSLHFPLNNTIVEQPTEPSETINLIEDSQ RISTSQDPKMKKAKKPSRTDRHSKIKTAKGTRDRRMRLSLDVAKELFGLQDMLGFDKASKT VEWLLTQAKPEIHKIATLSSHGCFSSGDESHIRPVLGSMDSLCELASMWTVDDRGSNNTN TTETRGNKVDGRSMRGKRKRPEPRTPIKKLSKEERAKARERAKGRTMEKMMMKMKGRSQ LVKVVEEDAHDHGEIHKNNNRSQVNRSSFEMTHCEDKIEELCKNDRFAVCNEFIMNKKDHS NESYDLVNYKPNSSFPVINHHRSQGAANSIEQHQTDLHYSFGAKPRDLMHNYQNMV
AtTCP19	AT5G51910.1	MESNHEGNAIQVIDQVTMTHLSDPNPKTKPGMMLMKQEDGYLPVKTTPAPKRPTSKDRH TKVEGRRRIRMPAGCAARVFQLTRELGHKSDGETIRWLLERAEPPIEATGTGTVPPIAVSV NGTLKIPTSSPVLNDGGRDGDGLIKRRKRKNTSDFVDVNDSSCHSSVTSGLPITASNYGVN ILNVTQGFVPFWPMGMGTAFVTGGPDQMGQMWAIPTVATAPFLNVGARPVSSYVSNASD AEAEMETSGGGTTQPLRDFSLEIYDKRELQFLGGSGNSSPSSCHET
AtTCP20	AT3G27010.1	MDPKNLNRHQVPNPLNPPPPPRNQGLVDDDAASAVVSDENRKPTEIKDFQIVVSASDKEPN KKSQNQNQLGPKRSSNDRHTKVEGRRRIRMPALCAARIFQLTRELGHKSDGETIQWLLQQ AEPPIAATGSGTIPASALASSAATSNHHQGGSLTAGLMISHDLGGSSSSGRPLNWGIGGGEG VSRSSLPTGLWPNVAGFGSGVPTTGLMSEGAGYRIGFPGDFPGVGHMSFASILGGNHNQMP GLEGLSQEGNVGVLPNPQSFQIYQQMGQAQAQAQGRVLHMHMHNEEHQQESGEKDDSQ GSGR
AtTCP21	AT5G08330.1	MADNDGAVSNGIIVEQTSNKGPLNAVKKPPSKDRHSKVDGRGRRRIRMPIICARVFQLTREL GHKSDGQTIEWLLRQAEPPIAATGTGTTASFTASLSTSSPFTLGKRVVRAEEGESGGGGGG GLTVGHTMGTSMLGGGGSGGFVAVPARPDFGQVWSFATGAPPEMVFAQQQPPATLFFVRHQ QQQASAAAAAAMGEASAARVGNYPGLHHLNLLASLGGANGSGRREDDHEPR
AtTCP22	AT1G72010.1	MNQNSSVAEATLQLNSGEKPSPGSIPFISSQGHGNISTSATSSTSTSSGSALAVVKSAAVKKPTK DRHTKVDGRGRRRIRMPAMCAARVFQLTRELGHKSDGETIEWLLQQAEPPIASTGTGTIPANF STLNASLRSGGSTLFSQASKSSSSPLSFHSTGMSLYEDNNGTNGSSVDPSRKLNSAANA FGFHHQMYPPIMSTERNPNTLVKPYREDYFKEPSSAAEPSESSQKASQFQEQLAQGRGTAN VVPQPMWAVAPGTTNGGSFAFWMLPMMSGGREGMQQQPGHQMWAFFNPNYPVGTGRVV TAPMGSMMLGGQQLGLGVAEGNMAAAMRSGRGLAMTLDQHQHQLHQEPNQSQASE NGDDDK
AtTCP23	AT1G35560.1	MESHNNNQSNNTTGSAPLHVPMSGPISGSVSLTTTAPNSTTTTVAATPAKRPSKDRHIKVD GRGRRRIRMPAICARVFQLTRELGHKSDGETIEWLLQQAEPPIAATGTGTIPANISTLNISLRS SGSTLSAPLSKSFHMGRAAQAQAVFGFQQQLYHPHHITDSSSSSLPKTFREEDLFKDPNFLD QEPGSRSPKPGSEAPDQDPGSTRSRTQNMIPPMWALAPTASTNGGSFAFWMLPVGGGGGPA NVQDPSQHMWAFNPGHYPRIGSVQLGSMLVGGQQLGLGVAENNNLGLFSGGGGGDGRV GLGMSLEQKPHQVSDHATRDQNPTIDGSP
AtTCP24	AT1G30210.1	MEVDEDIELQKHQEQSRKLQRFSEDNTGLMRNWNPNSSRIIRVSRASGGKDRHSKVLTSKG LRDRRIRLSVATAIQFYDLQDRLGFDQPSKAVEWLINAASDSITDLPLNNTNFDHLDQNNQNT KSACSSGTSESSLLSLRTEIRGKARERARERTAKDRDKDLQNAHSFTQLLTGGFDQQPSNR NWTGGSDCFNPVQLQIPNSSSQEPNMNHPFSFVPDYNFGISSSSAINGGYSSRGLTQSNSQSLF LNNNNNITQRSSISSSSSSSPMDSQSISSFMATPPPLDHHNHQLPETFDGRLYLYYGEGNRSSD DKAKERR

We performed an orthologous alignment between *PpTCPs*/*PeTCPs* and *AtTCPs* to explore the potential functions of *PpTCPs*/*PeTCPs* and found that 12 *PpTCPs* (*PpTCP3*, *PpTCP4*, *PpTCP5*, *PpTCP7*, *PpTCP10*, *PpTCP11*, *PpTCP12*, *PpTCP13*, *PpTCP19*, *PpTCP23*, *PpTCP27*, and *PpTCP28*) and 15 *PeTCPs* (*PeTCP2*, *PeTCP3*, *PeTCP7*, *PeTCP9*, *PeTCP10*, *PeTCP11*, *PeTCP12*, *PeTCP13*, *PeTCP16*, *PeTCP19*, *PeTCP23*, *PeTCP25*, *PeTCP26*, *PeTCP28*, and *PeTCP29*) were orthologous genes in *Arabidopsis*. These results supported the reliability of the evolutionary tree of *TCPs* between the *Populus* sect. *Turanga* and *Arabidopsis*.

Expression patterns of TCPs in different leaf morphology in *Populus* sect. *Turanga*

The gene expression levels of *PpTCPs*/*PeTCPs* were analyzed by RNA-Seq sequencing to explore the expression patterns of *PpTCPs*/*PeTCPs* in different leaf morphology. After removing low-quality reads, a total of 396,962,572 clean reads were obtained in *P. pruinosa*. The percentages of Q30 and GC were 91.7%–94.63% and 43.6%–45.6%, respectively. Meanwhile, a total of 681,315,674 clean reads were obtained in *P. euphratica*. The percentages of Q30 and GC were 93.49%–95.41% and

43.3%–44.8%, respectively. This indicated that the quality of the transcriptome sequencing data of *P. pruinosa* and *P. euphratica* was high enough for subsequent analysis.

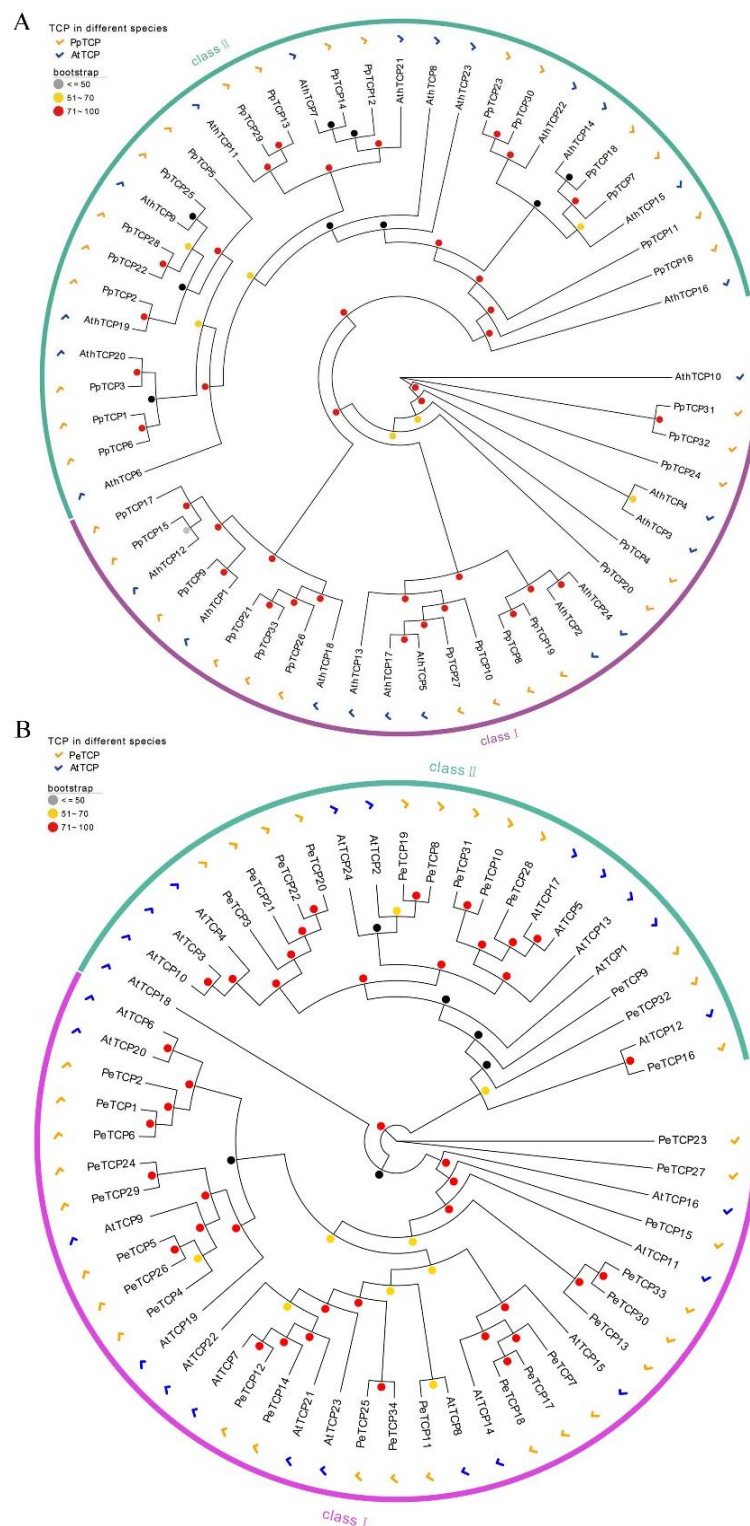


Figure 6. A neighbor-joining phylogenetic tree of *Populus* sect. *Turanga* and *A. thaliana*. (A) A neighbor-joining phylogenetic tree of *P. pruinosa* and *A. thaliana*. (B) A neighbor-joining phylogenetic tree of *P. euphratica* and *A. thaliana*

The analysis of the expression patterns of *TCPs* in the *Populus* sect. *Turanga* showed that the expression levels of *PpTCP9*, *PpTCP15*, and *PpTCP27* (homologous to *AtTCP5*) in the oblong and round leaves were lower than broad ovate, and five *PpTCPs* expression levels in the round leaves were higher than that in the oblong and broad ovate leaves, such as *PpTCP12* (homologous to *AtTCP7*), *PpTCP23* (homologous to *AtTCP23*) (Fig. 7C; Table 5a). In *P. euphratica*, the expressions of 11 *PeTCPs*, such as *PeTCP11* (homologous to *AtTCP8*) and *PeTCP28* (homologous to *AtTCP5*), expression levels in broad ovate leaves were higher than that in oblong and ovate leaves (Fig. 7D; Table 5b). A total of 11 *PpTCPs* contained CAT-box, of which seven *PpTCPs* expression levels were upregulated in oblong leaves (e.g., *PpTCP1*, *PpTCP3*, *PpTCP8*, *PpTCP13*, *PpTCP14*, *PpTCP19*, and *PpTCP30*). In addition, 16 *PpTCPs* contained TC-rich repeats in *cis*-acting elements, six *PpTCPs* (*PpTCP3*, *PpTCP6*, *PpTCP8*, *PpTCP10*, *PpTCP13*, and *PpTCP20*) expression levels were upregulated in oblong leaves, and three *PpTCPs* (*PpTCP12*, *PpTCP22*, and *PpTCP31*) were upregulated in round leaves; 18 *PpTCPs* contained ABRE *cis*-acting elements, of which seven *PpTCPs* (*PpTCP1*, *PpTCP2*, *PpTCP6*, *PpTCP10*, *PpTCP16*, *PpTCP17*, and *PpTCP18*) expression levels were upregulated in oblong leaves. Moreover, two *PpTCPs* (*PpTCP21* and *PpTCP23*) expression levels were upregulated in round leaves, and two *PpTCPs* (*PpTCP9* and *PpTCP15*) expression levels were upregulated in broad ovate leaves (Electronic Appendix 3a). Furthermore, nine *PeTCPs* contained CAT-box, among which four *PeTCPs* expression levels were upregulated in broad ovate leaves (for example, *PeTCP1*, *PeTCP5*, *PeTCP25*, and *PeTCP34*). A total of 12 *PeTCPs* contained TC-rich repeats *cis*-acting elements, of which two *PeTCPs* (*PeTCP2* and *PeTCP6*) expression levels were upregulated in linear leaves, three *PeTCPs* (*PeTCP20*, *PeTCP23*, and *PeTCP24*) expression levels were upregulated in broad ovate leaves, and 20 *PeTCPs* (*PeTCP20*, *PeTCP23*, and *PeTCP24*) contain ABRE *cis*-acting elements, of which two *PeTCPs* (*PeTCP6* and *PpTCP31*) expression levels were upregulated in linear leaves, one *PeTCP* (*PeTCP13*) expression level was upregulated in ovate leaves, and 10 *PeTCPs* (*PeTCP1*, *PeTCP4*, *PeTCP5*, *PeTCP7*, *PeTCP11*, *PeTCP14*, *PeTCP23*, *PeTCP24*, *PeTCP25*, and *PeTCP32*) expression levels were upregulated in broad ovate leaves (Electronic Appendix 3b). Among them, there are CAT-box and TC-rich repeats *cis*-acting elements in the promoters of *PpTCP8*, *PpTCP13*, and *PeTCP2*, ABRE and TGA-element *cis*-acting elements in the promoters of *PpTCP18*, CAT-box, and ABRE *cis*-acting elements in the promoters of *PpTCP1*, *PeTCP1*, *PeTCP5*, and *PeTCP25*. These results indicate that *PpTCPs/PeTCPs* have dynamic changes in the process of poplar leaf morphology. Moreover, *PpTCPs/PeTCPs* may be involved in the regulation of leaf morphology in the *Populus* sect. *Turanga*. In addition, some *PpTCPs* and *PeTCPs* lack expression information (e.g., *PpTCP4*, *PpTCP11*, and *PpTCP22*, etc.), which possibly indicates that these are pseudogenes or are expressed only under special conditions.

Table 5a. Expression of *PpTCPs* in three leaf shapes of *P. pruinosa*

<i>PpTCP</i> gene	Gene ID in genome	Pp_LOF_1	Pp_LOF_2	Pp_LOF_3	Pp_CF_1	Pp_CF_2	Pp_CF_3	Pp_BOF_1	Pp_BOF_2	Pp_BOF_3
<i>PpTCP1</i>	PprTF01G0485	0.496527	0.304383	0.238924	0.573882	0.468835	0.368809	0.209621	1.355704	0.746733
<i>PpTCP2</i>	PprTF01G0945	8.100595	6.407289	13.83313	7.914284	8.822757	12.46217	8.703259	11.98129	18.01558
<i>PpTCP3</i>	PprTF01G2752	32.65648	14.62331	25.06645	24.00908	23.99753	26.20363	19.93702	23.94489	14.41347
<i>PpTCP4</i>	PprTF01G3161	118.0275	96.23908	70.7172	100.1984	81.42838	58.97735	121.8302	109.8492	105.9065
<i>PpTCP5</i>	PprTF02G1396	4.266036	9.185717	9.50161	1.274154	3.985363	3.827708	1.123666	0.604428	2.979659
<i>PpTCP6</i>	PprTF03G1426	7.628499	6.828698	9.582638	6.723079	6.656298	9.007969	6.579758	7.501992	9.439015
<i>PpTCP7</i>	PprTF04G0329	38.84488	38.96642	26.79684	34.76294	35.04028	21.74104	28.20439	19.6759	14.56901
<i>PpTCP8</i>	PprTF04G0501	43.30738	40.90691	53.88897	30.38738	31.20461	40.94036	28.85948	31.23158	36.62663

PpTCP9	PprTF04G0806	0	0	0	0	0	0	0	0	0
PpTCP10	PprTF04G0910	15.82712	16.9993	35.67007	8.530254	12.36972	32.87297	8.3376	12.09226	21.35519
PpTCP11	PprTF04G1758	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A
PpTCP12	PprTF05G0677	63.58367	82.98757	54.76671	62.35713	60.75347	33.22623	39.44052	35.94786	22.33412
PpTCP13	PprTF06G1062	2.371225	3.385167	1.950956	3.242545	3.994873	1.77038	2.751948	1.3306	1.457915
PpTCP14	PprTF07G0637	27.22991	39.17136	35.94096	19.48389	26.64474	24.58399	16.81429	16.11779	14.83702
PpTCP15	PprTF08G1035	1.079699	0.610352	1.258749	0	0	0	0	0	0
PpTCP16	PprTF09G0110	0	0	0	0	0	0	0	0	0
PpTCP17	PprTF10G1116	0	0	0	0	0	0	0	0	0
PpTCP18	PprTF11G0334	9.406084	14.95391	4.522309	7.487578	10.60931	2.439363	4.491916	1.944481	1.229755
PpTCP19	PprTF11G0540	58.24596	42.73195	43.30865	48.2766	46.00862	46.914	56.31674	48.11705	56.29636
PpTCP20	PprTF11G0622	14.60224	20.44063	10.26936	10.95716	19.8027	9.395383	9.615374	18.07886	11.61753
PpTCP21	PprTF12G0077	0.234139	0.133707	0.270613	0	0	0	0	0	0
PpTCP22	PprTF12G0730	1.337184	0.503704	0.720352	0.468177	0.870743	0.704534	0.4952	0.934392	1.128291
PpTCP23	PprTF13G0866	14.00889	10.89207	11.52742	11.68833	16.98406	7.250685	10.20131	15.08822	5.385047
PpTCP24	PprTF13G0942	31.72127	23.14506	21.58299	24.0809	18.2058	20.54935	17.27254	20.07247	11.19798
PpTCP25	PprTF14G1024	15.08943	10.75743	7.696964	8.891618	14.2918	6.566007	7.965655	6.250122	5.660923
PpTCP26	PprTF15G0431	0.519123	0.047527	0.451777	0	0	0	0	0	0
PpTCP27	PprTF15G0488	1.688541	1.287277	1.58112	0.910027	0.908178	1.873439	0.483369	1.140617	1.225727
PpTCP28	PprTF15G1133	1.02372	0.588163	1.271452	0.520864	0.583492	0.638101	0.874001	1.242964	1.597083
PpTCP29	PprTF16G0759	1.186226	2.05425	3.728416	0.698987	1.328511	2.027888	0.356574	0.931482	2.297893
PpTCP30	PprTF19G0500	5.621068	7.807122	6.493599	5.243568	4.601569	4.247828	4.042048	3.061675	2.315325
PpTCP31	PprTF19G0575	0.067657	3.117332	1.6662	0.138588	2.741526	1.483087	0.100104	1.789406	1.469542
PpTCP32	PprTF19G0791	4.621033	4.171856	2.203539	3.752666	2.23274	0.913259	4.315103	1.063693	1.128077
PpTCP33	PprTF001Sca0109	0.548383	0.08442	0.634428	0.042393	0	0	0	0	0

Table 5b. Expression of *PeTCPs* in four leaf shapes of *P. euphratica*

PeTCP gene	Gene ID in genome	Pe_Li_1	Pe_Li_2	Pe_Li_3	Pe_La_1	Pe_La_2	Pe_La_3	Pe_Ov_1	Pe_Ov_2	Pe_Ov_3	Pe_Bo_1	Pe_Bo_2	Pe_Bo_3
PeTCP1	PeuTF01G00582.1	0.304071	0.984902	1.238447	0.562928	0.748769	1.135153	0.457452	1.032856	0.940477	0.569987	1.33492	1.256
PeTCP2	PeuTF01G03221.1	6.949008	11.20156	11.32982	5.749684	6.900757	11.98944	5.565426	6.368849	9.349638	5.784057	9.075234	9.458012
PeTCP3	PeuTF01G03710.1	51.0102	62.40197	75.90199	48.783806	53.877903	76.41217	42.60829	44.09178	52.44685	71.51007	55.10472	59.31002
PeTCP4	PeuTF02G01346.1	5.055426	3.28977	2.849618	4.516585	5.008121	2.566369	14.3685	3.247395	4.723223	16.83519	4.506668	4.865767
PeTCP5	PeuTF03G01171.1	1.743325	0.510393	0.755927	1.446756	1.273301	1.261506	0.984041	1.087611	2.076365	1.569819	2.53856	2.912438
PeTCP6	PeuTF03G01663.1	12.08354	14.19123	12.14559	10.320365	9.896369	12.0996	5.136673	10.954	10.67792	5.114893	12.13881	13.49411
PeTCP7	PeuTF04G00384.1	18.47634	14.33412	13.32567	18.857622	17.39164	15.29495	19.22354	8.816384	15.88135	22.35197	25.09613	23.31814
PeTCP8	PeuTF04G00594.1	34.04805	36.13252	33.7178	34.283978	30.518648	34.49505	43.69861	28.90287	30.0119	53.32311	22.6951	22.35347
PeTCP9	PeuTF04G00957.1	0	0	0.082321	0	0	0	0	0	0	0	0	0
PeTCP10	PeuTF04G01093.1	10.40833	14.76655	13.87081	10.482673	16.024248	11.48337	14.02793	6.063411	7.535802	18.66303	9.496696	8.390815
PeTCP11	PeuTF04G02091.1	8.961303	7.732124	9.102211	7.196257	6.947812	7.759266	11.41423	4.584918	8.276561	16.0028	8.142462	9.210629
PeTCP12	PeuTF05G00694.1	22.72137	18.67276	15.78229	19.814938	16.357466	19.48415	26.61314	8.777682	17.57686	38.25324	28.12774	28.92195
PeTCP13	PeuTF06G01253.1	5.425749	5.708979	5.725539	5.591628	6.93172	5.154604	6.521687	8.42092	6.632027	4.096058	7.171546	5.257028
PeTCP14	PeuTF07G00787.1	17.16137	10.72899	10.79263	15.599195	12.420944	14.87629	23.46	7.579904	15.25771	30.06738	15.87016	15.56577
PeTCP15	PeuTF09G00105.1	0	0	0	0	0	0	0	0	0	0	0	0
PeTCP16	PeuTF10G01164.1	0	0	0	0	0	0	0	0	0	0	0	0
PeTCP17	PeuTF11G00417.1	0.137636	0.075044	0.07778	0.086503	0.160897	0.111263	0.082854	0	0.087225	0.239137	0.12518	0.102898
PeTCP18	PeuTF11G00427.1	0.011021	0	0	0	0	0	0.063528	0	0	0.055137	0	0.012923
PeTCP19	PeuTF11G00676.1	54.54897	78.11594	78.88257	65.800888	68.86171	78.06641	49.6069	72.50123	71.56714	63.3456	48.28832	50.72112
PeTCP20	PeuTF11G00791.1	1.038181	0.722904	0.77641	1.712669	1.223883	1.432978	0.961055	0.896648	1.267905	1.785305	1.501222	1.448139
PeTCP21	PeuTF11G00795.1	0.034574	0.056936	0.095563	0.104911	0.068887	0.07642	0.066028	0	0.085115	0.151817	0.055595	0.023401
PeTCP22	PeuTF11G00796.1	0.05198	0.045962	0.032175	0	0.050648	0	0.05674	0	0.064716	0.076155	0.081586	0.047297
PeTCP23	PeuTF12G00485.1	0.048775	0.13337	0.023376	0.230859	0.023842	0.149175	1.171197	0	0.194215	1.880142	0.888387	0.539653
PeTCP24	PeuTF12G01222.1	0.908249	1.463258	1.414938	1.240543	1.006204	1.913451	1.095124	1.79366	1.339229	1.095393	3.217885	2.247777
PeTCP25	PeuTF13G01061.1	2.925897	3.714387	3.309559	1.81531	2.592296	4.376925	3.98086	3.303941	6.735123	7.290371	12.29699	10.92197
PeTCP26	PeuTF14G01204.1	4.455343	4.775588	4.619996	3.333934	3.47712	4.682336	18.83096	3.948987	6.007374	13.40371	7.854282	7.483635
PeTCP27	PeuTF15G00494.1	0.038576	0.06997	0	0	0	0	0	0	0	0	0	0
PeTCP28	PeuTF15G00571.1	0.657138	1.069491	1.626706	0.659954	0.766355	1.152483	1.085595	0.764052	0.822464	1.260431	1.393118	1.099532
PeTCP29	PeuTF15G01225.1	0.893361	0.245083	0.521051	0.922237	0.44548	0.920372	1.787049	0.337631	0.689299	1.730397	1.227753	1.017828
PeTCP30	PeuTF16G00852.1	1.186988	0.595808	1.006618	0.085163	0.521124	0.893687	1.010312	0.204293	0.560201	1.196984	0.506899	0.362103
PeTCP31	PeuTF17G00828.1	1.595642	2.905339	2.310266	3.076492	1.662182	1.317447	0.999868	1.319317	1.166479	0.663169	1.178787	1.95512
PeTCP32	PeuTF17G00950.1	0.703651	0.747716	0.335722	0.604033	0.239575	0.276962	3.695337	0.401759	0.07607	6.054657	1.893268	1.747961
PeTCP33	PeuTF18G00522.1	0	0.0369	0	0	0	0	0	0	0	0	0	0
PeTCP34	PeuTF19G00734.1	3.914328	3.331409	3.3398	2.772216	2.903973	3.826566	2.570396	2.204173	3.83076	4.312703	3.832491	3.891502

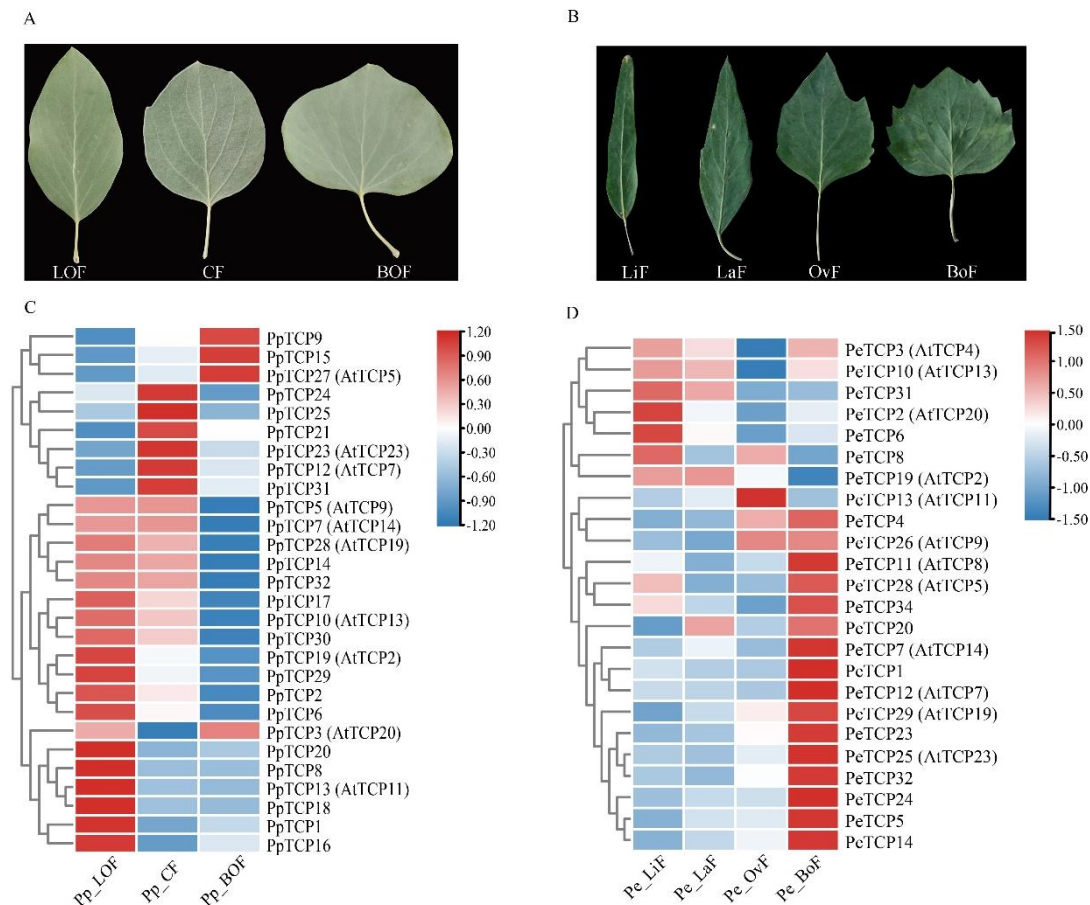


Figure 7. Expression profiles of TCP genes in the *Populus* sect. *Turanga*. (A) Photographs of leaf shape in *P. pruinosa*. (B) Photographs of leaf shape in *P. euphratica*. (C) Expression profiles of TCP genes in the *P. pruinosa* base on the transcriptome data. (D) Expression profiles of TCP genes in the *P. euphratica* base on the transcriptome data. The colour bar represents the normalized FPKM values as follows: red, high expression level; blue, low expression level; and white, no expression. Detail FPKM values is listed in Tables 5a, b

Discussion

P. pruinosa and *P. euphratica* as two sister plant species with morphologically well differentiated provide a good model for elucidating physiological and molecular mechanisms of tolerance in tree species. Plant leaves, as the main organ for photosynthesis and respiration, show continuous and discrete morphological changes during stage transitions: leaf size shows continuous changes, while several different shapes can be seen at different plant developmental stages (Wang and Wang, 1989). In addition to differences in types of habitat, they also occur in different shapes of leaves between *P. pruinosa* and *P. euphratica*. *P. pruinosa* shows three kinds of heteromorphic leaves, including oblong, round, and broad leaves, while *P. euphratica* have four kinds, which are linear, lanceolate, ovate, and broad ovate leaves. The TCP transcription factors play important roles in plant growth and development (Viola et al., 2011; Martín-Trillo and Cubas, 2010), and have been identified in various plants (Yao et al., 2007; Wei et al., 2016; Chen et al., 2016). However, the members of TCPS of *P.*

pruinosa remain unclear, and the comparative investigation of TCPs between *P. pruinosa* and its relatives has not been carried out.

In this study, we performed a comprehensive analysis of *PpTCPs*/*PeTCPs* using chromosome-level *P. pruinosa*/*P. euphratica* genome data, thereby identifying 33 and 34 *PpTCPs* and *PeTCPs*, respectively. Gene duplication events, such as tetraploidization (whole-genome duplication, WGD) and hexaploidization (whole-genome triplication, WGT), occur frequently in plants and are the major sources of evolutionary changes that enable rapid adaptation to different environments (Chen et al., 2016). Furthermore, WDG/segmental duplications are the main drivers of the expansion of *PeTCPs*. Identification and collinearity analysis of multispecies TCPs show that TCPs among Salicaceae species exhibit stronger collinearity as compared with *Arabidopsis*. Therefore, we speculate that the WGD event unique to Salicaceae causes the expansion of *PeTCPs* after differentiation from *Arabidopsis*.

Phylogenetic analysis and sequence alignment show that the genome of *P. euphratica* contains all the classes of TCPs, namely classes I and II. The alignment of these multiple sequences supports the classification of the phylogenetic tree, which is consistent with the TCP subclasses reported in plants, thereby indicating that this gene family is relatively conserved in plants and may have similar functions (Lin et al., 2016). Transcription factors from classes I and II are involved in the regulation of various growth and developmental processes in *Arabidopsis*. *AtTCP2*, *AtTCP3*, *AtTCP4*, *AtTCP10*, and *AtTCP24* were targeted by miR319 and involved in regulating leaf morphology (Palatnik et al., 2003). In addition, *AtTCP4* can regulate leaf margin serrations by affecting auxin distribution through the miR164-CUC pathway (Schommer et al., 2014), and its over-expression will lead to reduced cell division in *Arabidopsis*, thereby leading to smaller leaf size (Sarvepalli and Nath, 2011). In this study, *PpTCP19*, the orthologous gene of *AtTCP2*, is highly expressed in oblong leaves of *P. pruinosa*, and linear and lanceolate leaves of *P. euphratica*. Therefore, we speculate that *PpTCP19* and *PeTCP19* may affect the leaf morphology and “narrow leaves” (oblong, linear, and lanceolate) in the *Populus* sect. *Turanga* by participating in the regulation of leaf cell division. In addition, Ka/Ks analysis found that *PpTCP19* was a positively selected gene, and we speculated that the emergence of the oblong leaves of *P. pruinosa* may be the result of adaptive evolution.

The *AtTCP14* mutants show that *AtTCP14* is strongly expressed at the boundaries of leaves and sepals, wherein the cell division is highly restricted (Kieffer et al., 2011). The orthologous genes of *AtTCP14* in *P. pruinosa* and *P. euphratica* are *PpTCP7* and *PeTCP7*, respectively, which are lowly expressed in the broad ovate leaves of *P. pruinosa* and *P. euphratica*, respectively. Therefore, we speculate that *PpTCP7* and *PeTCP7* may participate in the regulation of “broad ovate leaves” of *P. euphratica* and *P. pruinosa* by inhibiting cell proliferation.

Conclusions

A total of 33 and 34 *PpTCPs* and *PeTCPs* were respectively identified based on the chromosome level genome of *P. pruinosa* and *P. euphratica*, and they were unevenly distributed on 19 chromosomes of *P. pruinosa* and *P. euphratica*. The main driving force of TCPs expansion in the *Populus* sect. *Turanga* was the WGD/fragmental duplication event. In addition, *PpTCP7*, *PpTCP19*, *PeTCP7*, and *PeTCP19* may play important roles in the leaf morphology of the *Populus* sect. *Turanga*. Overall, this study

is a valuable resource for the functional characterization of *TCPs* in the *Populus* sect. *Turanga* and lay further understanding of the structure-function relationship among these *TCP* members. Our study also provides comprehensive information and novel insights into the roles of *TCP* family genes in the regulation and heteromorphic leaves.

Availability of data and materials. The datasets supporting the results of *P. pruinosa* and *P. euphratica* in this article are available at the National Center for Biotechnology Information (*P. pruinosa*) and National Genome Data Center (*P. euphratica*) (NCBI, <https://www.ncbi.nlm.nih.gov>; NGDC, <https://ngdc.cncb.ac.cn/>) under project accession number PRJNA890019 and PRJCA005959.

Competing interests. The authors declared that they had no conflict of interests.

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REFERENCES

- [1] Chang, S. H., Meng, T. C., Wu, C. T., Lin, T. H., Jiang, S. Y., Liu, R. C., Tsai, M. C., Su, L. W., Yang, J. Y. (2018): Alterations of plant architecture and phase transition by the phytoplasma virulence factor SAP11. – *Journal of Experimental Botany* 69(22): 5389-5401.
- [2] Chen, L., Chen, Y. Q., Ding, A. M., Chen, H., Xia, F., Wang, W. F., Sun, Y. H. (2016): Genome-wide analysis of *TCP* family in tobacco. – *Genetics & Molecular Research* 15(2).
- [3] Chen, Y. X., Chen, Y. S., Shi, C. M., Huang, Z. B., Zhang, Y., Li, S. K., Li, Y., Yu, C., Li, Z., Wang, J., Yang, H. M., Fang, L., Chen, Q. (2018): SOAPnuke: a MapReduce acceleration-supported software for integrated quality control and preprocessing of high-throughput sequencing data. – *Oxford Open* 7(1).
- [4] Chen, C. J., Chen, H., Zhang, Y., Thomas, H. R., Frank, M. H., He, Y. H., Xia, R. (2020): TBtools: an integrative toolkit developed for interactive analyses of big biological data. – *Molecular Plant* 13(8): 1194-1202.
- [5] Cubas, P., Lauter, N., Doebley, J., Coen, E. (1999): The *TCP* domain: a motif found in proteins regulating plant growth and development. – *Plant Journal* 18(2): 215-222.
- [6] Doebley, J., Stec, A., Hubbard, L. (1997): The evolution of apical dominance in maize. – *Nature* 386(6624): 485-488.
- [7] Duvaud, S., Gabella, C., Lisacek, F., Stockinger, H., Ioannidis, V., Durinx, C. (2021): ExPASy, the Swiss Bioinformatics Resource Portal, as designed by its users. – *Nucleic Acids Research* 29(W1): W216-W227.
- [8] Gai, Z. S., Zhai, J. T., Chen, X. X., Jiao, P. P., Zhang, S. H., Sun, J. H., Qin, R., Liu, H., Wu, Z. H., Li, Z. J. (2021): Phylogeography reveals geographic and environmental factors driving genetic differentiation of sect. *Turanga* in Northwest China. – *Front Plant Sci* 12: 705083.
- [9] Han, Y., Wang, Y., Jing, H., Wang, M., Korpelainen, H., Li, C., Y. (2013): Reciprocal grafting separates the roles of the root and shoot in sex-related drought responses in *Populus cathayana* males and females. – *Plant Cell Environ* 36: 356-364.
- [10] Hao, J., Yue, N., Zheng, C. (2017): Analysis of changes in anatomical characteristics and physiologic features of heteromorphic leaves in a desert tree, *Populus euphratica*. – *Acta Physiologiae Plantarum* 39: 1-11.
- [11] Hervé, C., Dabos, P., Bardet, C., Jauneau, A., Auriac, M. C., Ramboer, A., Lacout, F., Tremousaygue, D. (2009): In vivo interference with *AtTCP20* function induces severe

- plant growth alterations and deregulates the expression of many genes important for development. – *Plant Physiology* 149(3): 1462-1477.
- [12] Horton, P., Park, K. J., Obayashi, T., Fujita, N., Harada, H., Adams-Collier, C. J., Nakai, K. (2007): WoLF PSORT: protein localization predictor. – *Nucleic Acids Res* 35: W585-W587.
- [13] Huo, Y., Xiong, W., Su, K., Li Yu, Yang, Y., Fu, C., Wu, Z., Sun, Z. (2019): Genome-wide analysis of the *TCP* gene family in Switchgrass (*Panicum virgatum*, L.). *International Journal of Genomics* 1: 1-13.
- [14] Hurst, L. D. (2002): The Ka/Ks ratio: diagnosing the form of sequence evolution. – *Trends in Genetics* 18(9): 486.
- [15] Jin, J. P., Tian, F., Yang, D. C., Meng, Y. Q., Kong, L., Luo, J. C., Gao, G. (2017): PlantTFDB 4.0: toward a central hub for transcription factors and regulatory interactions in plants. – *Nucleic Acids Research* 45(D1): D1040-D1045.
- [16] Kieffer, M., Master, V., Waites, R., Davies, B. (2011): TCP14 and TCP15 affect internode length and leaf shape in *Arabidopsis*. – *Plant Journal for Cell & Molecular Biology* 68(1): 147-158.
- [17] Kim, D., Paggi, J. M., Park, C., Bennett, C., Salzberg, S. L. (2019): Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. – *Nature Biotechnology* 37(8): 907-915.
- [18] Kosugi, S., Ohashi, Y. (1997): PCF1 and PCF2 specifically bind to cis elements in the rice proliferating cell nuclear antigen gene. – *Plant Cell* 9(9): 1607-1619.
- [19] Kosugi, S., Ohashi, Y. (2002): DNA binding and dimerization specificity and potential targets for the TCP protein family. – *Plant Journal* 30(3): 337-348.
- [20] Koyama, T., Mitsuda, N., Seki, M., Shinozaki, K., Ohme-Takagi, M. (2010): TCP Transcription factors regulate the activities of ASYMMETRIC LEAVES1 and miR164, as well as the auxin response, during differentiation of leaves in *Arabidopsis*. – *Plant Cell* 22(11): 3574-3588.
- [21] Koyama, T., Ohme-Takagi, M., Sato, F. (2011): Generation of serrated and wavy petals by inhibition of the activity of TCP transcription factors in *Arabidopsis thaliana*. – *Plant Signaling & Behavior* 6(5): 697-699.
- [22] Li, C. X., Potuschak, T., Colón-Carmona, A., Gutiérrez, R. A., Doerner, P. (2005): *Arabidopsis* TCP20 links regulation of growth and cell division control pathways. – *Proc Natl Acad Sci USA* 102(36): 12978-12983.
- [23] Li, H., Wen, X., Huang, X., Wei, M., Chen, H., Yu, Y., Dai, S. (2022): Genome-wide identification and characterization of *TCP* gene family members in *Melastoma candidum*. – *Molecules* 27(24): 9036.
- [24] Lin, Y. F., Chen, Y. Y., Hsiao, Y. Y., Shen, C. Y., Hsu, J. L., Yeh, C. M., Mitsuda, N., Ohme-Takagi, M., Liu, Z. J., Tsai, W. C. (2016): Genome-wide identification and characterization of TCP genes involved in ovule development of *Phalaenopsis equestris*. – *Journal of Experimental Botany* 67(17): 5051-5066.
- [25] Liu, S., Jiao, P., Li, Z. (2016): The types and temporal and spatial characteristics of heteromorphic leaves of *Populus euphratica*. – *Study on Arid Areas* 33(5): 6.
- [26] Luo, D., Carpenter, R., Vincent, C., Copsey, L., Coen, E. (1996): Origin of floral asymmetry in *Antirrhinum*. – *Nature* 383(6603): 794-799.
- [27] Martín-Trillo, M., Cubas, P. (2010): TCP genes: a family snapshot ten years later. – *Trends in Plant Science* 15(1): 31-39.
- [28] Nag, A., King, S., Jack, T. (2009): miR319a targeting of TCP4 is critical for petal growth and development in *Arabidopsis*. – *Proc Natl Acad Sci U S A* 106(52): 22534-22539.
- [29] Palatnik, J. F., Allen, E., Wu, X. L., Schommer, C., Schwab, R., Carrington, J. C., Weigel, D. (2003): Control of leaf morphogenesis by microRNAs. – *Nature* 425(6955): 257-263.

- [30] Pertea, M., Kim, D., Pertea, G. M., Leek, J. T., Salzberg, S. L. (2016): Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown. – Nature Protocols 11(9): 1650-1667.
- [31] Potter, S. C., Luciani, A., Eddy, S. R., Park, Y., Lopez, R., Finn, R. D. (2018): HMMER web server: 2018 update. – Nucleic Acids Research 46(W1): W200-W204.
- [32] Qi, X., Qu, Y., Gao, R., Jiang, J., Fang, W., Guan, Z., Zhang, F., Zhao, S., Chen, S., Chen, F., Wang, H. (2019): The heterologous expression of a Chrysanthemum nankingense TCP transcription factor blocks cell division in yeast and Arabidopsis thaliana. – Int J Mol Sci 20(19): 4848.
- [33] Sarvepalli, K., Nath, U. (2011): Hyper-activation of the TCP4 transcription factor in *Arabidopsis thaliana* accelerates multiple aspects of plant maturation. – Plant Journal 67(4): 595-607.
- [34] Schommer, C., Palatnik, J. F., Aggarwal, P., Chételat, A., Cubas, P., Farmer, E. E., Nath, U., Weigel, D. (2008): Control of jasmonate biosynthesis and senescence by miR319 Targets. – Plos Biology 6(9): e230.
- [35] Schommer, C., Debernardi, J. M., Bresso, E. G., Rodriguez, R. E., Palatnik, J. F. (2014): Repression of cell proliferation by miR319-regulated TCP4. – Molecular Plant 7(10): 1533-1544.
- [36] Steiner, E., Efroni, I., Gopalraj, M., Saathoff, K., Tseng, T. S., Kieffer, M., Eshed, Y., Olszewski, N., Weiss, D. (2012): The *Arabidopsis* O-linked N-acetylglucosamine Transferase SPINDLY interacts with class I TCPs to facilitate cytokinin responses in leaves and flowers. – Plant Cell 24(1): 96-108.
- [37] Sun, J., Xu, J., Qu, W., Han, X., Qiu, C., Gai, Z., Zhai, J., Qin, R., Liu, H., Wu, Z., Li, Z. (2023): Genome-wide analysis of R2R3-MYB transcription factors reveals their differential responses to drought stress and ABA treatment in desert poplar (*Populus euphratica*). – Gene 855: 147124.
- [38] Vadde, B. V. L., Challa, K. R., Nath, U. (2018): The TCP4 transcription factor regulates trichome cell differentiation by directly activating *GLABROUS INFLORESCENCE STEMS* in *Arabidopsis thaliana*. – Plant Journal 93(2): 259-269.
- [39] Viola, I. L., Manassero, N. G. U., Ripoll, R., Gonzalez, D. H. (2011): The *Arabidopsis* class I TCP transcription factor *AtTCP11* is a developmental regulator with distinct DNA-binding properties due to the presence of a threonine residue at position 15 of the TCP domain. – Biochemical Journal 435(1): 143-155.
- [40] Viola, I. L., Camoirano, A., Gonzalez, D. H. (2016): Redox-dependent modulation of anthocyanin biosynthesis by the TCP transcription factor TCP15 during exposure to high light intensity conditions in *Arabidopsis*. – Plant Physiology 170: 74-85.
- [41] Wang, X., Wang, J. (1989): Plant Morphology and Environment.
- [42] Wang, H. L., Yang, S. D., Zhang, C. L. (1998): The photosynthetic characteristics of differently shaped leaves in *Populus euphratica* Olivier. – Photosynthetica 34(4): 545-553.
- [43] Wang, D., Zhang, Y., Zhang, Z., Zhu, J. (2010): KaKs_Calculator 2.0: A Toolkit incorporating Gamma-series methods and sliding window strategies. – Genomics Proteomics & Bioinformatics 8(1): 77-80.
- [44] Wang, J. W., Park, M. Y., Wang, L. J., Koo, Y., Chen, X. Y., Weigel, D., Poethig, R. S. (2011): MiRNA control of vegetative phase change in trees. – PLoS Genetics 7(2): e1002012.
- [45] Wei, W., Hu, Y., Cui, M. Y., Han, Y. T., Gao, K., Feng, J. Y. (2016): Identification and transcript analysis of the TCP transcription factors in the diploid woodland strawberry *Fragaria vesca*. – Front Plant Sci 7: 1937.
- [46] Wu, G., Park, M. Y., Conway, S. R., Wang, J. W., Weigel, D., Poethig, R. S. (2009): The sequential action of miR156 and miR172 regulates developmental timing in *Arabidopsis*. – Cell 138(4): 750-759.

- [47] Wu, N., Li, Z., Wu, F., Zhen, L. (2022): Sex-specific photosynthetic capacity and Na⁺ homeostasis in *Populus euphratica* exposed to NaCl stress and AMF inoculation. – *Front Plant Sci* 13: 1066954.
- [48] Yang, L., Conway, S. R., Poethig, R. S. (2011): Vegetative phase change is mediated by a leaf-derived signal that represses the transcription of miR156. – *Development* 138(2): 245-249.
- [49] 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*. – *Journal of Integrative Plant Biology* 49: 885-897.
- [50] Yu, Z., Tian, C., Guan, Y., He, J., Wang, Z., Wang, L., Lin, S., Guan, Z., Fang, W., Chen, S., Zhang, F., Jiang, J., Chen, F., Wang, H. (2022): Expression analysis of *TCP* transcription factor family in autopolyploids of *Chrysanthemum nankingense*. – *Front Plant Sci* 13: 860956.
- [51] Zeng, M. (2020): Molecular mechanism of environmental adaptation of heterophylly in *Populus euphratica* Oliv. – Beijing Forestry University.
- [52] Zhang, L., Li, C., Yang, D., Wang, Y., Yang, Y., and Sun, X. (2021): Genomewide analysis of the *tcp* transcription factor genes in *Dendrobium catenatum* lindl. – *Int. J. Mol. Sci.* 22: 10269.
- [53] Zhao, L., Qin, S. (2017): Expression profiles of miRNAs in the genesis of *Populus euphratica* Oliv. heteromorphic leaves. – *Plant Growth Regulation* 81(2): 231-242.
- [54] Zheng, C., Qiu, J., Jiang, C. N., Yue, N., Wang, X. Q., Wang, W. F. (2007): Comparison of stomatal characteristics and photosynthesis of polymorphic *Populus euphratica* leaves. – *Frontiers of Forestry in China* 2(1): 87-93.

APPENDIX

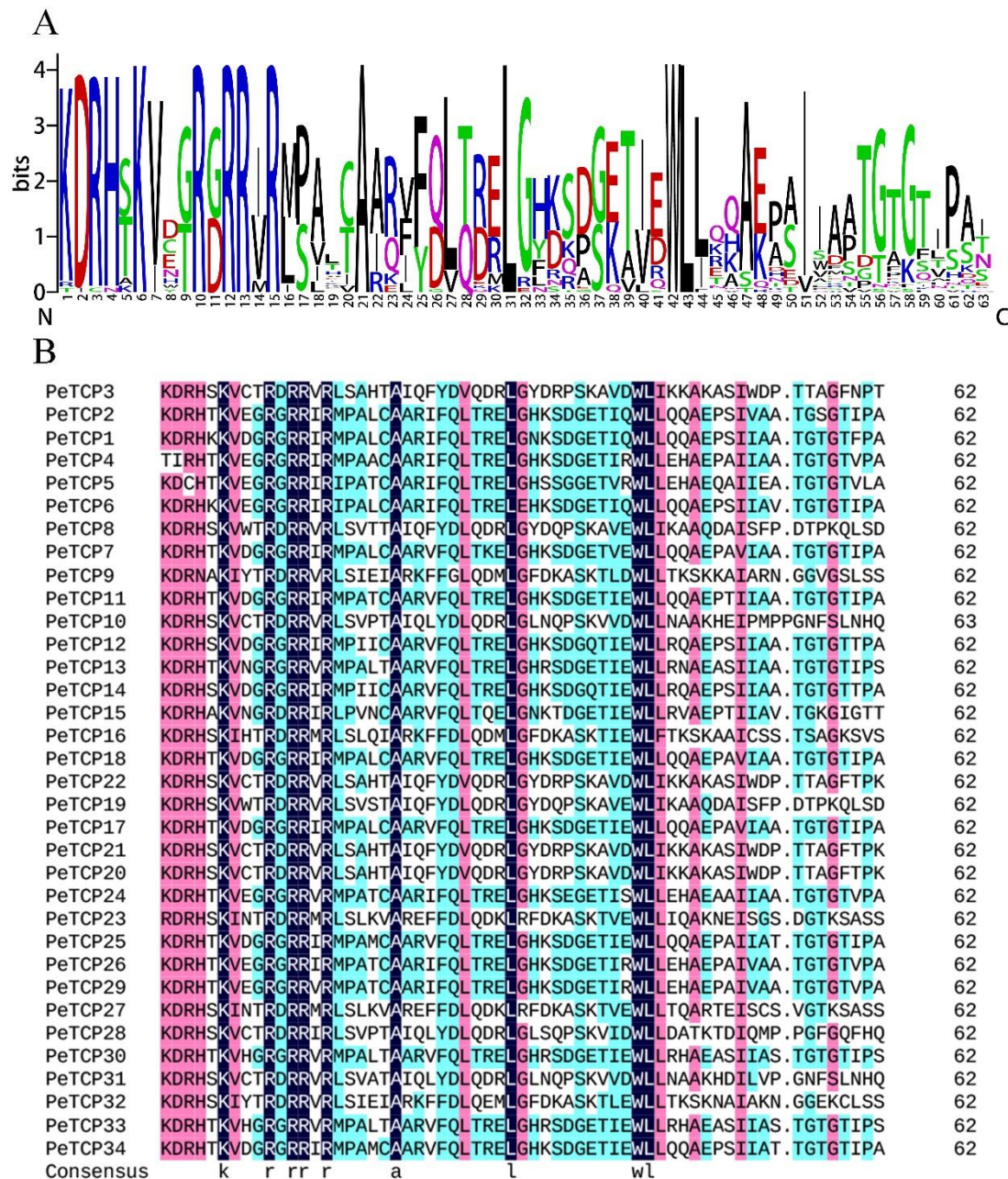


Figure A1. Conserved domains of TCP gene family in *P. euphratica*. (A) Amino acids are expressed in the standard single letter code. The size of the letters at each position represents their frequency. Numbers in the horizontal axis indicate the position of amino acids. (B) The conserved motif of PeTCs predicted by MEME5.4.1 online tools (<http://meme-suite.org/tools/meme>). The conserved amino acids are indicated with colored box. Numbers in the vertical axis indicate the total number of amino acids in this TCP domains

ELECTRONIC APPENDICES

Electronic Appendix 1. The identification and character analysis of *PeTCPs*

Electronic Appendix 2. The collinearity analysis of *TCPs* between *P. pruinosa* and other three plants (*P. euphratica*, *S. brachista* and *A. thaliana*)

Electronic Appendix 3a. The cis-element analysis of the *PpTCPs*

Electronic Appendix 3b. The cis-element analysis of the *PeTCPs*