horticulturae

MDPI

Article

Genome-Wide Identification, Evolution, and Expression Analysis of the *TCP* Gene Family in Rose (*Rosa chinensis* Jacq.)

Yi Hou †, Chunguo Fan †, Jingrui Sun †, Yufei Chang, Jun Lu, Jingjing Sun, Changquan Wang and Jinyi Liu *

College of Horticulture, Nanjing Agricultural University, Nanjing 210095, China
* Correspondence: jyl@njau.edu.cn
† These authors contributed equally to this work.

Abstract: Roses have not only high ornamental and economic values but also cultural importance worldwide. As a plant-specific transcription factor gene family, the *TCP* (*TEOSINTE BRANCHED 1*, *CYCLOIDEA*, *PROLIFERATING CELL FACTOR LAND 2*) genes have been indicated to be involved in various aspects of plant biological processes, such as leaf morphogenesis and senescence, lateral branching, flower development, stress response and hormone signaling. Currently, *TCP* genes have been identified and analyzed in many plants, yet there is no systematic analysis in *Rosa chinensis*. Here, we identified 16 *RcTCP* genes from *R. chinensis* genome, which were unevenly distributed in five out of all seven chromosomes. Phylogenetic and structural analyses showed that RcTCP family could be classified into two classes, I (namely PCF) and II, and class II genes can be further divided into CIN and CyC/TB1 subclasses. The different classes of *TCP* genes were showed to have undergone different evolutionary processes, and genes in the same branch shared similar motifs, gene structures and conserved structural domains. Promoter analysis showed that *RcTCPs* had many *cis*-acting elements that are mainly associated with plant growth and development, plant hormones and abiotic/biotic stress responses. Furthermore, the expression levels of *RcTCPs* under vegetative and reproductive growth and drought stress treatments were analyzed based on public RNA-seq dataset, and it was shown that *RcTCPs* exhibited serious tissue-specific expression, with most of them dominantly expressed in flowers, leaves and stems, with high levels of expression at different stages of flower and bud differentiation, particularly during petal formation and gametophyte development. The high inducement of seven *RcTCP* genes from PCF class in drought stress indicated their important roles in biological processes against drought stress. Our results provide valuable information for the evolution and functional characterization of *TCP* genes in *R. chinensis*.

Keywords: transcription factors; TCP; *Rosa chinensis*; expression analysis

Citation: Hou, Y.; Fan, C.; Sun, J.; Chang, Y.; Lu, J.; Sun, J.; Wang, C.; Liu, J. Genome-Wide Identification, Evolution, and Expression Analysis of the *TCP* Gene Family in Rose (*Rosa chinensis* Jacq.). *Horticulturae* **2022**, *8*, 961. https://doi.org/10.3390/horticulturae8100961

Academic Editors: Dilip R. Panthee and Jose V. Die

Received: 15 August 2022
Accepted: 13 October 2022
Published: 18 October 2022

1. Introduction

TCP transcription factors belong to a plant-specific gene family, which play significant roles in the regulation of plant growth and development and stress response [1]. In 1999, the TCP family was first defined as a new transcription factor family with few members [2]. This family was identified from four unrelated genes encoding proteins and named by their initials: TB1 (TEOSINTE BRANCHED 1), CyC (CYCLOIDEA), PCF1 (PROLIFERATING CELL FACTORS 1) and PCF2 (PROLIFERATING CELL FACTORS 2) [3]. An atypical helix-loop-helix (bHLH) structure forms at the N-terminus of the TCP domain, which is highly conserved [2]. TCP proteins are divided into two subfamilies, class I and class II. Class I, the TCP-P subfamily, is also called PCF subfamily. Class II, called TCP-C subfamily, includes CYC/TB1, and CIN subclades. There is a difference between the two classes in that the TCP domain in class I has four amino acids deletion compare to that in class II [4]. Additionally, the members of CYC/TB1 specifically contain a hydrophilic α-helix (R domain) structure which is rich in polar amino acids, while it does not exist in other classes [5]. The CIN is widespread in plants, but CyC/TB1 only occurs in angiosperms.

TCPs have been characterized to be involved in a variety of growth-related processes, including embryonic growth, leaf development, branching, floral organ morphogenesis, pollen development, germination, senescence, circadian rhythms, cell cycle regulation, and hormone signaling [6]. However, the majority of the functional features were only from model species, such as *Arabidopsis* and rice. Currently, even the genome-wide analysis of *TCP* gene family have been performed for many species, such as in *Arabidopsis thaliana* (24 *TCPs*) [3], soybean (*Glycine max*) (54) [7], cotton (*Gossypium hirsutum*) (74) [8], tomato (*Solanum lycopersicum*) (30) [9], grapevine (*Vitis vinifera*) (15) [10], plum (*Prunus mume)* (19) [11], but not in roses yet.

Roses are the most well-known and beloved ornamental plant genus worldwide, with not only high ornamental and economic value, but also cultural importance. Chinese old rose contributed a continuous flowering trait, double petals and its signature tea scent to modern roses [12]. Along with the genome release of *R. chinensis* cv. 'Old Blush', one of the important progenitors of modern roses, roses have been proposed to be an ideal model species for studying the molecular basis of woody plants [13]. Currently, although different studies have been conducted on the transcriptome, gene family functions, and genetic diversity of various genes and traits in roses [14], there is no comprehensive characterization of the *TCP* gene family in *R. chinensis*. Here, we performed genome-wide identification, evolution and functional characterizations of *TCP* gene family in *R. chinensis*. Our results provided insights into the evolution and functional profiles of *TCP* genes, and will be of benefit for the functional characterizations of individual *TCP* genes in roses.

2. Materials and Methods

2.1. Identification of RcTCPs Genes in R. chinensis

The sequences of *R. chinensis* genes were obtained from GDR (Genome Database for Rosaceae, https://www.rosaceae.org (accessed on 15 April 2022)). The sequences of *A. thaliana TCP* genes were downloaded from phytozome (https://phytozome-next.jgi.doe.gov/ (accessed on 15 April 2022)). Subsequently, the possible TCP proteins were retrieved from *R. chinensis* by using the BLAST Wrapper function in TBtools (https://github.com/CJ-Chen/TBtools (accessed on 16 April 2022)) with the sequences of AtTCP protein sequences as queries, and followed by verification of TCP domain existence and integrity using HMMER searching (http://www.ebi.ac.uk/Tools/hmmer/ (accessed on 16 April 2022)), SMART (https://smart.embl-heidelberg.de/ (accessed on 16 April 2022)), Pfam database (PF03634) (https://pfam.xfam.org/ (accessed on 17 April 2022)), and CDD/NCBI (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi (accessed on 17 April 2022)), and removing redundant and short sequences manually. Finally, all *RcTCP* genes were obtained for further analysis.

2.2. Chromosome Distribution and Synteny Analysis of RcTCP Genes

The *RcTCPs* were mapped to the chromosome based on the *R. chinensis* genome information obtained from the gff files and visualized by MG2C (http://mg2c.iask.in/mg2c_v2.0/ (accessed on 20 April 2022)). MCScanx (https://github.com/vidsvur/MCScanX-tutorial (accessed on 21 April 2022)) was used to analyze the gene collinearity and tandem duplication events between *RcTCP* genes. Tbtools was used to visualize the homology relationships of *RcTCPs*.

2.3. Physicochemical Properties and Subcellular Localization of RcTCP Genes

Protparam (http://expasy.org/tools/protparam.html (accessed on 25 April 2022)) was employed to predict molecular mass, isoelectric point, amino acid, average hydrophilic coefficient aliphatic index and instability index of RcTCP proteins. Cell-PLoc-2 (http://www.csbio.sjtu.edu.cn/bioinf/Cell-PLoc-2/ (accessed on 27 April 2022)) was used to predict the subcellular localization of RcTCP proteins.

2.4. Phylogenetic Analysis

The genome sequences of *Prunus mume*, *Malus domestica*, and *Fragaria vesca* were obtained from GDR. Phylogenetic trees were constructed using all protein sequences of TCP domain using MEGA11.0 (https://www.megasoftware.net/ (accessed on 8 May 2022)). All TCP proteins were aligned using ClustalW (https://www.genome.jp/tools-bin/clustalw (accessed on 8 May 2022)). The phylogenetic tree was constructed using the Maximum-likelihood (ML) method with 1000 bootstrap replicates.

2.5. Motif, Domain and Gene Structure Analysis

The conserved motifs of the TCP proteins were identified using MEME suite (http://meme-suit.org/tools/meme (accessed on 10 May 2022)). The exon-intron organizations were identified by comparing the coding sequences with their corresponding genomic sequences using the TBtools v1.0987663 software (https://github.com/CJ-Chen/TBtools (accessed on 11 May 2022)).

2.6. RcTCP Cis-Regulatory Element (CRE) Analysis

The promoters (2000 bp upstream sequences from ATG) of *RcTCP* genes were used to analyze the *cis*-acting elements using PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/ (accessed on 12 May 2022)). The results of the analysis were compiled by Adobe Illustrator 2021 software (https://www.adobe.com/products/illustrator/free-trial-download.html (accessed on 12 May 2022)), and a heatmap of *cis*-acting element copies in promoter region of *RcTCP* genes was visualized by TBtools.

2.7. Collinearity Analysis

The syntenic relationships among the *TCP* genes of *R.chinenisis* and other species was investigated using MCScanX (https://github.com/vidsvur/MCScanX-tutorial (accessed on 18 May 2022)) with the default parameters. *Oryza sativa* genome sequences were obtained from Ensembl Genomes (https://plants.ensembl.org/Oryza_sativa/Info/Index (accessed on 16 May 2022)). *Malus domestica* genome sequences were obtained from GDR (https://www.rosaceae.org (accessed on 16 May 2022)).

2.8. Expression Patterns of RcTCP Genes

The expression levels of the *RcTCPs* were analyzed using transcriptome datasets that retrieved from ROSAseq web interface database [15] (http://iant.toulouse.inra.fr/R.chinensis (accessed on 17 May 2022)) and NCBI, including datasets of drought treatment (PRJNA486271, PRJNA663119) [16,17], flower organ development (PRJNA351281, PRJNA398090, PRJNA325324, PRJCA000258) [18–21]. The expression levels were illustrated based on the log2 transformed FPKM values using Kallisto (https://kallisto/download.html (accessed on 22 May 2022)) and visualized by TBtools (https://github.com/CJ-Chen/TBtools (accessed on 23 May 2022)).

2.9. Plant Material and PEG Treatments

The plants of *R. chinensis* 'Old Blush' were grown in the rose resource nursery of Nanjing Agricultural University. The stems with at least one node were cut and used as explants, and cultured on Murashige and Skoog (MS) medium (Duchefa Biochemie, Haarlem, The Netherlands) supplemented with 1.0 mg/L 6-benzyl aminopurine (6-BA), and 0.05 mg/L 1-naphthaleneacetic acid (NAA) for 30 d at 22 ± 1 °C, under a 16 h light/8 h dark photoperiod. The shoots were then transferred to half strength MS medium supplemented with 0.1 mg/L NAA for 25 d for rooting. Then, the plants were transferred to Hoagland's nutrient solution and were grown in controlled conditions (25 °C, 40% relative humidity, and 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$) under LDs (16:8 h, light:dark) for 14 days. To simulate drought stress, the *R. chinensis* seedlings were transferred to Hoagland's nutrient solution containing 20% *w*/*v* PEG6000 and cultured for 24 h, taking purity Hoagland's nutrient solution as control. The treatments were repeated three times, and at least nine seedlings

were used for each treatment. The samples were collected after treatment and immediately frozen in liquid nitrogen and stored at −80 °C until RNA isolation.

2.10. RNA Isolation and Real-Time Quantitative PCR Analysis

Total RNA of the samples were extracted using the BioTeke Quick RNA isolation Kit (Cat. #: RP3301, BioTeke Corporation, Beijing, China) and 1 μg of high-quality total RNA was reverse transcribed using the PrimeScriptTM RT reagent Kit (Cat. #: RR047A, TaKaRa, Dalian, China) according to the manufacturer's instructions. Gene-specific primers for *RcTCP* genes were designed using GenScript (https://www.genscript.com/ (accessed on 3 June 2022)) and the IDT (http://sg.idtdna.com/scitools/Applications/RealTimePCR/ (accessed on 3 June 2022)) online server. RT-qPCR assays were carried out by the QuantStudio 6 Real Time PCR System (Thermo Fisher Scientifific, Carlsbad, CA, USA) using SYBR® Premix Ex TaqTM (Tli RNaseH Plus) (catalog number: RR420A, TaKaRa) according to the manufacturer's instructions. Expression levels of *RcTCP* genes were normalized by using *RcGAPDH* gene as reference, and the $2^{-\Delta\Delta CT}$ method was used to calculate the relative expression of *RcTCP* genes [22]. Three biological replicates and three technical replicates were used for each experiment. The primers used in RT-qPCR are listed in Supplemental Table S1.

3. Results

3.1. Identification and Characteristics of RcTCP Genes

To identify *TCP* genes in *R. chinensis*, we firstly retrieved all candidate genes using the BLAST Wrapper function in TBtools; 17 candidate genes were obtained. Then, HMMER searching, SMART, Pfam database (PF03634), and CDD/NCBI were further employed to verify the existence and integrity of TCP domains within the candidate genes, the genes with integrity of TCP domain less than 50% and short sequences were removed manually. Finally, all 16 *RcTCP* genes were obtained and named according to chromosome positions for further analysis (Table 1). Furthermore, the amino acid number, molecular weight (MW), isoelectric point (PI), average hydrophilicity coefficient, lipid coefficient, instability coefficient and subcellular localization of the 16 RcTCP proteins were analyzed (Table 1), and it showed that a total of 262 (RcTCP07)-466 (RcTCP15) amino acids make up the RcTCP protein, with an average of 373.8 amino acids. The molecular weight ranged from 27.59 kDa (RcTCP07) to 52.44 kDa (RcTCP15). The isoelectric point (PI) ranged from 6.4 (RcTCP16) to 9.45 (RcTCP09). The average hydrophilic coefficients of all proteins were less than zero, indicating that RcTCP proteins are hydrophilic. The aliphatic index ranged from 54.39 to 80.41. Each RcTCP protein has an instability index greater than 40, indicating its instability. Subcellular localization predictions showed that all RcTCP proteins were located in the nucleus.

3.2. Chromosome Distribution and Synteny Analysis of RcTCP Genes

In order to gain more insight into the evolution of *RcTCP* genes, the distributions of *RcTCP* genes on chromosomes were surveyed. The results showed that *RcTCP* genes are unevenly distributed on *R. chinensis* chromosomes with only five out of seven chromosomes containing *TCP* genes (Figure 1A), and no putative *TCP* genes were found on chromosomes three and six, indicating their biased evolution profiles during whole genome evolution. Furthermore, gene duplication events were detected by using MCScanX 3-28-2013 software (Figure 1B). Six pairs of *RcTCP* genes were found to be evolved from segmental duplications, while no tandem duplication events were found. These results suggested that the evolution of the *TCP* gene family was highly associated with whole-genome duplication events during evolution in *R. chinensis*.

Table 1. Physicochemical properties and subcellular localization of RcTCP proteins.

Protein Name	Gene ID	Molecular Mass (Da)	Isoelectric Point	Amino Acid (aa)	Average Hydrophilic Coefficient	Aliphatic Index	Instability Index	Subcellular Localization Prediction
RcTCP01	*RC1G0213700*	39,668.36	8.40	360	−0.510	70.19	50.85	Nucleus.
RcTCP02	*RC1G0312100*	32,831.23	8.57	307	−0.391	66.81	60.89	Nucleus.
RcTCP03	*RC1G0531500*	34,056.20	6.60	328	−0.226	76.13	47.93	Nucleus.
RcTCP04	*RC2G0577900*	39,859.44	9.17	360	−0.632	64.78	53.90	Nucleus.
RcTCP05	*RC2G0599700*	39,158.12	6.62	355	−0.800	63.46	55.30	Nucleus.
RcTCP06	*RC2G0685400*	32,176.69	9.27	304	−0.648	65.89	57.49	Nucleus.
RcTCP07	*RC4G0096800*	27,591.84	8.82	262	−0.445	63.82	41.51	Nucleus.
RcTCP08	*RC4G0266300*	32,732.35	9.08	290	−0.064	80.41	44.72	Nucleus.
RcTCP09	*RC4G0400400*	49,609.69	9.45	444	−0.837	58.90	48.24	Nucleus.
RcTCP10	*RC4G0435200*	44,618.17	6.57	423	−0.598	60.28	56.15	Nucleus.
RcTCP11	*RC5G0134300*	47,923.63	8.42	441	−0.826	57.82	49.47	Nucleus.
RcTCP12	*RC5G0279600*	48,250.47	6.68	438	−0.807	57.56	54.65	Nucleus.
RcTCP13	*RC7G0042700*	45,529.80	6.81	426	−0.734	54.39	64.76	Nucleus.
RcTCP14	*RC7G0061200*	42,654.47	8.93	384	−0.685	65.76	51.74	Nucleus.
RcTCP15	*RC7G0073800*	52,440.83	7.04	466	−0.869	59.27	52.95	Nucleus.
RcTCP16	*RC7G0209000*	40,894.44	6.40	393	−0.474	60.38	62.10	Nucleus.

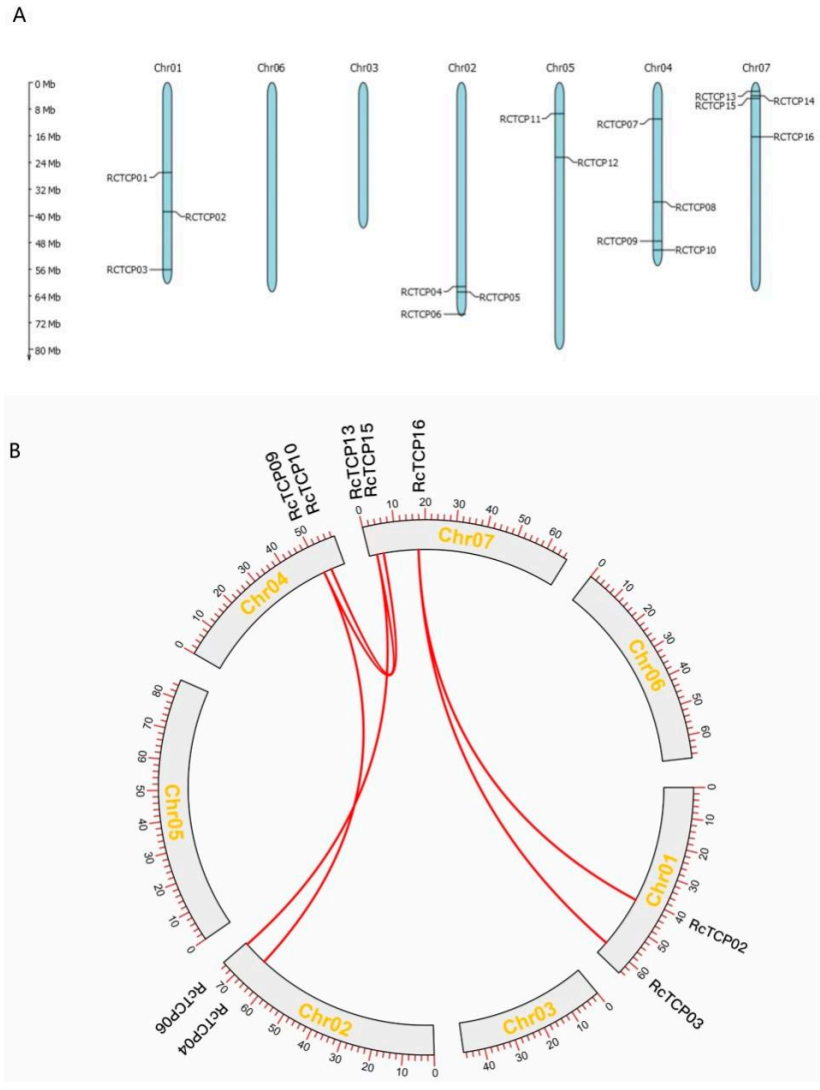
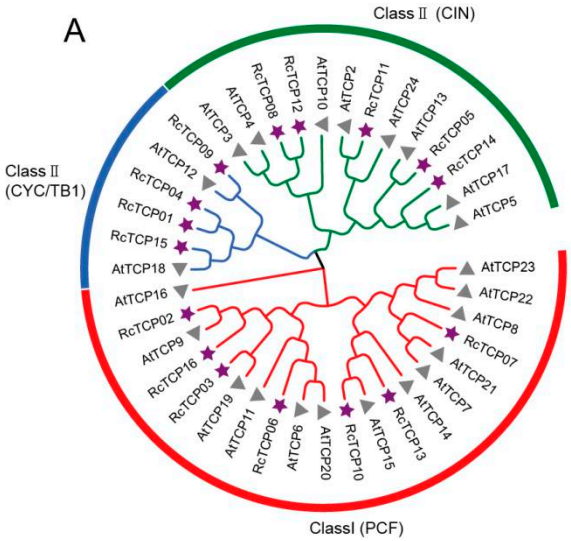


Figure 1. Distribution on chromosomes and homology analysis of *RcTCP* gene family. (**A**) The distribution of *RcTCP* gene family on chromosomes; (**B**) the chromosome distributions and syntenic analysis of *RcTCP* genes. The approximate positions of *RcTCP* gene were marked on the circle. The red curve denotes the syntenic relationships between *RcTCP* genes.

3.3. Phylogenetic Analysis of RcTCP Proteins

In order to explore the evolutionary and phylogenetic relationship between the TCP proteins of *R. Chinensis* and other known TCP proteins, a phylogenetic tree was constructed for 130 TCP protein sequences from *R. chinensis*, *A. thaliana*, *P. mume*, *M. domestica* and *F. vesca* using MEGA11.0 (Supplemental Figure S1). The phylogenetic tree showed that the *TCP* genes from all species can be divided into two major classes, class I, TCP-P/PCF, and class II, TCP-C, and in which, Class II was further divided into two subclasses, namely CyC/TB1 and CIN, according to previous study [6]. The phylogenetic tree of *TCP* genes from *R. chinensis* and *A. thaliana* were represented for classification of *R. chinensis* (Figure 2A). Interestingly, for each subclass, the copy number of *TCP* genes varied significantly (Figure 2B). The copy number of *TCP* genes in PCF and CIN subclass from apple (which suffered recent whole genome duplication event, WGD) is at least 2.2- and 4.3-fold higher than that of other Rosaceae species. However, the copy number of *TCP* genes in CyC/Tb1 is relatively well-conserved. This result further indicated that the evolution of *TCP* genes from PCF and CIN subclass is highly associated with whole-genome duplication events during evolution in Rosaceae, while it is not in CyC/Tb1. Furthermore, the relatively lower copy number of *TCP* genes from PCF and CIN subclass in *R. chinensis* suggests there might be gene loss during evolution.



B

	PCF	CyC/Tb1	CIN	All
Arabidopsis thaliana	13	3	8	24
Fragaria vesca	10	3	6	19
Malus domestica	22	4	26	52
Prunus mume	10	3	6	19
Rosa chinensis	7	4	5	16

Figure 2. The phylogenetic tree of RcTCPs. (**A**) The phylogenetic tree of TCP proteins from *R. chinensis* and *A. thaliana*. (**B**) Number of each subclass of *TCP* genes in Rosaceae family and *A. thaliana*.

3.4. Conservative Motifs, Structural Domains, Gene Structure Analysis

An analysis of the conserved motifs, domains, and gene structures of *R. chinensis* and *A. thaliana* was conducted to determine the structural differences between the TCP proteins of *R. chinensis* along with the phylogenetic tree (Figure 3A). Six conserved motifs were identified from *R. chinensis* and *A. thaliana* TCP proteins by using MEME online service. Among them, all TCP proteins share the conserved motif 1 which corresponds to the TCP domain. While motif 2 were found to be specific to PCF subclass, and motif 3 is specific to CIN and CyC/TB1 subclass, and motif 4 is very conserved within the CyC/TB1 subclass (Figure 3B). A high degree of similarity between protein motifs in a subfamily indicates that the subfamily division is consistent with the distribution of conserved motifs. A subfamily of PCF proteins is only represented by five proteins exhibiting motif 6, which indicates that some motifs play an important role in subfamily-specific function. As *R. chinensis* and *A. thaliana* have conserved motifs with similar compositions and positions, they may have similar structural characteristics and a similar function as well. Based on domain predictions, we defined these proteins as TCP proteins since they shared a common TCP protein domain (Figure 3C). The domain of TCP protein in CIN branch of

class II is closer to that of TCP protein of class I, which means that CIN branch may be older than CyC/TB1 branch. Furthermore, the domain regions and some motifs could be approximately overlapped when the conserved motif and domain maps were combined, indicating that the conserved motifs of TCP proteins corresponded to the domains, and that TCP proteins had additional motifs in addition to the reported bHLH and R-domains.

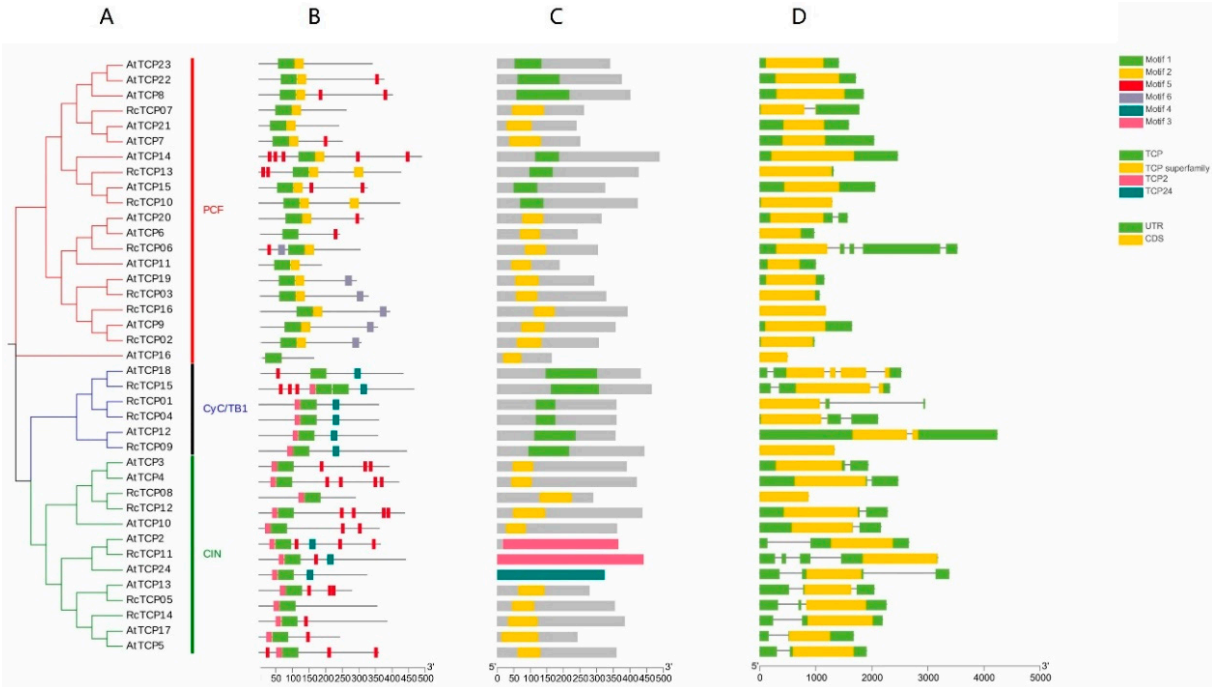


Figure 3. The phylogenetic relationships, distribution of conserved motif, domain prediction and gene structure analysis of *Arabidopsis* and *R. chinensis TCP* genes. (**A**) Phylogenetic tree of 23 *Arabidopsis. TCP* and 16 *R. chinensis TCP* genes. (**B**) Distribution of conserved motifs in TCP proteins. Six conservative motifs in TCP are displayed in different color boxes. (**C**) TCP domain of TCP proteins. This denoted the matching types that represent various confidence levels (specific matching, non-specific matching) and domain model ranges (superfamily, multi-domain). (**D**) *TCP* gene structures. The description of exons and introns adopts image tools. The green, yellow boxes and black lines represent the non-coding region (UTR), exon and intron, respectively.

Genetic structure analysis showed that almost all *TCP* genes in *Arabidopsis* and *R. chinensis* exhibit a highly conserved exon-intron organization, but with differences (Figure 3D). Interestingly, 19 *TCP* genes were found to be intronless, and most of the *RcTCP* genes show similar exon intron arrangement and number in the same subfamily, which supports the classification and evolutionary relationship of subclasses, indicating that the biological functions of these genes might be highly conserved.

3.5. Promoter Cis-Regulatory Elements Analysis of RcTCP Genes

To further understand the function and regulatory mechanism of the *RcTCP* genes, the *cis*-acting elements within promoter region (2000 bp upstream sequences from ATG) of 16 *RcTCP* genes were analyzed (Figure 4, Supplemental Table S2). Out of the basic *cis*-acting elements, a large number of *cis*-acting elements in the promoter of *RcTCP* genes were found to be involved in light response, phytohormone response, plant growth and development and stress response. As shown, light-responsive elements were observed in all *RcTCP* genes, counting for a total of 182 times. The Auxin response element, the salicylic acid response element, the abscisic acid response element, the methyl jasmonate (MeJA) response element, and the gibberellin response element were found in the promoter regions of 6, 9, 13, 13 and 8 *RcTCP* genes, respectively, indicating that plant hormones play important roles in plant growth and development. *Cis*-acting regulatory elements

involved in seed-specific regulation and endosperm expression were identified in the promoter regions of *RcTCP01*, *RcTCP03* and *RcTCP01*, *RcTCP16*, respectively, indicating that some genes of the *RcTCP* family are involved in the regulation of seed growth and development. Furthermore, eight and three *RcTCP* genes contain *cis*-regulatory elements for plant meristematic tissue expression and circadian rhythm regulation, respectively.

Furthermore, several *cis*-acting elements associated with abiotic and biotic stresses have been identified in the promoter region of the *RcTCP* genes. It was found that 7, 6 and 10 genes contain *cis*-acting elements involved in defense and stress responses, low-temperature responsiveness and MYB binding site involved in drought-inducibility. In addition, 13 and 1 genes were observed for anaerobically induced and related stress elements involved in hypoxia-specific induction of enhancer-like elements. As a result, it appears that the *RcTCP* genes may be involved in the transmission of defense signals.

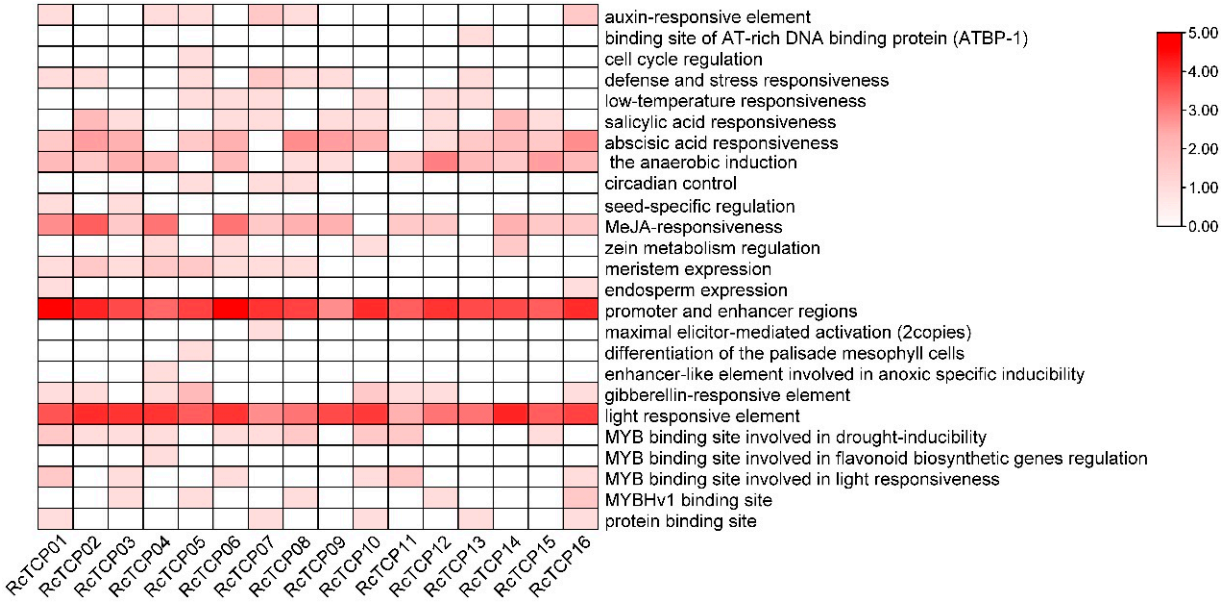


Figure 4. The *cis*-acting elements in the promoters of *RcTCP* genes. PlantCARE predicted *cis*-regulatory elements (CRES) in the *RcTCP* gene promoter region (upstream 2000 bp). The heatmap represent the abundance of the copy number of *cis*-regulatory elements.

3.6. Collinearity Analysis of RcTCPs

During evolution, gene families may suffer gene gain or loss within different species after genome shuffling. In order to gain more insights into the evolutionary profiles of *TCP* genes, collinearity analysis was performed on *TCP* genes from *R. chinensis*, rice and apple. As shown in Figure 5, six pairs of collinearity events were found between *R. chinensis* and *O. sativa TCP* genes, accounting for 37.5% of the total *RcTCPs*, and 14 pairs of collinearity events were found between *R. chinensis* and *M. pumila TCP* genes, accounting for 87.5%. Accordingly, *R. chinensis* and *M. pumila* appear to be more evolutionarily conservative, which is in accordance with their phylogenetic relationships. Furthermore, by comparing with *R. chinensis TCP* genes, most of the *TCP* genes in *M. pumila* were duplicated, indicating their retention after whole-genome duplication events.

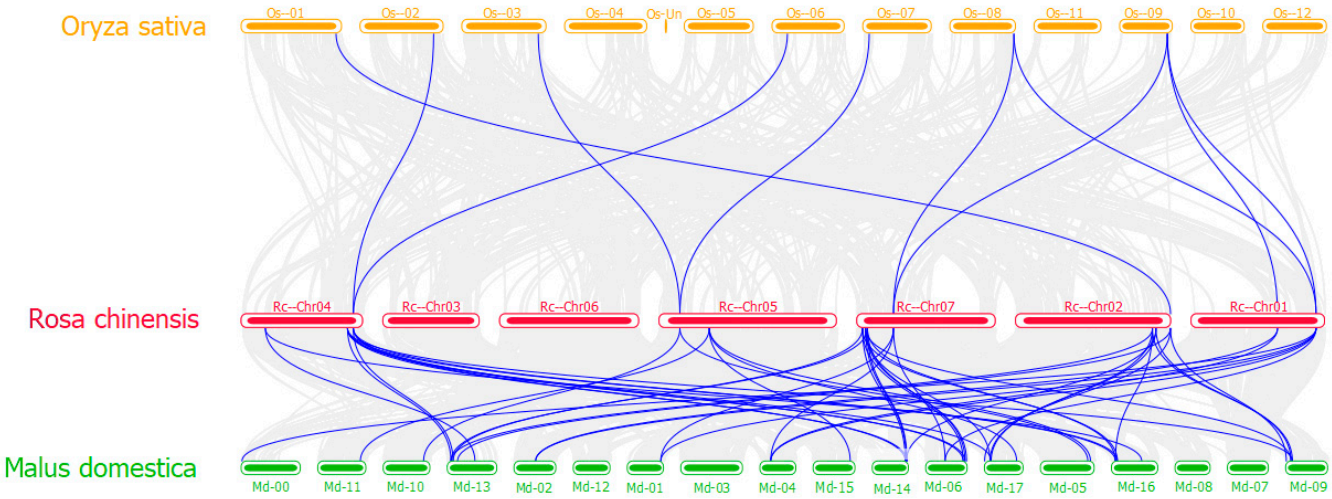


Figure 5. The collinearity relationships between *TCP* genes in *R. chinensis*, rice and apple. The red block represents the chromosome of *R. chinensis*, the orange block represents the chromosome of *O. sativa*, the green block represents the chromosome of *M. domestica*, the gray line represents the synteny blocks, and the blue line represents the collinearity relationships between *TCP* gene pairs.

3.7. Expression Patterns of RcTCP Genes

To better understand the functional profiles of *RcTCP* genes in *R. chinensis*, transcript levels of *RcTCP* genes in different tissues were analyzed based on various public RNA-seq datasets (Supplemental Table S3). As shown in Figure 6, most of the *RcTCP* genes from the PCF subclass, and three out of five *RcTCP* genes from the CIN subclass were highly expressed or induced in majority of the tissues and treatments. In contrast, the expression levels of *RcTCP* genes from CyC/TB1 subclass were generally low, except *RcTCP5* which was specifically highly expressed in dominant and active axillary buds. These results suggested the functional diversification of *RcTCP* between different subclasses. Interestingly, although *RcTCP* genes were barely expressed under drought treatments, three genes (*RcTCP05*, *RcTCP11* and *RcTCP12*) from the CIN subclass and six genes (*RcTCP06*, *RcTCP10*, *RcTCP13*, *RcTCP07*, *RcTCP02* and *RcTCP16*) from the PCF subclass were highly expressed under the condition of phosphorylation of RhPIP2; 1 (Figure 6B), which controls the transmembrane transport of water and plays a crucial role in petal expansion and stress response [23,24]. These results suggested that there are functional differences of *RcTCP* genes in the response to drought stress, and the CIN and PCF subclasses play an indirect role in the regulation of drought stress rather than being directly involved.

Furthermore, to investigate the expression pattern of *TCP* genes in the growth and development of rose organs, transcriptome data from the database were used to analyze the expression profile of *TCP* genes during the differentiation to full opening of *R. chinensis* floral organs (Figure 6D). Similarly to the previous findings, the genes with higher expression in rose organs were mainly found in the PCF and CIN, while the CyC/TB1 genes were barely expressed. *RcTCP16* was expressed at very high levels throughout the growth and development of the floral organ. During the period from meristematic development to full opening of flower organs, *RcTCP10*, *RcTCP13*, *RcTCP07* and *RcTCP02* of PCF subclasses have relatively high expression levels, but their expression levels decrease with the development of flower organs, suggesting their involvement in the early development of flower organs. The expression of *RcTCP05* in the phloem tissue of a flower was barely detectable, but it was relatively high from the beginning of flower opening to full open discoloration. Both *RcTCP11* and *RcTCP12* were highly expressed in the tested tissues except for partially open flowers and fully open-pink flowers. In general, different subgroups of *RcTCPs* play a conservative and diverse role in the process of rose floral organ differentiation to their full opening.

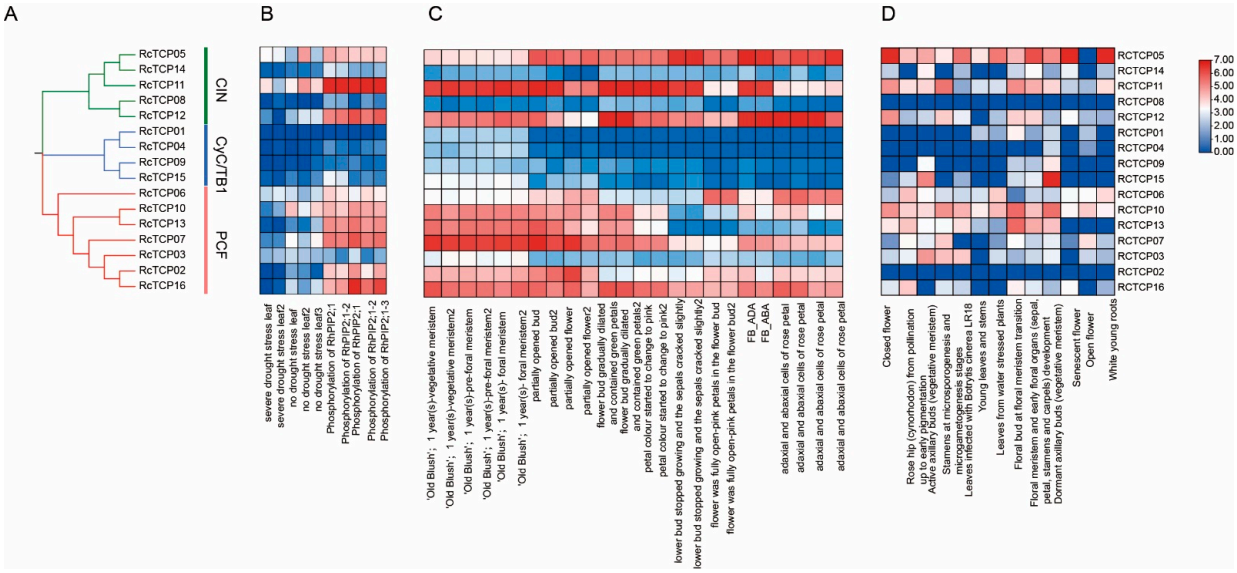


Figure 6. The gene expression pattern of *RcTCP* genes in different tissues and developmental stages. (**A**) Phylogenetic tree of 16 *R. chinensis TCP* genes. (**B**) Use TBtools software to take the log (log 2) of the FPKM value to convert the expression value for normalization. (**C**) *RcTCP* genes expression of leaves and aquaporin RrhPIP2;1 with drought stress. (**D**) *RcTCP* genes expression in floral organs.

3.8. Expression Pattern of RcTCP Genes under PEG Treatments

As described above, some *RcTCP* genes were showed to be involved drought stress. Seven *RcTCP* genes were then selected for futher expression validations under PEG (20% *w*/*v* PEG6000) treatments by using RT-qPCR method (Figure 7, Supplemental Table S4). Our results showed that all of the seven *RcTCP* genes were significantly induced by PEG treatments, especially after 24 h treatment, indicating that these seven *RcTCP* genes may play important roles in the response of drought stress in *R. chinensis*.

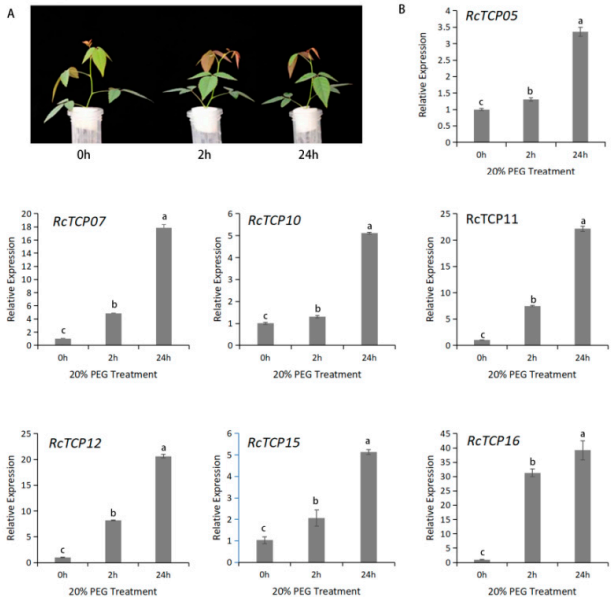


Figure 7. Expression patterns of *RcTCP* genes under PEG treatments (determined by RT-qPCR). (**A**) The method of PEG treatments. (**B**) The expression of *RcTCP* genes under 0 h, 2 h and 24 h of PEG treatments determined by RT-qPCR. The *RcGAPDH* gene was used as the internal control, and three biological and technical replicates were conducted. The relative expression levels of each gene were calculated using the $2^{-\Delta\Delta CT}$ method. Each bar shows the mean ± SE of the triplicate assay, and different letters indicates significant difference at $p < 0.05$ according to Duncan's multiple range test.

4. Discussion

The plant transcription factors play key roles in all stages of plant development, signaling pathways, stress responses and biosynthesis of bioactive metabolites [25]. TCPs are plant-specific transcription factors that participate in various biological processes such as leaf development, flower symmetry, flowering process, stem branching, and ultimately affects plant growth and development [6]. The genome-wide analysis of *TCP* genes has been reported in *Arabidopsis* [26], rice [27], apple [28], grape [29], and other species. Roses, known as the queen of flowers, not only have great ornamental value and economic value but also have cultural value, and which was proposed to be an ideal model species for studying the molecular basis of woody plants [13]. However, systematic and comprehensive analysis on *TCP* gene family in *R. chinensis* will facilitate the understanding of flower evolution in plants.

In this study, a total of 16 *RcTCP* genes were identified in *R. chinensis*, which are unevenly distributed in five out of seven chromosomes (Figure 1A), suggesting that origination of *RcTCP* genes might be bias to some kind of subgenomes. Synteny analysis revealed that six pairs of *RcTCP* genes (containing nine genes) were evolved from segmental duplications, and no tandem duplication events were detected, suggesting that the expansion of *TCP* gene family was contributed by segmental duplications and might be associated with whole-genome duplication events during evolution of *R. chinensis*. Phylogenetic analysis indicated that the 16 *RcTCP* genes could be divided into two subfamilies, namely class I, TCP-P/PCF, and class II, TCP-C, and in which, class II was further divided into two subclasses, namely CyC/TB1 and CIN, which was consistent with the previous studies in *Arabidopsis* [27], rice [27], tomato [9] and strawberry [5]. Interestingly, the copy number of *TCP* genes in PCF and CIN subclass from apple (which suffered recent whole genome duplication event, WGD) is much higher than that of other Rosaceae species. By contrast, the copy number of *TCP* genes in CyC/Tb1 is relatively conserved. This result suggested that there were differences of evolution pressures between those subclasses.

The gene structure analysis showed that *RcTCP* members from the same group or subgroup have similar motif composition and intron/exon organization but differ between different groups, and motif 2 is only present in class I and motif 3 is only present in class II, suggesting their important roles in diversification and functions of *RcTCP* gene during gene family evolution. Furthermore, the *cis*-acting element analysis showed that the cis-acting elements identified from the promoter regions of *RcTCPs* were classified into four categories. The first is the elements of plant growth and development, including circadian rhythm and endosperm-specific expression, such as circadian rhythm elements detected in *RcTCP05*, *RcTCP07*, and *RcTCP08*. The second is related to plant hormones such as ABA, SA and MeJA. These elements may be involved in the signaling process of hormones. The third is associated with responses to biotic stresses, such as drought, anaerobic and low temperature. The fourth is light-responsive elements, which were detected in all *RcTCP* genes. The above conclusions are mainly based on data analysis and further studies are needed to elucidate the regulatory mechanisms of *TCP* genes in *R. chinensis*.

Expression analysis showed that the *RcTCP* genes from CyC/TB1 subclass were barely expressed in all detected samples, while *RcTCP* genes from PCF and CIN subclasses showed very active expression profiles in both of different plant tissues and conditions, suggesting their robust roles in regulations of plant developments and stress response. These results were inconsistent with previous studies, in which *RcTCP* genes from PCF subclass played a positive cell division regulatory role mainly in different biological processes such as seed germination, leaf and flower organ development, gametophyte development and senescence [3,30–32]. A gene that encodes a CIN-like TCP is involved in the morphogenesis of monolearies in *Arabidopsis* [33]. A single mutant of *attcp5* produces wider petals than a wild-type plant [30]. Transient overexpression of *FvTCP9* in strawberry fruit significantly promoted the expression of a series of genes related to fruit color and aroma metabolism [34]. *PfTCP17* and *PfTCP27* of *Paulownia fortunei* may be associated with re-

sistance to bush disease and drought [33]. In this study, *RcTCP05* and *RcTCP11* of the CIN subclass and *RcTCP10* and *RcTCP13* genes of the PCF subclass showed high levels of expression in all plant tissues, suggesting that they might play important roles in the growth and development of rose and the regulation of stress. The *RcTCP* genes were barely expressed under drought treatment, while most *RcTCP* genes from PCF subclass and CIN subclass showed higher expression in the phosphorylation of RhPIP2; 1, an aquaporin from rose under drought stress conditions, suggesting that these genes might play indirect roles in the regulation of drought stress response in *R. chinensis*. Four *RcTCP* genes from PCF subclasses, *RcTCP10*, *RcTCP13*, *RcTCP07*, and *RcTCP02*, were expressed at high levels during floral organ differentiation to complete opening, and *RcTCP05* from CIN subclass was expressed at a high level during the period from flower initiation to complete open discoloration. *RcTCP11* and *RcTCP12* were highly expressed in the tested tissues of all but partially open flowers and fully open pink flowers, and both genes were on chromosome five. These results suggested the important roles of *RcTCP* genes in the regulations of flower organ development.

Drought stress is a common problem in horticultural production, which affects many aspects of plant growth and development. Previous studies have shown that *TCP* gene plays regulatory roles in drought stress [34]. Overexpression of *ZmTCP42* increased the inducibility of ABA or drought tolerance related genes, thereby improving drought tolerance in *Zea mays* under drought stress [35]. PEG-6000 is an ideal osmotic regulator, which can simulate drought stress by reducing the water potential of the solution to make the root system difficult to absorb water. In our study, there were differences between the PCR results and transcriptome data, perhaps due to the difference between drought and PEG treatment. The previous study has shown that drought stress significantly upregulates aquaporin expression [16]. The phosphorylation of aquaporins decreases with decreasing water potential, thereby reducing the permeability of the water. Based on the drought transcriptome analysis, *RcTCP* genes from PCF and CIN subclasses are highly induced when the water channel protein RhPIP2; 1 was overexpressed in phosphorylation state under drought conditions, speculating that *RcTCPs* might be involved in the regulation of water channel proteins via plant hormones and signal transduction, thus responding to the expression of drought stress in plants. In summary, *RcTCP* genes are mainly involved in plant development, hormonal processes and plant defense. PCF and CIN subclass genes have multiple biological functions, while the function of CyC/TB1 subclass genes is unclear.

5. Conclusions

In this study, 16 *RcTCP* genes were identified in the *R. chinensis* genome, and which were classified into class I (PCF class) and class II, where class II genes were further classified into CIN subclass and CyC/TB1 subclass. Subsequently, the analyses of phylogenetic, gene structure, synteny, *cis*-acting elements and expression analyses based on RNA-seq data and RT-qPCR method provide valuable information for understanding the evolution and functional profiles of *TCP* genes in *R. chinensis*.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae8100961/s1, Table S1: primer sequences of *RcTCP* genes for RT-qPCR; Table S2: the *cis*-acting elements of *RcTCP* genes; Table S3: the information of the *TCP* gene family in *R. chinensis*; Table S4: the expression of *RcTCP* genes; Figure S1: the phylogenetic tree of *RcTCP* genes from *R. chinensis*, *A. thaliana*, *P. mume*, *M. domestica* and *F. vesca*.

Author Contributions: Conceptualization, J.L. (Jinyi Liu) and C.W., methodology, Y.H., J.S. (Jingrui Sun) and C.F., software, J.S. (Jingjing Sun) and J.L. (Jun Lu), validation, J.L. (Jun Lu), Y.C. and C.F.; investigation, J.L. (Jinyi Liu) and C.W., resources, Y.H., Y.C. and C.F., data curation, Y.H., J.L. (Jinyi Liu) and C.F., writing—original draft preparation, Y.H., writing—review and editing, J.L. (Jun Lu), visualization, Y.H. and C.F., supervision, J.L.; project administration, J.L. (Jinyi Liu) and C.W.; funding acquisition, J.L. (Jinyi Liu) and C.W. All authors have read and agreed to the published version of the manuscript.

Funding: This work was financially supported by National Key R&D Program of China (No. 2021YFD1200200), The "JBGS" Project of Seed Industry Revitalization in Jiangsu Province (JBGS (2021) 034), Fundamental Research Funds for the Central Universities (KYZZ2022004), NSFC (32172615, 31972449) and the Priority Academic Program Development of Jiangsu Higher Education Institutions. This work was also supported by the high-performance computing platform of Bioinformatics Center, Nanjing Agricultural University.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained in the article.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Dhaka, N.; Bhardwaj, V.; Sharma, M.K.; Sharma, R. Evolving Tale of TCPs: New Paradigms and Old Lacunae. *Front. Plant Sci.* **2017**, *8*, 479. [CrossRef]
2. Cubas, P.; Lauter, N.; Doebley, J.; Coen, E. The TCP domain: A Motif Found in Proteins Regulating Plant Growth and Development. *Plant J.* **1999**, *18*, 215–222. [CrossRef]
3. Shutian, L. The *Arabidopsis thaliana* TCP Transcription Factors: A Broadening Horizon Beyond Development. *Plant Signal. Behav.* **2015**, *10*, e1044192.
4. Manassero, N.G.; Viola, I.L.; Welchen, E.; Gonzalez, D.H. TCP Transcription Factors: Architectures of Plant Form. *Biomol. Concepts.* **2013**, *4*, 111–127. [CrossRef]
5. Zhao, Y.; Su, X.; Wang, X.; Wang, M.; Chi, X.; Aamir Manzoor, M.; Li, G.; Cai, Y. Comparative Genomic Analysis of *TCP* Genes in Six Rosaceae Species and Expression Pattern Analysis in *Pyrus bretschneideri*. *Front. Genet.* **2021**, *12*, 669959. [CrossRef]
6. Liu, M.M.; Wang, M.M.; Yang, J.; Wen, J.; Guo, P.C.; Wu, Y.W.; Ke, Y.Z.; Li, P.F.; Li, J.N.; Du, H. Evolutionary and Comparative Expression Analyses of TCP Transcription Factor Gene Family in Land Plants. *Int. J. Mol. Sci.* **2019**, *20*, 3591. [CrossRef]
7. Citerne, H.L.; Luo, D.; Pennington, R.T.; Coen, E.; Cronk, Q.C. A phylogenomic investigation of *CYCLOIDEA*-like TCP genes in the Leguminosae. *Plant Physiol.* **2003**, *131*, 1042–1053. [CrossRef] [PubMed]
8. Han, L. Genome-wide identification and characterization of TCP transcription factors and study on the role of EIN3 in upland cotton (*Gossypium hirsutum*). *Sci. Rep.* **2017**, *7*, 10118.
9. Parapunova, V.; Busscher, M.; Busscher-Lange, J.; Lammers, M.; Karlova, R.; Bovy, A.G.; Angenent, G.C.; de Maagd, R.A. Identification, cloning and characterization of the tomato TCP transcription factor family. *BMC Plant Biol.* **2014**, *14*, 157. [CrossRef] [PubMed]
10. Jiu, S.; Xu, Y.; Wang, J.; Wang, L.; Wang, S.; Ma, C.; Guan, L.; Abdullah, M.; Zhao, M.; Xu, W. Genome-Wide Identification, Characterization, and Transcript Analysis of the TCP Transcription Factors in Vitis vinifera. *Front. Genet.* **2019**, *10*, 1276. [CrossRef]
11. Zhou, Y.; Xu, Z.; Zhao, K.; Yang, W.; Cheng, T.; Wang, J.; Zhang, Q. Genome-Wide Identification, Characterization and Expression Analysis of the TCP Gene Family in Prunus mume. *Front. Plant Sci.* **2016**, *7*, 1301. [CrossRef]
12. Bendahmane, M.; Dubois, A.; Raymond, O.; Bris, M.L. Genetics and Genomics of Flower Initiation and Development in Roses. *J. Exp. Bot.* **2013**, *64*, 847–857. [CrossRef]
13. Smulders, M.J.M.; Arens, P.; Bourke, P.M.; Debener, T.; Linde, M.; Riek, J.; Leus, L.; Ruttink, T.; Baudino, S.; Hibrant Saint-Oyant, L.; et al. In the Name of the Rose: A Roadmap for Rose Research in the Genome Era. *Hortic. Res.* **2019**, *6*, 65. [CrossRef]
14. Liu, J.; Wu, S.; Sun, J.J.; Sun, J.R.; Wang, H.; Cao, X.; Lu, J.; Jalal, A.; Wang, C. Genome-Wide Analysis Reveals Widespread Roles for Rcrem Genes in Floral Organ Development in Rosa Chinensis. *Genomics* **2021**, *113*, 3881–3894. [CrossRef]
15. Annick, D.; Sebastien, C.; Olivier, R.; Benjamin, P.; Ludovic, C.; Aymeric, R.; Jean-Paul, O.; Soulaiman, S.; Rossitza, A.; Sylvie, B. Transcriptome Database Resource and Gene Expression Atlas for the Rose. *BMC Genom.* **2012**, *13*, 638.
16. Zhang, S.; Feng, M.; Chen, W.; Zhou, X.; Lu, J.; Wang, Y.; Li, Y.; Jiang, C.Z.; Gan, S.S.; Ma, N.; et al. In rose, transcription factor PTM balances growth and drought survival via PIP2;1 aquaporin. *Nat. Plants* **2019**, *5*, 290–299. [CrossRef]
17. Li, W.; Fu, L.; Geng, Z.; Zhao, X.; Liu, Q.; Jiang, X. Physiological Characteristic Changes and Full-Length Transcriptome of Rose (Rosa chinensis) Roots and Leaves in Response to Drought Stress. *Plant Cell Physiol.* **2021**, *61*, 2153–2166. [CrossRef]
18. Han, Y.; Yong, X.; Yu, J.; Cheng, T.; Wang, J.; Yang, W.; Pan, H.; Zhang, Q. Identification of Candidate Adaxial-Abaxial-Related Genes Regulating Petal Expansion During Flower Opening in *Rosa chinensis* "Old Blush". *Front. Plant Sci.* **2019**, *10*, 1098. [CrossRef]
19. Gao, Y.; Liu, C.; Li, X.; Xu, H.; Liang, Y.; Ma, N.; Fei, Z.; Gao, J.; Jiang, C.Z.; Ma, C. Transcriptome Profiling of Petal Abscission Zone and Functional Analysis of an Aux/IAA Family Gene *RhIAA16* Involved in Petal Shedding in Rose. *Front. Plant Sci.* **2016**, *7*, 1375. [CrossRef]
20. Han, Y.; Wan, H.; Cheng, T.; Wang, J.; Yang, W.; Pan, H.; Zhang, Q. Comparative RNA-seq Analysis of Transcriptome Dynamics During Petal Development in *Rosa chinensis*. *Sci. Rep.* **2017**, *7*, 43382. [CrossRef]
21. Guo, X.; Yu, C.; Luo, L.; Wan, H.; Zhen, N.; Xu, T.; Tan, J.; Pan, H.; Zhang, Q. Transcriptome of the floral transition in *Rosa chinensis* 'Old Blush'. *BMC Genom.* **2017**, *18*, 199. [CrossRef]

22. Liu, J.; Fu, X.; Dong, Y.; Lu, J.; Ren, M.; Zhou, N.; Wang, C. MIK^{C}-Type Mads-Box Genes in *Rosa chinensis*: The Remarkable Expansion of ABCDE Model Genes and Their Roles in Floral Organogenesis. *Hortic. Res.* **2018**, *5*, 25. [CrossRef]
23. Li, Y.; Wu, Z.; Ma, N.; Gao, J. Regulation of the Rose Rh-PIP2;1 Promoter by Hormones and Abiotic Stresses in *Arabidopsis*. *Plant Cell Rep.* **2009**, *28*, 185–196. [CrossRef]
24. Ma, N.; Xue, J.; Li, Y.; Liu, X.; Dai, F.; Jia, W.; Luo, Y.; Gao, J. Rh-PIP2;1, a Rose Aquaporin Gene, Is Involved in Ethylene-Regulated Petal Expansion. *Plant Physiol.* **2008**, *148*, 894–907. [CrossRef]
25. Lehti-Shiu, M.D.; Panchy, N.; Wang, P.; Uygun, S.; Shiu, S.-H. Diversity, Expansion, and Evolutionary Novelty of Plant DNA-binding Transcription Factor Families. *BBA Gene Regul. Mech.* **2017**, *1860*, 3–20. [CrossRef]
26. Danisman, S.; van der Wal, F.; Dhondt, S.; Waites, R.; de Folter, S.; Bimbo, A.; van Dijk, A.D.; Muino, J.M.; Cutri, L.; Dornelas, M.C. *Arabidopsis* Class I and Class II TCP Transcription Factors Regulate Jasmonic Acid Metabolism and Leaf Development Antagonistically. *Plant Physiol.* **2012**, *159*, 1511–1523. [CrossRef]
27. Yao, X.; Ma, H.; Wang, J.; Zhang, D. Genome-Wide Comparative Analysis and Expression Pattern of TCP Gene Families in *Arabidopsis thaliana* and *Oryza sativa*. *J. Integr. Plant Biol.* **2007**, *49*, 885–897. [CrossRef]
28. Xu, R.; Sun, P.; Jia, F.; Lu, L.; Li, Y.; Zhang, S.; Huang, J. Genomewide Analysis of TCP Transcription Factor Gene Family in *Malus domestica*. *J. Genet.* **2014**, *93*, 733–746. [CrossRef]
29. Leng, X.; Wei, H.; Xu, X.; Ghuge, S.A.; Jia, D.; Liu, G.; Wang, Y.; Yuan, Y. Genome-wide Identification and Transcript Analysis of TCP Transcription Factors in Grapevine. *BMC Genom.* **2019**, *20*, 786. [CrossRef]
30. Ma, X.; Ma, J.; Fan, D.; Li, C.; Jiang, Y.; Luo, K. Genome-wide Identification of TCP Family Transcription Factors from *Populus euphratica* and Their Involvement in Leaf Shape Regulation. *Sci. Rep.* **2016**, *6*, 32795. [CrossRef]
31. Zhou, J.; Li, J.; Li, X.; Xiao, D.; Li, M. Genome-Wide Identification, Evolution and Expression Analysis of TCP Gene Family in Celery (*Apium graveolens* L.). *J. Sichuan Agric. Univ.* **2022**, *40*, 145–155.
32. Rath, M.; Challa, K.R.; Sarvepalli, K.; Nath, U. CINCINNATA-Like TCP Transcription Factors in Cell Growth—An Expanding Portfolio. *Front. Plant Sci.* **2022**, *13*, 825341. [CrossRef]
33. Huang, T.; Irish, V.F. Temporal Control of Plant Organ Growth by TCP Transcription Factors. *Curr. Biol.* **2015**, *25*, 1765–1770. [CrossRef]
34. Zhang, Z.; Lu, S.; Ma, Z.; Zhou, Q.; Hw, H.; Chen, B.; Mao, J. Bioinformatics Identifcation and Expression Analysis of TCP Transcription Factor Family in Strawberry. *Acta Bot. Boreal.* **2020**, *40*, 2031–2043.
35. Han, J.; Cao, X.; Liu, H.; Fan, G. Analysis Genes in the *Paulownia fortunei* TCP Family and Their Response to Witches' Broom and Drought Stress. *J. For. Environ.* **2022**, *42*, 337–345.