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Article

Genome-Scale Characterization, and Expression Profiling of TCP Gene Family in Cold Stress Tolerance of Passion Fruit (*Passiflora edulis*)

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Abstract: Passion fruit is a valued tropical fruit crop that faces environment-related growth strains. TCP genes are important for both growth modulation and stress prevention in plants. Herein, we systematically analyzed the TCP gene family in passion fruit, recognizing 30 members. Genes exhibiting closer phylogenetic relationships exhibited similar protein and gene structures. Gene members of the *TCP* family showed developmental stage or tissue-specific expression profiles during the passion fruit life cycle. Transcriptome data also demonstrated that many *PeTCPs* showed induced expression in response to hormonal treatments and cold, heat, and salt stress. Based on transcriptomics data, eight candidate genes were chosen for preferential gene expression confirmation under cold stress conditions. Additionally, four TCP genes exhibited *in silico* binding with cold stress-related miRNA319s. This study will aid in the establishment of novel germplasm, as well as the further investigation of the roles of *PeTCPs* and their cold stress resistance characteristics.

Keywords: passion fruit; *TCP* transcription factors; cold stress; gene expressions; miRNA319s

1. Introduction

Since passion fruit (*Passiflora edulis*) has significant edible, medicinal, and ornamental value, it is widely grown in tropical and subtropical regions worldwide. Many consumers relish its egg-shaped fruit due to its unique flavor, rich aroma, acid pulp, and yellow juice [1]. Owing to the abundance of alkaloids, flavonoids, and other physiologically active components found in passion fruit, and extracts derived from its leaves, fruits, peels, and seeds have therapeutic properties that include anti-inflammatory, soothing, antioxidant, and anticancer characteristics [2]. Because most passion fruit varieties have large floral organs, bright coronal filaments, a rich fragrance, and lush branches and leaves, they are used as ornamental plants for flower racks due to their ornamental value [3]. Identifying and characterizing important gene families in passion fruit might help boost the growth of the world's agricultural economy.

Transcription factors (TFs) are major elements of the genetic foundation for phenotypic evolution. TCP proteins (TCPs) are a class of plant-specific TFs, that was first identified and designated as *TEOSINTE BRANCHED 1* (*TB1*) in *Zea mays* [4], *PROLIFERATING CELL FACTORS 1* and *2* (*PCF1* and *PCF2*) in rice [5], *CYCLOIDEA* (*CYC*) in *Antirrhinum majus* [6]. TCP domains are defined by an atypical 59-amino acid basic helix-loop-helix motif structural feature and are not related to the DNA-binding bHLH domain. Based on TCP domains, this class of proteins is

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partitioned into Class I (TCP-P or PCF type) and Class II (TCP-C type) [7]. Class I is distinguished by a four-amino-acid deletion which is a conserved feature. Class II is further divided into CIN clade and CYC clade [8]. Two duplication events in the CYC clade among the main eudicots led to three subgroups designated as CYC1, 2, and 3 [9]. The accumulated evidence of research suggested that TCP genes were implicated in many growth-related mechanisms including axillary meristem development, flower and leaf morphology, circadian rhythm regulation, hormone signaling, seed germination, and defense [10,11].

Plants are subjected to adverse environmental conditions constantly. A correlation between TCPs and plant response to abiotic stresses has been discovered by researchers [12]. Knock-down of two rice TCP genes *OsPCF5* and *OsPCF8* resulted in enhanced tolerance to cold stress after chilling treatment [13]. Still, the other two *TCPs, OsPCF6* and *OsTCP21* exhibited significant cold-induced expression and their knockdown plants exhibited enhanced cold tolerance compared to wild-type plants due to amended reactive oxygen species scavenging [14]. *PCF6* expression in sugarcane seedlings exposed to cold stress for 24 hours was reduced to 50 percent [15]. Similarly, cassava *TCP* gene *MeTCP3a* and *MeTCP4* expressions were reduced after their seedlings were treated with 4 ^{0}C cold stress [16]. In transgenic creeping bentgrass four members of the TCP gene family namely *AsPCF5/6/8/14* exhibited expressional depression in conjunction with the increased salt and drought tolerance linked with enhanced water retention and leaf wax contents [17]. Contrarily, overexpression of the rice *OsTCP19* resulted in abnormal development including reduced formation of lateral roots and enhanced abiotic stress tolerance [18]. *ZmTCP14* overexpression under drought conditions led to a significant reduction of drought tolerance, while gene-edited lines of *ZmTCP14* demonstrated enhanced drought tolerance, suggesting it acts as a negative regulator of drought stress [19]. *TCP10* from *Moso bamboo* exhibited induced expressions under drought stress and its overexpression in rice and *Arabidopsis* enhanced drought tolerance in transgenic plants [20].

Owing to the development of genome sequencing technologies *TCP* gene families have been studied in various modal organisms of agro-botanical importance like cultivated rye [21], switchgrass [22], potato [23], and *Arabidopsis* [24]. As of right now, the passion fruit genome sequence has been made public [16,25,26] offering valuable genome resources for the discovery of related genes. In this work, the genomic and transcriptomic data of diploid passion fruit were utilized to identify the sequences of the TCP TFs family of *P. edulis* using bioinformatics techniques and to investigate the function of TCP family genes in response to cold stress. To our knowledge, this is the first report, herein we performed identification of the TCP gene family members in passion fruit through a genome-wide survey, estimated their evolutionary interrelationships, and analyzed their functions employing publicly available RNA-seq datasets in compliance with abiotic stresses and expressional validation of few promising candidate genes. These candidate genes will serve as raw materials for further molecular characterization and stress breeding of passion fruit.

2. Results

2.1. Identification and Phylogeny of TCP Proteins in Passion Fruit

A total of 30 *PeTCP* genes that encode TCP proteins were identified in the yellow passionfruit genome. The genes were renamed from PeTCP1 to PeTCP30 based on their ascending order of chromosomal locations (Table 1). Furthermore, 10 and 24 *PeTCP* genes were identified from GWHAZTM00000000 (Purple type) and GWHANWG00000000 genome assemblies respectively (Table S1). To study how all identified 30 TCP proteins are related to each other neighbor-joining phylogenetic tree was constructed along with 24 TCPs of *Arabidopsis* (Figure 1). Passionfruit TCP members were classified into two classes and three subfamilies. Among passion fruit TCP proteins 15 were Class (I) members of the PCF subfamily. The rest of the fifteen PeTCPs of Class (II) were distributed into CIN and CYC subfamilies having eight and seven proteins respectively.

Additionally, we calculated the physiochemical properties of 30 TCP proteins (Table 1). The amino acids in the proteins encoded by the 30 TCP genes ranged from 164 (PeTCP1) to 759 (PeTCP16), while their molecular weights (MW) varied from 17985.36 Da (PeTCP1) to 83909.83 Da (PeTCP16). Protein isoelectric points (pI) were predicted ranging from 5.61 (PeTCP6) to 8.86 (PeTCP10). Proteins' thermal stability varied little among TCP proteins, with the aliphatic amino index (A.I.) revealing it ranged from 52.83 (PeTCP28) to 86.96 (PeTCP16). All of the TCP proteins had a negative grand

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average of hydropathicity score (GRAVY), suggesting that they are primarily hydrophilic. Finally, the subcellular localization prediction analysis revealed that except for membrane-localized PeTCP6/15/16 and cytoplasm-localized PeTCP1/3/13/23/26, the rest of TCP proteins were predicted to be localized in the nucleus.

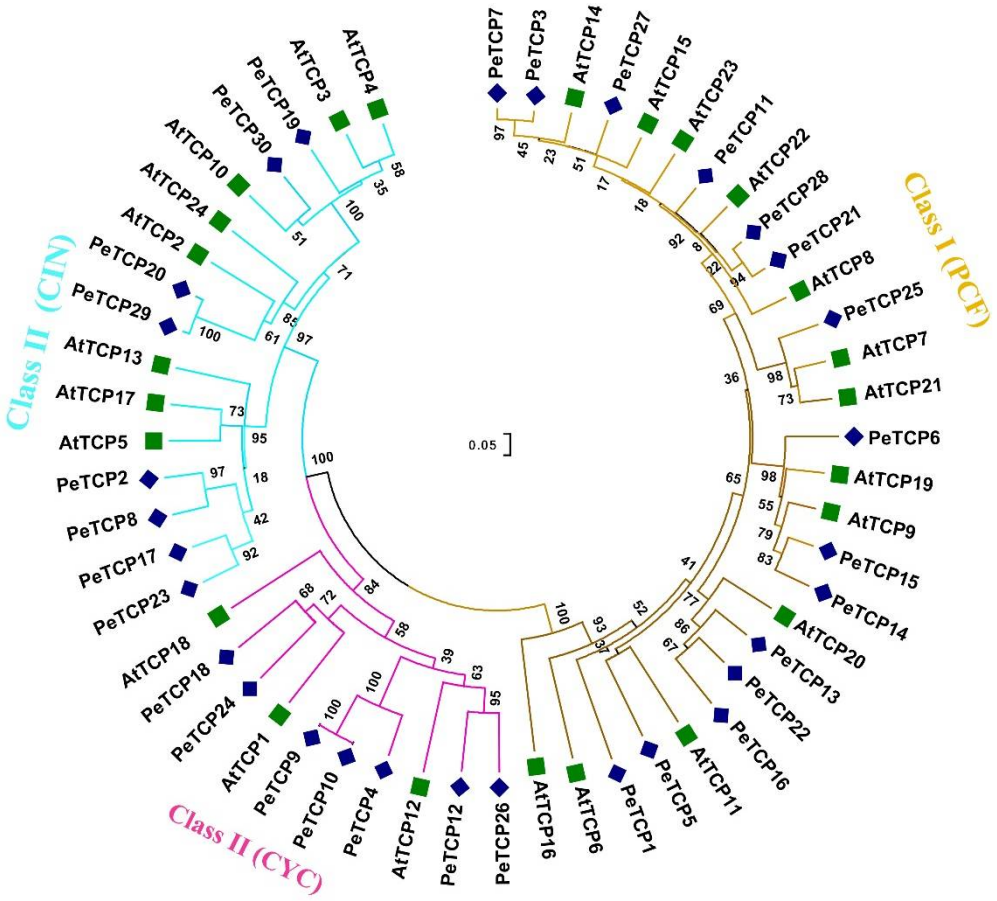

Figure 1. Phylogenetic analysis of TCP gene family proteins among passion fruit and *Arabidopsis*. The phylogenetic tree was built using MEGA-X software employing the neighbor-joining tree method with 1000 bootstrap replicates. Passion fruit TCP proteins are designated by "Pe" while *Arabidopsis* proteins are depicted with the "At" prefix.

Table 1. Information of TCP family in passion fruit.

Gene Name	Gene ID	Chromosome	Size (aa)	MW (Da)	pI	A.I.	Stability	GRAVY	Predicted location
PeTCP1	Pe1g00801	LG01	164	17985.36	6.1	58.35	U	-0.438	Cytoplasm
PeTCP2	Pe2g00351	LG02	364	40493.27	6.31	68.32	U	-0.665	Nucleus
PeTCP3	Pe2g00400	LG02	400	42807.17	7.91	62	U	-0.65	Cytoplasm
PeTCP4	Pe2g00517	LG02	385	43712.73	7.21	55.53	U	-0.876	Nucleus
PeTCP5	Pe2g01516	LG02	215	23168.9	6.71	65.95	U	-0.641	Nucleus
PeTCP6	Pe2g02083	LG02	419	45554.9	5.61	74.99	U	-0.472	membrane
PeTCP7	Pe2g02474	LG02	407	43474.67	7.44	58.06	U	-0.73	Nucleus
PeTCP8	Pe2g02555	LG02	357	39723.46	6.51	64.23	U	-0.622	Nucleus
PeTCP9	Pe2g02621	LG02	404	45981.81	8.67	62.82	U	-0.725	Nucleus
PeTCP10	Pe2g02632	LG02	299	34140.84	9.86	58.8	U	-0.894	Nucleus
PeTCP11	Pe2g03973	LG02	431	45408.01	6.56	57.61	U	-0.572	Nucleus
PeTCP12	Pe3g00812	LG03	486	54179.6	6.01	54.09	U	-0.9	Nucleus
PeTCP13	Pe3g01513	LG03	307	32776.29	9.16	58.47	U	-0.739	Cytoplasm
PeTCP14	Pe3g01959	LG03	411	42952	6.37	67.98	U	-0.43	Nucleus
PeTCP15	Pe4g04349	LG04	327	34638.45	9.26	81.19	U	-0.169	Membrane
PeTCP16	Pe5g00391	LG05	759	83909.83	6.34	86.96	U	-0.219	Chloroplast, membrane
PeTCP17	Pe5g00587	LG05	373	40572.4	6.88	77.94	U	-0.489	Nucleus
PeTCP18	Pe5g00665	LG05	396	44016.51	9.29	64.29	U	-0.685	Nucleus

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PeTCP19	Pe6g00604	LG06	349	38377.12	6.08	57.68	U	-0.718	Nucleus
PeTCP20	Pe6g01133	LG06	465	50516.54	8.7	60.86	U	-0.808	Nucleus
PeTCP21	Pe6g02163	LG06	543	56938.36	6.69	53.9	U	-0.702	Nucleus
PeTCP22	Pe8g00389	LG08	313	33367.08	7.99	62.75	U	-0.718	Nucleus
PeTCP23	Pe8g00755	LG08	367	40359.99	8.44	68.26	U	-0.6	Cytoplasm
PeTCP24	Pe8g00826	LG08	397	44330.83	8.46	70	U	-0.626	Nucleus
PeTCP25	Pe8g01074	LG08	276	28139.36	9.72	70.51	U	0.353	Nucleus
PeTCP26	Pe8g02699	LG08	491	54810.96	9.16	62	U	-0.754	Cytoplasm
PeTCP27	Pe8g03645	LG08	408	44573.37	6.7	62.5	U	-0.688	Nucleus
PeTCP28	Pe9g00054	LG09	552	58023.4	6.65	52.83	U	-0.743	Nucleus
PeTCP29	Pe9g01413	LG09	471	51180.1	8.84	59.68	U	-0.856	Nucleus
PeTCP30	Pe9g02247	LG09	420	45596.03	6.51	58.88	U	-0.65	Nucleus

After ascertaining the phylogenetic classification and distribution of TCP proteins in above mentioned three subfamilies we performed the multiple sequence alignment (MSA) of bHLH domains in MEGA-11 software and depicted it through the ESPript online server (Figure 2). The results indicated that the characteristic 4 amino acid deletions in the basic region led to the diversification of PeTCP into two major phylogenetic classes.

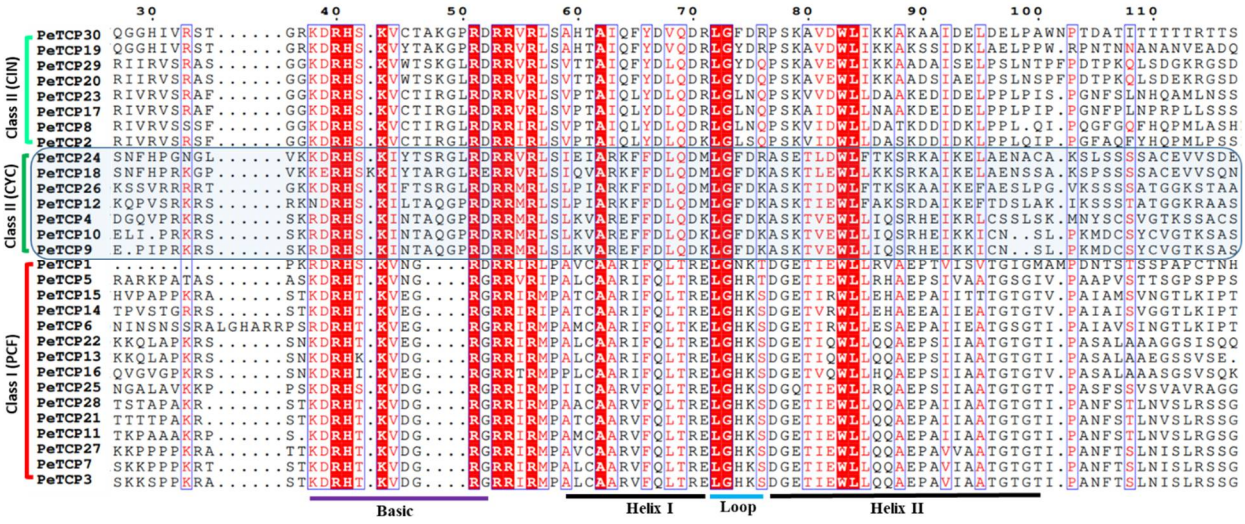

Figure 2. Multiple sequence alignment of passion fruit TCP proteins. Sequences were aligned in the MEGA software and sequence alignment was visualized in the ESPript 3.0 web tool.

2.2. Gene Structural Analysis and Domain Organization of PeTCP Genes

TCP gene family of passion fruit was examined for its conserved domain composition and gene structure features, and their phylogenetic connections were exhibited (Fig. 3 A, B, C). The domain analysis revealed all the TCP proteins possess only TCP domains however two members of Class (I) PeTCP22 and PeTCP16 contained additional domains called SKN1 and ACT respectively (Fig 3A). Gene structure organization depicted *PeTCP6* had the highest number of exons (4), whereas five genes of CYC subfamily *PeTCP4/9/10/18/24* possessed two exons each (Fig. 3B). The rest of *PeTCPs* contained only a single exon. Gene structures and domain composition of PeTCP genes clustered in the same subclasses are comparable, suggesting a strong correlation between gene structures and evolutionary relationships among PeTCPs.

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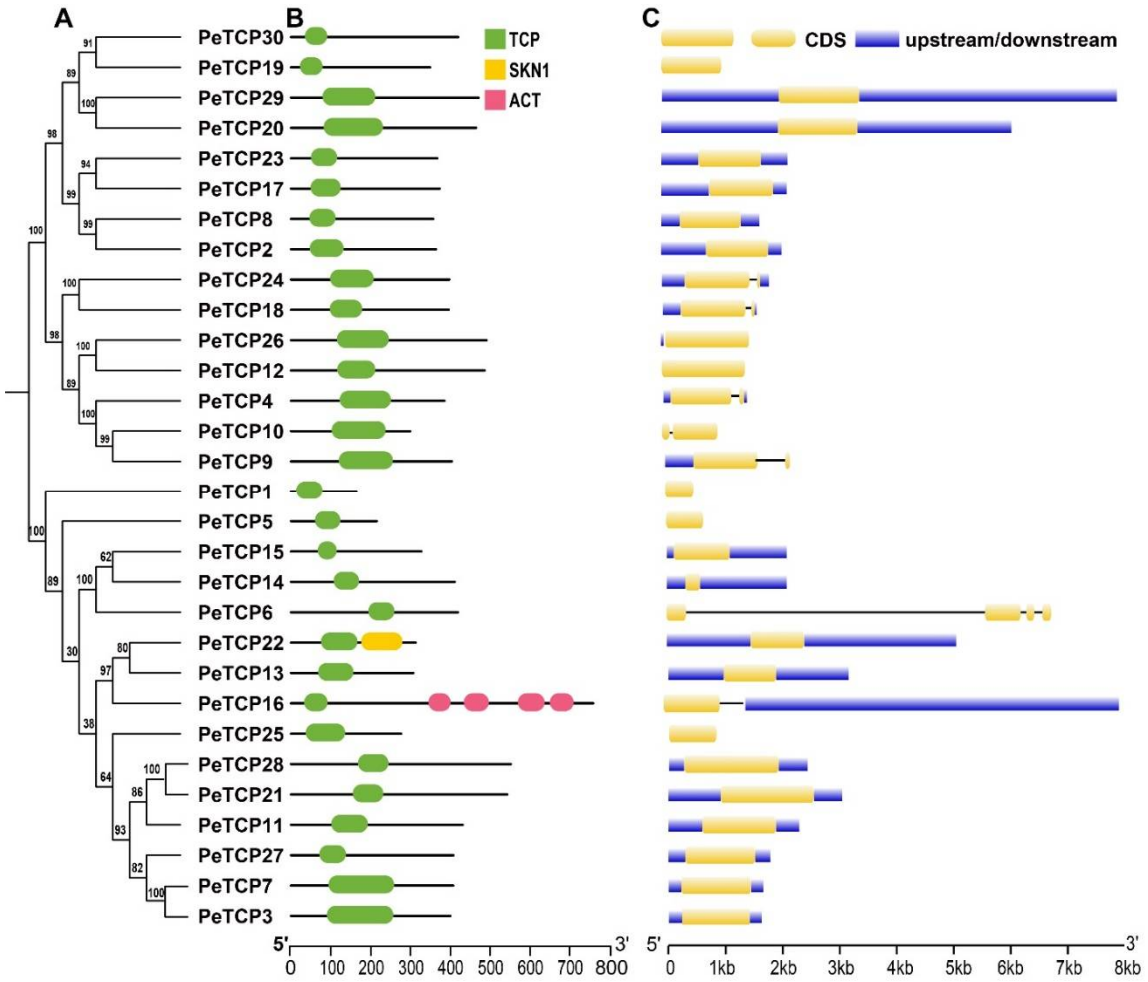

Figure 3. TCP protein domain composition and gene structure organization. (**A**) The phylogenetic tree was generated in MEGA-X software. (**B**) Domain attributes were downloaded from the NCBI batch CD server. TCP, SKN1, and ACT domains are depicted in green, yellow, and pink respectively. (**C**) The gene coordinate information was drawn through the TB tool. CDS, introns, and upstream/downstream regions of gene structure are shown in yellow, black, and blue respectively.

2.3. Homology Modeling and 3D Structural Comparisons of PeTCP Proteins

Structural examination of proteins has significant effects on comprehending their functions. We performed the homology modeling of PeTCP9 from CYC, PeTCP16 from CIN, and PeTCP25 from PCF subfamilies (Figure 4). Each subfamily protein was made up of single chains, consisting of typical DNA binding 59 amino-acid basic helix-loop-helix motif. The first helix was small with 3 turns and highly similar in all three representative proteins along with the loop region suggesting this region is highly conserved. Contrarily the 2nd helix exhibited structural differences such as PeTCP9 (CYC) had the longest alpha-helix with 7 turns while PeTCP25 (PCF) and PeTCP17 (CIN) possessed 6 and 5 turns respectively. These differences in 2nd helix length might be attributed to the functional variation of these proteins either through homodimerization with other TCP proteins or DNA-binding. Taken together, these proteins' homology models offer a foundational framework for delving deeper into the molecular roles of TCP proteins.

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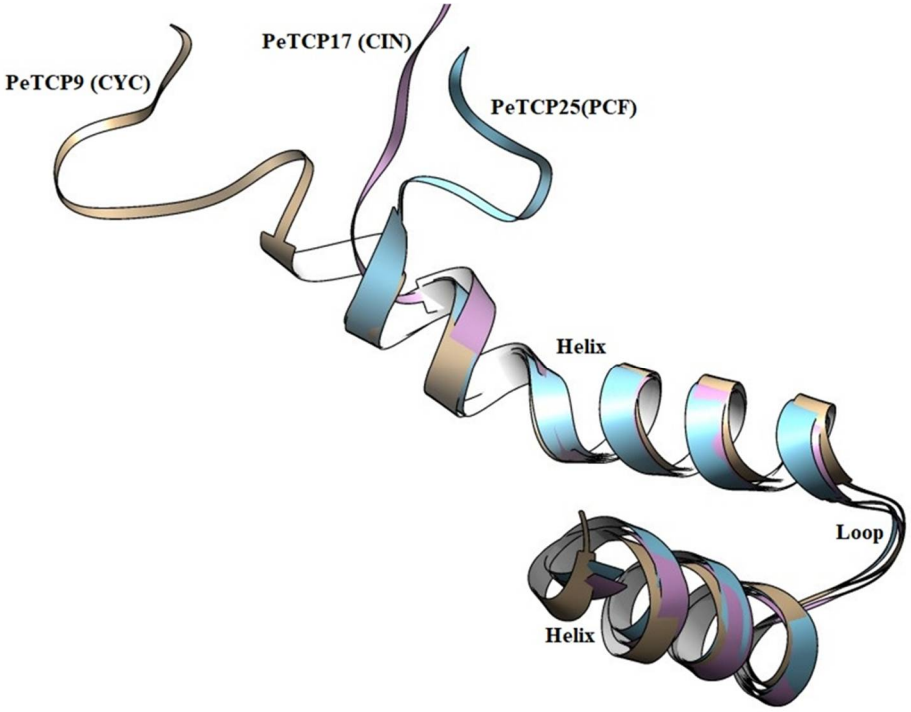

Figure 4. Structural modeling and superimposition of bHLH domains of three representative proteins from each subfamily of PeTCP. PeTCP9 depicted in brown, PeTCP17 shown in magenta, and PeTCP25 exhibited in blue represent CYC, CIN, and PCF subfamilies respectively. Protein 3D modeling was performed using the SWISS-MODEL employing orthologous Arabidopsis TCP proteins as templates. Modeled proteins were visualized and structurally aligned using the Chimera software.

2.4. Chromosomal Distributions and Gene Duplication Analysis of PeTCPs

In principle, different gene duplication patterns are assumed as the driving forces for gene family formation and evolution of species. Among all nine passionfruit linkage groups the *PeTCPs* were unevenly distributed (Fig. 5). Chromose 2 possessed the highest (10) number of *PeTCPs*, followed by chromosome 8 which contained 6 genes. Chromosomes 3, 5, 6, and 9 each had 3 *PeTCPs*, while chromosomes 1 and 4 each possessed a single gene. Duplicated genes were determined through reciprocal BLAST approaches. The location of duplicated genes on different chromosomes implied that *PeTCPs* might have arisen majorly through segmental gene duplications. Substitution ratio Ka/Ks has the functionality to describe evolutionary processes and the nature of selection or selection pressure, therefore we estimated the Ka/Ks ratios for all duplicated *PeTCPs* gene pairs (Table S2). Ka/Ks estimates indicated for all duplicated gene pairs the values were less than 1 which indicated that the TCP gene family might have experienced purifying selection during evolution. It's possible that the purifying selection was crucial in preserving the TCP genes' conserved structure over time.

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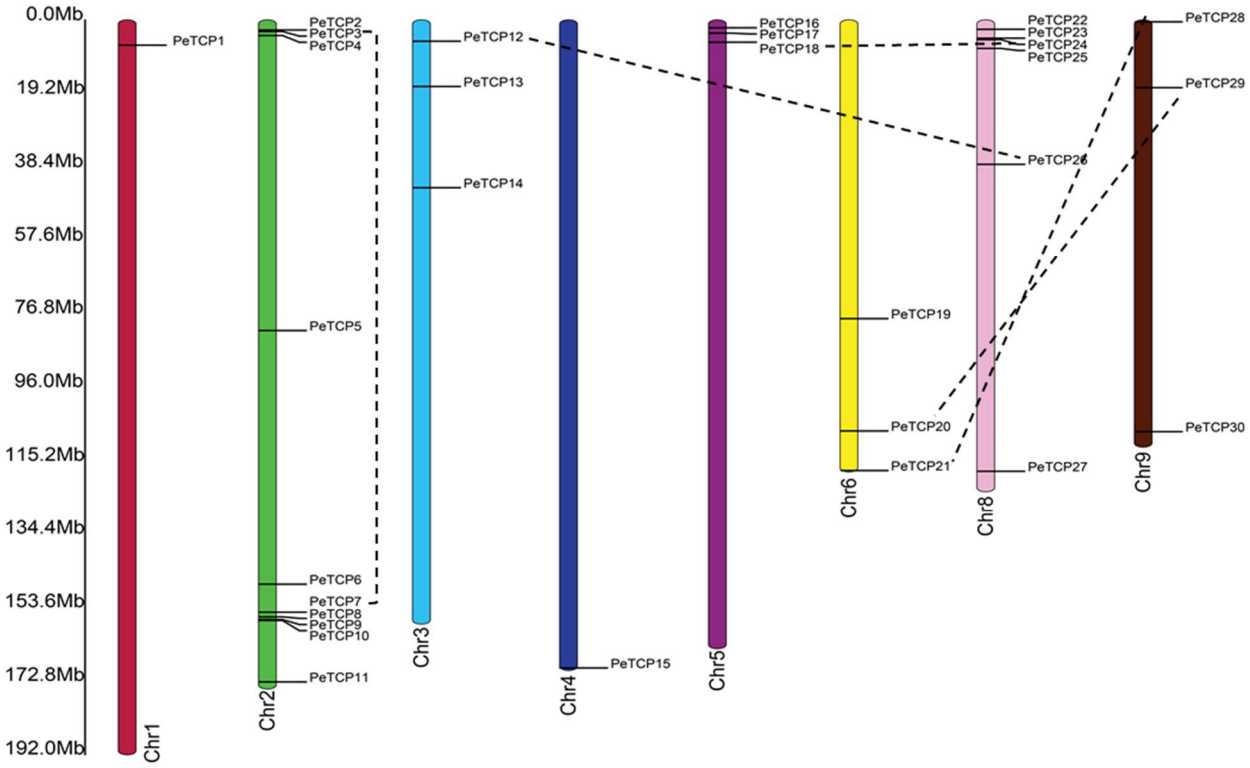

Figure 5. Distribution of 30 TCP genes on passion fruit genome. The chromosome structures and gene positions were depicted in TB tools employing the genomic information from the Passionfruit Genomics Network database. The scale on the left indicates the chromosome size in Mbps. The colored bars indicate chromosomes, while blue lines with double arrows show the duplicated genes.

2.5. Prediction of Cis-Regulatory Elements in PeTCP Promoters

Probable roles of *PeTCP* genes with the phytohormone responses, growth and development, and abiotic stresses were examined by analyzing the *cis-regulatory* elements (CREs) inside their promoter regions (Fig. 6). A total of 291 CREs were estimated on 16 *PeTCP* genes. The predicted 291 CREs could be distributed into 134, 94, and 63 related to growth, hormones, and stress respectively. Among all the genes *PeTCP15* had the highest 25 CREs, whereas *PeTCP22* possessed the lowest with 13 CREs. Abscisic acid (ABA) responsiveness (ABRE, 33, 35%) and MeJA-responsiveness (CGGTA-motif and TGACG-motif, 27, 28%) were relatively abundant among CREs related to hormonal responsiveness in the promoter regions of PeTCPs (Fig. 6A). On the other hand, CREs associated with salicylic acid (SA) responsiveness (16, 17%), gibberellin (GA)-responsiveness (12, 12.7%), and CREs linked to auxin (IAA)-responsiveness (6, 6.8%) were sporadically distributed. For CREs involved in regulating growth and development (Fig. 6B), such as CAT-box for meristem expression (10, 7.4%), GCN4_motif for endosperm expression (1, 0.73%), RY-element for seed-specific regulation (1, 0.73%), and circadian for circadian control (3, 2.23%), were relatively insufficient compared to the light-responsive elements (116, 86.5%). Regarding CREs associated with stress responsiveness (Fig.6 C), the anaerobic induction ARE (38, 60.3%) was found to be prevalent across all genes. Still, low-temperature responsiveness (9, 14%), defense, and stress responsiveness (10, 15%), and drought responsiveness (6, 9.5%) CREs were relatively unevenly distributed across different genes. In summary, these results indicated that there was significant diversity in both the makeup and quantity of CREs in the promoter regions of *PeTCP*, indicating that different CREs regulate the expression of TCP genes in passion fruit.

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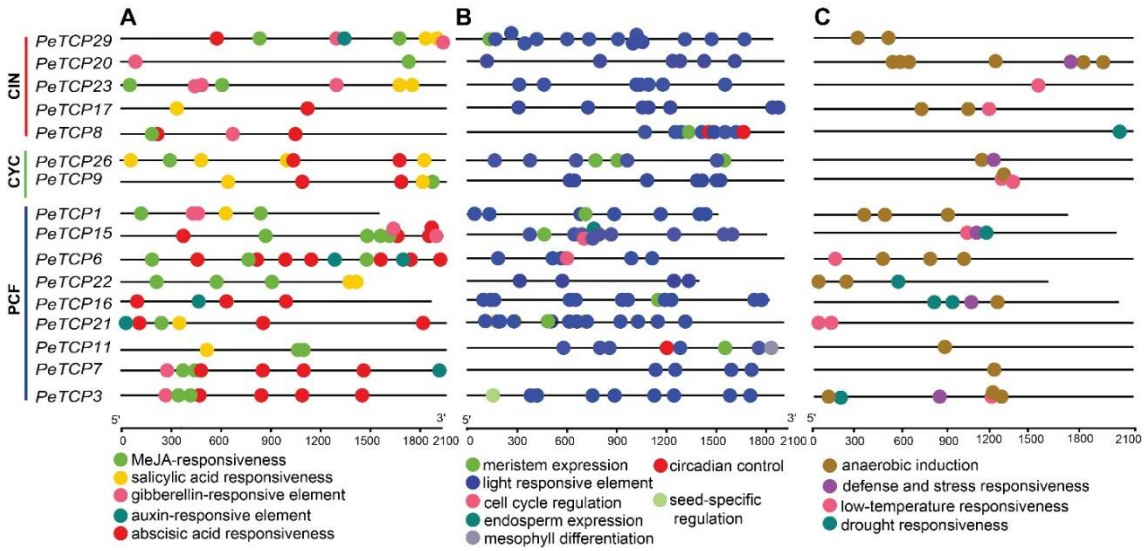

Figure 6. *Cis*-regulatory elements (CREs) distribution on the predicted promoter regions of *PeTCPs*. (**A**) Hormonal responsiveness. (**B**) Growth and development related. (**C**) Stress responsiveness.

2.6. Prediction of Putative TCP Genes Targeted by miRNA319

We tested the insilico binding of miRNA319 which is a conserved class of plant cold-stress-related microRNA with TCP genes. Our analysis indicated that both miRNA319a and miRNA319b can bind with *TCP19/20/29/30* genes (Figure 7). Interestingly all four genes were phylogenetically conserved belonging to the CIN subfamily of Class (II).

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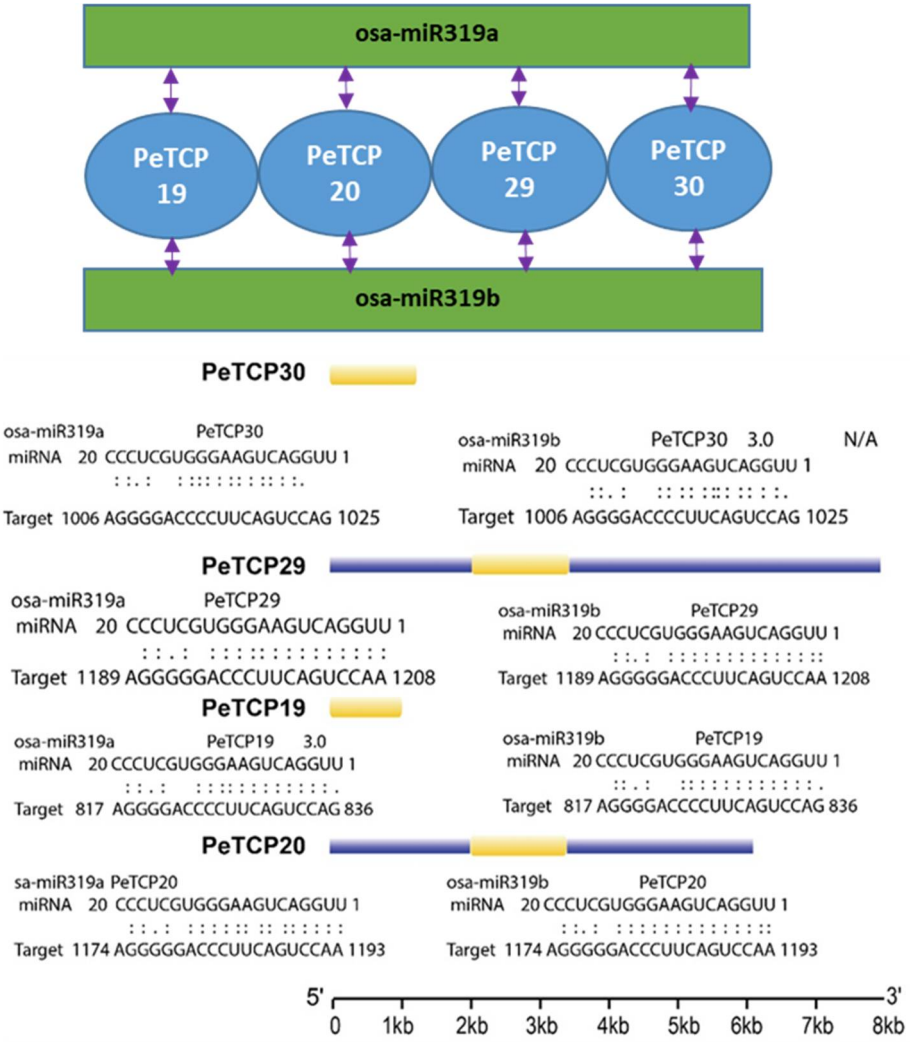

Figure 7. Predicted miRNA319a/b –TCP binding modules.

2.7. Expression and GO/KEGG Enrichment analysis of TCP genes

Transcriptomic data was used to characterize the expression profiles of the *PeTCPs* genes at different developmental stages to investigate the potential functions of these genes (Figure 8A, Table S3). The hierarchical clustering of expression patterns allowed the *PeTCP* genes to be sorted into different groups. Different groups of *PeTCP* genes had unique patterns of temporal and spatial expressions. The heatmap clustering indicated that *PeTCP17/23* in petals and stamens, *PeTCP11/29* in root, *PeTCP19/29* in leaf, and *PeTCP1/15* in immature fruit tissue exhibited high preferential gene expressions.

To anticipate potential roles for *TCP* genes in hormonal regulation, we examined the expression profile of *TCP* genes in response to a range of **Phytohormones,** such as ABA, ethylene, GA, Auxin, and MeJA (Figure 8B, Table S4). In reaction to ABA's hormonal treatments *PeTCP17/23/30/15/21* showed instantaneous induction. On ethylene treatments, *PeTCP15/17* exhibited preferential upregulation. Treatments with auxin resulted in elevated *PeTCP21/27/28/29/30* gene expression. In reaction to the GA treatment, *PeTCP15/17/22* showed induced expressions, while *PeTCP15/17/27* exhibited preferential upregulation in response to MeJA treatments.

TCP genes are critical for protecting cells from stress-induced oxidative damage. The expression profiles of the TCP genes of passion fruit under heat, cold (Figure 8C, Table S5), salt, and drought stress (Figure 8D, Table S6) conditions were also investigated using transcriptome data. Many genes, including *PeTCP15/16/17/19*, showed increased gene expressions and responded immediately to the cold treatments. *PeTCP16/17/20/25* exhibited quick accumulation of mRNA transcript in a brief amount of time after being rapidly induced under heat stress, indicating their speedy response to heat stress conditions. Furthermore, elevated gene expression was observed in CIN-type

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PeTCP17/19/29/30 and PCF-type *PeTCP11/15/16/25* in response to salt stress. The gene expression of *PeTCP15/16/17/19//25* increased in response to the drought stress.

GO and KEGG enrichment analyses of passion fruit were carried out for 30 TCP genes (Figure 9). The *PeTCPs* were majorly enriched with Go terms such as regulation of transcription (*PeTCP7/25*), response to lipids (*PeTCP3*) and hormones (*PeTCP7*), inflorescence development (*PeTCP7*), defense responses (*PeTCP6/7*), response to stimulus (*PeTCP27*), amino acid binding (*PeTCP16*), while only a single gene *PeTCP25* was significantly enriched for KEGG pathways for plant circadian rhythms.

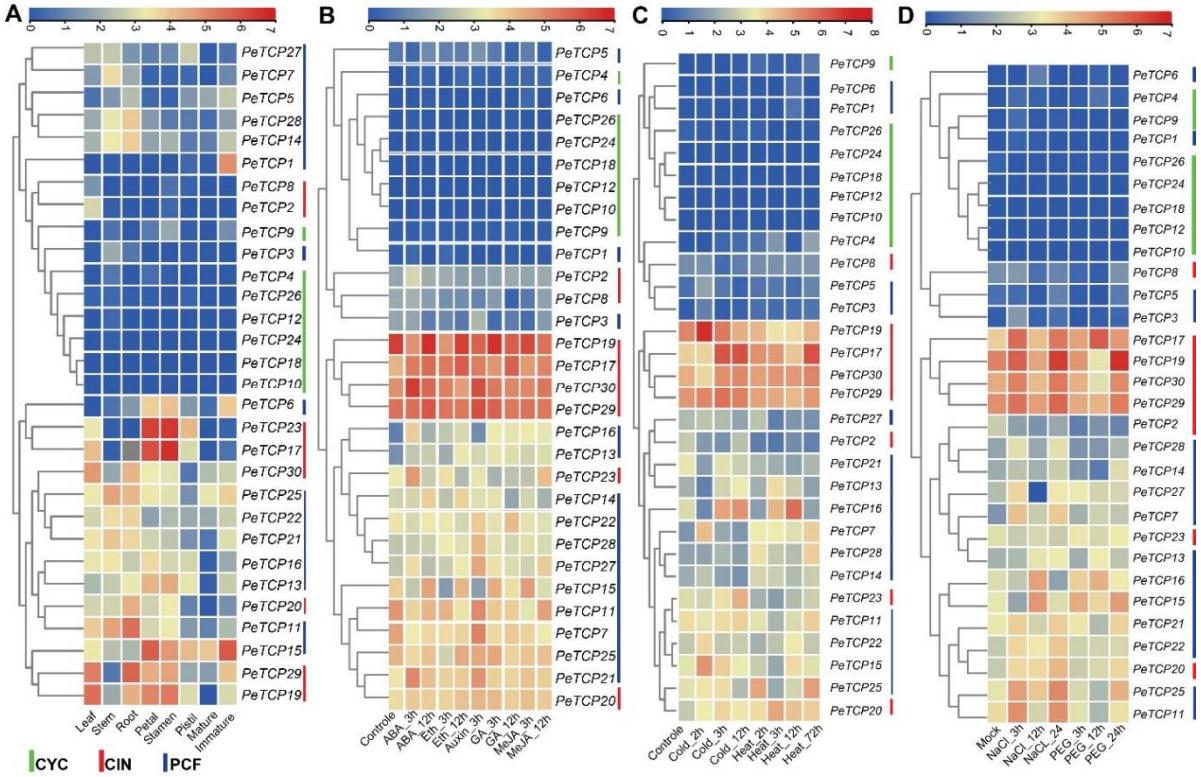

Figure 8. Heatmaps of TCP gene transcripts expression levels. (**A**) Different growth stages and tissues. (**B**) Hormonal treatments. (**C**) Cold and heat stress conditions. (**D**) Salt and drought stress.

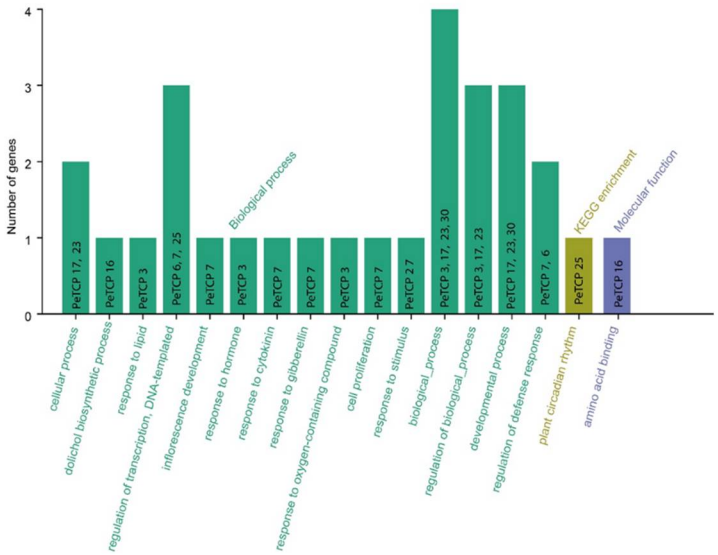

Figure 9. GO annotations and KEGG analysis of TCP gene family. The GO enrichment terms' names are on the X axis while the number of genes belonging to each category is along the Y axis.

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To further assess the expression profiles of the passion fruit TCP genes under cold stress conditions eight notable genes were chosen for qRT-PCR analysis based on their significantly varied expressions from transcriptome results. In conformation to the transcriptomics data, CIN-type *PeTCP19/17/23* and PCF-type *PeTCP16 /15* expressions were significantly increased by application of cold stress treatment at 6hr intervals, declined sharply at 12 hr intervals, and steadied at 24 hr (Figure 10), suggesting expressional induction of these genes. Contrarily PCF-type *PeTCP25/1* and CYC-type *PeTCP11* exhibited decreased expressions with subsequent cold treatments compared to controle (Figure 10). Interestingly among treatment intervals 6 hr interval was the most influential. Taken together these results indicate TCP genes of passionfruit play vital roles in the regulation of cold stress.

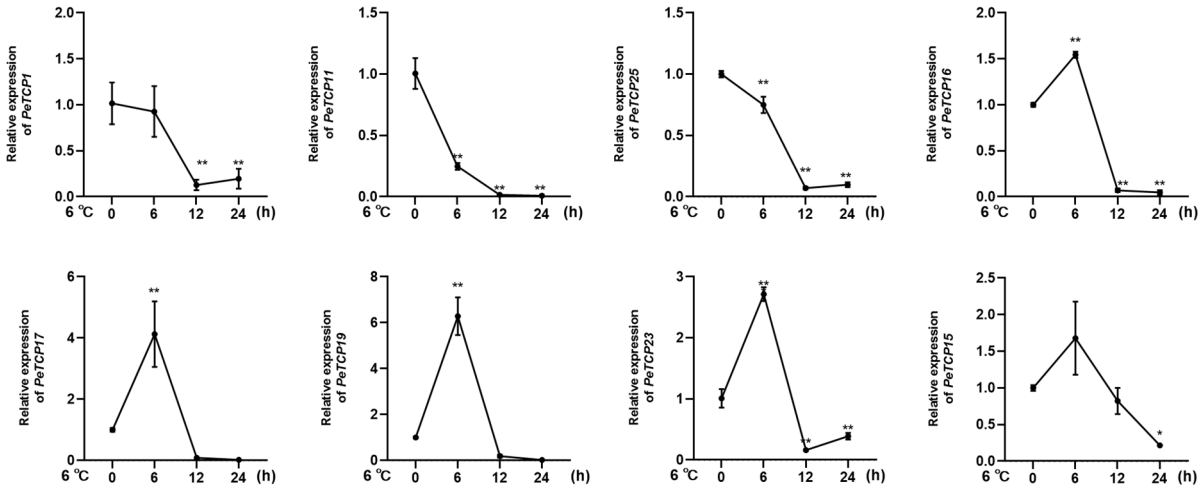

Figure 10. Expression profiles of 8 TCP genes (*PeTCP1, 11, 25, 16, 17, 19, 23, 15*) in response to the cold stress treatments. Three independent biological replicates' standard deviations of means are represented by error bars. Significant variations of the transcript levels between treatments and blank control (0 h) are indicated by asterisks. (*$p < 0.05$, **$p < 0.01$).

3. Discussion

Tropical fruit crops like passion fruit have significant agricultural, commercial, and ornamental value, however, environmental conditions have a big impact on the fruit's growth and development. TCP transcription factors play pivotal roles in growth and development and coping with biotic and abiotic stresses.

In the current study, a sum of 30 members of the TCP transcription factor gene family in the passion fruit genome were identified. Using homologous genes from *Arabidopsis*, phylogenetic analysis exhibited TCP gene family divergence into two classes and three subfamilies. The results of the phylogenetic analysis and classification were independently supported by domain architecture, gene exon/intron structure analysis indicated that closely related gene members typically exhibit similar structural characteristics, as observed in other plants like rice [30] and rye [21]. We noticed that most of the TCP proteins were located in the nucleus except a few in the cytoplasm. Furthermore, the results of homologous protein modeling the representative proteins of each gene subfamily exhibited divergent 3D structures and distinctive features associated with their varied functions. The structural diversity of these proteins may contribute to the functional diversity of TCP gene subfamilies.

Passion fruit had a slightly higher number of TCP proteins than *Arabidopsis* 24 protein members. Among 30 total TCP genes in passion fruit, five pairs of segmental duplication were identified, largely responsible for the expansion of the TCP family. The Ka/Ks ratios of all the duplicated gene pairs were smaller than 1 (Table S2), suggesting that these gene pairs underwent negative or purifying selection over their evolutionary history. Except for the lowly expressed duplicated pairs *PeTCP18/24* and *12/26*, the other members of duplicated gene pairs exhibited opposite or varied gene expressions, suggesting these genes following duplication might have experienced sub-functionalization or neo-functionalization. In contrast to the model laboratory species such as *Arabidopsis*, passion fruit had to

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adapt to a greater variety of abiotic stressors to flourish during its growth and development. The proliferation of these TCP genes and the variety of roles they play may offer plants additional resilience to a range of adverse conditions, hence improving passion fruit's capacity to adapt to shifting environmental conditions.

In the meantime, 4 genes (*PeTCP19/20/29/30*) potentially targeting miRNA319 in passion fruit were identified. Interestingly, all four genes belonged to the CIN subfamily and showed similar expression profiles except *PeTCP20*. miR319 is one of the primitive and the most evolutionarily conserved miRNAs in plants [34]. Growing data indicates that miR319-regulated TCPs (MRTCP) genes play a major role in the development of plants and stress response to the environment [12]. It appears that miR319 may have conserved roles in the modulation of stress responses in passion fruit as well, as all three of the genes *PeTCP19/29/30* targeted by miR319 were differentially expressed during stress conditions. These findings showed that, by altering the transcriptional level of *TCP* genes in passion fruit, the miRNA39 might have significant effects on the modulation of growth/development and stress responses.

Although TCP genes' involvement in growth/development and stress management have been reported in other plant species, studies of the TCP gene family in passion fruit are missing. The functional variety of the TCP genes was identified by analysis of the gene expression of the passion fruit. Some genes showed tissue-specific expression. For instance, *PeTCP17/23* exhibited obvious high expressions in petals and stamen tissues, whereas *PeTCP29* showed preferential upregulation in leaf and root tissues. Additionally, *PeTCP15* exhibited obvious high expressions in petals and immature fruit tissues.

Members of the TCP gene family have been demonstrated to be essential components of multiple hormonal signaling networks in various plant species [35] and in response to hormonal treatments of ABA, *PeTCP15/17/21/30* genes exhibited immediate induction. *PeTCP15/17* were up-regulated upon ethylene treatments. Auxin treatments led to increased gene expression of *PeTCP21/27/28/29/30*. Similarly, the snapdragon *CIN* gene has been linked to the control of the genes involved in the cytokinin and auxin signaling pathways as well as the formation of lateral organs [36]. *PeTCP15/22/17* exhibited expressional induction responding to the GA treatment, meanwhile, *PeTCP15/17/27* showed preferential upregulation in response to methyl jasmonate (MeJA) treatments. The altered gene expression levels measured during MeJA treatment in Senna tora exhibited *StTCP11* and *StTCP4.1* and may be implicated in jasmonic acid (JA) response signaling [37].

Passion fruit's growth and development are extremely susceptible to fluctuations in the climate. The expression of some gene members, such as *PeTCP15/16/17/19*, increased rapidly with the cold treatments. It is well documented that the miR319-TCP module regulates cold stress in rice [13,14,30], sugarcane [15], and cassava [16]. Similarly, *PeTCP16/17/20/25* were induced quickly under heat stress and showed high transcript accumulation in a short period. Additionally, CIN-type genes *PeTCP17/19/29/30* and PCF-type *PeTCP11/15/16/25* exhibited enhanced mRNA transcripts in response to salt stress. A comparative transcriptome investigation between salt-sensitive and salt-tolerant genotypes of common beans demonstrated salt-responsive expression patterns for *Pvul-TCP1/11/13/22/27*, which are targets of miR319 [38]. In response to the drought stress, *PeTCP17/19/15/16/25* showed increased gene expression. Drought treatments induced *ZmTCP42 and ZmTCP32* expressions, and overexpression of *ZmTCP42* in *Arabidopsis* resulted in enhanced drought tolerance [39], however in another study overexpression of *ZmTCP14* showed opposite results [19] confirming *TCP* roles in drought resistance. Interestingly, all the *PeTCPs* belonging to the CYC subfamily exhibited low expression across all the samples, while four CIN subfamily genes *PeTCP17/19/29/30* exhibited strong differential expressions in reaction to the stress conditions.

Some of these promising genes such as *PeTCP15/17/19/29/30* may have significant application potential for the genetic improvement of passion fruit with improved tolerance to abiotic stress because they respond to a variety of stimuli. In their promoter regions, they had a variety of cis-acting elements (CREs) linked to hormonal responses (ABA-responsiveness, MeJA-responsiveness), growth (light responsive element, meristem expression), and stress responses (anaerobic induction, low-temperature responsiveness), which plays a significant role in the transduction of biological information [31,32]. In rice, OsTCP19 has a role in modulating drought-induced ABA signaling because of its interaction with OsABI4, which encodes a TF implicated in the transmission of the ABA signaling [18]. AtTCP14 inhibits ABA signaling by interacting with the DOF6 (DNA binding with one

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finger) TF, preventing the induction of additional ABA-responsive genes in *Arabidopsis* as well as the downstream ABA biosynthesis gene *ABA1* (ABA deficient 1) [33]. In addition, the co-occurrence of several CREs in the promoter regions of *PeTCP* genes may be intimately associated with the functions of these genes in the growth and development of passion fruit in response to various environmental modifications [40]. Further studies are required to help establish links between *PeTCP* genes and stress responses.

4. Material and Methods

4.1. Identification and Sequence Analysis of Passion Fruit TCP Proteins

Firstly TCP protein sequences were retrieved from the yellow passion fruit genome at the Passionfruit Genomic Networks database (http://passionfruit.fafu.edu.cn/motif_search.html) using the TCP PFAM accession ID (PF03634). Later on, two published genomic assemblies named GWHAZTM00000000 and GWHANWG00000000 of purple passion fruit types were downloaded from Genome Warehouse (https://ngdc.cncb.ac.cn/gwh/Genome/557/show). Similarly, the latest whole genome protein sequences of rice and Arabidopsis were downloaded from the Phytozome database (https://phytozome-next.jgi.doe.gov/) and uploaded on Bioedit software to generate four local genome files. The yellow passionfruit TCP protein sequences were used as queries to perform a local BLAST against the rest of above mentioned four genomes in Bioedit software. The online Conserved Domains search tool in the NCBI database (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) was utilized to affirm the protein sequences that were obtained. The physicochemical properties of yellow passionfruit TCP proteins were computed employing the Expasy ProtParam (https://web.expasy.org/protparam/). The Euk-mPLoc (http://www.csbio.sjtu.edu.cn/bioinf/euk-multi/) tool was used to predict the sub-cellular localization of all putative TCPs.

4.2. Multiple Sequence Alignment and Phylogenetic Tree Construction

Using MEGA11 software [27], the full-length protein sequences of Arabidopsis (24), and PeTCPs (30) were aligned to construct a neighbor-joining phylogenetic tree. The criteria were adopted using 1000 bootstrap replications of the Poisson correction model and a pairwise deletion option. Gene structures of the identified PeTCPs were determined by the Gene Structure Display Server 2.0 tool (http://gsds.gao-lab.org/). Domain organizations of the probable PeTCPs were first determined in the NCBI Batch CDD server (https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi) and later on, depicted through TBtools software [28]. Using the TBtools program, the arrangement of PebTCPs throughout the nine chromosomes of the P. edulis genome as well as the duplications were mapped, and Ka/Ks values were computed.

4.3. Structural Analysis of TCP Proteins

Homology modeling of PeTCP proteins was performed and a single representative member structure of three subfamilies was modeled based on the SWISS-MODEL database in user-specified template mode. The Arabidopsis TCP proteins representing each subfamily AlphaFold structures were downloaded from UniProt and used as a template. Then these templates were used in SWISS-MODEL (https://swissmodel.expasy.org/) to model the characteristic DNA-binding 59 amino acid bHLH domains of PeTCPs. Eventually, the modeled domains were visualized and superimposed in Chimera software [29] to observe the structural differences.

4.4. Insilico Binding Prediction between TCP Genes and miRNA319

Rice microRNA319 positively regulates cold stress by repressing the expression of its target OsTCPs, and since microRNA sequences are conserved among plant species, we tested whether OsmiRNA319 could target PeTCPs. Rice miR319, miR319a, and miR319b sequences were downloaded from the plant microRNA database (PMRD) (http://bioinformatics.cau.edu.cn/PMRD)

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and later on were analyzed with PeTCPs CDS sequences as target genes in psRNATarget (https://www.zhaolab.org/psRNATarget/).

4.5. Expression Profile Analysis Based on RNA-seq Data

RNA-seq datasets of all the putative PeTCPs were downloaded from the Passionfruit Genomics Network database (http://passionfruit.fafu.edu.cn/search_genes_expression.html) for eight tissue samples (leaf, stem, root, petal, stamen, pistil, mature and immature fruit), abiotic stresses (heat, cold, salt, and drought), and hormonal treatments (ABA, Eth, Auxin, GA, and MeJA). The gene expressions were normalized and heatmaps were generated using the Tbtools software.

4.6. GO Annotation and KEGG Analysis of PeTCP Genes

The Go annotation and KEGG analysis of identified PeTCPs were performed through the Passionfruit Genomic Networks database and graphical depictions were performed through the https://bioinformatics.com.cn/ web tool.

4.7. Functional Validation of the Candidate PeTCP Genes through qRT-PCR

To validate the transcriptome gene expressions qRT-PCR analysis was used. Abiotic stress treatments were applied to the two-month-old healthy passionfruit seedlings with fully developed roots and shoots along with one control with three biological replicates. For cold abiotic stress treatment, 8-10 seedlings were used. To apply cold stress healthy plants were put in a growth chamber with temperatures set at 6°C. The samples under stress treatments were taken at 0, 6, 12, and 24 hour intervals, respectively. After stress treatments, the leaves were frozen and RNA was extracted employing a Vazyme RNA isolation kit with three biological replicates. Aidlab Truescript 1st Strand cDNA Synthesis Kit was used to synthesize cDNA from extracted RNA. Real-time PCR was done using the PC59-2 x SYBR Green qPCR Mix (Aidlab Biotechnologies., Ltd) in the DLAB accurate96 system and primers are listed in Table S7. The qRT-PCR conditions were 95 °C for 2 min; 40 cycles of 95 °C for 15 s, 60 °C for 30 s; and 72 °C for 30 s. Three technical replicates from three biological replicates were used for each analysis, and the $2^{-\Delta\Delta Ct}$ method was used to determine the fold change of each gene.

5. Conclusions

This study is the first time deciphering the phylogeny, gene structure, cis-regulatory elements, and expression of the TCP family, one of the important regulatory factors, in passion fruits. A total of 30 *PeTCPs* were identified distributed on nine chromosomes, and can be classified into three subgroups. The motif composition and the 3D models of the TCP proteins further exhibited similarity at the basic helix I and loop regions and the variations at the helix II. The differential expression patterns of *PeTCPs* across the tissues and organs were revealed, some of which were tissue-specific. In addition, expression analysis of *PeTCPs* under cold, heart, salts, and PEG treatments highlighted their predominant roles under stress stimulation. Our results provide insights into functional analyses of TCP genes and some of them (*PeTCP17/19/15/16/23* & *PeTCP1/11/25*) would be promising targets for the genetic improvement of stress tolerance of passion fruits.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

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References

1. Teixeira, N.; Melo, J.C.S.; Batista, L.F.; Paula-Souza, J.; Fronza, P.; Brandão, M.G.L. Edible Fruits from Brazilian Biodiversity: A Review on Their Sensorial Characteristics versus Bioactivity as Tool to Select Research. *Food Res. Int.* **2019**, *119*, 325–348, doi:https://doi.org/10.1016/j.foodres.2019.01.058.
2. Vuolo, M.M.; Lima, G.C.; Batista, Â.G.; Carazin, C.B.B.; Cintra, D.E.; Prado, M.A.; Júnior, M.R.M. Passion Fruit Peel Intake Decreases Inflammatory Response and Reverts Lipid Peroxidation and Adiposity in Diet-Induced Obese Rats. *Nutr. Res.* **2020**, *76*, 106–117, doi https://doi.org/10.1016/j.nutres.2019.08.007.
3. da Silva Nóbrega, D.; Peixoto, J.R.; Vilela, M.S.; Faleiro, F.G.; de Paula Silva Gomes, K.; de Deus Sousa, R.M.; Nogueira, I. Agronomic Descriptors and Ornamental Potential of Passion Fruit Species. *Ornam. Hortic.* **2017**, *23*, 357–362.
4. Doebley, J.; Stec, A.; Hubbard, L. The Evolution of Apical Dominance in Maize. *Nature* **1997**, *386*, 485–488, doi:10.1038/386485a0.
5. Kosugi, S.; Ohashi, Y. DNA Binding and Dimerization Specificity and Potential Targets for the TCP Protein Family. *Plant J.* **2002**, *30*, 337–348, doi:10.1046/j.1365-313x.2002.01294.x.
6. Luo, D.; Carpenter, R.; Vincent, C.; Copsey, L.; Coen, E. Origin of Floral Asymmetry in Antirrhinum. *Nature* **1996**, *383*, 794–799, doi:10.1038/383794a0.
7. Navaud, O.; Dabos, P.; Carnus, E.; Tremousaygue, D.; Hervé, C. TCP Transcription Factors Predate the Emergence of Land Plants. *J. Mol. Evol.* **2007**, *65*, 23–33, doi:10.1007/s00239-006-0174-z.
8. 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*, doi:10.3390/ijms20143591.
9. Chapman, M.A.; Leebens-Mack, J.H.; Burke, J.M. Positive Selection and Expression Divergence Following Gene Duplication in the Sunflower CYCLOIDEA Gene Family. *Mol. Biol. Evol.* **2008**, *25*, 1260–1273, doi:10.1093/molbev/msn001.
10. Lucero, L.E.; Manavella, P.A.; Gras, D.E.; Ariel, F.D.; Gonzalez, D.H. Class I and Class II TCP Transcription Factors Modulate SOC1-Dependent Flowering at Multiple Levels. *Mol. Plant* 2017, *10*, 1571–1574.
11. Shang, Y.; Yuan, L.; Di, Z.; Jia, Y.; Zhang, Z.; Li, S.; Xing, L.; Qi, Z.; Wang, X.; Zhu, J.; et al. A CYC/TB1-Type TCP Transcription Factor Controls Spikelet Meristem Identity in Barley. *J. Exp. Bot.* **2020**, *71*, 7118–7131, doi:10.1093/jxb/eraa416.
12. Fang, Y.; Zheng, Y.; Lu, W.; Li, J.; Duan, Y.; Zhang, S.; Wang, Y. Roles of MiR319-Regulated TCPs in Plant Development and Response to Abiotic Stress. *Crop J.* **2021**, *9*, 17–28, doi:https://doi.org/10.1016/j.cj.2020.07.007.
13. Yang, C.; Li, D.; Mao, D.; Liu, X.; Ji, C.; Li, X.; Zhao, X.; Cheng, Z.; Chen, C.; Zhu, L. Overexpression of MicroRNA319 Impacts Leaf Morphogenesis and Leads to Enhanced Cold Tolerance in Rice (Oryza Sativa L.). *Plant. Cell Environ.* **2013**, *36*, 2207–2218, doi:10.1111/pce.12130.
14. Wang, S.; Sun, X.; Hoshino, Y.; Yu, Y.; Jia, B.; Sun, Z.; Sun, M.; Duan, X.; Zhu, Y. MicroRNA319 Positively Regulates Cold Tolerance by Targeting OsPCF6 and OsTCP21 in Rice (Oryza Sativa L.). *PLoS One* **2014**, *9*, e91357, doi:10.1371/journal.pone.0091357.
15. Thiebaut, F.; Rojas, C.A.; Almeida, K.L.; Grativol, C.; Domiciano, G.C.; Lamb, C.R.C.; Engler, J. de A.; Hemerly, A.S.; Ferreira, P.C.G. Regulation of MiR319 during Cold Stress in Sugarcane. *Plant. Cell Environ.* **2012**, *35*, 502–512, doi:10.1111/j.1365-3040.2011.02430.x.
16. Lei, N.; Yu, X.; Li, S.; Zeng, C.; Zou, L.; Liao, W.; Peng, M. Phylogeny and Expression Pattern Analysis of TCP Transcription Factors in Cassava Seedlings Exposed to Cold and/or Drought Stress. *Sci. Rep.* **2017**, *7*, 10016, doi:10.1038/s41598-017-09398-5.
17. Zhou, M.; Li, D.; Li, Z.; Hu, Q.; Yang, C.; Zhu, L.; Luo, H. Constitutive Expression of a MiR319 Gene Alters Plant Development and Enhances Salt and Drought Tolerance in Transgenic Creeping Bentgrass. *Plant Physiol.* **2013**, *161*, 1375–1391, doi:10.1104/pp.112.208702.
18. Mukhopadhyay, P.; Tyagi, A.K. OsTCP19 Influences Developmental and Abiotic Stress Signaling by Modulating ABI4-Mediated Pathways. *Sci. Rep.* **2015**, *5*, 9998, doi:10.1038/srep09998.
19. Jiao, P.; Liu, T.; Zhao, C.; Fei, J.; Guan, S.; Ma, Y. ZmTCP14, a TCP Transcription Factor, Modulates Drought Stress Response in Zea Mays L. *Environ. Exp. Bot.* **2023**, *208*, 105232, doi:https://doi.org/10.1016/j.envexpbot.2023.105232.
20. Liu, H.; Gao, Y.; Wu, M.; Shi, Y.; Wang, H.; Wu, L.; Xiang, Y. TCP10, a TCP Transcription Factor in Moso Bamboo (Phyllostachys Edulis), Confers Drought Tolerance to Transgenic Plants. *Environ. Exp. Bot.* **2020**, *172*, 104002, doi:https://doi.org/10.1016/j.envexpbot.2020.104002.
21. Zhan, W.; Cui, L.; Guo, G.; Zhang, Y. Genome-Wide Identification and Functional Analysis of the TCP Gene Family in Rye (Secale Cereale L.). *Gene* **2023**, *854*, 147104, doi:https://doi.org/10.1016/j.gene.2022.147104.
22. Zheng, A.; Sun, F.; Cheng, T.; Wang, Y.; Xie, K.; Zhang, C.; Xi, Y. Genome-Wide Identification of Members of the TCP Gene Family in Switchgrass (Panicum Virgatum L.) and Analysis of Their Expression. *Gene* **2019**, *702*, 89–98, doi:https://doi.org/10.1016/j.gene.2019.03.059.

23. Wang, Y.; Zhang, N.; Li, T.; Yang, J.; Zhu, X.; Fang, C.; Li, S.; Si, H. Genome-Wide Identification and Expression Analysis of StTCP Transcription Factors of Potato (Solanum Tuberosum L.). *Comput. Biol. Chem.* **2019**, *78*, 53–63, doi:https://doi.org/10.1016/j.compbiolchem.2018.11.009.
24. Xu, R.; Gao, H.; Zhang, S.; Liu, P.; Wang, X.; Hao, Y. Genome-Wide Identification and Phylogenetic, Comparative Genomic, Alternative Splicing, and Expression Analyses of TCP Genes in Plants. *Plant Gene* **2017**, *12*, 23–32, doi:https://doi.org/10.1016/j.plgene.2017.05.004.
25. Ma, D.; Dong, S.; Zhang, S.; Wei, X.; Xie, Q.; Ding, Q.; Xia, R.; Zhang, X. Chromosome-Level Reference Genome Assembly Provides Insights into Aroma Biosynthesis in Passion Fruit (Passiflora Edulis). *Mol. Ecol. Resour.* **2021**, *21*, 955–968, doi:10.1111/1755-0998.13310.
26. Xia, Z.; Huang, D.; Zhang, S.; Wang, W.; Ma, F.; Wu, B.; Xu, Y.; Xu, B.; Chen, D.; Zou, M.; et al. Chromosome-Scale Genome Assembly Provides Insights into the Evolution and Flavor Synthesis of Passion Fruit (Passiflora Edulis Sims). *Hortic. Res.* **2021**, *8*, 14, doi:10.1038/s41438-020-00455-1.
27. Tamura, K.; Stecher, G.; Kumar, S. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Mol. Biol. Evol.* **2021**, *38*, 3022–3027, doi:10.1093/molbev/msab120.
28. Chen, C.; Chen, H.; Zhang, Y.; Thomas, H.R.; Frank, M.H.; He, Y.; Xia, R. TBtools: An Integrative Toolkit Developed for Interactive Analyses of Big Biological Data. *Mol. Plant* **2020**, *13*, 1194–1202, doi:10.1016/j.molp.2020.06.009.
29. Pettersen, E.F.; Goddard, T.D.; Huang, C.C.; Couch, G.S.; Greenblatt, D.M.; Meng, E.C.; Ferrin, T.E. UCSF Chimera--a Visualization System for Exploratory Research and Analysis. *J. Comput. Chem.* **2004**, *25*, 1605–1612, doi:10.1002/jcc.20084.
30. Gull, S.; Uddin, S.; Hussain, H.A.; Wang, S.; Bayar, J.; Liu, J. Genome-Wide Analysis Reveals the TCP-MiR159-MiR319 Module Is Crucial for Rice (Oryza Sativa L.) Growth and Response to Drought and Salinity. *Plant Stress* **2023**, *10*, 100215, doi:https://doi.org/10.1016/j.stress.2023.100215.
31. Inukai, S.; Kock, K.H.; Bulyk, M.L. Transcription Factor-DNA Binding: Beyond Binding Site Motifs. *Curr. Opin. Genet. Dev.* **2017**, *43*, 110–119, doi:10.1016/j.gde.2017.02.007.
32. Liang, J.; Hou, Z.; Liao, J.; Qin, Y.; Wang, L.; Wang, X.; Su, W.; Cai, Z.; Fang, Y.; Aslam, M.; et al. Genome-Wide Identification and Expression Analysis of LBD Transcription Factor Genes in Passion Fruit (Passiflora Edulis). *Int. J. Mol. Sci.* **2022**, *23*, doi:10.3390/ijms23094700.
33. Rueda-Romero, P.; Barrero-Sicilia, C.; Gómez-Cadenas, A.; Carbonero, P.; Oñate-Sánchez, L. Arabidopsis Thaliana DOF6 Negatively Affects Germination in Non-after-Ripened Seeds and Interacts with TCP14. *J. Exp. Bot.* **2012**, *63*, 1937–1949, doi:10.1093/jxb/err388.
34. Koyama, T.; Sato, F.; Ohme-Takagi, M. Roles of MiR319 and TCP Transcription Factors in Leaf Development. *Plant Physiol.* **2017**, *175*, 874–885, doi:10.1104/pp.17.00732.
35. Bao, S.; Zhang, Z.; Lian, Q.; Sun, Q.; Zhang, R. Evolution and Expression of Genes Encoding TCP Transcription Factors in Solanum Tuberosum Reveal the Involvement of StTCP23 in Plant Defence. *BMC Genet.* **2019**, *20*, 91, doi:10.1186/s12863-019-0793-1.
36. Das Gupta, M.; Aggarwal, P.; Nath, U. CINCINNATA in Antirrhinum Majus Directly Modulates Genes Involved in Cytokinin and Auxin Signaling. *New Phytol.* **2014**, *204*, 901–912, doi:10.1111/nph.12963.
37. Liu, S.; Yin, X.; Feng, T.; Kang, Z.; Zhang, X.; Dong, J.; Liang, Z. Genome-Wide Identification and Expression Analysis of the TCP Genes in Senna Tora Reveal the Regulatory Mechanism of Their Response to MeJA. *Ind. Crops Prod.* **2022**, *187*, 115511, doi:https://doi.org/10.1016/j.indcrop.2022.115511.
38. İlhan, E.; Büyük, İ.; İnal, B. Transcriptome – Scale Characterization of Salt Responsive Bean TCP Transcription Factors. *Gene* **2018**, *642*, 64–73, doi:https://doi.org/10.1016/j.gene.2017.11.021.
39. Ding, S.; Cai, Z.; Du, H.; Wang, H. Genome-Wide Analysis of TCP Family Genes in Zea Mays L. Identified a Role for ZmTCP42 in Drought Tolerance. *Int. J. Mol. Sci.* **2019**, *20*, doi:10.3390/ijms20112762.
40. Liang, J.; Lu, L.; Zhang, W.; Chi, M.; Shen, M.; An, C.; Chen, S.; Wang, X.; Liu, R.; Qin, Y.; et al. Comprehensive Characterization and Expression Analysis of Enzymatic Antioxidant Gene Families in Passion Fruit (Passiflora Edulis). *iScience* **2023**, *26*, 108329, doi:https://doi.org/10.1016/j.isci.2023.108329.