Comparative genomic investigation of TCP gene family in eggplant (Solanum melongena L.) and expression analysis under divergent treatments

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Abstract

Key message The putative TCP genes and their responses to abiotic stress in eggplant were comprehensively characterized, and SmTCP genes (Smechr0202855.1 and Smechr0602431.1) may be involved in anthocyanin synthesis.

Abstract The Teosinte branched1/Cycloidea/Proliferating cell factors (TCPs), a family of plant-specific transcription factors, plays paramount roles in a plethora of developmental and physiological processes. We here systematically characterized putative TCP genes and their response to abiotic stress in eggplant. In total, 30 SmTCP genes were categorized into two subfamilies based on the classical TCP conserved domains. Chromosomal location analysis illustrated the random distribution of putative SmTCP genes along 12 eggplant chromosomes. Cis-acting elements and miRNA target prediction suggested that versatile and complicated regulatory mechanisms that control SmTCPs gene expression, and 3 miRNAs (miR319a, miR319b, and miR319c-3p) might act as major regulators targeting SmTCPs. Tissue expression profiles indicated divergent spatiotemporal expression patterns of SmTCPs. qRT-PCR assays demonstrated different expression profiles of SmTCP under 4°C, drought and ABA treatment conditions, suggesting the possible participation of SmTCP genes in multiple signaling pathways. Furthermore, RNA-seq data of eggplant anthocyanin synthesis coupled with yeast one-hybrid and dual-luciferase assays suggested the involvement of SmTCP genes (Smechr0202855.1 and Smechr0602431.1) in the mediation of anthocyanin synthesis. Our study will facilitate further investigation on the putative functional characterization of eggplant TCP genes and lay a solid foundation for the in-depth study of the involvement of SmTCP genes in the regulation of anthocyanin synthesis.

Keywords TCP genes · Eggplant (Solanum melongena L.) · Genome-wide investigation · Expression analysis, Anthocyanin synthesis

Abbreviations

ABA Abscisic acid
bHLH Basic-helix-loop-helix
CAREs Cis-acting regulatory elements
DEGs Differentially expressed genes

Dual-LUC Dual luciferase
MW Molecular weight
TCPs Teosinte branched1/Cycloidea/Proliferating cell factors
TFs Transcription factors
PEG Polyethylene glycol
PIs Isoelectric points
Y1H Yeast one hybrid

Introduction

The Teosinte branched1/Cycloidea/Proliferating cell factor (TCP) gene family belongs to the plant-specific family of transcription factors. The name was derived from the initial four letters of identified and characterized members: TB1 (TEOSINTE BRANCHED 1) in maize (Doebble...
et al. 1997), CYC (CYCLOIDEA) in Anthirrinum majus (Luo et al., 1996), and PCF1 and PCF2 (PROLIFERATING CELL FACTORS 1 and 2) in Oryza sativa (Kosugi et al. 1997). TCP transcription factors contain a conserved TCP domain that forms a basic helix-loop-helix (bHLH) structure composed of approximately 59 amino acids at the N-terminus. This domain is crucial for DNA binding and is engaged in protein–protein interactions and protein localization (Schommer et al. 2008). Based on the sequence characteristic of the TCP domain, the TCP gene family is categorized into two subfamilies: Class I and Class II. Class I, also called the PCF class or TCP-P class, can bind to the GGNCCAC sequence. Class II, also named the TCP-C class, can bind to the GTGGNCCC sequence and is further subdivided into CIN and CYC/TB1 subclasses. The most remarkable distinction between the two subfamilies lies in the absence of four amino acids in the TCP domain of Class I. In addition, Class II members share a unique R domain (rich arginine motifs with 18–20 residues) and the ECE motif (glutamic acid-cysteine-glutamic acid stretch), whose function may be involved in protein–protein interactions (Howarth and Donoghue 2006; Li 2015).

Mounting evidence has indicated that TCP family members play critical roles in multiple processes of plant growth and development, as well as in responses to biotic and abiotic stresses (Cubas and Martí 2009; Nicolas and Cubas 2016), including plant cell wall formation (Sun et al. 2017), leaf morphogenesis (Palatnik et al. 2003; Koyama et al. 2010; Schommer et al. 2014; Ma et al. 2016; Sun et al. 2017), flower development (Palatnik et al. 2003; Crawford et al. 2004; Schommer et al. 2008; Nag et al. 2009; Rubio-somoza and Weigel 2013), biosynthesis and/or signaling of hormones (hormone pathways) (Efroni et al. 2008; Schommer et al. 2008; Osnat et al. 2011; Danisman et al. 2012; González-grandío et al. 2017; Liu et al. 2019), adversity stress responses (Mukhopadhyay and Tyagi 2015; Liu et al. 2019, 2020), and flavonoid synthesis (Li and Zachgo 2013; Viola et al. 2016). For Class I TCP members, Arabidopsis AtTCP14 and AtTCP15 affected internode length and leaf morphogenesis (Kieffer et al. 2011) and mediated gibberellin for seed germination (Resentini et al. 2015); AtTCP16 played a vital role in microspore development (Takeda et al. 2006); and AtTCP23 participated in flowering time and plant development (Balsemão-pires et al. 2013). Tomato TCP members (SITCP12, SITCP15, and SITCP18) are preferentially expressed in tomato fruit, revealing that they function in the regulation of tomato fruit development and ripening (Parapunova et al. 2014; Guo et al. 2018). For CYC/TB1 subclade TCP members, Arabidopsis AtBRC1 and AtBRC2 and tomato SIBRC1b (SITCP7) contribute to regulate the branch number by mediating gibberellin signals (Aguilar-Martínez et al. 2007; Martin-Trillo et al. 2011). Chrysanthemum GhCYC2 is involved in regulating petal development (Broholm et al. 2008). For CIN subclade TCP members, Arabidopsis AtTCP2, AtTCP3, AtTCP4, AtTCP10, and AtTCP24 have been shown to partake in the regulation of leaf and flower development (Ori et al. 2007; Schommer et al. 2008; Nag et al. 2009; Zhou and Hong 2014). Additionally, Arabidopsis AtTCP3 can enhance flavonoid biosynthesis by interacting with R2R3MYB protein (Li and Zachgo 2013). These investigations unveiled the functional divergence of diverse TCP members during plant development.

In addition to their significant as transcriptional regulators of plant growth and development, TCP genes play a pivotal role in response to abiotic stresses. For Class I TCP members, Arabidopsis AtTCP20 regulates nitrate assimilation and signaling by interacting with NLP6&7 (Guan et al. 2017). Overexpression of the rice OsPCF2 gene enhanced salt tolerance (Almeida et al. 2017). For Class II TCP members, PeTCP10 overexpression in mos bamboo increased drought tolerance (Liu et al. 2020). These studies demonstrated that the roles of TCP genes in response to abiotic stresses are well established.

The development and application of whole-genome high-throughput sequencing technologies have provided an excellent platform to comprehensively characterize TCP members in multiple plants, such as Arabidopsis (Yao et al. 2007), rice (Yao et al. 2007), tomato (Parapunova et al. 2014), potato (Bao et al. 2019), and grape (Leng et al. 2019a). At present, AP2/ERF, HSF and WRKY gene families have been well characterized in eggplant (Li et al., 2021; Wang et al., 2020; Yang et al., 2020). However, no comprehensive identification and systematic investigations regarding the eggplant TCP gene family have been conducted. Eggplant is an economically significant crop cultivated worldwide and rich in anthocyanins. We here screened 30 eggplant TCP genes and characterized their phylogenetic relationship, chromosomal location, gene duplications, structure composition, conserved domain, predicted cis-acting regulatory elements (CAREs) and miRNA-SmTCP interactions. Moreover, we analyzed SmTCP expression profiles in distinct eggplant organs and in response to various stresses and plant hormones through quantitative real-time PCR (qRT-PCR). Three genes were obtained by analyzing and screening previous transcriptomic data (Li et al. 2018). The possible participation of 2 out of the 3 genes in anthocyanin synthesis was verified by through the yeast one-hybrid (Y1H) and dual-luciferase (Dual-LUC) assays. The study results will contribute to further investigation on the functional characterization of SmTCPs and the determination of crucial SmTCPs that participate in growth development and stress responses.
Materials and methods

Characterization and categorization of eggplant TCP genes

To access the complete sequences of all eggplant TCP genes, the genome sequences of the model plant Arabidopsis thaliana, rice, tomato and eggplant genome sequences were extracted from the following corresponding online websites: (TAIR; https://www.arabidopsis.org/index.jsp), (https://phytozome.jgi.doe.gov/pz/portal.html) and (http://eggplant-hq.cn/Eggplant/home/index). The two-way blast method was then applied to extract and screen putative eggplant TCP genes from these sequences using TBtootls software (Chen et al. 2020). Subsequently, all non-redundant TCP sequences in eggplant were corroborated using NCBI (https://www.ncbi.nlm.nih.gov/) BLASTP. Next, these sequences identified were further proofed and confirmed using SMART (http://smart.embl-heidelberg.de/) and NCBI-CDD (https://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml) online tools. The molecular weight (MW) and theoretical isoelectric point (PI) of SmTCP proteins were obtained using the ExPasy website (https://web.expasy.org/compute_pi/).

Phylogenetic and multiple sequence alignment analysis of eggplant TCP genes

Multiple alignments of full-length TCP proteins from eggplant and Arabidopsis, rice, and tomato were aligned using ClustalW with default pairwise settings. According to alignment-based outcomes, the phylogenetic tree was generated through the maximum likelihood method by using MEGAX software (1000 bootstrap replicates). The results were embellished and presented using the iTOL (https://itol.embl.de/onlinetool) online website (Letunic and Bork 2007). According to the TCP conserved domain, TCP genes characterized in eggplant were subgrouped into diverse groups. Amino acid sequence alignment analysis of TCP conserved domains in eggplant was performed using DNASAN software.

Gene structure, conserved domains, and motif analysis

The exon–intron assembly of eggplant TCP members was analyzed using TBtools software. The conserved domains of SmTCPs were acquired using the Batch Web CD Online Tool (https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi). The online MEME website (http://meme-suite.org/tools/meme) was applied to ascertain conserved motifs of SmTCP genes.

Promoter element analysis and miRNA-TCP prediction

The promoter sequences of 2000-bp upstream of the SmTCP coding sequences were retrieved from the eggplant genome website (http://eggplant-hq.cn/Eggplant/home/index). Then, these sequences were submitted and predicted for putative CAREs through PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/). To forecast the putative miRNA target sites in SmTCP genes, we employed all CDS sequences of SmTCPs as candidate targets for predicting putative miRNAs. Subsequently, we queried against the candidate targets using the available Solanaceae miRNA mature sequences acquired from the miRBase database (http://www.mirbase.org/) via the psRNATarget server with default parameters (Dai and Zhao 2011). The Sankey plot was employed to present the associations of the putative miRNAs and the corresponding target genes through the Tutools platform (https://www.cloudtutu.com).

Chromosomal location and duplication analysis of SmTCP genes

The chromosomal location and visualization of all SmTCP family members were performed by using TBtools. Tandem duplicated genes were investigated artificially and displayed on the eggplant physical map by using MCScanX software (Wang et al. 2012).

The Ka (non-synonymous substitution rate) and Ks (synonymous substitution rate) of SmTCP gene pairs were calculated using the Simple Ka/Ks Calculator by the method of Nei and Gojobor (NG) (Li et al. 2006). Divergence times were determined according to the formula \( T = \frac{K_s}{2r} \), where \( K_s \) was the synonymous substitution per site and \( r \) was the divergence rate of plant nuclear genes. For dicotyledons, \( r \) was regarded as 1.5 × 10^{-8} synonymous substitutions per site per year (Koch et al. 2000).

Syntenic analysis

To exhibit the synthetic relationships of TCP genes between A. thaliana and tomato (Solanum lycopersicum), syntenic maps were plotted using Multiple Synteny Plot software from TBtools with default parameters.

Plant material growth condition and abiotic stress response

The eggplant variety ‘LSHX’ was used to determine the expression profiles of TCP responding to phytohormone and
Results

Identification and categorization of SmTCP family members

All putative TCPs were retrieved from the eggplant genome by querying Arabidopsis, rice and tomato TCPs against the eggplant genome using BLASTP. Altogether 30 presumed SmTCP members were determined and estimated using SMART and Pfam online tools to verify the existence of the unique TCP domains. The presumed TCP genes were derived from the phylogenetic categorization of the Arabidopsis, rice, and tomato TCP family. The 30 TCP transcription factors were featured by a highly conserved TCP domain at the N-terminus. The primary information for these SmTCP sequences was summarized and tabulated in Table S2. The results of this analysis suggested the SmTCP protein length varied from 201 amino acid residues (Smechr0900228.1) to 538 (Smechr0100729.1) aa. The potential TCP genes encoded proteins with the predicted MW ranging from 21.27 (Smechr0900228.1) to 57.64 (Smechr0100729.1) kDa and the PIs varying between 5.25 (Smechr0201631.1) and 9.84 (Smechr0101558.1). Prediction of subcellular localization indicated that most SmTCP proteins (29 of 30, 96.7%) were localized in the nucleus (Table S2).

As per the differences in TCP domains, 30 SmTCP proteins were categorized into two classes (Table S2). Concretely, 13 SmTCP proteins were classified into Class I, which contained the TCP domain with a four-amino-acid deletion compared with other SmTCPs. The remaining 17 genes were attributed to the Class II, and were further divided into the CIN subclade (13 SmTCPs) and the CYC/TB1 subclade (4 SmTCPs).

Phylogenetic analysis and categorization of SmTCP family

To assess the phylogenetic association and possible evolutionary history among identified genes in different TCP gene families, 30 eggplant, 24 Arabidopsis, 24 rice and 37 tomato TCP proteins were aligned and a phylogenetic tree was constructed using the maximum likelihood method (Fig. 1). Using phylogenetic distribution, 30 TCPs were primarily divided into 11 subgroups (Groups A–K) based on their sequence features. Groups A–F belonged to Class I TCP members. Groups I–K were assigned to Class II (CIN) TCP members. The remaining Groups G–H belonged to Class II CYC/TB1–type members.

Multiple sequence alignment

To characterize the sequence features of TCP domain regions in SmTCP proteins, multiple sequence alignment was conducted using DNAMAN software on the basis of the amino acid sequence of each SmTCP conserved domain.
The alignment outcomes highlighted divergences and similarities among these SmTCP proteins. For instance, the intact bHLH structure can be observed in all Class II TCP members, thereby indicating the conservation of this group. In comparison, Class I TCP members performed a higher level of diversity. For instance, Smechr0101558.1 only had a helix-loop-helix (HLH) structure without a basic region among Class I members. Additionally, in the basic region, Class II members contained a 4-amino-acid insertion compared with Class I.

**Domains and gene structure analysis**

To better understand the diversity and similarity of SmTCPs, the conserved domains and exon–intron structure were analyzed according to the phylogenetic distribution of SmTCP genes from Arabidopsis, rice, eggplant and tomato. Visualization of TCP domains indicated that all SmTCPs consisted of 39–59 amino-acid residues, which was a conserved TCP domain feature.

Gene structure analysis can help reveal the evolutionary relationships underlying the genesis of the TCP family. Diverse SmTCP members were inclined to contribute to various structural organizations. As shown in Fig. 3, Class I genes had no introns, apart from Smechr0101558.1, Smechr0201168.1, Smechr1102385.1 and Smechr0100729.1; all of them had one intron. Compared with Class I SmTCP genes, Class II genes were relatively longer and contained more exons. Class II CIN-type TCP genes had 0–2 introns. Class II CYC/TB1-type TCP genes had one intron, with the exception of Smechr0602362.1, which had two introns.

**Motif distribution and promoter region structures of SmTCP family members**

The 30 SmTCP-predicted ORFs were subjected to MEME analysis to investigate the motif composition of the deduced
polypeptides. In total, 10 conserved motifs, named as motifs 1–10, were identified in Fig. 3 and Table S3. Motif 1 was annotated as a conserved bHLH structure, which was present in almost all SmTCPs, except Smechr0101558.1, demonstrating that it may be essential for SmTCPs to perform their functions. Motifs 2 and 6 appeared only in Class I, while motifs 4, 5, and 9 appeared only in Class II. Consequently, SmTCPs in the same class had a similar motif composition, whereas differences were observed in the two classes, indicating that SmTCPs in the same class may have similar functions, while some of the patterns may play a crucial role in particular functions.

To investigate the regulatory features of SmTCPs, the isolated 2.0-kb sequence upstream of the translational start site of SmTCPs was selected and assessed for the presence and constitutions of CAREs using the PlantCARE tool (Fig. 4, Table S4). The result indicated that well-characterized CAREs can be mainly categorized into three types: light-responsive, phytohormone-related, and abiotic stress.

Several types of phytohormone-related CAREs were well represented among SmTCP promoter regions, with ABA-, MeJA-, SA-, GA- and auxin-responsive elements identified widely in the promoter regions of eggplant SmTCPs, such as ABRE, TGACG-motif, TCA-element, GARE-motif, and AuxRE. As for the light-responsive element category, abundant CAREs were distributed throughout the promoter region, including I-box, AE-box, Box II, Box 4, MRE, GT1-motif, and G-box. Abiotic stress-related CAREs mainly had defense- and stress-responsive elements (e.g., TC-rich repeats), drought-inducibility elements (e.g., MBS), low-temperature-responsive elements (e.g., LTR), and wounding-responsive element (e.g., WUN-motif).

Prediction of putative miRNA–target interactions

The putative miRNAs targeting SmTCPs were forecasted to perform the perspective relationship between plant growth, development, and stress responses and miRNA...
regulation (Fig. 5). The outcomes indicated that a total of 21 SmTCP genes were targeted by 28 putative miRNAs, of which 10 SmTCPs were targeted by a single miRNA and 3 (Smchr0101558.1, Smchr0201631.1, and Smchr1200724.1) were targeted by double miRNAs. Moreover, Smchr0303405.1, Smchr0800708.1, 3 genes (Smchr0201631.1, Smchr0702127.1, and Smchr0800707.1), and 3 genes (Smchr0602388.1, Smchr1200724.1, and Smchr1200724.1) were targeted by double miRNAs. Moreover, Smchr0303405.1, Smchr0800708.1, 3 genes (Smchr0201631.1, Smchr0702127.1, and Smchr0800707.1), and 3 genes (Smchr0602388.1, Smchr1200724.1, and Smchr1200724.1) were targeted by double miRNAs.

Fig. 3 Schematic phylogenetic association, motif, domain, and exon-intron structure analysis of SmTCP genes in eggplant. A The unrooted phylogenetic tree was generated using the maximum likelihood method. B The conserved motifs were predicted using the MEME program. Ten motifs were displayed in different colored boxes. C The conserved TCP domains were identified using the batch CD-Search online tool and represented by pink and blue boxes. D Exon-intron structures of SmTCP genes: exons, untranslated regions (UTRs), and introns were represented by light-blue boxes, yellow boxes, and black lines, respectively (colour figure online).

Fig. 4 Analysis of CAREs in the promoter regions of SmTCP genes. A Distribution of CAREs in the promoter regions of SmTCPs. B Number of each CAREs in the promoter region (2.0-kb upstream of translational of start site) of SmTCPs were displayed in different colors (red: high, white: low) (colour figure online).
Smechr0702382.1, and Smechr1000441.1) were targeted by 3, 4, 5, and 6 miRNAs, respectively. Meanwhile, multiple miRNAs targeted only 1–3 SmTCP genes, whereas 3 miRNAs (miR319a, miR319b, and miR319c-3p) were able to target 7 SmTCPs, which may be critical for the regulation of plant growth, development and stress responses. These putative miRNA gene interaction networks of eggplant TCPs may provide significant insights into their functions and contribute to the determination of candidates for prospective investigations.

**Chromosomal localization and gene duplication event of SmTCP genes**

The 30 genes that encode SmTCPs were distributed unevenly over 12 chromosomes, except chromosome 5, ranging...
between 1 and 6 members on each chromosome (Fig. 6). The E2 contained the maximum number of SmTCP genes (20%, 6 genes), with most belonging to Class II CIN-type. The E4, E9, and E12 all shared the lowest number of SmTCP genes (3.3%, 1 gene). The rest of the chromosomes harbored 2–4 SmTCP genes.

Gene families may be generated by tandem duplication and segmental duplication during biological evolution (Lv et al. 2020). To explore whether the SmTCP gene family had a duplication-based expansion, we analyzed the duplication events of these genes (Fig. 6, Table S5). Smechr0800707.1 and Smechr0800708.1 were a pair of tandem duplicated genes, and their divergence might date approximately 10.88 million years ago. Thus, the duplication events played a major role in the expansion of the SmTCP gene family.

Evolutionary association of TCP genes among eggplant, Arabidopsis and tomato

The genomes of eggplant, tomato, and Arabidopsis were compared to determine SmTCP genes’ collinear relationship among various plant species (Fig. 7, Table S6). According to the results eggplant and Arabidopsis genomes had 5 (13.3%) syntenic gene pairs. Arabidopsis chromosomes occupied minor genes that had collinear relationships within SmTCPs in the eggplant genome with the exception for E1, E3, E4, E5, E6, E8, and E12 chromosomes. A total of 28 (93.3%) gene pairs of SmTCPs between eggplant and tomato were syntenic. Consequently, according to the results of this analysis, a strong collinear correlation was observed between eggplant and tomato. These outcomes suggest that eggplant...
and tomato exhibit compatible phylogenetic conservation of TCP genes.

**Expression profiles of eggplant TCP in different tissues**

Using qRT-PCR, an expression analysis was performed for 30 eggplant TCP genes in various eggplant organs, including root, stem, leaf, flower, peel, and septal (Fig. 8, Table S7). The results revealed that SmTCPs had distinct expression patterns in diverse organs, suggesting the divergent roles of SmTCPs in plant growth and development. Several Class II SmTCP genes were expressed at a high level in the leaf, flower, and septal. Class I SmTCP genes were mainly highly expressed in the root, stem, and peel. Examples for genes highly expressed in specific organs included gene (Smechr1102385.1) in the root, 4 genes (Smechr0103973.1, Smechr0202786.1, Smechr0303405.1, and Smechr1002163.1) in the stem, 9 genes (Smechr1001558.1, Smechr0400435.1, Smechr0602362.1, Smechr0602431.1, Smechr0702382.1, Smechr0800707.1, Smechr0800708.1, Smechr0900228.1, and Smechr1102037.1) in the leaf, 6 genes (Smechr0201023.1, Smechr0201631.1, Smechr0300562.1, Smechr0602388.1, Smechr0702127.1, and Smechr1000441.1) in the flower, 1 gene (Smechr0201168.1) in the peel, and 4 genes (Smechr0202855.1, Smechr0602003.1, Smechr0802294.1, and Smechr1000174.1) in the septal. Furthermore, some genes had the lowest expression level in different tissues, such as Smechr0203181.1 in the root, Smechr1200724.1 in the stem, Smechr0103973.1 in the leaf, Smechr0201168.1 in the flower, Smechr1002163.1 in the peel, and Smechr1102385.1 in the septal. Based on the aforementioned results, it was inferred that SmTCP genes may play a momentous role in plant developmental processes.

**Expression pattern of SmTCPs under diverse treatments**

To discern the possible roles of eggplant TCP genes in plant stress responses, the expression level of SmTCP genes in response to the 4 °C, PEG, and ABA was examined using qRT-PCR. All SmTCP genes responded to at least

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**Fig. 8** Expression profiles of SmTCPs in diverse tissues determined through qRT-PCR. The mean expression values were obtained from three replicates and visualized using TBtools software. The red color indicates a high expression level, and the green color represents a low expression level (colour figure online)
one type of treatment, and 21 genes responded to all treatments (Fig. 9).

In total, 25 genes (Smechr0100729.1, Smechr0103973.1, Smechr0201023.1, Smechr0201168.1, Smechr0201631.1, Smechr0202855.1, Smechr0203181.1, Smechr0303082.1, Smechr0303405.1, Smechr04000435.1, Smechr0602003.1, Smechr0602362.1, Smechr0602388.1, Smechr0602431.1, Smechr0702382.1, Smechr0800707.1, Smechr0802294.1, Smechr0900228.1, Smechr100174.1, Smechr1102037.1, Smechr1102385.1, and Smechr1200724.1) were downregulated. Interestingly, most of the upregulated genes belonged to Class I, whereas the downregulated genes mainly belonged to Class II. Furthermore, 9 of the upregulated genes were remarkably induced (> 15 fold) with PEG treatment.

Of the 24 genes induced by ABA treatment, namely Smechr0100729.1, Smechr0103973.1, Smechr0201023.1, Smechr0201168.1, Smechr0201631.1, Smechr0302978.1, Smechr0303082.1, Smechr0303405.1, Smechr04000435.1, Smechr0602003.1, Smechr0602362.1, Smechr0602388.1, Smechr0602431.1, Smechr0702382.1, Smechr0800707.1, Smechr0802294.1, Smechr0900228.1, Smechr100174.1, Smechr1102037.1, Smechr1102385.1, and Smechr1200724.1, 15 genes were upregulated, with more than half of them being Class II members, and 9 genes (Smechr0103973.1, Smechr0303082.1, Smechr0303405.1, Smechr0702127.1, Smechr0800708.1, Smechr0802294.1, Smechr0900228.1, Smechr1102037.1, Smechr1102385.1, and Smechr1200724.1) were downregulated. The transcript level of Smechr04000435.1 was greatly upregulated (> 350 fold) at 1 h.

A total 24 genes (Smechr0100729.1, Smechr0103973.1, Smechr0201023.1, Smechr0201168.1, Smechr0201631.1, Smechr0203181.1, Smechr0303082.1, Smechr0303405.1, Smechr04000435.1, Smechr0602003.1, Smechr0602362.1, Smechr0602388.1, Smechr0602431.1, Smechr0702127.1, Smechr0702382.1, Smechr0800708.1, Smechr0802294.1, Smechr0900228.1, Smechr1000174.1, Smechr1000441.1, Smechr1102037.1, Smechr1102385.1, and Smechr1200724.1) were induced by PEG treatment, of them, the transcript levels of 13 genes were upregulated, and the remaining genes (Smechr0103973.1, Smechr0201023.1, Smechr0201631.1, Smechr0303082.1, Smechr0303405.1, Smechr0602431.1, Smechr0702127.1, Smechr0802294.1, Smechr100174.1, Smechr1102385.1, and Smechr1200724.1) were downregulated. The transcript level of Smechr0602362.1 was significantly upregulated (> 15 fold) with PEG treatment.

Of the 28 SmTCP genes, 19 genes (Smechr0100729.1, Smechr0103973.1, Smechr0201023.1, Smechr0303082.1, Smechr0303405.1, Smechr04000435.1, Smechr0602003.1, Smechr0602362.1, Smechr0602388.1, Smechr0602431.1, Smechr0702382.1, Smechr0800708.1, Smechr0802294.1, Smechr0900228.1, Smechr1000174.1, Smechr1102037.1, Smechr1102385.1, and Smechr1200724.1) were mediated by all three treatments, and 6 genes (Smechr0201631.1, Smechr0203181.1, Smechr0702127.1, Smechr0800707.1, Smechr1000174.1, and Smechr1000441.1) were induced by two treatments. The remaining 3 genes (Smechr0101558.1, Smechr0202855.1, and Smechr0302978.1) were activated by one treatment. Taken together, the expression profiles indicated that SmTCP genes might be involved in abiotic stress, especially under the 4 °C and ABA stress conditions.

Smechr0202855.1 and Smechr0602431.1 could involve in the regulation of anthocyanin synthesis

According to our previous investigations of short-term transcriptomes, we were inclined to select 3 SmTCP differentially expressed genes (Smechr0201023.1, Smechr0202855.1, and Smechr0602431.1) to determine their engagement in the regulation of anthocyanin synthesis (Figure S1). Moreover, comparative analysis of RNA-seq and qRT-PCR showed that there is a relatively consistent trend of expression between them (Figure S3). The subcellular localization outcomes of the 3 SmTCPs indicated that they were located in the nucleus, whereas the empty vector was located both in the nucleus and cell membrane, which was in accordance with the predicted outcomes (Fig. S2). Moreover, Y1H and Dual-LUC assays were utilized to gauge the binding of 3 transcription factors to structural genes promoters related to anthocyanin synthesis, and activation of gene expression. The results demonstrated that Smechr0202855.1 and Smechr0602431.1 could directly bind to the SmCHS promoter and activate its expression, whereas Smechr0201023.1 could not (Fig. 10).

Discussion

The TCP gene family is a group of plant-specific transcription factors that play pivotal and multiples roles in plant growth, development, and stress responses. As the genomes of plant species have recently been sequenced extensively, genome-wide identification of the TCP gene family has been conducted for numerous plants, including monocotyledons such as rice (Yao et al. 2007), maize (Chai et al. 2017), and sorghum (Francis et al. 2016), and dicotyledons, such as Arabidopsis (Danisman et al. 2012), tomato (Parapunova et al. 2014), and potato (Bao et al. 2019). However, only few
Fig. 9 Expression pattern analysis of SmTCP genes in response to diverse treatments (4 °C, ABA, and PEG) based on qRT-PCR. Relative gene expression levels in leaves were determined at 0, 1, 3, 6, 12, and 24 h under different stress conditions. Error bars indicate the standard deviations from three independent biological replicates. Asterisks indicate a significant difference in transcript abundance between diverse treatments and the control (*p<0.05, and, **p<0.01)
Fig. 10  Presentation of yeast one-hybrid (Y1H) and Dual-LUC assays. A A presentation of Y1H results. Blue precipitates show the corresponding beta-galactosidase activity. B A presentation of Dual-LUC results. Error bars represent the standard error of five biological replicates. Asterisks indicate a statistically dramatic difference in relative LUC activity between SmTCPs and the vector (*p < 0.05 and **p < 0.01)

studies have investigated eggplant TCP genes. In this study, a comprehensive query for genes encoding for SmTCP transcription factors against the eggplant genome was performed, which led to the characterization of 30 members, of which 13 members pertain to Class I, and 17 to Class II (Table S2). Studies have shown that most plants harbored a relatively consistent number of TCP genes between monocotyledons and dicotyledons, for example, rice 22, maize 29, sorghum 20, Arabidopsis 24, tomato 30 and potato 23. Interestingly, the genomic size of these plants diverged enormously, that is, 389 Mb for rice (Matsumoto et al. 2005), 2.25 Gb in maize (Schnable et al. 2009), 730 Mb in sorghum (Paterson et al. 2009), 125 Mb in Arabidopsis (Kaul et al. 2000), 900 Mb in tomato (Sato et al. 2012), 844 Mb in potato (Xu et al. 2011), and 1.07 Gb in eggplant (Wei et al. 2016). Based on the aforementioned results, these plants can be inferred to comprise nearly the identical number of TCP family members, irrelevant of their genomic size. Furthermore, comparative analysis reveals that the structure, evolution, and function of the TCP gene family is relatively conserved among monocot-dicot plants.

Domain investigation and structural assembly of the SmTCP gene family revealed that all SmTCP proteins contained a typical TCP domain with the exception for Smecn0101558.1, which only contained a partial TCP domain (HLH structure). Most genes belonging to Class I appeared to be intronless, while Class II members had 0–2 introns (Fig. 3). The structural assembly of TCP members in eggplant is comparable to that of TCP members in Vitis vinifera (Leng et al. 2019b; Jiu et al. 2019a) and Broussonetia papyrifera (Zhao et al. 2020). This demonstrates genomic divergence during evolution, as evidenced by discrepancies in the structural assembly between Class I and Class II.

Customarily, conserved motifs in TFs are critical for their protein–protein interactions, transcriptional activity, and DNA-binding activity. In this investigation, the analysis of motifs illustrated that motif 1 was predominantly implicated in the TCP domain in eggplant, whereas motifs 6–7 were specifically associated with the Class I subfamily. Motifs 5 and 8–10 were exclusively related to the Class II (CIN) subfamily. In addition, motif 2 was only present in the Class I subfamily, indicating its pivotal role in this subfamily. Collectively, these outcomes connote that although multiple motifs in the TCP gene family are highly conserved, the newly evolved motifs may be related to neo-functions in plants, which need to be delved into.

Analysis of subcellular localization clarified that 29 of the 30 SmTCP genes of eggplant were localized in the nucleus, and the remaining one was localized in the chloroplast (Fig. S2, Table S2). This outcome was in accordance with those of the previous investigations, which documented the localization of TCP transcription factors preferentially in the nucleus (Wei et al. 2016; Leng et al. 2019b).

Accumulating studies have shown that miRNAs play critical roles in plant growth and development, hormone metabolism as well as biotic and abiotic stresses by targeting specific genes, such as miRNA319-AtTCPs mediating leaf morphogenesis in Arabidopsis (Palatnik et al. 2003; Bresso et al. 2018), miRNA828-SIMyb7-like inhabiting anthocyanin biosynthesis in Arabidopsis (Gou et al. 2011), miRNA319-AsTCPs conferring its tolerance to drought and salinity in creeping bentgrass (Zhou and Hong 2014), and miRNA319-PvPCF5 enhancing ethylene accumulation and salt tolerance in switchgrass (Liu et al. 2019). In this study, the miRNA-targeted SmTCPs were predicted and 21 SmTCPs were modulated by 28 miRNAs, of which 11 SmTCPs were targeted by multiple miRNAs (Fig. 5), indicating that SmTCPs might be involved in the sophisticated regulatory network. The miRNA–SmTCP interaction will contribute to further investigation of the putative roles of miRNA–SmTCP in eggplant growth and development as well as stress responses.

The collinearity block analysis regarding the evolutionary associations of SmTCP family genes in various plant species (Arabidopsis, tomato, and eggplant) revealed that the SmTCP genes were strongly homologous to SITCP genes in tomato and less homologous to AtTCP genes in Arabidopsis (Fig. 7). Such results were consistent with the results of the phylogenetic analysis among Arabidopsis, tomato, and eggplant. Furthermore, the ortholog between SmTCP and SITCP genes was investigated. The results
indicated the collinearity between eggplant chromosomes 1, 2, 3, 4, 6, 7, 8, 9, 10, and 11 and tomato chromosomes 1, 2, 3/6, 10, 3/6, 7, 8, 9, 4/5/12, and 5, which suggested that the TCP gene family in eggplant and tomato might have evolved in a similar pattern and derived from a shared progenitor.

Gene expression profiles commonly reflect significant reference information concerning their functions (Pontes et al. 2013). As shown in Fig. 8, gene expression pattern analysis revealed that the expression of these SmTCP genes is omnipresent and varied greatly, and their transcriptional levels in leaf, flower and septal were comparatively higher than those in other organs. Moreover, some genes exhibited preferential expression in a specific tissue, such as Smechr1102385.1 for root, Smechr202786 for stem, Smechr0602362.1 for leaf, Smechr0300562.1 for flower, and Smechr0602003.1 for septal. The divergent tissue expression profile of eggplant TCPs might be attributed to their functional discrepancies.

Numerous studies have evidenced that TCP TFs play a significant role in response to multiple stresses, including phytohormones, biotic stress, and abiotic stress (Danisman 2016; Feng et al. 2018; Bao et al. 2019; Jiu et al. 2019b). The stress-related CARE analysis and qRT-PCR outcomes suggested that SmTCPs potentially function during plant growth, development and stress responses (Fig. 9). For example, 3 SmTCPs (Smechr0103973.1, Smechr0202855.1 and Smechr0800707.1), 9 SmTCPs (Smechr0103973.1, Smechr0303082.1, Smechr0303405.1, Smechr0702127.1, Smechr0800708.1, Smechr0900228.1, Smechr1102037.1, Smechr1102385.1 and Smechr1200724.1), and 11 SmTCPs (Smechr0103973.1, Smechr0201023.1, Smechr0201631.1, Smechr0303082.1, Smechr0303405.1, Smechr0602431.1, Smechr0702127.1, Smechr0800294.1, Smechr1002163.1, Smechr1102385.1, and Smechr1200724.1) appeared to be suppressed by low-temperature, ABA and drought stresses, respectively, owing to the presence of corresponding CAREs in their promoter regions. Additionally, 19 out of 28 SmTCP genes were induced by low-temperature, ABA, and drought treatments because their promoter regions contained multiple stress-related CAREs. Further integrated investigations of expression profiles and CARE characteristics also verified that low-temperature, ABA, and drought stresses might markedly induce SmTCP expression. Among them, activation of 3 SmTCPs (Smechr0101558.1, Smechr0202855.1, and Smechr0302978.1) was induced by particular stress. Distinct SmTCPs displayed diverse transcriptional profiles, activation, or suppression, signifying that they shared varied physiological functions. Taken together, SmTCP genes are believed to participate in different stress responses, which requires the full use of potential tools to further explore and evaluate the functional role of TCPs in plants.

Conclusions
This is a pioneering systematic identification of the eggplant TCP gene family to illuminate their evolution, expression patterns, and plausible functions. The study outcomes will help to unveil the functions of TCP genes engaged in plant growth and development as well as stress responses. The putative miRNA-SmTCP regulatory associations provide the referenced cues for the miRNA-mediated gene regulation. Additionally, in light of RNA-seq data, Y1H and Dual-LUC analysis, two candidate SmTCP members are probably involved in anthocyanin synthesis. In summary, our outcomes will lay out a solid foundation for gene selection, further functional characterization of SmTCPs in eggplant growth and development, as well as stress-related signal transduction.

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Author contributions HYC, HYG and YL provided the experimental materials. DLL conceived the experiments and wrote the manuscript. HYC, HYG and DLL revised the manuscript. DLL, XT, YXD, YYW, SLS, SHL performed the experiments. All authors approved the manuscript.

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Declarations
Conflict of interest The authors declare that they have no conflict of interest related to the contents of this article.

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