Genome-wide analysis of citrus TCP transcription factors and their responses to abiotic stresses

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Abstract
Background: Citrus is one of the most important fruit crops in the world, and it is worthy to conduct more research on artificially controlling citrus plant growth and development to adapt to different cultivation patterns and environmental conditions. The plant-specific TEOSINTE BRANCHED1, CYCOLOIDEA, and PROLIFERATING CELL FACTORS (TCP) transcription factors are crucial regulators controlling plant growth and development, as well as responding to abiotic stresses. However, the information about citrus TCP transcription factors remains unclear.

Results: In this study, twenty putative TCP genes (CsTCPs) with the TCP domain were explored from Citrus sinensis genome, of which eleven (CsTCP3, −4, −5, −6, −10, −11, −15, −16, −18, −19, −20), five (CsTCP1, −2, −7, −9, −13), and four genes (CsTCP8, −12, −14, −17) were unevenly distributed on chromosomes and divided into three subclades. cis-acting element analysis indicated that most CsTCPs contained many phytohormone- and environment-responsive elements in promoter regions. All of CsTCPs were predominantly expressed in vegetative tissues or organs (stem, leaf, thorn, and bud) instead of reproductive tissues or organs (flower, fruit, and seed). Combined with collinearity analysis, CsTCP3, CsTCP9, and CsTCP13 may take part in leaf development; CsTCP12 and CsTCP14 may function in shoot branching, leaf development, or thorn development; CsTCP15 may participate in the development of stem, leaf, or thorn. In mature leaf, transcript levels of two CsTCPs (CsTCP19, −20) were significantly increased while transcript levels of eight CsTCPs (CsTCP2, −5, −6, −7, −8, −9, −10, −13) were significantly decreased by shading; except for two CsTCPs (CsTCP11, −19), CsTCPs' transcript levels were significantly influenced by low temperature; moreover, transcript levels of two CsTCPs (CsTCP11, −12) were significantly increased while five CsTCPs' transcript levels were significantly reduced by drought.

Conclusions: This study provides significant clues for research on roles of CsTCPs in regulating citrus plant growth and development, as well as responding to abiotic stresses.

Keywords: TCP family, Citrus sinensis, Abiotic stress, Genome-wide analysis, Expression pattern

Background
Transcription factors are proteins that play a pivotal role in plant growth and development by binding to promoter or enhancer regions of specific genes [1]. TCP family, a plant-specific transcription factor family, is originally named from the first four family members, TEOSINTE BRANCHED1 (TB1) in Zea mays, CYCOLOIDEA (CYC) in Antirrhinum majus, PROLIFERATING CELL FACTORS 1 and 2 (PCF1 and PCF2) in Oryza sativa, which
contains a highly conserved non-canonical basic helix-loop-helix motif designated as the TCP domain with about 59 amino acids at the N-terminus; TCP domain is involved in nuclear targeting, DNA binding, and pairwise protein-protein interaction [2].

TCP family exists widely in plants. There are five to six members in pluricellular green algae, mosses, ferns, and lycophytes [3, 4], and over ten members in angiosperms [2]. They are generally divided into two subfamilies, CLASS I (PCF or TCP-P subclass) specifically binding to GGNCCAC, and CLASS II (TCP-C subclass) specifically binding to G(T/C)GGNCC, based on the homology and variation of TCP domain [5]. Moreover, CLASS II subfamily can be further divided into CINNINATAC (CIN) and CYC/TB1 subclades [6]; several TCP members in CLASS II subfamily contain another conserved region named as the R domain, which is an arginine-rich motif and includes polar residues with hypothetical functions related to protein-protein interaction by forming a coiled coil [7].

As plant-specific transcription factors, TCP members, especially CLASS II subfamily members, play a crucial role in plant growth and development, such as plant height regulation [8, 9], lateral bud outgrowth [10, 11], thorn conversion [12], leaf morphogenesis [13, 14], trichome formation [15, 16], floral asymmetry [17, 18], pollen development [19, 20], embryo growth [21], seed germination [22, 23], and circadian rhythm [24]. On the other hand, TCP members can be regulated by endogenous signals such as phytohormones [25], and can also respond to exogenous factors such as abiotic stresses [26]. Moreover, previous studies indicated that some CIN subclade members can be mediated by miR319 [27, 28].

As one of the most important fruit crops in the world, citrus produces fruits supplying not only different and vital nutrition for human health, but also tremendously delicious flavor for consumers [29]. With the update of labor-saving cultivation pattern, it is necessary to conduct more research on artificially controlling citrus plant growth and development. As mentioned above, some TCP members play a key role in regulation of plant height, lateral bud outgrowth, and leaf development. However, the information about citrus TCP genes is scarce, although complete genome sequence data of many citrus cultivars are released [30, 31]. In this study, a total of twenty putative TCP genes were explored from Citrus sinensis genome. The basic characteristics, gene duplication, phylogenetic relationships, cis-acting elements, gene ontology (GO) annotations, and protein-protein interaction were systematically analyzed for such TCP genes. In addition, their expression patterns were investigated in different tissues or organs as well as samples treated with shading, low temperature, and drought. Specially, CsTCP3, CsTCP9, and CsTCP13 may function in leaf development; CsTCP12 and CsTCP14 may participate in the regulation of shoot branching, leaf development, or thorn development, respectively; CsTCP15 may act as a regulator in the development of stem, leaf, or thorn; they possibly take part in the response of leaf to shading, low temperature, or drought, respectively. Overall, the present results suggested possible roles of some TCP genes and provided background information for further research on the specific role and mechanism of each TCP gene in citrus plant growth and development, as well as in response to abiotic stresses.

Results
Identification and basic characteristics of Citrus sinensis TCP genes
A total of twenty putative TCP genes were screened out from Citrus sinensis genome and named as CsTCP1-20 according to their distribution order on chromosomes (Table 1). It was found that CsTCPs were unevenly distributed on chromosomes. In detail, chromosome 7 contained five CsTCPs, chromosomes 2 and 5 contained three CsTCPs, chromosomes 6, 8, and 9 contained two CsTCPs, chromosome 3 contained one CsTCP, and chromosomes 1 and 4 contained no CsTCP. However, the location of CsTCP19 and CsTCP20 on chromosomes was unclear. On the other hand, the number of amino acids (aa), molecular weight (Mw), and theoretical isoelectric point (pl) varied among CsTCPs (Table 1). The number of amino acids of CsTCPs was between 174 and 577. In detail, five CsTCPs contained less than 300 aa, thirteen CsTCPs contained 300 to 500 aa, and two CsTCPs contained more than 500 aa. Accordingly, their Mw varied largely either, from 19,507.20 Da (Da) to 61,307.81 Da. As for theoretical pl, it was between 4.44 and 9.47. The theoretical pl of eight CsTCPs was lower than 7, seven CsTCPs were from 7 to 9, and five CsTCPs were higher than 9. Moreover, no signal peptide was found in all CsTCP protein sequences, and the predicted subcellular localization was in nucleus (Table 1).

In addition, these twenty TCP members could be divided into three subclades based on the homology and variation of their protein sequences by aligning with the protein sequences of Arabidopsis thaliana TCP (AtTCP) and Solanum lycopersicum TCP (SitTCP) transcription factors; eleven (CsTCP3, −4, −5, −6, −10, −11, −15, −16, −18, −19, −20), five (CsTCP1, −2, −7, −9, −13), and four members (CsTCP8, −12, −14, −17) belonged to subclades PCF, CIN, and CYC/TB1, respectively (Fig. 1A). All the sequences contained the TCP domain by further aligning with AtTCP protein sequences (Fig. 1B). In BASIC region, Asp (D), His (H), Lys (K), and Arg (R) amino acid residues were completely
conserved in the sequences of all members; the sequence of each PCF subclade member possessed a four-amino-acid deletion. In HELIX I and HELIX II regions, Leu (L) and Trp (W), two hydrophobic amino acid residues were fully conserved. In LOOP region, Gly (G), a hydrophilic amino acid residue was highly conserved. Moreover, the R domain with relatively conserved Ala (A) and Arg (R) amino acid residues was found in the sequences of one CIN subclade member (CsTCP1) and all CYC/TB1 subclade members (Fig. 1C); the putative miR319-binding sites were only found in the sequences of three CIN-type genes (CsTCP1, − 2, − 13) (Fig. 1D).

**Table 1** Basic characteristics of *Citrus sinensis* TCP genes

| Gene name | Gene ID    | Chromosome location | Strand | Length of CDS (bp) | Number of amino acids | Molecular weight (Da) | Theoretical pI | Signal peptide length (aa) | Predicted subcellular localization |
|-----------|------------|---------------------|--------|-------------------|-----------------------|-----------------------|----------------|---------------------------|-----------------------------------|
| CsTCP1    | Cs2g08080.1| chr2: 4868829..4853255 | −      | 1515              | 504                   | 55,291.28             | 7.16           | −                         | Nuclear                           |
| CsTCP2    | Cs2g15820.1| chr2: 12548474..12550882 | +      | 1005              | 334                   | 36,343.85             | 6.53           | −                         | Nuclear                           |
| CsTCP3    | Cs2g25640.1| chr2: 24866364..24868750 | +      | 954               | 317                   | 33,912.73             | 9.01           | −                         | Nuclear                           |
| CsTCP4    | Cs3g22260.1| chr3: 24926039..24927954 | −      | 963               | 320                   | 33,181.31             | 7.79           | −                         | Nuclear                           |
| CsTCP5    | Cs5g03980.1| chr5: 2195508..21974785 | +      | 1281              | 426                   | 46,428.83             | 7.10           | −                         | Nuclear                           |
| CsTCP6    | Cs5g10130.1| chr5: 6994168..6996231 | −      | 1107              | 368                   | 39,316.14             | 6.72           | −                         | Nuclear                           |
| CsTCP7    | Cs5g12070.1| chr5: 9052963..9054997 | +      | 1098              | 365                   | 40,856.09             | 6.74           | −                         | Nuclear                           |
| CsTCP8    | Cs6g18940.1| chr6: 18950739..18952107 | +      | 897               | 298                   | 34,061.36             | 9.38           | −                         | Nuclear                           |
| CsTCP9    | Cs6g22270.1| chr6: 2107808..21080763 | +      | 1203              | 400                   | 43,616.64             | 8.88           | −                         | Nuclear                           |
| CsTCP10   | Cs7g03980.1| chr7: 1887520..1889541 | +      | 1185              | 394                   | 42,105.60             | 7.78           | −                         | Nuclear                           |
| CsTCP11   | Cs7g11120.1| chr7: 7320850..7323133 | +      | 825               | 274                   | 29,286.77             | 9.44           | −                         | Nuclear                           |
| CsTCP12   | Cs7g12770.1| chr7: 8725990..8727399 | −      | 1410              | 469                   | 52,553.18             | 6.62           | −                         | Nuclear                           |
| CsTCP13   | Cs7g25460.1| chr7: 25950030..25952883 | +     | 1281              | 426                   | 46,861.16             | 6.78           | −                         | Nuclear                           |
| CsTCP14   | Cs7g26250.1| chr7: 26806458..26809706 | −     | 1095              | 364                   | 41,735.96             | 6.29           | −                         | Nuclear                           |
| CsTCP15   | Cs8g16060.1| chr8: 19154530..19155054 | +     | 525               | 174                   | 19,507.20             | 9.03           | −                         | Nuclear                           |
| CsTCP16   | Cs8g16080.1| chr8: 19169920..19171017 | +     | 675               | 224                   | 24,176.45             | 4.44           | −                         | Nuclear                           |
| CsTCP17   | Cs9g12640.1| chr9: 11059738..11061409 | +     | 957               | 318                   | 36,575.30             | 9.47           | −                         | Nuclear                           |
| CsTCP18   | Cs9g16600.1| chr9: 16080000..16081746 | −      | 1017              | 338                   | 35,966.34             | 8.84           | −                         | Nuclear                           |
| CsTCP19   | orange1.1:0.24281.1| chrUn: 36970387..36971007 | +     | 621               | 206                   | 21,666.35             | 7.00           | −                         | Nuclear                           |
| CsTCP20   | orange1.1:0.3861.1| chrUn: 59999994..60002615 | +     | 1734              | 577                   | 61,307.81             | 6.70           | −                         | Nuclear                           |

*aa* amino acid(s), *CDS* Coding sequence(s), *Da* Dalton(s), *pI* Isoelectric point

**Sequence features and protein-protein interaction of *Citrus sinensis* TCP members**

Some conserved motifs were found in each CsTCP protein sequence, and their type or quantity varied greatly among subclades or members; gene structure, namely, the component and size of exon and intron was also different from each other (Fig. S1). Notably, thirty-four special cis-acting elements related to phytohormones and environmental signals were found in 2kb promoter regions of twenty CsTCPS. However, each gene owned the different profile of cis-acting elements, and the number of cis-acting elements in the promoter region of
each gene ranged from ten to thirty (Fig. 2A). Specially, CsTCP19 contained 30 cis-acting elements, including ten methyl jasmonate (MeJA)-responsive elements, ten light-responsive elements, five abscisic acid (ABA)-responsive elements, three salicylic acid (SA)-responsive elements, one drought-responsive element, and one anaerobism-responsive element; whereas CsTCP16 contained ten cis-acting elements, including four MeJA-responsive elements, three light-responsive elements, two anaerobism-responsive elements, and one drought-responsive element.

In addition, the interaction relationships among twenty CsTCPs were complex and sixteen members interacted with each other (Fig. 2B). Of them, CsTCP13 interacted with nine members; CsTCP1 and CsTCP5 interacted with eight members; CsTCP2, CsTCP10, CsTCP18, and CsTCP20 interacted with seven members; CsTCP6, CsTCP7, and CsTCP9 interacted with six members; CsTCP4 interacted with five members; CsTCP3 and CsTCP11 interacted with four members; CsTCP12 and CsTCP14 interacted with three members; CsTCP19 interacted with two members. Moreover, the highest interaction score (0.861) was found between CsTCP6 and CsTCP13.

Collinearity analysis and GO annotation of Citrus sinensis TCP genes
A total of eleven CsTCPs belonging to paralogs were distributed on chromosomes 2, 5, 6, 7, and 9, respectively (Fig. 3A). Of them, CsTCP3 and CsTCP14, CsTCP5 and CsTCP10, CsTCP6 and CsTCP18, CsTCP7 and CsTCP9, CsTCP8 and CsTCP12, as well as CsTCP12 and CsTCP14 were paralogous genes, respectively. Moreover, the Ka (nonsynonymous substitution rate)/Ks (synonymous substitution rate) ratio of each pair was lower than 0.3 (Table S1). On the other hand, large-scale orthologous TCP genes including sixteen CsTCPs and eighteen AtTCPs were found between Citrus sinensis and Arabidopsis thaliana genomes (Fig. 3B). Specially, a sole orthologous relationship could be found in eight pairs of TCP genes, including CsTCP3 and AtTCP20, CsTCP8 and AtTCP11, CsTCP9 and AtTCP13, CsTCP11 and AtTCP15, CsTCP12
and AtTCP12, CsTCP13 and AtTCP4, CsTCP14 and AtTCP18, as well as CsTCP15 and AtTCP11.

In addition, a total of twelve CsTCPs (CsTCP1, −2, −3, −4, −5, −8, −13, −14, −17, −18, −19, −20) were annotated (Table S2). They could be classified into three classes, including molecular function, cellular component, and biological process (Fig. 3C). Of them, twelve CsTCPs were involved in transcription regulator activity, regulation of biological process, biological regulation, cellular process, and metabolic process; nine CsTCPs (CsTCP1, −2, −3, −4, −5, −8, −13, −14, −17) were related to developmental process; four CsTCPs (CsTCP1, −5, −13, −18) were involved in response to stimulus; two CsTCPs (CsTCP1, −5) were related to cell proliferation; one CsTCP (CsTCP19) was involved in rhythmic process.

Spatio-temporal expression analysis of Citrus sinensis TCP genes

In different tissues or organs of Poncirus trifoliata, including root, stem, leaf, thorn, bud, flower, peel, juice sac, and seed (Fig. 4A), twenty CsTCPs exhibited multifarious spatio-temporal expression profiles. Most genes were highly expressed in stem, leaf, thorn, and bud, but were lowly expressed in root, flower, peel, juice sac, and seed (Fig. 4B). In detail, ten CsTCPs (CsTCP1, −2, −3, −4, −6, −7, −9, −11, −12, −13), six CsTCPs (CsTCP5, −8, −10, −15, −16, −17), three CsTCPs (CsTCP14, −18, −19), and one CsTCP (CsTCP20) showed the highest expression level in leaf, stem, thorn, and bud, respectively. On the other hand, eight CsTCPs (CsTCP3, −9, −11, −14, −15, −17, −19, −20), six CsTCPs (CsTCPs, −6, −7, −8, −16, −18), three CsTCPs (CsTCP1, −2, −13), two CsTCPs (CsTCP4, −10), and one CsTCP (CsTCP12) were expressed with the lowest level in flower, juice sac, root, seed, and peel, respectively. Moreover, CsTCPs from the same subclade also exhibited different expression profiles (Fig. 4B). For example, in CIN subclade, the expression level of CsTCP1 in seed was similar to that in middle stem, while expression levels of the other genes in seed were lower than those in middle stem; on the other hand, expression levels of CsTCP2 and CsTCP9 were relatively high in peel and root, respectively, whereas expression levels of the other genes were relatively low in these two tissues or organs. In addition, with the development of some tissues or organs, some genes presented different expression trends (Fig. 4B). Expression levels of five CsTCPs (CsTCP3, −5,
Fig. 3 Collinearity analysis and gene ontology (GO) annotation of CsTCPs. A Paralogous relationships between CsTCPs. The twenty TCP genes were mapped on the chromosomes. Paralogs are shown in red and connected with red lines. Orange bands represent Citrus sinensis chromosomes. Tick labels represent chromosome length (Mb). B Orthologous relationships between CsTCPs and AtTCPs. The forty-four TCP genes were mapped on the chromosomes. Orthologs between two species are shown in red and connected with red lines. Orange and green bands represent Citrus sinensis and Arabidopsis thaliana chromosomes, respectively. Tick labels represent chromosome length (Mb). C GO annotations of CsTCPs. The sequences of twenty TCP proteins were submitted to EGGNOG-Mapper to perform GO annotation which was visualized by WEGO v.2.0. Blue, green, and red bars represent GO terms of molecular function, cellular component, and biological process, respectively.
Expression profiles of *CsTCPs* in different tissues or organs. A: Illustration of tissues or organs from *Poncirus trifoliata*. PR: primary root; LR, lateral root; TS: tip stem; MS, middle stem; BS: base stem; YL: young leaf; ML: mature leaf; OL: old leaf; YT: young thorn; OT: old thorn; YB: young bud; OB: old bud; FL, flower; PE, peel; JS, juice sac; SE, seed. Scale bars represent 5 cm. B: Heat map of expression patterns. The results were the mean of three independent biological replicates with quantitative real-time PCR (qRT-PCR) technology and transformed by log2 fold change. Color scale represents relative expression level. Red represents high expression level, and blue represents low expression level.

Expression patterns of *Citrus sinensis* TCP genes responding to shading, low temperature, and drought

Shading significantly decreased the light intensity in the canopy (Fig. S2A), and influenced expression levels of ten *CsTCPs* in mature leaf of *Citrus reticulata* cv. Kinokuni (Fig. 5A). Of them, expression levels of two *CsTCPs* (*CsTCP9, −20*) were significantly increased and were about 31.0- and 11.7-fold higher than those in the control, respectively; on the contrary, expression levels of eight *CsTCPs* (*CsTCP2, −5, −6, −7, −8, −9, −10, −13*) were significantly decreased and were about 0.4, 0.5, 0.5, 0.4, 0.4, 0.3, 0.6, and 0.4 times of those in the control, respectively. On the other hand, expression levels of four *CsTCPs* (*CsTCP3, −4, −11, −12*) were down-regulated without significance (Fig. 5A).

Moreover, transcript levels of most *CsTCPs* could be obviously influenced by low temperature (Fig. 5B). Specifically, transcript levels of six *CsTCPs* were reduced at 2 h after 5 °C treatment and then were increased at 6 h after 5 °C treatment. Of them, expression levels of five *CsTCPs* (*CsTCP1, −3, −4, −7, −12*) were significantly fluctuated along with the extension of 5 °C treatment; the expression level of one *CsTCP* (*CsTCP9*) was just significantly increased at 6 h after 5 °C treatment. However, transcript levels of eight *CsTCPs* (*CsTCP5, −8, −11, −13, −15, −16, −17, −20*) presented contrary expression trends, namely, were increased at 2 h after 5 °C treatment and then were decreased at 6 h after 5 °C treatment. Of them, expression levels of six *CsTCPs* (*CsTCP5, −8, −13, −15, −17, −20*) were significantly fluctuated along with the extension of 5 °C treatment; the expression level of one *CsTCP* (*CsTCP16*) was just significantly decreased at 6 h after 5 °C treatment. On the other hand, transcript levels of six *CsTCPs* (*CsTCP2, −6, −10, −14, −18, −19*) were up-regulated after 5 °C treatments. Of them, expression levels of three *CsTCPs* (*CsTCP2, −6, −14*) were
significantly increased after 5 °C treatments; the expression level of one CsTCP (CsTCP10) was significantly increased at 2 h after 5 °C treatment and then was almost kept stable at 6 h after 5 °C treatment; the expression level of one CsTCP (CsTCP18) was just significantly increased at 6 h after 5 °C treatment.

In addition, the effect of drought on expression patterns of twenty CsTCPs were also investigated (Fig. 5C). After drought treatment, the soil water content was significantly decreased and the proline content in mature leaf was significantly increased (Fig. S2B, C); transcript levels of seven CsTCPs were also significantly influenced. Of them, expression levels of two CsTCPs (CsTCP11, −12)
exhibited significant increase and were about 1.4- and 7.3-fold higher than those in the control, respectively; by contrast, expression levels of five CsTCPs (CsTCP14, −16, −18, −19, −20) exhibited significant decrease and were about 0.6, 0.2, 0.4, 0.4, and 0.2 times of those in the control, respectively. Moreover, expression levels of two CsTCPs (CsTCP15, −17) were reduced to less than half of those in the control (Fig. 5C).

Discussion
Plant-specific TCP family, widely distributed in plants but with different numbers [3, 4, 11, 32], is well known as a group of transcription factors to regulate plant growth and development; its members contain a highly conserved TCP domain with about 59 amino acids at the N-terminus [2]. Moreover, TCP members are generally divided into three groups, including PCF, CIN, and CYC/TB1 subclades, based on the homology and variation of TCP domain [5, 6]. In this study, a total of twenty putative TCP members with the TCP domain were explored from Citrus sinensis genome by systematical alignment and screening (Table 1, Fig. 1B); they were divided into three subclades, of which eleven, five, and four members belonged to subclades PCF, CIN, and CYC/TB1, respectively (Fig. 1A, B). These results suggested that there are at least twenty TCP genes in Citrus sinensis genome. In general, duplicated genes are considered to be paralogs forming a gene family, and they are thought to provide raw materials for the generation of new genes, which can facilitate the generation of new functions in turn [33]. As found in twenty CsTCPs, six pairs of paralogous genes, including CsTCP3 and CsTCP11, CsTCP5 and CsTCP10, CsTCP6 and CsTCP18, CsTCP7 and CsTCP9, CsTCP8 and CsTCP12, as well as CsTCP12 and CsTCP14 (Fig. 3A), suggested that the segmental duplication may contribute to the amplification of TCP gene family in Citrus sinensis genome. In addition, the Ka/Ks ratio of each pair was far lower than 1 (Table S1). Based on the viewpoint of the previous study [34], the purifying selection may play a major role in the evolution of CsTCPs.

To date, TCP genes have been reported to function largely in many aspects of plant growth and development [2]. Gene expression is a biological process by which the genetic information in DNA is converted to mRNA and then translated to protein, namely, gene function is eventually performed in form of protein [35]. Herein, physical and chemical properties of proteins, gene structure, and motif composition of Citrus sinensis TCP members were different even in the same subclade (Table 1, Fig. S1), suggesting that twenty CsTCPs may have different functions. The functions of some CsTCPs were discussed in the following.

CsTCP3, a PCF-type gene, was reeled to AtTCP20 in phylogenetic relationships and was the ortholog of AtTCP20 (Fig. 1A, Fig. 3B). Previous study indicated that AtTCP20 can regulate leaf development via the jasmonate signalling pathway, especially during early leaf developmental stages in Arabidopsis thaliana [13]. Given the viewpoint that collinear genes in relative species contain a lot of homologous functions [36], CsTCP3 might have functions similar to AtTCP20. Indeed, the highest transcript level of CsTCP3 was observed in leaf and two MeJA-responsive elements were found in its promoter region (Fig. 2A, Fig. 4B). These results suggested that CsTCP3 may also participate in leaf development. Moreover, CsTCP15 was another PCF subclade member and was orthologous to AtTCP11 (Fig. 1A, Fig. 3B). Previous study demonstrated that AtTCP11 can influence the development of leaf, stem, petiole, and pollen in Arabidopsis thaliana [20]. The present study indicated that transcript levels of CsTCP15 in stem, mature leaf, and old thorn were higher than those in other tissues or organs (Fig. 4B), and cis-acting elements, such as gibberellin (GA)- and ABA-responsive elements, were found in its promoter region (Fig. 2A), further suggesting that CsTCP15 possibly functions in the development of stem, leaf, or thorn (Fig. 4B).

CsTCP9, belonging to CIN subclade, was closely related to AtTCP13 in phylogenetic relationships (Fig. 1A). AtTCP13 is a regulator mediating leaf development in Arabidopsis thaliana [14]. Herein, CsTCP9 was the ortholog of AtTCP13 and was highly expressed in leaf (Fig. 3B, Fig. 4B), and it contained some phytohormone-responsive elements (Fig. 2A), suggesting that CsTCP9 may be involved in regulating leaf development. On the other hand, another CIN-type gene, CsTCP13 was closely related to AtTCP4 in phylogenetic relationships and was orthologous to AtTCP4 (Fig. 1A, Fig. 3B). AtTCP4 was confirmed to modulate cell proliferation at margins of the developing leaf in Arabidopsis thaliana by antagonizing miR319 [27, 28], and its ortholog LsTCP4 can participate in affecting the leaf shape phenotype of Lactuca sativa [37]. Notably, CsTCP13 also contained the miR319-binding site (Fig. 1D). The highest transcript level of CsTCP13 was observed in leaf and some cis-acting elements related to phytohormones were found in its promoter region (Fig. 2A, Fig. 4B). Hence, these results suggested that CsTCP13 possibly takes part in leaf development by antagonizing miR319. In addition, homodimers and heterodimers can be formed among TCP proteins, and these oligomerization combinations possess different affinity to bind various DNA components to regulate plant growth and development [5]. Herein, the protein-protein interaction network showed that CsTCP13 interacted with many other TCP members (Fig. 2B), suggesting that CsTCP13...
probably plays an important role in leaf development presumably by forming protein complexes.

Shoot branching determines plant architecture, which is essential to maintain yield in many crops [38]. *AtTCP18* (*BRC1*) and *AtTCP12* (*BRC2*), belonging to *CYC/TB1* subclade, were confirmed to control axillary bud outgrowth in *Arabidopsis thaliana* [11]; especially, *BRC1* and its orthologs in many species are generally regarded as an integrator of branching signals regulating bud outgrowth [39]. In this study, two *CYC/TB1*-type genes, *CsTCP14* and *CsTCP12* were closely related to *BRC1* and *BRC2* in phyllogenetic relationships, respectively (Fig. 1A), and collinearity analysis indicated that they were orthologous to *BRC1* and *BRC2*, respectively (Fig. 3B). Moreover, *CsTCP14* and *CsTCP12* were highly expressed in bud, and they both contained some phytohormone-responsive elements (Fig. 2A, Fig. 4B). These results suggested that *CsTCP14* and *CsTCP12* may function in shoot branching, similar to *BRC1* and *BRC2*, respectively. On the other hand, *TI1* (the ortholog of *BRC1* in *citrange*) is required in thorn conversion, and *p*C*BR2* (the ortholog of *BRC2* in poplar) was confirmed to play a key role in leaf development [12, 40]. The present study found that the highest transcript levels of *CsTCP14* and *CsTCP12* were observed in thorn and leaf, respectively (Fig. 4B), and they were fluctuated with the development of thorn and leaf, respectively (Fig. 4B), suggesting that *CsTCP14* and *CsTCP12* may participate in regulating the development of thorn and leaf, respectively.

Low intensity of light, abnormal temperature, and drought are three abiotic stresses that plants endure frequently in the process of growth and development [41]. Previous reports indicated that *TCP* genes can be involved in response to abiotic stresses. For example, *BRC1* was confirmed to promote axillary bud dormancy responding to shading in *Arabidopsis thaliana* [42]; *OsPCF6* and *OsTCP21* were found to influence the sensitivity to low temperature in *Oryza sativa* [43]; moreover, *ZmTCP32* and *ZmTCP42* were confirmed to associate with drought tolerance, and *ZmTCP42* acted as a positive regulator responding to drought in *Zea mays* [44]. In this study, expression levels of *CsTCP3* and *CsTCP15* were significantly fluctuated in mature leaf under low temperature (Fig. 5B), and low temperature-responsive elements were found in their promoter regions (Fig. 2A), suggesting that *CsTCP3* and *CsTCP15* may take part in the response of leaf to low temperature. On the other hand, *CsTCP9* and *CsTCP13* both contained at least five light-responsive elements (Fig. 2A), and their expression levels were significantly reduced in mature leaf by shading (Fig. 5A), suggesting that *CsTCP9* and *CsTCP13* are possibly involved in response to shading besides the regulation of leaf development. In addition, *CsTCP12* contained ABA- and drought-responsive elements, and its expression level was significantly increased in mature leaf by drought; *CsTCP14* contained seven ABA-responsive elements, but its expression level in mature leaf was significantly decreased by drought (Fig. 2A, Fig. 5C); moreover, the opposite trend in the change of their expression levels was also observed in mature leaf by shading and low temperature (Fig. 5A, B). These results suggested that these two genes may function differently in the response of leaf to shading, low temperature, and drought, and are worthy of further study in the future.

**Conclusions**

In this study, twenty putative *CsTCPs* with the TCP domain were explored from *Citrus sinensis* genome, of which eleven, five, and four *CsTCPs* were clustered into subclades *PCF*, *CIN*, and *CYC/TB1*, respectively. The segmental duplication may promote the amplification of *TCP* gene family in *Citrus sinensis* genome, and the purifying selection majorly contributes to the evolution of *CsTCPs*. The twenty *CsTCPs* may have their own functions due to their different protein properties, gene structure, motif composition, and their varied expression profiles in tissues or organs, as well as in response to abiotic stresses. *CsTCP3*, *CsTCP9*, and *CsTCP13* are probably involved in the regulation of leaf development; specially, *CsTCP13* may perform its function by antagonizing *miR319* or by forming protein complexes. *CsTCP12* and *CsTCP14* possibly function in shoot branching; specially, *CsTCP12* may also act as a regulator in leaf development, and *CsTCP14* may also play an important role in thorn development. *CsTCP15* may take part in the development of stem, leaf, or thorn. *CsTCP3* and *CsTCP15*, *CsTCP9* and *CsTCP13*, as well as *CsTCP12* and *CsTCP14* are probably involved in the response of leaf to low temperature, shading, and drought, respectively. Altogether, the present results suggested possible roles of some *TCP* genes, and their specific roles and potential mechanisms during citrus plant growth and development as well as in response to abiotic stresses are required to be further studied in the future.

**Methods**

**Plant materials**

Roots, stems, leaves, thorns, and buds were collected from two-year-old seedlings of *Poncirus trifoliata*. In detail, roots included primary roots and lateral roots; stems included tip stems (non-lignified), middle stems (semi-lignified), and base stems (lignified); leaves included young leaves (3 weeks old), mature leaves (3 months old), and old leaves (6 months old) of autumn shoots; thorns or buds included young thorns or young buds (from non-lignified shoots) and old thorns or old
buds (from lignified shoots). In this study, fifteen healthy seedlings were randomly selected and divided into three groups as three biological replicates for sample collection. Moreover, flowers were harvested at full flowering stage, as well as fruits and seeds were harvested at 190 days after flowering (DAF) from adult trees of *Poncirus trifoliata*. In addition, *Citrus reticulata* cv. Kinokuni adult trees grafted on *Poncirus trifoliata* were used to investigate the expression patterns of TCP genes responding to shade, low temperature, and drought treatments.

All the plant materials were located in citrus germplasm orchard of Huazhong Agricultural University (Wuhan, Hubei Province, China). Harvested samples were rapidly frozen by liquid nitrogen and immediately stored at −80°C.

**Abiotic stress treatments**

Shade and drought treatments were applied to adult trees of *Citrus reticulata* cv. Kinokuni at 135 DAF. For shade treatment, three healthy adult trees were randomly selected as three biological replicates. On each tree, two robust branches at the top of the same crown were regarded as one comparison. Of them, one branch was covered with a black shading net which transmits about 10% of incident light [45]; the other branch was not covered as the control. The light intensity of crown was measured by digital illuminance meter (GM1010; BENETECH, Shenzhen, China) at 14:00 and 18:00 on a sunny day. One week later, healthy mature leaves from the third to the sixth node of spring shoots were collected.

For drought treatment, six healthy adult trees were randomly selected and the soil was covered by black plastic films. Of them, three trees as three biological replicates were irrigated once per week (20 L of water per tree) as the control [46]; the three other trees were not irrigated. After 2 weeks, soil water content at 30 cm below the surface was detected by the oven-drying method [47], and healthy mature leaves from the third to the sixth node of spring shoots were collected. The proline content of mature leaves was determined by Proline Assay Kit (vis-spectrophotometry; Solarbio, Beijing, China). On the other hand, healthy mature leaves without shade and drought treatments were stored together with the shoots at 5°C for zero hour (Control), 2 h (T1), and 6 h (T2), respectively. Each treatment contained at least 30 leaves. Then, all the collected samples were rapidly frozen by liquid nitrogen and immediately stored at −80°C.

**Identification of TCP genes from Citrus sinensis genome**

The complete genome sequence data of *Citrus sinensis* v1.0 were downloaded from Citrus Pan-genome to Breeding Database (http://citrus.hzau.edu.cn/). The protein sequences of AtTCP transcription factors were retrieved from The Arabidopsis Information Resource (https://www.arabidopsis.org/). Based on such two files, the two-step BLAST method was used to explore TCP genes from *Citrus sinensis* genome with the Blast Compare Two Seqs program of TBtools [48]. In detail, AtTCPs were used as query sequences to search all possible CsTCPs (e-value, 1e−10) from subject sequences, which were translated by representative mRNA sequences from *Citrus sinensis* genome. Moreover, the Hidden Markov Model profile of TCP domain (PF03634) retrieved from Pfam database (http://pfam.xfam.org/) was used as the standard; all candidate CsTCPs were further screened out according to this standard by applying the phmmer program (https://www.ebi.ac.uk/Tools/hmmer/search/phmmer), the hmmscan program (https://www.ebi.ac.uk/Tools/hmmer/search/hmmscan), and Batch Web CD-Search Tool (https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi).

**Gene location, duplication, structure, and characterization**

The genomic distribution of putative CsTCPs on chromosomes, the chromosomal repeat fragment information, and gene structure were visualized by the Advanced Circos program and the Gene Structure View (Advanced) program of TBtools, respectively. The length of coding sequences (CDS), the size of proteins, and the Ka/Ks ratio were calculated by the Fasta Stats program and the Simple Ka/Ks Calculator (NG) program of TBtools, respectively; the Ka/Ks ratio was used to analyze the trend of gene divergence after duplication events with the criteria that Ka/Ks<1 means the purifying selection, Ka/Ks=1 means the neutral selection, and Ka/Ks>1 means the positive selection leading to the accelerated evolution [34].

In addition, Mw and theoretical pI of CsTCPs were computed by the Compute pI/Mw tool (https://web.expasy.org/compute_pi/); the length of signal peptide was calculated with the SignalP-5.0 server (http://www.cbs.dtu.dk/services/SignalP/); the predicted subcellular location information was retrieved with CELLO v2.5 (http://cello.life.nctu.edu.tw/).

**Phylogenetic analysis, conserved domain identification, and miR319-binding site recognition**

The protein sequences of CsTCPs, AtTCPs, and SITCPs [32] were used to construct phylogenetic tree by the neighbor-joining method with MEGA X (https://www.megasoftware.net/) and iTOL v6 (https://itol.embl.de/); the bootstrap test was implemented with 1000 iterations. On the other hand, the protein sequences of CsTCPs and AtTCPs were aligned by the Muscle method with MEGA X, and the overall conserved amino acids were
visualized with Jalview v.2.11.1.4 (http://www.jalview.org/). Moreover, miR319-binding sites of CsTCPs were predicted by psRNATarget (https://www.zhaolab.org/psRNATarget/).

**Analysis of conserved motif, cis-acting element, collinearity relationship, GO annotation, and protein-protein interaction**

The conserved motif composition of CsTCP protein sequences was analyzed by online program MEME v.5.4.1 (https://meme-suite.org/meme/tools/meme) and visualized by the Gene Structure View (Advanced) program of TBtools. cis-acting elements in promoter regions of 2 kb upstream of translation initiation sites of CsTCPs were screened out in PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/).

Moreover, the collinearity relationships between CsTCPs and A. thaliana TCPs were analyzed by the One Step MScAnX-Super Fast program of TBtools. The GO annotations of CsTCPs were performed with EGGNOG-Mapper (http://egg nog-mapper.embl.de/) and visualized by WEGO v.2.0 (https://wego.genomics.cn/). The protein-protein interaction network of CsTCPs was constructed by STRING v.11.5 (https://string-db.org/) and Cytoscape v.3.6.1 (https://cytoscape.org/).

**RNA extraction and quantitative real-time PCR (qRT-PCR)**

Total RNA of each sample was extracted by OmniPlant RNA Kit (CWBJO, Beijing, China). One microgram (μg) of high-quality total RNA was used for the first-strand cDNA synthesis by Transcript One-step gDNA Removal and cDNA Synthesis SuperMix (TransGen Biotech, Beijing, China). The qRT-PCR was conducted with three biological replicates, and each biological replicate was technically performed for three times in a 10 μL reaction volume using Hieff qPCR SYBR Green Master Mix (YEASEN, Shanghai, China) on the QuantStudio™ 6 Flex Real-Time PCR System (Thermo Fisher Scientific, USA). The reaction started with 95 °C for 5 min, then followed by 40 cycles of 95 °C for 10 s, 60 °C for 20 s and 72 °C for 20 s. In this study, CsActin (Gene ID: Cs1g05000.1) was used as the internal control, and specific primers of target genes for qRT-PCR were designed by Primer Premier 5 (http://www.premierbiosoft.com/primerdesign/) and listed in Table S3. The relative mRNA expression values were calculated with the Livak method [49].

**Statistical analysis**

The data were analyzed by t-test or by Tukey test in ANOVA program of IBM SPSS Statistics v.26 (https://www.ibm.com/cn-zh/analytics/spss-statistics-software); the level of significance was set at $P < 0.05$. The graphs were created by SigmaPlot v.12.5 (https://systatsoftware.com/products/sigmaplot/), RStudio (https://rstudio.com/), or TBtools.

**Abbreviations**

aa: Amino acid(s); ABA: Abscisic acid; CDS: Coding sequence(s); CIN: CINNAMONATA; CYC/TB1: CYCOLOIDEA and TEOSINTE BRANCHED1; Da: Dalton(s); DAF: Days after flowering; GA: Gibberellin; GO: Gene ontology; Ka: Non-synonymous substitution rate; Ks: Synonymous substitution rate; MeJA: Methyl jasmonate; Mw: Molecular weight; PCF: PROLIFERATING CELL FACTORS; pI: Isoelectric point; qRT-PCR: Quantitative real-time PCR; SA: Salicylic acid; TCP: TEOSINTE BRANCHED1; CYCOLOIDEA, and PROLIFERATING CELL FACTORS; UTR: Untranslated region(s); μg: Microgram(s).

**Supplementary Information**

The online version contains supplementary material available at https://doi.org/10.1186/s12870-022-03709-3.

**Additional file 1:** Fig. S1. Motif composition and gene structure of *Citrus sinensis* TCP members. A total of twenty motifs in *Citrus sinensis* TCP protein sequences were analyzed by MEME algorithm v.5.4.1. The structure of twenty TCP genes was visualized by the Gene Structure View (Advanced) program of TBtools. Different motifs, coding sequences (CDS), and untranslated regions (UTR) are represented by colored boxes. Introns are represented by gray lines. Tick labels represent protein length (aa) and gene length (bp).

**Additional file 2:** Table S1. Ka/Ks of TCP gene pairs in *Citrus sinensis* genome.

**Additional file 3:** Table S2. Gene ontology (GO) of *Citrus sinensis* TCP genes.

**Additional file 4:** Fig. S2. Illustration of differential conditions under shade and drought treatments. A. Light intensity in the canopy under shade treatment. The data were collected by digital illuminance meter at 14:00 and 18:00 on a sunny day. B. Absolute rate of water to soil under drought treatment. C. Proline content in mature leaf of *Citrus reticulata* cv. Kinokuni under drought treatment: Results are the mean of three independent biological replicates. Error bars represent the standard deviation of replicates. The asterisk indicates statistically significant difference between groups at $P < 0.05$ by t-test.

**Additional file 5:** Table S3. Primers for quantitative real-time PCR (qRT-PCR).

**Additional file 6:** Table S4. List of accession IDs or numbers of all sequences used in this study.

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Not applicable.

**Authors’ contributions**

YZL and DHL designed the experiments. DHL and YL performed bioinformatics analysis. YL and HH conducted abiotic stress treatments, and also collected the manuscript. YZL and DHL designed the experiments. DHL and YL performed bioinformatics analysis. YZL and DHL designed the experiments. YL and HH conducted abiotic stress treatments, and also collected the manuscript. YZL, SMA, and YTL polished the manuscript. All authors reviewed and approved the manuscript.

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**Availability of data and materials**

The whole genome data of *Citrus sinensis* v1.0 were downloaded from Citrus Pan-genome to Breeding Database (http://citrus.hzau.edu.cn/), and the
published TCP sequences of Arabidopsis thaliana and Solanum lycopersicum were acquired from The Arabidopsis Information Resource database (https://www.arabidopsis.org/) and Solanum lycopersicum ITAG2.4 of Phytozome genome database (https://phytozome-next.jgi.doe.gov/), respectively. The accession IDs or numbers of all sequences used in the present study are listed in Table S4, and all databases used in this study are available to the public. All other data are contained within the article or its supplementary information, and they are available upon reasonable request.

Declarations

Ethics approval and consent to participate

Experimental research and field studies on plants including the collection of plant material are comply with relevant guidelines and regulation.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. Katagiri F, Chua N-H. Plant transcription factors: present knowledge and future challenges. Trends Genet. 1992;8(2):12–7. https://doi.org/10.1016/0168-9525(92)90020-5.

2. Martín-Trillo M, Cubas P. TCP genes: a family snapshot ten years later. Trends Plant Sci. 2010;15(1):31–9. https://doi.org/10.1016/j.tplants.2009.11.003.

3. Floyd Sandra K, Bowman JL. The ancestral developmental tool kit of land plants. Int J Plant Sci. 2007;168(1):1–55. https://doi.org/10.1086/509079.

4. Navaud O, Dabos P, Campus E, Tremousaygue D, Hervé C. TCP transcription factors predate the emergence of land plants. J Mol Evol. 2007;65(1):23–33. https://doi.org/10.1007/s00239-006-0174-z.

5. Manassero NGU, Viola IL, Welchen E, Gonzalez DH. TCP transcription factors act as an integrator of branching signals within axillary buds. Plant Cell. 2012;159(4):1511–23. https://doi.org/10.1104/pp.112.200303.

6. Lan J, Zhang J, Yuan R, Yu H, Sun L, et al. TCP transcription factors suppress cotyledon trichomes by impeding a cell differentiation-regulating complex. Plant Physiol. 2021;186(1):434–51. https://doi.org/10.1093/phymolgs/kab053.

7. Cubas P, Lauter N, Doebley J, Coen E. The TCP domain: a motif found in proteins regulating plant growth and development. Plant J. 2002;30(3):337–48. https://doi.org/10.1046/j.1365-313X.2002.01294.x.

8. Davière J-M, Wild M, Regnault T, Baumberger N, Eisler H, Genschik P, et al. TCP transcription factor family. Plant J. 2019;100(4):677–92. https://doi.org/10.1111/tpj.14461.

9. Zhang W, Cochet F, Ponnaiah M, Lebreton S, Matheron L, Pionneau C, et al. The pea TCP transcription factor PiBRc1 acts downstream of Strigolactones to control shoot branching. Plant Physiol. 2011;158(1):225–38. https://doi.org/10.1104/pp.111.187275.

10. Tatematsu K, Nakabayashi K, Kamiya Y, Nambara E. Transcription factor AtTCP14 regulates embryonic growth potential during seed germination. Arabidopsis thaliana. Plant J. 2008;53(1):42–52. https://doi.org/10.1111/j.1365-313X.2007.03308.x.

11. Resentini F, Felipo-Benavent A, Colombo L, Blázquez MA, Alabadí D, Masi ero S. TCP14 and TCP15 mediate the promotion of seed germination by gibberellins in Arabidopsis thaliana. Mol Plant. 2015;8(3):482–5. https://doi.org/10.1016/j.molp.2014.11.018.

12. Zhang J, Wang Y, Luo D. LcYCYC genes constitute floral Dorsoventral asymmetry in Lotus japonicus. J Integr Plant Biol. 2010;52(11):959–70. https://doi.org/10.1111/j.1744-7909.2010.00926.x.

13. Takeda T, Amano K, Ohto M-a, Nakamura K, Sato S, Kato T, et al. RNA interference of the Arabidopsis putative transcription factor TCP16 gene results in abortion of early pollen development. Plant Mol Biol. 2006;61(1):165–77. https://doi.org/10.1007/s11103-006-6265-9.

14. Hur Y-S, Kim J, Kim S, Son Q, Kim W-Y, Kim G-T, et al. Identification of TCP13 as an upstream regulator of ATHB12 during leaf development. Genes. 2019;10(9):644. https://doi.org/10.3390 gen10090644.

15. Vadde BVl, Challia KR, Nath U. The TCP4 transcription factor regulates trichome cell differentiation by directly activating GLABRUS INFLORES CENCE STEMS in Arabidopsis thaliana. Plant J. 2018;93(2):259–69. https://doi.org/10.1111/tpj.13772.

16. Liu Y, Heying E, Tanumihardjo SA. History, global distribution, and nutritional importance of Citrus fruits. Compr Rev Food Sci Food Saf. 2012;11(6):530–45. https://doi.org/10.1111/j.1541-4337.2012.00201.x.

17. Goodstein DM, Shu S, Howson R, Neupane R, Hayes RD, Fazo J, et al. Phytozome: a comparative platform for green plant genomics. Nucleic Acids Res. 2011;40(D1):D1178–86. https://doi.org/10.1093/nar/gkr944.

18. Xu Q, Chen L-L, Ruan X, Chen D, Zhu H, Chen C, et al. The draft genome of Citrus sinensis. Nat Genet. 2013;45(1):59–66. https://doi.org/10.1038/nrg.2472.

19. Parapunova V, Busscher M, Busscher-Lange J, Lammers M, Karlova R, Bovy AG, et al. Identification, cloning and characterization of the tomato TCP transcription factor family. BMC Plant Biol. 2014;14(1):157. https://doi.org/10.1186/1471-2229-14-157.

20. Zhang J. Evolution by gene duplication: an update. Trends Ecol Evol. 2003;18(6):292–8. https://doi.org/10.1016/S0169-5347(03)00033-8.
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34. Hurst LD. The Ka/Ks ratio: diagnosing the form of sequence evolution. Trends Genet. 2002;18(9):486. https://doi.org/10.1016/S0168-9525(02)02722-1.
35. Uzman A. Fundamental molecular biology. Biochem Mol Biol Educ. 2007;35(6):481–2. https://doi.org/10.1002/bmb.110.
36. Angiuoli SV, Salzberg SL. Mugsy: fast multiple alignment of closely related whole genomes. Bioinformatics. 2010;27(3):334–42. https://doi.org/10.1093/bioinformatics/bttq665.
37. Seki K, Komatsu K, Tanaka K, Hiraga M, Kajiya-Kanegae H, Matsumura H, et al. A CIN-like TCP transcription factor (LsTCP4) having retrotransposon insertion associates with a shift from Salinas type to empire type in crisphead lettuce (Lactuca sativa L.). Horticulture. 2020;7(1):15. https://doi.org/10.1038/s41438-020-0241-4.
38. Teichmann T, Muhr M. Shaping plant architecture. Front Plant Sci. 2015;6:233. https://doi.org/10.3389/fpls.2015.00233.
39. Barbier FF, Dun EA, Kerr SC, Chabikwa TG, Beveridge CA. An update on the signals controlling shoot branching. Trends Plant Sci. 2019;24(3):220–36. https://doi.org/10.1016/j.tplants.2018.12.001.
40. Muhr M, Paulat M, Awwanah M, Binkkötter M, Teichmann T. CRISPR/Cas9-mediated knockout of Populus BRANCHED1 and BRANCHED2 orthologs reveals a major function in bud outgrowth control. Tree Physiol. 2018;38(10):1588–97. https://doi.org/10.1093/treephys/typ088.
41. Gong Z, Xiong L, Shi H, Yang S, Herrera-Estrella LR, Xu G, et al. Plant abiotic stress response and nutrient use efficiency. Sci China Life Sci. 2020;63(5):635–74. https://doi.org/10.1007/s11427-020-1683-x.
42. González-Grandío E, Poza-Carrión C, Sorzano COS, Cubas P. BRANCHED1 promotes axillary bud dormancy in response to shade in Arabidopsis. Plant Cell. 2013;25(3):834–50. https://doi.org/10.1105/tpc.112.108480.
43. Wang S-t, Sun X-i, Hoshino Y, Yu Y, Jia B, Sun Z-w, et al. MicroRNA319 positively regulates cold tolerance by targeting OsPCF6 and OsTCP21 in rice (Oryza sativa L.). PLoS One. 2014;9(3):e91357. https://doi.org/10.1371/journal.pone.0091357.
44. 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(11):2762. https://doi.org/10.3390/ijms20112762.
45. Yang X-y, Xie J-x, Lu X-p, Liu Y-z, Peng S-a. Isolation of a citrus ethylene-responsive element binding factor gene and its expression in response to abiotic stress, girdling and shading. Sci Hort. 2011;127(3):275–81. https://doi.org/10.1016/j.scienta.2010.07.008.
46. Jiang N, Jin L-f, Teixeira da Silva JA, Islam MDZ, Gao H-w, Liu Y-z, et al. Activities of enzymes directly related with sucrose and citric acid metabolism in citrus fruit in response to soil plastic film mulch. Sci Hort. 2014;168:73–80. https://doi.org/10.1016/j.scienta.2014.01.021.
47. Jin L-f, Guo D-y, Ning D-y, Hussain SB, Liu Y-z. Covering the trees of Kinokuni tangerine with plastic film during fruit ripening improves sweetness and alters the metabolism of cell wall components. Acta Physiol Plant. 2018;40(10):182. https://doi.org/10.1007/s11738-018-2761-1.
48. Chen C, Chen H, Zhang Y, Thomas HR, Frank MH, He Y, et al. TBtools: An integrative toolkit developed for interactive analyses of big biological data. Mol Plant. 2020;13(8):1194–202. https://doi.org/10.1016/j.molp.2020.06.009.
49. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods. 2001;25(4):402–8. https://doi.org/10.1016/S0165-6147(01)00407-2.