CsTCPs regulate shoot tip development and catechin biosynthesis in tea plant (*Camellia sinensis*)

Shuwei Yu1, Penghui Li1, Xuecheng Zhao1, Mangmang Tan1, Muhammad Zulfqar Ahmad1, Yujie Xu1, Million Tadege2 and Jian Zhao1

**Abstract**

The growth of leaves and biosynthesis of characteristic secondary metabolites are critically important for tea production and quality control. However, little is known about the coordinated regulation of leaf development and catechin biosynthesis in tea plants. Here, we reported that TCP TFs are involved in both catechin biosynthesis and leaf development. An integrated analysis of catechin profiling and CsTCP expression in different tissues of plants under various environmental conditions at different developmental stages indicated significant correlations between the transcript levels of CIN-type CsTCPs and catechin production. CIN-type CsTCP3 and CsTCP4 and PCF-type CsTCP14 interacted with the MYB-bHLH-WD40 repeat (MBW) complex by forming a CsTCP3-CsTT8 heterodimer and modulating the transactivation activity of the promoters of anthocyanin synthase (*CsANS1*) and anthocyanidin reductase (*CsANR1*). Four types of microRNA/target modules, miR319b/CsTCP3-4, miR164b/CsCUC, miR396/CsGRF-GIF, and miR165b/HD-ZIPIII ones, were also identified and characterized for their functions in the regulation of the development of tea plant shoot tips and leaf shape. The results of these modules were reflected by their different expression patterns in developing buds and leaves that had distinctly different morphologies in three different tea plant varieties. Their roles in the regulation of catechin biosynthesis were also further verified by manipulation of microRNA319b (miR319b), which targets the transcripts of CsTCP3 and CsTCP4. Thus, CsTCPs represent at least one of these important groups of TFs that can integrate tea plant leaf development together with secondary metabolite biosynthesis. Our study provides new insight into shoot tip development and catechin production in tea plants and lays a foundation for further mechanistic understanding of the regulation of tea plant leaf development and secondary metabolism.

**Introduction**

Tea plant (*Camellia sinensis* (L.) O. Kuntze) is a perennial evergreen tree or shrub species cultivated worldwide. Its tender shoot tips, including apical buds, young leaves, and stems are used for making various types of teas due to the concentrated presence of secondary metabolites such as catechins, caffeine, theanine, and terpenoid volatiles. These characteristic secondary metabolites largely determine the pleasant flavors and numerous health benefits of tea beverages. Therefore, the production of these tender shoot tips with appropriate contents and composition of secondary metabolites is extremely important for tea production. While many studies in recent decades have mainly focused on plant secondary metabolites in tea plant leaves with regard to the biochemical pathways and molecular mechanisms underlying their biosynthesis and regulation and their health benefits, the growth and production of tender shoot tips is not well understood, including shoot apical meristem (SAM) development, leaf initiation and development, internode elongation, trichome and stomata formation, branching, and the integrated control of secondary metabolite production during shoot tip development. Several MYB transcription factors (TFs), such as CsAN1, CsTT2, and CsMYB75, have been reported to regulate...
The TFs that regulate the growth and development of shoot tips and the formation of tissues and organs in tea plants are largely unknown. Aboveground plant growth and development are obviously orchestrated by the SAM; leaves and branches arise from apical and axillary meristems, respectively, the process of which is controlled by multiple complex regulatory networks. Such networks are also believed to operate in the shoot tips of tea plants, although the molecular coordination of this complex developmental process in terms of secondary metabolite production is poorly understood. Phytohormones such as auxin, cytokinin (CK), jasmonic acid (JA), and gibberellic acid (GA) and environmental factors can affect and alter shoot tip development and secondary metabolite production and are likely to be the key signaling integrators of growth and secondary metabolite accumulation in tea plants.

While a wide array of TFs may coordinate the development and biosynthesis of specialized metabolites in shoot tips, the members of the TEOSINTE BRANCHED 1 (TB1)/CYCLOIDEA (CYC)/PROLIFERATING CELL NUCLEAR ANTIGEN FACTOR 1 (PCF1) (TCP) family are particularly important. TCPs are important plant growth regulators involved in cell proliferation in various organs and tissues, hormone synthesis and signaling, plant responses to abiotic and biotic stresses, and biosynthesis of plant secondary metabolites. The plant TCP TF family comprises two distinct classes: class-I TCP PCFs and class-II TCPs, which in turn can be divided into CIN-TCPs and CYC-TCP subclades. Class-I TCPs regulate plant cell proliferation, leaf development, and stem elongation and modulate hormone biosynthesis and signaling.

Class-I TCP TFs redundantly promote plant growth, as reflected by OsPCF1/2 and AtTCP20 activation of CYCB1;1 genes. The onther hand, class-II TCP TFs often inhibit growth and proliferation. CIN-TCPs are involved in lateral organ development, and CYC/TB1 TCP TFs regulate the development of the axillary meristem (AM) into either flower petals or lateral shoots.

CIN-TCP TFs repress cell proliferation in developing leaf primordia, and compared with the wild type, mutants defective in the genes responsible for this phenomena displayed longer periods of cell division and larger leaves with altered shapes and/or crinkled surfaces. CYC-TCPs, such as Arabidopsis BRANCHED 1 (AtBRC1) and rice TEOSINTE BRANCHED 1 (OsTB1), act as repressors of axillary bud outgrowth and branching. The Arabidopsis class-I TCPs AtTCP14 and AtTCP15 redundantly function in the regulation of plant development, including seed germination, leaf shape, inflorescence stem growth, and inhibition of anthocyanin synthesis during exposure to high-light intensity, by modulating PRODUCTION OF ANTHOCYANIN PIGMENT 1 (PAP1) and TRANSPARENT TESTA 8 (TT8) expression. By contrast, the class-II AtTCP3 acts as an activator of anthocyanin biosynthesis-related genes by altering the Myb-bHLH-WD40 (MBW) complex, indicating that the function of class-II TCPs is usually opposite that of class-I TCPs in regulating plant growth and development, organogenesis, and hormone responses.

MicroRNAs (miRNAs), together with their target genes, essentially regulate leaf differentiation and growth. These miRNAs include miR319 and its target CIN-TCPs (abbreviated as miR319/CIN-TCPs), miR164/CLIP-SHAPED COTYLEDON 1 (CUL1), miR165/class-III homeodomain-leucine zipper (HD-ZIPIII), miR396/GROWTH REGULATING FACTORS (GRFs), and GRF-INTERACTING FACTORS (GIFs). Mutants with altered miRNA metabolism have pleiotropic developmental defects. For example, the JAW locus, which encodes microRNA319 (miRNA319) and represses expression of the AtTCP3, AtTCP4, AtTCP10 genes, controls leaf development.

The miRNA319-guided cleavage of AtTCP4 and its homologs is necessary to prevent aberrant activity of AtTCP4 due to improper gene expression. Overexpression of wild-type and miRNA-resistant AtTCP4 demonstrated that miRNA319 regulation is sufficient to restrict AtTCP4 function to its normal activity, and such regulatory module-mediated leaf morphogenesis is usually conserved. The concurrence of characteristic secondary metabolite biosynthesis with the growth and development of apical buds and young leaves suggests that these processes may be coordinately regulated in tea plants, although the underlying regulatory mechanisms remain unknown. It is highly important to understand these associations, since both the growth of shoot tips and accumulation of valuable secondary metabolites are major traits in terms of tea production and the nutritional quality of tea. Given the various and essential roles of TCP TFs in the regulation of the development of leaf shape, stem branching, and secondary metabolite biosynthesis, we conducted an expression analysis of tea plant CsTCP family genes and functionally characterized them. Their expression profiles in comparison with those of functionally known homologs in Arabidopsis and correlation analyses integrating transcriptome and catechin profiling of shoot tips at various developmental stages and under various environmental and hormone stresses enabled us to dissect the putative functions of CsTCP genes. The functions of miRNAs together with their target TFs, such as CsmiR319/CsTCP3 and CsTCP4, in the regulation of leaf development and catechin biosynthesis were determined. Our study provides new insight into the coordinated regulation of shoot tip development and secondary metabolite accumulation and lays a foundation for the future elucidation of the mechanisms underlying tea plant leaf development and the development of markers for breeding better tea plant varieties.
Results
Identification of 35 TCP genes in the tea plant genome

By using Arabidopsis and rice TCP protein sequences as queries for local BLAST searches against the tea plant genome database (http://tpia.teaplant.org/), we identified a total of 35 putative CsTCP proteins that contain conserved TCP domains and whose sequences are most similar to those of Arabidopsis and rice TCP TFs (Fig. 1 and Supplementary Fig. S1). For our functional study, we annotated tea plant CsTCP TFs in accordance with the homology and gene classification terminology used for the Arabidopsis TCP family (Supplementary Dataset S1). Essentially, most C. sinensis CsTCP TFs have corresponding counterparts in the Arabidopsis genome. However, the tea plant genome apparently contains more CsTCP genes than does Arabidopsis. These CsTCP genes were predicted to encode TF proteins of 172–544 amino acids with a putative molecular weight (MW) ranging from 19.84 to 60.32 and an isoelectric point (pI) ranging from 5.64 to 10.52 (Supplementary Dataset S1).

Phylogenetic analysis of the CsTCP TF family together with other functionally known Arabidopsis and rice homologs revealed two distinct subfamilies, class-I and class-II TCPs, using NJ tree topology. Class-I CsTCPs are PCF types and include 19 members, and class-II CsTCPs include 16 members referred to as CYC/TB1 and CIN types. The class-II CsTCPs could thus be further divided into subclades CIN (10 members) and CYC/TB1 (6 members) (Fig. 1a and Supplementary Fig. S2a). Multiple sequence alignment of the CsTCP proteins revealed that the basic helix–loop–helix (bHLH) domain-like TCP domain was present in most class-I CsTCPs, excluding CsTCP8a, which does not have a TCP domain but whose sequence is highly similar to that of most TCPs (Supplementary Fig. S1a). A four-amino acid deletion in the TCP domain was found in class-I TCPs compared to the class-II CYC/TB1 and CIN-TCPs. The slightly different but partly overlapping DNA-binding sequences, GGNCCCA for class I and GTGGGNCC for class-II, are apparent. The R domain, which comprises an

Fig. 1 Genome-wide analysis of CsTCP family genes in the tea plant genome. Phylogenetic analysis of CsTCPs from Camellia sinensis in comparison with TCPs from Arabidopsis and rice. The amino acid sequences were aligned using ClustalW, and MEGA 6.0 software was used to construct the phylogenetic tree by the NJ method with 1000 bootstrap replicates. The antagonistic but coordinately balanced functions of class-I TCPs and class-II TCPs (including CIN and CYC types) in regulating stem cell division and differentiation during plant tissue and organ growth and development are represented in a Yin (shade) and Yang (light) system.
~18-residue arginine-rich motif, is present only in class-II CYC/TB1 proteins at the C-terminus of the TCP domain, with the exception of CsTCP12, which had no R domain (Supplementary Fig. S1b).

Exon/intron analysis showed that the number of exons ranged from one to four in CsTCP genes. The majority of CsTCP genes contained one exon, and only 5 CsTCP genes had two exons, 3 had three exons, and 3 had four exons (Supplementary Fig. S2b). Most CsTCP genes clustered together with the Arabidopsis and rice genes in the phylogenetic tree, consistent with the exon/intron structures, indicating the evolutionary conservation of TCP gene structure. The conserved motifs were also analyzed, and fifteen motifs in CsTCPs were identified using the MEME program (http://meme-suite.org/) (Supplementary Fig. S2c). All CsTCP family proteins contained motif 1. Motif 15 was present in CsTCPs only of the CIN subclade, including CsTCP5a, CsTCP5b, CsTCP17a, and CsTCP17b. Only PCF-type TFs contained motifs 4, 8, 10, and 14. Motif 6 was conserved only in the CYC subclade, with the exception of CsTCP12. These analysis results suggest that TCP TFs are evolutionarily diverse in C. sinensis, which may indicate their largely different roles.

### Expression patterns of TCP genes in developing leaves and stems of tea plants

To explore the function of CsTCP TFs, we examined the abundance of 35 CsTCP transcripts in eight representative tissues of C. sinensis cv. Shuchazao (Fig. 2a, b and Supplementary Dataset S2). Interestingly, CsTCP genes in every class or subclade displayed distinct and characteristic expression patterns. Most class-II CIN subclade CsTCP genes were more highly expressed in the apical bud, young leaf, and mature leaf tissues than in other tissues, consistent with the well-known functions of these genes in regulating leaf development. The expression of these genes either was not detectable or was much lower in the roots, fruits, or stems (Fig. 2b). Most CYC/TB1 subclade genes were highly expressed in the leaves, stems, and apical buds. For example, CsTCP12 was highly expressed only in the stems and apical buds and was expressed at relatively low levels in the roots, young leaves, mature leaves, old leaves, flowers, and fruits, strongly suggesting its function in regulating stem branching. The rice OsTB1, maize ZmTB1, and Arabidopsis AtBRC1 (TCP18) and AtBRC2 (TCP12) homologs are mainly expressed in auxillary buds and negatively regulate lateral branching by suppressing axillary bud outgrowth. As the expression patterns of these homologous genes (CsBRC1a and CsBRC1b) in tea plants are similar, CsBRC1s may also have regulatory functions in repressing bud outgrowth (Fig. 2b) The tea plant genes CsBRC1a, CsBRC1b, CsCYC1, and CsCYC2 are highly expressed in the stems (1st, 2nd, and 3rd stems); CsBRC1a and b also show expression at relatively high levels in fruits and flowers (Fig. 2b).

Class-II TCPs are generally thought to act as repressors of cell division and inducers of cell differentiation. The dynamic expression of CIN-type TCP genes affects leaf shape by regulating the timing of leaf maturation. Mutation of the CIN-TCP gene LANCEOLATE (LA) in tomato (Solanum lycopersicum) or Arabidopsis alters leaf growth and maturation, leading to curled, crinkled, or rolled leaves due to overgrowth of and cell proliferation in certain leaf areas. Like their Arabidopsis counterparts AtTCP3, -4, and -10, CIN-type CsTCPs (specifically CsTCP2, -3, and -4; CsTCP5a and CsTCP5b; CsTCP17a and CsTCP17b; and CsTCP10a and CsTCP10b) are expressed in apical buds and young leaves. These TCPs likely act as negative regulators of leaf development, playing a pivotal role in the control of morphogenesis by negatively regulating the expression of boundary-specific genes.

Class-I PCF TCP genes displayed more extensive tissue-specific expression patterns. The expression patterns of AtTCP7, AtTCP8, AtTCP22, and AtTCP23 are similar in young Arabidopsis leaves, and these genes are functionally redundant with respect to leaf development. In tea plants, both CIN-type and some PCF-type TCP genes are highly expressed in apical buds and developing leaves. AtTCP15 regulates the expression of boundary-specific genes, and the function of this protein partially overlaps with the functions modulated by class-II CIN-like TCP proteins with opposite effects. AtTCP14 and AtTCP15 redundantly regulate internode development by promoting cell proliferation. Class-I PCF genes, including CsTCP15a, CsTCP15b, CsTCP9b, CsTCP19b, CsTCP19c, and CsTCP7 were highly expressed in both young leaves and mature leaves. In addition, CsTCP7/21 and CsTCP8b were highly expressed in the stem, young leaf, and mature leaf tissues, whereas CsTCP14a and CsTCP22/23 were highly expressed in the apical bud and young leaf tissues. We also observed that CsTCP19a, CsTCP20a, CsTCP20b, and CsTCP20c were highly expressed in old leaves and that the expression of CsTCP19a was upregulated in response to MeJA treatment (Supplementary Dataset S3 and S6).

### Expression patterns of CsTCPs in response to hormones and shade treatment

A large number of cis-acting elements involved in plant growth and development and hormone responses were identified in the promoters of CsTCP genes (Supplementary Fig. S4 and Supplementary Dataset S4). The cis-elements in the 1.50 kb promoter region of each CsTCP gene contained various types of cis-acting elements (Supplementary Dataset S4). The regions included elements responsive to light (37%), hormones (27.8%),
Fig. 2 Expression patterns of CsTCP family genes in tea plant tissues at various developmental stages or under light/shade conditions. 

a. Phylogenetic classification of CsTCPs from Camellia sinensis. 
b. Expression of CsTCPs in different tissues of Camellia sinensis plants. Shown are the expression levels of CsTCP genes in eight tissues. AB, apical buds of unopened leaves at the top of actively growing shoots; YL, first and second young leaves below the apical buds; ML, mature leaves geminated in the spring and harvested in the autumn; OL, old leaves at the bottom of tea tree plant; FL, flowers; FR, fruits of tea plants; ST, stem tissues at the 2nd and 3rd internodes; RT, roots. The expression levels were calculated on the basis of log10(FPKM) values. 
c. Expression patterns of CsTCP genes in developing leaves and stems of tea plants. L1–L5 refer to the 1st, 2nd, 3rd, 4th, and 5th leaves, respectively. 
d, e. Expression patterns of CsTCPs in tea plants under regular light treatment (d) and shade treatment with a black net that allows only 10–20% sunlight to pass (e). 
f. Comparative expression of tea shoot tips under regular light and shade treatments. The ratio of shade/light is shown in the heatmap analysis.
environmental stress (25.50%), and plant growth (9.70%) (Supplementary Fig. S4a). Along with these reported regions, there were some TF-binding sites that were also found to be responsive to hormones such as abscisic acid (ABA), GA, auxin, JA, salicylic acid, and ethylene (Supplementary Fig. S4b) or to stresses such as pathogen infection, heat, low temperature, and drought (Supplementary Fig. S4c, d). The expression of all development-related TCP TF genes, such as CsTCP2, CsTCP10a, CsTCP3, CsTCP20/6c, CsTCP14a, CsTCP22/23, and CsTCP8a, was repressed by drought and NaCl stresses (Supplementary Fig. S5a). The expression levels of most CsTCPs were unchanged or slightly repressed, and the expression of only CsTCP10a, CsTCP11b, and CsTCP5a was upregulated by MeJA treatment (Supplementary Fig. S5a–c). The patterns of cis-acting elements differed among CsTCP members (Supplementary Dataset S4). The promoter of CsTCP4 contains the maximum number (18) of hormone-responsive elements recorded, followed by the promoters of CsTCP11a (15) and CsTCP14b (14) (Supplementary Dataset S4). The CsTCP4 and CsTCP10b promoters contain the most (6) ABA-responsive elements, while the promoters of CsTCP9a and CsTCP14b contain at most (6) ERE motifs (Supplementary Dataset S4). CsTCP4 also contains (5) TCA elements that are related to the salicylic acid response. Most of the CsTCPs, namely, CsTCP9, -10, and -11, contained an equal number of environmental stress-responsive elements, but CsTCP14b contained the maximum number of environmental stress-responsive elements (only 2) (Supplementary Dataset S4). CsTCP19b contained the most (11) MYB-related elements (Supplementary Dataset S4).

Light is a key factor affecting plant growth, development, and metabolism. PHYTOCHROME INTERACTING FACTORS (PIFs) are key factors involved in the light signaling network. AtTCP5, AtTCP13, and AtTCP17 were reported to be involved in plant responses to light, temperature, and shade in both PIF-dependent and PIF-independent pathways. Our previous study showed that shade treatment drastically affects tea plant shoot tip growth, morphology, and secondary metabolism. Under shade treatment, the expression levels of the CsBRC1 genes were upregulated significantly at 2 d and the expression of the CsBRC1b and CsCYC2 genes was upregulated significantly at 4 h and 8 h but then repressed at 14 d. Similarly, the expression of CsTCP9a and CsTCP9b was upregulated at 2 d and 4 h, respectively (Fig. S4f and Supplementary Dataset S5). AtTCP17 interacts with the blue light receptor CRYPTOCHROME 1 (CRY1) at low temperatures, leading to reduced TCP1742 activity. Under shade treatment, the expression levels of CsTCP5a, CsTCP5b, and CsTCP17b, together with their putative target CsPIF4, and another CsTCP TF, CsTCP10a, were significantly upregulated 8 d after shading compared to normal light. Similar to AtTCP14/15 inhibition of anthocyanin accumulation during exposure of plants to high-light intensity, the CsTCP14a, CsTCP15a, and CsTCP15b gene expression patterns were consistent with the total catechin content (Fig. 3a).

Relationship between CsTCP expression and metabolite accumulation

Transcriptome and metabolome profiling of eight representative tissues [buds, leaves, stems, roots, flowers, fruits, leaves at six different developmental stages, and four internodes (stems) from the shoot tip to lower levels of tea plants] were used to determine the possible correlations between CsTCP TFs and tea plant secondary metabolite biosynthesis. Through an integration of the metabolite profiling and transcriptome data, a gene-to-metabolite correlation analysis of 16 flavonoid metabolites, including total catechins, caffeine, and theanine, with the expression patterns of all CsTCP genes in these specific tissues was conducted (Fig. 3a). The expression patterns of CsTCP2, -3, -4, -5, -10, and -17 were positively correlated with gallic acid (GC), gallogealocatechin (EGC), gallogealocatechin gallate (EGCG), epicatechin (EC), epicatechin gallate (ECC), and caffeic acid contents but negatively correlated with theanine contents (Fig. 3a). The PCF-type CsTCP22/23 genes were also positively correlated with the GC, EGC, EGCG, EC, ECG, and caffeic acids in developing leaves and stems (Fig. 3a). The expression patterns of CYC-type CsTCP genes were not significantly correlated with the metabolite levels in the eight tissues (Fig. 3a). However, in the developing leaves and stems at various stages, CYC-type CsTCP genes (except for CsTCP12) and PCF-type CsTCPs, such as CsTCP11a and CsTCP8a, showed a clearly negative correlation with these metabolites (Fig. 3b).

PCF-type CsTCP20/6b, CsTCP20/6a, and CsTCP11a expression was negatively correlated with galloylated catechins (EGC, EGCG, and cis-flavan-3-ols), total catechins, and caffeine. CsTCP15a and CsTCP15b expression was positively correlated with the contents of galloylated catechins (ECG, EGCG, CG), total catechins, and caffeine (Fig. 3a). Their opposite correlations with catechins were similar to those concerning theanine contents in these tissues. Moreover, CsTCP14b expression showed a clearly negative correlation with theanine, but CsTCP19b expression had a positive correlation with theanine (Fig. 3a). Specifically, CsTCP7/21 expression was negatively correlated with CG level; CsTCP20/6b, CsTCP20/6a, CsTCP20/6c, and CsTCP19b expression was positively correlated with CG content; CsTCP8b expression was negatively correlated with C contents; and CsTCP4...
Fig. 3 Correlation analysis of CsTCP genes with the accumulation of major tea plant secondary metabolites. a, b Integrative analysis of CsTCP expression and secondary metabolite accumulation patterns in eight representative tissues of tea plants (a) or in six representative leaf tissues and four representative stem tissues at various developmental stages (b). Correlation analysis conducted with 16 metabolites and 35 transcripts of CsTCPs. $R > 0.5$: Positive correlations; $R < -0.5$: negative correlation. c–e Correlation analysis of CsTCP expression with secondary metabolite accumulation in shoot tips under regular sunlight treatment (c), shade treatment (50–70% of transmitted sunlight blocked) (d), and shade treatment (80–90% of transmitted sunlight blocked) (e). The shoot tip (including apical buds and the 1st leaf) tissues of the Shuchazao variety were treated with shading as previously described (Liu et al., 2018). Shoot tips of tea plants under regular sunlight were set as controls, and four representative stem tissues at various developmental stages were used.
expression seemed to be closely correlated with C contents (Fig. 3b). Moreover, the CIN-type and CYC-type class-II TCP genes showed opposite correlations with the accumulation of catechins in the leaf and stem tissues at various developmental stages.

Our previous study showed that shade treatment of tea plant shoot tips enhanced chlorophyll accumulation but significantly decreased the contents of major catechins, including C, EC, GC, and EGC, under shade treatment compared with light treatment. The total catechin contents displayed a significant decrease from 4 h to 14 d in the S80–90% treatments, and the ECG content decreased until 14 d of treatment, whereas the GCG and EGCG contents showed only a minor decrease in the shade treatment. Under regular light conditions, the expression patterns of CsCYC2 were correlated with EGC content variations over two weeks. Similarly, CsTCP11b expression was correlated with total catechins, whereas the expression of CsTCP8b, CsTCP5a, CsTCP5b, CsTCP10a, and CsTCP22/23 was correlated with content variations of at least two of ECs, EGCs, and GCs (Fig. 3c). Under the shade treatment that blocked 50–30% of sunlight through the net, the EGC content variations in tea plant shoot tips (apical buds and the first fully open leaf) were significantly correlated with the expression of CsTCP8b, CsTCP5b, CsTCP10a, CsTCP10b, and CsTCP22/23. Moreover, CsCYC2 was also correlated with EGC contents, and CsTCP14a was correlated with ECG contents (Fig. 3d). Under the shade treatment that allowed 10–20% of sunlight to pass through the net, CsCYC2 expression was also correlated with most types of catechins, and the total catechin content, except for C, and CsTCP14a expression was correlated with the C content. In addition, CsTCP11b and CsTCP15a expression was correlated with total catechins, and CsTCP15a expression was also significantly correlated with EGC content (Fig. 3e). CsTCP7/21 expression was weakly induced under 80–90% shading (Fig. 2f), which may enhance chlorophyll accumulation. In MeJA-treated shoot tips, the expression levels of CsTCP5a, CsTCP5b, CsTCP10a, CsTCP11a, CsTCP17a, CsCYC2, CsTCP3, CsTCP19a, and CsTCP14b were upregulated compared with those of the controls (Supplementary Dataset S6).

CsTCPs are involved in the regulation of catechin biosynthesis

To verify the functions of CsTCPs in the regulation of tea plant secondary metabolism, we further studied the regulatory functions of the CIN-type TCP TFs CsTCP3 and CsTCP4, as well as PCF-type CsTCP14, in flavonoid biosynthesis. Yeast two-hybrid assays indicated that CsTCP3 and -4 interacted with several flavonoid regulators, including the anthocyanin-specific MYB regulator CsMYB75, the proanthocyanidin (PA)-specific regulators CsTT2a, CsTTG1, and CsTT8, and a negative regulator of anthocyanin CsMYBL2. Moreover, the bHLH TFs CsTCP3 and CsTCP4 interact with themselves to form homo- and heterodimers, which is consistent with their interactions with CsTT8 to form heterodimers (Fig. 4a). Furthermore, bimolecular fluorescence complementation (BiFC) assays in tobacco cells also confirmed that CsTCP3 N-GFP interacted with CsTT8a-C-GFP to form a heterodimer in planta (Fig. 4c). These data suggest that these MYBs, bHLHs, and WD40 proteins could interact, likely forming an MBW complex, similar to previously reported phenomena in other plant species. To test this hypothesis, we examined whether these interacting TFs could form an active regulatory complex by using a transactivation assay with a dual-luciferase reporter system. Transactivation assays using tea plant anthocyanidin synthase (CsANS1) and anthocyanidin reductase (CsANR1) promoters showed that adding CsTCP3 to the MBW complex activation system further enhanced CsANS and CsANR promoter activities. However, the addition of CsTCP14 to the promoter activation system inhibited the MBW-induced activation of the CsANS1 and CsANR1 promoters (Fig. 4d–f). Since CsTT2a or CsMYB75 could form the MYB-bHLH-WD40 (MBW) regulatory complex to regulate PA and anthocyanin biosynthesis in tea plants, our data are in line with those of a previous report showing that AtTCP3 regulates flavonoid biosynthesis.

CsTCPs and microRNAs regulate tea plant leaf development

We further identified several sets of genes that have been demonstrated to be critical players in regulating leaf and shoot development in Arabidopsis and other plant species. These included the following: genes that encode GRFs and GIFs that positively promote leaf cell proliferation; CUCs that regulate embryonic shoot meristem and leaf boundary formation; the HD-ZIPIII proteins PHAVOLUTA (PHV) and REVOLUTA (REV), which are required to establish leaf adaxial identity and patterning; and other related genes, such as those that encode cell division-related proteins including Cyclin Ds, ASYMMETRIC LEAVES 1/2 (AS1/2), and HOMEODOMAIN LEUCINE ZIPPER 2 (HAT2) (Figs. 5a, Supplementary Datasets S8 and S9, and Figs. S7–10).

Many miRNAs have been identified from tea plant microRNA transcriptome studies, and they are essentially conserved in other plant species (Supplementary Dataset S7). Five CIN-type CsTCP genes (CsTCP2, -3, -4, -10a, and -10b) contain a putative miR319 target site (Fig. 5b), suggesting that the CsmiR319/CsTCP module may be conserved in terms of its ability to regulate leaf development in C. sinensis. CsmiR319 transcript levels decreased as the leaf aged during development,
since expression in the 3rd leaf was much lower than that in the 2nd or 1st leaf (Fig. 5b). From the miRNA database, we also identified a CsmiR396b homolog whose sequence was complementary to the target sequence sites of the CsGRF and CsGIF genes in tea plant (Fig. 5c, Supplementary Figs. S7–10 and Supplementary Dataset S7). The CsmiR396b transcript level also slightly decreased with leaf age (Fig. 5c). In addition, these CIN-type TCPs also...
Fig. 5 Expression patterns of miR319-CsTCP and other miRNA modules in the developing leaves of tea plants. **a** Expression of development-related genes at different stages of tea plant leaf or stem growth and development in comparison with the expression of CsTCP3, CsTCP4, CsTCP10a, and CsTCP10b. **b** Top panel: alignment of CsmiR319b with its target genes CsTCP2 (1048–1067 bp), CsTCP3 (922–941 bp), CsTCP4 (1345–1364 bp), CsTCP10a (772–79 bp), and CsTCP10b (1396–1415 bp), which are complementary to CsmiR319b (1–21 bp). Bottom panel: RT-PCR-based assessment of CsmiR165b transcripts in apical buds (AB) and in the 1st, 2nd, and 3rd leaves from the shoot tips. A representative image is shown. **c** Top panel: alignment of CsmiR396b with its target genes CsGRF1 (870–891 bp), CsGRF2 (755–776 bp), CsGRF3 (342–363 bp), CsGRF4 (348–369 bp), CsGRF5 (339–360 bp), CsGRF6 (360–381 bp), and CsGRF9 (420–441 bp), which are complementary to CsmiR396b (1–21 bp). Bottom panel: RT-PCR-based assessment of CsmiR396b transcripts in apical buds (AB) and in the 1st, 2nd, and 3rd leaves from the shoot tips. A representative image is shown. **d** Top panel: alignment of CsmiR164b with its target genes CsCUC2a (454–475 bp), CsCUC2b (637–660 bp), CsCUC2c (619–642 bp), and CsCUC2d (652–675 bp), which are complementary to CsmiR164b (1–21 bp). Bottom panel: RT-PCR-based assessment of CsmiR164b transcripts in apical buds (AB) and in the 1st, 2nd, and 3rd leaves from the shoot tips. A representative image is shown. **e** Top panel: alignment of CsmiR165b with its target genes CsCUC2a (564–583 bp), CsCUC2b (939–958 bp), and CsPHV (357–386 bp), which are complementary to CsmiR165b (1–19 bp). Bottom panel: RT-PCR-based assessment of CsmiR165b transcripts in apical buds (AB) and in the 1st, 2nd, and 3rd leaves from the shoot tips. A representative image is shown. **f** Expression patterns of development-related genes in different tissues of tea plants.
induced the expression of AS1 and AS2, which are involved in adaxial polarity patterning, and of miR164, which targets CUC genes involved in the regulation of leaf boundary region development (Fig. 5d). CsmiR164a expression was the lowest recorded in the 3rd leaf, indicating a decrease with leaf age (Fig. 5d). AS1/2 regulates the expression of miR165, which cleaves its target HDII genes, CsPHV and CsREV1a (Fig. 5e). The levels of CsmiR165b transcripts, in contrast, increased with leaf age, as the highest levels were detected in the 3rd leaf (Fig. 5e).

We examined the expression patterns of miRNAs and their target genes in three varieties with distinct leaf shapes during leaf development (Fig. 6d). Shuchazao plants have round 1st leaves (Fig. 6a); Baihaozao plants have slightly long and curled 1st leaves (Fig. 6b); and Sidamingjia plants have long, narrow, curled 1st leaves (Fig. 6c). In addition, the 2nd and 3rd leaves of the Baihaozao variety were flat, with no clear crinkled leaf surface between veins (Fig. 6b). However, Sidamingjia has curled or crinkled leaves (Fig. 6c), and Shuchazao also has moderately curled or crinkled leaves (Fig. 6a). They all have jagged leaf edges.

Interestingly, a similarly decreasing pattern was observed for miR319 transcripts from the apical bud to the 3rd leaf in all three tea plant varieties (Fig. 6e). However, the Shuchazao leaves had a much lower miR319 expression level, approximately one-third of that in Sidamingjia leaves, while Baihaozao 1st leaves presented approximately one-half of that presented by the Sidamingjia leaves. Correspondingly, the transcripts of CsTCP3 and CsTCP4 gradually increased with leaf age in all three varieties (Fig. 6e). The transcript levels of CsTCP3 and CsTCP4 in Sidamingjia leaves were 2- to 4-fold lower than those in Shuchazao and Baihaozao leaves. These results suggest that CsmiR319b is likely involved in the cleavage of CsTCP3 and CsTCP4 transcripts.

The miR165/166 targets CsPHV and CsREV1a, miR164/ CUCs, and miR396/GRF-GIF modules were examined (Fig. 6f–h). The expression of CsmiR164a displayed similar patterns: it steadily decreased in apical buds and the 1st, 2nd, and 3rd leaves of Shuchazao and Baihaozao but continuously increased in Sidamingjia (Fig. 6h). In round-leaf Shuchazao plants, the overall expression levels of miR164a in the apical buds and developing leaves were approximately half of those of the long, narrow-leaf varieties Sidamingjia and Baihaozao. Correspondingly, the transcripts of miR164a target genes such as CsCUC2a and CsCUC2d, two major CUC genes highly expressed in 3rd leaves, were also at much higher levels in Sidamingjia than in Shuchazao and Baihaozao.

CUC1-3 NAC TFs form a critical boundary domain that delimits leaflets. These TFs promote leaf marginal outgrowth, local leaflet separation, and distal leaflet formation. By contrast, reduced CUC1-3 gene expression leads to fewer and fused leaflets. Relatively low expression levels of CsCUC2a and CsCUC2d genes in Shuchazao leaves partly explained the more extended outgrowth of leaflets, whereas Baihaozao and Sidamingjia had relatively high expression levels of CsCUC2a and CsCUC2d, coinciding with their narrow and long 1st leaves (Fig. 6h).

The miR165b transcripts in all three varieties showed similarly increasing patterns from ABs to the 1st, 2nd, and 3rd leaves. However, miR165b transcript levels were generally higher in Sidamingjia and Baihaozao buds and young leaves than in those of Shuchazao (Fig. 6f). Their target genes, CsREV1a and CsPHV, showed opposite changes in expression: their expression decreased slowly throughout leaf development. These target genes are required to establish adaxial identity in plant lateral organ primordia. Both CsREV1a and CsPHV transcript levels decrease throughout leaf development more rapidly in the long, narrow-leaf varieties Sidamingjia and Baihaozao than in the round-leaf variety Shuchazao, suggesting that miR165b modulates leaf shape regulation in these three tea varieties.

The miR396b transcripts rapidly decreased from the highest level in the apical buds to the lowest levels in the 2nd and 3rd leaves. The level of miR396b was significantly higher in the Shuchazao buds and leaves—approximately 3- and 9-fold higher than in those of Baihaozao and Sidamingjia, respectively (Fig. 6g). This was consistent with rapidly increasing expression levels of cell growth- and proliferation-promoting genes, such as CsGRF1 and CsGIF1c. CsGRF1 was also expressed at much higher levels in Shuchazao than in Baihaozao.

The transcript levels of two developmental repressors, CsAS1a and CsAS1b, continuously decreased in the 1st, 2nd, and 3rd leaves, with relatively higher levels detected in Shuchazao than in Baihaozao and Sidamingjia (Fig. 7d). The transcripts of two CYC-type TCPs, CsCYC1 and -2, also decreased throughout leaf development. While Sidamingjia had higher CsCYC2 expression levels, Shuchazao had higher CsCYC1 expression levels in the 2nd and 3rd leaves (Fig. 7d, e). Five class-I TCP genes were highly expressed in young leaves and may play roles in regulating leaf development. The transcript levels of most of these TCPs, e.g., CsTCP8b, CsTCP9a, CsTCP22, CsTCP15a, and CsTCP19c, increase during leaf growth and development to different extents. We found that the expression of most of these genes in the 1st and 2nd leaves was higher in Shuchazao and Sidamingjia than in Baihaozao; however, in the 3rd leaf, most PCF-type genes (CsTCP8b, CsTCP9a, and CsTCP22) were expressed at higher levels in Baihaozao and Sidamingjia. CsTCP15a was expressed at a higher level in the 3rd leaf of Baihaozao than in those of the other two varieties (Fig. 7e, f).
Fig. 6 Involvement of miR319/CsTCP and other miRNA/target modules in leaf development of different tea plant varieties. 

- **a–c** Leaf shape and patterns of the apical bud (AB) and the 1st, 2nd, and 3rd leaves of three tea plant varieties: Shuchazao (a), Baihaozao (b), and Sidamingjia (c).

- **d** Ratios of leaf width to length of leaves from the apical bud (AB) and 1st, 2nd, and 3rd leaves of three tea plant varieties.

- **e** Expression patterns of CsmiR319b with its target genes CsTCP3 and CsTCP4 in the apical bud (AB) and 1st, 2nd, and 3rd leaves.

- **f** Expression patterns of CsmiR165b with its target genes CsREV1a and CsPHV in the apical bud (AB) and 1st, 2nd, and 3rd leaves.

- **g** Expression patterns of CsmiR396b with its target genes CsGRF1 and CsGIF1c in the apical bud (AB) and 1st, 2nd, and 3rd leaves.

- **h** Expression patterns of CsmiR164b with its target genes CsCUC2a and CsCUC2d in the apical bud (AB) and 1st, 2nd, and 3rd leaves.

The relative expression levels of the miRNA genes were measured via qRT-PCR and normalized to the average expression level of CsACTIN. The data are expressed as the means ± SDs of three independent experiments, each with multiple biological replicates. Differences between tea plant varieties were analyzed with two-factor ANOVA using the LSD0.05 method.
Fig. 7 Involvement of miR319-CsTCP and other genes in leaf development of tea plant variety. a–c Shoot tip of tea plant varieties Shuchazao (a), Baihaozao (b), and Sidamingjia (c). From the top are the apical buds (ABs) and the 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, and 9th leaves. The short, middle, and long internodes are shown. Bar = 1 cm. d Expression patterns of CsAS1, CsAS2, and CsCYC2 in the apical bud (AB) and 1st, 2nd, and 3rd leaves of three tea plant varieties. e Expression patterns of CsCYC1, CsTCP8b, and CsTCP9a in the apical bud (AB) and 1st, 2nd, and 3rd leaves of three tea plant varieties. f Expression patterns of CsTCP22, CsTCP15a, and CsTCP19c in the apical bud (AB) and 1st, 2nd, and 3rd leaves of three tea plant varieties. The relative expression level of the CsmiR319b gene was measured via qRT-PCR and normalized to the average expression level of CsACTIN. The data are expressed as the means ± SDs of three independent experiments, each with multiple biological replicates. Two-factor ANOVA was performed on the data, and the differences were analyzed using the LSD0.05 method.
The CsmicroR319b/CsTCP3-4 module affects catechin biosynthesis in tea plant leaves

We further explored the relationship between CsmicroR319b/CsTCP3-4 expression and catechin biosynthesis. The major catechins, such as EGCG, ECG, GC, and EGC, as well as total catechins decreased throughout leaf development. Among those of the three varieties, the leaves of Baihaaoza had the lowest catechins, whereas catechin contents in the Shuchazao and Sidamingia leaves were comparable (Fig. 8a).

Although no transgenic techniques have yet been developed specifically for tea plants, we knocked down the CsmR319b transcript and monitored the effect on CsTCP3 and -4 expression and catechin levels by incubating the shoot tips in accordance with the antisense oligonucleotide (asODN) technique (Fig. 8b). Over longer incubation times, CsmR319b transcript levels clearly decreased significantly (Fig. 8c). Knockdown of the csmR319b transcript led to increased levels of CsTCP3 and CsTCP4 transcripts in the shoot tips after incubation, suggesting that the CsmR319b/CsTCP3-4 module can be manipulated (Fig. 8c). Interestingly, catechins in these treated samples also showed that CsmR319b knockdown was accompanied by increased catechin content (Fig. 8d and Supplementary Fig. S11). Overall, this indicated an inverse relationship between CsmR319b transcript levels and catechin accumulation. Further RT-PCR data showed that the expression of CsANS1, CsANR1, and CsTT8a was upregulated, indicating that it is likely that downregulation of CsmR319b expression may upregulate CsTCP3-4 expression, which then activates their target MBW-related and structural genes and ultimately alters the catechin content. Taken together, our results demonstrate that TCP TFs and their cognate microRNAs connect leaf and shoot development to catechin biosynthesis in the phenylpropanoid pathway in tea plant (C. sinensis), opening a new research avenue for improving tea productivity in terms of both yield and quality.

Discussion

Plant-specific TCP TFs play various roles in plant organ growth and development, cell proliferation, and biosynthesis of flavonoids. Apical buds and young leaves are the major parts of tea plants used for making various types of teas, such as green, white, and black teas. The shape of the apical buds and young leaves together with their yields is one of the major factors affecting tea production. The accumulation of characteristic secondary metabolites such as catechins, theanine, and caffeine in the apical buds and young leaves is critical for tea quality. Nevertheless, the molecular connections between shoot development and the accumulation of these secondary metabolites in tea plants remain unclear. By combining transcriptome and metabolite profiling with leaf morphology, miRNA expression analysis, and biochemical assays, our studies on CsTCP family TFs revealed some of their roles in determining tea plant leaf shape and regulating the biosynthesis of catechins.

Roles of CsTCPs in the determination of tea plant leaf and shoot development

Like in other plant species, the development of tea plant shoot tips from the apical meristem is regulated by TFs in a spatiotemporal manner and is an integration of both endogenous hormone signals and environmental factors. These conserved regulatory mechanisms that precisely control leaf initiation, polarity establishment and maintenance, leaf flattening, and intercalary growth have been extensively studied. Leaves initiate from the primordia on the ends of the SAM, and the leaf primordia are composed of adaxial and abaxial tissues that give rise to future upper and lower tissues of leaves (Fig. 9). The transcriptional regulatory networks controlling leaf and shoot development include many components: WUSCHEL (WUS)/CLAVATA (CLV) regulatory loop components; the auxin-, CK-, and GA-controlled KNOX regulatory module for meristem maintenance; and the HD-ZIPIII/AS1/2-KANADI (KAN)/ASYMMETRIC LEAVES 1/ROUGH SHEATH 2/PHANTASTICA (ARP) module for leaf adaxial/abaxial polarity specification and maintenance. TCPs play critical and multifaceted roles in leaf growth and shape determination. Two cellular processes, division, and expansion, that are spatially and temporally coordinated in leaf morphogenetic development have been recognized. Of them, CIN-type TCPs are key regulators of the timing of the transition from division to expansion within eudicot leaves. The expression and activity patterns of these TCPs mediate phytohormone responses during the spatiotemporal control of the cell fate transition through transactivation of cell cycle regulators, growth-repressing miRNAs, and/or interactions with chromatin remodeling machinery.

The profiling of more than 40 developmental-related genes essentially proved their high expression levels in developing tea plant buds and leaves (Figs. 2 and 5). Tea plant leaf shape should also be the outcome of the combined actions of multiple regulatory networks on the early development of leaf primordia (Fig. 9). WUS-like homebox (WOX) TFs such as STENOFOLIA (STF) and WOX9 were shown to be critical regulators of leaf outgrowth in a regulatory network connecting AS and TCP. NGATHA (NGA) TFs mediate the functions of AtTCP2 and AtTCP3 in leaf development. NGA and STYLISH family gene transcripts are abundant in leaf margins, perhaps regulating leaf marginal shapes such as smooth, serrated (toothed), or lobed ones. Thus, it is proposed that members of multiple TF families
Fig. 8 Involvement of CsmiR319b/CsTCP modules in catechin biosynthesis in tea plant leaves. 

(a) Contents of catechins in the apical bud (AB) and 1st, 2nd, and 3rd leaves of Shuchazao, Baihaozao, and Sidamingjia plants. Differences between tea plant varieties were analyzed via two-factor ANOVA using the LSD0.05 method.

(b) Knockdown of CsmiR319b (csmiR319b-KD) with asODN and incubation, with a solution containing sense oligonucleotide serving as a control.

(c) qRT-PCR verification of CsmiR319b knockdown (KD) and changes in the expression levels of CsTCP3 and CsTCP4 corresponding to treatment time.

(d) Changes in catechin contents in csmiR319b-KD shoot tips treated with asOND.

(e) Altered expression levels of CsANR1, CsANS1, and CsTT8a in csmiR319b-KD shoot tips treated with asOND. Significant differences between csmiR319b-KD and sODN controls were analyzed by using Student’s t-test in a two-tailed comparison (*P < 0.05 and **P < 0.01).
and hormones involved in the regulatory network specifically control tea plant leaf development (Fig. 9).

Class-I TCPs in Arabidopsis play positive roles in leaf growth and cell proliferation. AtTCP7 and AtTCP23 are involved in cell proliferation39. Correspondingly, CsTCP19 was reported to regulate leaf senescence in a manner that is redundant with CsTCP20 in C. sinensis, similar to that in Arabidopsis55. It was proposed that class-I and class-II TCP TFs usually have antagonistic functions17 in the regulation of senescence and leaf development associated with JA biosynthesis and cell proliferation. Class-I and class-II TCP TFs could antagonistically regulate the same target, balancing the regulation of development, growth, and other physiological functions or cellular processes, similar to the concept of Yin-Yang (Fig. 1). For example, in JA biosynthesis, class-I and class-II TCPs regulate the same target gene, LIPOXGENASE 2 (AtLOX2), by binding to different promoter elements56. AtTCP20 regulates leaf development by modifying LOX2. LOX2 is also targeted by AtTCP3, -4, and -10 under the control of miR319, and the manner of this targeting is antagonistic to that of AtTCP2056. Both the growth- and senescence-promoting effects of class-I TCPs are opposite those of the known class-II TCP mutants in JAW plants. The antagonistic regulatory effects of class-I and class-II TCP TFs on leaf development are likely to be mediated via the JA signaling pathway57.

Involvement of CsmiRNA/CsTCP modules in the regulation of leaf development

Plants with altered miRNA expression have pleiotropic developmental defects20–22,35. The JAW locus encoding miR319 represses AtTCP3, -4, and -10 gene expression and controls leaf development; normal expression of miR319 is required to prevent aberrant activity of AtTCP4 in leaf development23. For instance, high levels of miR319 downregulate these TCP transcript levels and alter Arabidopsis leaf morphogenesis, leading to the production of crinkled leaves18,22,35. In addition to miR319/CIN-TCPs, the miR396/HD-ZIPIII, miR164b/CUCs, and miR396b/GRF-GIF modules also critically regulate leaf development by modulating the differentiation or dedifferentiation balance in the shoot meristem or leaf cells33,36. This downregulation of CIN-TCP expression delays the arrest of cell proliferation within the margins and distal ends of
leaves and petals, resulting in overproduction of cells in these regions. Thus, downregulation of CIN-TCPs or overexpression of miR319 caused curled, crinkled, or rolled leaves due to overgrowth and cell proliferation in certain leaf areas. We also observed higher expression levels of Csmir319b but lower levels of CsTCP3 and -4 in Sidamingjia and, to a lesser extent, in Baihaozao, corresponding to crinkled or rolled leaves (Fig. 6). The Arabidopsis tcp2tcp4 double mutant has enlarged flat leaves, and the tcp2tcp3tcp4tcp10 mutant has highly crinkled leaves due to delayed differentiation, upregulation of cyclin and other cell division gene expression, and extended mitotic activity in the marginal regions of the leaves. miR319-mediated repression of CIN-TCP genes is required for normal organ development. The CsTCP2, -3, -4, or -10 genes also have complementary miR319 recognition sites, indicating that the expression of these genes could also be regulated by miR319 and affect leaf development in tea plants.

The miR164/CUC module regulates leaf margin dissection. AtTCP3 directly activates the expression of miR164, ASI, INDOLE-3-ACETIC ACID 3/SHORT HYPOCOTYL 2, and SMALL ALIXIN UP RNA (SAUR) genes. In Arabidopsis, redundant but partially distinct functions of CUC1, CUC2, and CUC3 control the formation of embryonic shoot meristems and boundaries between meristems and emerging organ primordia throughout the plant life cycle. miR164 negatively regulates AtCUC1 and AtCUC2, whose mutants fail to establish organ boundaries and show severe developmental defects. Compared with those of Shuchazao, developing leaves of both Sidamingjia and byahaozao had higher expression levels of CsCUC genes, corresponding to their curved and extended length, as well as longer internodes (Fig. 6). By contrast, overexpression of AtTCP3 activates miR164 and suppresses AtCUC1 gene expression, resulting in the fusion of cotyledons and formation of defective shoot meristems. These CIN-like TCP genes thus act redundantly in leaf differentiation in a dose-dependent manner, and their diversity has important roles in the signaling pathways that generate different leaf forms.

The miR396/GRF-GIF regulatory module also plays crucial roles in controlling the development of plant tissues and organs. The HD-ZIPII protein HAT3 physically interacts with HD-ZIPIII proteins and directly represses miR165/166 expression. AtTCP4 imparts differentiation competence by promoting the auxin response and activating HD-ZIP II HAT2 downstream of the auxin response to restrict the cell number and final size of leaves. Most tea plant leaves had similar sawtooth margins; thus, the miR396/CsPHV-CsREVIa modules in these varieties did not show drastic changes. Compared with those of the third variety, crinkled leaves of both Shuchazao and Sidamingjia had higher expression levels of CsGRF1 (Fig. 6).

A previous transcriptome study implied that miR319c/CsTCP2 regulates apical bud burst in tea plants, and other miRNAs play roles in tea plant bud dormancy and hormone responses. All these data, together with those of our study, strongly indicate the essential roles of miRNAs in the regulation of tea plant shoot tip activities and leaf development.

CsTCPs are involved in regulating tea plant secondary metabolism

The correlation analysis of metabolite-CsTCP gene expression in tea plant tissues of different developmental stages and under light/shade conditions supported the close relationships between CsTCP genes and catechin production in tea plants. CIN-type TCPs and some PCF-type TCPs were closely related to catechin biosynthesis. However, our study further demonstrated that CsTCP3 and CsTCP4 physically interacted with CsTT8 and even with CsTT2, CsTTG1, and CsMYB75. A bHLH-domain-containing TF, CsTCP3, interacted with CsTT8 to form a heterodimer. A PCF-type CsTCP14 also interacted with CsTT8, which is probably similar to CsTCP3. This is consistent with the fact that TT8s in various plant species form homodimers, which are assumed to bridge the MBW interaction complexes. The conserved Cys in AtTCP15 or AtTCP14 leads to inhibition of DNA binding when oxidized and thereby inhibits anthocyanin accumulation during exposure to high-light stress. These genes affect the expression of PAP1/2 and TT8 and anthocyanin biosynthesis-related genes in Arabidopsis.

Overexpression of AtTCP3 significantly promotes the transcriptional activation complex of MBW5s by interacting with TT8 to form a heterodimer. MBW ternary complexes were also confirmed in tea plants to have regulatory functions in both anthocyanin and catechin biosynthesis. CsTT2, CsMYB75, CsAN1, CsTT8, and CsTTG1 were identified as components of MBW complexes that activate flavonoid biosynthesis-related genes such as CsANS1 and CsANR1. Our study showed that CsTCP3 and CsTCP14 modified the MBW complex by interacting with their components and by strengthening or interfering with the activity of those complexes. This has further expanded the concept that CsTCP3 interacts with CsTT8 and CsTT2 but not with the repressor CsMYBL2. Our study indicated that CsTCP3, CsTCP4, or, likely, CsTCP14 could form homodimers by themselves and heterodimers with CsTT8a. Furthermore, both CsTCP3 and CsTCP14 acted through modification of the activity of MBW complexes via interactions with the components of those complexes. Interestingly, the Medicago leaf development regulator STF also activated flavonoid biosynthesis and MtTCP3.
Apparently, the catechin content in tea plant leaves is a quantitative trait that is determined by not only more than 30 structural genes but also a number of endogenous regulatory factors, as well as environmental cues. CsTCP3/4 and CsTCP14 are transcriptional regulators that can mediate endogenous signals, such as hormones, or environmental factors, such as light, to alter catechin biosynthesis in young tea plant leaves. As reported previously, TCP14 mediates the alteration of anthocyanins in response to high light, and CsTCPs may be involved in the regulation of catechin levels in different tea plant varieties under different environmental conditions. Such functional variations in these CsTCPs with regard to tea plant varietal differences based on different locations and environments will need further validation under multi-environmental conditions.

Conclusions

We identified 35 CsTCP TFs from the tea plant genome, and two classes of CsTCP TFs were expressed in tea plant tissues and organs to varying degrees, with expression patterns similar to those of their counterparts in Arabidopsis with conserved functions. We demonstrated that CsTCP3 and CsTCP4 are, at a minimum, TFs that can integrate the regulation of shoot tip and leaf developmental processes together with the biosynthesis of catechins in tea plants. Furthermore, our study revealed the regulatory modules of various sets of CsmiRNA/target CsTCPs or other regulatory genes, such as CsmiR319/CIN-CsTCPs, CsmiR396/CsGRFs/GIFs, CsmiR164/CsCuC, and CsmiR165/CsPHB-CsPHV, involved in tea plant leaf development. Moreover, the CsmiR319b/CsTCP3-4 modules are correlated not only with leaf development but also with catechin biosynthesis. These results were shown by using biochemical assays to form regulatory complexes together with MBWs that functionally regulate target metabolic gene promoters and by knocking down CsmiR319b in shoot tips for effective control of CsTCP3/4 expression and catechin accumulation. This study also revealed that TCPs are among the important regulators involved in the complex regulation of tea plant shoot tips and leaf development and demonstrated that they coordinate the regulation of leaf morphology with catechin biosynthesis in the developing leaves of tea plants in response to endogenous and environmental signals.

Materials and methods

Plant materials, growth conditions, and experimental treatments

Twenty-five-year-old tea plants produced by cuttings in the town of Dayang (31°55’ north, 117°12’ east; Hefei, Anhui Province, China) were used for phenotyping leaf shape, stem branching, and shoot tip development. The following tissues types were sampled during the spring and summer at 20–27 °C under a 12 h/12 h light/dark photoperiod: shoot tips; apical buds; 1st, 2nd, 3rd, 4th, and 5th leaves; and 1st, 2nd, 3rd, and 4th internodes of the stems. The apical buds, leaves, and stems were sampled from at least nine individual tea plants of three different tea plant (C. sinensis) varieties, Shuchazao, Baihaozao, and Sidamingjia, which were bulked into three pools as biological repeats. A similar sampling method was used for the treatment experiments. Each treatment was replicated three times. These materials were used for metabolite analysis, RNA extraction for transcriptome sequencing or qRT-PCR analysis. Transcriptomic data from various experimental treatments (MeA, polyethylene glycol, NaCl and cold, and shade) were retrieved from previous studies and the Tea Plant Information Archive (http://tpia.teaplant.org/index.html).

RNA extraction, transcriptome sequencing, and quantitative real-time polymerase chain reaction (qRT-PCR) analysis

For transcriptome analysis, total RNA was isolated from tissues using an RNAprep Pure Plant Kit and treated with DNase I (Tiangen; http://www.tiangen.com). The RNA concentration and quality were assessed using a Thermo 2000 Bioanalyzer and an RNA NanoDrop ND-2000 Spectrophotometer (Thermo Fisher Scientific, Co., Ltd., Shanghai, China). The purified RNA was reverse transcribed to cDNA and then sequenced on an Illumina HiSeq 2500 platform by BGI Shenzhen Biotechnology Company according to routine processes as described previously. The reads per kilobase per million mapped reads (RPKM) and read counts were calculated using eXpress.

For miRNA analysis, total RNA was extracted from tea plant tissues using a TRizol kit (Transgen Biotechnology Co., Ltd., Beijing, China) and reverse transcribed into cDNA using a PrimeScript™ RT Reagent Kit together with gDNA Eraser (Takara Biotechnology Co., Ltd., Dalian, China) according to the manufacturer’s instructions. Information about the mature microRNAs in tea plant buds and young leaf tissues, including miR319s, miR164s, miR396s, and miR165/166s, was obtained from previous microRNA sequencing results. These mature miRNAs were reverse transcribed and measured using a PrimeScript™ RT Reagent Kit (Perfect Real Time) (Takara Biotechnology Co., Ltd., Dalian, China). For miRNA, 0.5 μg of total RNA in a 10-μL volume, 2 μL of 5× PrimeScript buffer, 0.5 μL of PrimeScript RT Enzyme Mix I, 0.5 μL of miRNA-RT primer, 0.5 μL of random hexamers, and RNase-free dH2O were used. The PCR conditions were 42 °C for 60 min followed by 95 °C for 3 min, after which dilution to 150 ng/μL with water was performed. A SYBR Premix Ex Taq Kit (Takara Biotechnology Dalian...
Co., Ltd., Dalian, China) was used for qRT-PCR on a Bio-Rad qPCR fluorescence quantitative PCR platform. Five microliters of qRT-PCR product was subjected to 2% agarose gel electrophoresis. CsmiR222 was used as an internal reference for normalization of miRNA expression levels. The primers used for qRT-PCR of miRNAs are listed in Supplementary Table S1. For qRT-PCR, CsACTIN was used as the reference for normalization using the primer pairs listed in Supplementary Table S1. A SYBR Premix Ex Taq kit was used for qRT-PCR on a Bio-Rad iQ5 fluorescence quantitative PCR platform according to a previously described method.

Identification of CsTCP family genes in the tea plant genome

The sequences of the TCP proteins of Arabidopsis and rice were retrieved from the TAIR (https://www.arabidopsis.org/) and rice genome databases (http://rice.plantbiology.msu.edu/), respectively. The sequences from Arabidopsis were subjected to a multiple-database search against the tea plant genome sequence, which was downloaded from the Tea Plant Information Archive (http://tpia.teaplant.org/index.html). The amino acid sequences were aligned using ClustalW, and MEGA 6.0 software was used to construct a phylogenetic tree by the NJ method, with 1000 bootstrap replicates. The ExPASy proteomics server (http://expasy.org/) was used to predict the isoelectric point and molecular weight of the CsTCP proteins. The exon/intron structures of individual CsTCP genes were defined by comparing the coding sequences and corresponding genomic sequences via the Gene Structure Display Server (http://gsds1.cbi.pku.edu.cn/), and the conserved motifs were analyzed using the MEME program (http://meme-suite.org/). PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) was used to determine the distribution of putative cis-acting elements in the 1400-bp promoter sequence of 35 CsTCPs. The Pfam (http://pfam.xfam.org) and SMART (http://smart.embl-heidelberg.de) tools were subsequently used to identify conserved TCP domains and R domains. The identification of miRNA-targeted genes was performed according to a previously described method. To visualize the protein domain structures, IBS 1.0 software (http://www.mybiosoftware.com/ibs-illustrator-of-biological-sequences.html) was used.

Correlations of gene expression and metabolite accumulation

The transcriptomic data and metabolite data from 8 or 12 different tissues of the tea varieties Shuchazao and Longjin 43 were downloaded from the Tea Plant Information Archive (http://tpia.teaplant.org/index.html). The transcript levels of the CsTCP genes in each tissue were calculated using the log10(FPKM) value, after which R software was used to visualize the CsTCP expression patterns. The transcriptomic data concerning the expression level in ten tea plant tissues (first, second, third, fourth, and fifth leaves; old leaves; and first, second, third, and fourth internodes of the stems) were used for correlation analysis according to the method above. Metabolite profiling was performed by the use of high-performance liquid chromatography (HPLC). To identify TCPs associated with flavonoids, caffeine, and theaflavins, correlation analysis between the CsTCP genes and these metabolites was performed by the use of Pearson’s correlation coefficients. Correlations whose correlation coefficient (r) was >0.5 and whose p-value was <0.05 were considered statistically significant. In the representative figure, blue color means a positive correlation, and red color means a negative correlation. Transcriptome and metabolic profiling data sets were obtained from different tissues of tea plants.

Yeast two-hybrid assays and BiFC assays

The ORFs of CsTCP3 (GenBank accession No. MW071231), CsTCP4 (GenBank accession No. MW071232), CsTCP14 (GenBank accession No. MW071233), CsTT8a, CsTT2a, CsMYBL2, CsMYB75, and CsTTG1 were cloned via pairs of gene-specific primers that have specific restriction sites (Supplementary Table S1). Protein–protein interaction assays were conducted according to previously described methods. For subcellular localization assays, the ORFs of CsTCP3 and CsTT8a were cloned and inserted into pCAMBIA1300 (CAMBIA, Canberra, Australia) vectors by the in-fusion technique. The constructs were subsequently fused to the N-terminus of nCherry, after which the transformants were transformed into Agrobacterium tumefaciens strain GV3101. Nicotiana benthamiana leaves were infiltrated with agrobacteria harboring the constructs for transient expression of fusion proteins as described previously.

For BiFC assays, CsTCP3 and CsTT8a ORFs were amplified and subcloned into pCambia1300-eYFPN (the YFP N-terminal region) and pCambia1300-eYFPc (the YFP C-terminal region) vectors according to the in-fusion technique. The resulting constructs were then transformed into A. tumefaciens strain GV3101, the cells of which were infiltrated into N. benthamiana leaves separately or in different combinations. A Leica DMi8 M laser scanning confocal microscopy system (Leica Microsystems, Wetzlar, Germany) was used for fluorescence observations, according to previously described methods.

Transactivation assays of CsANS1 and CsANR1 promoters

Genomic sequences that were ~2 kb upstream of the translation start codon of both the CsANR1 and CsANS1 genes were amplified from Camellia sinensis genomic DNA.
by the use of primers (Supplementary Table S1), after which the sequences were subcloned into p2GW17 (http://gateway.psb.ugent.be) vectors, resulting in promoter::luciferase reporter constructs. Similarly, effectors (CsTT2a, CsMYB75, CsTTG1, CsTT2a, CsTCP3, CsTCP14) were cloned into p2GW7 vectors by LR reactions to form 35S::effector constructs by the use of previously constructed vectors. Promoter transactivation assays were conducted according to described previously methods.

**Suppression of CsmiR319b in tea shoot tips**

Since transformation techniques have not yet been developed specifically for tea plant, the knockdown of candidate genes with antisense oligonucleotides (asODNs) containing complementary segments of the target gene was performed to determine how CsmiR319b affects CsTCP3 and -4 expression and catechin production in tea shoot tips, according to previously described method. The antisense oligonucleotides for CsmiR319b were designed according to Soligo software (http://sfold.wadsworth.org/cgi-bin/soligo.pl), with the CsmiR319b sequence used as an input sequence (Supplementary Table S1). To silence CsmiR319b expression, freshly detached healthy apical buds and 1st leaves from at least 5 tea plants of the variety Baihaozao were incubated in 1.5 mL Eppendorf tubes that contained 33 least 5 tea plants of the variety Baihaozao were incubated in a fresh 10 mM sucrose solution together with sense ODNs solution for various times. Shoot tips incubated in a 10 mM sucrose solution together with sense ODNs (sODNs) were used as controls. The shoot tips were sampled at different time intervals to analyze the RNA and catechin levels.

**Data analysis**

The experiments were performed for at least three biological repeats. Statistical analysis was performed using either Student’s two-tailed t-test when comparing treatments with controls or multiple comparisons together with the ANOVA multiple range test at the 0.05 probability level (p < 0.05).

**Acknowledgements**

The authors acknowledge support from the National Key Research and Development Program of China (2018YFD1000601), the Key Research and Development (R&D) Program of Anhui Province (18030701155), funding from Anhui Agricultural University, and funding from the State Key Laboratory of Tea Plant Biology and Utilization.

**Author contributions**

J.Z. planned and designed the research. S.Y., X.Z., Y.X., and Q.W. performed the experiments. S.Y., X.Z., and X.S.Z. analyzed the data. J.Z., S.Y., and M.T. wrote and revised the article.

**Data availability**

The author responsible for the distribution of materials integral to the findings presented in this article in accordance with the policy described in the instructions for authors is Jan Zhao (jianzhao@ahau.edu.cn).

**Conflict of interest**

The authors declare no competing interests.

**Supplementary information**

The online version contains supplementary material available at https://doi.org/10.1038/s41438-021-00538-7.

Received: 11 October 2020 Revised: 1 January 2021 Accepted: 24 February 2021 Published online: 01 May 2021

**References**

1. Zhao, J. Li, P. H., Xia, T. & Wán, X. C. Exploring plant metabolic genomics: chemical diversity, metabolic complexity in the biosynthesis and transport of specialized metabolites with the tea plant as a model. Crit. Rev. Biotechnol. **40**, 667–688 (2020).
2. Yang, C. S. & Hong, J. Prevention of chronic diseases by tea: possible mechanisms and human relevance. Annu. Rev. Nutr. **33**, 161–181 (2013).
3. Sun, B. et al. Purple foliage coloration in tea (Camellia sinensis L.) arises from activation of the R2R3-MYB transcription factor Csmi1. Sci. Rep. **6**, 32534 (2016).
4. Liu, Y. J. et al. A WD40 repeat protein from Camellia sinensis regulates anthocyanin and proanthocyanidin accumulation through the formation of MYB-BiHL-WD40 ternary complexes. *Int. J. Mol. Sci.* **19**, 1688 (2018).
5. Wei, K. et al. A coupled role for CsmiR319b and CsGT5F1 in anthocyanin hyperaccumulation in purple tea. *Plant J.* **97**, 825–840 (2019).
6. Rodriguez, R. E. et al. Control of cell proliferation in Arabidopsis thaliana by microRNA miR396. Development **137**, 103–112 (2010).
7. Effron, I., Esheid, Y. & Lifshitz, E. Morphogenesis of simple and compound leaves: a critical review. *Plant Cell.* **22**, 1019–1032 (2010).
8. Challa, K. R., Rath, M. & Nath, U. The CIN-TCP transcription factors promote commitment to differentiation in Arabidopsis leaf pavement cells via both auxin-dependent and independent pathways. *PLoS Genet.* **15**, e1007988 (2019).
9. Wu, Z., Wang, W. & Zhuang, J. TCP family genes control leaf development and its responses to hormonal stimuli in tea plant [Camellia sinensis (L.) O. Kuntze]. Plant Growth Regul. **83**, 45–53 (2017).
10. Doebley, J., Stec, A. & Hubbard, L. The evolution of apical dominance in maize. *Nature* **386**, 485–488 (1997).
11. Kosugi, S. & Ohashi, Y. PCF1 and PCF2 specifically bind to cis elements in the rice proliferating cell nucleotide antigen gene. *Plant Cell.* **9**, 1607–1619 (1997).
12. Cubas, P., Lauter, N., Doebely, J. & Coen, E. The TCP domain: a motif found in proteins regulating plant growth and development. *Plant J.* **18**, 215–222 (1999).
13. Manassero, N. G., Viola, I. L., Welchen, E. F. & Gonzalez, D. H. TCP transcription factors architectures of plant form. *Biomol. Concepts* **4**, 111–127 (2013).
14. Kosugi, S. & Ohashi, Y. DNA binding and dimerization specificity and potential targets for the TCP protein family. *Plant J.* **30**, 337–348 (2002).
15. Li, C., Potuschak, T., Colón-Carmona, A., Gutierrez, R. A. & Doerner, P. Arabidopsis TCP20 links regulation of growthand cell division control pathways. *Proc. Natl Acad. Sci. USA* **102**, 12978–12983 (2005).
16. Nath, U., Crawford, B., Carpenter, R. & Coen, E. Genetic control of surface curvature. *Science* **299**, 1404–1407 (2003).
17. Crawford, B., Nath, U., Carpenter, R. & Coen, E. CINNACINA controls both cell differentiation and growth in petal lobes and leaves of Antirrhinum. *Plant Physiol.* **135**, 244–253 (2004).
18. Koyama, T., Futurani, M., Tasa, K. & Ohme-Takagi, M. TCP transcription factors control the morphology of shoot lateral organs via negative regulation of the expression of boundary-specific genes in Arabidopsis. *Plant Cell.* **19**, 475–484 (2007).
19. Koyama, T., Mitsuda, N., Seki, M., Shinohzaki, K. & Ohme-Takagi, M. TCP transcription factors regulate the activities of ASYMMETRIC LEAVES1 and miR164, as well as the auxin response, during differentiation of leaves in Arabidopsis. *Plant Cell.* **22**, 3574–3588 (2010).
20. Schommer, C. et al. Control of jasmonate biosynthesis and senescence by miR319 targets. *PLoS Biol.* **6**, e230 (2008).
21. Nag, A. & King, S. Jack T miR319a targeting of TCP4 is critical for petal growth and development in Arabidopsis. *Proc. Natl Acad. Sci. USA* **106**, 22534–22539 (2009).
22. Palatnik, J. et al. Control of leaf morphogenesis by microRNAs. *Nature* **425**, 257–263 (2003).

23. Aguilar-Martínez, J., Poza-Camion, C. & Cubas, P. Arabidopsis BRANCHED1 acts as an integrator of branching signals within axillary buds. *Plant Cell* **19**, 458–472 (2007).

24. Takeda, T. et al. The OsTB1 gene negatively regulates lateral branching in rice. *Plant J.* **33**, 513–520 (2003).

25. Resentini, F. et al. TCP14 and TCP15 mediate the promotion of seed germination by gibberellins in Arabidopsis thaliana. *Mol. Plant* **8**, 482–485 (2015).

26. Kieffer, M., Master, V., Waites, R. & Davies, B. TCP14 and TCP15 affect internode length and leaf shape in Arabidopsis. *Plant J.* **68**, 147–158 (2011).

27. Davière, J. M. et al. TCP-DELLA interactions in inflorescence shoot apex determine plant height. *Curr. Biol.* **24**, 1923–1928 (2014).

28. Viola, I. L., Camoriano, A. & Gonzalez, D. H. Redox-dependent modulation of anthocyanin biosynthesis by the TCP transcription factor TCP15 during exposure to high light intensity conditions in Arabidopsis. *Plant Physiol.* **170**, 74–85 (2016).

29. Li, S. & Zachgo, S. TCP3 interacts with RORS-MVB proteins, promotes flavonoid biosynthesis and negatively regulates the auxin response in Arabidopsis thaliana. *Plant J.* **76**, 901–913 (2013).

30. Debernardi, J. M. et al. Post-transcriptional control of GFF transcription factors by microRNA miR396 and GFF co-activator affects leaf size and longevity. *Plant J.* **79**, 413–426 (2014).

31. Micale, P. et al. Regulation of MR165/166 by class II and class III homeodomain leucine zipper proteins establishes leaf polarity. *Proc. Natl Acad. Sci. USA* **113**, 11973–11978 (2016).

32. Liebisch, D., Javier, F. & Palatnik, J. F. MicroRNA miR396, GFF transcription factors and GFF co-regulators: a conserved plant growth regulatory module with potential for breeding and biotechnology. *Curr. Opin. Plant Biol.* **53**, 31–42 (2019).

33. Palatnik, J. et al. Sequence and expression differences underlie functional specialization of Arabidopsis microRNAs miR159 and miR319. *Dev. Cell* **13**, 115–125 (2007).

34. Koyama, T., Sato, F. & Ohme-Takagi, M. Roles of miR319 and TCP transcription factors in leaf development. *Plant Physiol.* **175**, 874–885 (2017).

35. Ori, N. et al. Regulation of LANCEOLEATE by miR319 is required for compound leaf development in Arabidopsis. *Nat. Genet.* **39**, 787–791 (2007).

36. Maugerny-Calès, A. & Laufs, P. Getting leaves into shape: a molecular, cellular, environmental and evolutionary view. *Develop. Growth* **45**, dev161646 (2018).

37. Shkleier-Burko, S., Burko, Y., Ben-Hezil, O. & Ori, N. Dynamic growth program regulated by LANCEOLEATE enables flexible leaf patternning. *Development* **138**, 695–704 (2011).

38. Yanai, O., Shani, E., Russ, D. & Ori, N. Gibberellin partly mediates LANCEOLEATE expression by miR319-regulated TCP transcription factor family with potential for breeding and biotechnology. *Curr. Opin. Plant Biol.* **53**, 31–42 (2019).

39. Koyama, T., Sato, F. & Ohme-Takagi, M. Roles of miR319 and TCP transcription factors in leaf development. *Plant Physiol.* **175**, 874–885 (2017).

40. Ori, N. et al. Regulation of LANCEOLEATE by miR319 is required for compound leaf development in Arabidopsis. *Nat. Genet.* **39**, 787–791 (2007).

41. Shkleier-Burko, S., Burko, Y., Ben-Hezil, O. & Ori, N. Dynamic growth program regulated by LANCEOLEATE enables flexible leaf patternning. *Development* **138**, 695–704 (2011).

42. Yanai, O., Shani, E., Russ, D. & Ori, N. Gibberellin partly mediates LANCEOLEATE expression by miR319-regulated TCP transcription factor family with potential for breeding and biotechnology. *Curr. Opin. Plant Biol.* **53**, 31–42 (2019).

43. Liu, L. et al. Growth alterations and deregulates the expression of many genes important for development. *Plant Physiol.* **176**, 1694–1708 (2018).

44. Hervé, C. et al. In vivo interaction with ATTCP20 function induces severe plant growth alterations and deregulates the expression of many genes important for development. *Plant Physiol.* **149**, 1462–1477 (2009).

45. Danisman, S. et al. Analysis of functional redundancies within the Arabidopsis TCP transcription factor family. *J. Exp. Bot.* **64**, 5673–5685 (2013).

46. Danisman, S. et al. Arabidopsis class I and II TCP transcription factors regulate jasmonic acid metabolism and leaf development antagonistically. *Plant Physiol.* **159**, 1511–1523 (2012).

47. Zhang, Y. et al. Identification and characterization of cold-responsive microRNAs in tea plant (*Camellia sinensis*) and their targets using high-throughput sequencing and degradation analysis. *BMC Plant Biol.* **21**, 271 (2014).

48. Jeyaraj, A., Chandran, V. & Gajjeraman, P. Differential expression of microRNAs in dormant bud of tea (*Camellia sinensis*) (L.) O. Kuntze. *Plant Cell Rep.* **33**, 1053–1069 (2014).

49. Yeh, I. et al. Expression of seed flavonoids. *Ann. Rev. Plant Biol.* **57**, 405–430 (2006).

50. Shi, J. et al. Transcriptional responses and flavor volatiles biosynthesis in methyl jasmonate-treated tea leaves. *BMC Plant Biol.* **15**, 233 (2015).

51. Zhang, Q. et al. Transcriptome dynamics of Medicago truncatula under continuous salinity and drought stress. *Plant J.* **84**, 405–416 (2015).

52. Wolak, T. W. et al. WOX9 functions antagonistic to STF and LAM1 to regulate leaf blade expansion in Medicago truncatula and Nicotiana sylvestris. *New Phytol.* https://doi.org/10.1111/nph.16994 (2020).

53. Ballester, P., Navarrete-Gómez, M., Carbonero, P., Orueta-Sánchez, L. & Ferrándiz, C. Leaf expansion in Arabidopsis is controlled by a TCP-NGA regulatory module likely conserved in distantly related species. *Physiol. Plant.* **155**, 21–32 (2015).

54. Dong, L. et al. Transcriptome analysis of tea processing suitability. *Plant Cell, Tissue & Organ Culture* **121**, 91–102 (2016).

55. Palatnik, J. et al. Control of leaf morphogenesis by microRNAs. *Nature* **425**, 257–263 (2003).

56. Aguilar-Martínez, J., Poza-Camion, C. & Cubas, P. Arabidopsis BRANCHED1 acts as an integrator of branching signals within axillary buds. *Plant Cell* **19**, 458–472 (2007).

57. Takeda, T. et al. The OsTB1 gene negatively regulates lateral branching in rice. *Plant J.* **33**, 513–520 (2003).

58. Resentini, F. et al. TCP14 and TCP15 mediate the promotion of seed germination by gibberellins in Arabidopsis thaliana. *Mol. Plant* **8**, 482–485 (2015).

59. Liu, L. et al. Growth alterations and deregulates the expression of many genes important for development. *Plant Physiol.* **176**, 1694–1708 (2018).

60. Hervé, C. et al. In vivo interaction with ATTCP20 function induces severe plant growth alterations and deregulates the expression of many genes important for development. *Plant Physiol.* **149**, 1462–1477 (2009).

61. Danisman, S. et al. Analysis of functional redundancies within the Arabidopsis TCP transcription factor family. *J. Exp. Bot.* **64**, 5673–5685 (2013).

62. Danisman, S. et al. Arabidopsis class I and II TCP transcription factors regulate jasmonic acid metabolism and leaf development antagonistically. *Plant Physiol.* **159**, 1511–1523 (2012).

63. Zhang, Y. et al. Identification and characterization of cold-responsive microRNAs in tea plant (*Camellia sinensis*) and their targets using high-throughput sequencing and degradation analysis. *BMC Plant Biol.* **21**, 271 (2014).

64. Jeyaraj, A., Chandran, V. & Gajjeraman, P. Differential expression of microRNAs in dormant bud of tea (*Camellia sinensis*) (L.) O. Kuntze. *Plant Cell Rep.* **33**, 1053–1069 (2014).

65. Lepniec, I. et al. Genetics and biochemistry of seed flavonoids. *Ann. Rev. Plant Biol.* **57**, 405–430 (2006).