Abstract: Teosinte branched1/cycloidea/proliferating cell factor (TCP) gene family members are plant-specific transcription factors that regulate plant growth and development by controlling cell proliferation and differentiation. However, there are no reported studies on the TCP gene family in *Dendrobium catenatum* Lindl. Here, a genome-wide analysis of TCP genes was performed in *D. catenatum*, and 25 TCP genes were identified. A phylogenetic analysis classified the family into two clades: Class I and Class II. Genes in the same clade share similar conserved motifs. The GFP signals of the DcaTCP-GFPs were detected in the nuclei of tobacco leaf epidermal cells. The activity of DcaTCP4, which contains the miR319a-binding sequence, was reduced when combined with miR319a. A transient activity assay revealed antagonistic functions of Class I and Class II of the TCP proteins in controlling leaf development through the jasmonate-signaling pathway. After different phytohormone treatments, the DcaTCP genes showed varied expression patterns. In particular, DcaTCP4 and DcaTCP9 showed opposite trends after 3 h treatment with jasmonate. This comprehensive analysis provides a foundation for further studies on the roles of TCP genes in *D. catenatum*.

Keywords: *Dendrobium catenatum* Lindl.; TCP transcription factor family; phylogenetic analysis; expression profiles; phytohormone response

1. Introduction

The TCP transcription factor family was designated on the basis of its original identified members, TEOSINTE BRANCHED1 (TB1) in maize (*Zea mays*) that functions to maintain apical dominance [1], CYCLOIDEA (CYC) in snapdragon (*Antirrhinum majus*) that controls floral symmetry [2], and PROLIFERATING CELL FACTORS 1/2 in rice (*Oryza sativa*) that is associated with the cell cycle, regulates DNA replication and repair, maintains chromosomal morphology and structure, and controls chromosomal segregation [3,4]. The family members contain a highly conserved non-canonical basic helix–loop–helix motif that is involved in DNA-binding and protein–protein interactions. It occurs in 59 amino acids at the N-terminus within the designated TCP domain [5]. The TCP family members are classified into two subfamilies on the basis of their TCP domains, Class I (PCF or TCP-P class) and Class II (TCP-C class), and the members of class II are divided into the CIN and CYC/TB1 [5–7]. Compared with Class II, Class I members have 4-aa deletions in their TCP domains. In addition, some members of Class II have an arginine-enriched motif of 18 to 20 aa residues, referred to as an R domain, and an ECE motif, which is composed of a glutamic acid–cysteine-glutamic acid stretch.

The TCP transcription factor family has been identified in many plant species. For example, *Arabidopsis thaliana* has 24 TCP genes, *Oryza sativa* has 28 TCP genes, *Lycopersicon esculentum* has 30 TCP genes, *Populus euphratica* has 33 TCP genes, *Populus trichocarpa* has 36 TCP genes, *Citrullus lanatus* has 27 TCP genes, *Prunus mume* has 19 TCP genes,
and *Petunia inflata* has 34 TCP genes [5,8–12]. The TCP genes have a variety of functions, participating in the regulation of numerous growth and developmental processes, such as the coordination of cell proliferation, inflorescence development, shoot branching, leaf development, phytohormone biosynthesis, and the circadian clock regulation [13–24].

*Dendrobium catenatum* Lindl. belongs to the Orchidaceae family, and it is a rare and precious Chinese herbal medicinal plant. It is mainly distributed in Southeast, South, and Southwest China, and it usually grows on cliffs to receive sufficient sunlight and adequate water in the wild [25,26]. Here, we identified 25 TCP genes in *D. catenatum* and analyzed their phylogenetic relationships, gene architectures, conserved domain profiles, and subcellular localizations. Furthermore, the expression levels of *DcaTCP* genes after different phytohormone treatment were examined.

2. Results

2.1. Identification of TCP Family Genes in *D. catenatum*

The release of the complete *D. catenatum* genome allowed the genome-wide identification of genes. In the present study, BLAST searches were used to identify *DcaTCP*s in the *D. catenatum* genome. The obtained sequences were further verified using the HMM in SMART and Pfam. In total, 25 TCP-like sequences that possessed the conserved TCP motif were identified from the *D. catenatum* genome. The *DcaTCP* genes were annotated following the nomenclature of *A. thaliana* in accordance with protein sequence similarities (Figure 1).

![Figure 1. Phylogenetic tree of TCP proteins from *D. catenatum* and *Arabidopsis*. The phylogenetic tree was generated using the neighbor-joining (NJ) method implemented in the MEGA 7.0 software with JTT model and the pairwise gap deletion option. Bootstrap analysis was conducted with 1000 iterations.

The *DcaTCP* proteins were classified into two subclades (subclade PCF within Class I, and subclades CYC/TB1 and CIN within Class II). Among them, 11 and 14 *DcaTCP*s clustered into Class I (PCF) and Class II, respectively. A sequence analysis revealed that *AtTCP8, -10, -22*, and -24 had no orthologs in *D. catenatum*, whereas *AtTCP2* had more than one ortholog in the genome.
The lengths of the DcaTCPs ranged from 178 (DcaTCP1) to 450 (DcaTCP2b) aa, the molecular weights ranged from 20.03 (DcaTCP1) to 49.60 (DcaTCP2b) kDa, and the theoretical isoelectric point values ranged from 4.808 (DcaTCP2e) to 10.017 (DcaTCP7). The calculated grand average of hydrophobicity values ranged from $-13.867$ (DcaTCP2e) to $12.969$ (DcaTCP1), indicating that most of the DcaTCP proteins were hydrophobic, except for DcaTCP2a, -2b, -2e, -5, -6, and -13 (Table 1).

| Gene Name | Accession Number | CDS Length (bp) | Protein Size (aa) | MW (kD) | PI | GRAVY |
|-----------|------------------|-----------------|-------------------|---------|----|-------|
| DenTCP1   | XP_028548590     | 537             | 178               | 20.03   | 9.809 | 12.969 |
| DenTCP2   | XP_020673786     | 1170            | 389               | 42.07   | 8.417 | 5.644 |
| DenTCP2a  | XP_028551220     | 1221            | 406               | 44.45   | 6.723 | 1.370 |
| DenTCP2b  | XP_020679251     | 1353            | 450               | 49.60   | 6.875 | 0.761 |
| DenTCP2c  | XP_020703327     | 1122            | 373               | 40.56   | 8.798 | 7.145 |
| DenTCP2d  | XP_020675099     | 1110            | 369               | 40.64   | 8.402 | 3.993 |
| DenTCP2e  | XP_028551222     | 978             | 325               | 35.19   | 4.808 | 13.867 |
| DenTCP3   | XP_028553615     | 672             | 223               | 24.98   | 9.531 | 6.732 |
| DenTCP4   | XP_020674146     | 1236            | 411               | 45.23   | 9.346 | 9.242 |
| DenTCP5   | XP_028553819     | 927             | 308               | 34.67   | 6.765 | 0.807 |
| DenTCP6   | XP_028550088     | 903             | 300               | 31.82   | 6.740 | 1.591 |
| DenTCP7   | XP_020688226     | 837             | 278               | 29.78   | 10.017| 9.840 |
| DenTCP9   | XP_020701159     | 879             | 292               | 30.26   | 9.460 | 5.754 |
| DenTCP10  | XP_020699509     | 732             | 243               | 25.64   | 7.132 | 0.397 |
| DenTCP12  | XP_028556330     | 1026            | 341               | 37.73   | 7.472 | 1.493 |
| DenTCP13  | XP_020702002     | 828             | 275               | 30.77   | 6.019 | 3.960 |
| DenTCP14  | XP_020695302     | 801             | 266               | 28.62   | 8.720 | 3.578 |
| DenTCP15  | XP_020697754     | 1107            | 368               | 38.87   | 8.131 | 4.875 |
| DenTCP16  | XP_020686130     | 636             | 211               | 22.32   | 8.154 | 1.389 |
| DenTCP17  | XP_020702494     | 933             | 310               | 35.05   | 7.097 | 0.265 |
| DenTCP18  | XP_028550169     | 978             | 325               | 36.22   | 7.172 | 0.662 |
| DenTCP19  | XP_020673638     | 954             | 317               | 33.20   | 8.933 | 3.916 |
| DenTCP20  | XP_020693581     | 900             | 299               | 31.70   | 8.204 | 2.740 |
| DenTCP21  | XP_020682251     | 816             | 271               | 27.21   | 9.723 | 6.533 |
| DenTCP23  | XP_020696150     | 1053            | 350               | 35.96   | 9.514 | 6.902 |

Table 1. Identification and characteristics of TCP genes in D. catenatum.

2.2. Conserved Domain and Motif Analysis

To better reveal the diversification among the TCP genes in D. catenatum, the DcaTCPs’ conserved motifs were analyzed. An NJ phylogenetic tree was constructed using the DcaTCP protein sequences (Figure 2A). The MEME online tool was used to predict the conserved motif compositions of the DcaTCPs (Figure 2B). The number of motifs varied from 1 to 11. Motif 1, as the TCP domain, was identified in all the DcaTCPs. In addition, other motifs were only present in members of specific subclades, such as Motifs 3, 9, and 11 in DcaTCP2, -2a, -2b, -2c, -2d, and -2e; Motifs 8, 14, and 18 in DcaTCP6, -11, and -20; Motif 15 in DcaTCP1, -12, and -18; and Motif 20 in DcaTCP5 and -17, suggesting that they have subclade-specific functions. In Arabidopsis, miR319a controls JA biosynthesis and leaf senescence by cleaving TCP transcription factors [14,20]. AtTCP2, -3, -4, -10, and -24, which belong to the CIN clade, are miR319a-targeted genes in Arabidopsis. miR319a is a conserved microRNA that regulates the expression of CIN subclade members [3,18,27]. Therefore, we speculated that the DcaTCP genes were miR319a targets in D. catenatum. miRNA-target complementarity analysis showed that DcaTCP4, a member of CIN family, might be a target gene of miR319a (Figure 2C).
miRNA-target complementarity analysis showed that DcaTCP4, a member of CIN family, might be a target gene of miR319a (Figure 2C).

2.3. Subcellular Localization

The known members of the TCP gene family function as transcription factors that regulate specific plant growth and developmental processes. Four DcaTCPs genes, DcaTCP9 and DcaTCP14 from Class I and DcaTCP2 and DcaTCP4 from Class II, were selected for the further analysis. The GFP gene was fused with each DcaTCP as a reporter. DcaTCP2, -4, -9, and -14 were all detected in nuclei (Figure 3), which suggests that they may function as transcription factors.

Figure 3. Subcellular localization of DcaTCP-GFP in Nicotiana benthamiana leaves. DcaTCP2-GFP, DcaTCP4-GFP, DcaTCP9-GFP and DcaTCP14-GFP were localized in the nucleus. GFP (Green Fluorescent Protein) is a versatile biological marker for visualizing protein localization. DAPI (4’,6-diamidino-2-phenylindole) is a blue-fluorescent DNA stain, which is used as a nuclear counterstain in fluorescence microscopy. Bar = 50 μm.
miRNA-target complementarity analysis indicated that DcaTCP4 may be a target gene of miR319a. To test this hypothesis, we co-overexpressed DcaTCP4-GFP with 35s:miR319a in accordance with the protocol of Liu [28]. The expression level of DcaTCP4-GFP co-injected with 35s:miR319a led to a significant downregulation compared with DcaTCP4-GFP alone (Figure 4). However, there was no obvious difference in expression between DcaTCP9-GFP and DcaTCP9-GFP with 35s:miR319a. Thus, miR319a appeared to recognize and cleave DcaTCP4, as in Arabidopsis.

Figure 3. Subcellular localization of DcaTCP-GFP in Nicotiana benthamiana leaves. DcaTCP2-GFP, DcaTCP4-GFP, DcaTCP9-GFP and DcaTCP14-GFP were localized in the nucleus. GFP (Green Fluorescent Protein) is a versatile biological marker. DAPI (4',6-diamidino-2-phenylindole) is a blue fluorescent DNA stain, which is used as a nuclear counterstain in fluorescence microscopy. Bar = 50 μm.

2.4. Class I and II TCPs Antagonistically Regulate LOX2 Expression

Class I and II TCP transcription factors in Arabidopsis have been reported to antagonistically control LOX2 expression [29]. Consequently, we speculated that Class I and II TCP transcription factors in D. catenatum regulated LOX2 expression by the same mechanism. To test this hypothesis, the AtLOX2 promoter contained the TCP protein binding site (GTG-GTCC and GGACCAC) (Figure 5A) was used to determine whether Class I (DcaTCP2 and DcaTCP4) and Class II (DcaTCP9) TCP transcription factors regulate the expression of the AtLOX2 gene. When the ProAtLOX2:LUC reporter plasmid was co-transfected with the 35S:DcaTCP2 or 35S:DcaTCP4 effector plasmid, a strong LUC activity was detected. However, in the absence of the effector 35S:DcaTCP2 or 35S:DcaTCP4 plasmid, the LUC activity was much lower (Figure 5B,C,E). Thus, DcaTCP2 and DcaTCP4 indeed activated the transcription of AtLOX2 in vivo. However, when the ProAtLOX2:LUC reporter plasmid was co-transfected with the 35S:DcaTCP9 effector plasmid, a weak LUC activity was detected, but in the absence of the effector 35S:DcaTCP9 plasmid, the LUC activity was much greater (Figure 5D,E). Thus, DcaTCP9 inhibited the transcription of AtLOX2 in vivo. In conclusion, the Class I DcaTCPs (DcaTCP9) inhibited the expression of AtLOX2, and the Class II DcaTCPs (DcaTCP2 and DcaTCP4) activated AtLOX2.
2.4. Class I and II TCPs Antagonistically Regulate LOX2 Expression

Class I and II TCP transcription factors in Arabidopsis have been reported to antagonistically control LOX2 expression [29]. Consequently, we speculated that Class I and II TCP transcription factors in D. catenatum regulated LOX2 expression by the same mechanism. To test this hypothesis, the AtLOX2 promoter contained the TCP protein binding site (GTGGTCC and GGACCAC) (Figure 5A) was used to determine whether Class I (DcaTCP2 and DcaTCP4) and Class II (DcaTCP9) TCP transcription factors regulate the expression of the AtLOX2 gene. When the ProAtLOX2:LUC reporter plasmid was co-transfected with the 35S:DcaTCP2 or 35S:DcaTCP4 effector plasmid, a strong LUC activity was detected. However, in the absence of the effector 35S:DcaTCP2 or 35S:DcaTCP4 plasmid, the LUC activity was much lower (Figure 5B, C, E). Thus, DcaTCP2 and DcaTCP4 indeed activated the transcription of AtLOX2 in vivo. However, when the ProAtLOX2:LUC reporter plasmid was co-transfected with the 35S:DcaTCP9 effector plasmid, a weak LUC activity was detected, but in the absence of the effector 35S:DcaTCP9 plasmid, the LUC activity was much greater (Figure 5D, E). Thus, DcaTCP9 inhibited the transcription of AtLOX2 in vivo. In conclusion, the Class I DcaTCPs (DcaTCP9) inhibited the expression of AtLOX2, and the Class II DcaTCPs (DcaTCP2 and DcaTCP4) activated AtLOX2.

2.5. Expression Profiles of TCP Genes in D. catenatum

Gene expression patterns are correlated with their functions [30]. To explore the possible functions of DcaTCP genes in D. catenatum, transcriptomic data were used to determine the expression changes of DcaTCP gene family members exposed to different phytohormone treatments.

As shown in Figure 6, at 3 h after the JA treatment, the expression of DcaTCP4 increased significantly, reaching the highest level, but the expression of DcaTCP9 decreased. These results are consistent with antagonistic functions of Class I and II TCP proteins in the control of JA biosynthesis. DcaTCP2 has six homologs in D. catenatum, and their expression levels varied. The expression of DcaTCP2 increased in response to ABA and IAA treatments. The expression of DcaTCP2d increased after exposure to the JA treatment, but decreased after exposure to other phytohormone treatments. DcaTCP2a, -2b, and -2e had similar responses to the different phytohormone treatments. Thus, the DcaTCP2 homologs appeared to have undergone functional differentiation during evolution.
2.5. Expression Profiles of TCP Genes in *D. catenatum*. To better understand the structural and functional features of DcaTCPs, 20 conserved motifs were investigated (Figure 2B), and the DcaTCP proteins within the same subclades shared similar motifs. These results might explain the fundamental redundancy that occurs among members of the same subclade.

Representative DcaTCP genes, DcaTCP9 and DcaTCP14 from Class I and DcaTCP2 and DcaTCP4 from Class II, were selected to analyze the functions of DcaTCPs in *D. catenatum*. They all localized in the nucleus (Figure 3), which demonstrated that the DcaTCPs might act as transcription factors to regulate plant growth and development. In *Arabidopsis*, Class I and II TCP proteins play opposite regulatory roles in the expression of *AtLOX2* by binding to its promoter. *AtTCP4* activates the expression of *AtLOX2*, whereas *AtTCP9* inhibits the expression of *AtLOX2* [20]. *AtLOX2* is a key enzyme in the JA-synthesis pathway, which regulates the synthesis of anthocyanins and the development of trichomes [33]. The antagonistic functions of Class I and II TCP proteins in the control of leaf development through the JA-signaling pathway have been documented previously [29]. Therefore, we investigated whether the functions of the two TCP subfamilies in *Dendrobium* were similar to those in *Arabidopsis*. DcaTCP2 and DcaTCP4 positively regulated the expression of *AtLOX2*, and DcaTCP9 negatively regulated the expression of *AtLOX2* (Figure 5), which was consistent with the regulation in *Arabidopsis*. This indicated that *Dendrobium* and *Arabidopsis* shared the same TCP regulatory element. This result also suggested that the functions of the TCP transcription factors were conserved between monocotyledons and dicotyledons.

Figure 6. Expression analysis of DcaTCP genes after treatment with ABA, IAA, JA and SA. ABA: abscisic acid, IAA: indole-3-acetic acid, JA: jasmonic acid, SA: salicylic acid. ck: control check, 3 h: phytohormone treatments after 3 h, 6 h: phytohormone treatments after 6 h.

3. Discussion

*D. catenatum* is the main component of several traditional medicines and health care products used to settle upset stomachs, promote body-fluid production, and nourish Yin [31,32]. The TCPs are crucial plant-specific transcription factors. The TCP transcription factors have been identified in many plant species. However, there are no reports on the TCP transcription factors in *D. catenatum*. Here, we identified 25 TCP members in the genome of the orchid *D. catenatum*. Using the sequences of 24 TCPs from *A. thaliana* and 25 TCPs from *D. catenatum*, we constructed a phylogenetic tree (Figure 1) and analyzed the phylogenetic relationships of DcaTCP genes (Figure 2A). To better understand the structural and functional features of DcaTCPs, 20 conserved motifs were investigated (Figure 2B), and the DcaTCP proteins within the same subclades shared similar motifs. These results might explain the fundamental redundancy that occurs among members of the same subclade.
Phytohormones are important signaling substances in plants. They play important roles in plant defense, fertilization, and growth and development. The TCP transcription factors are involved in the phytohormone-signaling pathways in some plant species [34]. To determine the expression profiles and understand the roles of DcaTCP genes in response to phytohormones, transcriptomic data after exposure to ABA, IAA, JA and SA treatments were used. The expression levels of DcaTCP genes were affected by the different phytohormone treatments (Figure 6). This indicated that DcaTCP transcription factors in D. catenatum were involved in phytohormone responses, which supported their roles in regulating plant growth and development. The mechanism behind the DcaTCP genes’ participation in various phytohormone responses need further study.

4. Materials and Methods

4.1. Plant Material and Phytohormone Treatments

Dendrobium catenatum Lindl. was cultivated in a 5:1 (w/w) soil: sand mixture. In a greenhouse, the plants were maintained at 24 °C with 60–80% relative humidity and a 16 h photoperiod (daytime, 06:00–20:00).

35s:miR319a was kindly provided by Diqiu Yu [35]. miR319a was cloned into binary vector pOCA30 to generate construct 35s:miR319a. ProLOX2:LUC was kindly provided by Chuanyou Li [36]. The LOX2 promoter was cloned into pENTR vector and then fused with the luciferase reporter binary vector pGWB35 using gateway reaction to generate the reporter construct ProLOX2:LUC.

Six-month-old plants were treated with 0.2 µM/L abscisic acid (ABA), 2 µM/L indole-3-acetic acid (IAA), 10 µM/L Jasmonate (JA) and 10 µM/L salicylic acid (SA) for 3 h or 6 h, respectively. Non-treatment plants were used as the control. Then, the samples were quickly frozen in liquid nitrogen and stored at −80 °C until use.

4.2. Identification of TCP Family Genes in D. catenatum

Data for D. catenatum proteins were downloaded from the D. catenatum Genome (http://orchidbase.itps.ncku.edu.tw/est/Dendrobium_2019.aspx, accessed on 5 June 2021). Using the following pipeline, TCP-like sequences were identified from publicly available D. catenatum sequences. First, the complete coding DNA sequences (CDSs) of 24 TCP genes from A. thaliana were extracted from the public database TAIR (https://www.arabidopsis.org/, accessed on 12 June 2021) (accession listed in Table S1), using local BLAST algorithm-based searches and a hidden Markov model (HMM) to identify the TCP genes [5,37]. Local BLASTN and BLASTP searches were conducted with known AtTCP sequences as the query to search the genome sequences of D. catenatum. These searches identified the initial candidate genes containing putative TCP domains in D. catenatum. The HMM profile (accession number PF03634) from the Pfam protein family database (http://pfam.sanger.ac.uk, accessed on 9 July 2021) was applied to confirm the presence of the conserved TCP domain in each candidate TCP protein [38]. Finally, the identified TCP-like sequences were double-checked for the presence of a functional TCP domain/motif using the NCBI Conserved Domains tool [39]. The retrieved sequences lacking a TCP domain were removed prior to further analyses.

4.3. Conserved Motif Analysis

The Multiple Em for Motif Elucidation (MEME) program (http://meme-suite.org/index.html, accessed on 16 July 2021) was used to analyze the conserved motifs of DcaTCP proteins, with the following site distribution parameters: (1) the optimum motif width was set from 6 to 50; and (2) the maximum number of motifs to identify was set as 20. Additionally, each sequence that appeared at least once was used [40].

4.4. Sequence Characterization and Phylogenetic Analysis

LaserGene7 was used to further analyze the identified DcaTCPs, including their CDS lengths, protein sizes, protein molecular weights, isoelectric points, and grand averages of
hydropathicity. All the amino acid sequence-based phylogenetic trees were constructed using the MEGA7.0 program, whereas the protein-based phylogenetic tree was constructed using the Neighbor-Joining (NJ) method with a bootstrap value of 1000 to test the reliability [41].

4.5. Subcellular Localization and miRNA-Target Interactions
The CDSs of DcaTCP2, -4, -9, and -14 were amplified using a Phanta® Max Super-Fidelity DNA Polymerase (Vazyme Biotech Co., Ltd., Nanjing, China) with relative primers (listed in Table S2), then using ClonExpress® II (Vazyme Biotech Co., Ltd., Nanjing, China) inserted into the pRI101-GFP vector to allow its sequencing-based validation. The 35S:GFP-DcaTCP2, -4, -9, and -14 were transferred into Agrobacterium tumefaciens EHA105 using electroporation and then injected into N. benthamiana leaves, respectively. The penetrated plants were cultivated for 3 days. Laser confocal microscopy (Olympus Optical Co., Ltd., Tokyo, Japan) was performed in accordance with Du [42].

miRNA319a-target interactions were analyzed according to Liu [28]. 35s:miR319a was introduced into Agrobacterium GV3101-pSoup and then co-injected with DcaTCP4-GFP and DcaTCP9-GFP into N. benthamiana leaves, separately.

4.6. Transient Expression in Nicotiana benthamiana
Transactivation activity detection assays were performed using 35S:GFP-DcaTCP2, -4, -9 as effector and ProLOX2:LUC as reporter [43]. 35S:GFP-DcaTCP2, -4 and -9 co-injected with ProLOX2:LUC into N. benthamiana leaves, respectively. The penetrated plants were cultivated for 3 days. Then, 1 mM luciferin was sprayed onto the injected N. benthamiana leaves, which were then placed in the dark for 3 min. Luciferase luminescence was captured using a Tanon-5200 Chemiluminescent Imaging System (Tanon Science & Technology Co., Ltd., Shanghai, China) automated chemiluminescence image analysis system with a low-light cooled CCD camera.

4.7. Transcriptomic Data
Transcriptomic data from phytohormone-treated D. catenatum were obtained from the Biodiversity Data Center (https://data.iflora.cn/Home/DataContent?data_gd=89131009-82d9-ad4b-faaa-1bad087095e2, accessed on 19 August 2021). The DcaTCP genes’ expression levels were analyzed with moderated t-statistics using deseq2, and fold-change values were also calculated [44]. A heatmap was constructed using Genesis.

5. Conclusions
Our study provided the first genome-wide analysis of the TCP gene family in D. catenatum. It revealed the difference of DcaTCP gene function in Class I and Class II. The expression of 25 DcaTCP in response to various phytohormones treatments was characterized. The results of this study lay the basis for further research on the functions of the TCP gene family members in growth and development process, which will promote their application in D. catenatum.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/ijms221910269/s1.

Author Contributions: X.S. and Y.Y. conceived and designed the experiments. L.Z., C.L. and D.Y. performed the experiments and collected the data. X.S., Y.W. and Y.Y. revised the paper. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by grants from the Beijing DR PLANT Biotechnology Co., Ltd. (2019H078), the Project of Science & Technology of Guizhou Province (NY [2017]2524).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.
27. Palatnik, J.F.; Wollmann, H.; Schommer, C.; Schwab, R.; Boisbouvier, J.; Rodriguez, R.; Warthmann, N.; Allen, E.; Dezulian, T.; Huson, D.; et al. Sequence and expression differences underlie functional specialization of Arabidopsis microRNAs miR159 and miR319. Dev. Cell 2007, 13, 115–125. [CrossRef]

28. Liu, Q.; Wang, F.; Axtell, M.J. Analysis of Complementarity Requirements for Plant MicroRNA Targeting Using a Nicotiana benthamiana Quantitative Transient Assay. Plant Cell 2014, 26, 741–753. [CrossRef]

29. Danisman, S.; van der Wal, F.; Dhonts, S.; Waite, R.; de Folter, S.; Bimbo, A.; van Dijk, A.-J.; Muino, J.M.; Cutri, L.; Dornelas, M.C.; et al. Arabidopsis Class I and Class II TCP Transcription Factors Regulate Jasmonic Acid Metabolism and Leaf Development Antagonistically. Plant Physiol. 2012, 159, 1511–1523. [CrossRef] [PubMed]

30. Xu, Z.D.; Sun, L.D.; Zhou, Y.Z.; Yang, W.R.; Cheng, T.R.; Wang, J.; Zhang, Q.X. Identification and expression analysis of the SQUAMOSA promoter-binding protein (SBP)-box gene family in Prunus mume. Mol. Genet. Genom. 2015, 290, 1701–1715. [CrossRef] [PubMed]

31. Yan, L.; Wang, X.; Liu, H.; Tian, Y.; Lian, J.; Yang, R.; Hao, S.; Wang, X.; Yang, S.; Li, Q.; et al. The Genome of Dendrobium officinale Illuminates the Biology of the Important Traditional Chinese Orchid Herb. Mol. Genet. Genom. 2015, 8, 922–934. [CrossRef] [PubMed]

32. Tang, H.; Zhao, T.; Sheng, Y.; Zheng, T.; Fu, L.; Zhang, Y. Dendrobium officinale Kimura et Migo: A Review on Its Ethnopharmacology, Phytochemistry, Pharmacology, and Industrialization. Evid. Based Complement. Altern. Med. 2017, 2017, 7436259. [CrossRef] [PubMed]

33. Vick, B.A.; Zimmerman, D.C. The biosynthesis of jasmonic acid—A physiological-role for plant lipoxygenase. Biochem. Biophys. Res. Commun. 1983, 111, 470–477. [CrossRef]

34. Feng, Z.J.; Xu, S.C.; Liu, N.; Zhang, G.W.; Hu, Q.Z.; Gong, Y.M. Soybean TCP transcription factors: Evolution, classification, protein interaction and stress and hormone responsiveness. Plant Physiol. 2018, 127, 129–142. [CrossRef] [PubMed]

35. Liang, G.; He, H.; Li, Y.; Yu, D.Q. A new strategy for construction of artificial miRNA vectors in Arabidopsis. Planta 2012, 233, 1421–1429. [CrossRef] [PubMed]

36. Zhai, Q.Z.; Yan, L.H.; Tan, D.; Chen, R.; Sun, J.Q.; Gao, L.Y.; Dong, M.Q.; Wang, Y.C.; Li, C.Y. Phosphorylation-Coupled Proteolysis of the Transcription Factor MYC2 Is Important for Jasmonate-Signaled Plant Immunity. PLoS Genet. 2013, 9, e1003422. [CrossRef] [PubMed]

37. Jin, J.P.; Tian, F.; Yang, D.C.; Meng, Y.Q.; Kong, L.; Luo, J.C.; Gao, G. PlantTFDB 4.0: Toward a central hub for transcription factors and regulatory interactions in plants. Nucleic Acids Res. 2017, 45, D1040–D1045. [CrossRef]

38. Finn, R.D.; Alex, B.; Jody, C.; Penelope, C.; Eberhardt, R.Y.; Eddy, S.R.; Andreas, H.; Kirstie, H.; Liisa, H.; Jaina, M. Pfam: The protein families database. Nucleic Acids Res. 2014, 42, D222–D230. [CrossRef]

39. Lu, S.; Wang, J.; Chitsaz, F.; Derbyshire, M.K.; Geer, R.C.; Gonzales, N.R.; Gwadz, M.; Hurwitz, D.I.; Marchler, G.H.; Song, J.S.; et al. CDD/SPARCLE: The conserved domain database in 2020. Nucleic Acids Res. 2020, 48, D265–D268. [CrossRef]

40. Bailey, T.L.; Johnson, J.; Grant, C.E.; Noble, W.S. The MEME Suite. Nucleic Acids Res. 2015, 43, W39–W49. [CrossRef]

41. Kumar, S.; Stecher, G.; Tamura, K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol. Biol. Evol. 2016, 33, 1870–1874. [CrossRef]

42. Du, J.; Hu, S.; Yu, Q.; Wang, C.; Yang, Y.; Sun, H.; Yang, Y.; Sun, X. Genome-Wide Identification and Characterization of BrrTCP Transcription Factors in Brassica rapa ssp. rapa. Front. Plant Sci. 2017, 8, 1588. [CrossRef] [PubMed]

43. Chen, H.; Zou, Y.; Shang, Y.; Lin, H.; Wang, Y.; Cai, R.; Tang, X.; Zhou, J.-M. Firefly luciferase complementation imaging assay for protein-protein interactions in plants. Plant Physiol. 2008, 146, 368–376. [CrossRef] [PubMed]

44. Anders, S.; Huber, W. Differential expression analysis for sequence count data. Genome Biol. 2010, 11, R106. [CrossRef] [PubMed]