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## Research Article

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# Genome-wide identification of Cellulose-like synthase D gene family in *Dendrobium catenatum*

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## Abstract

**Background:** *Dendrobium catenatum*, which grows on the semi humid rocks in the mountains, has been at the top of the "Nine Immortals of China" since ancient times. It is a kind of yin tonic medicine and its main active component is polysaccharide. Cellulose synthase-like D(*CsID*) genes were predicted to catalyse the biosynthesis of 1,4- $\beta$ -d-glycan backbone of hemicelluloses, which plays fundamental roles in plant development.

**Results:** To investigate the role of *CsID* in the development of *Dendrobium catenatum*, eight *CsID* genes (*DcCsID1, 2a, 2b, 3a, 3b, 4a, 4b, 5*) were identified. The results of protein prediction and analysis showed that *CsID2a/2b/4a/4b* proteins were acidic proteins, the rest were basic proteins; Leu, Ser, Ala, Gly, Arg, Pro and Asp were the main amino acids. All the proteins had obvious hydrophobic or hydrophilic regions, and had transmembrane structure. The main elements of protein secondary structure were  $\alpha$ -helix, random coil and extended chain. Phylogenetic analysis revealed that the *DcCsID* family could be divided into I, II, III and IV groups. *DcCsID* proteins had typical Cellulose synthase domain and similar protein structures to the *CsIDs* of other plants. Their promoter regions contain cis regulatory elements related to stress and hormone. The results of qRT-PCR showed that the identified *DcCsIDs* were differentially

expressed in roots, stems and leaves. Most of them highly expressed in stems and leaves. What's more, the environmental stresses examination showed that the expressions of *DcCslD5* were closely associated with drought-recovery treatment, the expression of *DcCslD1*, *DcCslD2a*, *DcCslD2b*, *DcCslD3a*, and *DcCslD5* were significantly influenced by low temperature.

**Conclusions:** This study systematically analyzed the sequence characteristics of CslD protein of *D. catenatum*, which can provide reference for further study on the function of CslD protein in polysaccharide metabolism of *D. catenatum*.

**Keywords** *Dendrobium catenatum*, Cellulose synthase-like D, Gene family, Polysaccharid biosynthesis, expression patterns

## Introduction

*Dendrobium catenatum* (*D. catenatum*), a perennial herb of Dendrobium in Orchidaceae, is a valuable traditional Chinese medicine and has the effects of nourishing the stomach and promoting hydration, nourishing yin and antipyretic, etc [1-3]. The principal functional substances of *D. catenatum* are polysaccharides, alkaloids, flavonoids, terpenes, fluorenones, phenanthrenes and bibenzyls. *D. catenatum* polysaccharide (DOP), which is mainly composed of mannose and glucose, and a minor amount of galactose, are especially abundant in *D. catenatum* stems[4, 5]. Structurally, DOPs are 2-*O*-acetylglucomannan or 2,3-*O*-acetyl-glucomannan, made up primarily of  $\beta$ -1,4-linked mannose and  $\beta$ -1,4-linked glucose[6, 7]. And it has showed antioxidant, antitumor and anti-inflammatory bioactivities[8]. For example, D-Mannose played an immunomodulatory role in T cell immune response[9]; the *D. catenatum* polysaccharides could stimulate splenocytes, T-lymphocytes and B-lymphocytes, promote the cell viability and NO production of RAW 264.7 macrophages[10, 11]. But the structures of polysaccharide were complex, and the functions of genes in the polysaccharide synthesis pathway were not very clear.

Cellulose synthase A (*CESA*) superfamily genes which includes cellulose synthase (*CESA*) gene and cellulose synthase like gene (*Csl*), are related to mannan synthesis[12, 13]. The first plant *CesA* gene was reported in cotton in 1996[14]. Based on this, Cutler and Somerville[15] identified the *Csl* gene family in *Arabidopsis* and speculated that some of members might function as different types of  $\beta$ -1,4-glucan synthase. These provided the basis for the further study of *Csl* gene family.

Studies have shown that three *CsIA* genes (*CsIA2*, *CsIA7* and *CsIA9*) and three *CsID* genes (*CsID2*, *CsID3* and *CsID5*) were participated in the synthesis of mannose-containing polysaccharides in *Arabidopsis thaliana*[16, 17]; *CsIC* genes were responsible for the biosynthesis of xyloglucan in *Ananas com osus*[18, 19]; *CsIF* and *CsIH* gene families could mediate the synthesis of (1,3;1,4)- $\beta$ -glucan which was a polysaccharide characteristic of the evolutionarily successful grasses that was not widely distributed elsewhere in the plant kingdom[20-22]; the *CsIM* lineage was widely distributed in eudicots, and the *CsIJ* clade which also capable of directing (1,3;1,4)- $\beta$ -glucan biosynthesis, were widely distributed in monocots[12]. Cellulose synthase-like D (*CsID*) genes were predicted to be located on Golgi membrane and catalyzes the synthesis of 1,4-  $\beta$ - D- glucomannan from GDP glucose and GDP mannose (**Fig. 1**)[23, 24]. It is conserved in all land plants, and among the ten *CsI* subfamilies, it shows the highest sequence similarity to the cellulose synthase genes, suggesting that it plays fundamental roles in plant development[25, 26]. Many studies have described that *CsIDs* are involved in various aspects of the plant life cycle, including synthesis of polysaccharides[27-29], environmental stimuli response[30], the expansion and division of cells[31-33], plant development[34-37] and so on. Over the past few years, the *CsID* family genes have been identified from the genome of various species, such as *Arabidopsis thaliana*[17, 32], *Populus trichocarpa*[38], *Zea mays L*[31], *Dendrobium catenatum*[28, 29], *Phalaenopsis stuartiana*[39], *Gossypium hirsutum*[40, 41] and *Oryza sativa*[42, 43]. This indicates that *CsID* genes have a wide range of functions, and more and more researchers pay attention to it.

In this study, we analyzed the physicochemical and domain of eight complete DcCsID proteins from *D. catenatum* and their homology with other plants. Through the analysis of expression patterns, the functions of *DcCsID* genes in the polysaccharide synthesis pathway of *D. catenatum* were characterized. These results can provide theoretical basis for the further study of polysaccharide synthesis and *DcCsID* genes function of *D. catenatum*.

## Results

### Identification and Characterization of DcCsID Genes in *D. catenatum*.

Through HMMER analysis and BLAST search, we identified Eight *DcCsID* genes which were named as *DcCsID1*, *DcCsID2a*, *DcCsID2b*, *DcCsID3a*, *DcCsID3b*, *DcCsID4a*, *DcCsID4b*,

*DcCslD5*. The physicochemical properties of DcCslD protein showed that, the size of deduced DcCslD proteins varied between 740 and 1180 amino acids (aa), with an average of 1009.75 aa; the molecular weight (MW) varied from 82.39 to 131.73 kDa, and the theoretical pI of these genes ranged from 5.68 to 8.84, DcCslD2a, DcCslD2b, DcCslD4a and DcCslD4b were acidic protein and the rest were basic protein. The instability index was greater than 40, indicated that they were unstable proteins. And from the analysis of the grand average hydrophobicity of the protein, CslD3a was hydrophobic, while other proteins were hydrophilic (**Table 1**).

### **Conducting peptide, signal peptide and transmembrane domain of CslD protein from *D. catenatum*.**

Prediction analysis showed that the CslD protein of *D. catenatum* had no obvious signal peptide and guiding peptide. The transmembrane domain of DcCslD was predicted using TMHMM server v. 2.0. The results showed, except for DcCslD4b protein, the other DcCslD proteins had 6-8 transmembrane domains, among which 1-2 transmembrane domains were in the N region of the protein, and 5-6 transmembrane domains were at the C-terminal of the protein (**Table 2**).

### **Prediction and analysis of secondary structure of CslD protein in *D. catenatum*.**

The secondary structure of the protein mainly includes  $\alpha$ -helix,  $\beta$ -fold,  $\beta$ -turn, random coil and extended chain. The prediction results showed that the DcCslD protein sequences were composed of four structural components ( $\alpha$ -helix,  $\beta$ -fold, extended chain and random coil), and the proportions of this structures were very similar. Among them,  $\alpha$ -helix, extended chain and random coil were the main elements, and  $\beta$ -fold accounted for the least proportion (**Table 3**).

### **Prediction and analysis of the tertiary structure of CslD protein in *D. catenatum*.**

The DcCslD protein tertiary structures were predicted and compared by using the dormal model in Phyre2 structure prediction server, and the rationality of them were detected by the protein structure testing tool PDBsum Generate (**Fig. 2**). All proteins except DcCslD4b had similar tertiary structures. And some differences of structures might be responsible for the different functions of proteins.

The proportion of all DcCslD proteins in the disallowed regions were less than 2%, indicating that the spatial structures were stable. More than 80% of the amino acid residues were in the most

favorable region, means the conformation was reasonable. The values of generation factors were all greater than -0.5, which indicated that all DcCslD had normal spatial structures (**Table 4**).

### **Phylogenetic analysis and classification of CslD proteins in *D. catenatum*.**

Due to the high sequence homology of the *DcCslD* genes, we investigated their evolutionary relationship. To understand dynamic topological evolution, a neighbor joining phylogenetic tree was constructed by MEGA 7.0 using CslD protein sequences, including the above *D. catenatum* CslD proteins and *Arabidopsis thaliana*, *Oryza sativa*, *Phalaenopsis aphrodite*, *Zea mays* and *Populus trichocarpa* (**Fig. 3**).

The result showed that phylogenetic tree analysis among these 8 DcCslD proteins belong to three clades (I–III). On phyletic lineage, DcCslD2a, DcCslD2b, DcCslD3a and DcCslD3b shared the same clade I with ZmCslD5, OsCslD1, OsCslD2, AtCslD2, AtCslD3, PtrCslD5, PtrCslD6, PAXXG120890, PAXXG156700, PAXXG000590 and PAXXG228570. In this clade, AtCslD2, AtCslD3, OsCslD1 are required for root hair morphogenesis and the formation of cell wall, and ZmCslD1 is essential for cell division of rapidly growing tissues[35, 37, 42, 44-46]. There were 15 proteins in clade II, DcCslD1, DcCslD4a and DcCslD4b shared high similarity with AtCslD1, AtCslD4, OsCslD3, OsCslD5, ZmCslD3, ZmCslD4, PtrCslD7, PtrCslD8, PtrCslD9, PtrCslD10, PAXXG228240 and PAXXG023130, Among these proteins, AtCslD1 and AtCslD4 caused abnormal flowers, pollen tubes, and pollen grains; PtrCslD7, PtrCslD8, PtrCslD9 and PtrCslD10 may participate in flower and pollen tube development[34, 47, 48]. In addition, DcCslD5 was in clade III as well as functions of OsCslD4, PtrCslD2 and AtCslD5 were studied, which mutants displayed reduce stem growth and synthesis of polysaccharides[49, 50]. In clade IV, AtCslD6, PtrCslD3, PtrCslD4 and PAXXG254610 shared the same lineage; none of those were identified for their functions[33]. Based on the phylogenetic tree analysis, DcCslD2a, DcCslD2b, DcCslD3a and DcCslD3b may function in root hair formation, cell wall formation and cell elongation; DcCslD1, DcCslD4a and DcCslD4b may participate in flower and pollen tube development, and DcCslD5 may involve in the formation of cell wall, the growth and development of plants and the synthesis of polysaccharides.

### **Analysis of gene structures, conserved domains and motifs of CslD proteins in *D.***

### ***catenatum*.**

The exon/intron structures and intron numbers of the most closely related members in the same sub-families were not very similar. For example, the *DcCsID3b* had seven exons while *DcCsID3a* just had one; the *DcCsID1* had three exons while *DcCsID4a* and *DcCsID4a* had five.

The functional domain of DcCsID proteins were analyzed by HMMER online tool. It was found that all 8 DcCsID proteins contained Cellulose synthase domain. The results showed that all DcCsID proteins contained 12 motifs, except that DcCsID3a lacked motif 7 in the C-terminal region and DcCsID 4b lacked motif 2, 6, 12, 8, 9 in the N-terminal region (**Fig. 4**). According to the multiple sequence alignment results of DcCsID protein domain, they had a large number of amino acid conservative sites, and the sequence of Cellulose synthase domain had the ability to bind nucleoside sugar and the conserved motifs of D, D, D, Q,X,X,R,W (**Fig. 5**), indicating the highly conservative function of DcCsID protein.

### **Analysis of cis elements in *DcCsID* promoter**

Plant gene promoter is an important cis-acting element which is the control center of gene transcription. Cis-acting elements analysis revealed that the promoter regions of these eight *DcCsID* contain more than three cis-acting elements (**Fig. 6**). These cis-regulatory elements could be broadly divided into eight categories: Light responsive elements, Hormone responsive elements, Promoter related elements, Development related elements, Environmental stress-related elements, Site-binding related elements and other elements[51, 52]. The light responsive category contains nineteen kinds of cis-regulatory elements and twelve types of cis-regulatory elements were found to be involved in plant hormone responsiveness, which including TATC-box, p-box, GARE-motif, gibberellins responsive element; ERE, ethylene responsive element; ABRE, abscisic acid responsive element; TGA-element, auxin-responsive element; TCA-element, salicylic acid responsiveness; the MeJA-responsiveness; AuxRR-core, auxin responsiveness element, CGTCA-motif, TATC-box, and TGACG-motif. The third category was related to plant growth and development which contains five types of cis-regulatory elements: CCAAT-box, CAT-box GCN4-motif, HD-Zip3, and MSA-like. The flowing category might respond to environmental stress, such as ARE, the anaerobic induction; GC-motif, anoxic specific inducibility; TC-rich repeats, defense and stress responsiveness; MBS, MYB binding site involved in drought-responsiveness; LTR, low-temperature responsiveness; and

W-box, wound-responsive element. In addition, two categories were promoter related elements and site-binding related elements; they both had two types of cis-regulatory elements, respectively. Finally, the functions of the remaining three types of cis-regulatory elements were unclear.

### **Analysis of *CsID* gene expression pattern in *D. catenatum*.**

#### **Expression patterns of *DcCsID* gene in different organs**

*DcCsID* genes are important for plant growth and development[53, 54]. In order to preliminarily elucidate the function of *DcCsIDs* in *D. catenatum*, we used qRT-PCR examine the expression of eight *DcCsIDs* in roots, stems and leaves. Overall, most *DcCsIDs* genes were highly expressed in stems and leaves, while *DcCsID4b* gene was highly expressed in root. But the expression level of *DcCsID4b* was lower compared with others. The expression of *DcCsID1*, *DcCsID2a*, *DcCsID3a*, *DcCsID4a*, and *DcCsID5* were higher than that in leaf, and *DcCsID5* showed the highest expression in *D. catenatum* stem (**Fig. 7** and Additional file 1).

#### **Expression of *DcCsIDs* in response to drought and low-temperature treatments**

It has been shown that *DcCsIDs* are involved in plant responses to environmental stresses[55-57]. In order to investigate the response of *DcCsIDs* under stress conditions, we used the transcriptome data to analyze the levels of transcripts in tissues treated with drought and low-temperature. Under drought treatment, the expression of *DcCsID5* gene changed most obviously, and increased with the extension of drought treatment time while decreased after rewatering (**Fig. 8A** and Additional file 1). Compared with the control group (20°C, 20 h), *DcCsID1*, *DcCsID2a*, *DcCsID2b*, *DcCsID3a*, and *DcCsID5* were significantly up-regulated and *DcCsID3b*, *DcCsID4a*, *DcCsID4b* were significantly down regulated after 20 h treatment at 0 °C (**Fig. 8B** and Additional file 2). The results indicated that *DcCsIDs* may play different physiological roles under different stress conditions.

### **Discussion**

*D. catenatum* has been known as the first of "nine magic herbs in China" since ancient times. Because of its high medicinal value and wide application, it has been described as "giant panda of pharmaceutical kingdom" in the international medicinal plant community[58, 59]. DOP which mainly composed of mannose and glucose, is one of the main active components in *D.*

*catenatum*[60]. Studies had reported the first draft genome sequence of *D. catenatum* and discovered extensive duplication of genes included *DcCsID* gene family, involved in glucomannan synthase activities, likely related to the synthesis of medicinal polysaccharides. These allow us to systematically analyze the *CsID* gene family in *D. catenatum*.

In this study, a total of eight *DcCsID* genes were identified and characterized. The physicochemical properties of amino acids showed that all *DcCsID* proteins had long sequences, and they were unstable proteins; *DcCsID2a/2b/4a/4b* proteins were acidic proteins, the rest were basic proteins, what might be related to the function of protein. Except for *DcCsID3a*, the other proteins had obvious hydrophilic regions, it could be let them bind to the substrate and function better. Each protein contain several transmembrane structures, suggesting that they might be transported to the outside of the cell membrane after synthesis. In the prediction of protein secondary structure, it could be seen that the main components of *DcCsID* protein secondary structure were  $\alpha$ -helix, extended chain and random coil and  $\alpha$ -helix was the main part of transmembrane structure. In this study, the high-level structure model of *DcCsID* protein was established by homologous modeling method, and the model was tested by PROCHECK. It was found that all *DcCsID* proteins had normal spatial structure and conformation of proteins were reasonable. The prediction and analysis of *DcCsID* protein sequence structure can guide the expression and modification of protein; in addition, the prediction and analysis of secondary and tertiary structures will be helpful to further explore the relationship between structure and function and the mechanism of action.

According to phylogenetic analysis, *CsID* proteins could be divided into four clades(I–IV), and *DcCsID* proteins was distributed in clades I–III. We found that *DcCsID2a/2b/3a/3b*, which showed close phylogenetic relationships with *AtCsID2/3*, *OsCsID1* and *ZmCsID1*, were required for plant growth and development such as root hair morphogenesis and the accumulation of mannose in internode fiber. *DcCsID1/4a/4b* shared high similarity with *AtCsID1/4* and *PtrCsID7/8/9/10*, mean that they might participate in flower and pollen tube development. From the clade III of the phylogenetic tree, *DcCsID5* might have the same function as *AtCsID5* in promoting plant stem growth and polysaccharide synthesis. The functional differences among the three clades of proteins indicated that they were obviously divergent in the process of evolution. This might be related to the growth environment and internal genetic transformation of plants.

Based on the phylogenetic, gene structures, and motif analysis of the *DcCsID* protein, we

discovered that the exon/intron structures and intron numbers of the most closely related members in the same sub-families were not very similar. For example, the sister pairs DcCslD1, DcCslD4a and DcCslD4b had different intron/exon structures and numbers. These findings indicated that some intron loss, along with intron gain events, might have occurred during the structural evolution in the gene family of DcCslD encoding genes. In contrast, the DcCslD proteins had similar motif distributions. The clade I members (DcCslD2a/2b/3b), clade II DcCslD1 member and clade III member DcCslD5 had 12 distinct motifs, while the clade I group members DcCslD3a and clade II DcCslD4b were lost motif 1 and motif 2, 6, 8, 9, 12, respectively. All of them contained the marker Cellulose synthase domain and highly conserved D, D, D, QXXRW motifs. It was essential for the activation of the monosaccharide binding enzyme[61-63].

Analyses of cis-elements in the promoters suggest that *DcCslDs* might respond to regulate plant growth and development, as well as different environmental stresses and stimuli. To determine where the *DcCslD* genes were expressed in *D. catenatum*, qRT-PCR analysis was performed on RNA extracted from various organs. As shown in **Fig. 6**, *DcCslD* genes accumulated in *D. catenatum* tissues tested, but with different relative expression levels. Except for *DcCslD4b*, the expression of other genes in stem and leaf was higher than that in root, it might be that the six-month-old seedlings were in the vigorous growth period, and the formation of stems and leaves required the expression of *DcCslD* genes. And the high expression of *DcCslD* gene in stem also confirmed this point. *DcCslD* genes had the function of synthesizing polysaccharides, which provided conditions for the formation of plant cell wall. Similarly, multiple genes expressed ubiquitously in *D. catenatum* leaves and stems, but especially in stems, mainly because stems are the principal storage organs for DOPs in *D. catenatum*.

Heat map data showed that drought could induce the expression of *DcCslD5* gene while the effect on other genes was not obvious. This result is similar to that reported by Wan et al[55] whose results showed that moderate drought would not lead to a significant change in *D. catenatum*'s gene expression because of its strong ability to adapt to drought. The expression of 5 *DcCslD* genes (*DcCslD1*, *DcCslD2a*, *DcCslD2b*, *DcCslD3a*, and *DcCslD5*) which promoters contain low temperature response element, were up-regulated induced by low temperature. It was also reported that low temperature induced the expression of *DcCSLA5* which belong to the same subfamily as the *DcCslD*[64]. That might be related to the adaptation of *D. catenatum* to the harsh environment

such as cliffs and cold. What's more, some studies speculated that the polysaccharide produced by *CsIDs* might have a signaling role in plants development. For example, the plasma-membrane-bound receptor-like kinase (THESEUS1), which is present in Arabidopsis elongating cells, could not only mediate cell response to disturbance of cellulose synthesis, but also act as a sensor for cell wall integrity[65]. OsCSLD4 mutant could significantly slow down plant growth by delaying cell cycle progression and it was closely related to several genes involved in cell cycle regulation[66]. Based on the above results and our prediction analysis, we speculated that the growth of *D. catenatum* was inhibited under low temperature. In order to resist the environmental stress, the expression of *DcCsIDs* were up-regulated, which promoted the synthesis of polysaccharides, and then made the cell cycle and growth process of *D. catenatum* normal. This results also provided a good basis for further analysis of the function of this gene family in the growth and development and glucomannan synthesis of *D. catenatum*.

## **Conclusions**

In this study, we identified 8 *CsID* proteins in *D. catenatum* and analyzed its biochemical characteristics, structures and phylogeny of them. In addition, we analyzed the expression profiles of *DcCsID* genes in different tissues and organs and their responses to diverse environmental stresses. Our findings provide comprehensive information on the classification and expression profiles on *DcCsID* genes, and will lay the foundation for the functional characterization of the *DcCsID* genes family in orchids.

## **Materials and methods**

### **Plant Materials**

*D. catenatum* cultivar "Jingpin NO. 1" (Breed NO. Zhe R-SV-DO-015-2014) was from the State Key Laboratory of Subtropical Silviculture in Zhejiang Province, China. Fresh roots, stems and leaves from the plants were collected and frozen in liquid nitrogen for subsequent RNA isolation.

### **Genome-wide identification of *CsIDs* proteins in *D. catenatum***

The complete *CsIDs* protein sequence of *D. catenatum* was downloaded from NCBI. Then we

download the HMM (hidden Markov model) profile of Cellulose synthase domain (PF03552) from Pfam database[67]. And this HMM profile was used to identify for all of Cellulose synthase domain in *D. catenatum* genome by HMMER 3.0 software with the E value cutoff set at  $10^{-5}$ . At the same time, we used 6 *A.thaliana* CslD protein sequences and 5 *Oryza sativa* CslD protein sequences to conduct local BLAST search on *D. catenatum* protein database. The obtained gene ID was compared with the former, and finally obtained 8 *DcCslD* genes. It was found that there were multiple sequences in the same gene, so the suffixes were distinguished by a and b.

### **Bioinformatics analysis of CslD family proteins in *D. catenatum***

The basic physicochemical properties (including amino acid length, PI, molecular weight and hydrophobic/hydrophilic) of protein were determined by ProtParam ([http://web.expasy.org/compute\\_pi/](http://web.expasy.org/compute_pi/)) online website. The leading peptide and signal peptide were predicted by TargetP1.1 Server (<http://www.cbs.dtu.dk/services/TargetP/>) and SignalP 4.1 Server (<http://www.cbs.dtu.dk/services/SignalP/>) respectively; the transmembrane structure was predicted by TMHMM Server v. 2.0(<http://www.cbs.dtu.dk/services/TMHMM/>); the protein secondary and tertiary structures were predicted by SOPMA (<http://nhjy.hzau.edu.cn/kech/swxxx/jakj/dianzi/Bioinf7/Expasy/Expasy8.htm>) and Phyre2 (<http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index>); the protein tertiary structure was tested by PDBsum Generate (<https://www.ebi.ac.uk/thornton-srv/databases/pdbsum/Generate.html>); and TMHMM (<http://www.cbs.dtu.dk/services/TMHMM/>) was used to analyze the DcCslD protein domain.

### **Multiple alignment and phylogenetic analysis of CslD family proteins in *D. catenatum***

Used 6 species including *D. catenatum*, *Arabidopsis thaliana* (<https://www.arabidopsis.org/>), *Phalaenopsis Aphrodite* (<http://tagrc.org/orchidstra2/genesearch.php>), *Oryza sativa*, *Zea mays* and *Populus trichocarpa* CslD proteins to do phylogenetic analysis. Phylogenetic tree was constructed by neighbor joining method (NJ) and completed by MEGA 7.0.

### **Gene structure analysis and identification of conserved motifs**

Used TBtools to compare CD's sequence and analyze the exon intron structure of *DcCsID* gene. The conserved motif of DcCsID proteins were analyzed by MEME suite(<http://meme-suite.org/tools/meme>), Homology alignment of *DcCsID* family was performed by DNAMAN software. At the same time, we used TBtools to extract the sequence of 2000 base pairs (bp) upstream of the start codon (ATG) of *DcCsIDs* gene, and then through the PlantCARE database (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>) to search for CIS components of promoters.

## **Analysis of gene expression patterns**

### **Real-Time quantitative RT-PCR (RT-qPCR)**

For the *DcCsID* gene expression pattern analysis, roots, stems and leaves were collected from six-month-old *D.catenatum* seedlings and put in liquid nitrogen immediately. The total RNA from all of the samples was extracted by using TaKaRa MiniBEST Plant RNA Extraction Kit. The first-strand cDNA synthesis was performed using the PrimeScript™ RT reagent Kit with gDNA Eraser (Petfect Real Time). Primer design with Primer Premier 5 (Additional file 3). The real-time quantitative reverse transcription-polymerase chain reaction (PCR) was conducted using CFX96™ Real-Time PCR System. The reaction volume consisted of 10 μL TB Green *Premix Ex Taq* II (Tli RNaseH Plus) (2X), 2 μL cDNA, 0.8 μL upstream primer (10 μM), 0.8 μL downstream primer (10 μM) and 6.4 μL ddH<sub>2</sub>O (20 μL in total). The reaction was performed with the following cycling profile: 95 °C for 3 min, 40 cycles of 94 °C for 20s, 60 °C for 20s, 72°C for 20s. Three technical replicates were performed for each sample. The calculation of the gene expression levels followed the 2- $\Delta\Delta$ CT method described by Livak and Schmittgen[68].

### **In silico expression profiling of *DcCsID* genes**

Based on the transcriptome data of *D.catenatum*, we screened out the expression of *DcCsID* gene family under drought and low temperature conditions and then used TBtools to draw the heat map[69]. Vigorous 8-month-old *D. catenatum* plants with a height of ~12 cm were chosen for the drought stress and stress removal experiment[70]. Irrigation was performed on the 1<sup>st</sup> day, omitted from the 2<sup>nd</sup> to the 7<sup>th</sup> day, and resumed on the 8<sup>th</sup> day, watering every two days at 15:30. Finally, the raw RNA-seq reads were obtained from the leaves that were harvested at both 06:30 and 18:30 on

the 2<sup>nd</sup> [DR5 (NCBI: SRR7223299) and DR8 (SRR7223300)], 7<sup>th</sup> [DR6 (SRR7223298) and DR10 (SRR7223296)], and 9<sup>th</sup> [DR7 (SRR7223301) and DR15 (SRR7223297)] days, respectively, and at 18:30 on the 8<sup>th</sup> day [DR11 (SRR7223295)]. In order to analyze the expression of *DcCslD* genes in response to cold stress, the raw RNA-seq reads of leaves under 20 °C control condition (SRR3210630, SRR3210635 and SRR3210636) and 0°C cold treatment for 20 h (SRR3210613, SRR3210621 and SRR3210626) were obtained from NCBI provided by Wu *et al*[71]. The HISAT package[72] was used to align the readings of all samples with the reference genome of NCBI *Dendrobium*. Used StringTie[73] to assemble the mapping readings for each sample. Then, used the Perl script to merge all transcriptome from the samples to reconstruct a comprehensive transcriptome. After the final transcriptome was generated, StringTie and edgeR were used to estimate the expression levels of all transcripts. StringTie calculated FPKM to perform expression level for mRNAs. After screening the expression level data of *DcCslD* genes, used TBtools to generate the Heatmap.

Accession numbers for each of the gene sequences referred to in this work are as follows (Additional file 4): *DcCslD1*, LOC110112325; *DcCslD2a*, LOC110097221; *DcCslD2b*, LOC110101672; *DcCslD3a*, LOC11011629; *DcCslD3b*, LOC110109313; *DcCslD4a*, LOC110115924; *DcCslD4b*, LOC110109154; *DcCslD5*, LOC110104407; *AtCslD1*, AT2G33100.1; *AtCslD2*, AT5G16910.1; *AtCslD3*, AT3G03050.1; *AtCslD4*, AT4G38190.1; *AtCslD5*, AT1G02730; *AtCslD6*, AT1G32180.1; *OsCslD1*, AC027037.6; *OsCslD3*, AC091687.1; *OsCslD4*, AK242601.1; *OsCslD5*, Os06g0336500; *ZmCslD1*, GRMZM2G015886; *ZmCslD2*, GRMZM2G052149; *ZmCslD3*, GRMZM2G061764; *ZmCslD4*, GRMZM2G044269; *ZmCslD5*, GRMZM2G436299; *PtrCslD1*, Potri.002G200300; *PtrCslD2*, Potri.014G125100; *PtrCslD3*, Potri.003G097100; *PtrCslD4*, Potri.001G136200; *PtrCslD5*, Potri.019G046700; *PtrCslD6*, Potri.013G082200; *PtrCslD7*, Potri.004G208800; *PtrCslD8*, Potri.009G170000; *PtrCslD9*, Potri.003G177800; *PtrCslD10*, Potri.001G050200; PAXXG023130; PAXXG228240; PAXXG000590; PAXXG120890; PAXXG156700; PAXXG228570; PAXXG188550; PAXXG254610.

## **Declarations**

## **Ethics approval and consent to participate**

The *D. catenatum* used in this study is a commercial cultivar "Jingpin NO. 1", which was cultivated by Prof Jinping Si (Zhejiang A&F University), was authorized by Zhejiang Province with Breed NO. Zhe R-SV-DO-015-2014. It does not require ethical approval.

### **Consent to publish**

Not applicable.

### **Availability of data and materials**

All data generated or analyzed during this study are included in this published article and its Additional files. The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

### **Competing interests**

The authors declare that they have no competing interests.

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### **Authors' contributions**

HXX, QL, LZ, JLL and JPS planned and designed the research. HXX performed the experiments. HXX, QL, XLC, CL, YXZ, JBY and DHC analyzed the data. HXX, LZ, JLL and JPS wrote the article. All the authors approved the manuscript.

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## Figure Legend

**Figure 1 Catalytic synthesis of glucomannan in plants.**

**Figure 2 3D structures prediction of CslD proteins of *D. catenatum*.**

**Figure 3 Phylogenetic relationship of CslD proteins in six plant species.** Phylogenetic tree of CslD proteins. A neighbor-joining (NJ) tree was constructed by MEGA 7.0 using 41 CslD proteins, including 8 proteins from *Dendrobium catenatum*, 10 proteins from *Populus trichocarpa*, 6 proteins from *Arabidopsis thaliana*, 4 proteins from *Oryza sativa*, 5 proteins from *Zea mays*, and 8 genes from *Phalaenopsis aphrodite*. The tree shows 4 distinct clades.

**Figure 4 Distribution of conserved motif of CslD protein in *D. catenatum*.**

**Figure 5 Multiple sequence alignment of CslD protein domain in *D. catenatum*.**

**Figure 6 Analysis of CIS sequence of *CsID* gene promoter in *D. catenatum*.**

**Figure 7 Expression of *CsID* gene family in organs of *D. catenatum*.** We used six-month old seedlings of *D. catenatum* to extract RNA from roots, stems and leaves for qRT-PCR. During this period, to ensure the accuracy of the experiment we designed three biological and three technical repeats for each sample.

**Figure 8 Heatmaps showing member of *CsID* gene family in different treatment. (A)** Expression of *DcCsID* after drought treatment, DR5/DR6: The 2<sup>nd</sup> day treatment at 06:30 and 18:30; DR7/DR8: The 7<sup>th</sup> day treatment at 06:30 and 18:30; DR10: The 8<sup>th</sup> day treatment at 18:30 after watering the seedlings at 15:30; DR11/DR15: The 9<sup>th</sup> day treatment at 06:30 and 18:30. **(B)** Expression of *DcCsID* after low temperature treatment. Control 1~3: 20 °C, 20 hours; Cold 1~3: 0 °C, 20 hours. Each group have three repetitions.

**Table 1 The physic-chemical analysis of CslD protein in *D. catenatum*.**

**Table 2 Prediction and analysis of CslD protein signal peptide, guiding peptide and transmembrane domains in *D. catenatum*.**

**Table 3 Main component proportion of secondary structure of CslD protein in *D. catenatum*.**

**Table 4 Stability prediction about CsID proteins 3D structures of *D. catenatum*.**

**Additional file 1 Transcriptome data of drought treatment.** Expression data of *DcCsID* genes from different drought treatments. The FPKM values of *DcCsID* genes in leaves under different drought treatments were used for expression analysis in **Fig. 8A**. The seedlings were watered on the 1<sup>st</sup> day, dried from the 2<sup>nd</sup> to the 7<sup>th</sup> day, and re-watered on the 8<sup>th</sup> day. DR5/ DR8, DR6/DR10, and DR7/DR15 indicate sampling at 06:30 and 18:30 on the 2<sup>nd</sup>, 7<sup>th</sup>, and 9<sup>th</sup> days, respectively, and DR11 indicates sampling at 18:30 on the 8<sup>th</sup> day

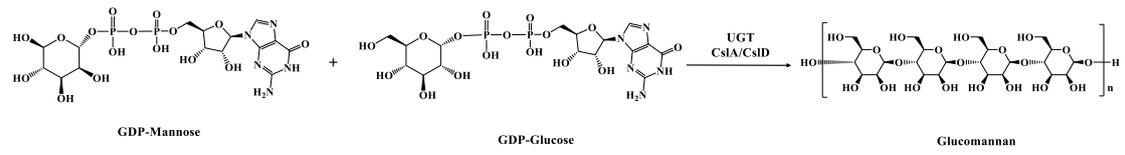
**Additional file 2 Transcriptome data of low-temperature treatment.** Expression data of *DcCsID* genes from cold treatments. The FPKM values of *DcCsID* genes in leaves under cold stress / 20°C (control) for 20 h were used for expression analysis in **Fig. 8B**.

**Additional file 3 Primers used in tissues expression analysis of in this study.**

**Additional file 4 Accession numbers for each of the gene sequences referred to in this work.**

The sequences included 8 proteins from *Dendrobium catenatum*, 10 proteins from *Populus trichocarpa*, 6 proteins from *Arabidopsis thaliana*, 4 proteins from *Oryza sativa*, 5 proteins from *Zea mays*, and 8 genes from *Phalaenopsis aphrodite*.

**Figure 1**



**Figure 2**

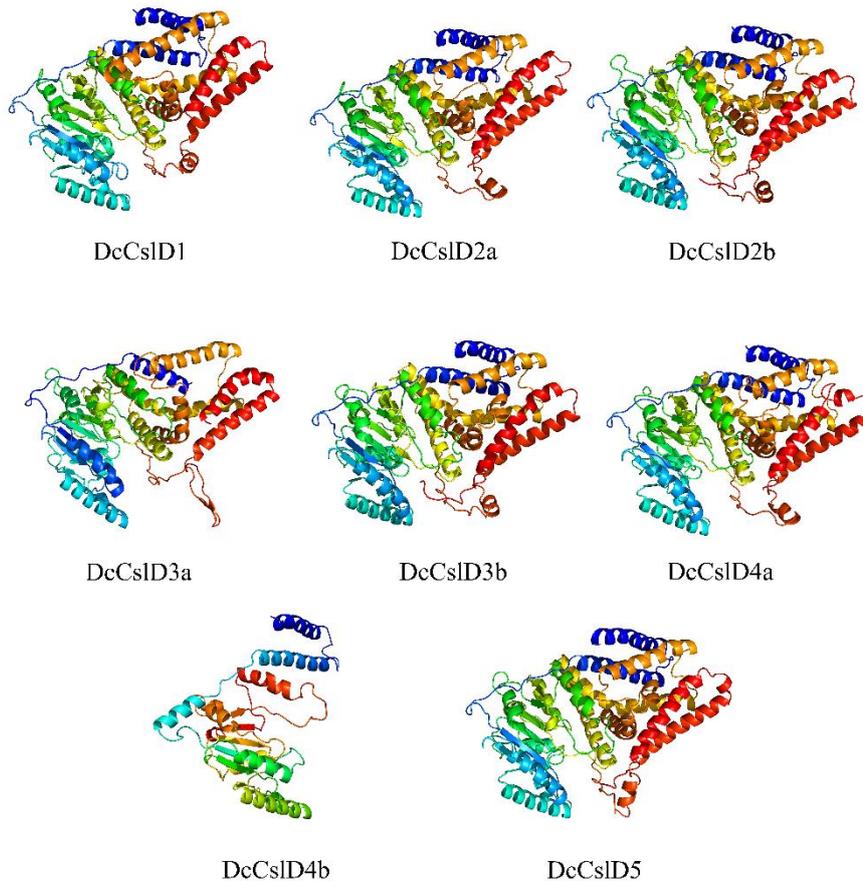
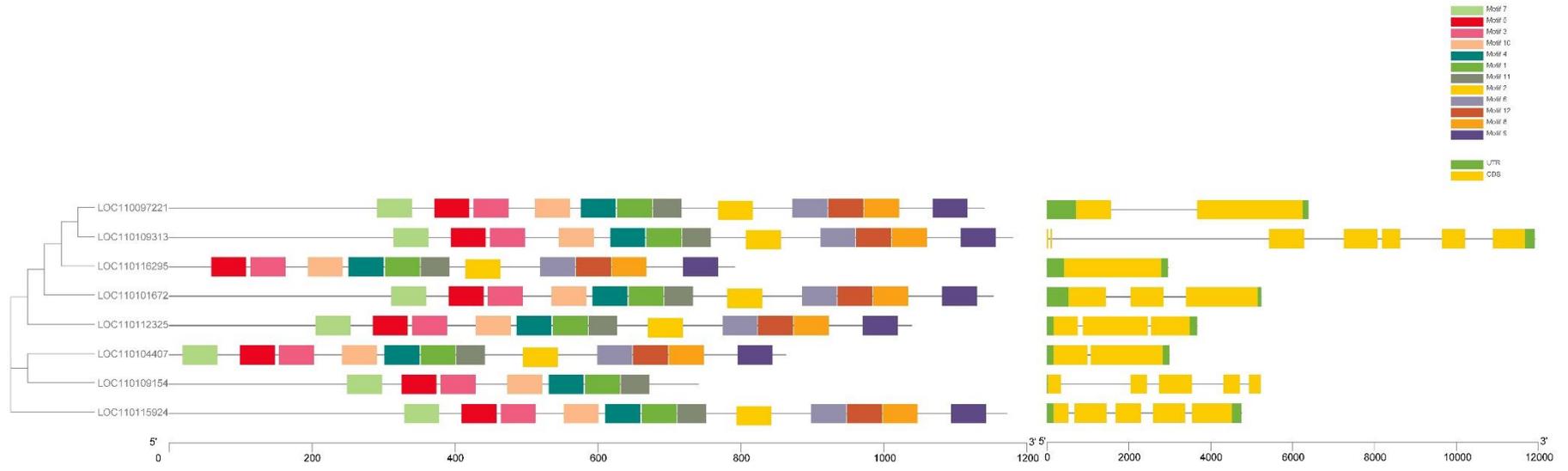
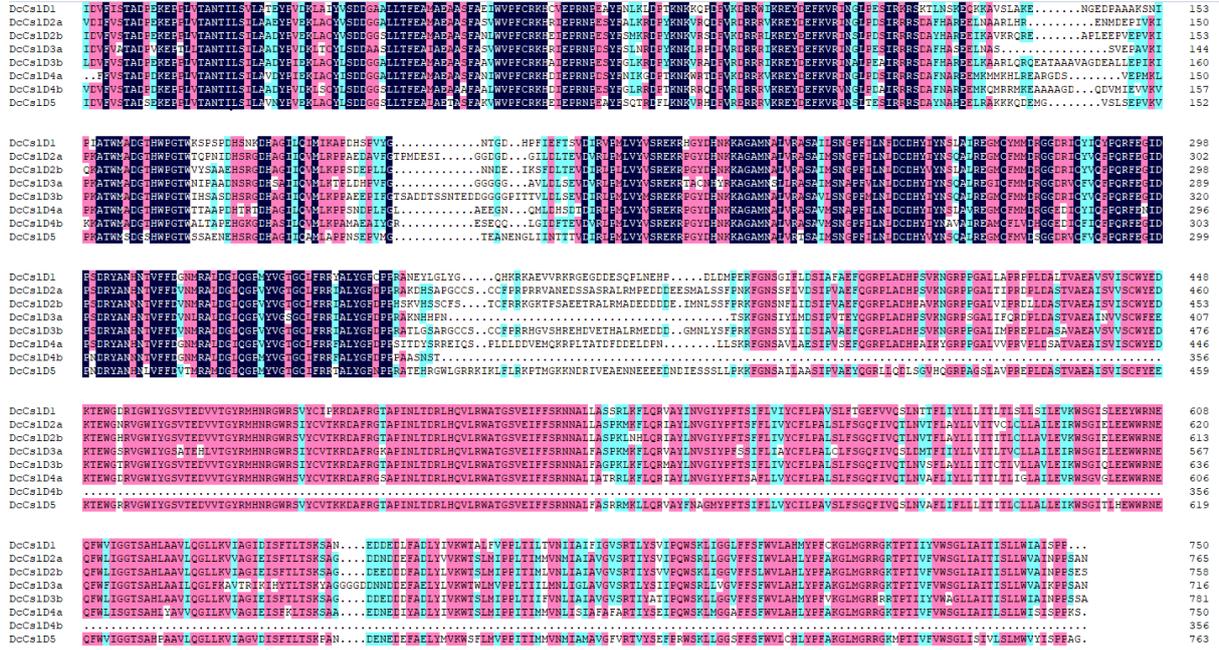




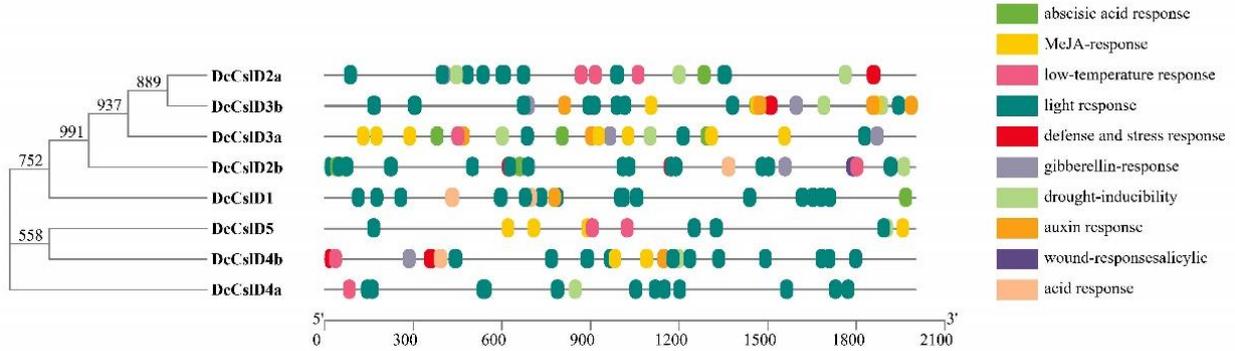
Figure 4



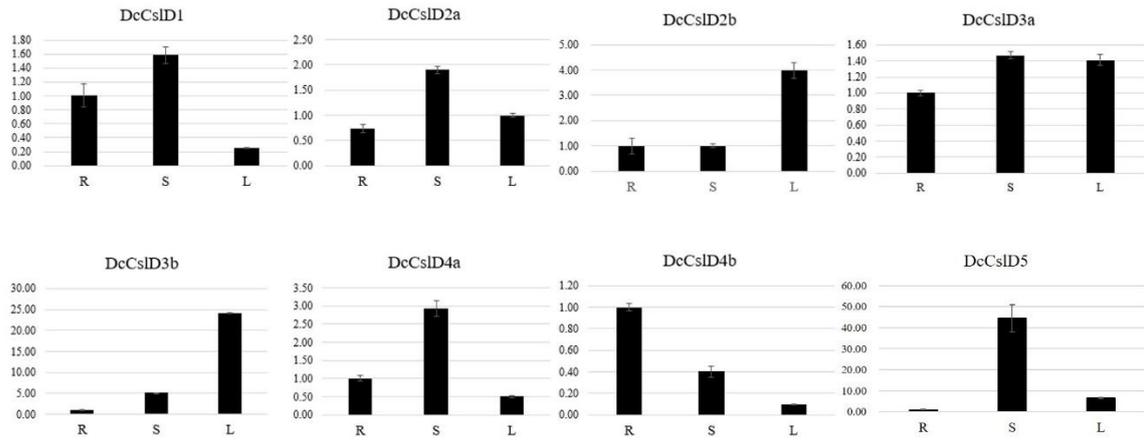
**Figure 5**



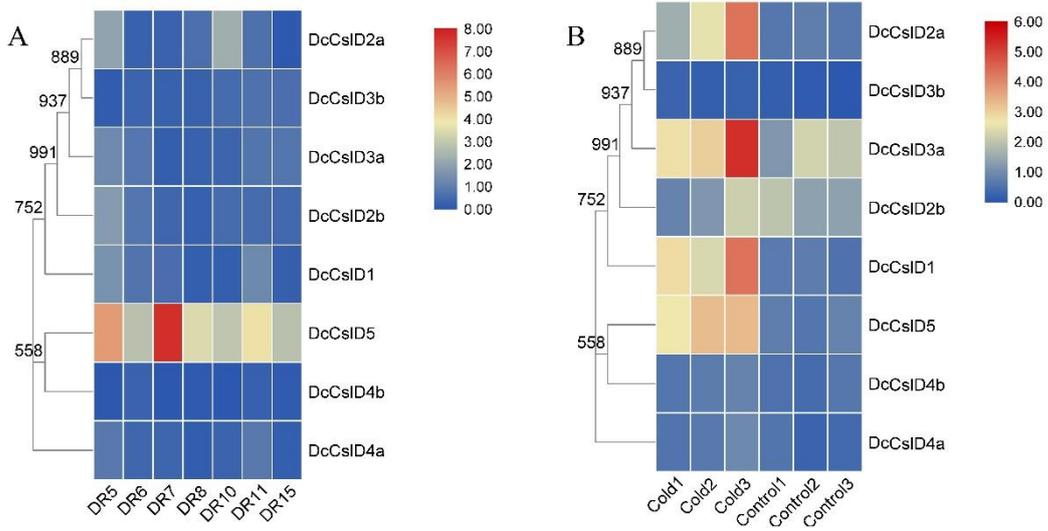
**Figure 6**



**Figure 7**



**Figure 8**



**Table 1 The physico-chemical analysis of CslD protein in *D. catenatum***

Gene symbol	Gene name	Amino acid/AA	Molecular weight/kDa	Isoelectric point/pI	Instability index	Grand average of hydropathicity
LOC110112325	<i>CslD1</i>	1039	115.82	8.43	45.82	-0.193
LOC110097221	<i>CslD2a</i>	1140	127.81	6.76	41.89	-0.171
LOC110101672	<i>CslD2b</i>	1153	129.06	6.50	44.51	-0.199
LOC110116295	<i>CslD3a</i>	791	88.88	8.84	46.11	0.020
LOC110109313	<i>CslD3b</i>	1180	131.73	7.89	45.52	-0.176
LOC110115924	<i>CslD4a</i>	1172	130.38	5.68	42.28	-0.243
LOC110109154	<i>CslD4b</i>	740	82.38	5.75	52.42	-0.455
LOC110104407	<i>CslD5</i>	863	97.99	8.57	47.09	-0.054

**Table 2 Prediction and analysis of CslD protein signal peptide, guiding peptide and transmembrane domains in *D. catenatum***

Name	Signal peptide	Mitochondrial transfer peptide	Chloroplast transfer peptide	Thylakoid luminal transfer peptide	transmembrane domain
CslD1	0.0005	0	0	0	8
CslD2a	0.0117	0.0119	0.0205	0.0002	8
CslD2b	0.0002	0.0001	0.0001	0	8
CslD3a	0.0357	0.0008	0.015	0.0001	6
CslD3b	0.0004	0.0789	0	0	8
CslD4a	0.0029	0.0001	0.0043	0.0001	6
CslD4b	0.0034	0.0001	0.0002	0	2
CslD5	0.0075	0.0007	0	0	7

**Table 3 Main component proportion of secondary structure of CslD protein in *D. catenatum***

Gene symbol	Name	Alpha helix (%)	Beta turn (%)	Random coil (%)	Extended strand (%)
LOC110112325	<i>CslD1</i>	30.51	3.08	50.91	15.50
LOC110097221	<i>CslD2a</i>	30.79	3.86	51.49	13.86
LOC110101672	<i>CslD2b</i>	31.57	3.38	50.30	14.74
LOC110116295	<i>CslD3a</i>	34.64	3.54	46.52	15.30
LOC110109313	<i>CslD3b</i>	32.63	4.24	49.83	13.31
LOC110115924	<i>CslD4a</i>	29.27	3.58	51.79	15.36
LOC110109154	<i>CslD4b</i>	27.03	4.86	53.11	15.00
LOC110104407	<i>CslD5</i>	38.24	3.94	42.76	15.06

**Table 4 Stability prediction about CslD proteins 3D structures of *D. catenatum***

Name	Most favoured regions (%)	Additional allowed regions (%)	Generously allowed regions (%)	disallowed regions (%)	G-factor
<i>CslD1</i>	88.1	8.3	2.8	0.9	-0.14
<i>CslD2a</i>	86.9	9.9	2.5	0.6	-0.26
<i>CslD2b</i>	84.9	11.3	2.3	1.5	-0.22
<i>CslD3a</i>	85.9	11.7	1.8	0.7	-0.26

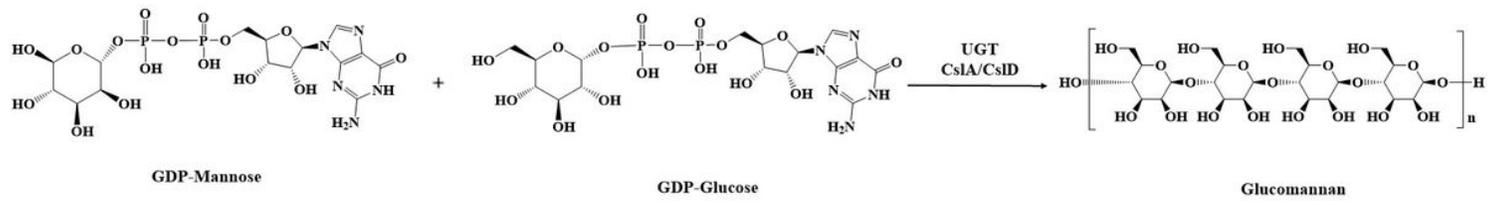
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<i>CsID3b</i>	86.4	10.6	2.1	0.8	-0.21
<i>CsID4a</i>	85.3	11.3	2.5	0.8	-0.25
<i>CsID4b</i>	87.2	9.5	2.5	0.8	-0.43
<i>CsID5</i>	85.4	11.9	1.9	0.8	-0.23

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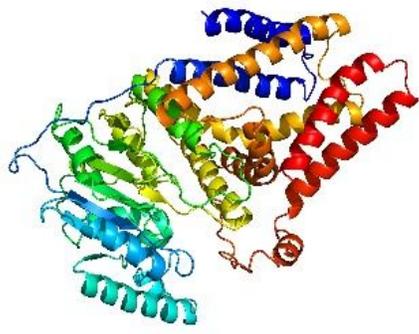
Note: G-Factor<-0.5 unusual; G-factor<-1.0 highly unusual

# Figures

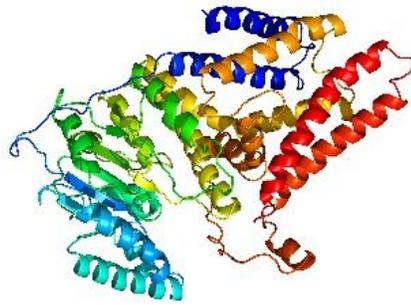


**Figure 1**

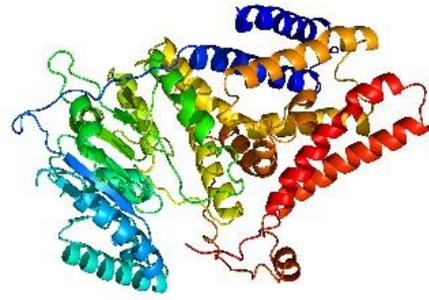
Catalytic synthesis of glucomannan in plants.



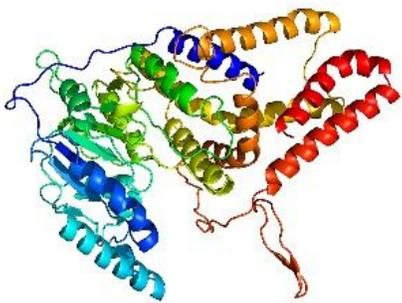
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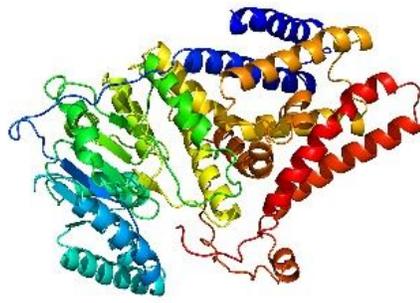
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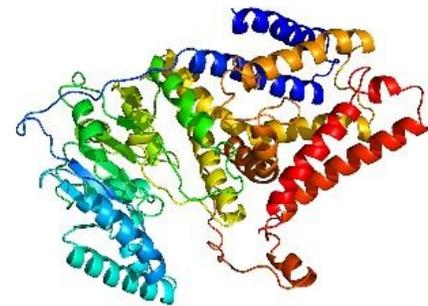
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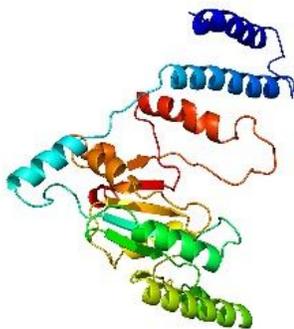
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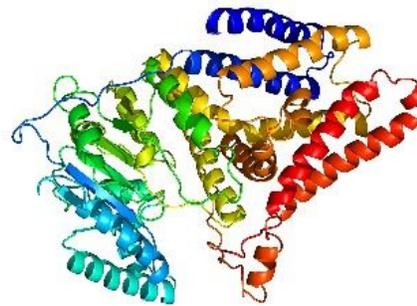
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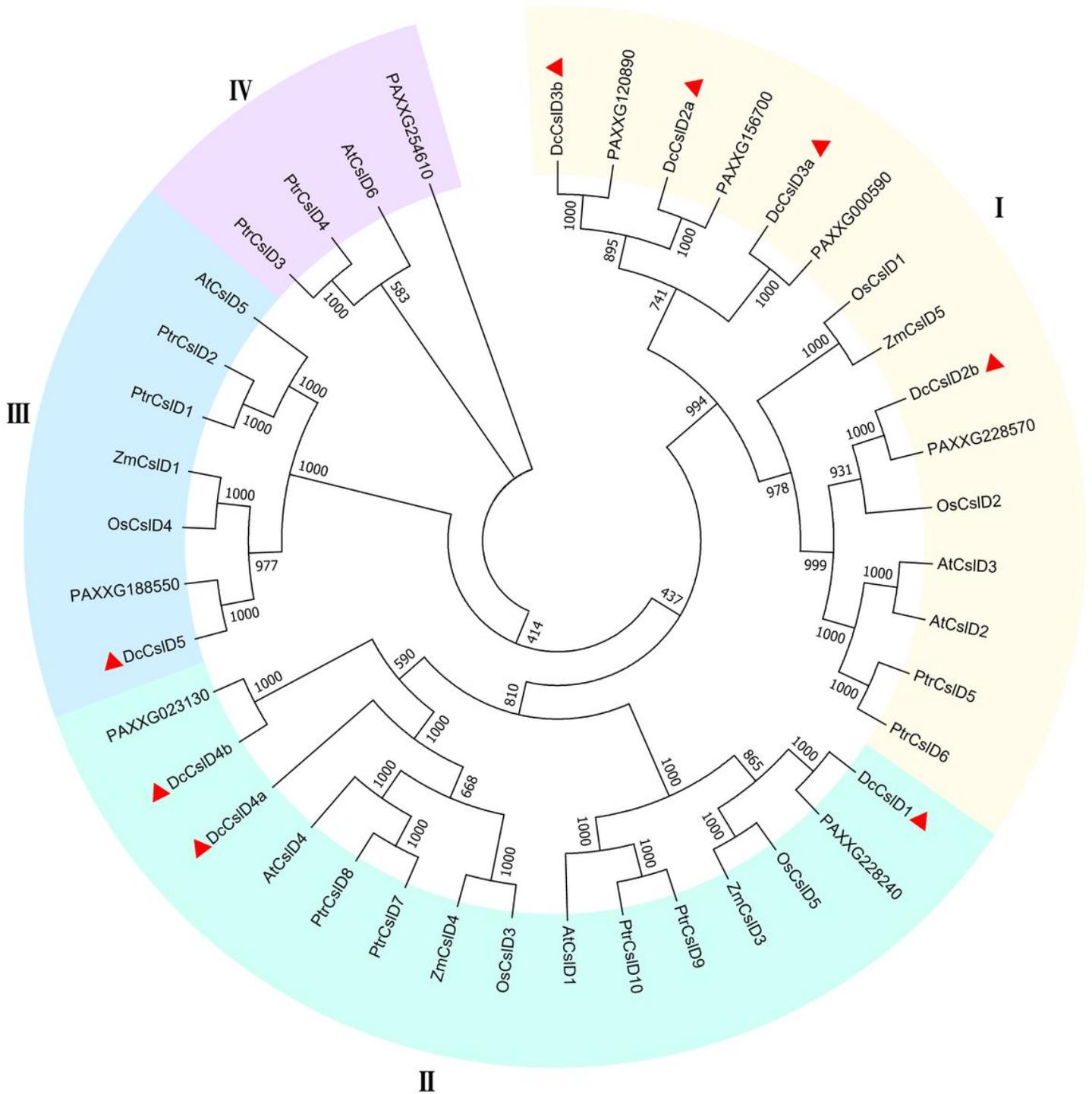
DcCslD4b



DcCslD5

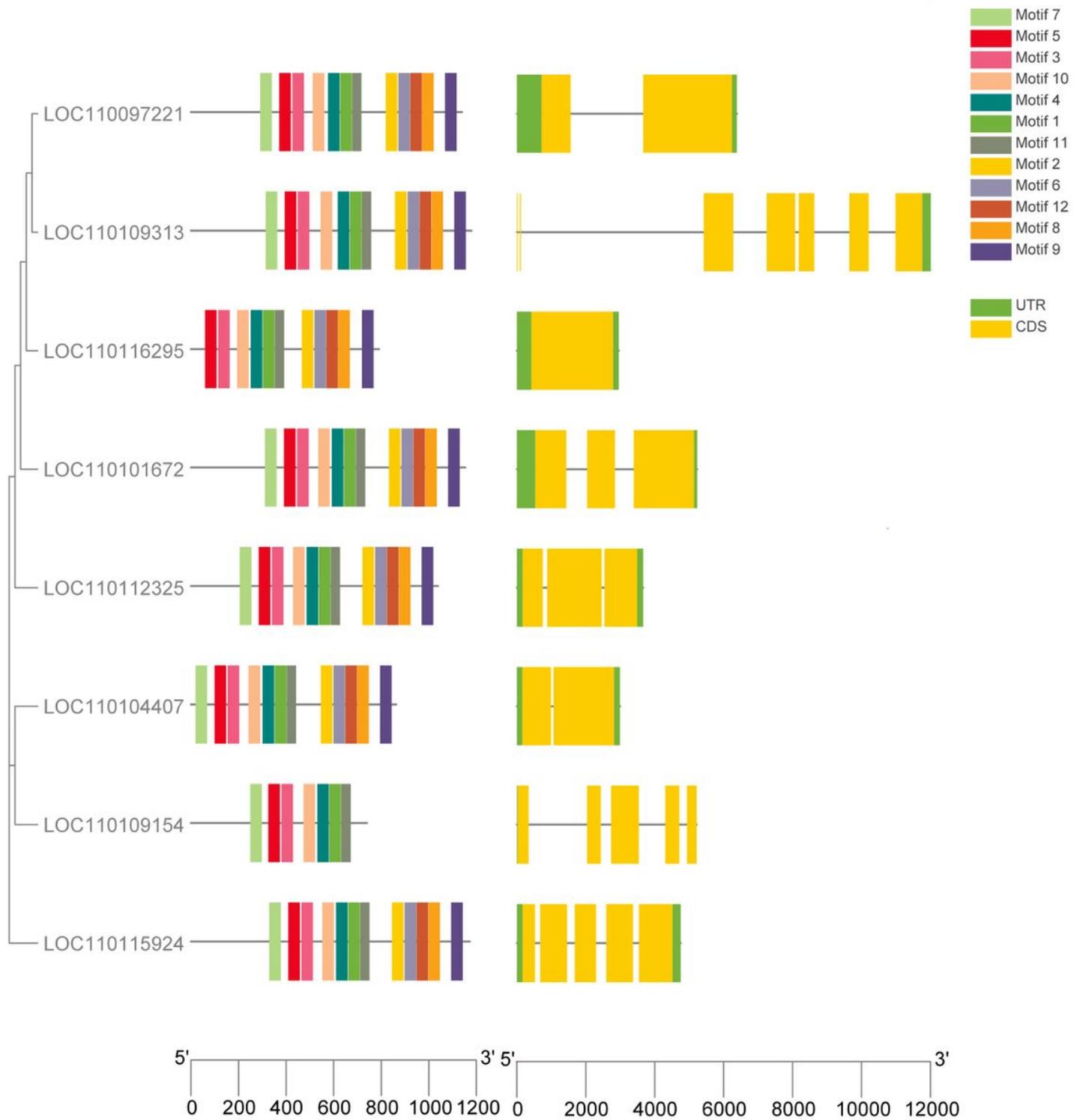
**Figure 2**

3D structures prediction of CslD proteins of *D. catenatum*.



**Figure 3**

Phylogenetic relationship of CsID proteins in six plant species. Phylogenetic tree of CsID proteins. A neighbor-joining (NJ) tree was constructed by MEGA 7.0 using 41 CsID proteins, including 8 proteins from *Dendrobium catenatum*. 10 proteins from *Populus trichocarpa*, 6 proteins from *Arabidopsis thaliana*, 4 proteins from *Oryza sativa*, 5 proteins from *Zea mays*, and 8 genes from *Phalaenopsis aphrodite*. The tree shows 4 distinct clades.



**Figure 4**

Distribution of conserved motif of CsLD protein in *D. catenatum*.

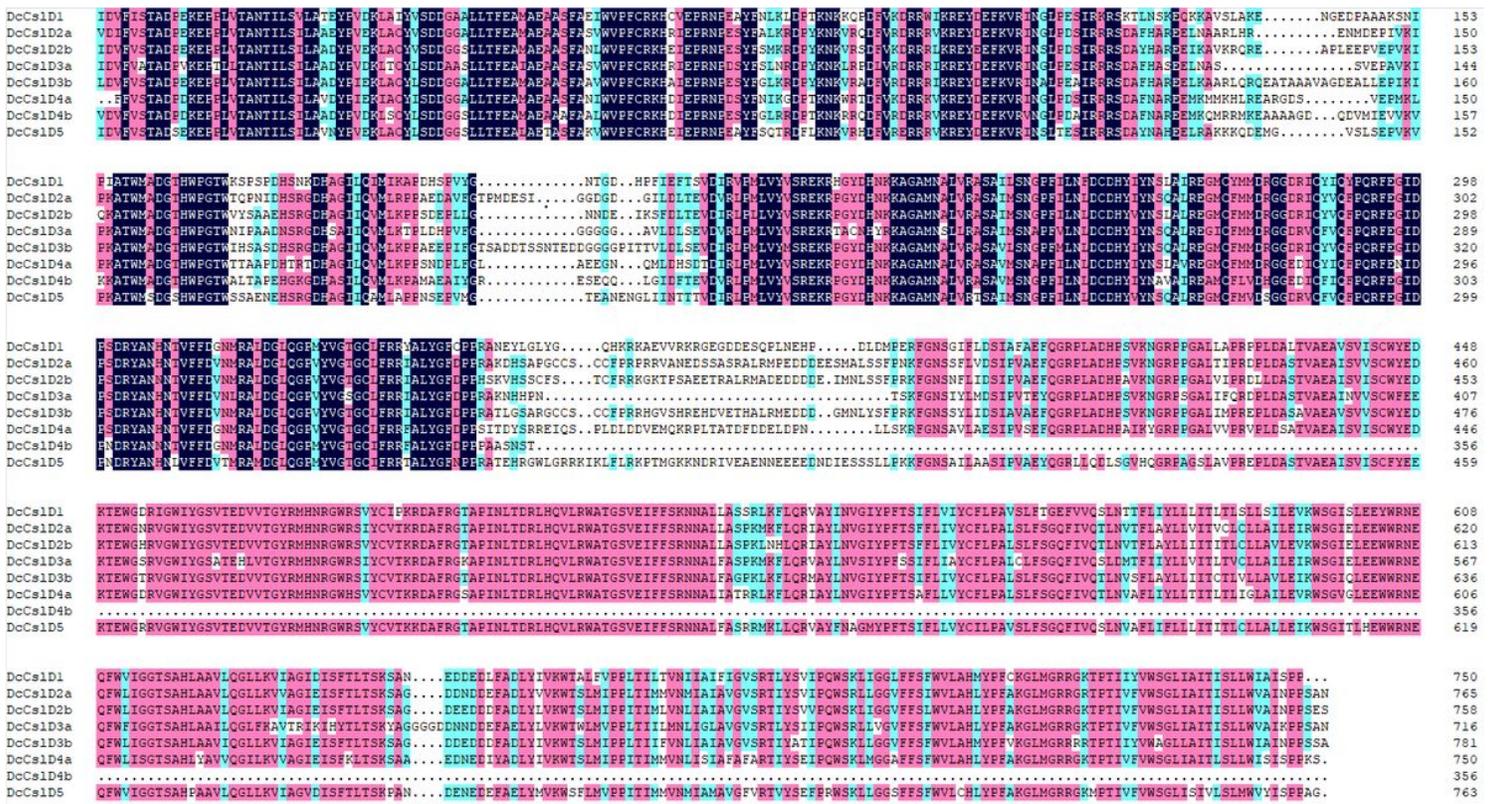


Figure 5

Multiple sequence alignment of CslD protein domain in *D. catenatum*.

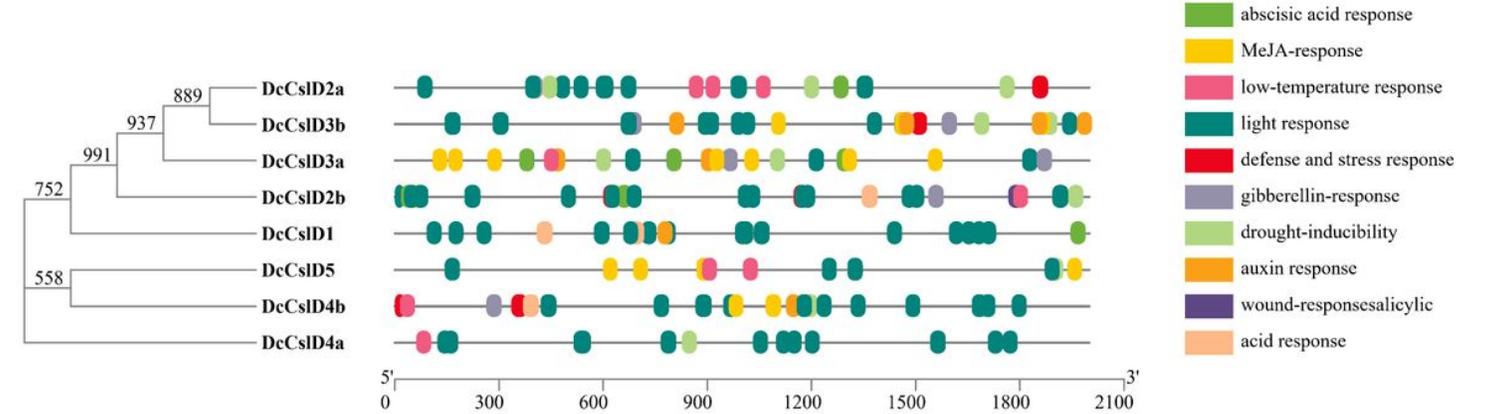
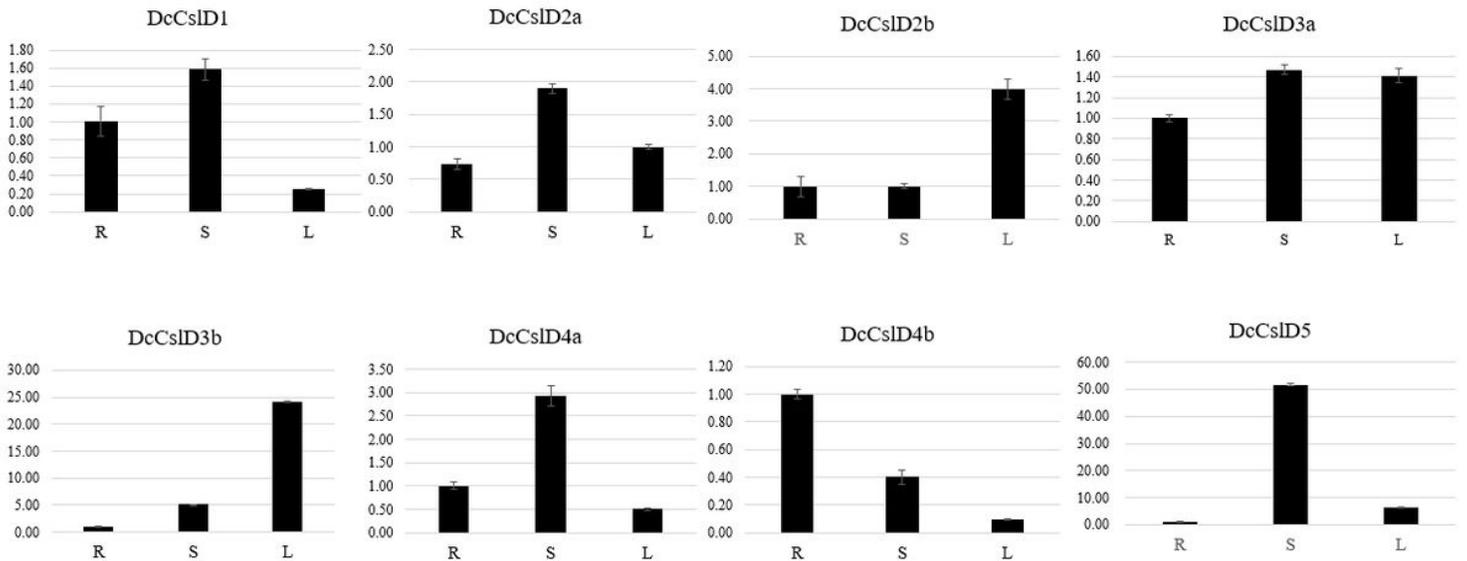


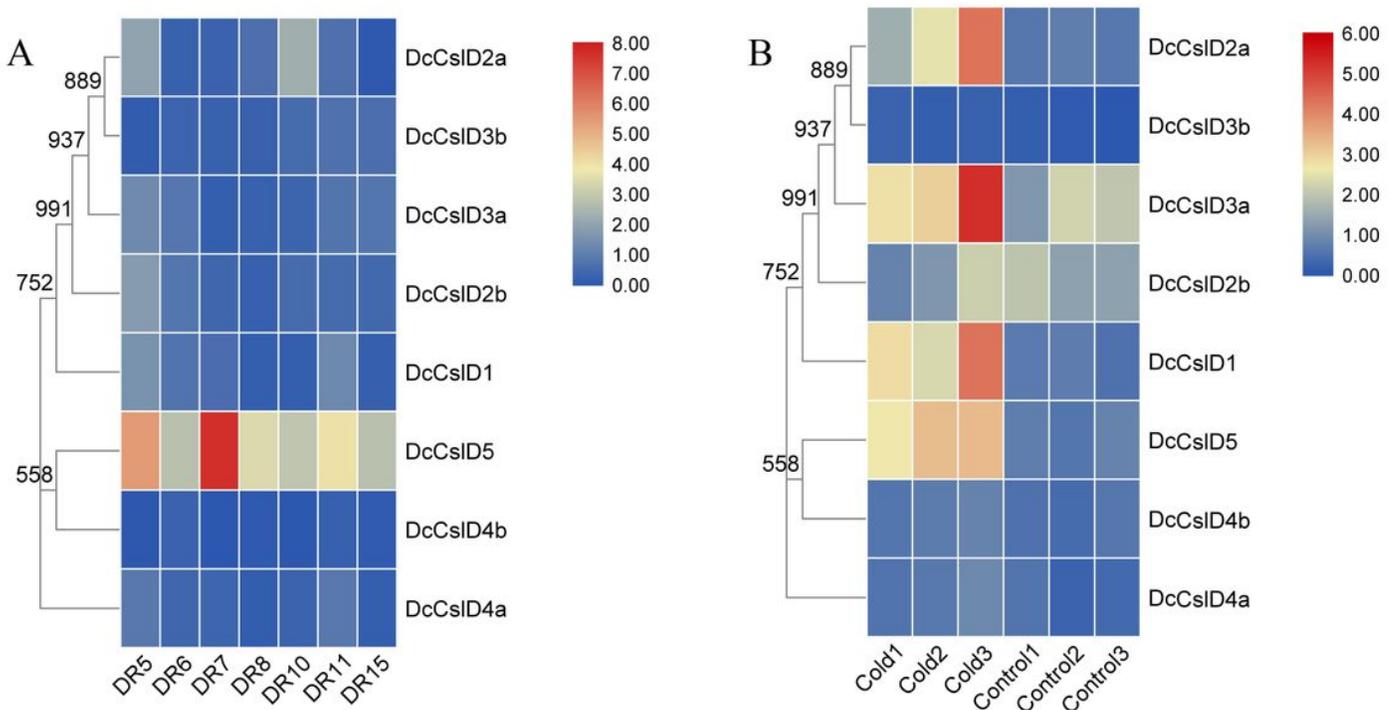
Figure 6

Analysis of CIS sequence of CslD gene promoter in *D. catenatum*.



**Figure 7**

Expression of CsID gene family in organs of *D. catenatum*. We used six-month old seedlings of *D. catenatum* to extract RNA from roots, stems and leaves for qRT-PCR. During this period, to ensure the accuracy of the experiment we designed three biological and three technical repeats for each sample.



**Figure 8**

Heatmaps showing member of CsID gene family in different treatment. (A) Expression of DcCsID after drought treatment, DR5/DR6: The 2nd day treatment at 06:30 and 18:30; DR7/DR8: The 7th day treatment at 06:30 and 18:30; DR10: The 8th day treatment at 18:30 after watering the seedlings at 15:30; DR11/DR15: The 9th day treatment at 06:30 and 18:30. (B) Expression of DcCsID after low temperature treatment. Control 1~3: 20 °C, 20 hours; Cold 1~3: 0 °C, 20 hours. Each group have three repetitions.

## Supplementary Files

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