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ARTICLE

Genome-Wide Characterization of the Cellulose Synthase Gene Superfamily in Tea Plants (*Camellia sinensis*)

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ABSTRACT

The cellulose synthase gene superfamily, including *Cellulose synthase A* (*CesA*) and *cellulose synthase-like* (*Csl*) gene families, is responsible for the synthesis of cellulose and hemicellulose, respectively. The *CesA/Csl* genes are vital for abiotic stress resistance and shoot tenderness regulation of tea plants (*Camellia sinensis*). However, the *CesA/Csl* gene family has not been extensively studied in tea plants. Here, we identified 53 *CsCesA/Csl* genes in tea plants. These genes were grouped into five subfamilies (*CsCesA*, *CsCslB*, *CsCslD*, *CsCslE*, *CsCslG*) based on the phylogenetic relationships with Arabidopsis and rice. The analysis of chromosome distribution, gene structure, protein domain and motif revealed that most genes in *CsCesA*, *CsCslD* and *CsCslE* subfamilies were conserved, whereas *CsCslB* and *CsCslG* subfamily members are highly diverged. The transcriptome analysis showed that most *CsCesA/Csl* genes displayed tissue-specific expression pattern. In addition, members of *CsCslB4*, *CsCesA1/3/6*, *CsCslB3/4*, *CsCslD3*, *CsCslE1* and *CsCslG2/3* subfamilies were up-regulated under drought and cold stresses, indicating their potential roles in regulating stress tolerance in tea plants. Furthermore, the expression levels of *CsCslG2_6* and *CsCslD3_5* in different tissues and cultivars, respectively, were positively correlated with the cellulose content that is negatively related with shoot tenderness. Thus, these two genes were speculated to be involved in the regulation of shoot tenderness in tea plants. Our findings may help elucidate the evolutionary relationships and expression patterns of the *CsCesA/Csl* genes in tea plants, and provide more candidate genes responsible for stress tolerance and tenderness regulation in tea plants for future functional research.

KEYWORDS

Tea plant (*Camellia sinensis*); cellulose synthase superfamily; phylogeny; stress resistance; shoot tenderness regulation

1 Introduction

The tea plant (*Camellia sinensis* (L.) O. Kuntze) is one of the most important economic crops in China. It has been cultivated in China for thousands of years [1], and now is planted in more than 50 countries worldwide [2]. Its buds and young leaves are generally used for manufacturing tea, which is one of the most widely consumed non-alcoholic health beverages in the world [3]. As a perennial evergreen woody crop, the tea plant is often exposed to various abiotic stresses such as drought, low temperature and high salt during its growth and development, which are the main environmental factors that significantly affect

the growth, survival and geographical distribution of tea plants, and seriously constrain the yield and quality of tea products [4–8]. Additionally, the quality and economic value of tea products are also affected by a number of other factors such as the tea plant cultivars, the types and tenderness of leaves, as well as the processing methods used to make tea products [9].

The cell wall acts as the first line of defense when plants encounter various stresses [10]. Cellulose and hemicellulose are the main components of plant cell walls, and they were synthesized by the cellulose synthase A (CesA) and cellulose synthase-like (Csl) enzymes, respectively. Celluloses are the main constituents of both primary and secondary cell walls, and provide the major structural rigidity of the cell-wall matrix [11,12]. Hemicelluloses are the main components of the primary cell wall contributing to strengthening the cell wall by interaction with cellulose and/or lignin [13]. A number of studies have reported the involvement of cellulose and hemicellulose in plant defensing against environmental stresses. For instance, cellulose deficient mutant plants lesioned in *CesA6*, *POM2/CSI1*, or *KOR1* led to enhanced sensitivity to salt stress [14–18]. Besides, mutation of hemicellulose biosynthesis-related genes such as *CslD4*, *CslD5* and *CslF6* significantly affect the abiotic stress-tolerance of plants [14,19,20]. These studies pointed out an important role of the cellulose and hemicellulose synthesis machinery in abiotic stress responses.

In addition to various environmental stresses, the tenderness of tea shoots is another important factor affecting the quality and economic value of tea products. The content of cellulose was considered to be negatively correlated with the tenderness of tea shoots [21,22]. The tender shoots generally contain lower amounts of cellulose than the older ones, and tea products made from tender shoots are usually more expensive than those made from older leaves [9,21]. Besides, the tenderness of new shoots declined gradually with the growth of tea plants, whereas the cellulose content increased during the maturity process of tea shoots [21,22]. Furthermore, 3 *CsCesA* genes involved in the second cell wall cellulose synthesis have been identified in the ‘Huangjinya’ and ‘Yujinxiang’ cultivars of tea plants. Their expression levels were positively correlated with the cellulose content in tea leaves and stems, as well as the thickness of leaf tissues, indicating their involvement in modifying the tenderness of new shoots via regulating cellulose biosynthesis [23].

The *CesA* and *Csl* genes belong to cellulose synthase superfamily which is classified as glycoside transferase gene family (GT2). Multiple isoforms of CesA enzymes form the cellulose synthase complex (CSC) and catalyze the assembly of β-(1,4)-glucan chains forming protofibrils, which are then transformed into cellulose microfibrils [24–26]. The Csl enzymes share sequence similarity with the CesA family [26]. The Arabidopsis genome encodes 40 *AtCesA/Csl* genes, including 10 *AtCesA* and 30 *AtCsl* genes [27–29]. The *AtCsl* genes are grouped into 6 subfamilies, including *AtCslA*, *AtCslB*, *AtCslC*, *AtCslD*, *AtCslE*, *AtCslG*. In addition, *CslF*, *CslH, CslJ* and *CslM* subfamilies exist in some plant genomes [30–32]. At present, the cellulose synthase superfamily has been extensively characterized in a variety of plant species, such as Arabidopsis [32], rice [33], maize [34], barley [35], cotton [36], pear [37], tomato [38], poplar [39,40], pine [41] and moss [42]. However, even after the whole-genome sequence for tea plants is available [43], the *CesA/Csl* family genes have not been fully characterized in tea plants.

In this work, we identified 53 *CsCesA/Csl* family members in tea plants at the whole-genome level and analyzed their evolutionary relationships with Arabidopsis and rice. Besides, we studied the expression patterns of *CsCesA/Csl* genes in different tissues and under abiotic stresses by analyzing the transcriptome data. Furthermore, we examined the distinctive expression patterns of several selected *CsCesA/Csl* genes in sampled tissues and cultivars. We also investigated the correlation between the expression patterns of these genes and cellulose content alterations in different tissues and cultivars. These results may present new insights into the evolution, expression profiles and functional divergence

of *CsCesA/Csl* genes, as well as provide more candidate genes for further study to improve the economic value of tea plants.

2 Materials and Methods

2.1 Plant Growth and Tissue Sampling

The tea plants were grown under standard field conditions in the tea plant resource garden of Guizhou University. Three biological replicates of the first three leaves and the tender stems of 'Fuding' cultivar, as well as the tender stems of 'Longjing', 'Wuniuzao', 'Zhonghuang', 'Naibai' and 'Shuijingbai' were collected to determine the cellulose content and expression patterns of *CsCesA/Csl* genes. All samples were stored at −80°C for further use.

2.2 Identification of CsCesA/Csl Genes in the Tea Genome and Physicochemical Property Analysis

The genome data and protein sequence of tea plant, Arabidopsis and rice were obtained from Tea Plant Information Archive (TPIA) (http://tpia.teaplant.org/), The Arabidopsis Information Resource (TAIR) (https://www.arabidopsis.org/) and Rice Genome Annotation Project (RGAP) (http://rice.plantbiology.msu.edu/index.shtml) database, respectively. To identify the tea *CsCesA/Csl* genes, we used the Arabidopsis AtCesA/Csl amino acid sequences to search against tea proteome with Basic Local Alignment Search Tool (BLAST-P) (E-value $< 10^{-5}$) and deleted the redundant sequences. All candidate CsCesA/Csl proteins were submitted to the online bioinformatics tool, the National Center of Biotechnology Information Conserved Domain-Search Tool (NCBI CD-Search Tool) (https://www.ncbi.nlm.nih.gov/Structure/index.shtml) (E-value $< 10^{-2}$) [44] to confirm CsCesA/Csl proteins that contained the core domains. The resulting candidate CsCesA/Csl proteins were further confirmed through BLAST-P against the Universal Protein Resource (UniProt) proteome databases (https://www.uniprot.org/) (E-value $< 10^{-10}$). The length of chromosome, exon and amino acid of CsCesA/Csl proteins were analyzed based on the annotated information downloaded from tea plant genome database. The molecular weight and isoelectric point of tea CsCesA/Csl amino acid sequences were predicted by using ExPASy (http://web.expasy.org/compute_pi/).

2.3 Phylogenetic Analysis of CsCesA/Csl Genes

DNAMAN software (Lynnon Biosoft, Quebec, Canada) was used to perform multiple sequence alignment with default settings for CesA/Csl protein sequences of tea plant, Arabidopsis and rice. The phylogenetic tree was constructed by MEGA X software (Mega Limited, Auckland, New Zealand) through the Neighbor joining (NJ) method with a bootstrap option of n = 1000.

2.4 Chromosomal Distribution and Gene Structure Analysis

The information of chromosome localization of *CsCesA/Csl* genes was downloaded from TPIA (http://tpia.teaplant.org/) and visualized by using TBtools (https://github.com/CJ-Chen/TBtools/releases), including the localization and length in corresponding chromosomes. The exon-intron structures about the quantity and distribution of the *CsCesA/Csl* genes based on the genome annotation were visualized by TBtools software.

2.5 Protein Domain and Conserved Motif Analysis

The conserved domains of CsCesA/Csl proteins were obtained by NCBI CD-Search Tool (https://www.ncbi.nlm.nih.gov/Structure/index.shtml) and visualized by TBtools. The conserved protein motifs of CsCesA/Csl proteins were analyzed through the Multiple Em for Motif Elicitation (MEME) program (http://meme-suite.org/) and visualized by TBtools.

2.6 Expression Pattern Analysis

The transcriptome data (TPM value) of *CsCesA/Csl* genes in different tissues (apical bud, young leaf, mature leaf, old leaf, stem, root, flower and fruit) of 'Shuchazao' cultivar, and transcriptome data in 'Longjing43' and 'Tieguanyin' in response to drought and cold stresses, respectively, were downloaded from TPIA (http://tpia.teaplant.org). The Log2-based-fold changes were used to create heatmaps via TBtools software.

2.7 RNA Extraction and qRT-PCR Analysis

Primers for the selected *CsCesA/Csl* genes were designed using the online program Integrated DNA Technologies (IDT) (https://sg.idtdna.com/pages) (Supplementary Table 1), the primer sequences were synthesized by Beijing Qingke Biotechnology Limited Company (China). Total RNA was extracted from the first, second and third leaves and tender stem segments of tea plants by cetyltrimethylammonium bromide (CTAB) method. The concentration of RNA was detected by a NanoPhotometer N50 Touch (Implen, Munich, Germany), and the RNA was then reversed transcribed into the first-strand cDNA by qRT-PCR kit (Tiangen, Beijing, China) according to the manufacturer's instructions. The cDNA was subsequently used as a template for qRT-PCR analysis. SYBR Green qPCR Mix (Genenode, Wuhan, China) reagent was used for qRT-PCR amplification. Each reaction system contains 10 μL SYBR Green qPCR Mix, 0.8 μL forward and reverse primers, 1.5 μL cDNA template, 7.7 μL ddH_2O. Reaction procedure was as follows: 95°C for 3 min; 40 cycles of 95°C for 10 s and 60°C for 20 s; 72°C for 30 s, *CsGAPDH* was used as the reference gene. The $2^{-\Delta\Delta CT}$ algorithm was used to calculate the relative expression of genes [45].

2.8 Determination of Cellulose Content

The tea samples were ground into fine powder and filtered through a 30-mesh sieve. The cell wall structural components were extracted with 70% ethyl alcohol and acetone reagent. The crystalline cellulose was hydrolyzed completely to glucose under highly acidic conditions. The glucose content in the supernatant was determined by anthrone-sulfuric colorimetry [46]. The crystallized cellulose was calculated according to the standard curve established by absorbance on the same 96-well polystyrene microtitration plate by the 1510 Multiskan GO spectrophotometer (Thermo Fisher Scientific, Vanta, Finland) under 620-nm wavelength [46]. All data were analyzed by Statistical Product and Service Solutions (SPSS) software (SPSS, Chicago, USA).

2.9 Statistical Analysis

All results were given as means ± standard deviation of at least three biological replicates. The data were subjected to one-way analysis of variance using SPSS software. A p value smaller than 0.05 was considered to be statistically significant.

3 Results

3.1 Identification and Phylogenetic Analysis of the CsCesA/Csl Genes in the Tea Genome

To identify the tea plant *CsCesA/Csl* genes, Arabidopsis AtCesA/Csl protein sequences were used to search against tea plant proteome with BLAST-P program (E-value $< 10^{-5}$). To confirm CsCesA/Csl proteins that contained the core domains, all candidate CsCesA/Csl proteins were submitted to the online bioinformatics tool, NCBI CD-Search Tool (https://www.ncbi.nlm.nih.gov/Structure/index.shtml) (E-value $< 10^{-2}$) [44]. The authenticity of the candidate CsCesA/Csl proteins was further confirmed through BLAST-P against the Universal Protein Resource (UniProt) proteome databases (https://www.uniprot.org/) (E-value $< 10^{-10}$). A total of 53 candidate *CsCesA/Csl* genes were identified from tea plants, and they were named after their corresponding orthologs in Arabidopsis and classified into five subfamilies, including *CsCesA*, *CsCslB*, *CsCslD*, *CsCslE*, *CsCslG* based on the phylogenetic relationships with Arabidopsis and rice (Fig. 1, Supplementary Table 2).

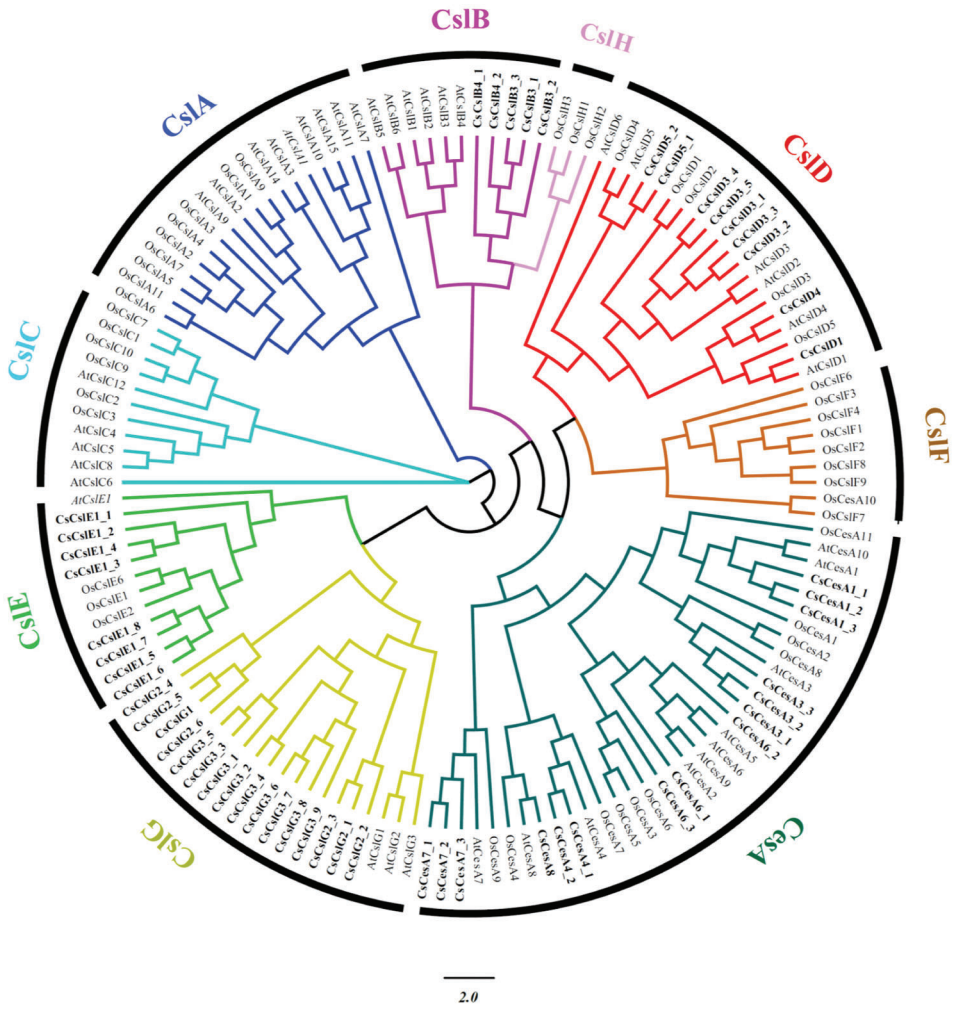

Figure 1: Phylogenetic relationships and subfamily designations of CesA/Csl proteins from the tea plant (*Camellia sinensis*), *Arabidopsis thaliana* and rice (*Oryza sativa*). The phylogenetic tree was constructed with MEGA X using the neighbor-joining (NJ) method and 1000 bootstrap replicates. The superfamily was divided into 9 subfamilies, including *CesA*, *CslA*, *CslB*, *CslC*, *CslD*, *CslE*, *CslF*, *CslH* and *CslG*. Prefix 'At', 'Os' and 'Cs' indicate CesA/Csl proteins from *Arabidopsis thaliana*, *Oryza sativa* and *Camellia sinensis*

To investigate the functional associations and evolutionary relationships of *CsCesA*/*Csl* genes, a multi-species phylogenetic tree of *CesA/Csl* genes from Arabidopsis, rice and tea plant were constructed. This tree comprised 9 subfamilies, including *CesA*, *CslA*, *CslB*, CslC, *CslD*, *CslE*, *CslF*, *CslG* and *CslH* (Fig. 1, Supplementary Table 2). Among them, the *CesA* subfamily was the largest subfamily, containing 15 tea *CsCesA* genes, 10 Arabidopsis genes and 11 rice genes, accounting for 26.1% of the total *CesA/Csl* genes. The second largest subfamily was the *CslD* subfamily, with 9, 6 and 5 *CslDs* from tea plant, Arabidopsis and rice, respectively. Since rice lacks any *CslG* gene [47], the third largest family, *CslG* subfamily, involved 16 tea genes and 3 Arabidopsis genes. The *CslH* subfamily was the smallest one with only 3 rice *OsCslH* genes. No tea plant genes were found in the subfamilies of *CslA*, *CslC*, *CslF* and *CslH*, and there were 9 *AtCslAs* and 5 *AtCslCs* from Arabidopsis, and 9 *OsCslAs* and 6 *OsCslCs* from rice, respectively. The *CslF* and *CslH* subfamilies only contained 8 and 3 genes from rice,

respectively. These results suggested evolutionary conservation and closer homology existed in closely related *CesA*/*Csl* gene subfamilies.

As shown in Table 1, the genomic DNA size of these genes varied from 448 bp (*CsCslG2_4*) to 27,703 bp (*CsCslB3_1*), and the average length was 9,512 bp. Among them, 18.9% *CsCesA/Csl* genes were <4000 bp, 24.5% of them were 4000–7000 bp, and 56.6% were >7000 bp. The encoded protein sequences consisted of 79 (CsCslG2_4) to 1,174 (CsCslD5_2) amino acids, with an average of 779 amino acids. Their corresponding molecular weights varied from 9 kDa (CsCslG2_4) to 132.1 kDa (CsCslD5_2), with the theoretical isoelectric points ranging from 6 (CsCesA1_2 and CsCesA1_3) to 9.51 (CsCslB4_1). The diverse physical and molecular properties of the 53 *CsCesA/Csl* genes might be resulted from the selection pressure during gene family evolution, as well as the genomic assembly and transcript annotation quality, indicating their different roles in various microenvironments.

Table 1: Physical and molecular properties of 53 CsCesA/Csl identified in tea plants (*Camellia sinensis*)

Gene ID	Gene name	Chromosome No.	Length (bp)	Introns	Exons	Amino acid (aa)	Molecular weight (Da)	Isoelectric point
CSS0012399.1	*CsCesA1_1*	Chr12	9647	13	14	1085	122049.67	6.04
CSS0010245.1	*CsCesA1_2*	Chr14	8456	13	14	1027	115468.09	6.00
CSS0034898.1	*CsCesA1_3*	Chr14	8548	13	14	1027	115468.09	6.00
CSS0008005.3	*CsCesA3_2*	Chr3	14867	14	15	1083	121034.60	6.86
CSS0038121.4	*CsCesA3_3*	Chr3	15241	14	15	1088	121945.62	6.84
CSS0031116.1	*CsCesA3_1*	Chr3	15331	14	15	1083	121061.63	6.86
CSS0016274.1	*CsCesA4_1*	Contig653	6792	10	11	1037	117820.59	7.78
CSS0012493.1	*CsCesA4_2*	Chr11	6859	10	11	1038	117950.69	7.41
CSS0025911.1	*CsCesA6_3*	Chr4	6558	15	16	1074	122079.74	7.61
CSS0009305.1	*CsCesA6_1*	Chr4	7668	12	13	1097	123465.15	6.65
CSS0028360.1	*CsCesA6_2*	Chr1	9241	13	14	1102	124259.58	6.09
CSS0005229.1	*CsCesA7_2*	Chr2	6670	12	13	1042	117993.78	6.12
CSS0014034.1	*CsCesA7_3*	Chr6	7687	12	13	1048	118380.00	6.06
CSS0012489.1	*CsCesA7_1*	Contig242	4000	7	8	788	89572.29	8.37
CSS0033685.1	*CsCesA8*	Chr8	9268	13	14	933	104455.27	6.88
CSS0005626.1	*CsCslB3_2*	Chr5	14189	8	9	744	83606.08	8.58
CSS0031882.1	*CsCslB3_3*	Chr5	26392	8	9	744	83585.95	8.48
CSS0015423.1	*CsCslB3_1*	Chr5	27703	8	9	632	71676.15	8.57
CSS0024869.1	*CsCslB4_1*	Chr7	1155	1	2	289	32127.85	9.51
CSS0006219.1	*CsCslB4_2*	Chr5	13050	3	4	312	34879.20	9.25
CSS0012214.1	*CsCslD1*	Contig313	12539	5	6	1057	117895.77	8.49
CSS0012996.1	*CsCslD3_1*	Chr1	5811	3	4	1139	128058.16	7.24
CSS0039274.1	*CsCslD3_3*	Chr7	6162	4	5	1146	128713.94	7.04
CSS0024657.1	*CsCslD3_2*	Chr7	9482	3	4	1146	128741.96	7.04
CSS0020384.1	*CsCslD3_4*	Chr12	7815	3	4	983	110562.94	8.15

(Continued)

Table 1 (continued)

Gene ID	Gene name	Chromosome No.	Length (bp)	Introns	Exons	Amino acid (aa)	Molecular weight (Da)	Isoelectric point
CSS0001659.1	*CsCslD3_5*	Contig539	5133	3	4	820	92013.29	6.39
CSS0009118.1	*CsCslD4*	Chr3	4583	3	4	1140	127504.81	6.38
CSS0001364.1	*CsCslD5_2*	Chr11	4538	2	3	1174	132134.46	8.75
CSS0045476.1	*CsCslD5_1*	Contig879	4755	2	3	1173	132051.38	8.75
CSS0008757.1	*CsCslE1_3*	Chr8	12743	7	8	741	84868.52	7.72
CSS0041502.1	*CsCslE1_4*	Chr8	13591	7	8	744	85312.96	7.14
CSS0027440.1	*CsCslE1_2*	Chr8	19230	7	8	725	83418.87	7.96
CSS0009487.1	*CsCslE1_1*	Chr5	9079	7	8	737	84214.29	8.00
CSS0038776.1	*CsCslE1_6*	Chr7	5806	7	8	732	83581.83	8.58
CSS0019900.1	*CsCslE1_5*	Chr7	5799	7	8	732	83411.67	8.58
CSS0045281.1	*CsCslE1_7*	Chr7	3370	7	8	734	83703.69	7.13
CSS0001964.1	*CsCslE1_8*	Chr7	3060	8	9	704	80196.95	8.17
CSS0018408.1	*CsCslG1*	Chr9	2279	6	7	488	55322.27	8.88
CSS0044240.1	*CsCslG2_1*	Chr12	24359	6	7	716	81530.02	7.95
CSS0019495.1	*CsCslG2_6*	Chr5	20909	8	9	689	78565.51	8.81
CSS0009342.1	*CsCslG2_5*	Chr5	2664	8	9	395	45047.40	8.93
CSS0049722.1	*CsCslG2_2*	Chr12	1773	3	4	355	40029.19	6.38
CSS0020707.1	*CsCslG2_3*	Chr12	1779	3	4	355	40525.08	8.58
CSS0000850.1	*CsCslG2_4*	Chr5	448	2	3	79	9015.66	9.15
CSS0020977.1	*CsCslG3_5*	Contig1173	19718	5	6	734	82980.35	8.37
CSS0041642.1	*CsCslG3_3*	Chr14	17169	4	5	473	53111.89	8.61
CSS0032646.1	*CsCslG3_7*	Chr14	13457	5	6	527	59503.87	8.77
CSS0032273.1	*CsCslG3_4*	Chr14	7396	5	6	528	59505.98	8.60
CSS0037731.1	*CsCslG3_6*	Chr14	16613	5	6	525	59368.74	8.68
CSS0036058.1	*CsCslG3_8*	Chr14	11903	5	6	518	58410.87	8.99
CSS0028184.1	*CsCslG3_1*	Contig931	1497	2	3	262	29507.05	6.97
CSS0026824.1	*CsCslG3_2*	Chr14	1436	1	2	264	29842.57	8.25
CSS0033694.1	*CsCslG3_9*	Chr14	7901	8	9	487	55390.08	7.17

3.2 Genomic Distribution and Structural Feature of the CsCesA/Csl Genes

To clarify the genomic distribution of the *CsCesA/Csl* genes, a map of chromosomal locations was constructed based on the information annotated in the tea plant genome. A total of 46 *CsCesA/Csl* genes were unevenly distributed on 12 of 15 chromosomes, with the exception being Cs_chr10, Cs_chr13 and Cs_chr15 (Table 1, Fig. 2). Chromosome 14 contained the most *CsCesA/Csl* gene members, including 2 *CsCesA1s* and 7 *CsCslG3s*, and each subfamily was presented in the form of gene clusters. Besides, Chromosome 5 included 8 genes, followed by chromosome 7 containing 7 genes. In addition, only one

gene was found on chromosome 2, 6 and 9, respectively. However, 7 out of 53 *CsCesA/Csl* genes were located on scaffolds, and were not presented in the figure (Table 1).

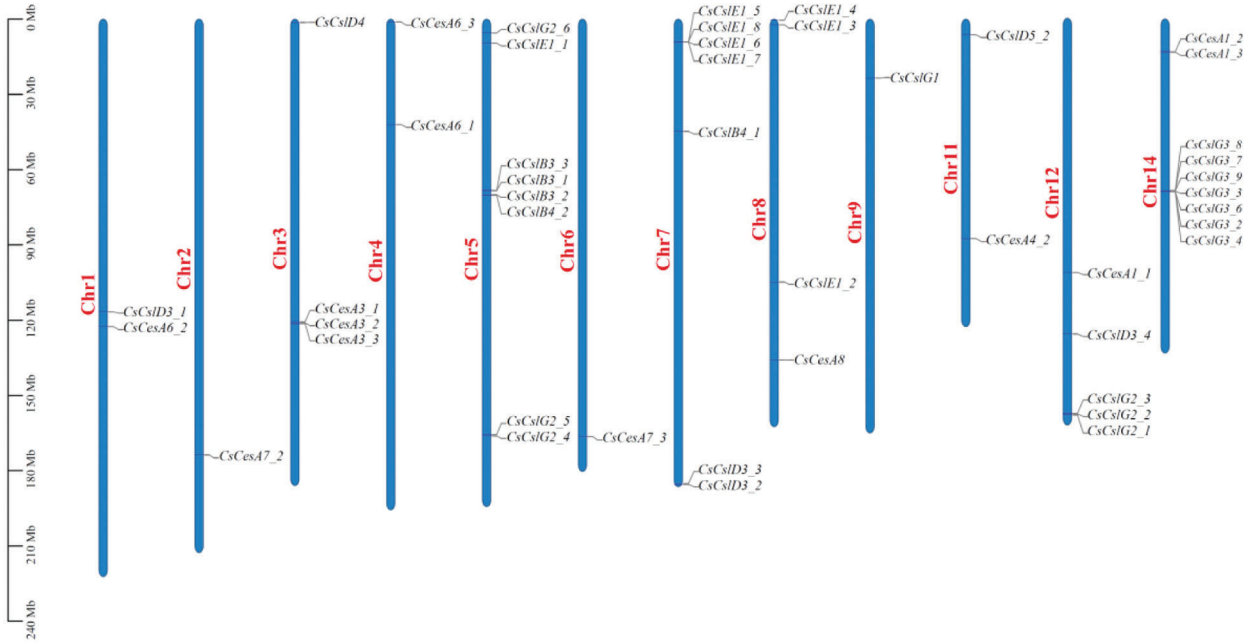

Figure 2: Distribution of *CsCesA/Csl* genes on the tea plant chromosomes. A total of 46 *CsCes/Csl* genes were localized to tea chromosomal regions, while the other genes were detected on scaffolds and were not presented in the figure

To provide more valuable information involved in evolutionary pattern and structural diversity of *CsCesA/Csl* genes, their exon-intron structures were analyzed. As shown in Fig. 3, most of the *CsCesA/Csl* genes in each of the *CsCesA*, *CsCslE* and *CsCslD* subfamilies showed similar exon-intron structure features. For instance, *CsCesA* genes had more than 10 exons (ranging from 11 to 16 exons), except for *CsCesA7_1* with 8 exons. In contrast, all of the remaining *CsCsl* genes contained fewer than 10 exons. Specifically, *CsCslE* subfamily had 8 to 9 exons, and *CsCslDs* included 3 to 6 exons. However, the numbers of exons in both *CsCslB* and *CsCslG* subfamilies varied from 2 to 9, indicating a higher degree of divergence might exist in these two subfamilies than others.

3.3 Protein Conserved Domains and Motifs Analysis of the CsCesA/Csl Family

All of the 53 identified CsCesA/Csl proteins contained one conserved cellulose synthase domain, most of which were located at the C-terminus (Fig. 4). In addition, several of the CsCesA/Csl proteins contained other domains. For instance, 13 CsCesA proteins included the Zinc finger-UDP domain located near the N-terminal, 5 CsCslD3 proteins and one CsCslD4 contained the Zinc finger-RING_4 domain, which existed upstream of the cellulose synthase domain, and 2 CsCslD5s and one CsCesA8 had the RING-Ubox superfamily domain, with the domain in CsCesA8 located at the N-terminal (Fig. 4).

To further explore the diversification of *CsCesA/Csl* gene family in tea plants, the putative conserved motifs of CsCesA/Csl proteins were predicted by the MEME program. A total of 15 distinct motifs were identified in the *CsCesA/Csl* gene family (Fig. 5). The number of different motifs was similar in each of the *CsCesA*, *CsCslD*, *CsCslE* subfamilies, but varied in the subfamilies of *CsCslB* and *CsCslG*. In the *CsCesA* subfamily, 14 out of the 15 (93.3%) members contained all of the 15 motifs, except for

CsCesA7_1 lacking motif 10. Similarly, the 15 different motifs were also predicted in 6 out of the 9 (66.7%) *CsCslD* genes. For the *CsCslE* genes, 8 out of the 9 (87.5%) members had 12 motifs. In contrast, the number of distinct motifs varied from 2 to 13 and 2 to 12 in the *CsCslB* and *CsCslG* subfamilies, respectively. Motif 7 and 10 were not predicted in the *CsCslB*, *CsCslE* or *CsCslG* subfamilies, indicating these motifs might be key players during the functional differentiation of *CsCesA/Csl* genes during evolution.

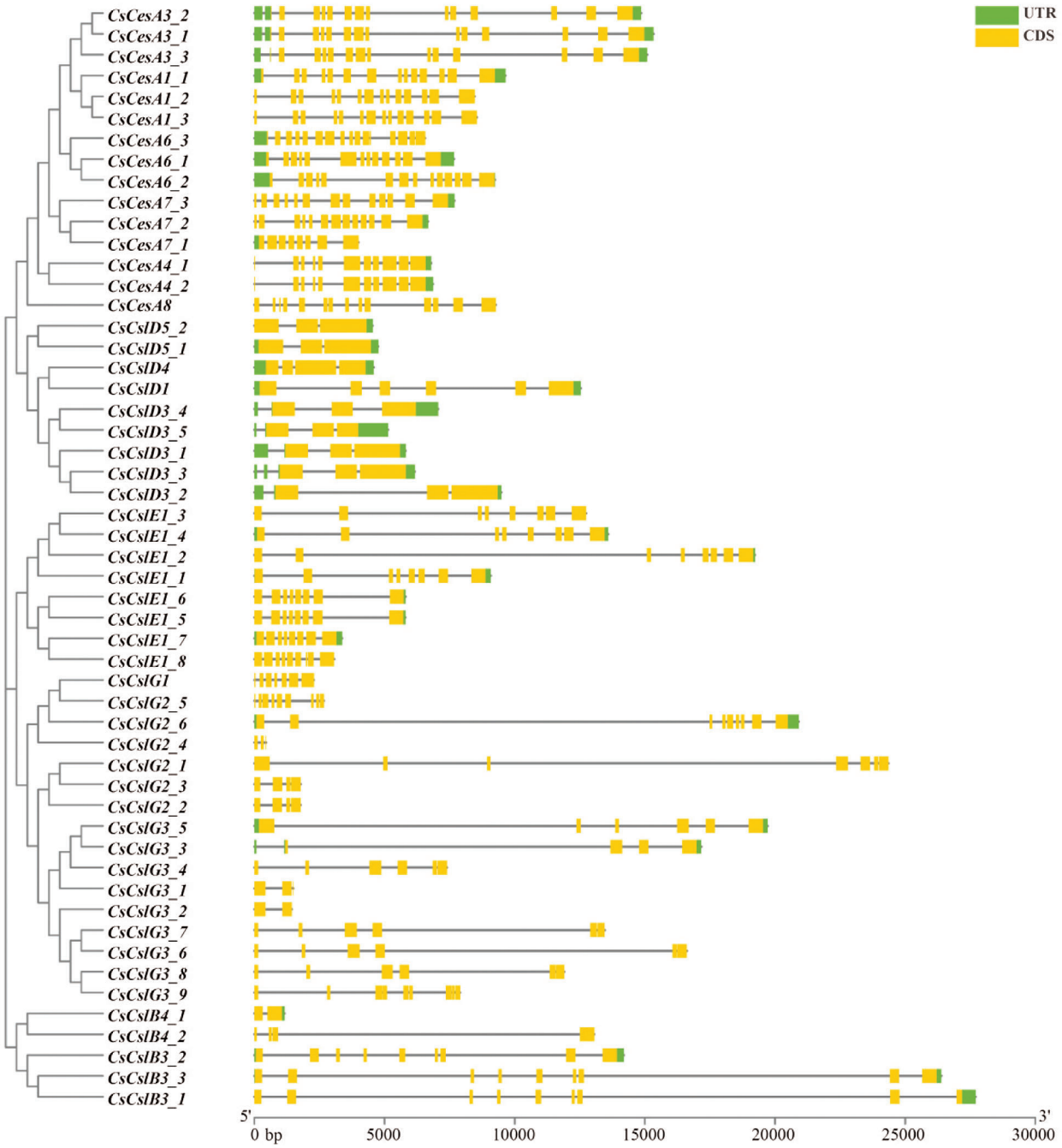

Figure 3: Exon-intron structure of tea plant *CsCesA/Csl* genes. Green boxes indicate untranslated 5'- and 3-regions, yellow boxes represent exons, and black lines indicate introns

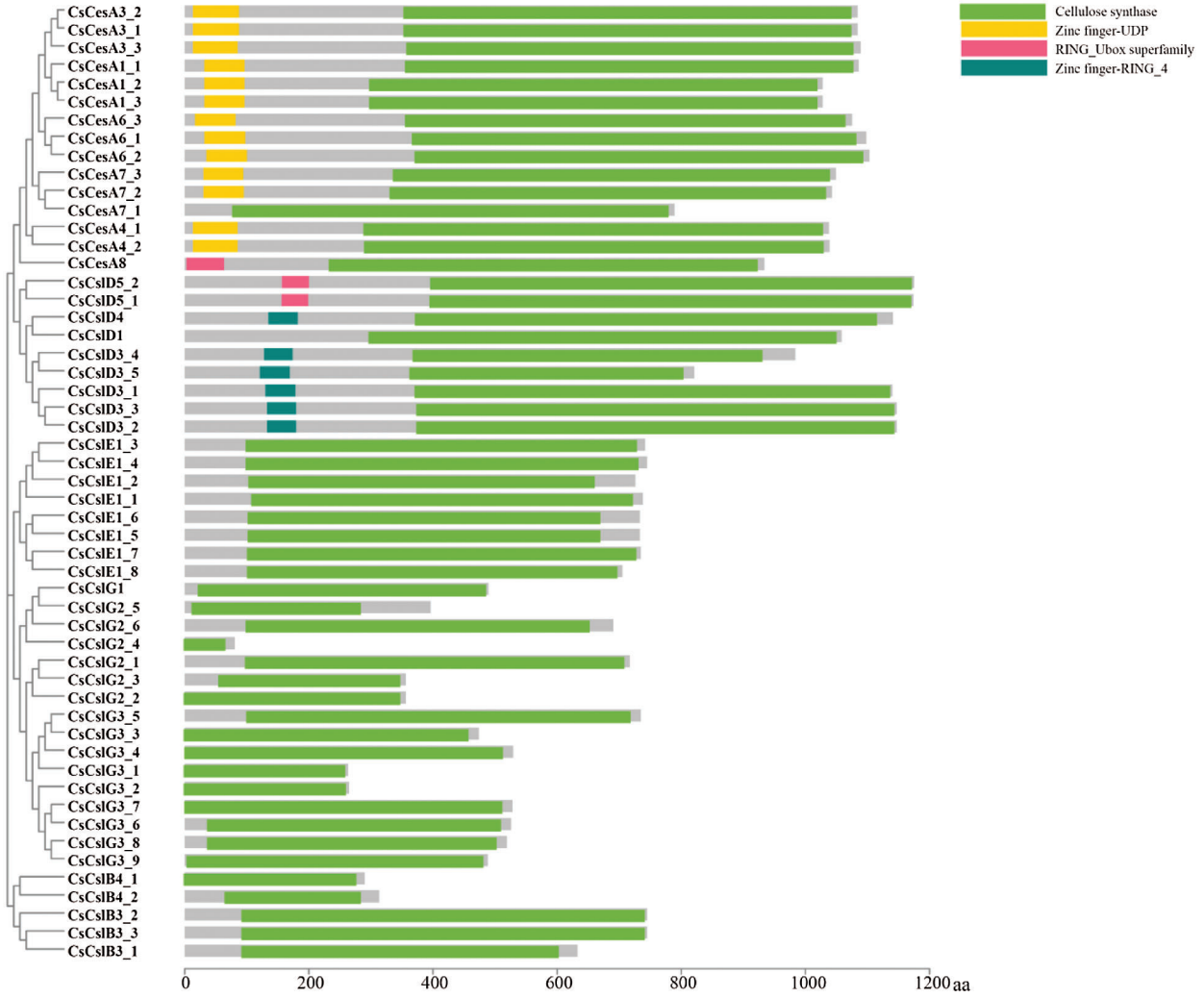

Figure 4: Conserved domains of tea plant CsCesA/Csl proteins. Green, yellow, pink and blue boxes indicate cellulose synthase, Zinc finger-UDP, RING_Ubox superfamily and Zinc finger-RING_4 domains, respectively

3.4 Expression Patterns of CsCesA/Csl Genes in Different Tissues

To further explore the particular function of the *CsCesA/Csl* genes in tea plants, we conducted a transcriptome analysis of the *CsCesA/Csl* genes in different tissues of the tea cultivar 'Shuchazao' of tea plant (apical bud, young leaf, mature leaf, old leaf, stem, root, flower and fruit) [43,48]. A heat map was used to display the *CsCesA/Csls* expression patterns in eight tissues (Fig. 6). A total of 17 genes (3 *CsCesA1s*, 3 *CsCesA3s*, 2 *CsCesA4s*, 3 *CsCesA6s*, a *CsCslD3*, and 5 *CsCslG3s*) were expressed in all sampled tissues at high levels, indicating that these genes might play crucial roles in the whole process of tea plant development. Besides, the tissue-specific expression patterns were identified in a number of tea *CsCesA/Csl* genes. For instance, 4 *CsCslG3s* (*CsCslG3_1*, *CsCslG3_3*, *CsCslG3_5* and *CsCslG3_8*) and 2 *CsCslE1s* (*CsCslE1_5* and *CsCslE1_6*) displayed moderate-to-high expression levels in 6 out of the 8 tissues, including mature leaves, old leaves, stems, roots, flowers and fruits, but expressed at low levels in the apical buds and young leaves, suggesting their potential tissue-specific functions in the corresponding developmental processes. In addition, 2 *CsCesA7s* (*CsCesA7_1* and *CsCesA7_2*) and 2

CsCslG2s (*CsCslG2_5* and *CsCslG2_6*) might be important for the development of the apical buds, young leaves, stems and roots because of their higher expression levels in these tissues than in others. Interestingly, it was found that several *CsCesA/Csl* gene family members displayed opposite expression patterns. For example, the *CsCslD1* and *CsCslD4* genes showed high expression levels in flowers and fruits and low expression in other tissues. In contrast, the expression levels of the *CsCslE1_1* and 4 *CsCslBs* (*CsCslB3_1*, *CsCslB3_2*, *CsCslB3_3* and *CsCslB4_2*) were low in flowers and fruits, but high in other sampled tissues. The contrary expression pattern was also found between 2 *CsCslE1s* (*CsCslE1_7* and *CsCslE1_8*) and *CsCslD3_1*. The former genes expressed at higher levels in mature and old leaves than in other tissues, whereas the latter showed lower transcript abundances in mature and old leaves than in other sampled tissues. Furthermore, the expression levels of 2 *CsCslE1s* (*CsCslE1_3* and *CsCslE1_4*) were lower in the root than in the other tissues, while *CsCslE1_2* and 3 *CsCslG2* genes (*CsCslG2_1*, *CsCslG2_2* and *CsCslG3*) showed high root-specific expression. These results suggested that these genes, especially *CsCslD* and *CsCslE1* subfamily members, might play antagonistic roles during tea plant development.

3.5 Expression Profiles of CsCesA/Csl Genes under Abiotic Stresses

To further describe the role of *CsCesA/Csl* genes, we performed a comparative transcriptome analysis of the *CsCesA/Csl* genes based on the downloaded abiotic (drought and cold) stress-responsive transcriptome data [3,48–50]. All of the 53 *CsCesA/Csl* genes were clustered into two main groups based on the gene expression pattern analysis of drought-responsive dataset (Fig. 7A). A total of 23 *CsCesA/Csl* genes, accounting for 56.6% of the whole *CsCesA/Csl* gene family, were clustered into Group I. Most of the genes in this group expressed at lower levels in the control and drought-stressed tea leaves than Group II members. In Group I, the expression level of *CsCslB4_1* increased first but then decreased to low level, peaking at 24 h. The transcript abundances of *CsCesA4_1* and 3 *CsCslGs* (*CsCslG2_1*, *CsCslG2_4* and *CsCslG3_5*) were decreased in response to drought treatment for 24, 48 and 72 h compared with control plants. Besides, 2 *CsCesAs* (*CsCesA1_2* and *CsCesA7_1*) showed down-regulated expression patterns under drought stress for 48 and 72 h. Besides, the expression levels of *CsCesA7_2*, *CsCslB3_1* and 2 *CsCslE1s* (*CsCslE1_2* and *CsCslE1_6*) were down-regulated in response to drought stress for 72 h. Other genes were not significantly affected by the drought stress. Among the *CsCesA/Csl* genes clustered in Group II, the expression level of *CsCslB4_2* was gradually increased with the duration of drought stress (from 24 to 72 h), which was very different from other differentially expressed *CsCesA/Csl* genes in the Group II (Fig. 7A). The up-regulated expression of the 2 *CsCslB4s* in each subgroup suggested their potential roles in increasing tea plant defense against drought stress. Besides, the transcript abundances of 7 genes, including 3 *CsCslG3s* (*CsCslG3_1*, *CsCslG3_4* and *CsCslG3_7*), 2 *CsCesAs* (*CsCesA3_1* and *CsCesA6_3*), *CsCslD5_2* and *CsCslE1_4*, were gradually decreased along with the duration of drought stress (from 24 to 72 h). Furthermore, drought stress for 72 h resulted in the reduced expression of 3 *CsCslGs* (*CsCslG2_5*, *CsCslG3_2* and *CsCslG3_6*), *CsCesA6_2 and CsCslD3_3*.

The expression patterns of the 53 *CsCesA/Csl* genes under cold stress were also analyzed based on the cold stress-responsive transcriptome data during three stages of tea plant cold acclimation, including non-acclimated (CK), fully acclimated (CA1) and de-acclimated (CA3) stages (Fig. 7B) [3,48,49]. All of the *CsCesA/Csl* genes were clustered into two main groups with most of the Group I members showing relatively higher expression levels than those in Group II. Nine genes displayed the highest expression levels at the CA1 stage compared with other two stages, including 5 *CsCesAs* (*CsCesA1_2*, *CsCesA1_3*, *CsCesA3_3*, *CsCesA6_1* and *CsCesA6_2*), 3 *CsCslGs* (*CsCslG3_6*, *CsCslG3_7* and *CsCslG3_9*) and *CsCslD3_4*. The expression levels of 2 *CsCslE1s* (*CsCslE1_5* and *CsCslE1_6*) peaked at the CA3 stage. Besides, 7 genes showed gradual up-regulated expression patterns across CA1 to CA3 stages compared with the control plants, including 3 *CsCslBs* (*CsCslB3_1*, *CsCslB3_3* and *CsCslB4_1*), 2 *CsCslGs*

(*CsCslG2_5* and *CsCslG2_6*), *CsCslD3_1* and *CsCslE1_2*. In contrast, the transcript level of *CsCslE1_1* was gradually down-regulated at CA1 and CA3 stages compared with the CK stage. These results indicated that genes in tea plant *CsCesA/Csl* subfamilies might have distinct functions in response to different abiotic environments.

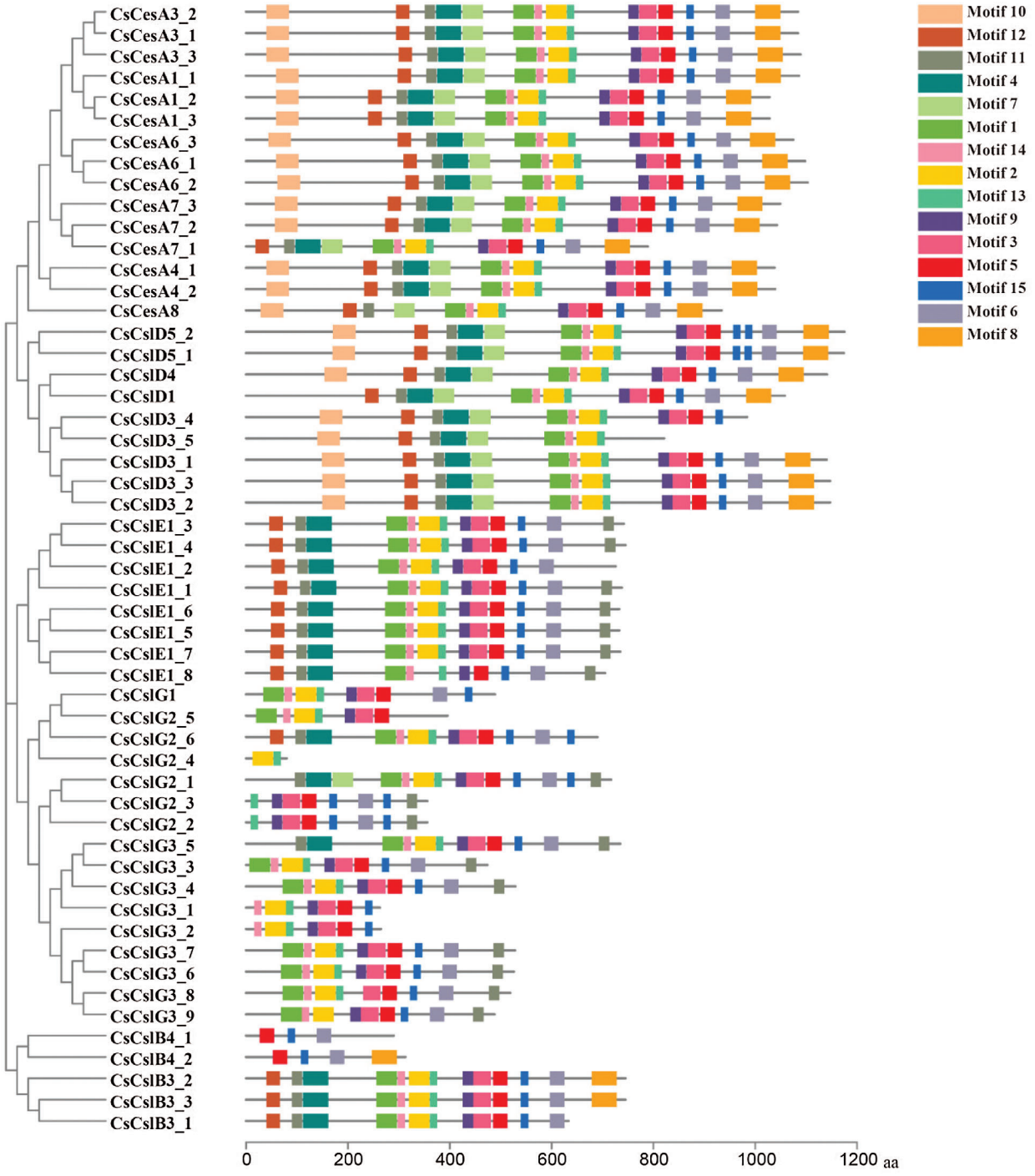

Figure 5: Motif analysis of the tea plant *CsCesA/Csl* proteins. Motif 1 to motif 15 displayed by different colors represent different conserved motifs. The order of the motifs corresponds to their position within individual protein sequence

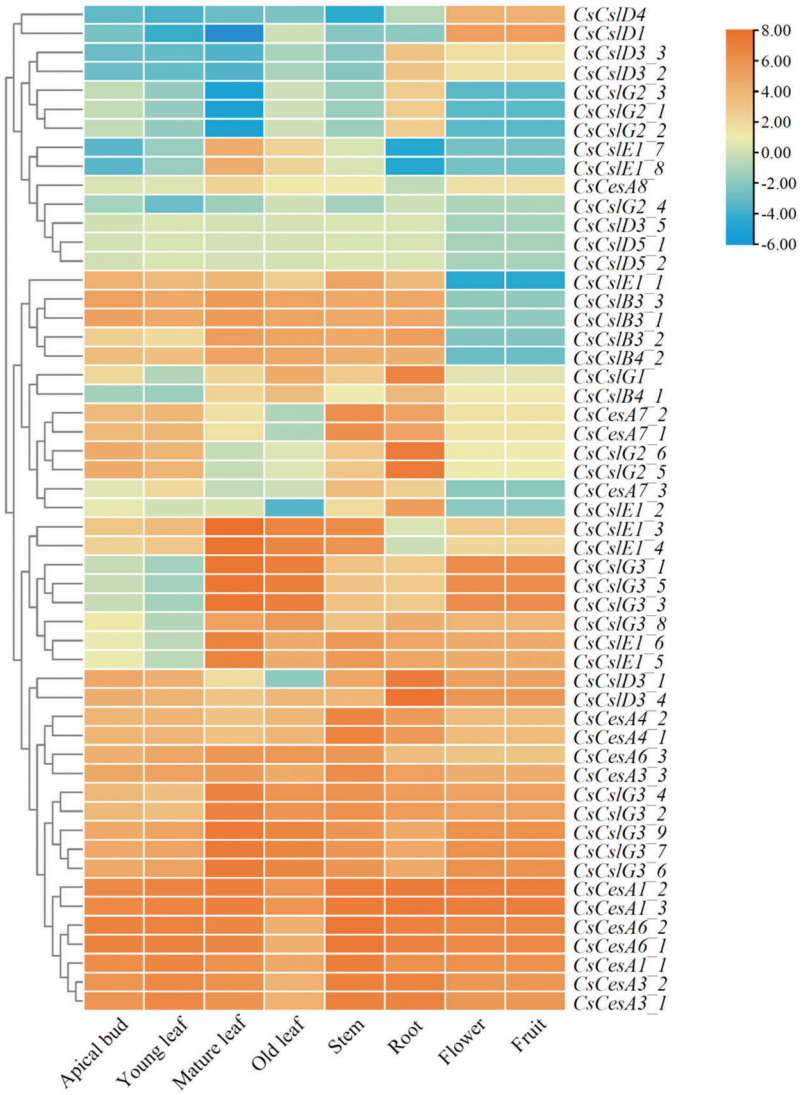

Figure 6: Expression profiles of tea plant *CsCesA/Csl* genes in different tissues. The heatmap was generated by TBtools software according to the RNA-seq database downloaded from TPIA (http://tpia.teaplant.org). Log2-based-fold changes were used to create a heatmap. The gene expression level is displayed in different colors on the map, as shown in the bar at the upper right corner. Three biological replicates of each tissue were used [3,43,48]

3.6 Analysis of Cellulose Content and Expression Patterns of the Selected CsCesA/Csl Genes in Different Tissues and Tea Plant Cultivars

Since the cellulose content is one of the major factors affecting the tenderness of tea plants, we measured the cellulose content in four tissues, as well as stems in five different cultivars of tea plants (Fig. 8). The cellulose content was gradually increased with the leaf maturity degree (Fig. 8A), and the third leaves from tea plants contained the highest amount of cellulose among the four tested tissues (Fig. 8A). The absolute amount of cellulose in stems was higher than those in the first and second leaves, and lower than that in the third leaves (Fig. 8A). The comparison of the cellulose content in stems of the five cultivars

revealed that the ‘Zhonghuang’ had a higher cellulose content than other cultivars (Fig. 8B), indicating a cultivar-specific accumulation of cellulose might exist in certain tea plant cultivars, such as ‘Zhonghuang’.

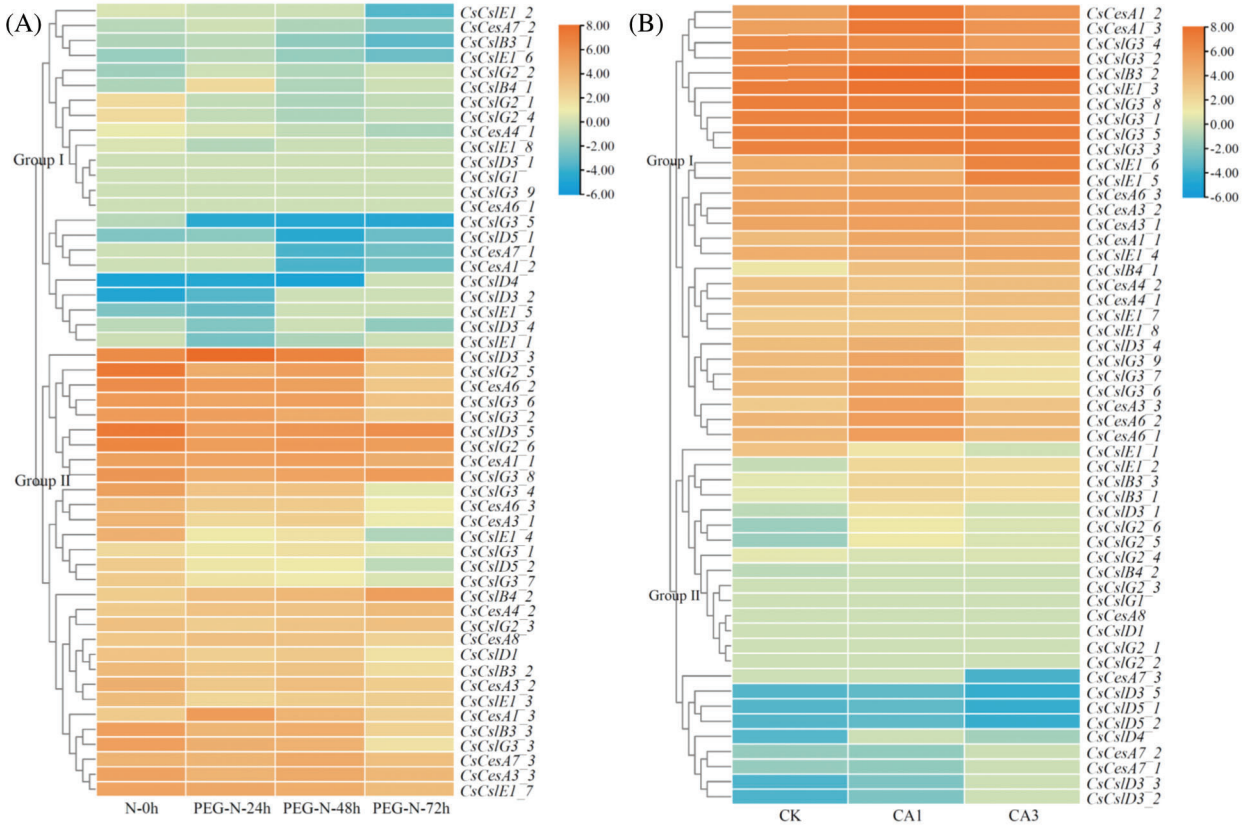

Figure 7: Expression profiles of tea plant *CsCesA/Csl* genes under drought (A) and cold (B) stresses. The heatmap was generated by TBtools software according to the RNA-seq database downloaded from TPIA (http://tpia.teaplant.org). Log2-based-fold changes were used to create a heatmap. The gene expression level is displayed in different colors on the map, as shown in the bar at the upper right corner. Drought treatments was included 25% polyethylene glycol (PEG) treatment for 0, 24, 48 and 72 h [50]. Cold treatments included non-acclimated (CK), fully acclimated (CA1) and de-acclimated stages (CA3) [49]. Three biological replicates were used in each experiment [49,50]

To further validate the roles of *CsCesA/Csl* genes in the development of tea plants and the relationship between cellulose contents and expression patterns of the *CsCesA/Csl* genes, 6 *CsCesA/Csl* family members, including *CsCesA6_2*, *CsCslD3_3*, *CsCslD3_5*, *CsCslE1_4*, *CsCslG2_5* and *CsCslG2_6,* were selected and their transcriptional activity in various tissue types and different tea plant cultivars were analyzed using qRT-PCR with gene-specific primers (Fig. 9). The expression level of the *CsCesA6_2* in the third leaves was significantly lower than in other tissues, which is correlated well with the transcriptomic findings that the old leaves expressed the lowest level of the *CsCesA6_2* compared with tested leaves and stems (Fig. 9). However, the expression patterns of other 5 genes were different from those in the transcriptomic results, suggesting these genes might adopt cultivar-specific expression strategies in different tea plant cultivars (Fig. 9). Besides, the *CsCslG2_6* showed a gradual elevation of cellulose amount in the first leaves, the second leaves, and the stems, which is in accordance with the increased trend of cellulose content in the corresponding tissues (Fig. 9). The expression levels of the *CsCesA6_2*, *CsCslD3_5*, *CsCslG2_5* and

CsCslG2_6 genes were significantly increased in the second leaves than those in the first leaves, which is also well correlated with the change patterns of cellulose content in these two tissues (Fig. 9). Furthermore, the transcript level of the *CsCslD3_5* in the stems of 'Zhonghuang' cultivar was the highest among all five cultivars. This result is in accordance with the highest cellulose contents in the same cultivar (Fig. 10). However, the remaining 5 *CsCesA/Csl* genes showed varied expression patterns in different cultivars (Fig. 10).

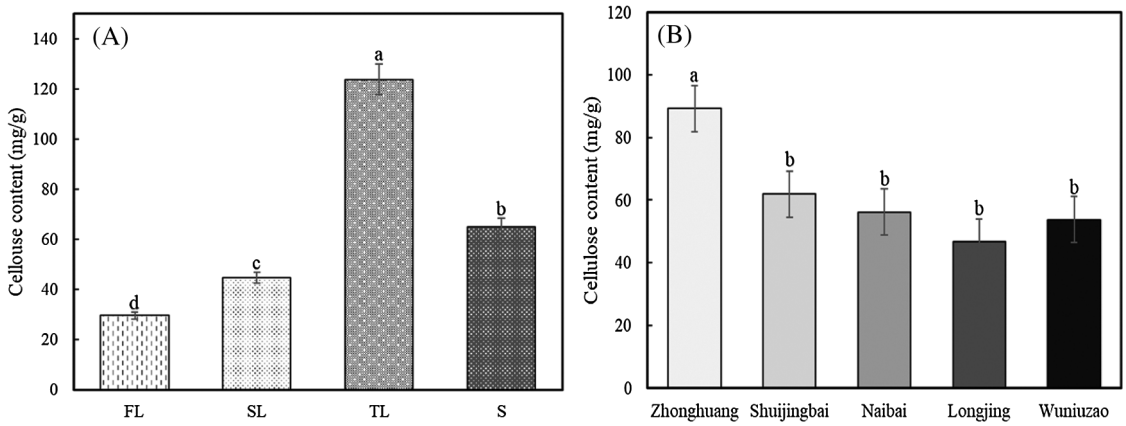

Figure 8: Cellulose content in different tissues of the 'Fuding' cultivar (A) and in stems of different tea cultivars (B). Error bars show the standard error among three replicates. Different letters above the columns indicate the significant differences calculated by one-way ANOVA method. L1: the first leaves; L2: the second leaves; L3: the third leaves; S: stems, respectively

4 Discussion

The cell wall is crucial for plants to adapt and survive through modulating plant growth and development, as well as by acting as the front line of plant immunity [51]. Cellulose and additional polysaccharides are the main components of plant cell walls. CesA and Csl enzymes are responsible for synthesizing cellulose and most hemicellulose polysaccharides, respectively, and they constitute the cellulose synthase superfamily that is classified as GT2 family [52]. Here, we conducted a genome-wide analysis and identified a total of 53 *CsCesA/Csl* genes with cellulose synthase domains in the tea plant genome database. Our results showed that the tea genome encodes 15 *CsCesA* genes, and they were classified into two distinct major groups based on the type of cell wall production and adaptive involvement in cellulose synthesis, with *CsCesA1s*, *CsCesA3s* and *CsCesA6s* in a group while *CsCesA4s*, *CsCesA7s*, and a *CsCesA8* in another group (Fig. 1), which is similar with the classification of Arabidopsis *AtCesA/Csl* genes. In Arabidopsis, AtCesA1, AtCesA3 and AtCesA6-like proteins (AtCesA2, AtCesA5, AtCesA6 and AtCesA9) participate in the primary wall cellulose synthesis, whereas AtCesA4, AtCesA7 and AtCesA8 are components of the secondary wall cellulose synthase complex [27–29,53]. Thus, the molecular function of the CsCesAs in tea plants may be similar with their corresponding orthologs in Arabidopsis.

In addition, we found that the tea plant lacks *CslA*/*C*/*F*/*H*/*J*/*M* subfamilies based on the phylogenetic comparison with Arabidopsis and rice (Fig. 1). Similarly, it was reported that pear also lacks *CslA* genes [37] and there is only one *SlCsl* gene identified in the subgroup of *SlCslA* and *SlCslC* genes in tomato [38]. These results indicate that *CsCslA* and *CsCslC* subfamilies may have been lost during the evolution of the tea genome. Furthermore, the composition of hemicellulose between monocots and dicots is highly diverged, which partially resulted from the fact that some specific *Csl* classes exist only in monocots or

dicots [54]. Specifically, the *CslB* and *CslG* classes were found exclusively in dicots. In contrast, the *CslF* and *CslH* classes were considered to be unique to monocots, thus they were not identified in both Arabidopsis and tea plants. The *CslJ/M* members were not identified in the genomes of Arabidopsis, rice and tea, but presented in a few monocot and eudicot species [31,33].

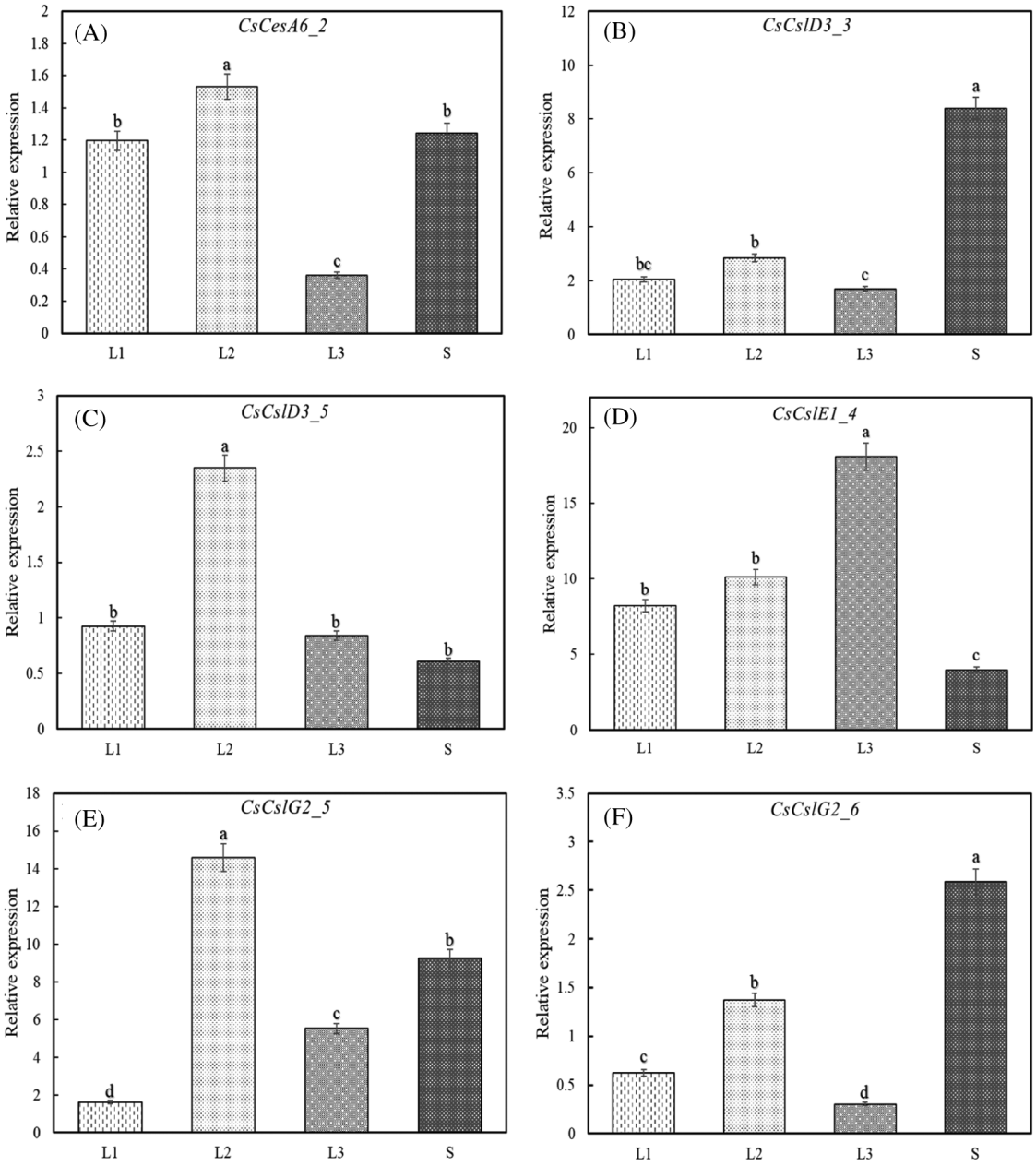

Figure 9: Expression profiles of selected *CsCes/Csl* genes in different tea tissues determined by qRT-PCR (A to F). Values represent the average ± standard error of three biological replicates with three technical replicates in each tissue. Different letters above the columns indicate the significant differences calculated by one-way ANOVA method. L1, L2, L3 and S represent the first leaves, the second leaves, the third leaves and stems, respectively

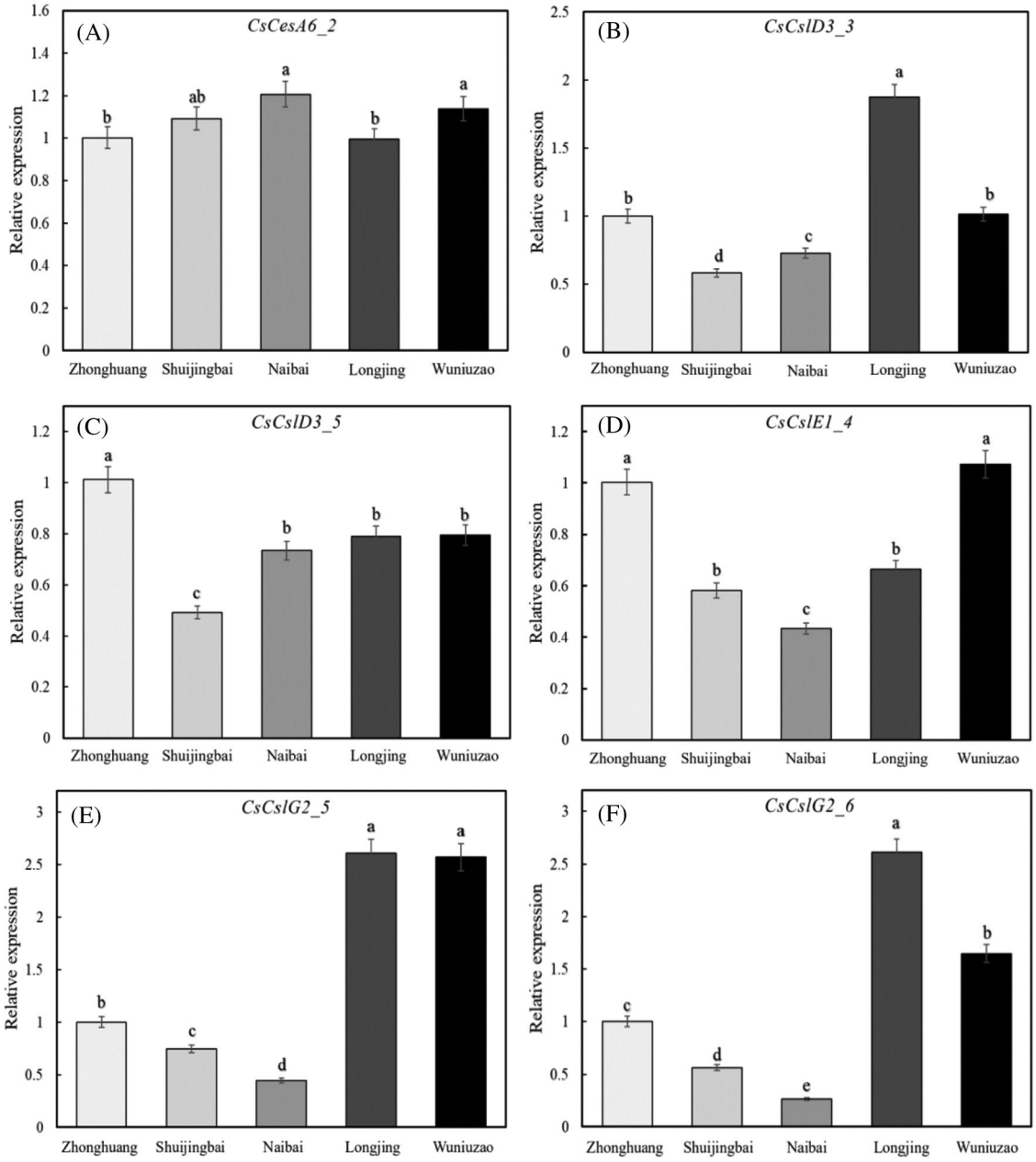

Figure 10: Expression profiles of selected *CsCes/Csl* genes in stems of different tea cultivars determined by qRT-PCR (A to F). Values represent the average ± standard error of three biological replicates with three technical replicates in each tissue. Different letters above the columns indicate the significant differences calculated by one-way ANOVA method. L1, L2, L3 and S represent the first leaves, the second leaves, the third leaves and stems, respectively

The gene structures and protein motif features are critical for elucidating the evolution, differentiation, or conservation of the function of gene family members. In the tea plant, most *CsCesA*, *CsCslE* and *CsCslD* genes have nearly the same gene structure and protein motifs in each subfamily, suggesting that these genes are conserved during evolution (Figs. 3 and 5). In contrast, *CsCslB* and *CsCslG* genes are highly diverged with different exon-intron structures and protein motifs existing in most family members (Figs.

3 and 5), probably due to chromosome fusion and/or rearrangement [55]. However, the gene structure of *CslD* was reported to be varied in other plant species, such as pear [37], pineapple [56] and tomato [38]. These results indicated that evolutionary mechanism of *CsCslD* subfamily genes might be special in tea plants and needs to be further elucidated.

Our analysis based on the transcriptomic data also provided valuable information on the potential function of *CsCesA/Csl* genes in tea plants. A number of *CsCesA/Csl* genes, including members of *CsCesA1/3/4/6*, *CsCslD3* and *CsCslG3s*, were found to be expressed highly in all sampled tissues, indicating these genes were necessary for the whole processes of tea plant growth (Fig. 6). Besides, members from *CsCesA7*, *CsCslE1*, *CsCslG2* and *CsCslG3* subfamilies expressed highly in one or more specific tissues (Fig. 6). Additionally, the expression pattern of *CsCesA6_2* verified by qRT-PCR displayed lower expression levels in the older leaves than in other tissues of the 'Fuding' cultivar (Fig. 9), which was in accordance with the changes of *CsCesA6_2* in the 'Shuchazao' cultivar revealed by the transcriptomic data (Fig. 6). These tissue-specific expressed genes were considered to be closely related to the biosynthesis of cellulose and hemicellulose polysaccharides during the corresponding tissue development. Interestingly, *CsCslD* and *CsCslE1* subfamily members displayed contrary tissue-specific expression patterns (Fig. 6), suggesting the potential antagonistic relationship between these two subfamilies. In Arabidopsis, the function of *AtCesA/Csl* genes has been extensively studied via gene mutation. For instance, null mutations in *AtCesAs* are usually lethal during embryogenesis or early seedling development [26]. Point mutations in *AtCesAs* often lead to abnormal phenotypes, such as a radial swelling phenotype caused by *rsw1-1* mutation and an abnormal swollen cell phenotype resulted from *rsw1-20* mutation [57,58], revealing the important roles of *CesA/Csl* genes in plant growth and development. Although the functional exploration of *CsCesA/Csl* genes in tea plants is still lacking, the analysis of their tissue expression patterns would provide more candidate genes for elucidating the specific and biologically important roles of *CsCesA/Csl* family members during tea plant growth and development.

The plant cell wall acts as the first line of defensing against a number of abiotic stresses, such as drought, low temperature and salinity [10]. Therefore, the cell wall-related genes are supposed to play important roles in regulating cell wall function under abiotic stresses. In tea plants, two members of *CsCslB4* subfamily involved in cell wall hemicellulose biosynthesis displayed increased expression patterns under drought stress (Fig. 7A), and a number of genes were found to be up-regulated in response to different cold treatments, including members of *CsCesA1/3/6*, *CsCslB3/4*, *CsCslD3*, *CsCslE1* and *CsCslG2/3* classes (Fig. 7B), suggesting that these genes would be required for tea plants to survive under abiotic stresses. In other plant species, a series of studies have been conducted in order to elucidate the specific function of *CesA/Csl* genes when plants confront environmental stresses due to their involvement in cell wall biosynthesis. However, most of the research only focused on a few *CesA/Csl* subfamilies. For instance, *OsCesA10* was found to exhibit a close correlation with rice drought tolerance [59]. Overexpression of *OsCslD4* can enhance rice salt tolerance by elevating the ABA synthesis gene expression and increasing ABA content [20]. Additionally, *AtCslD5* was confirmed to be required for Arabidopsis osmotic stress tolerance [60]. Our analysis of the expression pattern of tea *CsCesA/Csl* genes under drought and cold stresses provided more candidate stress-resistant genes, which would be valuable for a better understanding of tea plant stress-tolerant mechanism and the subsequent breeding of stress-tolerant tea plants.

The content of cellulose, one of the most important factors affecting the tenderness of new shoots, was considered to be positively correlated with the maturity of tea plant tissues [21–23]. This has been confirmed in our data that the cellulose content was gradually increased with the maturity of the leaves in the 'Fuding' cultivar (Fig. 8A), which was similar in both 'Huangjinya' and 'Yujinxiang' cultivars reported in previous studies [23]. However, the cellulose content in stems was not the highest among the four tested samples

(Fig. 8A), probably due to that the stems of the first three internodes were mixed together as an integral stem sample, within which the first two internodes were tender than the third one [21]. Additionally, among the selected *CsCesA/Csl* genes, we found that the expression level of *CsCslG2_6* in the 'Fuding' cultivar and *CsCslD3_5* in the 'Zhonghuang' cultivar were positively correlated with the change pattern of the cellulose contents among different tissues and different cultivars, respectively (Figs. 9 and 10). A previous study has found that the expression levels of 3 *CsCesAs* involved in the secondary cell wall cellulose synthesis were positively related to the cellulose content alteration, showing their potential regulatory roles in modulating shoot tenderness [23]. Furthermore, several *AtCsl* genes were also proved to be directly involved in the biosynthesis of cellulose in Arabidopsis [61]. For instance, *AtCslD1* and *AtCslD4* mutation caused significantly reduced cellulose deposition in the cell wall of pollen tube [61]. Thus, we speculate that *CsCslG2_6* and *CsCslD3_5* in tea plants might also be involved in the cellulose synthesis to some extent, and thereby affect the tenderness of the new shoots. Taken together, these two genes would act as valuable targets for the further study on deciphering the molecular mechanism of the regulatory roles of *CsCesA/Csl* superfamily in modulating the tenderness of tea shoots.

5 Conclusions

The *CsCesA/Csl* gene family involved in the biosynthesis of cellulose and hemicellulose is critical for tea plant stress tolerance and shoot tenderness regulation. Although the whole-genome sequence for tea plants is available, information about the *CsCesA/Csl* gene family is still lacking. In this study, a total of 53 *CsCesA/Csl* genes were identified and classified into five subfamilies. Their phylogenetic relationships with *CesA/Csl* genes in Arabidopsis and rice, chromosome location, gene structure, expression patterns in different tissues and under drought and cold stresses were analyzed, providing a number of candidate genes probably involved in the regulation of tea plant growth, development and stress resistance. The correlation between selected *CsCesA/Csl* genes and shoot cellulose contents in different tissues and cultivars were also studied and revealed that two genes, *CsCslG2_6* and *CsCslD3_5*, might be involved in regulating shoot tenderness by affecting the changes of cellulose content. Our results may be useful for further elucidating the function of candidate *CsCesA/Csl* genes in order to improve the stress-tolerance of tea plant, as well as the quality of tea products.

Authors' Contributions: Conceive the study and revise the manuscript: **Litang Lu** and **Qi Zhao**. Perform the bioinformatic analysis, qRT-PCR validation mainly and draft the manuscript: **Qianqian Li**. Collect the materials and participate in the qRT-PCR validation: **Xinzhuan Yao**. Participate in the bioinformatic analysis: **Baohui Zhang**. All authors reviewed the results and approved the final version of the manuscript.

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Table S1: List of the primers used for quantitative real-time PCR in present study

Gene ID	Forward Primer	Reverse Primer
GAPDH	GTGAGGCTGGTGCTGATTACG	TGGTGCAGCTAGCATTTGAGAC
CsCesA6_2	CCTTGATCGGCTATCACTTAGG	GAACGGTGTTTGCTGTGATTAG
CsCslD3_3	CAGCGTGTTGGATGGATTTATG	GAGCCTATCCGTGAGATTGATT
CsCslD3_5	GATGAGATCGGTGAGCCTAAAG	GAGAACAGCTATCCGGACAAATA
CsCslE1_4	ATGTCACTGTCCCATCTCTTTG	CGCCTAAGCTGTATCCGTATTT
CsCslG2_6	CACAACCTTCCACCAACATAAAC	CGTGAGGCGGTAGTTGAATAA
CsCslG2_5	AAGGATGGAGGTCTGTCTATCT	GAGAGGGCTAAACCTTGAGAAA

Table S2: The genes of *Ces/Csl* in Arabidopsis, rice and tea plant

Gene ID	Gene name
AT4G32410.1	AtCesA1
AT4G39350.1	AtCesA2
AT5G05170.1	AtCesA3
AT5G44030.1	AtCesA4
AT5G09870.1	AtCesA5
AT5G64740.1	AtCesA6
AT5G17420.1	AtCesA7
AT4G18780.1	AtCesA8
AT2G21770.1	AtCesA9
AT2G25540.1	AtCesA10
AT4G16590.1	AtCslA1
AT5G22740.1	AtCslA2
AT1G23480.1	AtCslA3
AT2G35650.1	AtCslA7
AT5G03760.1	AtCslA9
AT1G24070.1	AtCslA10
AT5G16190.1	AtCslA11
AT3G56000.1	AtCslA14
AT4G13410.1	AtCslA15
AT2G32610.1	AtCslB1
AT2G32620.1	AtCslB2
AT2G32530.1	AtCslB3
AT2G32540.1	AtCslB4
AT4G15290.1	AtCslB5

(Continued)

Table S2 (continued)

Gene ID	Gene name
AT4G15320.1	AtCslB6
AT3G28180.1	AtCslC4
AT4G31590.1	AtCslC5
AT3G07330.1	AtCslC6
AT2G24630.1	AtCslC8
AT4G07960.1	AtCslC12
AT2G33100.1	AtCslD1
AT5G16910.1	AtCslD2
AT3G03050.1	AtCslD3
AT4G38190.1	AtCslD4
AT1G02730.1	AtCslD5
AT1G32180.1	AtCslD6
AT1G55850.1	AtCslE1
AT4G24010.1	AtCslG1
AT4G24000.1	AtCslG2
AT4G23990.1	AtCslG3
LOC_Os05g08370.1	OsCesA1
LOC_Os03g59340.1	OsCesA2
LOC_Os07g24190.1	OsCesA3
LOC_Os01g54620.1	OsCesA4
LOC_Os03g62090.1	OsCesA5
LOC_Os07g14850.1	OsCesA6
LOC_Os10g32980.1	OsCesA7
LOC_Os07g10770.1	OsCesA8
LOC_Os09g25490.1	OsCesA9
LOC_Os12g29300.1	OsCesA10
LOC_Os06g39970.1	OsCesA11
LOC_Os02g09930.1	OsCslA1
LOC_Os10g26630.1	OsCslA2
LOC_Os06g12460.1	OsCslA3
LOC_Os03g07350.1	OsCslA4
LOC_Os03g26044.1	OsCslA5
LOC_Os02g51060.1	OsCslA6
LOC_Os07g43710.1	OsCslA7
LOC_Os06g42020.1	OsCslA9

(Continued)

Table S2 (continued)

Gene ID	Gene name
LOC_Os08g33740.1	OsCslA11
LOC_Os01g56130.1	OsCslC1
LOC_Os09g25900.1	OsCslC2
LOC_Os08g15420.1	OsCslC3
LOC_Os05g43530.1	OsCslC7
LOC_Os03g56060.1	OsCslC9
LOC_Os07g03260.1	OsCslC10
LOC_Os10g42750.1	OsCslD1
LOC_Os06g02180.1	OsCslD2
LOC_Os08g25710.1	OsCslD3
LOC_Os12g36890.1	OsCslD4
LOC_Os06g22980.1	OsCslD5
LOC_Os09g30120.1	OsCslE1
LOC_Os02g49332.1	OsCslE2
LOC_Os09g30130.1	OsCslE6
LOC_Os07g36700.1	OsCslF1
LOC_Os07g36690.1	OsCslF2
LOC_Os07g36750.1	OsCslF3
LOC_Os07g36740.1	OsCslF4
LOC_Os08g06380.1	OsCslF6
LOC_Os10g20260.1	OsCslF7
LOC_Os07g36630.1	OsCslF8
LOC_Os07g36610.1	OsCslF9
LOC_Os10g20090.1	OsCslH1
LOC_Os04g35020.1	OsCslH2
LOC_Os04g35030.1	OsCslH3
CSS0012399.1	CsCesA1_1
CSS0010245.1	CsCesA1_2
CSS0034898.1	CsCesA1_3
CSS0031116.1	CsCesA3_1
CSS0008005.3	CsCesA3_2
CSS0038121.4	CsCesA3_3
CSS0016274.1	CsCesA4_1
CSS0012493.1	CsCesA4_2
CSS0009305.1	CsCesA6_1

(Continued)

Table S2 (continued)

Gene ID	Gene name
CSS0028360.1	CsCesA6_2
CSS0025911.1	CsCesA6_3
CSS0012489.1	CsCesA7_1
CSS0005229.1	CsCesA7_2
CSS0014034.1	CsCesA7_3
CSS0033685.1	CsCesA8
CSS0015423.1	CsCslB3_1
CSS0005626.1	CsCslB3_2
CSS0031882.1	CsCslB3_3
CSS0024869.1	CsCslB4_1
CSS0006219.1	CsCslB4_2
CSS0012214.1	CsCslD1
CSS0012996.1	CsCslD3_1
CSS0024657.1	CsCslD3_2
CSS0039274.1	CsCslD3_3
CSS0020384.1	CsCslD3_4
CSS0001659.1	CsCslD3_5
CSS0009118.1	CsCslD4
CSS0045476.1	CsCslD5_1
CSS0001364.1	CsCslD5_2
CSS0009487.1	CsCslE1_1
CSS0027440.1	CsCslE1_2
CSS0008757.1	CsCslE1_3
CSS0041502.1	CsCslE1_4
CSS0019900.1	CsCslE1_5
CSS0038776.1	CsCslE1_6
CSS0045281.1	CsCslE1_7
CSS0001964.1	CsCslE1_8
CSS0018408.1	CsCslG1
CSS0044240.1	CsCslG2_1
CSS0049722.1	CsCslG2_2
CSS0020707.1	CsCslG2_3
CSS0000850.1	CsCslG2_4
CSS0009342.1	CsCslG2_5
CSS0019495.1	CsCslG2_6

(Continued)

Table S2 (continued)

Gene ID	Gene name
CSS0028184.1	CsCslG3_1
CSS0026824.1	CsCslG3_2
CSS0041642.1	CsCslG3_3
CSS0032273.1	CsCslG3_4
CSS0020977.1	CsCslG3_5
CSS0037731.1	CsCslG3_6
CSS0032646.1	CsCslG3_7
CSS0036058.1	CsCslG3_8
CSS0033694.1	CsCslG3_9