

Article

Genome-Wide Analysis of the *CesA/Csl* Family in *Chara braunii* Provides Insights into the Evolution of Plant Cell Wall Biosynthesis

Jian Zhou ¹ and Qianqian Li ^{2,*} 

¹ Southern Marine Science and Engineering Guangdong Laboratory, Zhuhai 519000, China; zhoujian@sml-zhuhai.cn

² State Key Laboratory of Plant Diversity and Specialty Crops, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China

* Correspondence: liqianqian@scbg.ac.cn; Tel.: +86-20-37252711

Abstract

The evolution of plant cell walls was critical for land colonization, yet its genetic basis remains incompletely understood. Charophyte green algae are the closest relatives of land plants and provide key insights into the origins of cell wall biosynthesis. Here, we conducted a genome-wide analysis of the *CesA/Csl* superfamily in the charophyte alga *Chara braunii* to investigate the evolutionary origins of plant cell wall formation. Thirty genes were identified and classified into three major clades: *CesA*, *CslA*, and *CslC*. *CesA* genes were exclusively associated with primary cell wall lineages, with no homologs of secondary wall-specific *CesA4/CesA7/CesA8*, indicating an early origin of cellulose biosynthesis followed by later functional specialization. In contrast, *Csl* genes were restricted to the *CslA* and *CslC* subfamilies, suggesting that diversification of hemicellulose biosynthesis occurred after the divergence of charophytes and land plants. Expression and promoter analyses revealed clade-specific regulatory patterns and stress-responsive expression, and the quantitative RT-PCR validated that *CesA*, *CslA*, and *CslC* genes respond differentially to NaCl and ABA treatments, with *CslA* genes strongly induced by ABA and *CslC* genes preferentially by salt. Together, these findings are consistent with a stepwise model of cell wall evolution and suggest that *C. braunii* may represent an important intermediate stage in the transition to land plants.

Keywords: cellulose synthase; cellulose synthase-like; *Chara braunii*; charophyte; cell wall evolution; gene family; plant terrestrialization



Academic Editor: Leonel Pereira

Received: 19 April 2026

Revised: 13 July 2026

Accepted: 13 July 2026

Published: 20 July 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and

conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

1. Introduction

The colonization of land by plants represents one of the most profound evolutionary transitions in Earth's history. It required coordinated adaptations to overcome desiccation, increased ultraviolet radiation, and mechanical stresses imposed by gravity. Central to this transition was the evolution of a structurally robust and chemically diverse cell wall, which provides mechanical support, regulates cell expansion, and mediates interactions with the environment [1]. Comparative studies have shown that many core components of land plant cell walls, including cellulose microfibrils, mannans, xyloglucans, and pectins, were already present in charophyte green algae, the closest extant relatives of land plants [2,3]. These findings suggest that key aspects of cell wall architecture were established before

terrestrialization, indicating a substantial degree of evolutionary pre-adaptation. Charophytes, particularly members of the Charophyceae, occupy a critical phylogenetic position for understanding the origin of plant cell wall complexity. They exhibit morphological and developmental features shared with land plants, including phragmoplast-mediated cytokinesis and aspects of hormone signaling [4,5]. Recently available high-quality genomes from representative streptophyte algae, including the charophycean alga *Chara braunii*, and the zygnematophycean species *Spirogloea muscicola* and *Mesotaenium endlicherianum*, have provided unprecedented opportunities to investigate the genetic basis underlying these traits [6,7]. Despite accumulating biochemical evidence for the presence of land plant-like polysaccharides in charophyte cell walls [3,8], the genetic mechanisms responsible for their biosynthesis remain poorly characterized, particularly with respect to the cellulose synthase (*CesA*) and cellulose synthase-like (*Csl*) superfamily.

The *CesA/Csl* superfamily constitutes the core enzymatic machinery for the biosynthesis of cellulose and hemicellulose, the principal structural polysaccharides of plant cell walls. In land plants such as *Arabidopsis thaliana*, *CesA* proteins assemble into rosette cellulose synthase complexes responsible for cellulose microfibril formation, whereas distinct *Csl* subfamilies synthesize the backbones of diverse hemicelluloses [9]. Functional studies have demonstrated clear specialization within this superfamily: for example, *CesA1*, *CesA3*, and *CesA6* are required for primary cell wall synthesis, while *CesA4*, *CesA7*, and *CesA8* are involved in secondary cell wall formation [10]. Similarly, different *Csl* subfamilies (e.g., *CslA*, *CslC* and *CslD*) are responsible for the synthesis of specific hemicellulose types such as mannans, xyloglucans, and xylans [11]. The diversification of these gene families is therefore considered a key factor underlying the increasing complexity of plant cell walls during evolution. However, the composition, evolutionary history, and functional differentiation of the *CesA/Csl* superfamily in charophytes remain largely unresolved.

Given that *Chara braunii* occupies a key phylogenetic position within Charophyceae and exhibits several streptophyte features, including phragmoplast-mediated cytokinesis, while retaining an aquatic lifestyle, it provides a valuable system for investigating the transitional state of cell wall biosynthesis [4,6]. Here, we hypothesize that the *CesA/Csl* repertoire of *Chara braunii* represents a transitional genomic architecture, retaining ancestral features while lacking key innovations associated with the complex cell walls of land plants. This leads to a central question: does *C. braunii* possess a fully diversified set of *CesA* and *Csl* genes comparable to that of terrestrial plants, or does it instead exhibit a simplified gene complement reflective of its aquatic lifestyle? To address this, we performed a comprehensive analysis of the *CesA/Csl* superfamily in *C. braunii* within a broad comparative framework across Viridiplantae. Specifically, we (i) identified and classified *CesA/Csl* genes in *C. braunii*, (ii) reconstructed their evolutionary relationships across major green plant lineages, and (iii) integrated structural and expression analyses to assess their functional differentiation. Our results suggest a simplified and lineage-specific gene repertoire that comprises *CesA* homologs associated with primary cell wall synthesis and a restricted set of *Csl* subfamilies (*CslA* and *CslC*), which is consistent with the view that charophytes represent an intermediate stage in the evolution of plant cell wall biosynthetic complexity. These findings provide new insights into the genetic innovations that underpinned plant terrestrialization.

2. Materials and Methods

2.1. Identification and Classification of *CesA/Csl* Genes in *C. braunii*

The *C. braunii* genome and its annotation were obtained from the ORCAE interface [6]. To identify members of the *CesA/Csl* superfamily, Hidden Markov Model (HMM) searches were performed using profile HMMs downloaded from the Pfam database (ver-

sion 35.0) [12]. The query set included the core cellulose synthase domain (PF03552) and three Glycosyltransferase Family 2 (GT2) related domains (PF00535, PF13641 and PF13632), which represent the key catalytic modules of the *CesA/Csl* superfamily as characterized in *A. thaliana*. An *E*-value cutoff of $<1 \times 10^{-3}$ was applied. Protein sequences containing one or more of these four PFAM domains were retained as initial candidates. These candidates were further screened for the plant-specific zinc-binding RING-finger domain (PF14569). A separate search for the bacterial-type cellulose synthase domain (PF03170, BcsB) served as a negative control and yielded no significant hits, even with a very permissive *E*-value threshold of <1.0 . Incomplete, low-quality, or redundant sequences (e.g., shorter splice variants), were manually removed. To validate the candidate genes and ensure functional relevance, all putative proteins were queried against the NCBI non-redundant (NR) protein database using NCBI BLASTP. online tool (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>, accessed on 1 June 2026). Only hits whose top matches were annotated as “cellulose synthase” or “cellulose synthase-like” and an *E*-value $<1 \times 10^{-5}$ were retained for subsequent analysis.

To classify the identified *C. braunii* *CesA/Csl* genes and infer their evolutionary relationships with *Arabidopsis* homologs, a phylogenetic tree was constructed. The analysis included the deduced protein sequences from *C. braunii* and the full set of *CesA/Csl* family members from *A. thaliana*. A maximum likelihood phylogenetic tree was constructed using MEGA 12 under the Jones–Taylor–Thornton (JTT) model, with branch support assessed by 1000 bootstrap replicates [13]. The resulting tree was visualized and annotated using the Interactive Tree of Life (iTOL) platform [14].

2.2. Phylogenetic Analysis of *CesA/Csl* Gene Family Across Viridiplantae

To reconstruct the macroevolutionary history of the *CesA/Csl* superfamily, we selected 26 species spanning eight major lineages. Three rhodophyte species (*Chondrus crispus*, *Porphyra umbilicalis*, *Cyanidioschyzon merolae* 10D) were used as outgroups. The remaining species belong to Viridiplantae, including representatives from Chlorophyta (*Volvox carteri*, *Chlamydomonas reinhardtii*, *Coccomyxa subellipsoidea* C-169, *Chlorella variabilis*, *Micrinomonas pusilla*—formerly *Micromonas pusilla*, *Ostreococcus tauri*), and streptophyte algae including Mesostigmatophyceae (*Mesostigma viride*), Chlorokybophyceae (*Chlorokybus atmophyticus*), Klebsormidiophyceae (*Klebsormidium nitens*), Charophyceae (*Chara braunii*), and Zygnematomphyceae (*Penium margaritaceum*, *Mesotaenium endlicherianum*, *Spiroglaea muscicola*), as well as bryophytes (*Anthoceros agrestis*, *Marchantia polymorpha*, *Physcomitrella patens*), lycophytes (*Selaginella moellendorffii*), ferns (*Azolla filiculoides*), gymnosperms (*Gnetum montanum*, *Cycas panzhihuaensis*), and angiosperms (*Amborella trichopoda*, *A. thaliana*, *Oryza sativa*). *CesA/Csl* proteins from these genomes were identified using the same HMM search criteria as described in Section 2.1. The amino acid sequences of the 411 orthologous genes were aligned using MAFFT v7.505 [15] with default parameters. Next, the alignments were trimmed using trimAl v1.4 with the automated heuristic method (-automated1). A maximum likelihood tree was then constructed in IQ-TREE 2.0 under the best-fit model with 1000 ultrafast bootstrap replicates [16]. The final tree was visualized and annotated in iTOL [14].

2.3. Integrated Bioinformatic Characterization of *CbCesA/Csl* Genes and Proteins

For all identified *CbCesA/Csl* genes, coding sequences (CDSs) and genomic coordinates were extracted from the ORCAE database. The deduced protein sequences were analyzed for key physicochemical properties using the ExPASy-ProtParam tool (Expasy—ProtParam, <https://web.expasy.org/protparam/>, accessed on 1 June 2026). These properties include molecular weight (MW), isoelectric point (pI), and grand average of hydropathy (GRAVY). Transmembrane domains were predicted with TMHMM Server v2.0, and subcellular

localizations were inferred using WOLF PSORT (<https://wolfpsort.hgc.jp/>, accessed on 1 June 2026). Multiple sequence alignment of the 30 CbCesA/Csl proteins was performed using the MAFFT v7.505 following a profile alignment strategy. Specifically, sequences belonging to different subfamilies were aligned separately using the L-INS-i algorithm, and the resulting sub-alignments were subsequently merged using the –merge option. Conserved protein motifs were identified using the MEME Suite 5.0.2 with original protein sequences, setting motif lengths between 6 and 300 residues and the maximum number of motifs to 20 [17]. Gene structures, including exon–intron organization, were determined by aligning genomic sequences with CDSs using Gene Structure Display Server (GSDS) [18].

2.4. Tissue-Specific Expression Analysis of CbCesA/Csl Genes by RNA-Seq

To examine the expression patterns of the identified *CesA/Csl* genes, we utilized publicly available RNA-seq data from *C. braunii* (NCBI SRA BioProject PRJNA445548) [6]. The dataset included four tissue types: whole plant, archegonia, antheridia, and zygote. The expression levels for each gene were quantified as FPKM (Fragments Per Kilobase per Million mapped reads) and visualized as a heatmap.

2.5. Cis-Regulatory Element Prediction in CbCesA/Csl Gene Promoters

To explore the transcriptional regulation of the *CbCesA/Csl* genes, we extracted the 2000 bp genomic sequences upstream of the translation start codon for each gene as putative promoter regions. These sequences were analyzed using the PlantCARE database to identify potential *cis*-regulatory elements [19]. The identified elements were systematically categorized into three major functional groups: responses to environmental stress, phytohormone responses, and regulation of plant growth and development. The distribution and abundance of these elements were visualized using MATLAB 7.12.0 (R2011a), and a comprehensive summary of their types and sequences was compiled.

2.6. Stress-Responsive Expression Analysis of CbCesA/Csl Genes Using qRT-PCR

Chara braunii plants were collected in June 2025 from Yancheng, Jiangsu Province, China (33°2' N, 120°3' E). Uniformly grown plants (15–20 cm in length) were subjected to stress treatments by incubating whole plants in solutions containing either 100 mM NaCl or 50 μ M abscisic acid (ABA), with untreated plants serving as controls. The NaCl concentration was chosen based on previous transcriptomic evidence in *C. braunii* and preliminary dose–response tests, which confirmed that this treatment induced no visible necrosis or chlorosis over 48 h, although a slight reduction in growth rate was noted [20]. Samples were collected at 0 (pre-treatment), 6, 12, 24, and 48 h post-treatment, with three biological replicates per time point. Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and reverse-transcribed into cDNA using a PrimeScript RT Reagent Kit (TaKaRa, Dalian, China) with gDNA Eraser. Gene-specific quantitative real-time PCR (qRT-PCR) primers are listed in Table S4. Real-time PCR was performed using a SYBR Premix ExTaq Kit (TaKaRa, Dalian, China) on a QuantStudio 5 Real-Time PCR System (Applied Biosystems, Foster, CA, USA). The actin gene (g30919.t1) was used as the internal reference. Relative expression levels were calculated using the $2^{-\Delta\Delta CT}$ method [21]. Three technical replicates were run for each biological replicate.

3. Results

3.1. The Cesa/Csl Superfamily in C. braunii Comprises 30 High-Confidence Members

Hidden Markov Model (HMM)-based searches were performed against the genome annotation using four Pfam domains, including the core cellulose synthase domain (PF03552) and three GT2-related domains (PF00535, PF13641, PF13632). A total of 41 candidate genes

were initially retrieved (E -value $< 1 \times 10^{-3}$). After removing incomplete, low-quality, and redundant sequences, and validating the remaining candidates by BLASTP against the NCBI non-redundant database (top hits annotated as “cellulose synthase” or “cellulose synthase-like”, E -value $< 1 \times 10^{-5}$), 30 high-confidence *CesA/Csl* genes were retained (Table S1).

Phylogenetic analysis of these 30 proteins, together with 40 well-characterized *CesA/Csl* proteins from *A. thaliana*, resolved three major clades (Figure 1A). Within Group I (*CesA*), ten genes contained the plant-specific zinc-binding RING-finger domain (PF14569), a characteristic feature of canonical *CesA* proteins. In contrast, two genes (g8591.t1 and g45235.t1) clustered within the *CesA* clade but lacked this domain. The placement of g8591.t1 within the *CesA* clade received bootstrap support of 85 (Figure 1A). However, despite this placement, g8591.t1 showed highest BLASTP similarity to *Arabidopsis* *CslD* proteins.

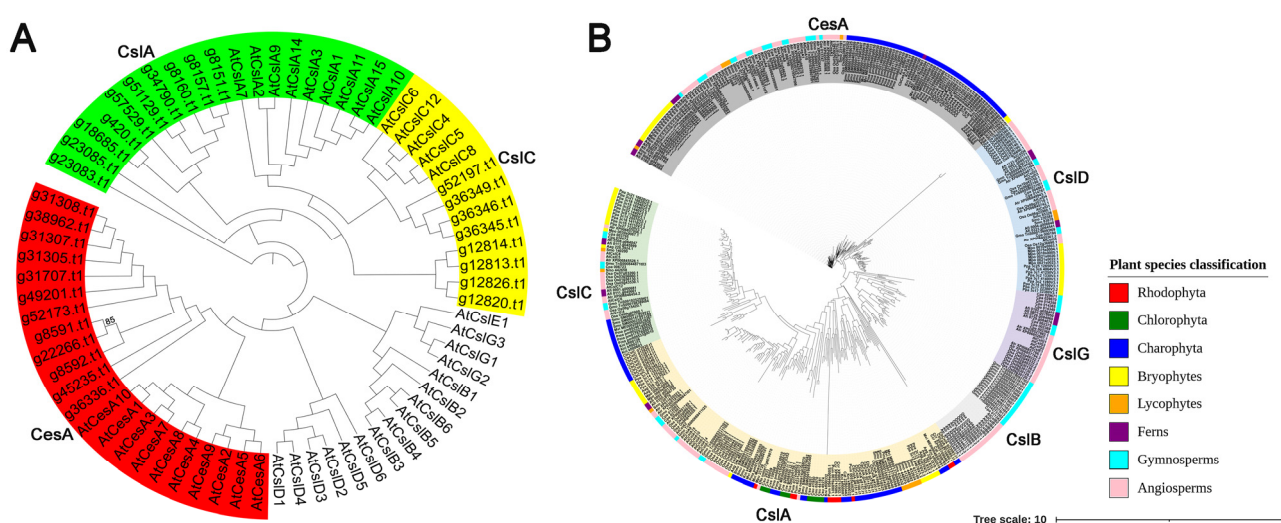


Figure 1. Phylogenetic analysis of the cellulose synthase (*CesA/Csl*) gene superfamily. **(A).** Maximum likelihood tree (1000 bootstrap replicates) of *CesA/Csl* families from *C. braunii* and *A. thaliana*. Branch support for g8591.t1 is 85. **(B).** A broader maximum likelihood tree of 411 sequences across Viridiplantae, with branches colored by gene subfamily (*CesA*, *CslA*, *CslB*, *CslC*, *CslD*, *CslG*) and an outer ring indicating major plant lineages.

3.2. Phylogenomic Analysis Reveals the Emergence of *CesA* Genes and Restricted *Csl* Diversification in Charophytes

CesA/Csl homologs were identified from 26 representative species spanning Rhodophyta, Chlorophyta, Charophyta, Bryophytes, Lycophytes, Ferns, Gymnosperms, and Angiosperms across Viridiplantae. A total of 411 sequences were retrieved using the same HMM-based criteria and subjected to maximum-likelihood phylogenetic analysis (Figure 1B). Two major evolutionary transitions were resolved. First, *CesA* genes were detected exclusively in Phragmoplastophyta (Charophytes and land plants) and were absent from Rhodophyta and Chlorophyta. This phylogenetic pattern is consistent with the emergence of phragmoplast-mediated cell division and upright growth, suggesting that *CesA* represents an early innovation associated with complex multicellularity and cell wall reinforcement in streptophytes. Second, the *Csl* gene family showed marked expansion and diversification in land plants relative to Charophytes. Within the charophyte genomes analyzed in this study (including *C. braunii*), we identified only members of the *CslA* and *CslC* subfamilies, whereas land plants additionally harbor *CslB*, *CslD*, *CslE*, and *CslG* subfamilies (Figure 1B). No homologs of *CslB*, *CslE*, or *CslG* were detected in our charophyte dataset. For *CslD*, previous evidence has suggested its presence in some charophyte

lineages [22]. However, we did not recover clear *CslD* orthologs under our phylogenetic criteria. Notably, one gene (g8591.t1) showed marginal BLAST similarity to *Arabidopsis* *CslD* but phylogenetically clustered within the *CesA* clade and lacked the characteristic RING-finger domain, implying that it may represent an intermediate or divergent form between *CesA* and *CslD*. The presence/absence of *CslD* thus likely varies across charophyte lineages, and further sampling will be needed to fully resolve its distribution.

3.3. Structural Features of *CbCesA/Csl* Proteins

We further analyzed the predicted physicochemical properties, transmembrane domains, subcellular localization, conserved motifs, and exon–intron organization of the 30 identified proteins (Table 1). Their lengths ranged from 234 to 1396 amino acids, with predicted molecular weights from 25.98 to 154.25 kDa, theoretical pI values from 4.43 to 9.46, and GRAVY scores from -0.563 to -0.209 . Subcellular localization prediction indicated that 27 of the 30 proteins are likely targeted to the plasma membrane, consistent with the presence of 3–14 predicted transmembrane helices. Three proteins (g8591.t1, g8157.t1, g23083.t1) showed fewer transmembrane helices (1–3) and were predicted to localize to the chloroplast. Phylogenetically, all *CesA*-group proteins clustered with the primary wall *CesA*s of *Arabidopsis* (AtCesA1, AtCesA3, AtCesA6), whereas *CslA* and *CslC* proteins grouped with AtCslA and AtCslC, respectively (Figure 1A; Table S2). Conserved motif analysis identified 20 motifs across the family (Figure 2A). Motifs 1 and 3 were present in nearly all proteins, whereas motifs 2, 4, 5, and 9 were restricted to the *CesA* group. Gene structure analysis revealed substantial variation in exon–intron organization, with intron numbers ranging from 0 to 22 (Figure 2B). Eleven genes lacked introns, ten contained one intron, and nine contained two or more introns. The two genes with the highest intron numbers (g8592.t1 and g22266.t1) both belonged to the *CesA* group. Multiple sequence alignment showed that the N-terminal zinc-binding RING-finger domain (PF14569), characterized by conserved cysteine-rich residues, was present in all *CesA* group proteins except g8591.t1 and g45235.t1. The canonical D,D,D,QxxRW catalytic motif was conserved across the *CesA*, *CslA*, and *CslC* clades, whereas sequence variation was concentrated in residues flanking the core motif (Figure S1).

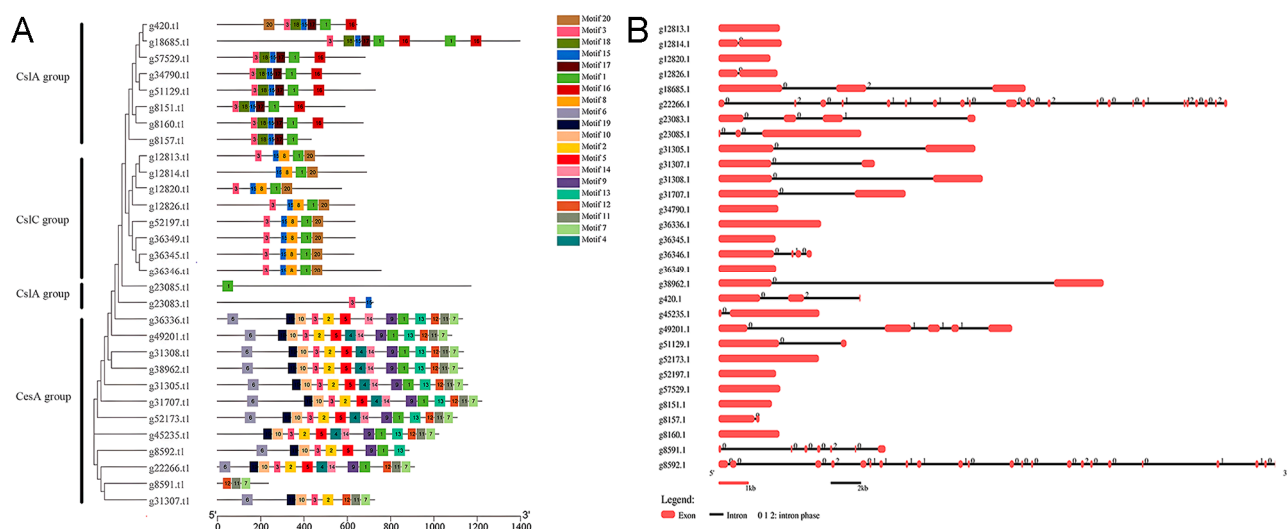


Figure 2. Conserved motif and gene structure analysis of the *CesA/Csl* family in *Chara braunii*. (A). Phylogenetic tree (left) and corresponding conserved protein motifs (right) identified by MEME. Motifs 1 and 3 are widely conserved across clades; motifs 2, 4, 5 and 9 are specific to the *CesA* clade. (B). Exon–intron structures of *CesA/Csl* genes. Exons are indicated by red boxes and introns by lines, with gene length scaled in base pairs.

Table 1. Summary of key features of *CesA/Csl* family members in *C. braunii*.

Classification	Gene ID	TMHs	Subcellular Localization	Protein (aa)	MW (kDa)	pI	GRAVY	Exon No.	Intron No.
<i>CesA</i>	g8591.t1	3	Chloroplast	234	25.982	4.43	−0.453	7	6
	g8592.t1	3	Plasma membrane	884	99.982	5.19	−0.347	23	22
	g22266.t1	3	Plasma membrane	909	103.462	8.00	−0.328	23	22
	g31305.t1	5	Plasma membrane	1155	128.622	7.23	−0.181	2	1
	g31307.t1	3	Plasma membrane	725	80.402	5.94	−0.225	2	1
	g31308.t1	5	Plasma membrane	1135	126.340	6.28	−0.190	2	1
	g31707.t1	5	Plasma membrane	1220	134.998	5.69	−0.248	2	1
	g36336.t1	5	Plasma membrane	1131	123.271	8.56	−0.172	1	0
	g38962.t1	5	Plasma membrane	1132	125.718	6.89	−0.174	2	1
	g45235.t1	5	Plasma membrane	1021	112.950	6.96	−0.100	2	1
	g49201.t1	5	Plasma membrane	1081	120.68	7.89	−0.177	5	4
	g52173.t1	7	Plasma membrane	1106	122.533	6.52	−0.161	1	0
<i>CslA</i>	g420.t1	4	Plasma membrane	643	72.109	9.27	−0.070	3	2
	g8151.t1	4	Plasma membrane	588	66.654	7.21	0.209	1	0
	g8157.t1	2	Chloroplast	432	49.277	8.96	−0.197	2	1
	g8160.t1	5	Plasma membrane	672	76.106	8.29	0.176	1	0
	g18685.t1	14	Plasma membrane	1396	154.249	8.40	−0.024	3	2
	g23083.t1	1	Chloroplast	718	79.076	4.76	−0.401	4	3
	g23085.t1	5	Plasma membrane	1170	129.186	7.67	−0.563	3	2
	g34790.t1	4	Plasma membrane	659	74.451	6.71	0.192	1	0
	g51129.t1	5	Plasma membrane	728	82.686	8.22	−0.074	2	1
	g57529.t1	7	Plasma membrane	681	77.272	8.91	0.111	1	0
<i>CslC</i>	g12813.t1	5	Plasma membrane	676	74.747	5.99	−0.082	1	0
	g12814.t1	6	Plasma membrane	687	76.64	6.63	−0.047	2	1
	g12820.t1	3	Plasma membrane	572	63.452	5.45	−0.11	1	0
	g12826.t1	3	Plasma membrane	633	70.667	5.40	−0.168	2	1
	g36345.t1	8	Plasma membrane	629	71.995	9.46	0.102	1	0
	g36346.t1	7	Plasma membrane	755	85.865	6.56	−0.018	4	3
	g36349.t1	8	Plasma membrane	635	72.567	9.37	0.145	1	0
	g52197.t1	6	Plasma membrane	634	72.617	9.41	0.134	1	0

TMHs, transmembrane helices; MW, molecular weight; pI, isoelectric point; GRAVY, grand average of hydropathy.

3.4. Tissue-Specific Expression and Promoter Architecture of *CbCesA/Csl* Genes

To investigate the transcriptional activity of the *CbCesA/Csl* genes, we first analyzed publicly available RNA-seq data from four distinct tissue types: whole plant, archegonia, antheridia, and zygote (Figure 3A). The 30 genes exhibited markedly distinct expression patterns that clustered by phylogenetic clades. Among the *CesA* group, most genes showed strong tissue specificity. For instance, g8592.t1 (*AtCesA6*-like) was predominantly expressed in whole plant tissue, whereas g22266.t1 (*AtCesA3*-like) peaked in zygotes. Genes g31305.t1 (*AtCesA1*-like) and g31707.t1 (*AtCesA3*-like) showed the highest expression in antheridia, while seven other *CesA* genes (*AtCesA1/3*-like) peaked in archegonia. Notably, genes g8591.t1 (*CesA* group) and g12813.t1 (*CslC* group) showed no detectable transcripts in any of the sampled tissues. In contrast, *CslA* genes exhibited moderate tissue preference, with higher expression in archegonia and zygotes. *CslC* genes displayed the broadest expression patterns, with the highest transcript abundance observed in whole plant tissue. These clade-specific expression patterns suggest that different branches of the *CesA/Csl* family

have evolved distinct developmental roles: *CesA* genes are more specialized for specific reproductive structures, whereas *CslC* genes may contribute to general vegetative cell wall maintenance.

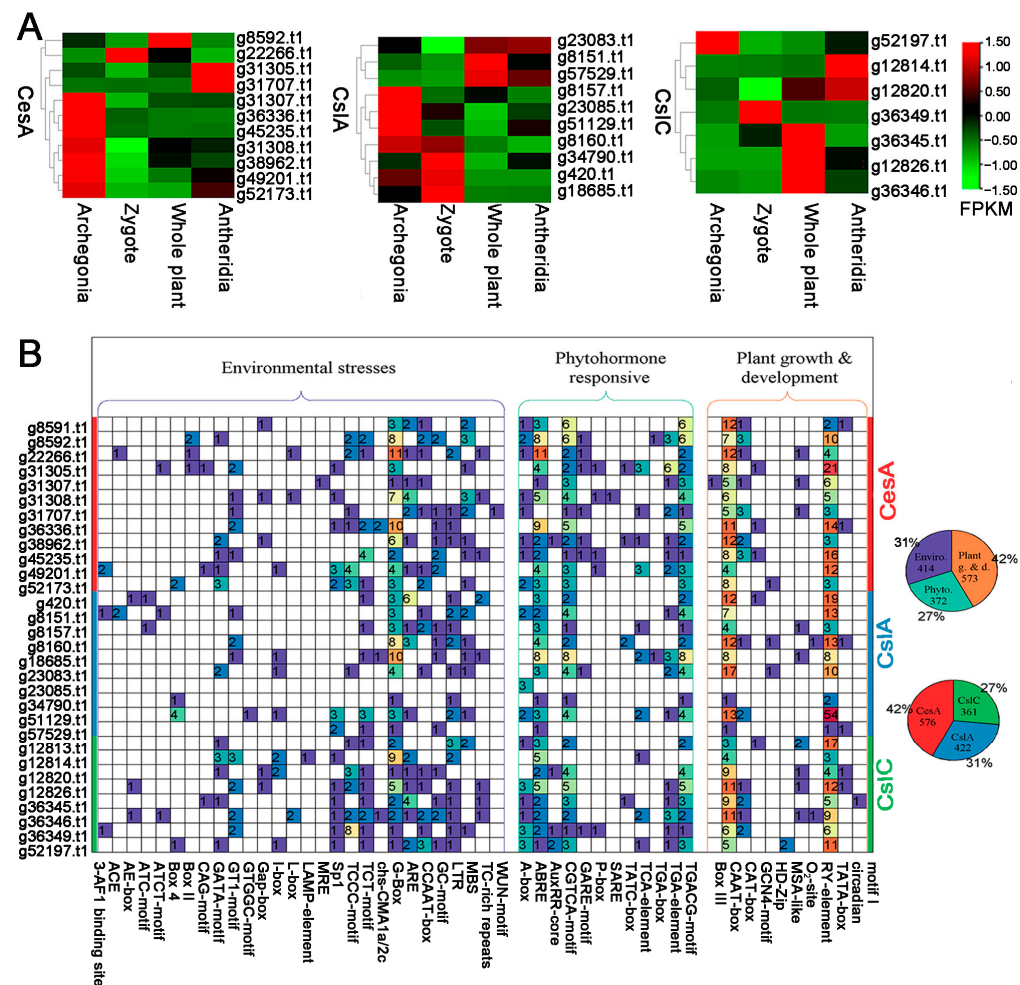


Figure 3. Expression profiles and promoter *cis*-acting element analysis of the *CesA/Csl* gene family in *C. braunii*. (A). Heat map showing tissue-specific expression patterns (whole plant, archegonia, antheridia, zygote) of *CesA*, *CslA* and *CslC* genes. (B). Distribution of predicted *cis*-acting elements in the 2 kb promoter regions of *CesA/Csl* genes, classified into three functional categories: environmental stress response, phytohormone response, and plant growth/development.

A total of 1359 *cis*-regulatory elements were identified in the 2000 bp upstream regions of all 30 genes and classified into three functional categories (Figure 3B). Growth- and development-related elements were most abundant (42.2%), followed by environmental stress-responsive (30.5%) and phytohormone-responsive (27.4%) elements. Among stress-responsive elements, light-responsive motifs (e.g., G-box, 8.61%) were predominant, while ABRE (ABA-responsive, 7.51%) was the most frequent hormone-related element (Figure 4). Promoter architectures differed among clades: *CesA* promoters showed a balanced distribution across categories, *CslA* promoters were slightly biased toward phytohormone-responsive elements, and *CslC* promoters were relatively enriched in stress-responsive motifs. This clade-specific promoter composition likely contributes to the observed differential expression patterns (Table S3).

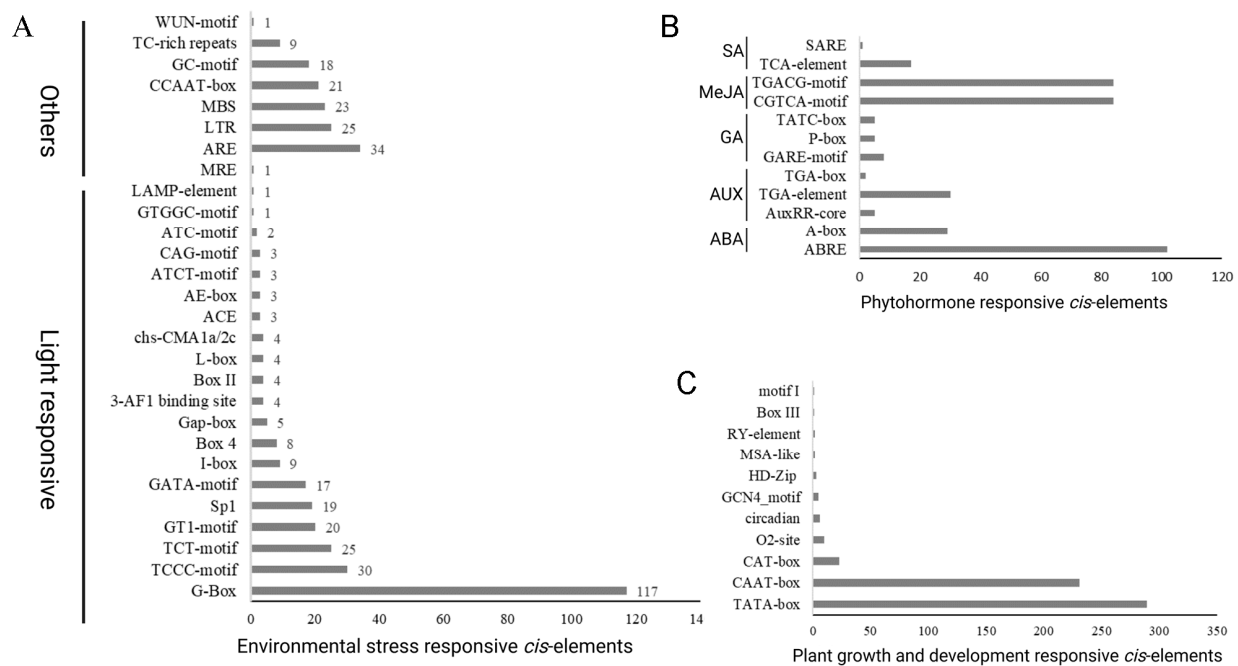


Figure 4. Distribution of *cis*-regulatory elements in the promoters of *CbCesA/Csl* genes. *Cis*-elements identified within the 2 kb upstream regions were classified into three functional categories and are presented as bar charts. (A). Environmental stress-responsive elements (28 types). Light-responsive elements are marked by vertical lines. (B). Phytohormone-responsive elements (12 types). Elements associated with salicylic acid (SA), methyl jasmonate (MeJA), gibberellin (GA), auxin (AUX), and ABA are indicated. (C) Plant growth and development-related elements (11 types), including core promoter elements such as CAAT-box and TATA-box. The x-axis represents the number of elements identified per category.

3.5. Differential Expression of *CbCesA/Csl* Genes Under Abiotic Stress Treatments

To further characterize stress responsiveness, we selected 12 representative genes spanning the *CesA*, *CslA*, and *CslC* clades and examined their temporal expression under two treatments: 100 mM NaCl (salt stress) and 50 μ M ABA (abscisic acid, a key stress hormone) using qRT-PCR (Figure 5). Genes could be grouped into three categories. The first category, comprising most *CesA* members (g8592.t1, g22266.t1, g31305.t1, g31707.t1, and g45235.t1), was responsive to both NaCl and ABA, albeit with different kinetics. Among these, g31305.t1 showed a notably stronger and more persistent induction by ABA than by NaCl, suggesting a preferential role in ABA-mediated stress signaling pathways. The second category included the three *CslA* genes (g8157.t1, g420.t1, and g23085.t1), which responded much more strongly to ABA than to NaCl. In particular, g23085.t1 exhibited the highest induction level among all tested genes, with a rapid and sustained increase under ABA treatment. This pattern aligns with the enrichment of ABA-responsive elements (e.g., ABRE) in *CslA* promoters (Table S3), supporting a direct regulatory link. The third category consisted of *CslC* genes (g12814.t1, g52197.t1, and g12826.t1), which were preferentially induced by NaCl. Among them, g12814.t1 showed the strongest and fastest salt response, consistent with the presence of multiple stress-related *cis*-elements (e.g., LTR, MBS, ARE) in its promoter region. Notably, g8591.t1, which showed no detectable expression in the RNA-seq data, remained largely unchanged under both treatments. These results suggest that the *CbCesA/Csl* gene family shows different stress-responsive patterns under the tested conditions, which are consistent with their clade-specific promoter architectures. The differential sensitivity to NaCl and ABA suggests that hemicellulose and cellulose synthesis in *C. braunii* is not a static process but is dynamically adjusted in response to abiotic stresses, likely through hormone-mediated signaling pathways.

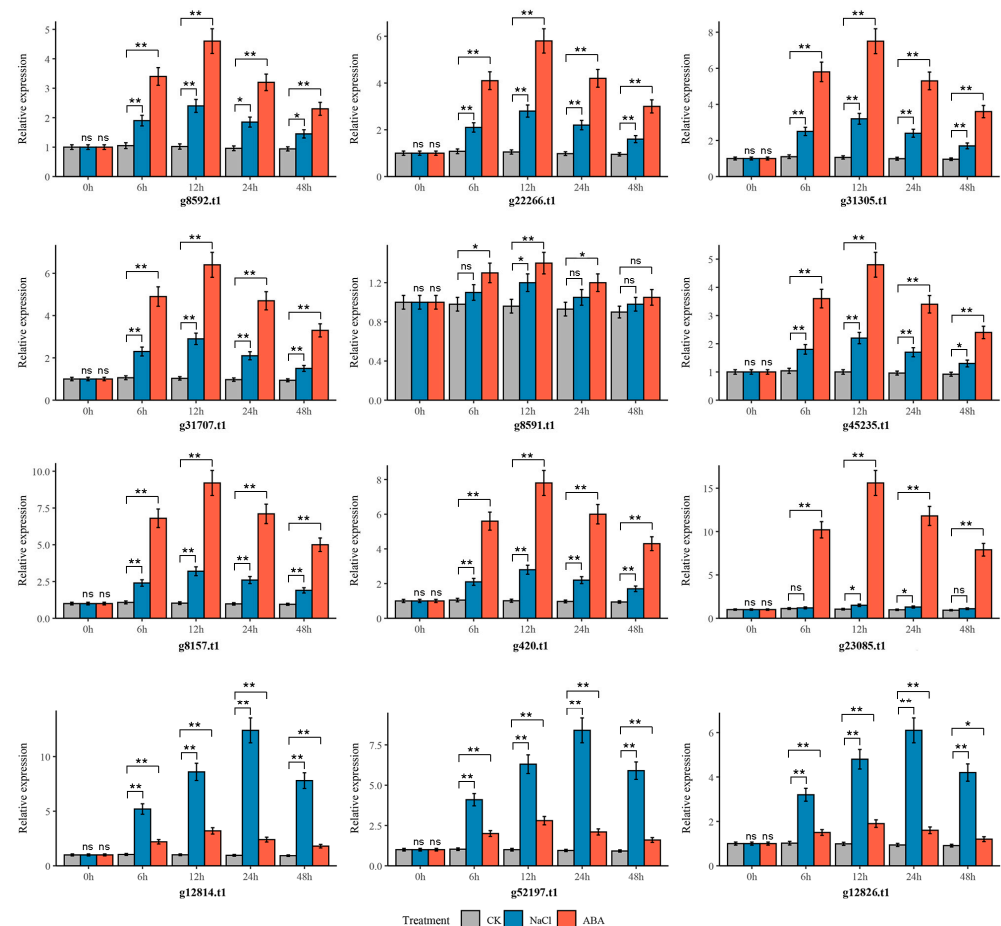


Figure 5. Expression of *CbCesA/Csl* genes under NaCl and ABA treatments. Twelve representative genes from the *CesA*, *CslA*, and *CslC* subfamilies were analyzed by qRT-PCR at 0–48 h after treatment with 100 mM NaCl or 50 μ M ABA. Values are mean $\pm$ SE ($n = 3$), normalized to actin as internal control. Asterisks indicate a significant difference between the treatment group and the control (CK) at the same time point (* $p < 0.05$, ** $p < 0.01$). Non-significant comparisons are labeled “ns”.

4. Discussion

The transition of plants from aquatic to terrestrial environments required profound innovations in cell wall architecture, yet the genetic and regulatory foundations underlying these changes remain incompletely understood [23]. As the closest extant relatives of land plants, streptophyte algae provide a critical evolutionary framework for reconstructing the origin of plant cell wall complexity [6]. In this study, our genome-wide analysis of the *CesA/Csl* superfamily in *Chara braunii* suggests a stepwise evolutionary trajectory for cellulose synthase diversification in streptophytes.

The presence of primary wall-associated *CesA* genes in *C. braunii* indicates that the ancestral framework for cellulose biosynthesis was already present in charophyte algae before the colonization of land, whereas the absence of secondary-wall-specific *CesA4/CesA7/CesA8* orthologs in our analysis is consistent with the later emergence of secondary wall specialization in land plants [24,25]. This pattern implies that the enzymatic capacity for primary wall cellulose synthesis preceded the regulatory and structural innovations required for mechanically reinforced secondary walls. In aquatic environments, where buoyancy reduces the need for extensive structural reinforcement, selection pressure on thickened secondary wall programs may have been reduced, potentially contributing to the retention of a more ancestral *CesA* repertoire [26,27]. Among them, g8591.t1 phylogenetically clusters within the *CesA* clade with 85 bootstrap supports but lacks the

canonical RING-finger domain, and showed no detectable expression across tissues and treatments. This combination of evidence suggests that g8591.t1 may represent a divergent or non-functional *CesA* paralog, though functional validation is required. More broadly, the presence of such atypical *CesA*-like variants points to early diversification of *CesA* architecture, though their precise functions remain unresolved [28,29]. The later assembly of secondary wall biosynthesis machinery likely accompanied vascular tissue evolution and increased mechanical demands on land. In land plants, secondary wall deposition is associated with *CesA4/CesA7/CesA8* complexes and upstream NAC- and MYB-type regulators [29,30]. Although our data do not directly test these regulatory connections in *C. braunii*, the absence of secondary-wall-associated *CesA* orthologs and the restricted *Csl* repertoire are consistent with the view that these enzymatic and regulatory components were assembled after charophytes diverged from the land plant lineage.

The second major insight concerns the restricted *Csl* repertoire in charophytes and its implications for the evolution of hemicellulose biosynthesis. In *C. braunii*, we identified only *CslA* (10 members) and *CslC* (8 members), with no detectable homologs of *CslB*, *CslE*, or *CslG*, and no clear *CslD* orthologs were recovered, with the closest sequence (g8591.t1) grouping within the *CesA* clade by phylogenetic and domain criteria. While the canonical land-plant toolkit for hemicellulose biosynthesis underwent significant expansion and functional specialization post-divergence, the capacity for complex polysaccharide synthesis (e.g., xylan) possibly originated in specific charophyte lineages [31]. Importantly, this genomic simplification does not equate to biochemical absence: xyloglucan and mannan presence in *Chara* cell walls aligns with functional *CslA/CslC* genes [3,8], while xylan detection [2,32] initially appear inconsistent with the lack of *CslD* homologs. Indeed, Jensen et al. identified a xylan synthase (KfXYS1) from the charophyte *Klebsormidium flaccidum* that is not a member of the *CslD* clade, suggesting that charophytes may synthesize xylan via alternative enzymes independent of *CslD* [33]. From an evolutionary perspective, *C. braunii*'s restricted *Csl* repertoire likely reflects both ancestral constraints and aquatic ecology. *Chara* cell walls contain ~13% hemicellulose versus $\geq 32\%$ in land plant [2,34], suggesting limited diversification in aquatic environments. In contrast, terrestrial adaptation would have imposed increased mechanical and desiccation stresses, favoring the expansion of *Csl* subfamilies and the elaboration of hemicellulose networks capable of enhancing wall rigidity and flexibility [35]. Recent phylogenomic analyses confirm most *Csl* subfamilies (*CslB*, *CslD*, *CslE*, *CslG*) originated within embryophytes [1,9], supporting a model where hemicellulose complexity arose through gene family expansion and enzymatic innovation.

A third key observation is the pronounced clade-specific expression and promoter architectures of *CbCesA/Csl* genes. *CesA* genes predominantly expressed in reproductive structures (archegonia, antheridia, zygotes), while *CslA* genes exhibited moderate archegonia/zygote preference and *CslC* genes showed broadest vegetative expression. This spatial differentiation reflects clade-level regulatory partitioning in *C. braunii*. These expression patterns closely correspond to distinct promoter *cis*-regulatory landscapes. Specifically, *CslA* promoters are enriched in ABA-responsive elements (e.g., ABRE) [36], *CslC* promoters contained stress-related motifs (e.g., G-box, LTR, MBS) [37], and *CesA* promoters exhibit a more balanced distribution of regulatory elements. This correspondence between promoter architecture and expression patterns is consistent with transcriptional divergence contributing to functional differentiation within the *CesA/Csl* family [38]. Consistent with these predictions, qRT-PCR analyses revealed distinct clade-specific stress responses. *CesA* genes responded to both NaCl and ABA treatments, *CslA* genes showed strong ABA induction (notably g23085.t1), and *CslC* genes exhibited preferential salt stress induction (e.g., g12814.t1). These results demonstrate that cell wall biosynthesis in *C. braunii* is dynamically regulated through integrated hormonal and environmental signaling pathways.

From an evolutionary perspective, these findings provide evidence that the regulatory coupling between cell wall biosynthesis and stress/developmental signaling predates land plants origins [39]. The widespread presence of light-responsive elements across promoters suggests early integration of metabolic and environmental signals, potentially linking energy availability to synthesis of structurally demanding cell wall components [40]. Such pre-existing regulatory complexity likely facilitated terrestrial adaptation, enabling rapid and coordinated remodeling under fluctuating abiotic stresses.

5. Conclusions

This study provides a comprehensive genome-wide analysis of the *CesA/Csl* superfamily in the charophyte alga *Chara braunii*. Our findings suggest that the foundational genetic toolkit for cellulose biosynthesis was already established in charophyte algae prior to the land colonization of plants, whereas the specialized machinery for secondary cell wall formation likely emerged later during land plant diversification. This evolutionary transition highlights the stepwise complexity of cell wall biosynthesis during the adaptation from aquatic environments to land. Two immediate next steps are to heterologously express these *CesA/Csl* genes in land plant mutants (e.g., *Arabidopsis*) to test their functional complementation and to couple transcriptional dynamics with immunochemical profiling of wall polymers to validate their enzymatic activities. Such approaches will bridge the gap between genomic presence and biological function, illuminating the early evolution of plant cell walls.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/phycolgy6030081/s1>: Figure S1: Multiple sequence alignment of the 30 CbCesA/Csl proteins. The N-terminal RING-finger domain (PF14569) is highlighted in light blue; the D,D,D,QxxRW catalytic motif is highlighted in light yellow; Table S1: Genomic locations, detailed sequence features, and PFAM domain positions of CbCesA/Csl genes; Table S2: Putative orthologs of CbCesA/Csl genes in *A. thaliana*; Table S3: Cis-regulatory elements identified in the 2000 bp upstream promoter regions of CbCesA/Csl genes; Table S4: Gene-specific qRT-PCR primers.

Author Contributions: Q.L. conceived and designed the study, acquired funding, supervised the project, and reviewed and edited the manuscript. J.Z. performed the experiments, analyzed the data, validated the results, and wrote the original draft. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Opening Foundation of the Key Laboratory of Smart Earth (KF2023YB03-08), the National Natural Science Foundation of China (NSFC, 42206029), the National Key R&D Program of China (2020YFA0608804) and the Southern Marine Science and Engineering Guangdong Laboratory (Zhuhai) (SML2024SP030).

Data Availability Statement: The authors confirm that the data supporting the findings of this study are available within the article and its Supplementary Materials. The Supplementary Materials, including the multiple sequence alignment (Figure S1) and supplementary tables (Tables S1–S4), are provided as part of the submission. Additional data supporting the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Fuertes-Rabanal, M.; Rebaque, D.; Largo-Gosens, A.; Encina, A.; Melida, H. Cell walls: A comparative view of the composition of cell surfaces of plants, algae, and microorganisms. *J. Exp. Bot.* **2025**, *76*, 2614–2645. [CrossRef] [PubMed]
2. Franková, L.; Fry, S.C. *Chara*—A living sister to the land plants with pivotal enzymic toolkit for mannan and xylan remodelling. *Physiol. Plant.* **2024**, *176*, e14134. [CrossRef]

3. Popper, Z.A.; Michel, G.; Hervé, C.; Domozych, D.S.; Willats, W.G.T.; Tuohy, M.G.; Kloareg, B.; Stengel, D.B. Evolution and diversity of plant cell walls: From algae to flowering plants. *Annu. Rev. Plant Biol.* **2011**, *62*, 567–590. [[CrossRef](#)] [[PubMed](#)]
4. Domozych, D.S.; Popper, Z.A.; Sørensen, I. Charophytes: Evolutionary giants and emerging model organisms. *Front. Plant Sci.* **2016**, *7*, 1470. [[CrossRef](#)] [[PubMed](#)]
5. Sørensen, I.; Pettolino, F.A.; Bacic, A.; Ralph, J.; Lu, F.C.; O'Neill, M.A.; Fei, Z.Z.; Rose, J.K.C.; Domozych, D.S.; Willats, W.G.T. The charophycean green algae provide insights into the early origins of plant cell walls. *Plant J.* **2011**, *68*, 201–211. [[CrossRef](#)] [[PubMed](#)]
6. Nishiyama, T.; Sakayama, H.; de Vries, J.; Buschmann, H.; Saint-Marcoux, D.; Ullrich, K.K.; Haas, F.B.; Vanderstraeten, L.; Becker, D.; Lang, D.; et al. The Chara genome: Secondary complexity and implications for plant terrestrialization. *Cell* **2018**, *174*, 448–464.e24. [[CrossRef](#)] [[PubMed](#)]
7. Cheng, S.; Xian, W.; Fu, Y.; Marin, B.; Keller, J.; Wu, T.; Sun, W.; Li, X.; Xu, Y.; Zhang, Y.; et al. Genomes of subaerial Zygnematophyceae provide insights into land plant evolution. *Cell* **2019**, *179*, 1057–1067.e14. [[CrossRef](#)] [[PubMed](#)]
8. Mikkelsen, M.D.; Harholt, J.; Westereng, B.; Domozych, D.; Fry, S.C.; Johansen, I.E.; Fangel, J.U.; Łężyk, M.; Feng, T.; Nancke, L.; et al. Ancient origin of fucosylated xyloglucan in charophycean green algae. *Commun. Biol.* **2021**, *4*, 754. [[CrossRef](#)] [[PubMed](#)]
9. Daras, G.; Templelexis, D.; Avgeri, F.; Tsitsekian, D.; Karamanou, K.; Rigas, S. Updating insights into the catalytic domain properties of plant cellulose synthase (CesA) and cellulose synthase-like (Csl) proteins. *Molecules* **2021**, *26*, 4335. [[CrossRef](#)] [[PubMed](#)]
10. Vandavasi, V.G.; Putnam, D.K.; Zhang, Q.; Petridis, L.; Heller, W.T.; Nixon, B.T.; Haigler, C.H.; Kalluri, U.; Coates, L.; Langan, P.; et al. A structural study of CESA1 catalytic domain of Arabidopsis cellulose synthesis complex: Evidence for CESA trimers. *Plant Physiol.* **2016**, *170*, 123–135. [[CrossRef](#)] [[PubMed](#)]
11. Little, A.; Schwerdt, J.G.; Shirley, N.J.; Khor, S.F.; Neumann, K.; O'Donovan, L.A.; Lahnstein, J.; Collins, H.M.; Henderson, M.; Fincher, G.B.; et al. Revised phylogeny of the cellulose synthase gene superfamily: Insights into cell wall evolution. *Plant Physiol.* **2018**, *177*, 1124–1141. [[CrossRef](#)] [[PubMed](#)]
12. Eddy, S.R. Accelerated profile HMM searches. *PLoS Comput. Biol.* **2011**, *7*, e1002195. [[CrossRef](#)] [[PubMed](#)]
13. Kumar, S.; Stecher, G.; Suleski, M.; Sanderford, M.; Sharma, S.; Tamura, K. MEGA12: Molecular evolutionary genetic analysis version 12 for adaptive and green computing. *Mol. Biol. Evol.* **2024**, *41*, msae263. [[CrossRef](#)] [[PubMed](#)]
14. Letunic, I.; Bork, P. Interactive Tree Of Life (iTOL) v5: An online tool for phylogenetic tree display and annotation. *Nucleic Acids Res.* **2021**, *49*, W293–W296. [[CrossRef](#)] [[PubMed](#)]
15. Katoh, K.; Standley, D.M. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol. Biol. Evol.* **2013**, *30*, 772–780. [[CrossRef](#)] [[PubMed](#)]
16. Minh, B.Q.; Schmidt, H.A.; Chernomor, O.; Schrempf, D.; Woodhams, M.D.; von Haeseler, A.; Lanfear, R. IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. *Mol. Biol. Evol.* **2020**, *37*, 1530–1534. [[CrossRef](#)] [[PubMed](#)]
17. Bailey, T.L.; Boden, M.; Buske, F.A.; Frith, M.; Grant, C.E.; Clementi, L.; Ren, J.Y.; Li, W.W.; Noble, W.S. MEME Suite: Tools for motif discovery and searching. *Nucleic Acids Res.* **2009**, *37*, W202–W208. [[CrossRef](#)] [[PubMed](#)]
18. Hu, B.; Jin, J.; Guo, A.Y.; Zhang, H.; Luo, J.; Gao, G. GSDS 2.0: An upgraded gene feature visualization server. *Bioinformatics* **2015**, *31*, 1296–1297. [[CrossRef](#)] [[PubMed](#)]
19. Lescot, M.; Dehais, P.; Thijs, G.; Marchal, K.; Moreau, Y.; Van de Peer, Y.; Rouzé, P.; Rombauts, S. PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. *Nucleic Acids Res.* **2002**, *30*, 325–327. [[CrossRef](#)] [[PubMed](#)]
20. Heß, D.; Heise, C.M.; Schubert, H.; Hess, W.R.; Hagemann, M. The impact of salt stress on the physiology and the transcriptome of the model streptophyte green alga *Chara braunii*. *Physiol. Plant.* **2023**, *175*, e14123. [[CrossRef](#)] [[PubMed](#)]
21. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* **2001**, *25*, 402–408. [[CrossRef](#)] [[PubMed](#)]
22. Roberts, E.M.; Whelan, K.A.; Gobell, M.; Haigler, C.H.; Roberts, A.W. Evolution of cellulose synthesis complexes in plants: Cellulose synthase-like D rosettes in the charophyte green alga *Coleochaete*. *J. Exp. Bot.* **2026**, erag176. [[CrossRef](#)] [[PubMed](#)]
23. Morris, J.L.; Puttick, M.N.; Clark, J.W.; Edwards, D.; Kenrick, P.; Pressel, S.; Wellman, C.H.; Yang, Z.; Schneider, H.; Donoghue, P.C.J. The timescale of early land plant evolution. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, E2274–E2283. [[CrossRef](#)] [[PubMed](#)]
24. Niklas, K.J. The cell walls that bind the tree of life. *BioScience* **2004**, *54*, 831–841. [[CrossRef](#)]
25. Yin, Y.; Johns, M.A.; Cao, H.; Rupani, M. A survey of plant and algal genomes and transcriptomes reveals new insights into the evolution and function of the cellulose synthase superfamily. *BMC Genom.* **2014**, *15*, 260. [[CrossRef](#)] [[PubMed](#)]
26. Hill, J.L., Jr.; Russo, D.A.; Sawada, D.; Pingali, S.V.; Kowalik, M.; Kiemle, S.N.; O'Neill, H.; Baskin, T.I. The three cellulose synthase isoforms for secondary cell wall make specific contributions to microfibril synthesis. *Plant J.* **2025**, *123*, e70344. [[CrossRef](#)] [[PubMed](#)]
27. Banasiak, A.J. Evolution of the cell wall components during terrestrialization. *Acta Soc. Bot. Pol.* **2014**, *83*, 349–362. [[CrossRef](#)]

28. Kurek, I.; Kawagoe, Y.; Jacob-Wilk, D.; Doblin, M.; Delmer, D. Dimerization of cotton fiber cellulose synthase catalytic subunits occurs via oxidation of the zinc-binding domains. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 11109–11114. [[CrossRef](#)] [[PubMed](#)]
29. Zhong, R.; Cui, D.; Ye, Z.H. Secondary cell wall biosynthesis. *New Phytol.* **2019**, *221*, 1703–1723. [[CrossRef](#)] [[PubMed](#)]
30. Zhong, R.; Ye, Z.-H. Secondary cell walls: Biosynthesis, patterned deposition and transcriptional regulation. *Plant Cell Physiol.* **2014**, *56*, 195–214. [[CrossRef](#)] [[PubMed](#)]
31. Mokshina, N. Functional specialization of cellulose synthase-like proteins (CSLs) in celery: Tissue-specific roles in hemicellulose biosynthesis and beyond. *Plant Physiol. Rep.* **2025**, *30*, 548–557. [[CrossRef](#)]
32. Bernal, A.J.; Jensen, J.K.; Harholt, J.; Sørensen, S.; Møller, I.; Blaukopf, C.; Johansen, B.; de Lotto, R.; Pauly, M.; Scheller, H.V.; et al. Disruption of ATCSLD5 results in reduced growth, reduced xylan and homogalacturonan synthase activity and altered xylan occurrence in Arabidopsis. *Plant J.* **2007**, *52*, 791–802. [[CrossRef](#)] [[PubMed](#)]
33. Jensen, J.K.; Busse-Wicher, M.; Poulsen, C.P.; Fangel, J.U.; Smith, P.J.; Yang, J.-Y.; Peña, M.-J.; Dinesen, M.H.; Martens, H.J.; Melkonian, M.; et al. Identification of an algal xylan synthase indicates that there is functional orthology between algal and plant cell wall biosynthesis. *New Phytol.* **2018**, *218*, 1049–1060. [[CrossRef](#)] [[PubMed](#)]
34. Anderson, D.M.; King, N.J. Polysaccharides of the Characeae. III. The carbohydrate content of *Chara australis*. *Biochim. Biophys. Acta* **1961**, *52*, 449–454. [[CrossRef](#)] [[PubMed](#)]
35. Cosgrove, D.J. Structure and growth of plant cell walls. *Nat. Rev. Mol. Cell Biol.* **2024**, *25*, 340–358. [[CrossRef](#)] [[PubMed](#)]
36. Zha, D.; He, Y.; Song, J. Regulatory role of ABA-responsive element binding factors in plant abiotic stress response. *Physiol. Plant.* **2025**, *177*, e70233. [[CrossRef](#)] [[PubMed](#)]
37. Jeevaraj, T.; Blessy, A.; Krishnamoorthy, S.; Sridhar, A.J. Histone demethylation by JMJ family genes: Insights into plant growth and adaptation. *Plant Mol. Biol.* **2025**, *115*, 118. [[CrossRef](#)] [[PubMed](#)]
38. Marand, A.P.; Eveland, A.L.; Kaufmann, K.; Springer, N.M. *cis*-Regulatory elements in plant development, adaptation, and evolution. *Annu. Rev. Plant Biol.* **2023**, *74*, 111–137. [[CrossRef](#)] [[PubMed](#)]
39. Donoghue, P.C.J.; Harrison, C.J.; Paps, J.; Schneider, H. The evolutionary emergence of land plants. *Curr. Biol.* **2021**, *31*, R1281–R1298. [[CrossRef](#)] [[PubMed](#)]
40. Zhang, B.; Gao, Y.; Zhang, L.; Zhou, Y. The plant cell wall: Biosynthesis, construction, and functions. *J. Integr. Plant Biol.* **2021**, *63*, 251–272. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.