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Genome-Wide Analysis of the Multidrug and Toxic Compound Extrusion Gene Family in the Tea Plant

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Abstract: The multidrug and toxic compound extrusion (MATE) family is the latest class of novel secondary transporters discovered in plants. However, there is currently no comprehensive analysis of the *MATE* gene family in the tea plant. In this study, 68 *CsMATE* genes were identified from the tea plant genome using bioinformatic methods. In general, we analyzed the evolutionary relationships, intron–exon structure, distribution in chromosomes, conserved domains, and gene expression patterns in different tissues and stresses of the *CsMATE* gene family. The 68 CsMATEs were phylogenetically divided into four major clusters (Class I to Class IV). The *CsMATE* genes within the same class exhibit similar structural features, while displaying certain distinctions across different classes. Evolutionary analysis indicated that the *CsMATE* gene family expanded mainly through gene duplication events, in addition to proximal duplication. Through the analysis of *cis*-acting elements, it was found that *CsMATE* genes may be involved in the growth, development, and stress response. Furthermore, we observed that certain *CsMATE* genes could be induced to exhibit expression under abiotic stress conditions such as low temperature, high salinity (NaCl), osmotic stress (PEG), and methyl jasmonate treatment (MeJA). The findings presented herein offer a crucial theoretical foundation for elucidating the biological functions of *CsMATE* genes, particularly in response to abiotic stress, and furnish valuable potential genetic resources for tea plant resistance breeding.

Keywords: multidrug and toxic compound extrusion; tea plant; genome-wide analysis; gene expression pattern; stress responses

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1. Introduction

The tea plant (*Camellia sinensis*) is a perennial economic crop that thrives in tropical, subtropical, and temperate regions and has its origins in southwest China [1]. Tea stands as the most widely consumed beverage globally and ranks among the top three non-alcoholic beverages worldwide [2]. The tea plant boasts an abundance of flavonoids, including catechins (flavanols), flavonoids, flavonols, and anthocyanidins. These compounds exhibit remarkable properties such as antioxidation, anti-inflammation, anticancer effects, cardiovascular protection, antibacterial activity, the regulation of hyperglycemia levels, as well as properties that combat obesity [3].

Flavonoids, as a crucial class of secondary metabolites in plants, are believed to exert a significant role in plant defense owing to the potent antioxidant properties [4]. Similarly to other plants, the biosynthesis of flavonoids in the tea plant involves the shikimic acid pathway, phenylpropanoid pathway, and flavonoid pathway [5–7]. The shikimate pathway provides the starting substrate phenylalanine for the phenylpropane pathway. Flavonoid biosynthesis occurs at different locations within plant cells' cytoplasm and is subsequently

transported to specific organelles for storage, such as vacuolar membranes for anthocyanins [8]. In contrast to the flavonoid biosynthesis pathway, there is limited knowledge about flavonoid transport [9,10]. The transportation of anthocyanins to vacuoles primarily occurs through vesicles and transporters [10,11]. Among these models, most scientists strongly support the transporter-mediated model which includes ABC transporters, the MSF superfamily, and MATE proteins [8–13].

The MATE family is the latest class of novel secondary transporters discovered. The first MATE protein was cloned from *Vibrio parahemolyticus*, and subsequent investigations have revealed its abundance across various species including bacteria, yeasts, animals, and plants [14,15]. Previous studies have demonstrated that MATE transporters play direct or indirect roles in numerous physiological processes within plants, encompassing aluminum stress tolerance, the detoxification of toxic complexes or heavy metals, metal ion uptake and transportation, and the transport of diverse plant hormones [16,17].

The expansion of the *MATE* gene family in plants leads to the differentiation of protein function. Compared with mammals and bacteria, plants exhibit a higher abundance of *MATE* genes. For instance, *Arabidopsis*, rice, tomato, alfalfa, and soybean genomes contain 56, 46, 67, 88, and 117 *MATE* genes, respectively, whereas bacterial and mouse genomes have less than 15 [15,18–21]. The polyploidy and tandem replication of plant genomes during evolution are the primary factors contributing to the expansion of the *MATE* gene family [20,22]. Among the plants studied thus far, *Arabidopsis* MATE proteins have been extensively researched with approximately 10 identified functions, including the regulation of plant organogenesis, ion and metabolite transportation, and conferring resistance to abiotic stress and aluminum stress [17,23,24]. The *ZRZ* gene in *Arabidopsis* belongs to the *MATE* gene family; the overexpression of *ZRZ* promotes leaf and axillary bud growth, thereby enhancing the overall growth rate [23]. Lu et al. found that the protein encoded by the cotton *MATE* gene *Gh_D06G0281* is located on the cell membrane, and the heterologous overexpression of *Gh_D06G0281* in *Arabidopsis* could increase the antioxidant oxidase content under different abiotic stress conditions [25]. Additionally, *Arabidopsis MATE45* is involved in the regulation of anthocyanin response to abiotic stress, while also mediating abiotic stress responses through antagonizing local abscisic acid signaling [26]. In addition, the analysis conducted on rapeseed, Chinese pear, cotton, rice, flax, soybean, and populous revealed that numerous *MATE* genes were involved in various abiotic stress processes including salt, drought, and cold stresses [18,20,22,27–30]. In addition to investigating abiotic stress, Chen et al. also conducted a study on the transport function of MATEs in flavonoids. By performing a correlation analysis between flavonoids and the *MATE* genes, it was discovered that the *MATE* genes may play a role in the process of flavonoid accumulation [31].

The involvement of MATE proteins in numerous vital physiological processes in plants renders them potential target genes for breeding programs aimed at enhancing important traits, such as nutrient uptake, secondary metabolite content, abiotic stress tolerance, and aluminum toxicity resistance [32]. Therefore, a comprehensive analysis of the *MATE* gene family in the tea plant is imperative for elucidating the potential functionalities of the *MATE* genes. Surprisingly, despite the identification of several plant MATE transporters thus far, complete functional identification is rarely studied in the tea plant. In this study, we conducted a comprehensive study on the structure, conserved domains, chromosome localization, subcellular localization, expression patterns, and collinearity of 68 *MATE* genes in the tea plant. This study lays a crucial foundation for future functional investigations on *CsMATEs* and may offer novel insights into enhancing the stress resistance of the tea plant.

2. Materials and Methods

2.1. Genome-Wide Identification of CsMATE Genes in Tea Plant

The complete protein sequences and genome sequences of the tea plant were downloaded from the Tea Plant Information Archive (TPIA, http://tpia.teaplants.cn/, accessed on 1 August 2024) [33,34]. Hidden Markov Model (HMM) profiles of conserved MATE

domain (MATE domain, pfam number: PF01554) acquired from InterPro (https://www.ebi.ac.uk/interpro/, accessed on 1 August 2024) were used as a query for blast to identify the *CsMATEs* in the tea plant [19,35]. HMMER v.2.39.0 software (https://www.ebi.ac.uk/Tools/hmmer/, accessed on 2 August 2024) was used to carry out a search of the whole genome protein sequences of the tea plant and obtained the candidate proteins of CsMATEs [36]. To improve the accuracy of subsequent analyses, we analyzed the protein length and transmembrane domain of the candidate *CsMATE* genes with TMHMM Server v.2.0 (https://services.healthtech.dtu.dk/services/TMHMM-2.0/, accessed on 5 August 2024) [37,38]. CsMATEs were further manually screened according to a previous study (protein length > 400 amino acid residues and 8-12 transmembrane domains) [19].

2.2. Physicochemical Parameter and Characteristic of CsMATEs

The physicochemical parameter of CsMATEs including molecular weight (MW) and theoretical isoelectric points (pI) were analyzed by the protein isoelectric point calculator v.2.0 (http://isoelectric.org/, accessed on 8 August 2024), and the subcellular localization analysis was predicted by Cell-PLoc 2.0 (http://www.csbio.sjtu.edu.cn/bioinf/Cell-PLoc-2/, accessed on 8 August 2024) [39,40]. Information about the initiation and termination sites of *CsMATEs* on chromosomes was extracted from GFF files from the TPIA, and location images were generated using MG2C v.2.0 (http://mg2c.iask.in/mg2c_v2.0/, accessed on 8 August 2024).

2.3. Construction of Phylogenetic Tree for CsMATE Proteins

The protein sequences of 68 CsMATEs and 56 *Arabidopsis* AtDTXs were, respectively, acquired from the TPIA and TAIR database (https://www.arabidopsis.org/, accessed on 9 August 2024). Multiple amino acid sequence alignments of these protein sequences were carried out by Clustal W (http://www.clustal.org/clustal2/, accessed on 12 August 2024) [41]. Phylogenetic tree was performed with neighbor-joining (NJ) method combined with 1000 bootstraps in MEGA 7.0 software [42].

2.4. Conserved Motif and Intron-Exon Composition Analysis of CsMATEs

The conserved motifs in CsMATE proteins were analyzed by the Multiple Em for Motif Elicitation website (MEME, http://meme-suite.org/tools/meme, accessed 13 August 2024) using the website default parameters and setting the maximum motifs to 10. The exon–intron compositions of *CsMATEs* were analyzed with the tea plant GFF profile by TBtools v.2.086 [43]. The *CsMATE* gene structure and conserved motif pattern were visualized with the Amazing Optional Gene Viewer function in TBtools.

2.5. Identification of MATE Homologs

The gene duplication events and synteny relationships among *Camellia sinensis* var. sinensis, *Coffea canephora,* and *Actinidia chinensis* were performed by Multiple Collinearity Scan toolkit X version (MCScanX) software with default parameter settings [44]. The relationship of paralogous *MATEs* in *C. sinensis* and the orthologous *MATEs* among *C. sinensis* and other species were visualized by TBtools [43]. The analysis of the duplication event was applied with the MCScanX duplicat_gene_classifier program.

2.6. Cis-Acting Element Analysis of CsMATE Genes

The SAMtools v.1.19.2 software was employed to extract the 2000 bp upstream sequence of the start codon of the *MATE* gene family members from the tea plant GFF file. Subsequently, these obtained sequences were uploaded to the PlantCatre database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/, accessed on 17 August 2024) for the prediction of *cis*-acting elements [45].

2.7. Tissue Expression Pattern of CsMATE Genes

The expression data of *CsMATE* genes in eight major tea plant developmental tissues and organs, including roots, stems, young leaves, mature leaves, old leaves, aptical buds, flowers, and fruits, were obtained from the TPIA [33,34]. The $\log_2$-transformed FPKM + 1 values were utilized to represent the expression levels of the *CsMATE* genes across different tissues and organs. These data were visualized using TBtools software.

2.8. Expression Patterns of CsMATE Genes Under Diverse Treatments

To investigate the response of the *CsMATE* genes under diverse abiotic stress treatments, the transcriptome data of tea plant genes under cold acclimation (CA1: fully acclimation at 10 °C, CA3: cold response at 4 °C), PEG, NaCl, and MeJA stress were downloaded from the TPIA [33,34]. The heat maps were constructed with TBtools by using the $\log_2$-transformed TPM ratio between the treatment group and the control group (CK). If the TPM value is lower than 1 under normal conditions and treatment conditions, the $\log_2$-transformed ratio is defined as 0, indicating that there is no difference after treatments.

3. Results

3.1. Genome-Wide Identification and Characteristics of MATE Gene Family Members in Tea Plant

To identify members of the *MATE* gene family in the tea plant, the MATE domain was used as a query for genome-wide analysis. A total of 98 *CsMATEs* were identified in the tea plant genome and named by sequence numbers from *CsMATE1* to *CsMATE98* according to the position on the chromosome (Tables S1 and S2). However, according to previous studies, 30 CsMATEs with a protein length < 400 amino acids or a number of transmembrane domains < 8 were excluded [27,46]. Finally, 68 CsMATE proteins were retained for further study (Table S2).

Detailed molecular characteristics of CsMATE proteins were analyzed including protein length, MW, pI, and predicted subcellular localization (Table S2). The protein length of CsMATE proteins consists of 401 (CsMATE29) to 987 (CsMATE15) amino acids. The pI of CsMATE proteins ranged from 4.73 (CsMATE17) to 9.22 (CsMATE86), and the MW ranged from 43.65 (CsMATE29) to 111.11 (CsMATE15) kDa. The results of subcellular localization prediction showed that all of the sixty-eight CsMATE proteins were cell membrane-localized, and two of them (CsMATE20 and CsMATE21) were also localized in te chloroplast, suggesting that CsMATE proteins may be involved in the membrane transport of molecular substances.

3.2. Phylogenetic Analysis of CsMATE Proteins

In order to understand the evolutionary relationship of CsMATE proteins, a neighbor-joining phylogenetic tree of MATE proteins in the tea plant and *Arabidopsis* was constructed. Here, 124 MATE proteins were classified into four classes named Class I to Class IV, which contain twenty-three CsMATEs and twenty-two AtDEXs, twenty CsMATEs and nineteen AtDEXs, fifteen CsMATEs and nine AtDEXs, and ten CsMATEs and six AtDEXs, respectively (Figure 1). Interestingly, parts of the MATEs of *Arabidopsis* or the tea plant were clustered on a single branch, such as CsMATE10, CsMATE81, CsMATE82, and CsMATE83 and AtDEX20, AtDEX21, AtDEX22, AtDEX23, AtDEX24, and AtDEX25, suggesting that both *Arabidopsis* and the tea plant have produced many new genes during evolution.

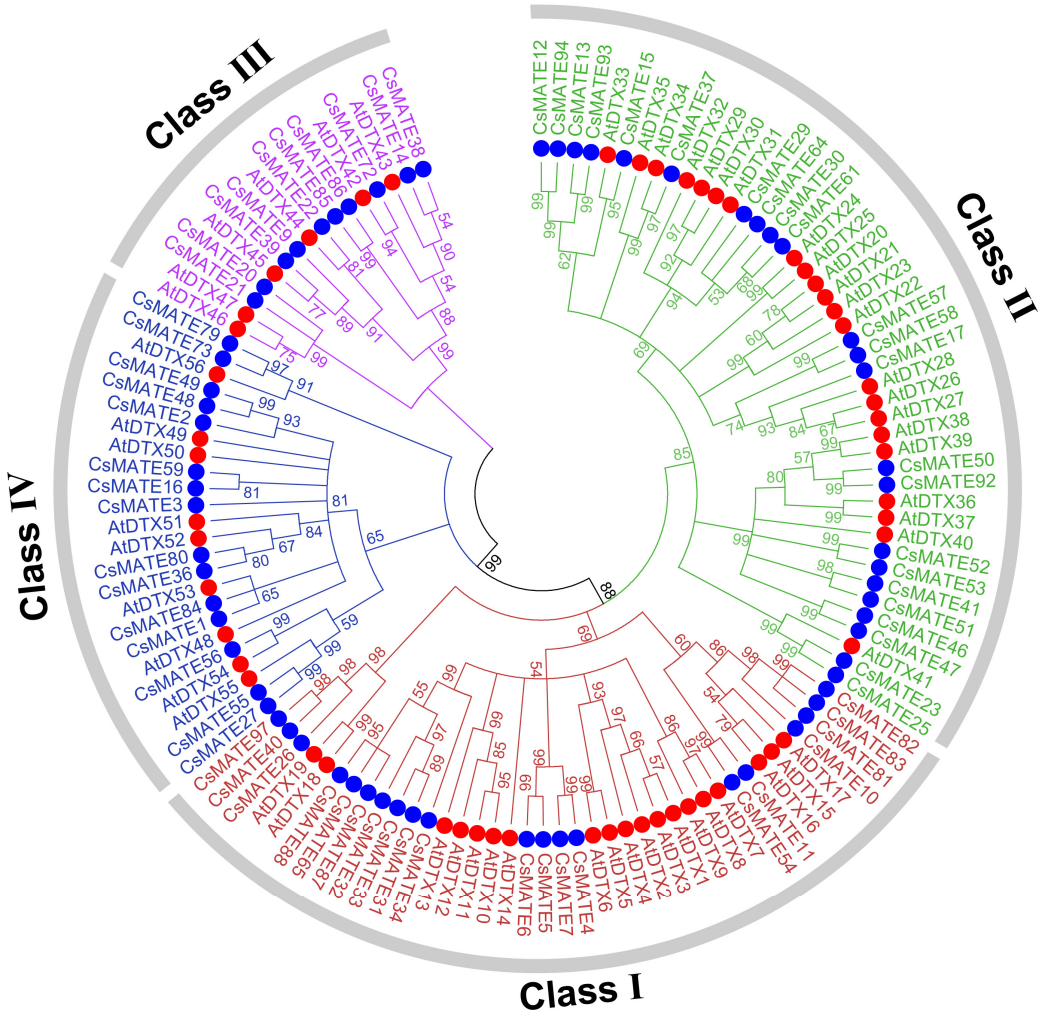

Figure 1. Phylogenetic relationships of MATE proteins of *Arabidopsis* and the tea plant. Blue dots represent MATE proteins from the tea plant and red dots represent MATE proteins from *Arabidopsis*. Branch colors represent different classes. The numbers assigned to branches indicate the reliability.

3.3. Conserved Motif, Gene Structure, and Chromosomal Localization Analysis in CsMATEs

A total of 10 conserved motif were identified in CsMATE proteins by MEME (Figure S1). An extremely conserved motif (motif 8) existed in all CsMATE proteins, and a relatively conserved motif (motif 7) existed in all CsMATE proteins except CsMATE6, CsMATE29, CsMATE34, CsMATE81, and CsMATE82 (Figure 2B). The distribution of conserved domains in CsMATE proteins was divided into two categories: CsMATE proteins with only two conserved motifs and CsMATE proteins with at least eight conserved motifs. Among them, only ten CsMATE proteins contained two conserved motifs, and the entirety of these CsMATE proteins belonged to Class IV. The remaining 58 CsMATE proteins exhibited the presence of nearly all conserved motifs, with only 10 CsMATEs (CsMATE1, CsMATE11, CsMATE12, CsMATE13, CsMATE16, CsMATE53, CsMATE59, CsMATE82, CsMATE88 and CsMATE93) lacking a single motif and 9 CsMATEs (CsMATE6, CsMATE29, CsMATE34, CsMATE46, CsMATE47, CsMATE55, CsMATE58, CsMATE83, and CsMATE97) lacking two motifs.

The variability of exon–intron structure pattern is closely related to functional disparities and gene expression [47]. In *C. sinensis*, the number of introns varies significantly among members of the *CsMATE* gene family, ranging from 0 to 16 (Figure 2C). Among them, only seven *CsMATEs* did not contain introns. Furthermore, genes with closer evolutionary relationships exhibited similar exon–intron structure patterns, suggesting potential

functional similarities. For instance, the 15 *CsMATE* members of Class III contain only 1–3 exons, while the 10 *CsMATE* members of Class IV contain 11–14 exons.

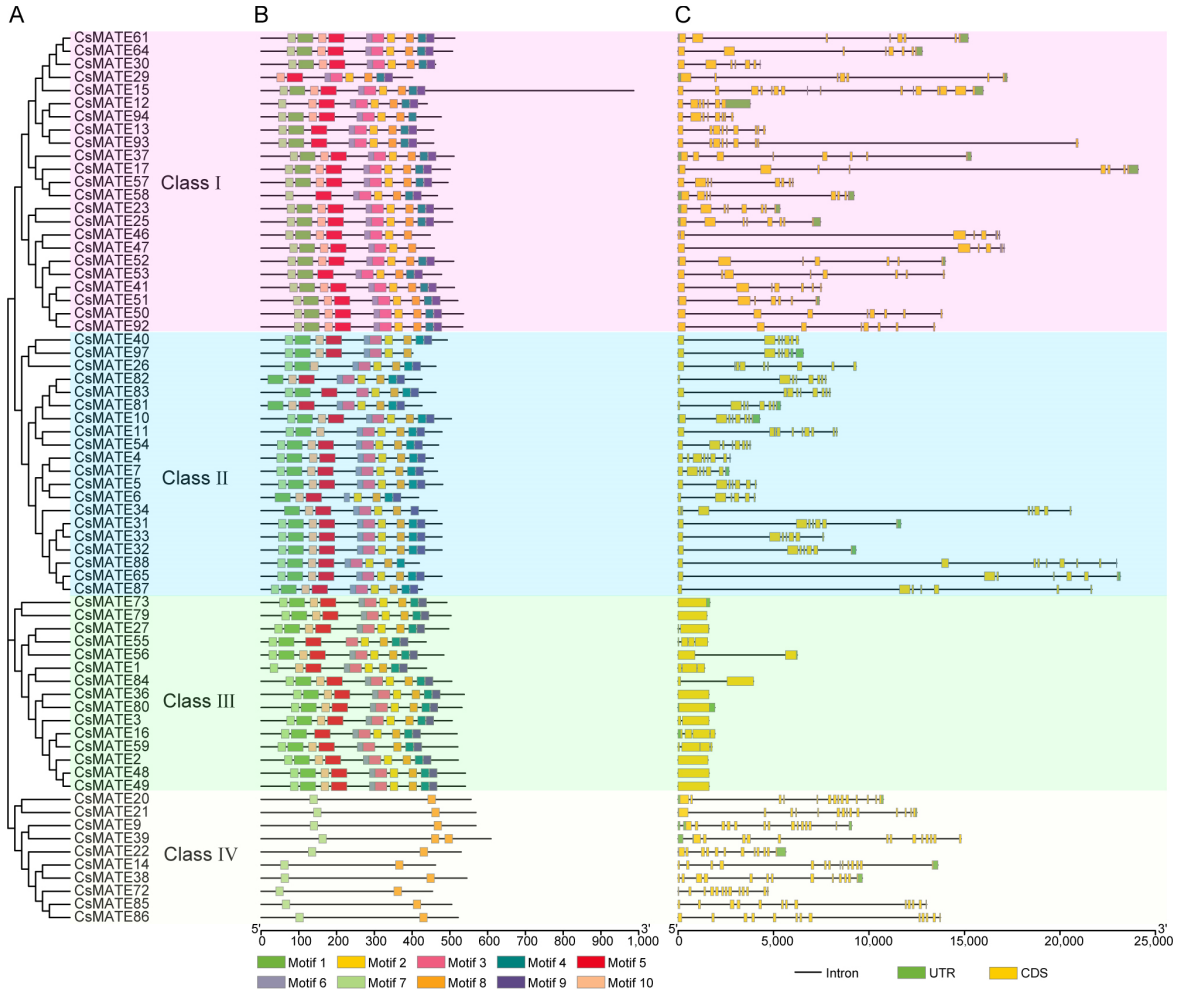

Figure 2. Intron–exon and motif composition of CsMATEs. (**A**) Phylogenetic tree of CsMATEs. (**B**) 10 conserved motifs in CsMATE proteins. (**C**) Intron–exon composition of *CsMATEs*.

In order to assess the chromosomal distribution of *CsMATEs*, the tea plant genome annotation file was used for the characterization of *CsMATEs*. The results revealed that *CsMATEs* exhibited an uneven distribution across the fourteen chromosomes and seven scaffolds of *C. sinensis* (Figure 3). From the published chromosome level genomes, a total of 59 *CsMATEs* were mapped to the fourteen chromosomes, which were assembled relatively completely, while nine *CsMATEs* still remained on scaffolds. Notably, chromosome 6 harbored the highest number of CsMATEs with a count of ten, followed by chromosome 1 with a count of seven. Both chromosomes 4 and 7 contained six CsMATEs each, whereas both chromosomes 8 and 9 also possessed six *CsMATEs* each. Except for chromosome 13, all other chromosomes encompassed varying numbers of CsMATEs ranging from 1 to 4. Additionally, nine CsMATEs were mapped to seven scaffolds (Contig 310, Contig 341, Contig 783, Contig 1054, Contig 1197, Contig 1221, and Contig 1303), averaging one or two *CsMATEs* per scaffold.

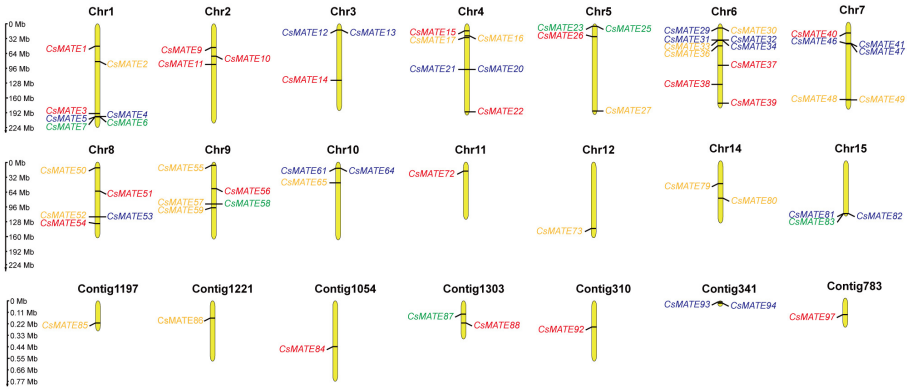

Figure 3. The distribution of *CsMATEs* on tea plant chromosomes. Font colors represent different classes. MB, megabase.

3.4. CsMATEs Gene Duplication Events and Collinearity Relationships Between Four Genomes

Using MCScanX software, we identified 13346 paralogs of 8446 gene pairs (approximately 26.41%) in the *C. sinensis* genome. Within the *CsMATE* gene family, we identified 20 paralogs of 13 gene pairs, accounting for about 29.41%. Next, we analyzed the gene duplication events in the *CsMATE* gene family in the tea plant and showed that all *CsMATEs* were the result of a gene duplication event (Figure 4, Table S3). Among them, seven *CsMATEs* originated from proximal duplication, which are nearby but not adjacent to the chromosomal region, twenty *CsMATEs* originated from tandem duplication that exhibit coherent repeats, twenty *CsMATEs* originated from the whole-genome duplication (WGD) which are collinear genes within collinear blocks, and twenty-one *CsMATEs* arose from dispersed duplication belonging to other types excluding segmental, tandem, and proximal duplication. These findings indicate that gene duplication events, in addition to proximal duplication, play a pivotal role in the formation and expansion of the *CsMATE* gene family in *C. sinensis*.

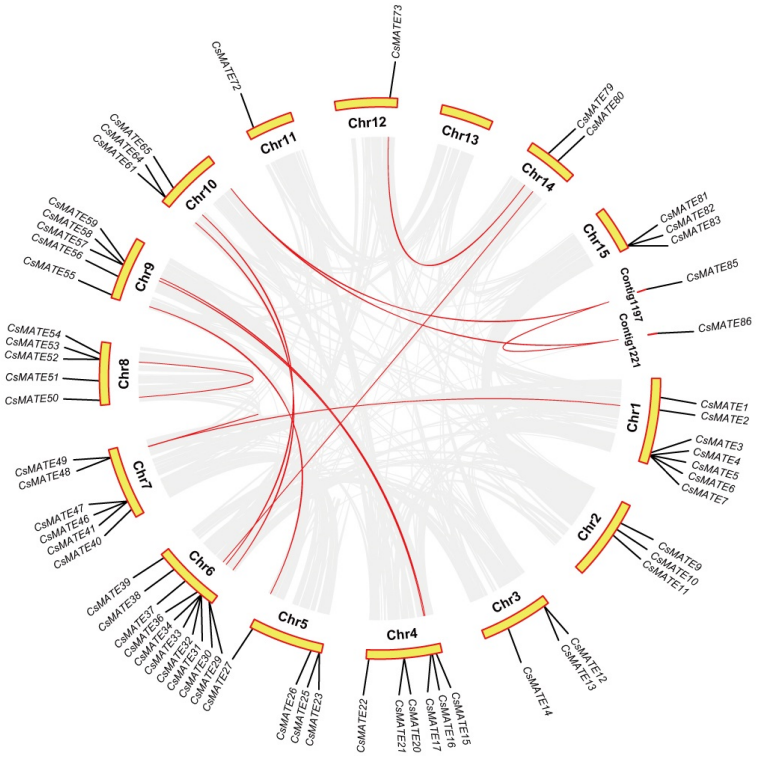

Figure 4. Synteny of *CsMATEs* in *C. sinensis*. Grey and red lines represent synteny blocks and *CsMATE* gene pairs, respectively.

To further understand the evolutionary relationships of the *CsMATE* genes, we conducted an analysis of the collinearity relationships between *Arabidopsis*, kiwifruit (*A. chinensis*), coffee (*C. canephora*), and the tea plant (Figure 5). The result revealed that a large number of orthologs exist between *C. sinensis* and other species. In total, forty-nine *CsMATEs* exhibited collinearity with *MATE* genes in three related species, including twenty with *Arabidopsis*, thirty-seven with coffee, and forty-three with kiwifruit. *MATE* genes in the tea plant and kiwifruit have more collinear gene pairs, indicating that they are more closely related. Interestingly, we observed that 12, 32, and 25 of the *CsMATEs* located in collinearity blocks between the tea plant and *Arabidopsis*, coffee, and kiwifruit, respectively, were single-copy genes. This result suggested that more than half of the *CsMATEs* in the tea plant genome had undergone loss or deletion events during evolution, a slightly higher proportion than that in the genome-wide level [33].

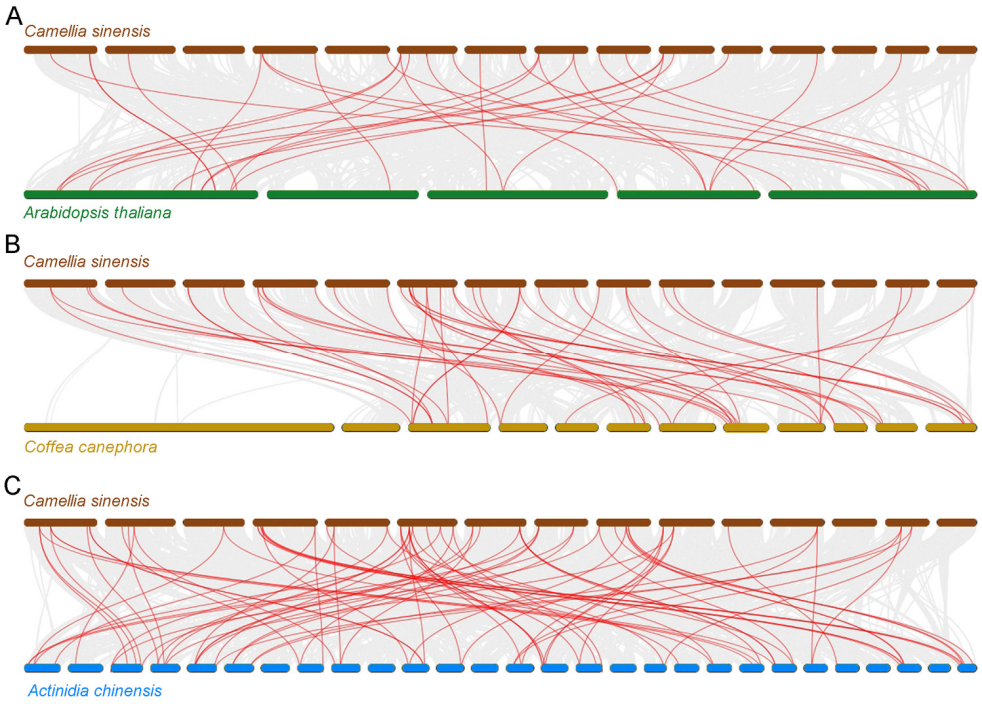

Figure 5. The collinearity of *MATE* genes in *C. sinensis* and three other species. (**A**) *C. sinensis* and *A. thaliana*; (**B**) *C. sinensis* and *C. canephora*; (**C**) *C. sinensis* and *A. chinensis*. Grey lines represent collinear blocks among *C. sinensis*, *A. thaliana*, *C. canephora*, and *A. chinensis*; red lines represent the syntenic *MATE* gene pairs.

3.5. Cis-Acting Element Analysis in the Promoter Regions of CsMATEs

Notably, 8150 *cis*-acting elements were identified in the promoter region of *CsMATEs* (Table S4). Based on previous study, these *cis*-elements mainly included gene transcription, site binding, tissue expression, secondary metabolism, circadian control, cell cycle, and various responsive progresses (Figure 6). Among them, there were 6537 gene transcription elements, accounting for 80.2% of the total number identified, predominantly composed of TATA-box (3931), CAAT-box (2591), and A-box (15). The second most abundant group was light-responsive elements, which accounted for 9.95% of the overall distribution among *CsMATE* promoter regions. Plant hormone-responsive regulatory elements were also highly prevalent, mainly including the MeJA-response element (TGACG-motif, 72; CGTCA-motif, 72), abscisic acid-response element (ABRE, 123), salicylic acid-response element (TCA-element, 24), gibberellin-response element (GARE-motif, 21), and auxin-response element (TGA-element, 23; P-box, 33). Furthermore, the majority of *CsMATEs* exhibit *cis*-regulatory elements associated with various abiotic stresses, such as ARE elements and GC motifs associated with hypoxia stress, LTR elements associated with low temperature stress, and

MBS elements associated with drought stress. The aforementioned results implied that the transcriptional level of *CsMATEs* may be subjected to diverse external stimuli, thereby potentially exerting significant influences on hormone signaling pathways and abiotic stress responses in the tea plant.

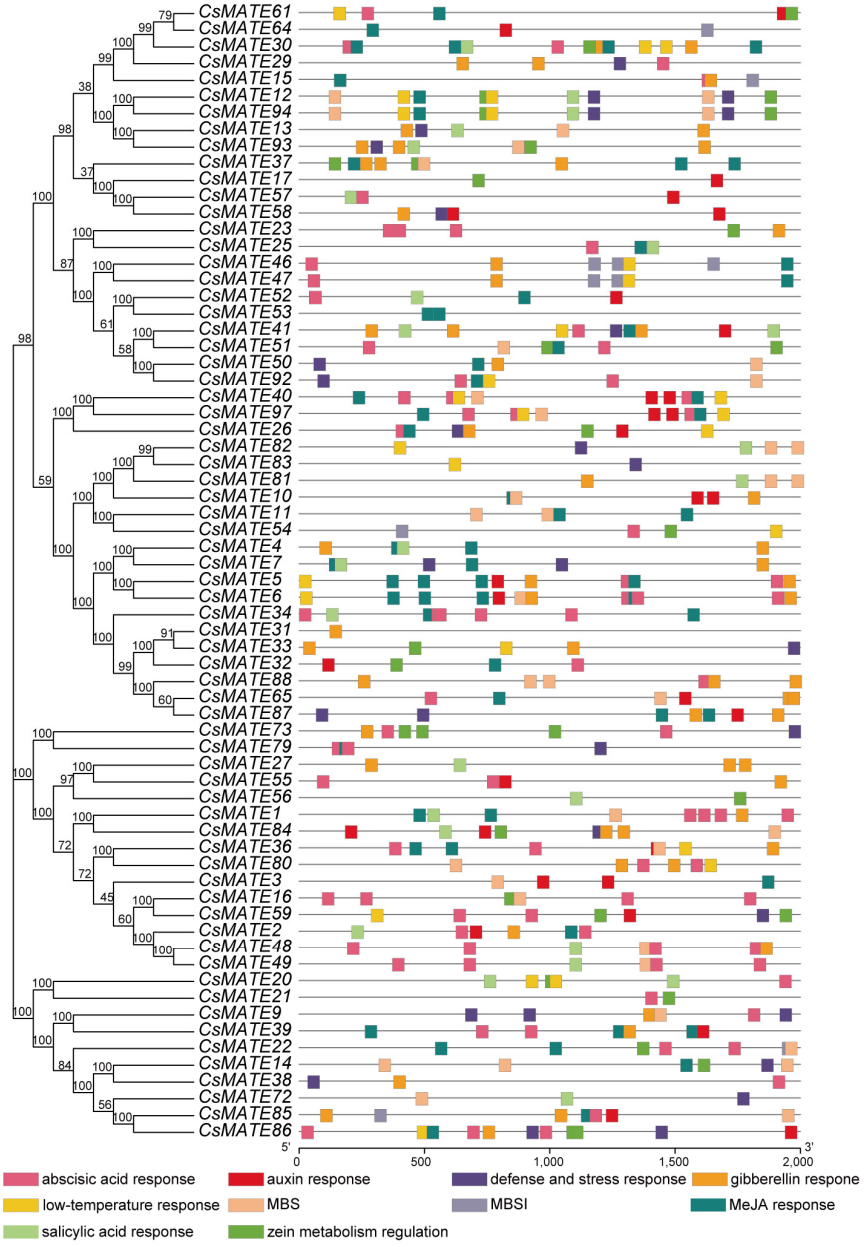

Figure 6. *Cis*-acting elements in *CsMATE* promoters.

3.6. Expression Profile of the CsMATE Genes in Different Tissues

To investigate the potential functions of *CsMATE* genes, tissue expression patterns were analyzed (Figure 7). Out of the 68 *CsMATE* genes examined, 10 showed no expression in any tested tissue or developmental stage. However, the remaining 58 *CsMATE* genes were expressed in at least one tissue. The majority of *CsMATE* genes displayed significant variations in their expression levels among different tissues and developmental stages, and the expression of some *CsMATE* genes demonstrated highly tissue-specific characteristics. For instance, the expression level of *CsMATE2*, *CsMATE5*, *CsMATE9*, *CsMATE14*, *CsMATE26*, *CsMATE29*, *CsMATE30*, *CsMATE41*, *CsMATE51*, and *CsMATE56* were higher in roots than other tissues. On the other hand, *CsMATE27*, *CsMATE37*, and *CsMATE55* were

highly expressed in flowers. Interestingly, the expression level of *CsMATE40*, *CsMATE50*, *CsMATE92*, and *CsMATE97* were higher in leaves, and the expression profiles gradually changed as the leaves matured and aged. Additionally, constitutive expression was observed in 15 *CsMATEs*, with *CsMATE10*, *CsMATE17*, *CsMATE25*, *CsMATE38*, *CsMATE61*, and *CsMATE85* exhibiting high expression levels across all tissues. The constitutive expression patterns of these CsMATEs suggest their potential involvement throughout the entire process of plant growth and development. Collectively, the above results indicate that different *CsMATE* genes may play functions in specific developmental stages and tissues of the tea plant.

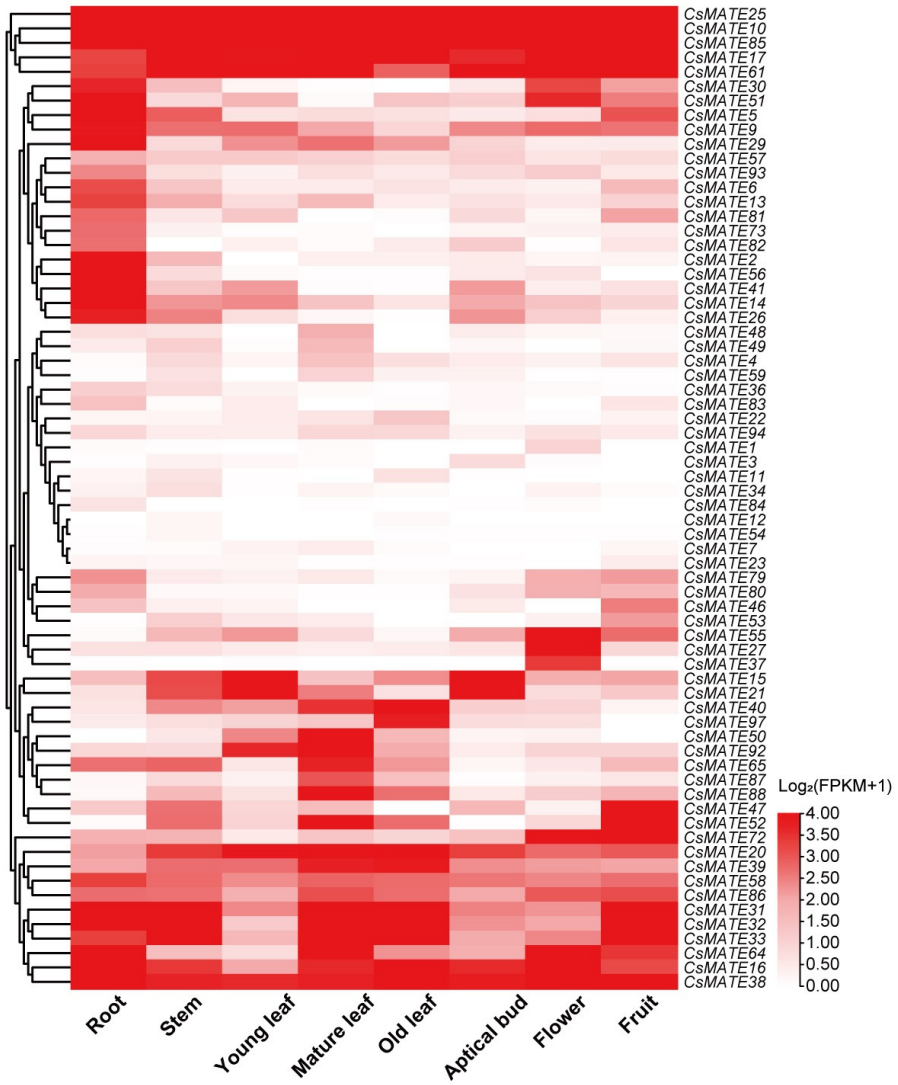

Figure 7. Spatiotemporal expression pattern of *CsMATE* genes. The heatmap of *CsMATEs* expression with $\log_2$ (FPKM + 1).

3.7. Expression Profile of CsMATE Genes Under Various Treatment

As previously mentioned, the promoters of *CsMATE* genes were found to contain numerous *cis*-acting elements related to abiotic stress and hormonal responses. Therefore, the expression profile of *CsMATE* genes following various abiotic stresses and hormone treatments were investigated. As shown in Figure 8, the transcription of some *CsMATE* genes were modulated by abiotic stress treatment. For example, *CsMATE5*, *CsMATE6*, *CsMATE7*, *CsMATE10*, *CsMATE11*, *CsMATE23*, *CsMATE40*, *CsMATE83*, and *CsMATE97* were significantly induced by cold acclimation ($\log_2$Foldchange > 2). Moreover, NaCl treatment resulted in the significant induction of *CsMATE1*, *CsMATE11*, *CsMATE41*, and

CsMATE51, with *CsMATE41* and *CsMATE51* exhibiting an over 20-fold increase in expression levels. Under PEG stress conditions, *CsMATEs* were not significantly induced, except for *CsMATE2*, which exhibited slight induction after 48 h of PEG treatment, and *CsMATE51*, which showed continuous strong induction under PEG treatment. Interestingly, the expression level of *CsMATE* genes also underwent significant changes following MeJA treatment. Among them, six *CsMATE* genes (*CsMATE4*, *CsMATE7*, *CsMATE41*, *CsMATE51*, *CsMATE61*, and *CsMATE64*) were significantly up-regulated by MeJA treatment. Thereinto, it was observed that *CsMATE51* was significantly induced by NaCl, PEG, and MeJA treatments, suggesting its potential involvement in different stress responses. Overall, these findings suggest that different *MATE* gene members in the tea plant possess diverse functions under numerous treatments.

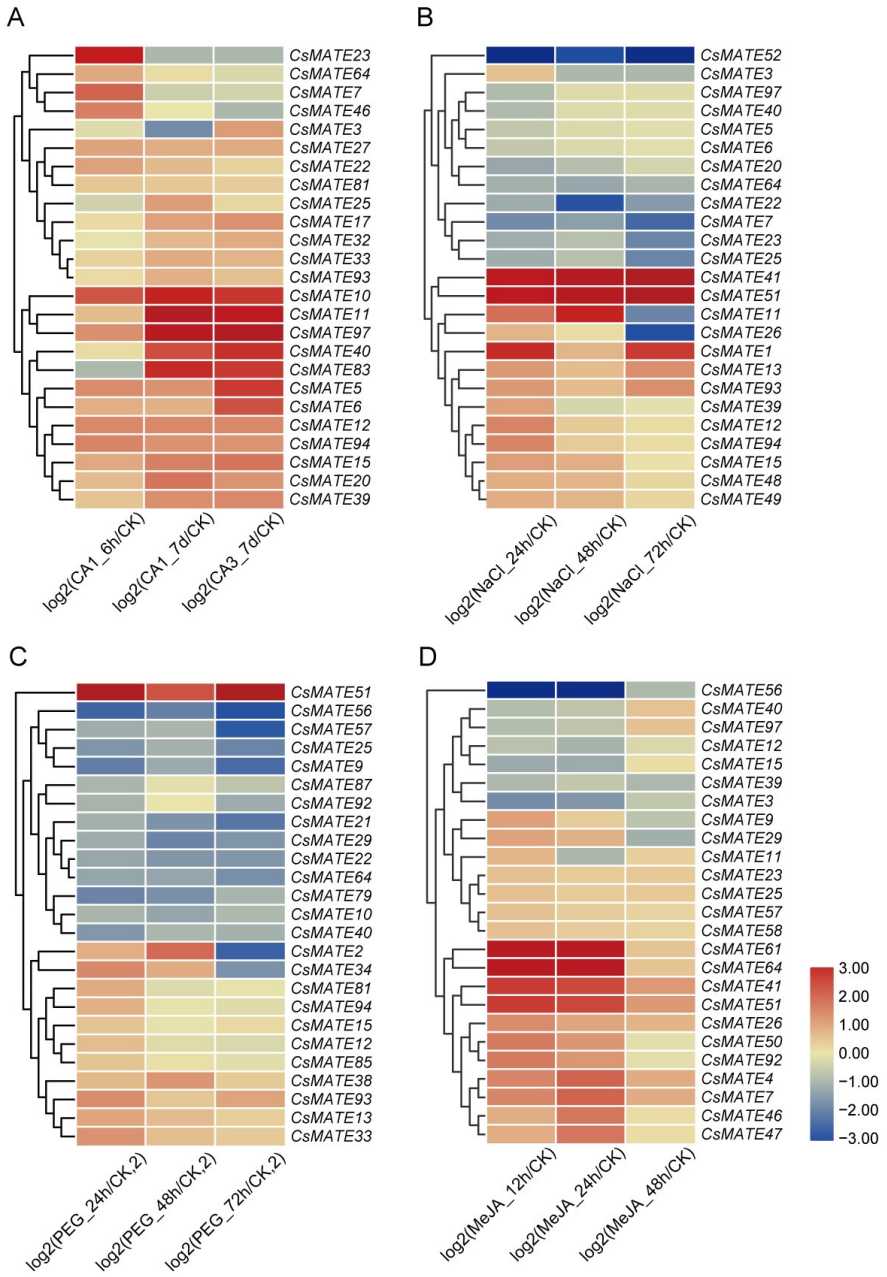

Figure 8. Expression analysis of *CsMATE* genes under diverse abiotic stress and hormone treatment (**A**–**D**).

4. Discussion

The tea plant (*Camellia sinensis*), the origin of the second most widely consumed beverage globally after water, is highly esteemed by consumers for its distinctive bioactivity and health benefits [48]. The secondary metabolites, particularly polyphenols, are pivotal in determining both the flavor and health-promoting properties of tea [49]. Among these compounds, the *MATE* gene family is prevalent across various plant species and plays a critical role in transporting diverse secondary metabolites, as well as enhancing plant resistance to stressors [17,29,50–52]. Consequently, conducting a comprehensive whole-genome identification and expression analysis of the tea *MATE* gene family holds significant scientific importance and practical implications.

While there have been some reports of *MATE* gene family studies across different plant taxa, a thorough investigation specifically focusing on tea plant *MATE* genes remains rarely reported [18,53]. In the current study, 68 *CsMATE* genes were identified by an exhaustive analysis of the tea plant genome. These genes exhibit uneven distribution throughout the genome across various chromosomes. For instance, there are ten *CsMATE* genes located on chromosome 6, while only one *CsMATE* gene resides on chromosomes 11 and 12—mirroring patterns observed in other plant species [54,55]. Phylogenetic analyses indicate that members of the *CsMATE* gene family likely originated from a common ancestral gene followed by lineage-specific divergence during evolutionary processes. The identified *CsMATE* gene members can be categorized into four distinct subgroups, consistent with findings reported for *MATE* genes in other organisms, such as *Arabidopsis* and apple [46]. Moreover, a gene structure analysis reveals the variability among these *CsMATE* genes concerning the intron number and positioning—ranging from 0 to 16—which may correlate with the functional diversity and regulatory mechanisms governing their expression. By examining conserved motifs within *CsMATE* gene members, we discovered that those within identical subgroups typically share similar motif compositions; notably, Class IV members predominantly contain motifs 7 and 8, which could account for the functional diversification among *CsMATE* gene members.

The tea plant is believed to have evolved from its ancestor, Ericales, through a whole-genome triplication (WGT) and WGD event, which may have resulted in an increased frequency of genomic rearrangements [56]. In comparison to the number of *CsMATE* gene families in the tea plant, potato, rice, mung bean, and *Daucus carota* exhibit 48, 46, 48, and 45 *MATE* gene family members, respectively [18,53,57,58]. This suggests that genome duplication during the evolution of the tea plant has significantly contributed to the diversity observed within the *CsMATE* gene family. Gene duplication primarily occurs through various mechanisms including WGD/segmental duplications, tandem duplications, proximal duplications, and dispersed duplications [59]. Among these mechanisms, WGD/segmental duplications (29.4%), tandem duplications (29.4%), and dispersed duplications (30.9%) are identified as the principal contributors to the formation and expansion of the *CsMATE* gene family; this aligns with previous research findings. Only about 10% of *CsMATE* genes arise from proximal duplication events. However, due to the limited studies on proximal duplication in plants thus far, its underlying causes remain unclear. Further evolutionary relationship analyses reveal that *MATE* gene family members from the tea plant share certain collinearity relationships with those from *C. sinensis*, coffee, and *A. chinensis*—wherein *A. chinensis* exhibits the highest degree at 63.2%, indicating a closer evolutionary relationship between the tea plant and *A. chinensis*.

The analysis of expression patterns has revealed the tissue-specificity of the *CsMATE* gene members in the tea plant. Studies have demonstrated that most *CsMATE* genes are expressed in various tissues, including the root, stem, and leaf, albeit at different levels. For example, *CsMATE10/26/31/32* exhibit high expression in the root, suggesting their potential involvement in substance absorption or expulsion processes. Research has shown that *AtDTX18*, the homologous gene of *CsMATE26*, exhibits increased expression in roots and is capable of coumaroylagmatine transport and extracellular accumulation [24].

Conversely, *CsMATE85*/38/33 show higher expression levels in the stem, indicating their possible role in transporting substances from underground to aboveground parts. The differential expression of these *CsMATE* gene members may be associated with distinct requirements for secondary metabolite synthesis and transport among different tissues. Furthermore, under stress conditions, such as cold temperatures, droughts, or high salt environments, significant changes are observed in *CsMATE* genes expression levels, which suggests an important role for the response to abiotic stress. For instance, AtDTX50, a homologous gene of CsMATE2, acts as an ABA effector transporter. *dtx50* mutants exhibit enhanced drought tolerance compared to wild-type plants [60]. Similarly, cotton *GhDTX27* and *CsMATE17* are homologous genes, and the heterologous overexpression of *GhDTX27* enhances plant resistance against droughts, salt, and cold stresses in *Arabidopsis* [25]. This observation aligns with the analysis results on *cis*-elements present within *CsMATE* genes. Many members of the *CsMATE* gene family contain stress-related *cis*-acting elements which further supports their crucial role.

Additionally, we have observed that *CsMATE51* exhibits responsiveness to various abiotic stresses, including NaCl, PEG, and MeJA. This phenomenon may be attributed to the presence of specific *cis*-acting elements within its structure. The identification of the ABA response element in the promoter region of *CsMATE51* implies potential regulation by genes involved in the ABA signaling pathway, consequently influencing tea plant's resistance to abiotic stresses such as drought stress. A previous study showed that the stresses can be alleviated by the application of MeJA, which is a hormone involved in plant signaling. MeJA can induce the synthesis of defensive compounds and initiate the expression of related genes, which can be used to combat the toxicity of salt stress, drought stress, low temperature stress, heavy metal stress, and other elements. Therefore, we speculated that *CsMATE51* may also participate in abiotic stress regulation in this way. The genomic annotation of the tea plant has revealed that *CsMATE51* encodes the Transparent testa 12 (TT12) protein. However, there is a lack of research on the involvement of *TT12* in plant abiotic stress, which primarily pertains to anthocyanin-related processes. Based on this, we are more interested in the subsequent study on whether *CsMATE51* participates in abiotic stress.

In addition to the relationship between abiotic stress and the *MATE* gene family, many studies have also studied the accumulation and transport of flavonoids by the *MATE* gene family. The analysis of the *MATE* gene family in upland cotton revealed that GhMATE12, GhMATE16, and GhMATE38 potentially participate in proanthocyanidins transport [35]. The investigation on *Daucus carota* demonstrated that *DcMATE21* may be involved in the process of anthocyanin accumulation [57]. Chen et al. only identified 41 members of the *MATE* gene family from the tea plant, which could be attributed to an incomplete genome assembly and varying screening standards [31]. They discovered that *TEA006173* plays a crucial role in flavonoid accumulation. Based on the aforementioned studies, it is speculated that the *MATE* gene family not only participates in the abiotic stress response, but also contributes to flavonoid transport and accumulation processes, thereby further elucidating the functional diversity of the *MATE* gene family.

In this study, certain advancements have been achieved in the whole-genome identification and expression analysis of the *CsMATE* gene family in the tea plant, but numerous issues require further exploration. Firstly, it is necessary to clarify the regulatory mechanism of *CsMATE* gene expressions in different tissues and development stages of the tea plant. Secondly, it is necessary to further explore the specific functions of *CsMATE* genes, especially the mechanism in plant stress resistance. Finally, through technological approaches such as gene editing, the expression levels of *CsMATE* genes can be modified to improve the stress tolerance of the tea plant.

5. Conclusions

In summary, 68 putative *CsMATEs* were identified from the tea plant genome. Subsequently, detailed descriptions of CsMATEs regarding fundamental characteristics, phylo-

genetic analysis, and expression profiles specific to different tissues and developmental stages were provided. Furthermore, we conducted an analysis on the expression patterns of *CsMATE* genes under various abiotic stress conditions and hormone treatments, leading us to identify several potential key genes involved in responses to different stress conditions. The results of this study lay an important theoretical basis for clarifying the biological functions of *CsMATEs*, especially in abiotic stress-related functions in the tea plant.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agronomy14112718/s1, Figure S1: Amino acid sequence logos of conserved domains in CsMATEs. The bit score exhibits the information content for each position in the sequence; Table S1: Manual filtering of 98 putative CsMATE proteins in tea plant; Table S2: Details of the 68 *CsMATE* genes in tea plant; Table S3: Duplication event of the 68 *CsMATEs*; Table S4: *Cis*-acting regulatory elements identified in the promoter regions of *CsMATE* genes; Table S5: The RNA-seq data (FPKM) of *CsMATEs* in different tissues and developmental stages; Table S6: The RNA-seq data (FPKM) of *CsMATEs* in different abiotic and hormone treatments.

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