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Genome-Wide Identification and Expression Analysis of the PME and PMEI Gene Families in *Diospyros kaki*: A Bioinformatics Study

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Abstract: Pectins are major components of cell walls in plants. Pectin methylesterases (PMEs) and pectin methylesterase inhibitors (PMEIs) play crucial roles in pectin synthesis and metabolism. Overall, 28 putative DkPMEs and 29 putative DkPMEIs were identified from the *D. kaki* genome. According to phylogenetic analysis, DkPME/DkPMEI proteins can be classified into four and five clades, respectively. Motif and gene structure analysis showed that DkPME/DkPMEI are highly conserved in the same clades, which indicates that the function of these DkPME/DkPMEI were similar. Besides, *DkPME/DkPMEI* genes were distributed unevenly on their corresponding chromosomes. Synteny analysis showed that *PME* or *PMEI* gene usually matched with more than one *DkPME/DkPMEI* in *D. oleifera*, *D. lotus*, and *A. thaliana*, implying that the function of these genes in *D. kaki* may be diverse. Expression analysis showed that *DkPME/DkPMEI* from the same clade exhibited diverse expression patterns, indicating that these genes might have diverse functions. Functional protein–protein interaction network analysis showed that DkPMEI21 and DkPMEI15 were core nodes and were, respectively, positive and negative regulators for carbohydrate metabolism, stress responses, and sugar signaling. This study provides a theoretical basis for the functional characteristics, evolutionary relationship, and role of these gene families in developing persimmon fruit.

Keywords: *Diospyros kaki*; PME; PMEI; fruit

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1. Introduction

Plant cell wall, synthesized from cellulose, hemicellulose, pectin, and several glycosylated proteins, plays multiple roles in physiological and developmental processes [1]. Pectin is a significant component of cell walls and mainly contains homogalacturonan (HG), xylogalacturonan (XGA), rhamnogalacturonan-I (RG-I), and rhamnogalacturonan-II (RG-II) [2,3]. The richest form of pectic synthesized in the Golgi apparatus is a highly methyl-esterified state. It is delivered to cell walls and de-methylesterified by pectin methylesterase (PME) [4,5]. Pectin methylesterase inhibitor (PMEI) inhibits PME activity by forming a non-covalent at a 1:1 ratio in plants [6]. PME and PMEI proteins play crucial roles in pectin synthesis and metabolism [7,8].

PME proteins are present widely in higher plants and some microorganisms [8]. For the protein structures, Type-I PME and Type-II PME proteins share the PME domains, and Type-I PME proteins also contains the PRO-region, an additional N-terminal region similar to the PMEI domain [9]. Recently, *PME* genes have been discovered in various plant species, such as 66 PME genes, which were identified from *Arabidopsis thaliana* [10], 53 from *Citrus sinensis* [11], 61 from *Glycine max* [12], 54 from *Fragaria vesca* [12], and 56 from *Solanum tuberosum* [13]. The *PME* genes are tightly linked to various functions of

plants, such as fruit maturity and softening [14,15], seed germination and development [16], pollen development [17,18], stem elongation [19], root hair formation [19], and stress response [20,21].

PMEI proteins belong to a large multigene family, containing a conserved PMEI domain (PF04043) [22,23]. The first PMEI protein was discovered in kiwi fruit and later identified in multiple plants [7]. Until now, 78 *PMEI* genes have been identified from *Arabidopsis thaliana* [24], 42 from *Pyrus bretschneideri* [25], 49 from *Oryza sativa* [26], 51 from *Camellia sinensis* [27], 55 from *Sorghum bicolor* [28], 83 from *Linum usitatissimum* [29], 95 from *Brassica oleracea* [30], and 100 from *Brassica campestris* [31]. *PMEI* family genes play multiple roles in plant growth, development, and stress response [32]. For example, *AtPMEI4* promotes root growth of *A. thaliana* [33]. *AtPMEI5* overexpression affected HG methyl esterification by repressing the PME activity and accelerating seed germination [34]. In rice, *OsPMEI28* overexpression suppressed the PME activity, impacting the growth process, resulting in dwarfed phenotypes [35]. Overexpression of *Camellia sinensis PMEI2/4* in *Arabidopsis* decreased PME activity but increased sugar content, promoting early flowering phenotypes [27].

Persimmon *(Diospyros kaki)* belongs to the family Ebenaceae and is widely distributed in the subtropics and tropics of East Asia [36]. An abundant tannin content characterizes it, and the high soluble tannin content leads to the astringency of persimmon fruit [37]. Previous studies have shown that the interaction of pectin and tannin might be essential in reducing astringency [38,39]. Methanol, which is produced by PMEs catalyzing the pectin de-methyl esterification, might be associated with the loss of astringency [40]. Based on the critical role of *PME* and *PMEI* genes in regulating pectin de-methylesterifcation [7], these genes might play essential roles in persimmon de-astringency. However, studies of the *PME* and *PMEI* gene families in persimmon are still limited.

The availability of the persimmon genome has facilitated the identification of *PME* and *PMEI* gene families. The present study reported a comprehensive analysis to examine their conserved protein motif, gene structure, phylogenetic and evolutionary relationship, chromosome location, collinearity and promoter, protein interaction, and in silico expression analysis of *PME* and *PMEI* genes during fruit development. This study provides a theoretical basis for the functional characteristics, evolutionary relationship, and the role of *DkPME* and *DkPMEI* genes in developing persimmon fruit.

2. Materials and Methods

2.1. Identification of PME and PMEI Genes

The most updated Hidden Markov Model (HMM) of the PME domain (PF01095) and PMEI domain (PF04043) were downloaded from the Pfam to identify *PME* and *PMEI* genes in the persimmon genome (unpublished) using HMMER 3.3 [41]. The presence and completeness of the PME and PMEI domains were evaluated using SMART tools with an E-value < 0.1 [42]. The gene length, molecular weights, and isoelectric point of putative *PME* and *PMEI* genes were predicted using the online ExPASy program (https://www.expasy.org/, accessed on 6 April 2022) [43].

2.2. Phylogenetic Analysis and Interaction Network Construction

PME and PMEI proteins sequences from persimmon and arabidopsis were used to investigate the phylogenetic relationships; among them, AtPME and AtPMEI proteins were downloaded from Tair (https://www.arabidopsis.org/index.jsp, accessed on 10 April 2022). Using Clustal X2 at the default parameters, multiple sequence alignments were aligned, and neighbor-joining (NJ) phylogenetic trees were constructed with 1000 bootstrap replicates using MEGA5.0 [44,45]. DkPMEs and DkPMEIs were classified according to their phylogenetic relationship with corresponding *A. thaliana PME* and *PMEI* genes. We analyzed the interaction network of DkPME and DkPMEI proteins using a model plant *A. thaliana* on the STRING protein interaction database (http://string--db.org/, accessed on 13 April 2022) [46].

2.3. Motif Prediction and Gene Structure Analysis of PME and PMEI Genes

The online MEME tool (http://meme.ebi.edu.au/, accessed on 15 April 2022) was used to identify unknown conserved motifs shared among persimmon PME and PMEI proteins [47]. The parameters were used as follows: a maximum number of motifs: 15 (DkPME) and 10 (DkPMEI); 6 $\leq$ optimum motif width $\leq$ 50. Gene Structure Display Server online software (http://gsds.cbi.pku.edu.cn/index.php, accessed on 20 April 2022) was used to display the structures of *PME* and *PMEI* genes. The motif and gene structure with the phylogenetic tree was shown using the TBtools [48].

2.4. Chromosomal Distribution, Gene Duplication, and Kaks Calculation Analysis

The positions of all *PME* and *PMEI* genes from the persimmon genome were extracted from the GFF3 file using TBtools [48]. The collinear relationship of *PME* and *PMEI* genes of persimmon and arabidopsis and gene duplication events of *PME* and *PMEI* genes were identified using the Multiple Collinearity Scan Toolkit (MCScanX) with default parameters [49]. The chromosomal locations and duplication events were visualized using TBtools [48]. The number of non-synonymous (Ka) and synonymous (Ks) substitutions of paralogous *DkPME* and *DkPMEI* gene pairs were computed using TBtools [48], and the divergence time of the duplication events was calculated using the formula T = Ks/(2 $\times$ 1.5 $\times$ 10^{-8}) $\times$ 10^{-6} million years ago (MYA).

2.5. Promoter Cis-Regulatory Elements Analysis

The upstream 2000 bp sequences of the *DkPME* and *DkPMEI* promoter regions were cut and extracted using TBtools [48]. PlantCARE (https://bioinformatics.psb.ugent.be/webtools/plantcare/html/, accessed on 22 April 2022) searched for plant growth and development, hormone-responsive, and abiotic stress cis-acting elements in the promoter sequences [50].

2.6. In Silico Expression Analysis of PME and PMEI Genes

RNA-seq data were available in the NCBI SRA under the Bioproject ID PRJNA771936. Five different stages (T1 = 70, T2 = 100, T3 = 120, T4 = 140, T5 = 160 days after flower (DAF)) of persimmon fruit were sampled, and three independent biological replicates were used. The expression levels of *DkPME* and *DkPMEI* genes were calculated as fragments per kilobase million (FPKM). The heat maps were created using the Heatmap tool in Hiplot (https://hiplot.com.cn, accessed on 24 April 2022) based on the transformed data of log2 (FPKM + 1) values [51].

3. Results

3.1. Identification and Phylogenetic Analysis of PME and PMEI Genes in D. kaki

After removing the redundant sequences, 28 putative *DkPME* genes and 29 putative *DkPMEI* genes were identified from the *D. kaki* genome. These genes were named *DkPME1* to *DkPME28* and *DkPMEI1* to *DkPMEI29* based on their physical chromosome location. Overall, 14 DkPME contained both PMEI and PME domain (Type-II PME) among these genes. As shown in Table S1, the length of DkPME proteins varied from 253 aa (DkPME9) to 1164 aa (DkPME21), the molecular weights (MW) ranged from 28,529.85 (DkPME9) to 126,409.97 (DkPME21) Da, and the theoretical isoelectric points (pIs) were changed from 5.75 (DkPME22) to 9.38 (DkPME19). The MW of DkPMEI was extended from 6762.71 (DkPMEI17) to 103,158.13 (DkPMEI29) Da, and the encoded amino acids were ranged from 66 (DkPMEI17) to 918 (DkPMEI29) aa, and the pI varied from 4.25 (DkPMEI17) to 11.07 (DkPMEI14) (Table S1).

NJ phylogenetic trees were constructed to verify the evolutionary relationship using the protein sequences of 66 published AtPMEs, 78 AtPMEIs, and putative DkPMEs and DkPMEIs (Figure 1). According to sequence similarity, the PMEs and PMEIs families in *D. kaki* were divided into 4 and 5 clades, respectively. The Type-II DkPME PMEs were clustered into Clade II, III, and IV, while Clade I only contained Type-I DkPMEs. The largest

subfamily of PME was Clade IV which had 14 DkPME proteins, while Clade I, II, and III held 12, 1, and 1 DkPME genes, respectively (Figure 1a). As for the DkPMEI family, CladeI contained 21 proteins, while Group III and IV only contained 1 PMEI protein (Figure 1b).

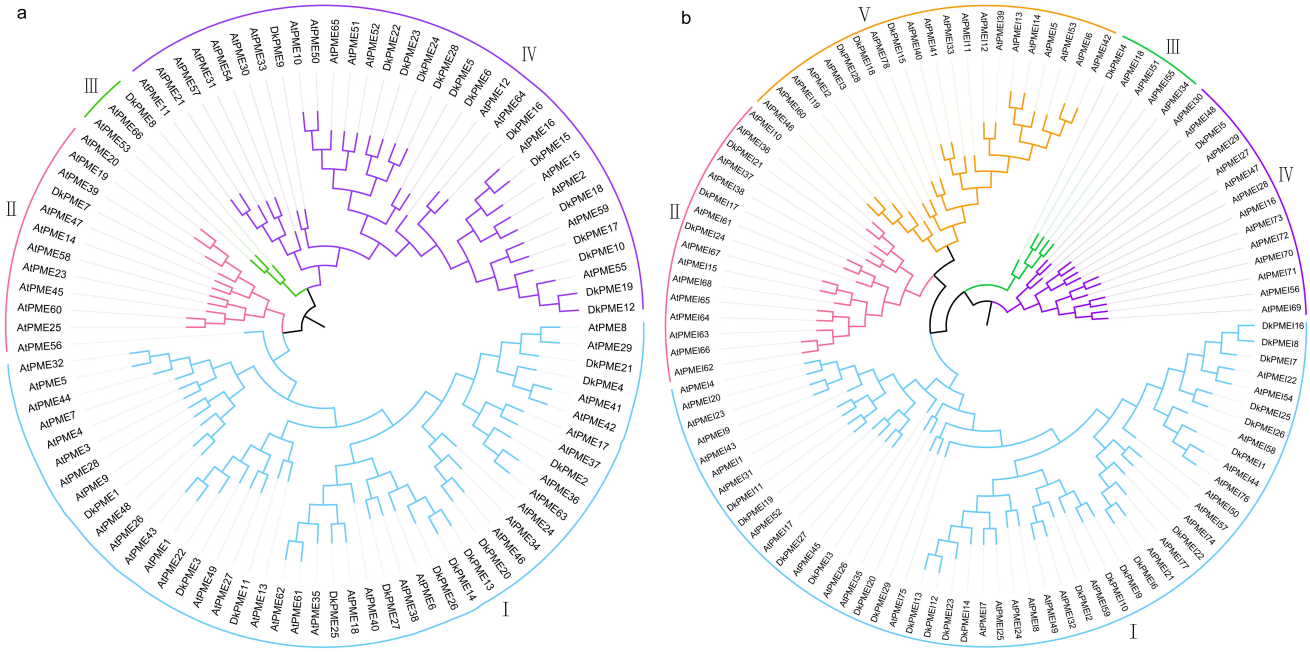

Figure 1. Phylogeny of the PME/PMEIs in *D. kaki*. (**a**) NJ phylogenetic tree of the DkPMEs; (**b**) NJ phylogenetic tree of the DkPMEIs.

3.2. Conserved Motifs and Structural Analysis

To analyze the structural characteristics of the DkPME and DkPMEI proteins, a total of 15 and 10 conserved motifs of DkPME and DkPMEI proteins were predicted using MEME online software. Of the DkPMEs, motifs 3, 4, 6, 7, and 9 were annotated as PME domains; these motifs were exhibited in 26, 27, 26, 26, and 27 DkPME proteins, respectively, indicating that these PME-related motifs were essential and highly conserved. Motif 13 contained the PMEI domain in DkPME proteins and was presented in 12 Clade I DkPME proteins (Figure 2a). For the DkPMEIs, motifs 1, 2, and 3 were annotated as PMEI domain and were presented in 20, 17, and 24 PMEI proteins, respectively, revealing that PMEI-related motifs were highly conserved in DkPMEI proteins (Figure 3a).

The DkPME and DkPMEI proteins in the same clade possessed the same conserved motifs, indicating functional similarity in paralogous gene pairs or genes in the same clades. For example, motif 2 of DkPME was prominent in Clade IV, and motifs 14 and 15 were only observed in Clade I (Figure 2a). Motifs 2 and 5 were unique to most of the DkPMEIs in Clade I, while motif 9 was mainly present in Clade V (Figure 3a). The conservation of conserved motifs of DkPME and DkPMEI members in the same clade supported the results of the phylogenetic analysis.

The exon–intron distribution of *DkPME* and *DkPMEI* genes was created based on the coding sequence. The *DkPME* gene structure is relatively conserved, with most having about 3–4 introns, while two genes of Clade IV have only 6 and 9 introns. The exon numbers of Clade IV genes (Type-II *PME*) were somewhat more extensive than that of Clade I members (Type-I *PME*), but the intron sizes of Clade IV genes were relatively smaller. Furthermore, two Clade I members had noncoding regions (3′UTR and 5′UTR) (Figure 2a). The number of exons in *DkPMEI* genes varied from 1 to 10. The introns were detected from only 7 out of 29 DkPMEI genes. Among them, 4 *DkPMEI* genes belonged to Clade I. Interestingly, most *DkPMEI* genes lacked introns and noncoding regions (Figure 3a).

The gene structures analysis of *DkPME* and *DkPMEI* genes also supported the reliability of the phylogenetic classification.

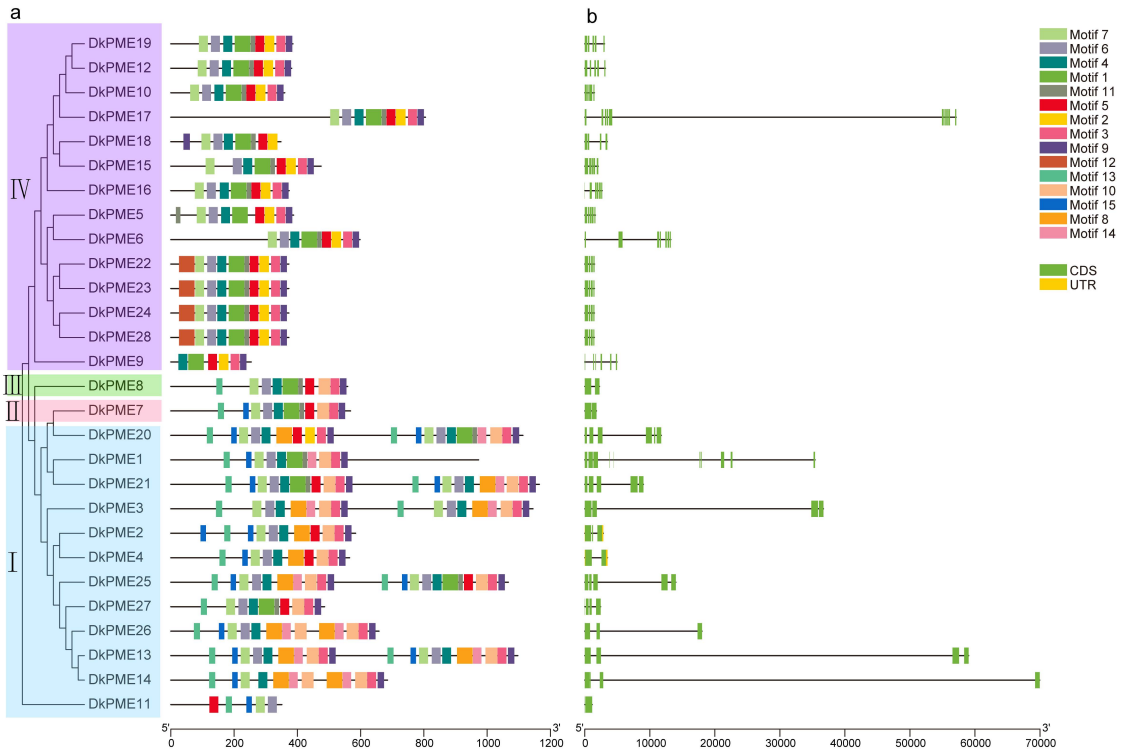

Figure 2. Conserved motif and gene structure analyses of *PME* genes in *D. kaki*. (**a**) Conserved motif distribution. (**b**) Exon–intron structure of *DkPME* genes.

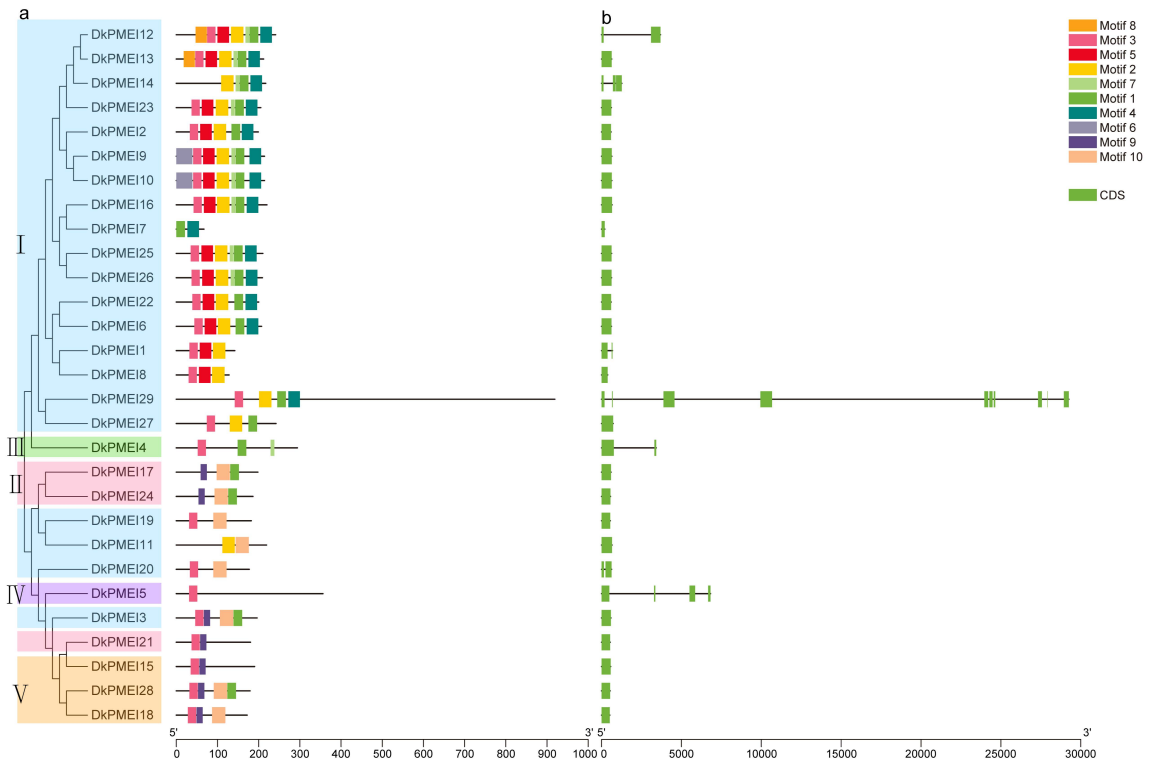

Figure 3. Conserved motif and gene structure analyses of *PMEI* genes in *D. kaki*. (**a**) Conserved motif distribution. (**b**) Exon–intron structure of *DkPMEI* genes.

3.3. Chromosomal Locations, Gene Duplication, and Ka/Ks of PME and PMEI Genes

Chromosomal localization analysis showed that *DkPME* and *DkPMEI* genes were distributed unevenly on their corresponding chromosomes (Table S1, Figures 4 and 5). *DkPME* and *DkPMEI* genes were identified in 13 and 9 of 15 *D. kaki* chromosomes, respectively (Table S1, Figures 4 and 5). For the *DkPMEs*, there were three gene clusters (*DkPME3-DkPME4*, *DkPME5-DkPME6-DkPME7*, *DkPME22-DkPME23-DkPME24*), respectively, located on chromosomes 1, 1, 13 (Figure 4). Of the *DkPMEIs*, there were four gene clusters (*DkPMEI6-DkPMEI7-DkPMEI8*, *DkPMEI9-DkPMEI10*, *DkPMEI13-DkPMEI14*, and *DkPMEI25-DkPMEI26*) located on chromosomes 3, 5, 6 and 14, respectively (Figure 4).

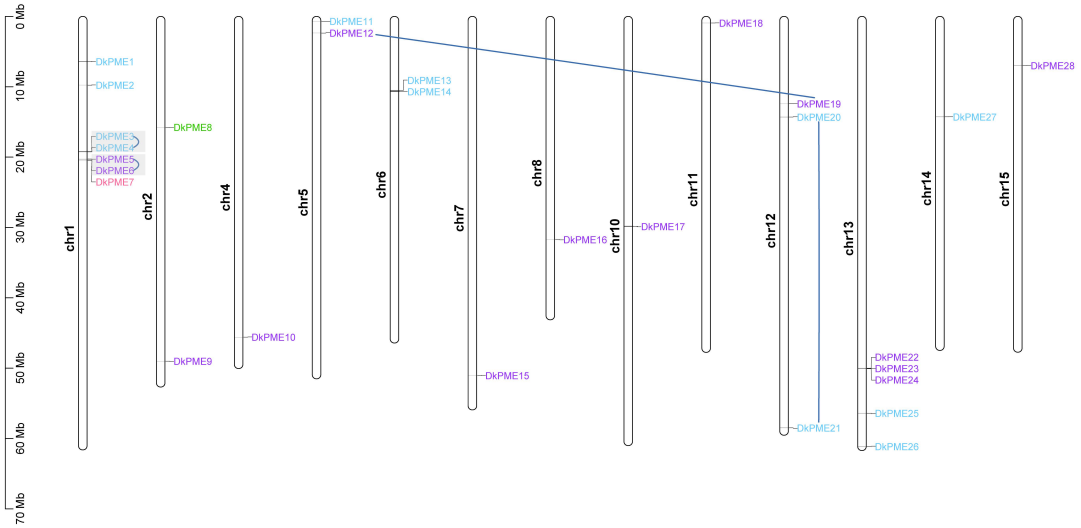

Figure 4. Chromosome location of *DkPME* genes in *D. kaki*. Genes of different groups are expressed in different colors. Darkblue lines showed fragment duplication events, and gray boxes presented tandem duplication events.

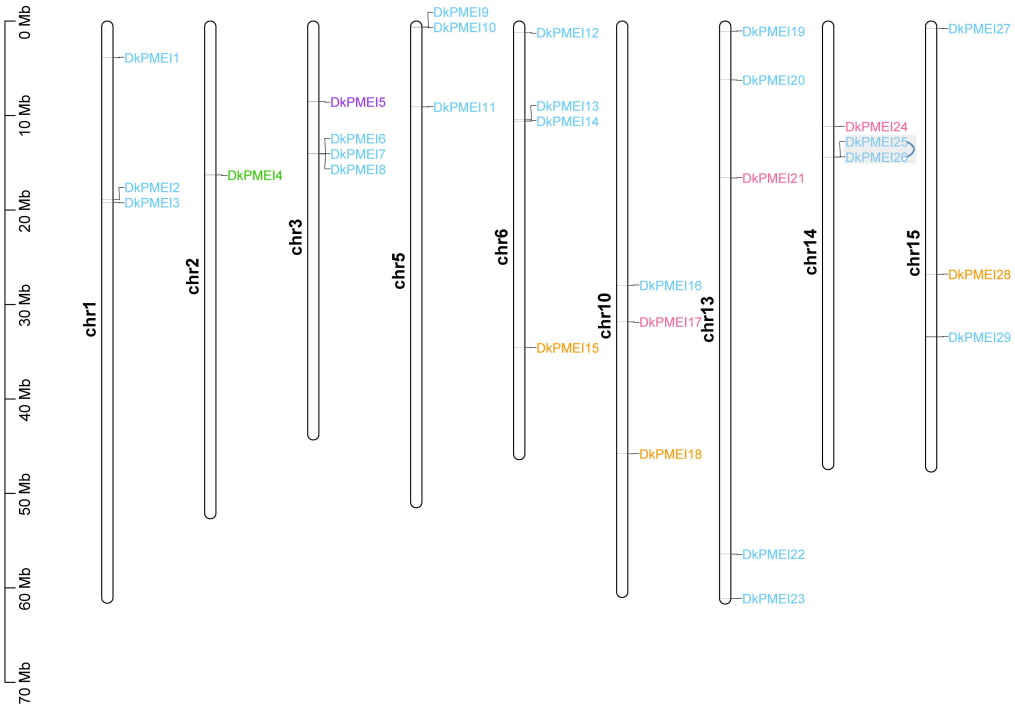

Figure 5. Chromosome location of *DkPMEI* genes in *D. kaki*. Genes of different groups are expressed in different colors. Gray boxes presented tandem duplication events.

The duplication analysis showed that 2 *DkPME* gene pairs were identified as segment duplication and 2 tandem duplication events. Two *DkPME* gene clusters were formed by tandem duplication located on chromosome 1. However, only one *DkPMEI* gene pair originated in tandem duplication and is located on chromosome 14. The Ka/Ks ratios of all tandem duplicated and segment duplicated *DkPME/DkPMEI* gene pairs were <1, indicating that these *DkPME/DkPMEI* genes evolved under purifying selection to reduce deleterious mutations after replication. The differentiation time of *DkPME* genes was primarily between 26.599 and 49.717 million years ago (MYA), and the differentiation time of the *DkPMEI* gene pair was 39.777 MYA (Table S2).

3.4. Synteny Analysis of PME and PMEI Genes

We analyzed the syntenic relationship between the *PME* and *PMEI* genes in *D. kaki*, its closely related species *Diospyros oleifera* and *Diospyros lotus*, and model plants *A. thaliana* (Figure 6). The results showed that the *PME* genes in *D. kaki* had the most homologous gene pairs with *PME* genes in *D. oleifera* (30), followed by *D. lotus* (22) and *A. thaliana* (21) (Figure 6a–c). However, the *PMEI* genes in *D. kaki* had more homologous gene pairs with *PMEI* genes in *D. lotus* (35) and *D. oleifera* (33), followed by *A. thaliana* (21) (Figure 6d–f). Notably, one *PME* or *PMEI* gene usually matched with more than one *PME* or *PMEI* in *D. oleifera*, *D. lotus*, and *A. thaliana*, implying that the function of certain *DkPME* or *DkPMEI* genes in *D. kaki* may be diverse.

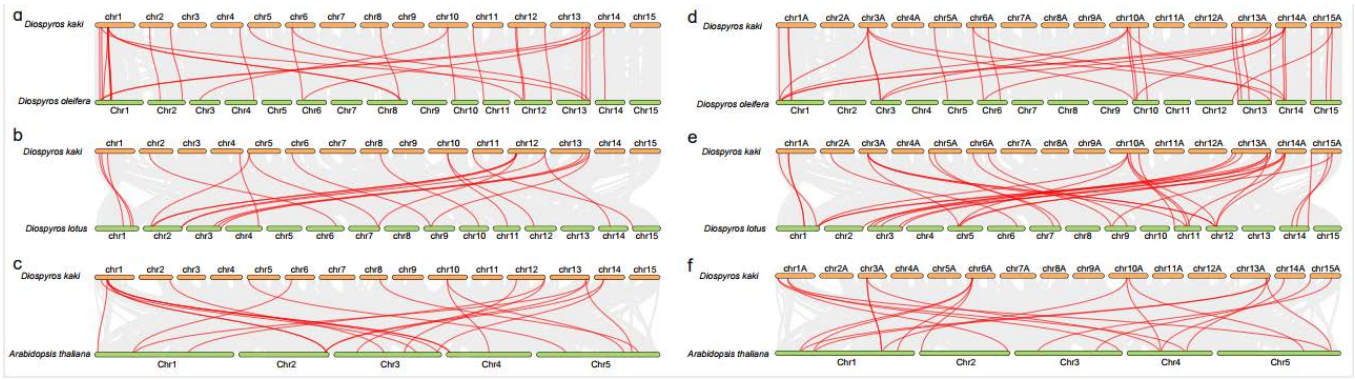

Figure 6. Synteny analysis of *PME* and *PMEI* genes between *D. kaki*, *D. oleifera*, and *D. lotus*, and *A. thaliana*. (**a**) *PME* genes between *D. kaki* and *D. oleifera*. (**b**) *PME* genes between *D. kaki* and *D. lotus*. (**c**) *PME* genes between *D. kaki* and *A. thaliana*. (**d**) *PMEI* genes between *D. kaki* and *D. oleifera*. (**e**) *PMEI* genes between *D. kaki* and *D. lotus*. (**f**) *PMEI* genes between *D. kaki* and *A. thaliana*.

3.5. Cis-Element Analysis of PME and PMEI Genes

To investigate the gene characteristics and function of *DkPME* and *DkPMEI* genes, cis-regulatory elements in the promoters were predicted using PlantCARE. Briefly, multiple light-related elements were identified, namely Box 4, G-box, GT1-motif, TCT-motif, GATA-motif, and so on. Other cis-elements could be categorized into plant growth and development, hormone-responsive, and abiotic stress. Eight cis-elements were involved in plant growth and development, including circadian, MSA-box, CAT-box, RY-element, etc. Ten cis-elements are related to hormone-responsive, i.e., ABRE, TATC-box, CGTCA-motif, AuxRR-core, and P-box. Six cis-elements participated in abiotic stress (e.g., LTR, ARE, and MBS). In addition, the MBSI cis-element was involved in flavonoid biosynthetic genes regulation and was identified in the promoter of *DkPMEI12*, *DkPMEI17*, *DkPMEI22*, and *DkPMEI29* (Figure 7 and Table S3).

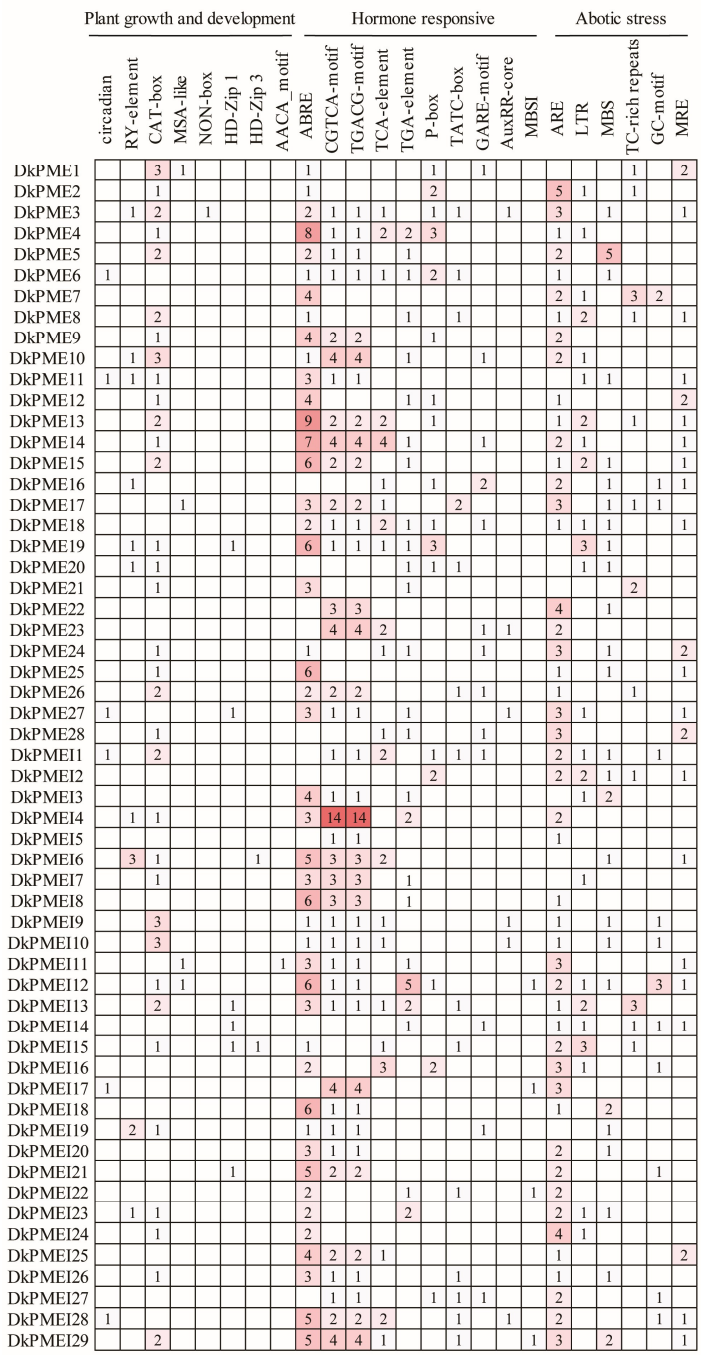

Figure 7. Cis-acting element in the promoter region of *DkPME* and *DkPMEI* genes.

3.6. Expression Patterns of DkPME and DkPMEI Genes during Fruit Development

The expression levels of *DkPME* and *DkPMEI* genes during fruit development were evaluated using high-throughput RNA-seq data (Figure 8). Overall, 22 *DkPME* and 23 *DkPMEI* genes were expressed in persimmon during fruit development. Seven *DkPME* and eight *DkPMEI* genes exhibited constitutive expression (FPKM > 1 in all tested developmental stages), showing an indispensable role of *DkPME* and *DkPMEI* genes during fruit development. Six *DkPME* genes from clade IV were highly expressed at 70 DAF, suggesting that these genes might be associated with fruit growth. Three *DkPME*s and two *DkPMEI*s were highly expressed in 160 DAF, including *DkPME8*, *DkPME9*, *DkPME20*, *DkPMEI22*, and *DkPMEI24*. Therefore, these genes might have essential roles in fruit maturation. Moreover, some *DkPMEs* and *DkPMEIs* from the same clade exhibited diverse expression patterns. For example, the expression of 7 *DkPMEI* genes from clade I peaked at 140 DAF, whereas

8 *DkPMEI* genes, also from clade I, expressed highly at 70 DAF, indicating that these *PMEI* genes might have diverse functions.

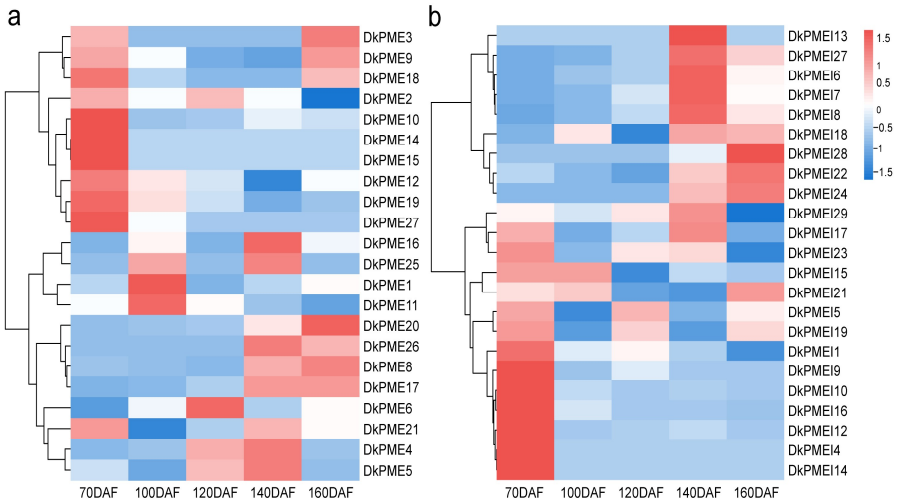

Figure 8. Gene expression of (**a**) *DkPME* and (**b**) *DkPMEI* genes during fruit development.

3.7. Protein Interaction Analysis of DkPMEs and DkPMEIs

To analyze the function of DkPMEs and DkPMEIs, the protein–protein interaction network was constructed using the STRING database according to *A. thaliana* homologous proteins (Figure 9a). As a result, DkPMEI21 and DkPMEI15 were prominent core nodes in this interaction network. The protein sequence of DkPMEI21 was highly similar to that of AtPMEI10, a negative transcriptional regulator related to carbohydrate metabolism, stress responses, and sugar signaling. Furthermore, the sequence of DkPMEI15 was homology to that of AtPMEI78, a crucial transcriptional activator related to carbohydrate metabolism, stress responses, and sugar signaling with a positive regulatory function. DkPMEI21 and DkPMEI15 were closely related to DkPMEI1 and DkPMEI8, which may form a solid interaction network (Figure 9b).

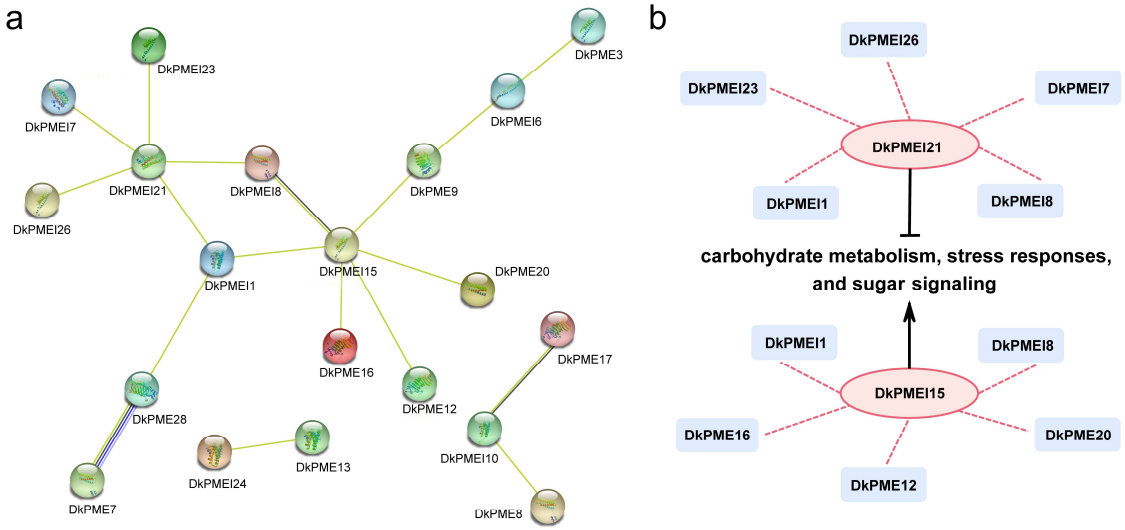

Figure 9. Functional network of DkPMEs and DkPMEIs: (**a**) Network of DkPMEs and DkPMEIs based on the orthologous genes in *A. thaliana*. (**b**) A schematic representation of a regulatory network among DkPMEs and DkPMEIs.

4. Discussion

The *PME* and *PMEI* gene families are involved in cell wall modifications, and thus play a crucial role in organ development, fruit maturation and softening, and responses to various biotic and abiotic stresses in plants [14,17,34]. Due to their essential functions, the P*ME* and *PMEI* gene families were identified in multiple plants [27,29]. However, systematic analysis of *PME* and *PMEI* genes is lacking in persimmon. This study identified 28 *PME* and 29 *PMEI* genes in *D. kaki* via genome-wide analysis. The number of *DkPME* genes (28) was comparable with that of Selaginella moellendorffii (23) [52] and *O. sativa* (30) [52] but was smaller than that of *Gossypium arboretum* (80) [53] and *A. thaliana* (66) [10]. The number of *DkPMEI* genes (29) was comparable to that observed in *O. sativa* (35) [52] and Carica papaya (23) [52] but smaller than that observed in *A. thaliana* (71) [34] and *C. sinensis* (45) [54]. The number of PMEs and PMEIs in persimmon was lower than that in *A. thaliana*, which might be associated with the genome of the persimmon (*D. oleifera*), only undergoing an ancient γ whole-genome duplication event [55], but *A. thaliana* genome underwent at least three duplication events [56].

Gene duplication events generate new gene copies that encode proteins with novel or improved functions, and it can increase genome evolution, diversity, and complexity [57]. Our study found two pairs of *DkPME* and two pairs of *DkPMEI* genes with tandem duplication events, two pairs of *DkPME* genes, and 1 *DkPMEI* gene pair with segmental duplication events. These results are similar to those found in A. thaliana, *V. vinifera*, and *O. sativa* [52], indicating that tandem and segmental duplication events are crucial in expanding *PME* and *PMEI* gene families. By measuring the Ka/Ks ratios between duplicated *DkPME* or *DkPMEI* gene copies, we observed that all duplicated *DkPME* or *DkPMEI* genes underwent purifying selection. The differentiation time of Ebenaceae with *Actinidia chinensis* and *C. sinensis* was approximately 73.6–95.6 MYA, and that of *Diospyros* species (*D. oleifera* and *D. lotus*) was nearly 2.9–18.3 [58]. However, the differentiation time of the *PME* and *PMEI* gene pairs in persimmon was primarily at 26.599–49.717 MYA and 39.777 MYA, respectively [58]. These results showed that the time of gene differentiation was later than that of Ebenaceae with the other plants (like *A. thaliana*) differentiation but earlier than the differentiation time between *Diospyros species*. Besides, synteny analysis showed that the *PME* and *PMEI* genes of persimmon had more homologous gene pairs in *Diospyros species* (*D. oleifera* and *D. lotus*) than *A. thaliana*. Thus, we inferred that the *PME* and *PMEI* genes were conserved between persimmon species but might show high inter-species polymorphism.

Analysis of gene structure and conserved motif provided potential feature information for resolving phylogenetic relationships [59]. *DkPMEs* and *DkPMEIs* could divide into 4 and 5 clades based on the phylogenetic analysis. *DkPME* and *DkPMEI* members of the same clade contained similar exon–intron and motifs compositions. For example, nearly all DkPMEI genes lacked introns and UTRs except genes in Clade I, which is in accordance with the results for *C. sinensis* [54], *Zea mays* [60], and *G. max* [61]. These intronless genes could reduce post-transcriptional processing and respond immediately to abiotic stresses [62]. PME-related motifs and PMEI-related motifs were distributed in most DkPME and DkPMEI proteins. Moreover, a unique motif was conserved across subgroups, like motif 2 of PMEs is unique in Clade IV, and motif 9 of DkPMEIs was mainly present in Clade V. The same phenomenon has been observed in other plants like *C. sinensis* [54]. These results showed that these conserved and clade clade-specific motifs keep the fundamental functions and improve the gene diversities during evolution [53].

Cis-elements are essential regulatory units as they regulate various biological processes, including developmental, hormone, and abiotic or biotic stress response [63]. In this study, the promoter of four *DkPMEI* genes (*DkPMEI12*, *DkPMEI17*, *DkPMEI22*, and *DkPMEI29*) contained a flavonoid biosynthetic MBSI cis-element. MYB family members could recognize the MBSI region [64]; in persimmon, tannin insolubilization, the MBSI, and the other motif participated in the trans-activating of the *DkERF19* promoter by *DkMYB6*, *DkERF18*, and *DkERF19* [65]. Thus, these four *DkPMEI* genes contained an MBSI cis-

element, which might have potential functions in tannin biosynthesis under MYB transcription factor regulation and need further confirmation. Six cis-regulatory elements (LTR, ARE, and MBS, etc.) were essential to stress response in the promoter of *DkPME* and *DkPMEI* genes, in accord with the vital role of these genes in the abiotic stress response [20,21,32]. Gene family members usually formed protein complexes during a long evolutionary process, and diverse PPI networks could represent protein physical relationships [66]. The interaction network found two key nodes, DkPMEI21 and DkPMEI15. They could be used as candidate genes for studying carbohydrate metabolism, sugar signaling, and stress responses of *DkPMEs* and *DkPMEIs*. These results further proved the function of *PME* and *PMEI* genes for regulating growth, development, and stress response.

Fruit development is complicated, directly affecting fruit qualitative traits such as appearance, flavor, and texture [67]. The development, ripening, and softening of fruits is regulated by environmental factors and transcription regulatory factors, such as PME, PMEI, etc. [68]. VvPMEI1 is expressed during fruit development and could control PME activity at the early developmental stage in grape berry [68]. The Tomato SlPMEI gene forms an inactive complex with the significant fruit-specific PME-1 subtype and inhibits pectin degradation during fruit ripening [69]. The temporal expression patterns provide essential information for gene function investigation. A large proportion of DkPME family members (around 79%, 22 out of 28) was expressed in persimmon than that in navel orange (about 64%, 34 out of 53) [11] and tomato (about 47%, 27 out of 57) [70] during fruit development. The same phenomenon was also found in *DkPMEI* genes. These results firmly indicated that *DkPME* and *DkPMEI* genes might play indispensable roles in persimmon fruit development.

In summary, 28 *DkPME* and 29 *DkPMEI* genes were identified in the *D. kaki* genome. Subsequently, comprehensive bioinformatics analyses of *DkPME* and *DkPMEI* genes/proteins on phylogeny, gene structure, conserved motif, chromosomal location, gene duplication, and collinearity, cis-elements, and temporal expression patterns in persimmon fruit were performed. This study provides a systematic and comprehensive analysis that may help elucidate the *DkPME* and *DkPMEI* genes for further structural and functional characterization.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae8121159/s1, Table S1: List of all PME and PMEI genes identified in the *Diospyros oleifera*; Table S2: Ka, Ks and Ka/Ks values calculated for homologous PME and PMEI gene pairs; Table S3: List of light responsive elements in the PME and PMEI genes.

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