Genome-Wide Identification of m^6^A Writers, Erasers and Readers in Poplar 84K

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Abstract: N^6^-methyladenosine (m^6^A) RNA modification is a conserved mechanism to regulate gene expression that plays vital roles in the development of plants. However, the m^6^A RNA modification in forest trees remains limited. Here, we performed a complete analysis of m^6^A writers, erasers and readers in Poplar 84K, including gene location, gene structures, conserved motifs, phylogenetic relationships, promoter analysis, expression profiles and the homology modeling. We have identified 61 m^6^A pathway genes in Poplar 84K (Populus alba × Populus glandulosa), including 14 m^6^A writers, 14 m^6^A erasers and 33 m^6^A readers. Phylogenetic analysis indicated that the m^6^A writers and erasers were clustered into four groups and m^6^A readers were clustered into two groups. Promoter analysis showed that m^6^A pathway genes were mainly responsive to low oxygen followed by ABA and ethylene. The expression of the identified m^6^A pathway genes showed tissue-specific expression patterns in leaves, xylem, phloem and roots. Moreover, 17 genes were significantly up-regulated and 13 genes were significantly down-regulated in poplar overexpressing the transcription factor LBD15. Homology modeling and molecular docking results suggested that PagFIP37b was most likely to be regulated by LBD15, and the qPCR showed that PagFIP37b were up-regulated in the LBD15-oe plants. The results provide insights that aid in the future elucidation of the functions of these m^6^A pathway genes and the epigenetic regulation mechanism of these genes in Poplar 84K.

Keywords: m^6^A modification; writers; erasers; readers; LBD 15; poplar 84K

1. Introduction

The RNA epigenetic modification plays a significant role in the regulation of gene expression [1,2]. Up to now, more than 100 modifications have been reported in RNA [3], and N^6^-methyladenosine (m^6^A) RNA methylation is the most abundant intermediate chemical modification of post-transcriptional gene regulation in eukaryotes. The m^6^A modification occurs at the sixth N atom of adenine, and the m^6^A accounts for up to 80% of RNA methylation modifications in eukaryotic cells and 50% of methylated modifications in mRNA [4]. m^6^A is widely studied in eukaryotes species, such as yeast, plants, flies, mammals and viral RNAs with a nuclear phase [5].

m^6^A modification affects almost every stage of mRNA metabolism, as it provides a binding site for effector proteins that regulate the stability, splicing and translation of mRNA [6,7]. Liu and Pan confirmed that the m^6^A could recognize RNAs all the time through identifying the m^6^A responsive RNA-binding protein [8]. The core proteins that participate in the m^6^A pathway are divided into three groups named writers (methyltransferases), erasers (demethylases) and readers [6,9]. In mammals, METTL3 (methyltransferase-like 3), METTL14 (methyltransferase-like 14) and WTAP serve as writers, FTO belongs to erasers, the readers included ALKBH5 and YTH (YTH domain family 2) [6,10,11]. In plants, the MTA (homologue of human METTL3), MTB (homologue of
human METTL14), VIR, HAKA, and FIP (ortholog of human WTAP) were identified as plant m^6^A writers [5,7,12–14]. ALKBH9B and ALKBH10B proteins were considered as erasers to remove the methylation modification in the nucleus [5,9,10]. The reader proteins mainly included ECT2/3/4 and CPSF30 [6,9]. m^6^A has been examined in many species, including Arabidopsis [11], maize [12], wheat [13], oat [14], rice [15], sea buckthorn and apple [16,17]. Recently, the function of some m^6^A pathway genes in plants has been studied. The inactivation of MTA could reduce m^6^A modification and lead to a failure of embryonic development and reduced apical dominance [11,18]. Shoot meristems require FIP37 to be maintained and are continuously produced in Arabidopsis [19]. ECT2/3 are necessary to regulate the formation and timing of leaf morphogenesis [6]. These studies indicated that the m^6^A modifications in mRNA play a crucial role in plant development.

m^6^A RNA methylation is a conserved mechanism to enrich and control gene expression and plays an important role in organisms. The distribution pattern of methylation sites and the consensus sequence of m^6^A seem to be conserved in human and yeast; both the modification sites are enriched at the 3′untranslated regions (3′UTRs). In addition, m^6^A in human is also enriched around stop codons and within internal long exons [5]. However, plants may have evolved with unique mechanisms in m^6^A methylation machinery. In plants, it has been shown that m^6^A is usually enriched around the stop codon, 3′UTRs, long exons, TSS (the transcription start site) and TES (transcription end site) [16,20–22]. The writers in Arabidopsis could recognize the consensus motif RRACH [23]. However, not all RRACH motifs in plants are associated with m^6^A modification, and the molecular mechanism of recognition is also undefined [8].

The m^6^A level was affected by the expression of m^6^A pathway genes. It has been shown that the m^6^A levels were reduced through down-regulation of the m^6^A pathway genes [23]. However, knowledge of RNA modification in forest trees remains limited. Poplar 84K has become a model plant for forest tree studies, since it is a fast-growing poplar with an available whole genome sequence, high transformability and economic and ecological value. In our previous studies, we have reported that an ortholog gene of AtLBD15 from Eucalyptus grandis takes part in the leaf development in Poplar 84K [24] and analyzed the differential m^6^A modification sites between Poplar 84K and the LBD15 overexpression plants [22]. In order to further elucidation of the molecular mechanism of m^6^A modification associated with LBD15, here, we systematically identified m^6^A writers, erasers and readers genome-wide in Poplar 84K. The gene structures, gene location, conserved motifs and phylogenetic relationships were identified, and the homology modeling of these proteins and LBD15 were analyzed. In addition, the tissue-specific expression profiles and the expression of these genes in Poplar 84K and the LBD15 overexpression plants were also investigated. The results provide insight to further elucidate the functions of the genes of m^6^A pathway and the epigenetic regulation mechanism of m^6^A in Poplar 84K.

2. Materials and Methods

2.1. Plant Materials

Poplar 84K was planted in the greenhouse located at the Chinese Academy Forestry, Beijing, China. Leaves, xylem, phloem and roots were sampled from 6-month-old plants and stored in liquid nitrogen for RNA isolation. The shoots cultivated for 6 weeks were collected from Poplar 84K and LBD15 overexpression plants and stored in liquid nitrogen until use. Three independent biological replicates were performed from three whole plants for each sample.

2.2. Identification of m^6^A Pathway Genes in Poplar 84K

All the m^6^A pathway genes from Arabidopsis and Oryza sativa protein sequences were downloaded from GenBank (Table S3) and used as a query to search for homologous genes in Poplar 84K genome through tBLASTn with an e-value of 1 × 10^-5 [25]. The conserved domains of the candidate genes were analyzed in NCBI-CDD [26]. The molecular weight (Mw) and isoelectric point (pl) of these proteins were investigated using the ExPaSy
online software [27]. Their subcellular localization was predicted based on the Busca online software [28].

2.3. Phylogenetic Tree, Gene Structure, Conserved Motifs, Promoter Prediction and Protein Interaction Analysis

The chromosomal location and gene structure of these m⁶A pathway genes were obtained from the genome annotation files, which were downloaded from (https://www.ncbi.nlm.nih.gov/genome/87686, accessed on 1 June 2022). The chromosome physical location of those genes was displayed by MapGene2Chromosome v2.0 [29], and the gene structure was shown using a Gene Structure Display Server [30]. The MEME tool was used to predict the conserved motif of the candidate protein sequences [31]. The maximum motif number was set to 5, and other parameters were left on the default settings. The phylogenetic trees and dN/dS analyzed were constructed by MEGA7.0 software [32] using the muscle and neighbor-joining (NJ) method with the bootstrap value of 1000 [33]. The upstream 2000 base pair (bp) genomic DNA sequences from the transcription start sites of m⁶A pathway genes were predicted in PlantCare database [34] to identify the putative cis-regulatory elements.

2.4. Quantitative Real-Time Reverse Transcription-PCR (qRT-PCR)

Total RNA of leaves, roots, xylem and phloem from Poplar 84K and LBD15-oe plants were isolated by RNA Easy Fast Plant Tissue Kit (TIANGEN, Beijing, China). The cDNA was generated by reverse transcription using FastKing RT Kit (TIANGEN, Beijing, China). The qRT-PCR analysis of m⁶A pathway genes were performed with SYBR® rapid quantitative PCR Kit (KAPA KK4601, Pleasanton, CA, USA) using the methods described previously [35]. Pagactin was used as a reference [24]. The primers of all the genes were listed in Table S5, and the results were analyzed using the 2^{−ΔΔCt} method [24].

2.5. Homology Modeling of 3D Structures, Molecular Docking and Protein Docking

The 3D structures of m⁶A pathway proteins are important for investigating their gene function. The 3D structure of m⁶A pathway proteins and LBD15 were predicted using the homology modeling method. The PDB database [36] was used to find the best template, and the model was built by Swiss-Model [37]. The predicted cis-elements of m⁶A pathway proteins that can interact with LBD15 were performed by JASPAR [38]. The highest scoring cis-element was used to dock with LBD15 through Autodock vina [39]. The protein docking of PagMT families was performed using ZDOCK [40], and PDBePISA [41] was used to analyze the docking results. Pymol software was used for evaluation of quality, and the equation linking affinity (Ka) and ligand free energy of binding ΔG were calculated by formula: ΔG = −RTln(KA/C) (T is temperature in kelvin, C is 1 M concentration and R = 8.314 J/mol/K) [42].

2.6. Statistical Methods

In this study, one-way ANOVA was used to perform the statistical analysis for the differential tissues expression of m⁶A pathway genes through IBM SPSS 19 software. We ranked all averages from highest to lowest and marked the letter a after the highest average; then, the mean was compared with the following means, and any mean that was not significantly different was marked with the letter a, while any mean that was significantly different was marked with the letter b, and so on until the smallest average has a marked letter and stops. Moreover, the statistical analysis of the expression between Poplar 84K and LBD15-oe plants were calculated by T-test was using IBM SPSS 19 software. p < 0.05 (*) was considered statistically significant, while p < 0.01 (**) was considered extremely significant.
3. Results

3.1. Genome-Wide Identification of m6A Pathway Genes in Poplar 84K

The protein sequences involved in the m6A pathway in plants including Arabidopsis and O.sativa were downloaded from GenBank. tBLASTn analysis of these protein sequences against the genome of Poplar 84K were performed to identify putative m6A pathway genes. As a result, a total of 61 m6A pathway-like genes were identified in Poplar 84K, including 14 m6A erasers (14 PagALKBHs), 14 m6A writers (8 PagMTs, 2 PagFIP37s, 2 PagVIRILIZERS and 2 PagHAKAIs), 33 m6A readers (28 PagECTs and 5 PagCPSF30s). Analysis of gene feature showed that the length of the ORF (open-reading frame) varied from 1783 to 7182 bp of m6A erasers, 1285 to 17,347 bp of m6A writers, and 3379 to 16,254 bp of m6A readers, respectively. The amino acid length varied from 240 to 770 aa for m6A erasers, 288 to 2179 aa for m6A writers, and 398 to 975 aa for m6A readers, respectively. The average molecular weight (Mw) is 44.41, 82.22 and 69.89 kDa, respectively, and the theoretical pl of m6A pathway genes ranged from 4.62 to 9.22. The prediction of subcellular localization displayed that 59 genes were located in the nucleus, and another 2 genes were located in cytoplasm (Table 1).

Table 1. Sequences feature of m6A pathway genes in Poplar 84K.

| Genes         | Length (bp) | Length (aa) | PI   | MW (kDa) | Subcellular Location |
|---------------|-------------|-------------|------|----------|----------------------|
| PagALKBH1Ba   | 6018        | 340         | 6.41 | 37.91    | nucleus              |
| PagALKBH1Bb   | 7182        | 346         | 6.51 | 38.59    | nucleus              |
| PagALKBH2Ba   | 3684        | 506         | 5.62 | 56.68    | nucleus              |
| PagALKBH2Bb   | 3280        | 506         | 5.72 | 56.74    | nucleus              |
| PagALKBH3Ba   | 2086        | 240         | 9.17 | 27.97    | nucleus              |
| PagALKBH3Bb   | 2646        | 240         | 9.22 | 28.03    | nucleus              |
| PagALKBH4Ba   | 3221        | 511         | 6.08 | 57.38    | nucleus              |
| PagALKBH4Bb   | 3091        | 511         | 5.98 | 57.48    | nucleus              |
| PagALKBH5Ba   | 2190        | 269         | 5.45 | 30.53    | nucleus              |
| PagALKBH5Bb   | 3731        | 263         | 5.37 | 29.96    | nucleus              |
| PagALKBH6Ba   | 6847        | 525         | 5.72 | 56.96    | nucleus              |
| PagALKBH6Bb   | 6870        | 770         | 6    | 84.95    | nucleus              |
| PagALKBH7Ba   | 2029        | 259         | 4.62 | 29.28    | nucleus              |
| PagALKBH7Bb   | 1783        | 259         | 4.7  | 29.33    | nucleus              |
| PagFIP37a     | 7503        | 336         | 5.22 | 38.04    | nucleus              |
| PagFIP37b     | 6770        | 336         | 5.22 | 38.08    | nucleus              |
| PagHAKAI1     | 3511        | 498         | 6.06 | 53.75    | nucleus              |
| PagHAKAI2     | 3162        | 498         | 6.15 | 53.74    | nucleus              |
| PagMTA1       | 1294        | 454         | 6.24 | 49.99    | nucleus              |
| PagMTA2       | 1285        | 288         | 5.54 | 32.56    | nucleus              |
| PagMTB1       | 4948        | 1185        | 7.18 | 131.99   | nucleus              |
| PagMTB2       | 4755        | 1193        | 7.4  | 132.93   | nucleus              |
| PagMTB3       | 4711        | 1181        | 7.4  | 131.55   | nucleus              |
| PagMTB4       | 5307        | 1188        | 7.18 | 132.34   | nucleus              |
| PagMTC1       | 3822        | 406         | 6.58 | 46.93    | nucleus              |
| PagMTC2       | 3812        | 406         | 6.77 | 46.94    | nucleus              |
| PagVIRILIZER1 | 17,347      | 2179        | 5.26 | 238.44   | nucleus              |
| PagVIRILIZER2 | 16,715      | 2179        | 5.28 | 23.83    | nucleus              |
| PagECT1       | 3401        | 616         | 6.9  | 68.49    | nucleus              |
| PagECT2       | 6030        | 588         | 6.25 | 64.73    | nucleus              |
| PagECT3       | 4030        | 739         | 8.67 | 81.95    | nucleus              |
| PagECT4       | 4325        | 619         | 7.17 | 68.65    | nucleus              |
| PagECT5       | 3379        | 625         | 6.44 | 69.20    | nucleus              |
| PagECT6       | 4628        | 653         | 5.48 | 71.62    | nucleus              |
| PagECT7       | 5476        | 591         | 6.48 | 65.24    | nucleus              |
| PagECT8       | 5224        | 602         | 5.94 | 66.12    | nucleus              |
Table 1. Cont.

| Genes       | Length (bp) | Length (aa) | PI  | MW (kDa) | Subcellular Location |
|-------------|-------------|-------------|-----|----------|----------------------|
| PagECT9     | 5342        | 583         | 7.21| 64.51    | nucleus              |
| PagECT10    | 6578        | 703         | 6.34| 78.35    | nucleus              |
| PagECT11    | 7680        | 761         | 6.1 | 84.21    | nucleus              |
| PagECT12    | 4211        | 603         | 5.72| 66.29    | nucleus              |
| PagECT13    | 3780        | 600         | 6.16| 66.44    | nucleus              |
| PagECT14    | 4156        | 766         | 5.95| 84.53    | nucleus              |
| PagECT15    | 4431        | 585         | 5.71| 64.22    | nucleus              |
| PagECT16    | 4089        | 601         | 6.22| 66.33    | nucleus              |
| PagECT17    | 5212        | 774         | 5.98| 85.56    | nucleus              |
| PagECT18    | 5324        | 654         | 5.33| 71.68    | nucleus              |
| PagECT19    | 5080        | 584         | 6.6 | 64.66    | nucleus              |
| PagECT20    | 4567        | 636         | 5.2 | 69.39    | nucleus              |
| PagECT21    | 9328        | 975         | 6.78| 108.47   | nucleus              |
| PagECT22    | 4478        | 629         | 5.54| 68.75    | nucleus              |
| PagECT23    | 4856        | 548         | 6.58| 60.02    | nucleus              |
| PagECT24    | 6050        | 584         | 6.68| 64.65    | nucleus              |
| PagECT25    | 4745        | 609         | 5.34| 66.39    | cytoplasm            |
| PagECT26    | 4476        | 639         | 5.43| 70.22    | nucleus              |
| PagECT27    | 4409        | 624         | 5.38| 68.26    | nucleus              |
| PagECT28    | 4672        | 540         | 6.25| 59.01    | cytoplasm            |
| PagCPSF30a  | 15,333      | 695         | 6.08| 76.07    | nucleus              |
| PagCPSF30b  | 16,254      | 684         | 6.2 | 75.03    | nucleus              |
| PagCPSF30c  | 6063        | 398         | 6.17| 44.82    | nucleus              |
| PagCPSF30d  | 15,158      | 684         | 6.2 | 74.99    | nucleus              |
| PagCPSF30e  | 3987        | 375         | 5.82| 42.11    | nucleus              |

3.2. Phylogenetic Analysis of m^6^A Pathway Genes in Poplar 84K

To investigate the phylogenetic relationship of m^6^A pathway genes in Poplar 84K, the phylogenetic tree was constructed using the protein sequence of the N^6^-methyladenosine writers, erasers and readers in Poplar 84K with the corresponding genes in *Arabidopsis* and *O. sativa*, respectively. The results showed that the writers in Poplar 84K were clustered into four groups, including PagMT, PagFIP37, PagVIRILIZER and PagHAKAI families (Figure 1A). The PagMT group was divided into three subgroups: PagMTA, PagMTB and PagMTC. The erasers in Poplar 84K only contained the PagALKBH family, which was clustered into four groups (Figure 1B). The erasers in Poplar 84K had a distribution in each group. The phylogenetic analysis showed that the readers in Poplar 84K were classified into two groups, PagECT and PagCPSF30 (Figure 1C). PagECT family contained a conserved YTHDF domain, whereas the PagCPSF30 family contained a conserved YTHDC domain. This is consistent with previous studies from other plants, suggesting that the readers have a conserved role in the plants.

The dN/dS value of the m^6^A pathway genes were calculated by the MEGA7.0 software to detect molecular selection effects (Table S1). As a result, the m^6^A pathway genes except for PagCPSF30s had a dN/dS < 1, indicating that they had undergone strong purifying selection. There were both dN/dS > 1 and dN/dS < 1 in the PagCPSF30 family, suggesting it had undergone purifying selection and position section. The results suggested that the m^6^A pathway genes were highly conserved during the evolutionary process with the exception of PagCPSF30s.
3.3. Analysis of Chromosomal Location

To further understand the evolutionary relationship of the 61 m^6_A pathway genes, the chromosomal location of these genes was determined. The result showed that these genes were distributed on 31 chromosomes (Figure 2). Among them, chromA01 and chromG01 contain the largest number of m^6_A pathway genes; PagCPSF30b, PagCPSF30c, PagMTA1, PagECT1 and PagECT19 were located on chromA01. Additionally, PagCPSF30d, PagCPSF30e, PagMTA2, PagECT5 and PagECT24 were also located on chromG01. ChromA14 and chromG14 contained four m^6_A pathway genes, respectively. PagALKBH6Bb, PagALKBH7Ba, PagMTA3 and PagECT21 were located on chromA14, and PagALKBH7Bb, PagALKBH6Ba, PagMTA4 and PagECT18 were located on chromG14. The result suggested that these genes were generated by segmental duplication, which was the single driving force in the evolutionary process of m^6_A pathway genes in Poplar 84K.

3.4. Gene Structure and Conserved Motif Analysis

The exon–intron pattern is an important feature for a gene and can provide important evidence for gene functional diversification, so the exon–intron patterns of m^6_A pathway genes were determined (Figure 3). The intron numbers in writers varied from 2 to 27. For erasers, the intron number varied from 3 to 6. The intron number of the readers clustered in the PagECT group varied from 5 to 11, and those clustered in PagCPSF30 group varied from 5 to 7. The PagCPSF30 gene family has the largest intron size. The results showed that
the genes clustered into the same clade in the phylogenetic tree had similar exon–intron patterns, indicating the conservation of these genes during evolution.

Figure 3. The gene structure and conserved motifs of m6A pathway genes. (A) Exons, UTRs, introns and intron phases are shown. (B) Motifs are represented by boxes.

The conserved motif analysis results showed that a total of 15 motifs were identified in the m6A pathway genes in Poplar 84K and the genes clustered with the same group had similar motifs, which is consistent with the results of the gene structure and the phylogenetic tree. We found that some motifs were highly conserved in the m6A pathway genes in Poplar 84K; for example, the motif 1 was found in all the erasers, motif 7, motif 9 and motif 10 were present in all the readers and motif 15 was present in all writers.

3.5. Promoter Analysis of m6A Pathway Genes

The cis-elements of these genes are shown in Figure 4 and Table S2. CAAT-box and TATA-box were found in all the m6A pathway genes. The main cis-acting elements were
predicted to respond to abiotic stress, hormones and inducers such as methyl jasmonate (MeJA), light, anaerobic, ethylene, salicylic acid (SA), drought, low temperature, abscisic acid (ABA), gibberellin and auxin. The largest number of cis-elements were light-response elements, and they were found in all the m^6A pathway genes. For erasers, 13 genes had ABA response elements, while 12 genes had anaerobic response and ethylene response elements. In readers, 33 genes have anaerobic response elements. In writers, anaerobic response elements were found in all the members. The results showed that m^6A pathway genes were mainly responsive to low oxygen followed by ABA and ethylene.

Figure 4. The cis-regulatory elements in the promoter of 61 m^6A pathway genes. The cis-regulatory elements were represented by triangle and rectangle in different colors.

3.6. Expression Profiles of m^6A Pathway Genes

The expression of m^6A pathway genes in differential tissues was investigated using qPCR (Figure 5). The results showed that the expression of these genes displayed differential expression patterns. All the writer genes were highly expressed in leaves,
especially, the PagFIP37 genes which had the highest expression in leaves. The readers that clustered into the same subgroup displayed similar expression patterns; for instance, PagECT1, PagECT3, PagECT4 and PagECT5 were mainly expressed in roots, while PagECT20, PagECT22, PagECT25 and PagECT27 showed the highest expression levels in leaves. PagECT19 had the highest expression level in the phloem. As for erasers, the different erasers showed divergent expression patterns, such as PagALKBH4s and PagALKBH5s, which were mainly expressed in phloem, and PagALKBH3s showed significantly high expression in leaves.

![Figure 5.](image)

Figure 5. qPCR validation of 61 m^6^A pathway genes in tissues. Figure (A)–(B1) showed the expression level of 61 genes in differential tissues. One-way ANOVA was calculated using IBM SPSS 19 software. The a, b, c and d indicated whether the difference was significant. The same letter marked in the same gene among different tissues indicated no significant difference, and different letters indicated significant difference.

The expression of the m^6^A pathway genes in Poplar 84K and LBD15-oe plants was also investigated by qPCR (Figure 6). The results showed that the expression of the m^6^A pathway genes was regulated in the LBD15-oe plants. There were 17 genes that were significantly up-regulated; among them, the expressions of PagALKBH4Ba, PagECT7 and PagECT13 were up-regulated more than 2-fold, while PagHAKAI2, PagFIP37b, PagALKBH2Ba, PagECT2, PagECT9, PagECT15, PagECT16, PagECT23, PagECT28 and PagCPSF30a were up-regulated more than 1.5-fold in the LBD15-oe plants. Conversely, 13 genes were significantly down-regulated; for example, PagECT12 and PagALKBH3Bb were down-regulated more than
2-fold in the LBD15-oe plants. The results implied that the regulatory role of m\(^6\)A modification may be associated with the expression of LBD15.

![Figure 6. qPCR validation of 61 m\(^6\)A pathway genes in Poplar 84K and LBD15-oe plants. Figure (A)–(B1) showed the expression level of 61 genes in Poplar 84K and LBD15-oe plants. A t-test was calculated using IBM SPSS 19 software. * represent p < 0.05 and ** represent p < 0.01.](image)

### 3.7. Homology Modeling, Molecular Docking and Protein Docking

The protein model of the m\(^6\)A pathway genes and LBD15 were built by homology modeling, as shown in Figures 7 and 8A. The template and RMSD are shown in Table S3. The RMSD (root-mean-square deviation) is less than 1Å with respect to the templates, suggesting that the homology modeling was reliable. As a result, we obtained the 3D structure of a total of 56 proteins of the m\(^6\)A pathway genes; five other proteins were not modeled, as there was no homologous template to build in the database. In the 3D structures, the α-helix, β-fold and random coil were signed in different colors. All the structures of readers contained five α-helices and four adjacent β-folds and formed highly similar spatial structures. The 3D structures of erasers varied among groups based on the metal ions; for example, PagALKBH1Ba contained one Mn ion, while PagALKBH1Bb contained one Mn ion and one Zn ion in the structure, and PagALKBH7Bs and PagALKBH3Bs contained one Fe ion. For the writers, PagMTCs displayed as dimers and contained two centrosymmetric monomeric proteins. The proteins that clustered into the same group had highly similar 3D structures, indicating that these genes were highly conserved in the plants.
Figure 7. The homology modeling of m^6A pathway genes in Poplar 84K. The α-helix, β-fold and random coil are shown, and the ball represents metal ions.
Figure 8. (A) The homology modeling of LBD15. (B) The structure of chain A in LBD15.

The LBD15 protein contains a typical LOB domain displayed as dimers, which contained two centrosymmetric monomeric chains (chain A and chain B) (Figure 8A), and the monomeric chain consisted of five α-helixes (Figure 8B). The most reliable cis-element in promoter of the m6A pathway genes that up-regulated more than 1.5-fold in the LBD15-oe plants were predicted, and the molecular docking of these cis-elements with LBD15 was performed. The results of cis-elements docking with LBD15 are shown in Table 2 and Figure S1. Among those 13 genes, PagFIP37b could be the most reliable to interact with LBD15 according to the Affinity.

Table 2. Amino acid residues participate in protein–ligand docking between LBD15 and predicted cis-elements in the promoters of m6A pathway genes.

| Receptor Protein | Ligand          | Predicted Cis-Element Sequence | Affinity (kcal/mol) | Amino Acid Residues in Docking |
|------------------|-----------------|--------------------------------|---------------------|--------------------------------|
| LBD15            | PagFIP37b       | CACCCCGAATT                 | −5.3                | Thr-44 Cys-56 Asn-103 Tyr-107 Arg-114 |
| LBD15            | PagALKBH48a     | AAACCGGAAAAAG              | −5                  | Arg-53 Gln-129 Gln-128 Gln-132 |
| LBD15            | PagECT2         | AAGCAGGAACTT                | −4.8                | Gln-55 Ser-84 |
| LBD15            | PagHAKAI2       | CAGCAGGAGACA                | −4.8                | Arg-55 Ser-84 |
| LBD15            | PagCPSF30a      | CCCCCAGAAAAT                | −4.7                | Pro-45 Arg-55 Cys-56 Gln-58 Ser-63 Pro-64 Asp-100 Asn-103 |
| LBD15            | PagALKBH28a     | CTGCCCTGAGACA               | −4.6                | Leu-52 Arg-55 Glu-95 |
| LBD15            | PagECT23        | TCCGAGGCAATG                | −4.5                | Try-107 Asn-110 Gln-128 |
| LBD15            | PagECT13        | GAGCCTGGAAAT                | −4.4                | Tyr-107 Tyr-107 Asn-139 Ser-143 |
| LBD15            | PagECT15        | GTCACCACCCCTG               | −4.3                | Glu-70 Asn-103 Asn-110 Ser-125 |
| LBD15            | PagECT28        | TGGCAGGCAATG                | −4.1                | Thr-44 Glu-59 Cys-60 Lys-73 Ser-84 |
| LBD15            | PagECT9         | TCTCAGGAAACAGA              | −4.1                | Lys-73 Asn-110 Arg-114 |
| LBD15            | PagECT16        | TCTCCCCGCGTCCC              | −4.0                | Phe-80 Ser-84 His-78 Asn-85 |
| LBD15            | PagECT7         | AAACCGGAAAT                 | −3.7                | His-69 Lys-73 Asn-103 Gln-128 |
The protein docking of PagMTs was performed. The results of protein docking are shown in Table 3; the docking of PagMTA1-PagMTC1 and PagMTA2-PagMTB1 are the most credible based on the score of $\Delta G$ (kcal/M) and $p$-value. The results revealed that PagMTA-PagMTB, PagMTA-PagMTC, and PagMTC-PagMTB could bind and formed a dimer, which could help us understand how these writers work.

### Table 3. Protein–protein docking of PagMTAs, PagMTBs and PagMTCs. NHB: number of potential hydrogen bonds. NSB: number of potential salt bridges.

| Structure 1 | Structure 2 | Interface Area, Å² | $\Delta G$ kcal/M | $p$-Value | NHB | NSB |
|-------------|-------------|--------------------|-------------------|-----------|-----|-----|
| PagMTA1     | PagMTC1     | 3151.8             | $-18.3$           | 0.471     | 34  | 12  |
| PagMTA1     | PagMTC2     | 2655.1             | $-9.2$            | 0.552     | 28  | 22  |
| PagMTA1     | PagMTB1     | 2326.9             | $-18.2$           | 0.160     | 25  | 19  |
| PagMTA1     | PagMTB2     | 2321.8             | $-15.9$           | 0.265     | 25  | 13  |
| PagMTA1     | PagMTB3     | 2326.7             | $-18.2$           | 0.162     | 25  | 19  |
| PagMTA1     | PagMTB4     | 2321.8             | $-15.9$           | 0.265     | 25  | 13  |
| PagMTA2     | PagMTC1     | 2613.1             | $-5.3$            | 0.769     | 33  | 20  |
| PagMTA2     | PagMTC2     | 3360.1             | $-14.0$           | 0.325     | 28  | 18  |
| PagMTA2     | PagMTB1     | 2326.9             | $-18.2$           | 0.160     | 25  | 19  |
| PagMTA2     | PagMTB2     | 2321.8             | $-15.9$           | 0.265     | 25  | 13  |
| PagMTA2     | PagMTB3     | 2326.7             | $-18.2$           | 0.162     | 25  | 19  |
| PagMTA2     | PagMTB4     | 2321.8             | $-15.9$           | 0.265     | 25  | 13  |
| PagMTC1     | PagMTB1     | 2281.4             | $-11.9$           | 0.479     | 28  | 9   |
| PagMTC1     | PagMTB2     | 2281.4             | $-11.9$           | 0.479     | 25  | 18  |
| PagMTC1     | PagMTB3     | 2445.5             | $-8.6$            | 0.398     | 25  | 22  |
| PagMTC1     | PagMTB4     | 2281.8             | $-11.8$           | 0.508     | 25  | 9   |
| PagMTC2     | PagMTB4     | 2391.4             | $-7.8$            | 0.442     | 25  | 18  |

### 4. Discussion

$m^6$A RNA methylation is the most abundant intermediate chemical modification of post-transcriptional gene regulation in eukaryotes. It is indispensable for plant growth and development through participating in the mRNA splicing, stability and translation [6,7]. In our previous study, the regulation of the $m^6$A modification between Poplar 84K and the LBD15 overexpression plants was analyzed [22]. In order to increase our understanding of the mechanism of $m^6$A modification and the epigenetic regulation of LBD15, here, we systematically at a genome level identified 61 $m^6$A pathway genes in poplar 84K, including 14 $m^6$A erasers, 14 $m^6$A writers and 33 $m^6$A readers. The gene structures, gene location, conserved motifs and phylogenetic tree were performed; the tissue-specific expression profiles and the expression of these genes in Poplar 84K and the LBD15 overexpression plants were investigated. In addition, the 3D structures and protein docking of the identified proteins were also analyzed. Our results provide insight into understanding the roles of these $m^6$A pathway genes and the epigenetic regulation mechanism of these genes in Poplar 84K.

In this study, in total, we identified 61 $m^6$A writers, erasers and readers in Poplar 84K, genome-wide, the number of the $m^6$A pathway genes was significantly greater than the number found in O.sativa and Arabidopsis, which both contain 28 genes. This may be partially explained by the fact that Poplar 84K is a hybrid of P.alba and P.glandulosa. It has two subgenomes, which have different gene numbers based on the chromosomal localization analysis. Based on the phylogenetic tree, the 14 $m^6$A writers in Poplar 84K were clustered into four groups, implying that the four types of writers have different functions. Among them, the PagMT group was the largest one, which contained eight members. This is distinct from its counterparts in Arabidopsis, suggesting that the PagMT
group proteins potentially have more functions in Poplar 84K. The eraser is a demethylase which could remove the m\textsuperscript{6}A modification in the nucleus. In Arabidopsis, the functions of \textit{ALKBH9B} and \textit{ALKBH10B} are well studied \cite{5,9,10}. Six \textit{ALKBH1} proteins in Poplar 84K were clustered into the same clades with \textit{AtALKBH9B} and \textit{AtALKBH10B}, including Pag\textit{ALKBH2}a, Pag\textit{ALKBH2}b, Pag\textit{ALKBH4}a, Pag\textit{ALKBH4}b, Pag\textit{ALKBH6}a and Pag\textit{ALKBH2}b, indicating they may have a role similar to m\textsuperscript{6}A demethylation. The 33 readers were classified into two groups according to the YTHDC domain and YTHDF domain; the result is consistent with other plants \cite{25}, suggesting that the readers are highly conserved in plants.

The m\textsuperscript{6}A modification controls gene expression through its writers, readers and erasers, so the expression of the writers, readers and erasers is very important for investigating their potential functions. In this work, the expression of m\textsuperscript{6}A pathway genes in four tissues including leaves, roots, xylem, and phloem was analyzed. The results revealed that all the identified genes were detected in the leaves, roots, xylem, and phloem of Poplar 84K, but they displayed differential expression profiles. The genes clustered into the same clade showed a similar expression pattern, suggesting they may play similar roles in the plant growth and development. Our previous study has reported that an ortholog of \textit{AtLBD15} is involved in the development of leaves \cite{24}. We found that the level of m\textsuperscript{6}A modification in \textit{LBD15} overexpression plants was altered. In order to further understand the mechanism of m\textsuperscript{6}A modification in \textit{LBD15} overexpression plants, we detected the specific expression of m\textsuperscript{6}A pathway genes in \textit{CK} and \textit{LBD15-oe} plants. As a result, we found that the expressions of the m\textsuperscript{6}A pathway genes were regulated in the \textit{LBD15-oe} plants. For example, some readers including Pag\textit{ECT7}, Pag\textit{ECT13}, Pag\textit{ECT15}, Pag\textit{ECT16}, Pag\textit{ECT2}, Pag\textit{ECT9}, Pag\textit{ECT23}, Pag\textit{ECT28} and Pag\textit{CPSF30a} were up-regulated more than 1.5-fold in \textit{LBD15-oe} plants. Moreover, these genes were mainly expressed in leaves, suggesting these genes are probably involved in the leaf development. It has been shown that proteins ECT2/3/4 are essential for leaf formation in \textit{Arabidopsis} \cite{8}, which is consistent with our results. On the contrary, Pag\textit{HAKAI2} was significantly down-regulated in the \textit{LBD15-oe} plants; this is consistent with our previous work, which showed that 4260 down-regulated m\textsuperscript{6}A peaks were detected in \textit{LBD15-oe} plants \cite{22}. Taken together, the level of m\textsuperscript{6}A in the \textit{LBD15-oe} plants was affected by \textit{LBD15} through regulating the expression of m\textsuperscript{6}A pathway genes.

The m\textsuperscript{6}A in the plants is a complex process; the functions of the m\textsuperscript{6}A writers, erasers and readers in plants are still unclear \cite{8}. The homology model and molecular docking could help us better understand the 3D structure of proteins and drive our future research. The results of molecular docking between the cis-element of m\textsuperscript{6}A pathway genes and \textit{LBD15} showed that Pag\textit{FIP37b} could be regulated by \textit{LBD15}, which will provide a direction for studying the epigenetic regulation mechanism associated with \textit{LBD15} in Poplar 84K. In \textit{Arabidopsis}, it has been shown that MTA and MTB could interact with each other and form homodimers \cite{43}. In this study, the protein–protein docking of PagMTs revealed that Pag\textit{MTA-PagMTB}, Pag\textit{MTA-PagMTC}, and Pag\textit{MTC-PagMTB} could bind and form a dimer. The \(\Delta\text{G} < 0\) indicated that the docking was reliable \cite{41}. The \(p\)-value \(< 0.5\), implying the interface surface can be interaction-specific. The results showed that two PagMTs could form a dimer, which is consistent with \textit{Arabidopsis}, suggesting this prediction was reliable. These results will provide valuable information for further study of the functions of the m\textsuperscript{6}A pathway genes in the Poplar 84K.

5. Conclusions

In this study, a thorough and systematic approach, combining phylogenetic analysis, chromosomal localization, gene structure, conserved motif and promoter analysis as well as expression and 3D structures, was performed to help characterize the 61 m\textsuperscript{6}A pathway genes identified in the genome of Poplar 84K. The results revealed that the m\textsuperscript{6}A pathway genes in Poplar 84K were highly evolutionary conserved. The expression of the identified m\textsuperscript{6}A pathway genes showed tissue-specific expression patterns in leaves, xylem, phloem and roots. These genes were regulated in the \textit{LBD15-oe} plants. The results of 3D structures
Genes 2022, 6, 10. Duan, H.-C.; Wei, L.-H.; Zhang, C.; Wang, Y.; Chen, L.; Lu, Z.; Chen, P.R.; He, C.; Jia, G. ALKBH10B is An RNA N

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/genes13061018/s1. Table S1: dn/ds analysis of writers, readers and erasers; Table S2: The cis-regulatory elements in the promoter of m6A pathway genes; Table S3: Accession numbers and amino acid sequences of m6A pathway genes from Arabidopsis thaliana and O.sativa; Table S4: The homology templates and docking score (RMSD) in protein homology modeling; Table S5: The primers used in qPCR; Figure S1: The results of molecular docking between cis-elements of m6A pathway genes and LBD15. The amino acid residues involved in docking are displayed in sticks and signed a label.

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References

1. Meyer, K.D.; Jaffrey, S.R. The dynamic epitranscriptome: N6-methyladenosine and gene expression control. Nat. Rev. Mol. Cell Biol. 2014, 15, 313–326. [CrossRef] [PubMed]

2. Wang, X.; Lu, Z.; Gomez, A.; Hon, G.C.; Yue, Y.; Han, D.; Fu, Y.; Parisien, M.; Dai, Q.; Jia, G.; et al. N6-methyladenosine-dependent regulation of messenger RNA stability. Nature 2014, 505, 117–120. [CrossRef]

3. Czerwoniec, A.; Dunin-Horkawicz, S.; Purta, E.; Kaminska, K.H.; Kasprzak, J.M.; Bujnicki, J.M.; Grosjean, H.; Rother, K. MO-DOMICS: A database of RNA modification pathways. 2008 update. Nucleic Acids Res. 2009, 37, 118–121. [CrossRef] [PubMed]

4. Kierzek, E.; Kierzek, R. The thermodynamic stability of RNA duplexes and hairpins containing N6-alkyladenosines and 2-methylthio-N6-alkyladenosines. Nucleic Acids Res. 2003, 31, 4472–4480. [CrossRef]

5. Fu, Y.; Dominissini, D.; Rechavi, G.; He, C. Gene expression regulation mediated through reversible m6A RNA methylation. Nat. Rev. Genet. 2014, 15, 293–306. [CrossRef]

6. Arribas-Hernández, L.; Bressendorff, S.; Hansen, M.H.; Poulsen, C.; Erdmann, S.; Brodersen, P. An m6A-YTH Module Controls Developmental Timing and Morphogenesis in Arabidopsis. Plant Cell 2018, 30, 952–967. [CrossRef]

7. Meyer, K.D. m6A-mediated translation regulation. Biochem 2018, 1862, 301–309. [CrossRef]

8. Liu, N.; Pan, T. N6-methyladenosine–encoded epitranscriptomics. Nat. Struct. Mol. Biol. 2016, 23, 98–102. [CrossRef] [PubMed]

9. Martinez-Pérez, M.; Aparicio, F.; López-Gresa, M.P.; Bellès, J.M.; Sánchez-Navarro, J.A.; Pallás, V. Arabidopsis m6A demethylase activity modulates viral infection of a plant virus and the m6A abundance in its genomic RNAs. Proc. Natl. Acad. Sci. USA 2017, 114, 10755–10760. [CrossRef]

10. Duan, H.-C.; Wei, L.-H.; Zhang, C.; Wang, Y.; Chen, L.; Lu, Z.; Chen, P.R.; He, C.; Jia, G. ALKBH10B is An RNA N6-Methyladenosine Demethylase Affecting Arabidopsis Floral Transition. Plant Cell 2017, 29, 2995–3011. [CrossRef] [PubMed]

11. Zhong, S.; Li, H.; Bodí, Z.; Button, J.; Vespa, L.; Herzog, M. MTA is an Arabidopsis messenger RNA adenosine methylase and interacts with a homolog of a sex-specific splicing factor. Plant Cell 2008, 20, 1278–1288. [CrossRef]

12. Nichols, J.L. ‘Cap’ structures in maize poly(A)-containing RNA. Biochim. Biophys. Acta 1979, 576, 490–495. [CrossRef]

13. Kennedy, T.D.; Lane, B.G. Wheat embryo ribonucleases. XIII. Methyl-substituted nucleoside constituents and 5′-terminal dinucleotide sequences in bulk poly (AR)-rich RNA from imbibing wheat embryos. Can. J. Biochem. Cell Biol. 1979, 57, 927–931.

14. Haugland, R.A.; Cline, M.G. Post-transcriptional modifications of oat coleoptile ribonucleic acids: 5′-terminal capping and methylation of internal nucleosides in poly(A)-rich RNA. Eur. J. Biochem. 1980, 104, 271–277. [CrossRef] [PubMed]

15. Li, Y.; Wang, X.; Li, C.; Hu, S.; Yu, J.; Song, S. Transcriptome-wide N6-methyladenosine profiling of rice callus and leaf reveals the presence of tissue-specific competitors involved in selective mRNA modification. RNA Biol. 2014, 11, 1180–1188. [CrossRef]

16. Zhang, G.; Lv, Z.; Diao, S.; Liu, H.; Duan, A.; He, C.; Zhang, J. Unique features of the m6A methylome and its response to drought stress in sea buckthorn (Hippophae rhamnoides Linn.). RNA Biol. 2021, 18, 794–803. [CrossRef] [PubMed]
17. Mao, X.; Hou, N.; Liu, Z.; He, J. Profiling of N6-Methyladenosine (m6A) Modification Landscape in Response to Drought Stress in Apple (Malus prunifolia (Willd.) Borkh.). Plants 2021, 11, 103. [CrossRef]

18. Bodi, Z.; Zhong, S.; Mehra, S.; Song, J.; Graham, N.; Li, H.; May, S.; Fray, R.G. Adenosine methylation in Arabidopsis mRNA is associated with the 3’ end and reduced levels cause developmental defects. Front. Plant Sci. 2012, 3, 48. [CrossRef]

19. Shen, L.; Liang, Z.; Wu, X.; Chen, Y.; Ye, Z.W.N.; Hou, X.; Cai, W.M.; Dedon, P.C.; Liu, L.; Yu, H. N6-Methyladenosine RNA Modification Regulates Shoot Stem Cell Fate in Arabidopsis. Dev. Cell 2016, 38, 186–200. [CrossRef]

20. Wang, Y.; Tang, K.; Zhang, D.; Xie, S.; Zhu, X.; Wang, Z.; Lang, Z. Transcriptome-wide high-throughput put deep m6A-seq reveals unique differential m6A methylation patterns between three organs in Arabidopsis thaliana. Genome Biol. 2015, 16, 272. [CrossRef] [PubMed]

21. Meyer, K.D.; Saletore, Y.; Zumbo, P.; Elemento, O.; Mason, C.E.; Jaffrey, S.R. Comprehensive analysis of mRNA methylation reveals enrichment in 3’ UTRs and near stop codons. Cell 2012, 149, 1635–1646. [CrossRef]

22. Zhao, F.J.; Sun, X.C.; Wu, W.L.; Lu, Q.; Wilson, I.W.; Qiu, D.Y. A comparative analysis of differential N6-methyladenosine (m6A) modification between non-transgenic and LBD15 overexpressing Poplar 84K plants. Tree Genet. Genomes 2021, 17, 39. [CrossRef]

23. Růžička, K.; Zhang, M.; Campilho, A.; Bodi, Z.; Kashif, M.; Saleh, M.; Eeckhout, D.; El-Showk, S.; Li, H.; Zhong, S.; et al. Identification of factors required for m6A mRNA methylation in Arabidopsis reveals a role for the conserved E3 ubiquitin ligase HAKAI. New Phytol. 2017, 215, 157–172. [CrossRef]

24. Lu, Q.; Zhao, F.J.; Macmillan, C.; Wilson, I.W.; Merwe, K.; Hussey, S.G.; Myburg, A.A.; Dong, X.; Qiu, D. Genome-wide analysis of the lateral organ boundaries domain gene family in Eucalyptus grandis reveals members that differentially impact secondary growth. Plant Biotechnol. J. 2018, 16, 124–136. [CrossRef] [PubMed]

25. Altschul, S.F.; Madden, T.L.; Schäffer, A.A.; Zhang, J.; Miller, W.; Lipman, D.J. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic Acids Res. 1997, 25, 3389–3402. [CrossRef] [PubMed]

26. Lu, S.; Wang, J.; Chitsaz, F.; Derbyshire, M.K.; Geer, R.C.; Gonzales, N.R.; Gwandz, M.; Hurwitz, D.I.; Marchler, G.H.; Song, J.S.; et al. CDD/SUPERCLUE: The conserved domain database in 2020. Nucleic Acids Res. 2020, 48, 265–268. [CrossRef] [PubMed]

27. Wilkins, M.R.; Gasteiger, E.; Bairoch, A.; Sanchez, J.C.; Williams, K.L.; Appel, R.D.; Hochstrasser, D.F. Protein identification and analysis tools in the ExPASy server. Methods Mol. Biol. 1999, 112, 531–552.

28. Savojardo, C.; Martelli, P.L.; Fariselli, P.; Profitti, G.; Casadio, R. BUSCA: An integrative web server to predict subcellular localization of proteins. Nucleic Acids Res. 2018, 46, 459–466. [CrossRef] [PubMed]

29. Jiangtao, C.; Yingzhen, K.; Qian, W.; Yuhe, S.; Daping, G.; Jing, L.; Guanshan, L. MapGene2Chrom, a tool to draw gene physical map based on Perl and SVG languages. Yi Chuan 2015, 37, 91–97.

30. 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]

31. Bailey, T.L.; Boden, M.; Buske, F.A.; Frith, M.; Grant, C.E.; Clementi, L.; Ren, J.; Li, W.W.; Noble, W.S. MEME Suite: Tools for motif discovery and searching. J. Mol. Biol. 2010, 399, 65–85. [CrossRef] [PubMed]

32. Berman, H.M.; Westbrook, J.; Feng, Z.; Gililland, G.; Bhat, T.N.; Weissig, H.; Shindyalov, I.N.; Bourne, P.E. The Protein Data Bank. Nucleic Acids Res. 2000, 28, 235–242. [CrossRef]

33. Waterhouse, A.; Berton, M.; Bienert, S.; Studer, G.; Tauriello, G.; Gumienny, R.; Heer, F.T.; De Beer, T.A.P.; Rempfer, C.; Bordoli, L.; et al. SWISS-MODEL: Homology modelling of protein structures and complexes. Nucleic Acids Res. 2018, 46, 296–303. [CrossRef]

34. Castro-Mondragon, J.A.; Ruudavets-Puig, R.; Raulusevicute, I.; Lemma, R.B.; Turchi, L.; Blanc-Mathieu, R.; Lucas, J.; Boddie, P.; Khan, A.; Pérez, N.M.; et al. JASPAR 2022: The 9th release of the open-access database of transcription factor binding profiles. Nucleic Acids Res. 2020, 28, 325–327. [CrossRef]

35. Li, Y.Y.; Yang, Y.F.; Wang, S.; Liu, H.; Qiu, D.Y. Cloning and Expression of Lateral Organ Boundaries Domain Genes (TcLBDs) in Taxus Chinensis. Sci. Silvae Sin. 2015, 51, 126–133.

36. Berman, H.M.; Westbrook, J.; Feng, Z.; Gililland, G.; Bhat, T.N.; Weissig, H.; Shindyalov, I.N.; Bourne, P.E. The Protein Data Bank. Nucleic Acids Res. 2000, 28, 235–242. [CrossRef]

37. Waterhouse, A.; Berton, M.; Bienert, S.; Studer, G.; Tauriello, G.; Gumienny, R.; Heer, F.T.; De Beer, T.A.P.; Rempfer, C.; Bordoli, L.; et al. SWISS-MODEL: Homology modelling of protein structures and complexes. Nucleic Acids Res. 2018, 46, 296–303. [CrossRef]

38. Castro-Mondragon, J.A.; Ruudavets-Puig, R.; Raulusevicute, I.; Lemma, R.B.; Turchi, L.; Blanc-Mathieu, R.; Lucas, J.; Boddie, P.; Khan, A.; Pérez, N.M.; et al. JASPAR 2022: The 9th release of the open-access database of transcription factor binding profiles. Nucleic Acids Res. 2020, 28, 325–327. [CrossRef]

39. Li, Y.Y.; Yang, Y.F.; Wang, S.; Liu, H.; Qiu, D.Y. Cloning and Expression of Lateral Organ Boundaries Domain Genes (TcLBDs) in Taxus Chinensis. Sci. Silvae Sin. 2015, 51, 126–133.

40. Berman, H.M.; Westbrook, J.; Feng, Z.; Gililland, G.; Bhat, T.N.; Weissig, H.; Shindyalov, I.N.; Bourne, P.E. The Protein Data Bank. Nucleic Acids Res. 2000, 28, 235–242. [CrossRef]

41. Waterhouse, A.; Berton, M.; Bienert, S.; Studer, G.; Tauriello, G.; Gumienny, R.; Heer, F.T.; De Beer, T.A.P.; Rempfer, C.; Bordoli, L.; et al. SWISS-MODEL: Homology modelling of protein structures and complexes. Nucleic Acids Res. 2018, 46, 296–303. [CrossRef]

42. Castro-Mondragon, J.A.; Ruudavets-Puig, R.; Raulusevicute, I.; Lemma, R.B.; Turchi, L.; Blanc-Mathieu, R.; Lucas, J.; Boddie, P.; Khan, A.; Pérez, N.M.; et al. JASPAR 2022: The 9th release of the open-access database of transcription factor binding profiles. Nucleic Acids Res. 2020, 28, 325–327. [CrossRef]

43. Bhat, S.S.; Bielewicz, D.; Jarmolowski, A.; Szweykowska-Kulinska, Z. N6-methyladenosine (m6A): Revisiting the Old with Focus on New, an Arabidopsis thaliana Centered Review. Genes 2018, 30, 596. [CrossRef] [PubMed]