





Article

Genome-Wide Analysis, Identification, and Characterization of the PFK Gene Family Members of *Populus deltoides*

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Abstract: Plants produce two phosphorylated enzymes: pyrophosphate-dependent fructose-6-phosphate phosphotransferase (PFP) and ATP-dependent phosphofructokinase (PFK). However, the characterization of phosphofructokinase (PFK) family members and their functions remains unexplored in Eastern Cottonwood (*Populus deltoides*). This study comprehensively analyzed 17 *phosphofructokinase* genes in the *P. deltoides* genome, including their gene structure, conserved domains, evolutionary relationships, and expression patterns. Genome-wide analysis of the poplar genome identified 11 genes belonging to *PdPFK* and 6 genes belonging to *PdPFP*. Phylogenetic, gene structure, and motif analyses revealed two main categories of *PFK* genes: *PFK* with eleven genes and *PFP* with six genes. Quantitative PCR (qPCR) analysis demonstrated that all 17 *phosphofructokinase* genes were induced in various tissues and developmental stages of *P. deltoides*. *PFPs* were strongly expressed throughout *P. deltoides*' growth and development, notably in the roots, according to the transcript analysis. This genome-wide analysis provides insights into *PFK* and *PFP* genes and establishes a foundation for future investigations into the role of genes in *P. deltoides*.

Keywords: PFK gene family; characterization; phylogenetic analysis; gene expression pattern; *Populus deltoides*



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1. Introduction

Glycolysis, which involves the conversion of glucose to pyruvic acid, is a crucial respiratory pathway in plants. Glycolysis provides ATP, reducing agents, and precursors for plant growth and development and helps cells adapt to cold, drought, and anoxia [1–4]. The enzymes PFP and PFK, which phosphorylate fructose-6-phosphate, are found in plants. Fructose-6-phosphate phosphotransferase (PFP, EC 1.7.1.90) catalyzes the phosphorylation of fructose-6-phosphate (F-6-P) into fructose-1,6-bisphosphate (F-1,6-BP) in the glycolytic pathway. It also facilitates the dephosphorylation of F-1,6-BP back to F-6-P in the gluconeogenic direction during primary carbohydrate metabolism in the cytosol [5]. In contrast with ATP-dependent phosphofructokinase (PFK, EC 2.7.1.11), which catalyzes the irreversible phosphorylation of F-6-P to F-1,6-BP, PFP is an enzyme that catalyzes the reversible phosphorylation of F-6-P to F-1,6-BP [4]. In contrast with plants that possess both PFP and PFK [6], bacteria and protozoans have only one type, while yeasts and animals exclusively rely on PFK [1,2,4].

Phosphofructokinase gene families have been extensively studied, and several genes have been identified in various plants. For instance, *Arabidopsis* has 11 PFK genes [7], *Saccharum* has 10 genes [8], *Oryza sativa* has 15 genes [9], and *Pyrus bretschneideri* has 14 genes [10]. PFK plays a central role in plant growth and development, serving multiple functions. The PFK family comprises more than ten members. Interestingly, the activity

of PFP is comparable to or sometimes even surpasses that of PFK, and it is present in diverse plant tissues [11]. The activity and composition of PFP enzymes, including subunits, are influenced by environmental factors, developmental stages, tissue specificity, species variation, and growth conditions [12–14]. In most plant tissues, PFP exists as a heterotetramer composed of two catalytic subunits and two regulatory subunits, typically β_2 and $\alpha_2\beta_2$ [15–17]. The β subunit of PFP acts as a catalyst, while the α subunit controls the catalytic activity of fructose-2,6-bisphosphate (Fru-2,6-BP) [11,18,19]. However, plants have been found to exhibit copy number differences in the genes that encode these subunits [20]. It has been reported that under a variety of circumstances, including anoxia, wounds, phosphate shortage, excessive salt, and dehydration, PFP is engaged in stress responses [2,4,21,22]. However, the specific function and relevance of PFP remain unclear.

Poplar is a representative model tree species that grows rapidly, exhibits strong adaptability, and is widely used for greening, afforestation, and production because of its excellent properties. *P. deltoides*, which is native to the southeastern United States and southern Canada, is a member of the section Aigeiros [23]. In South Korea, the Korea National Commission on Poplars and Other Fast-Growing Trees (KCFGT) reported the establishment of poplar plantations in the reclaimed tidelands of Saemangeum for restoration and timber production between 2013 and 2018. This involved a total of 156.5 hectares and included the planting of *P. euramericana*, *P. deltoides*, and *P. alba* × *P. tremula* var. *glandulosa* (source: <https://www.kcftg.org/>, accessed on 11 May 2023). Currently, *P. deltoides* is widely distributed in the forests of Korea. Genomic information on *P. deltoides* has been previously published [24]. As a species of significant ecological and economic value, it is characterized by rapid growth, resistance to disease, a moderate genome size, and easy experimental manipulation [24].

In this study, we carried out a systematic and thorough analysis of the poplar PFP and PFK families, including chromosomal location analysis, subcellular localization, family member identification and categorization, and phylogenetic analysis, using the published genome as a reference. These investigations serve as a solid foundation for understanding the functions of PFP and PFK in poplar and exploring the underlying regulatory mechanisms.

2. Materials and Methods

2.1. Tree Materials

The experimental material used in this study was the ‘Ay48’ clone of 12-year-old *P. deltoides*. These trees were planted at the National Institute of Forest Science in Korea, with the coordinates 37°15′04″ N, 136°57′59″ E. Plants aged 1 year were propagated from 15 cm long dormant cuttings and grown in pots filled with topsoil in a greenhouse. The greenhouse maintained a temperature range of 18–25 °C. Fresh tissue samples were collected from the poplar trees, immediately frozen in liquid nitrogen, and stored at –80 °C until further extraction of RNA and protein.

2.2. Identification of Phosphofructokinase Family Genes in *P. deltoides*

The *phosphofructokinase* gene family was identified based on the presence of a PFK domain. To identify the members of the *phosphofructokinase* gene family in *Populus* genomes, whole genome and proteome data on *P. deltoides* (var. WV94) genome assembly v2.1 were downloaded from Phytozome version V13 (https://phytozome-next.jgi.doe.gov/info/PdeltoidesWV94_v2_1, accessed on 24 April 2023). The hidden Markov model (HMM) profile of the conserved functional domain of phosphofructokinase (PFK) (PF00365) was obtained from InterPro 93.0 (<https://www.ebi.ac.uk/interpro/>, accessed on 24 April 2023). The HMM profile was then used to screen *Populus* proteomes using the *hmmsearch* software in the HMMER package v3.0 to identify potential members of the PFK gene family. After removing redundant and incomplete sequences, the conserved domain architectures of the recovered sequences were confirmed using the Pfam database in the Conserved Domain Interface (<https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>, accessed on 24 April

2023). Sequences exhibiting the typical functional domain of a phosphofructokinase were considered potential members of the *Populus* phosphofructokinase family.

2.3. Chromosomal Location and Collinearity Analyses of PdPFK Genes

The PFK-domain-containing PdPFPs and PdPFKs were mapped onto the chromosomes based on the poplar genome information from the Phytozome database and visualized using TB tools (ToolBox for Biologists) (Version 1.121) [25]. In addition, the genome data and gene annotation files from Phytozome version V13 (https://phytozome-next.jgi.doe.gov/info/PdeltoidesWV94_v2_1, accessed on 24 April 2023) were downloaded to investigate the syntenic relationship between PFK genes on poplar chromosomes. MCScanX (Multiple Collinearity Scan toolkit X version) [26] was used to detect gene synteny and collinearity using standard Pa parameters. A circular plot was constructed using TBTools (Version 1.121).

2.4. Conserved Motifs and Gene Structure Analysis

Gene features, such as the composition and positions of exons, introns, and conserved elements of PdPFPs and PdPFKs, were visualized by aligning coding sequence regions (CDSs) to genomic DNA sequences in the Gene Structure Display Server (GSDS) (<http://gsds.gao-lab.org/Gsd.sabout.Php>, accessed on 18 April 2023). Motifs were predicted using the online tool Multiple Expectation Maximization for Motif Elicitation (MEME) (<http://memesuite.org/tools/meme>, accessed on 18 April 2023). Based on the protein sequences of all PFK members, the online tool ExPASy (<https://www.expasy.org/tools/>, accessed on 18 April 2023) was used to determine the molecular weight (MW) and theoretical isoelectric point (pI).

2.5. Phylogenetic Analysis and Classification of the Phosphofructokinase Genes

Complete amino acid sequences of PFK from *Arabidopsis thaliana* (TAIR10.1), *Solanum tuberosum* (SolTub_3.0), *Ricinus communis* (ASM1957865v1), and *Oryza sativa* (IRGSP-1.0) were retrieved from the NCBI database. ClustalW (<https://www.genome.jp/tools-bin/clustalw>, accessed on 13 April 2023) was used to perform multiple sequence alignments between the PFK proteins of all five species, including *P. deltoides*. Phylogenetic trees were constructed based on protein alignment using iTOL software. The final phylogenetic tree was obtained using iTOL (<https://itol.embl.de/>, accessed on 13 April 2023) [27], and the output was established for visualization using Adobe Photoshop (Version 2020).

2.6. RNA Extraction and Quantitative PCR (qPCR)

To extract total RNA from poplar tissues, the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) was utilized. Three biological replicates were used for RNA extraction. Subsequently, single-stranded cDNA was synthesized from the RNA samples using the cDNA EcoDry™ Premix (TaKaRa, Shiga, Japan). IQtm SYBR Green Supermix and a CFX96 Touch Real-Time PCR Detection System from Bio-Rad, Hercules, CA, USA were used to conduct qPCR. The qPCR reaction consisted of an initial denaturation step at 95 °C for 3 min, followed by 36 cycles of amplification at 95 °C for 15 s and 60 °C for 60 s. Technical replicates were performed three times using samples derived from three independent biological replicates. The relative transcript abundance was analyzed using the $2^{-\Delta\Delta C_t}$ method [28]. *UBQ7* and *ACTIN* were used as reference genes to normalize the qPCR results [29]. In Supplementary Table S1, the gene-specific primers that were employed are provided.

2.7. Measurement of Phosphofructokinase Content

To determine the phosphofructokinase (PFK) content in homogenized leaf, stem, and root samples, a PFK ELISA kit (MyBioSource, San Diego, CA, USA) was used. We followed the manufacturer's directions for every procedure. After the completion of the substrate reaction, absorbance readings were taken at 450 nm using an automated plate reader (SpectraMax M2, Molecular Devices, San Jose, CA, USA).

2.8. Statistical Analysis

One-way ANOVA was used for the analyses, and Tukey's honest significant difference test (HSD) was used for multiple comparisons. For statistical significance, *p* values of less than 0.05 were considered significant. The results are presented as means with standard deviation (SD).

3. Results

3.1. Identification and Physicochemical Properties of Phosphofructokinase Gene Family in *P. deltooides*

A total of 17 PFK gene family members were downloaded from the InterPro database using PFK-domain-hidden Markov models (PF00365) (as of 28 March 2023) and used as queries to search in the local *P. deltooides* database using the hmmer program [30]. The identified PFK proteins were checked for the presence of redundant proteins using the NCBI Conserved Database (<https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi>, accessed on 18 April 2023). Seventeen PFK family members were found to have conserved domains in all protein sequences, leading to the final identification of PFKs (Table 1). The lengths of 17 PFK proteins, which is a crucial physicochemical characteristic, ranged from 86 amino acids (Podel.01G082600.1.p) to 617 amino acids (Podel.05G274300.1.p), with a mean length of 435 amino acids. The proteins were 9.74 (Podel.01G082600.1.p) to 67.24 (Podel.05G274300.1.p) kDa in relative molecular weight. The isoelectric points in theory ranged from 5.71 to 9.07. More than seven isoelectric points were present in five PFK genes. These proteins were alkaline, whereas the other proteins were acidic.

Table 1. Physicochemical properties of phosphofructokinase family members in *P. deltooides*.

Gene Name	Transcript ID	CDS (bp)	Protein Length (aa)	Molecular Weight (kDa)	Theoretical PI	Gravy	Predicted Localization
Podel.01G054800.1.p	Podel.01G054800.1	672	223	25.08	8.59	−0.437	Nucleus
Podel.01G082600.1.p	Podel.01G082600.1	261	86	9.74	8.21	0.029	Chloroplast
Podel.02G002900.1.p	Podel.02G002900.1	1590	529	57.94	6.78	−0.103	Cytoplasm
Podel.03G163700.1.p	Podel.03G163700.1	1515	504	55.83	7.65	−0.127	Cytoplasm
Podel.04G003100.1.p	Podel.04G003100.1	1686	516	61.28	6.00	−0.131	Peroxisome
Podel.05G274300.1.p	Podel.05G274300.1	1854	617	67.24	6.39	−0.115	Cytoplasm
Podel.06G165500.1.p	Podel.06G165500.1	1659	552	60.69	6.16	−0.217	Chloroplast
Podel.06G165500.2.p	Podel.06G165500.2	1497	498	54.92	5.73	−2.242	Cytoplasm
Podel.06G247900.1.p	Podel.06G247900.1	1443	480	52.72	8.74	−0.222	Cytoplasm
Podel.06G247900.2.p	Podel.06G247900.2	1296	431	47.45	9.07	−0.201	Chloroplast
Podel.07G012400.1.p	Podel.07G012400.1	1599	532	58.34	6.83	−0.112	Cytoplasm
Podel.11G024700.1.p	Podel.11G024700.1	1284	427	47.20	6.24	−0.22	Cytoplasm
Podel.16G002300.1.p	Podel.16G002300.1	1419	472	52.08	5.82	−0.161	Cytoskeleton
Podel.18G067900.1.p	Podel.18G067900.1	1479	492	54.57	5.73	−0.294	Cytoskeleton
Podel.18G067900.2.p	Podel.18G067900.2	1479	492	54.57	5.73	−0.294	Cytoskeleton
Podel.T151900.1.p	Podel.T151900.1	948	315	34.77	6.56	−0.121	Mitochondria
Podel.T152000.1.p	Podel.T152000.1	699	232	24.50	5.71	−0.189	Chloroplast

Molecular weight—molecular weight of the amino acid sequence; PI—isoelectric point of the PdPFKs; grand average of hydropathicity—hydrophilic mean; predicted localization—subcellular localization was predicted by analyzing the sequences.

3.2. Prediction of the Subcellular Localization of Phosphofructokinase Family Members of *P. deltooides*

In silico analysis revealed that Podel.01G054800.1.p is localized in the nucleus, with four proteins in the chloroplasts, seven in the cytoplasm, three in the cytoskeleton, one (Podel.T151900.1.p) in the mitochondria, and one (Podel.T151900.1.p) in peroxisomes. According to the projected subcellular distribution, the cytoplasm hosted 73% of the PFK proteins, chloroplasts hosted 54%, the cytoskeleton hosted 34%, and the nucleus hosted 33.5% (Figure 1, Table 1).

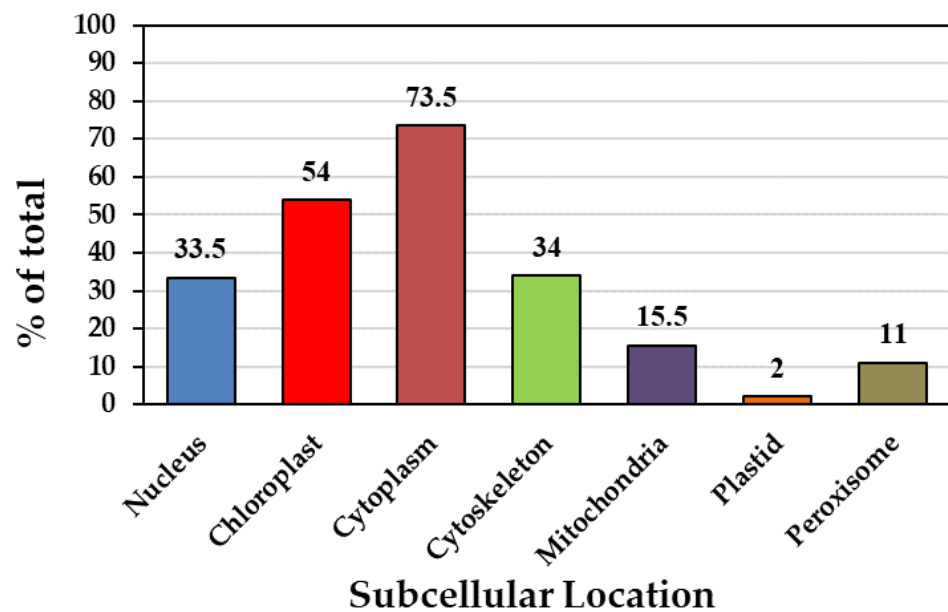


Figure 1. Prediction of subcellular localization.

3.3. Chromosomal Location and Collinearity Analysis of PdPFP and PdPFK Gene Families

We mapped phosphofructokinase proteins on *P. deltoides* chromosomes and found that *PdPFK* genes were mapped on chromosomes Chr01, Chr03, Chr04, Chr05, Chr06, Chr07, and Chr16, whereas six *PdPFPs* were located on Chr02, Chr11, Chr18, and scaffold 380 (Figure 2). Most of the *PdPFKs* (four genes) were mapped on chromosome Chr06 of *P. deltoides*. Chromosome Chr18 and the unplaced Scaffold_380 harbored several *PdPFPs* (two each). Chr01, Chr03–Chr07, and Chr16 were occupied by *PdPFKs*. In general, chromosome length did not positively correlate with gene distribution (Figure 2).

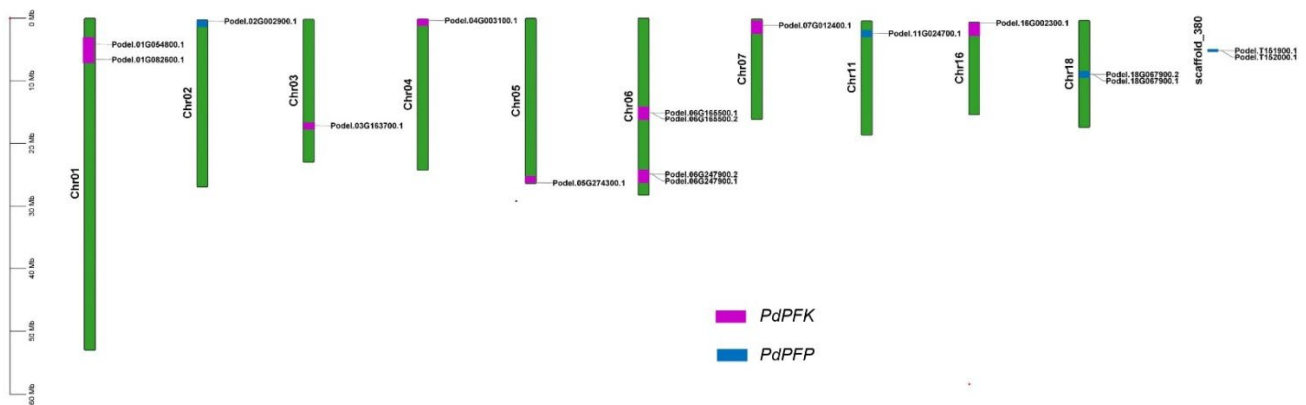


Figure 2. Chromosome location of phosphofructokinase gene family in *P. deltoides*. The length of chromosomes can be estimated with the scale on the side. The chromosome map was constructed using TBTools.

Gene duplication events in *P. deltoides* chromosomes lead to the expansion of gene families. These events were identified using the homology-based BLAST approach in the MCScanX toolkit. At the genome level of *P. deltoides*, 21,626 gene pairs and 1922 groupings of tandem duplication genes were discovered. The identified 17 *PdPFK* genes were unevenly distributed across the 19 chromosomes of *P. deltoides* (Figure 3). Using TBtools, the synteny gene pairs and tandem duplication gene pairs of the *PdPFK* gene family were chosen and visualized. (Figure 3). Using the MCscanX and Advanced Circos software from TBTools, we conducted synteny analysis to comprehend the duplication events of all *PdPFK* genes. On 10 of the 19 chromosomes of *P. deltoides*, there were a total of 85 pairs of duplicated

genes that were scattered irregularly. Additionally, genes scattered on chromosomes 1 and 2 were syntenic with genes on chromosomes 2, 4, 5, and 11 as well as the unplaced scaffold 380. Genes distributed on chromosome 1 were also syntenic with genes on chromosomes 3, 6, and 18 (Supplementary File S1). Overall, the results showed that syntenic genes from chromosomes 1, 6, and 18 were the most shared genes. These results indicate that a duplication event was crucial for the evolution of *PdPFK* genes in *P. deltoides*.

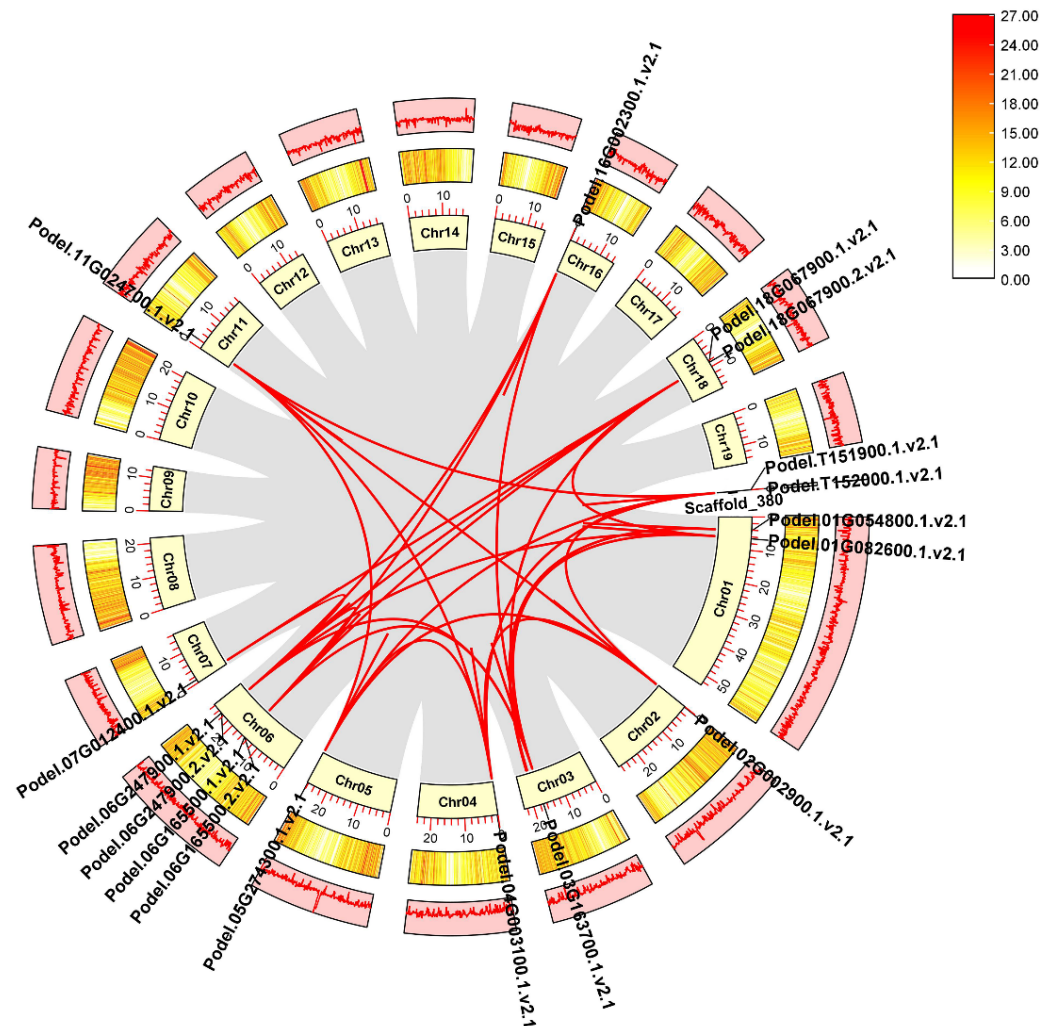


Figure 3. Collinearity analysis between phosphofructokinase genes in *P. deltoides*. The red lines indicate the collinearity of PFK genes in the poplar genome. The inner pale yellow layer represents the poplar chromosomes, where Chr stands for chromosomes, and the chromosome number can be found next to it. The length of each poplar chromosome is expressed in units of Mb with the labels of collinear genes. The middle layer represents the distribution of genes on individual poplar species chromosomes. The third, outermost layer represents the percentage of GC on each chromosome. The collinearity was identified using MCScanX and the circular plot was drawn using TBTools.

3.4. Conserved Motifs and Gene Structure Analysis of the *PdPFP* and *PdPFK* Gene Families

By analyzing the structural exon–intron distribution in *P. deltoides*, the gene architecture of *PdPFPs* and *PdPFKs* was revealed (Figure 4). The gene structure reflects the evolutionary relationship between *PdPFK* gene families. Comparing the length of the genes, *PdPFPs* were mostly longer than other genes, with an average length of greater than 5 kb. In comparison, *PdPFPs* had higher exon numbers than *PdPFKs*, especially Podel.05G274300.1, Podel.02G002900, and Podel.04G003100.1, which contained 19, 18, and 16 exons, respectively. The analysis of the motifs in PFK proteins was carried out using the MEME suite (Figure 5, Table 2). Of the 17 proteins subjected to motif analysis, only 14 showed motif

blocks and 3 did not. A total of ten motifs were analyzed, of which nine were observed in seven proteins. Two pro-proteins lacked motif 8, whereas five proteins only had four motifs. The number of conserved motifs was larger in *PdPFKs* than in *PdPFPs*. A common motif in *PdPFPs* was motif 1.

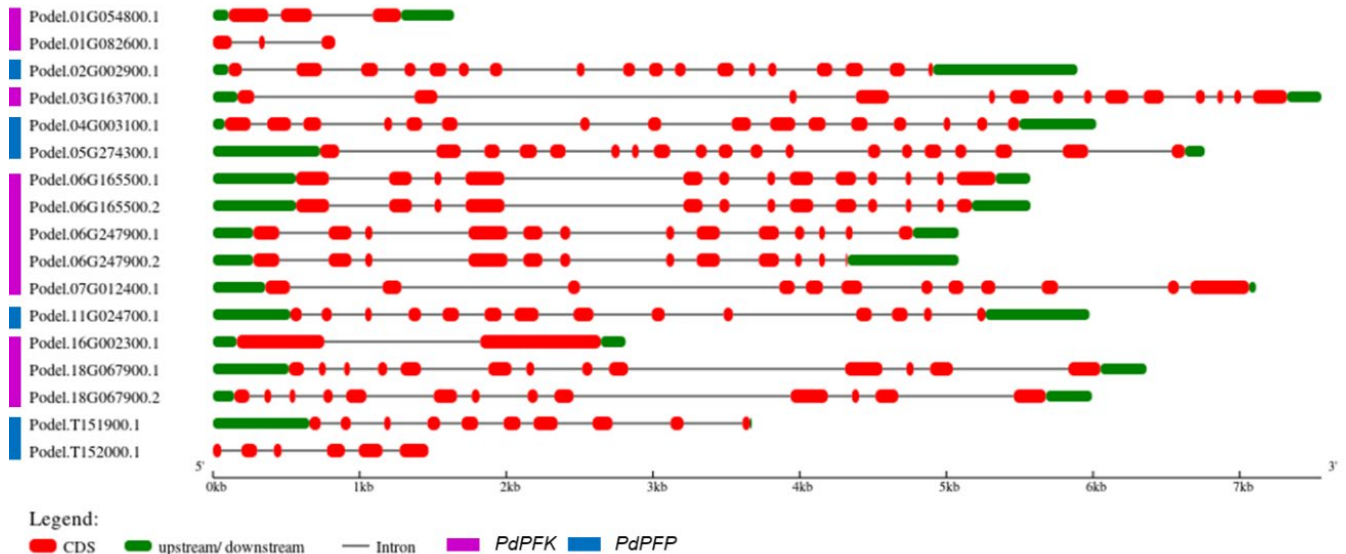


Figure 4. Gene structures of *phosphofruktokinase* gene family in *P. deltooides*. The exon–intron structure was constructed using the Gene Structure Display Server (GSDS 2.0) (<http://gsds.gao-lab.org/>, accessed on 18 April 2023).

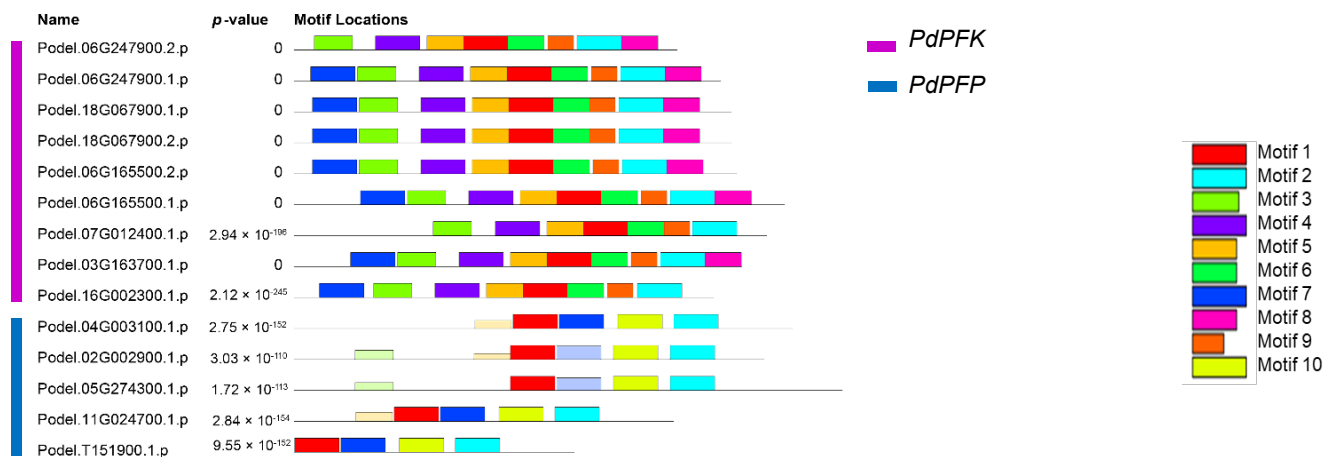












Figure 5. Analysis of conserved *phosphofruktokinase* gene family motifs in *P. deltooides*. Motifs were identified using the MEME suite available online (<https://meme-suite.org/meme/tools/meme>, accessed on 19 April 2023). There are 10 motifs in total, which are shown in different colors. The number of motifs in *PdPFKs* is higher than in *PdPFPs*.

3.5. Phylogenetic Analysis of the *PdPFP* and *PdPFK* Gene Families

The evolutionary analysis and patterns of the PFKs were compared with four species: *A. thaliana*, *R. communis*, *S. tuberosum*, and *O. sativa* (Figure 6). The phylogenetic tree clustered into two groups. ClustalW was used to align the proteins of all five species, including *P. deltooides*. A total of six *PdPFPs* were strongly clustered with four *AtPFPs*, two *RcPFPs*, and four *StPFPs*, while 11 *PdPFKs* were clustered with eight *AtPFKs*, seven *RcPFKs*, and 11 *StPFKs*. The number of *PdPFPs* was higher in *P. deltooides* than in the other species. The phylogenetic tree was constructed using iTOLv6, EMBL.

Table 2. Conserved motif information on phosphofructokinase family members in *P. deltooides*.

Motif	Symbol	Length	Amino Acid Sequence Information
motif 1		50	INAAHVEAESVENGIGLVKLMGRYSGFIAMEATLASRDVDCCLIPESPFY
motif 2		50	YIDPTYMIRAVPPNASDNVYCTLLAQSAVHGAMAGYTGFSTGVVNGRQPY
motif 3		43	HFRRAGPRQKVYFEPDEVRAICIVTCGGLCPGLNTVIREIVYSL
motif 4		50	LTPKVVNDIHKRGGTILGTSRGGHDTSKIVDSIQDRGINQVYIIGDGTQ
motif 5		41	EIRRRGLKVAVAGIPKTIDNDIPVIDRSFGFDTAVEEAQRA
motif 6		41	LEGKGLFEYIEKRLKENGHMVIVIAEGAGQELLESEMOSD
motif 7		50	DVPHLTDYIPLPTYSNPLQDNPAYSVVKQYFVHVDDSVQKIVVHKDSP
motif 8		41	IPFYRINEKQNKVVITDRMWARLLSSTNQPSFISNKEVIED
motif 9		29	ASGNKLLQDVGLWISQGIKDYFSRQKKMA
motif 10		50	NQSLQLFEFLPPAIQEQLMLERDPHGNVQVAKIETEKMLIQMVETELEKR

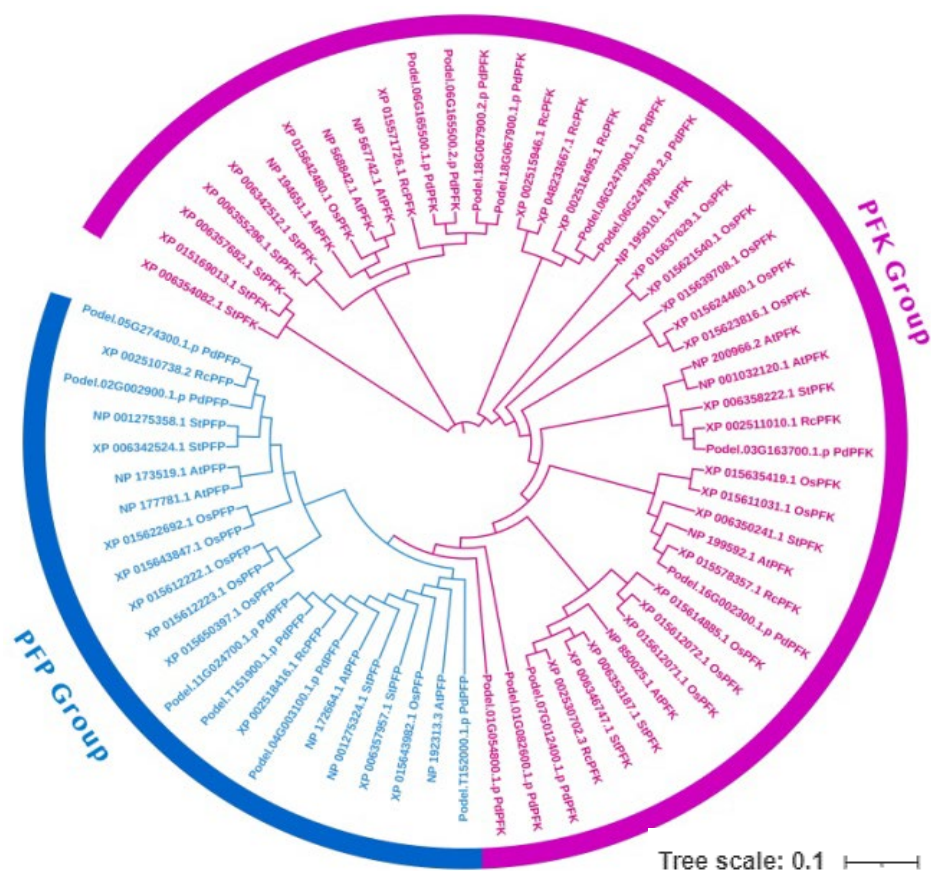


Figure 6. Phylogenetic analysis of phosphofructokinase genes of poplar and four other species (At—*Arabidopsis thaliana*, Rc—*Ricinus communis*, St—*Solanum tuberosum*, and Os—*Oryza sativa*). The two members of the PFK gene family are shown in two different colors. Pink and blue color represent the PFK and PFP members respectively. The phylogenetic tree was constructed using the iTOL server (v6), and the visualization was improved using Adobe Photoshop 2020. The tree scale bar represents 0.1 substitutions per amino acid.

3.6. Analysis of PdPFP and PdPFK Expression Patterns

To investigate the potential role of phosphofructokinase, the PFK content was measured in *P. deltooides*. PdPFK showed high content in all tissues, particularly in roots (Figure 7). To further investigate the expression of PFP and PFK genes in different tissues and ages, transcriptional analysis was conducted in *P. deltooides*. qPCR was utilized to assess gene expression and provide insights into the functions of PdPFP and PdPFK genes in different tissues and developmental stages of poplar. Phosphofructokinase is known to play a role in diverse processes, including growth and the response to biotic

and abiotic stresses [10]. Consistent with the protein assay findings, the expression of all 17 *phosphofructokinase* genes was detected in various tissues and ages of *P. deltoides* (Figure 8). The *PdPFK* genes Podel.02G002900.1, Podel.04G003100.1, Podel.05G274300.1, Podel.11G024700.1, Podel.T151900.1, and Podel.T152000.1 showed similar patterns in different tissues and ages, being more similar to PFK content patterns (Figure 8). This suggests that PdPFK is essential in the glycolytic pathway of poplar.

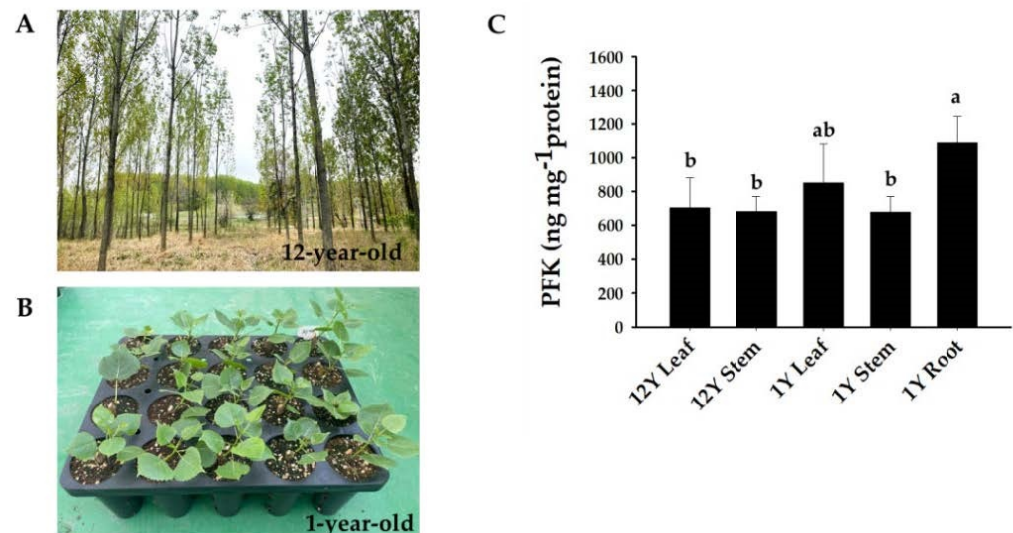


Figure 7. (A) Growth phenotypes of *P. deltoides* trees 12 years after planting. (B) Growth phenotypes of *P. deltoides* one year after planting. (C) Analysis of the phosphofructokinase (PFK) contents in different tissues (leaf, stem, and root) and ages (12-year-old and 1-year-old trees) of *P. deltoides*. The data are represented as means \pm SD of triplicate experiments. Significant differences are denoted with different lowercase letters (ANOVA with Tukey's HSD, $p < 0.05$).

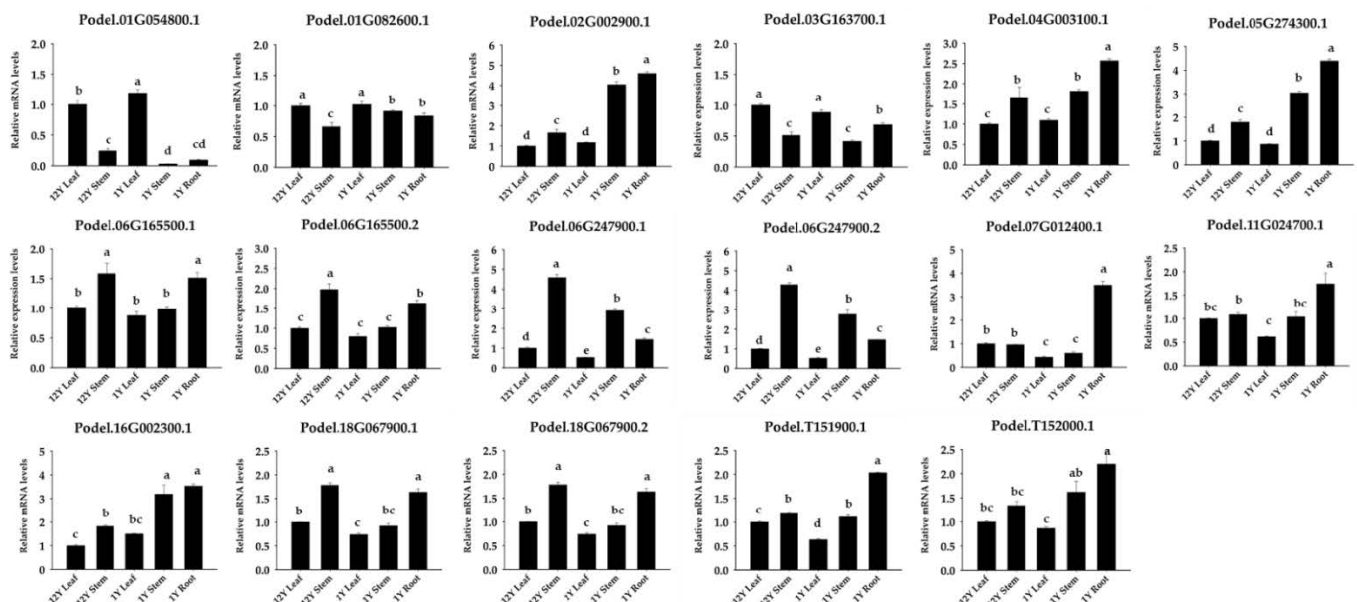


Figure 8. Expression analysis of 17 *phosphofructokinase* genes in different tissues (leaf, stem, and root tissues) and ages (12-year-old and 1-year-old trees) of *P. deltoides*. The statistically significant differences between expression levels of *phosphofructokinase* genes were tested using Tukey's test ($p < 0.05$). Error bars indicate the standard deviation calculated from three biological replicates. Different lowercase letters indicate significant differences ($p < 0.05$).

This study confirmed the tissue-specific expression patterns of *phosphofructokinase* genes. Podel.01G054800.1, Podel.01G082600.1, and Podel.03G163700.1 were expressed specifically in leaves, while Podel.06G247900.1 and Podel.06G247900.2 were expressed specifically in stems. In addition, Podel.05G274300.1, Podel.07G012400.1, Podel.11G024700.1, and Podel.T151900.1 were expressed specifically in roots. Podel.02G002900.1, Podel.05G274300.1, Podel.16G002300.1, and Podel.T152000.1 showed similar patterns of strong expression in one-year-old stems and roots (Figure 8). These genes may be used for the production of compounds that are beneficial for specific plant organs.

4. Discussion

Phosphofructokinase is essential in plant growth, metabolism, and stress responses; however, further research is required to confirm its exact mechanism and function. In particular, it has yet to be reported in poplar, which is a tree model plant. As far as we are aware, this study is the first to identify phosphofructokinase in *P. deltoides* through genome analysis.

We identified 17 *PFK* gene families in *P. deltoides*, with most *PFK* genes (11 genes) and only 6 genes belonging to the *PFP* subfamily. A similar division of the gene family into *PFK* and *PFP* groups was found in the other plants studied. The *PFK* gene family in *A. thaliana* consists of eleven members, with four members associated with *AtPFP* and seven members associated with *AtPFK*. Five of the fifteen *PFK* genes found in rice belonged to *OsPFP* and ten to *OsPFK*. Ten of the fourteen members of the *PFK* family in white pears (*P. bretschneideri*) are *PbPFKs*, while the other four are *PbPFPs* [31]. In cotton, 56 *PFKs* and 24 *PFPs* have been detected in 4 *Gossypium* species [32].

Based on our analysis of the subcellular localization of *PFK* genes, we found that of the six *PdPFPs*, three *PdPFPs*, and most *PdPFKs*, were localized in the cytoplasm. Four *PdPFKs* and one *PdPFP* were localized in the chloroplasts. It was hypothesized that five *MePFKs* are localized in the chloroplasts, two in the cytoplasm, and four in the cytoplasm of *Manihot esculenta*. To provide evidence for this assertion, *MePFK03* and *MePFP1*, which are presumed to be localized in the chloroplast and cytoplasm, respectively, were selected for the production of GFP fusion proteins. The localization of *PFK* and *PFP* in the cytoplasm and chloroplasts was confirmed using GFP images. Therefore, most *PFKs* and *PFPs* are expected to be in the cytoplasm and chloroplasts [32].

Under normal growth conditions, the cellular sugar status is essentially maintained but severely affected by numerous environmental stresses. One such negative environmental stress is drought, which reduces the cell's osmotic potential and hampers the photosynthetic fixation of carbon and the transportation of sugars. A specific category of proteins, referred to as sugar transporters, plays a crucial role in facilitating the transportation of cellular sugars. The transporter proteins play vital roles in regulating the influx and efflux of diverse sugars and their metabolic intermediates, thereby facilitating plant growth and development. Abiotic stresses, particularly drought stress, lead to the reprogramming of sugar distribution within cellular and subcellular compartments [33]. *PFP* is tightly controlled; however, its purpose remains unknown. There is no evidence that *PFP* controls glycolysis in transgenic potato plants, where *PFP* activity is reduced by more than 90%. However, *PFP* is implicated in the capacity of plants to adapt to adverse conditions, such as phosphate or oxygen deficiency [7].

In this study, we confirmed that *PdPFK* and *PdPFP* show tissue- or development-specific expression. We specifically looked into the function of *PdPFPs* during *P. deltoides'* developmental stages. Examination of the expression of *PFKs* and *PFPs* clearly showed that compared with *PdPFKs*, the expression of *PdPFP* was higher in developing *P. deltoides*. We found that the expression of *PdPFPs*, such as Podel.02G002900.1, Podel.04G003100.1, Podel.05G274300.1, Podel.11G024700.1, Podel.T151900.1, and Podel.T152000.1, was higher in roots than in other tissues. Previous studies have documented that *PdPFPs* are upregulated in conjunction with heat shock proteins (*HSPs*) when maize endosperm is subjected to heat, cold, and unfolded protein response stresses [34]. In *Arabidopsis*, the expression of

PFPA1 was found to be higher than *PFPA2* in leaves, flowers, and roots, while the *PFPB2* gene exhibited predominant expression in leaves [35]. Moreover, *AtPFPA2* had a trichome-specific expression promoter [36]. The expression levels of *PdPFK* and *PdPEK* in the stems differed according to age and developmental stage. In particular, the expression levels of Podel.02G002900.1 and Podel.06G165500.2 were more than twice as high according to age.

5. Conclusions

This study provided a bioinformatic analysis to identify 17 *phosphofructokinase* genes, which were further classified into 2 subfamilies based on phylogenetic analysis. The *PFK* subfamily comprised 11 genes, while the *PFPA* subfamily consisted of 6 genes. The subfamilies exhibited relatively conserved characteristics in terms of motif composition and exon/intron structures. The conserved motif 1, composed of 50 amino acids, was the center, and 8 motifs of *PFK* and 4 motifs of *PFPA* constituted the protein. *Phosphofructokinase* was unevenly distributed in the chromosomes and showed high synteny, especially on chromosomes 1, 6, and 18. Additionally, the expression patterns of *PFKs* in various tissues of *P. deltoides* were analyzed, revealing high gene expression and protein concentrations in the roots. This study establishes a foundation for investigating the *PdPFK* and *PdPFPA* gene families in poplar and provides valuable insights into their molecular mechanisms.

Supplementary Materials: The following supporting information can be downloaded from <https://www.mdpi.com/article/10.3390/f14061104/s1>: Table S1: Sequences of primers used for qPCR in *P. deltoides*; File S1: Collinearity of *P. deltoides*.

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