Article

Transcriptome Sequencing Analysis of Birch (*Betula platyphylla* Sukaczew) under Low-Temperature Stress

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Abstract: Low temperature is one of the common abiotic stresses that adversely affect the growth and development of plants. In this study, we used RNA-Seq to identify low-temperature-responsive genes in birch and further analyzed the underlying molecular mechanism. Birch seedlings were treated by the low temperature (6 °C) for 0, 1, 1.5, 2, 2.5, and 3 h, respectively. A total of 3491 genes were differentially expressed after low-temperature stress. Gene Ontology (GO) and Kyoto Encyclopedia of Gene and Genomes (KEGG) functional enrichment analysis were performed for the differentially expressed genes (DEGs). GO analysis indicated that 3491 DEGs were distributed into 1002 categories, and these DEGs were enriched in “cell process”, “metabolic process”, and “stimulus response”, under the “biological process” category; in “organelles” and “cell components”, under the “cell component” category; and in “catalytic activity” and “adhesion”, under the “molecular function” category. The KEGG enrichment indicated that 119 DEGs were involved in Ca^{2+} and plant hormone signal transduction; 205 DEGs were involved in secondary metabolic processes, such as lipid metabolism and phenylpropanoid biosynthesis pathway; and 20 DEGs were involved in photosynthesis. In addition, a total of 362 transcription factors (TFs) were differentially expressed under low-temperature stress, including AP2/ERF, C2H2, MYB-HB-like, WRKY, bHLH, WD40-like, and GRAS families. Gene *Bpev01.c0480.g0081* (calmodulin-like CML38), *Bpev01.c1074.g0005* (calmodulin-like CML25), *Bpev01.c1074.g0001* (Calcium-binding EF-hand family protein), *Bpev01.c2029.g0005* (calmodulin-like protein), *Bpev01.c0154.g0008* (POD), *Bpev01.c0015.g0143* (N-acetyl-1-glutamate synthase), and *Bpev01.c0148.g0010* (branched chain amino acid transferase) were up-regulated at a high level, under low-temperature stress.

Keywords: *Betula platyphylla*; low-temperature stress; transcriptome; differentially expressed genes

1. Introduction

Low temperature is one of the adverse environmental stresses which affects the growth, development, and geographical distribution of plants. Plants have evolved sophisticated molecular mechanisms to deal with low-temperature stress [1], such as synthesis of cryoprotectants [2], scavenging of reactive oxygen species [3], and cold acclimation [1].

The cell membranes of plants first recognize the low-temperature signal and transmit it into intracellular parts [4]. Low-temperature stress changes the cell membrane from the liquid state to the gel state. This leads to a decreased fluidity, increased permeability, and changes in the structure of membrane proteins, which in turn cause metabolic disorders of plant cells [4]. For example, the Ca^{2+} channel on the cell membrane is opened under low temperature, and the Ca^{2+} concentration in the membrane is rapidly increased [5]. Calmodulin (CaM) transmits the Ca^{2+} flow signals and

subsequently affects the expression of downstream genes and activates the MAPK pathway, as well [6]. At the same time, low-temperature stress destroys the membrane structure of plastid and affects photosynthetic electron transfer and energy metabolism [7,8]. The disorder of photosystem II (PSII) system leads to the accumulation of reactive oxygen species (ROS) in the plastid [9]. The excessive ROS will result in oxidative damage to biomolecules, including cell membranes, proteins, and DNA [6]. Meanwhile, low temperature will induce the accumulation of protective proteins, including heat-shock proteins (HSPs) [10], cold-regulatory proteins (COR), dehydrated proteins, cold-protected proteins [1], and various metabolites [11].

The key expression regulators of low-temperature-responsive genes in plants have been reported. C-repeat/dehydration-responsive element binding factors (CBFs) are the first identified transcription factors that regulate the expression of low-temperature-responsive genes in plants [12]. CBFs bind to the C-repeat/dehydration-responsive elements in promoters of cold-responsive genes [12,13]. Inducer of CBF expression 1 (ICE1) is a MYC-type bHLH transcription factor, which activates the expression of CBF genes by directly binding to their promoters [14]. ICE1 can interact with SNOW1, which activates CBF3 to adjust the tolerance of plants under low temperature [15]. A R2R3-MYB protein encoded by MYB15 can bind to promoters of CBF, to prevent their expression [16]. Ethylene insensitive 3 (EIN3) is a key transcription factor involved in transduction of ethylene signaling, which inhibits the expression of CBFs and is a negative regulator of low-temperature resistance [17].

In recent years, with the rapid development of sequencing technology, a large number of experiments have been carried out to study the mechanism of plants under low temperature, using high-throughput sequencing [18–23]. The RNA-Seq studies found that genes related to transcription factors [24], Ca^{2+} signaling [25], plant-hormone signaling [26], phenylalanine biosynthesis [27], starch and sucrose metabolism [28], and protein metabolism pathways may play important roles in the response to low-temperature stress. Birch is one of the most common tree species in the northern hemisphere [29]. In this study, we used RNA-Seq to identify the low-temperature-responsive genes in birch within a short time frame and analyze the responsive mechanism of birch exposed to low-temperature stress.

2. Materials and Methods

2.1. Plant-Material Treatment and RNA Isolation

Two-month-old birch (*Betula platyphylla* Sukaczew) were grown and maintained in the greenhouse, under a 16 h light/8 h dark photoperiod, at 25 °C, at Northeast Forestry University. For low-temperature stress treatment, plant materials were transferred to 6 °C and harvested after 1, 1.5, 2, 2.5, or 3 h treatment, for RNA sequencing. Plants grown at normal condition (0 h time point) were used as controls. Two biological replicates were performed for each time point, except for 1.5 h, and each biological replicate included at least ten individual plants. For 1.5 h, three cDNA libraries were qualified for RNA sequencing. Before RNA isolation, all the samples were cleaned with sterile water. Total RNAs of all the samples were extracted, using the CTAB [30] method. The quality and concentration of RNA were examined by NanoDrop 2000. The integrity of the RNA samples was examined with an Agilent 2100. The cDNA library was constructed at Beijing Biomarker Technology and sequenced on Illumina platform, with a 150 bp paired-end read length. The transcriptome data obtained in this study were submitted to the NCBI SRA database which is a database for storing the original data of second-generation sequencing. (NCBI accession number: PRJNA532995).

2.2. Transcriptome Analysis

Clean reads were obtained by filtration of low-quality reads, using fastp [31]. All the clean reads were mapped to the birch genome (v1.4c) [32], using HISAT2, with default parameters [33]. Differentially expressed genes (DEGs) between the control group and each treatment group were identified, using DEG discovery pipeline HTSFilter [34] and edgeR, with thresholds of false discovery rate (FDR) < 0.05

and $|\log_2$ of fold change| > 1 [35]. The genes expression abundance was normalized by FPKM (number of fragments per kilobase of transcript per million map reads) method. The hypergeometric test was used for GO (Gene Ontology) enrichment analysis. The Bonferroni method was used to correct the p -value. KOBAS2.0 [36] software was used to detect the significantly enriched Kyoto Encyclopedia of Gene and Genomes (KEGG) pathways. BLASTx (E-value < 1.0×10^{-5}) [37] was used for functional annotation of birch genome, using RefSeq non-redundant proteins (NR) [38], Swiss-Prot [39], and Kyoto Encyclopedia of Genes and Genomes (KEGG) [40] as databases. The transcription factors (TFs) were annotated, using a plant TFs database (<http://plntfdb.bio.uni-potsdam.de/v3.0/>).

2.3. Quantitative RT-PCR Analysis

The HiFiScript cDNA Synthesis Kit was used to reverse transcribe the extracted RNA samples for fluorescence quantitative detection [41]. Applied Biosystems 7500 Real-Time PCR System was used to perform qRT-PCR. A total of 12 DEGs were selected to validate the RNA-Seq results. The 18S of birch was used as an internal reference gene. All the primers were designed by using primer 3 software. The primer sequences are listed in Table A1.

3. Results

3.1. Statistics of Transcriptome Sequencing Data

A time series RNA-Seq experiment was carried out to reveal the transcriptomic response of birch to low-temperature stress, within a short time. Young leaves of birch exposed to 4 °C for 0, 1, 1.5, 2, 2.5, and 3 h were harvested for RNA sequencing on Illumina platform. After the filtration of low-quality reads, an average of 25,120,872 reads was obtained for each sample. The detailed information of the sequencing of reads is shown in Table A2. The results indicated that the obtained sequencing reads are qualified for subsequent analysis.

3.2. Analysis of Differentially Expressed Genes (DEGs)

In order to obtain low-temperature-responsive genes in birch, we first used HISAT2 [33] to align the sequencing reads to the birch reference genome. DEGs in the treatment groups relative to the control group were identified by using edgeR [42], with thresholds of FDR ≤ 0.05 and absolute value of $\log_2$ (fold-change) > 1. The results showed that 3197 (1656 up-regulated and 1541 down-regulated), 881 (494 up-regulated and 387 down-regulated), 79 (67 up-regulated and 12 down-regulated), 859 (470 up-regulated and 389 down-regulated), and 451 (378 up-regulated and 73 genes down-regulated) genes were differentially expressed after 1, 1.5, 2, 2.5, and 3 h exposure to low temperature, respectively (Table 1). In total, 3491 genes were differentially expressed in at least one time point.

Table 1. Differentially expressed genes (DEGs) under different low-temperature stress time points.

DEGs	1 h	1.5 h	2 h	2.5 h	3 h	Total
All_DEGs	3197	881	79	859	451	3491
Up-regulated	1656	494	67	470	378	1764
Down-regulated	1541	387	12	389	73	1727

3.3. Gene Ontology (GO) and KEGG Enrichment of the DEGs

In order to reveal the biological processes underlying the DEGs, we performed GO enrichment analysis for the 3491 DEGs. Using FDR values ≤ 0.05 as a threshold, a total of 1002 GOs of cellular component, molecular function, and biological process were identified as enriched GO terms (Figure 1).

Figure 1. Function annotation of the DEGs in Gene Ontology. The x-axis is the Gene Ontology (GO) terms, and the y-axis is the percent of genes in each GO term. The blue columns represent the percentage of DEGs in each GO term, and the red columns represent the percentage of reference in each GO term. This figure shows the enrichment of DEGs and reference.

In the category of biological process, a total of 610 GOs were enriched. GOs such as GO:0009987 (cell process), GO:0008152 (metabolic process), and GO:0050896 (response to stimulus) were significantly enriched (Figure 1). The results indicate that genes involved in these biological processes may play an important role in the response to low-temperature stress. Biological processes such as GO:0008152 (metabolic process), GO:0044237 (cell metabolic process), GO:0006793 (phosphorus metabolic process), GO:0006796 (phosphate metabolic process), GO:0016310 (phosphorylation), and GO:0006468 (protein Amino acid phosphorylation) were enriched as well (Figure A1). In the category of cell components, a total of 131 GOs were enriched. GOs including GO:0044464 (cell component) and GO:0043226 (organelle) were enriched (Figures 1 and A2). In the category of molecular function, a total of 261 GOs were enriched. Among them, GOs such as GO:0003824 (catalytic activity) and GO:0005488 (binding activity) were enriched (Figure 1). In addition, GO:0016740 (transferase activity), GO:0016772 (transferase activity, transfer phosphorus-containing groups), GO:0016301 (kinase activity), GO:0004672 (protein kinase activity), and GO:0004674 (protein serine/threonine kinase activity) were significantly enriched (Figure A3).

KEGG pathway enrichment analysis of the DEGs was performed by KOBAS 3.0. All DEGs were allocated to 121 metabolic pathways, 47 of which were significantly enriched ($p \leq 0.05$) (Table A3). The results indicated that the DEGs were mainly enriched in metabolic pathways, biosynthesis of secondary metabolites, ribosomes, amino acid biosynthesis, carbon metabolism, phenylpropanoid biosynthesis, plant hormone signal transduction, plant pathogenic bacteria interaction, and starch and sucrose metabolism. These enriched pathways may play important roles in the process of birch in response to low-temperature stress (Figure 2).

Figure 2. Pathway assignment of DEGs based on Kyoto Encyclopedia of Gene and Genomes (KEGG).

3.4. Analysis of DEGs Related to Calcium Signal Transduction Pathway

The calcium signal transduction pathway plays a vital role in perceiving the low-temperature stress of plants. In this study, a total of 50 DEGs related to calcium signal transduction were identified, of which 40 were up-regulated. The up-regulated genes include four calcium-dependent protein kinase (CDPK) genes, nine calmodulin-like protein (CML) genes, three calmodulin-binding protein-like genes, and four CBL-interacting protein kinase (CIPK) genes. The expression levels of these genes were significantly up-regulated after low-temperature treatment (Table A4). In addition, six EF-hand calcium-binding proteins (calcium-binding EF-hand family protein) were differentially expressed, of which five were up-regulated. Of the eight calcium-dependent lipid-binding (CaLB domain) family proteins, seven were up-regulated. All the results indicated that genes in the calcium signal transduction pathway of birch respond positively to low-temperature stress.

3.5. Analysis of Hormone and Signal Transduction Pathways-Related DEGs

Under low-temperature stress, the expression of genes related to plant hormone biosynthesis or signal transduction were significantly affected (Table A5). According to the KEGG results, we found that 23 genes related to abscisic acid (ABA) biosynthesis were differentially expressed, of which 15 were up-regulated. *Bpev01.c0268.g0003*, encoding a 9-cis-epoxy carotenoid dioxygenase, which is an important enzyme in ABA biosynthesis, was up-regulated under low-temperature stress. In addition, we identified 15 DEGs encoding protein phosphatase 2C (PP2C), and 11 of them were up-regulated. Studies have shown that PP2C in plants may play a role in the activation of signaling pathways by environmental stresses, such as cold damage [43]. An ABA 8'-hydroxylase encoding gene (*Bpev01.c0870.g0008*) was up-regulated by 4.01 times. *Bpev01.c0015.g0010*, encoding ABA1, which converts zeaxanthin to antheraxanthin and violaxanthin through the epoxidation, was up-regulated under the low-temperature stress.

Most ethylene-signal-related genes in birch were up-regulated under low-temperature stress. We identified a total of 12 ethylene-signal-transduction-related genes, including one ethylene synthase encoding gene (*Bpev01.c0299.g0032*), one ethylene insensitive 3 gene (EIN3, *Bpev01.c0523.g0001*), and ten ethylene response transcription factors (BpERFs). In addition, five DEGs related to the gibberellin signaling pathway were identified. Table A5 shows that the differential expression of the five genes (*Bpev01.c0118.g0033*, *Bpev01.c0094.g0034*, *Bpev01.c1673.g0004*, *Bpev01.c0411.g0004*, and *Bpev01.c1170.g0013*) may cause a decrease in gibberellin content.

3.6. Identification of DEGs Related to Starch and Sucrose Metabolic Pathways

When exposed to low-temperature stress, many metabolic processes of plants were affected. The enrichment analysis of GO and KEGG indicated that genes related to metabolism and biosynthesis were significantly differentially expressed. A total of 29 DEGs related to the “starch and sucrose metabolism” (Ko00500) pathway were identified, of which 15 were up-regulated and 14 were down-regulated (Table A6). These DEGs include five glucosidase genes, of which four were up-regulated and two up-regulated amylase genes. The pfkB-like carbohydrate kinase genes *Bpev01.c0080.g0100* and *Bpev01.c0762.g0008* were up-regulated by 4.58 times and down-regulated by 1.29 times, respectively. However, the reason for this remains to be further studied. The eight glycosyl hydrolase genes were significantly down-regulated, and one DEGs encoded to trehalose-6-phosphate synthase were up-regulated. Let us notice that the sugar contents in plants under low-temperature stress may increase, in this case.

3.7. Identification of DEGs Related to Lipid Metabolism

A total of 109 DEGs related to lipid metabolism were identified in this study, most of which were related to oxidation of lipids, biosynthesis, and metabolic processes of lipids and fatty acids (Table A7). The *Bpev01.c0190.g0079* gene encodes UDP-sulfoquinolone and participates in the biosynthesis of sulfide lipid, the *Bpev01.c1135.g0005* gene encodes a long-chain acyl-CoA synthetase, and the *Bpev01.c0364.g0019* gene encodes monogalactosyldiacylglycerol (MGDG) synthase; and these three genes were up-regulated in birch, under low-temperature stress. In addition, a total of 20 GDSL-like esterases/lipases were identified, including one up-regulated and 19 down-regulated DEGs, and the most expression level for down-regulated was up to 4.92 times of the control group. GDSL lipase is a multifunctional family of hydrolases that can hydrolyze a variety of substrates, such as thioesters, aryl esters, phospholipids, and amino acids [43]. In addition, the *Bpev01.c0569.g0008* gene encodes a plant ene desaturase, and it was also up-regulated. A total of three genes (*Bpev01.c1627.g0007*, *Bpev01.c2716.g0001*, and *Bpev01.c2716.g0001*) encode terpene synthase and were down-regulated.

3.8. Identification of DEGs Related to Amino Acid Biosynthetic Pathway

Low-temperature response of amino acids to plants is also important, such as the “amino acid biosynthesis” pathway (ko01230). A total of 40 DEGs were found in this pathway, including 17 up-regulated, such as the gene *Bpev01.c0015.g0143*, which encodes the N-acetyl-l-glutamic acid synthase and was up-regulated by 9.63 times (Table A8). In addition, six gene were also up-regulated, including one encoding pyridoxal-5'-phosphate-dependent enzyme (*Bpev01.c0112.g0007*), two encoding aldolase (*Bpev01.c0511.g0007* and *Bpev01.c0115.g0053*), one encoding ATP phosphotransferase (*Bpev01.c0274.g0001*), one encoding branch-chain amino acid transferase (*Bpev01.c0148.g0010*), and one encoding cysteine synthase (*Bpev01.c0137.g0017*) (Table A8). Gene *Bpev01.c3196.g0001* and *Bpev01.c0652.g0026* were down-regulated which encodes the dihydropyridine carboxylic acid reductase and shikimate kinase, respectively (Table A8).

3.9. Identification of DEGs Related to Photosynthesis

A total of 21 DEGs related to photosynthesis were identified, including 13 up-regulated and eight down-regulated (Table A9). Among this, genes (*Bpev01.c1891.g0002* and *Bpev01.c1891.g0006*)

that encoded photosystem II reaction centers A and C were up-regulated by 4.02 and 3.49 times, respectively. The gene (*Bpev01.c1891.g0007*) encoded photosystem I PsaA/PsaB protein was up-regulated by 3.05 times. The gene (*Bpev01.c0154.g0066*) encoded ferritin 1 was up-regulated by 4.19 times. Genes encoded phosphoenolpyruvate carboxykinase (*Bpev01.c1286.g0001*), photosystem II subunit R (*Bpev01.c1275.g0002*), and chloroplast-sensing kinase (*Bpev01.c0038.g0100*) were also up-regulated. Six genes were down-regulated, including one encoding chlorophyll A/B binding protein (*Bpev01.c0615.g0010*), one encoding ferredoxin 3 (*Bpev01.c0536.g0012*), one encoding tubulin FtsZ (*Bpev01.c0327.g0054*), one encoding membrane localization protein MinD (*Bpev01.c0298.g0026*), and two encoding the PsbP family proteins of the photosystem II reaction center (*Bpev01.c0118.g0021* and *Bpev01.c0142.g0012*).

3.10. Transcription Factors Involved in Low-Temperature Response

Transcription factors (TFs) play important roles in transcriptional regulation of plants under abiotic stress. TFs are also the core nodes that initiate downstream genes. In this study, a total of 362 TFs were found to be classified into 58 families, including the AP2/EREBP, C2H2, MYB-HB-like, WRKY, bHLH, bZIP, WD40-like, and GRAS families (Table 2). In the 1 h sample, 276 TFs that were classified into 51 TFs families were differentially expressed; among all the families, the C2H2 family contained the maximum DEGs, adding up to 53 TFs. It was followed by the AP2-EREBP family (29 TFs), MYB-HB-like families (21 TFs), NAM families (18 TFs), and WD40-like families (16 TFs). In the 1.5 h sample, 112 TFs belonged to 31 TFs families, including the AP2-EREBP family (25 TFs), Hap3/NF-YB family (13 TFs), and C2H2 families (11 TFs), MYB-HB-like family (7 TFs), Znf-B family (6 TFs), and NAM family (5 TFs). In the 2 h sample, 25 TFs belonged to seven TFs families; among them, the AP2-EREBP family has 14 TFs, the C2H2 family has six TFs, and the C3H, PHD, RAV, AUX-IAA, and Bromo-domain families have one TF, respectively. In the 2.5 h sample, 120 TFs belonged to 35 TFs families, including 24 AP2-EREBP, 16 C2H2, 10 Hap3/NF-YB, seven MYB-HB-like, six NAM, five GRAS, and five WRKY transcription factor genes. In the 3 h sample, 78 TFs belonged to 20 TFs families, including 24 AP2-EREBP, 14 C2H2, six MYB-HB-like, five WRKY, four Znf-B, three GRAS, and three NAM transcription factor genes.

Table 2. Differentially expressed transcription factors (TFs) under different low-temperature stress time points.

TF Family	Total	1 h	1.5 h	2 h	2.5 h	3 h
C2H2	58	53	11	6	16	14
AP2-EREBP	42	29	25	14	24	24
MYB-HB-like	27	21	7	0	7	6
NAM	20	18	5	0	6	3
WD40-like	20	16	5	0	4	0
WRKY	16	15	2	0	5	5
bHLH	15	12	1	0	2	1
Hap3/NF-YB	15	1	13	0	10	0
PHD	14	10	2	1	0	0
Homobox-WOX	10	10	3	0	1	2
bZIP	8	8	0	0	0	0
GRAS	8	7	4	0	5	3
Znf-B	7	5	6	0	4	4
C2C2-Dof	6	4	1	0	2	2
C2C2-GATA	6	4	1	0	2	2
BTB-POZ	5	4	1	0	2	0
C3H	5	4	2	1	3	2
C3H-WRC/GRF	5	2	3	0	3	0
HSF-type-DNA-binding	5	4	3	0	3	2
A20-like	4	4	1	0	2	0
HD-ZIP	4	2	2	0	2	1

Although the C2H2TFs family had the most DEGs (53) at the 1 h sample, the AP2/ERF family contained the most DEGs at the other four samples. Interestingly, 13 AP2/ERF family genes were up-regulated in all samples, which are the most among all the TFs families (Table A10).

3.11. Validation of RNA-Seq Based DEGs Results by qRT-PCR

To verify the accuracy of the RNA-Seq data, we analyzed the transcript abundance of the 12 randomly selected DEGs, using qRT-PCR. All 12 up-regulated genes were significantly induced by the low-temperature stress, which was consistent with the results of RNA-Seq data (Figures 3 and 4). This result confirmed the reliability of RNA-Seq analysis.

Figure 3. Validation of the RNA-Seq results by qRT-PCR. A total of 12 DEGs were selected for the validation.

Figure 4. Expression analysis of selected DEGs, using qRT-PCR.

4. Discussion

The research led us to find that, in the biological process under low temperatures, DEGs were abundantly enriched in the protein phosphorylation pathway downstream of the metabolic pathway. Protein phosphorylation pathways can regulate and control protein activity and function in order to better play the role of protein under low-temperature stress.

4.1. Ca^{2+} Signal Transduction under Low Temperature

Ca^{2+} is the second messenger in plant cells which plays an important role in many signaling pathways. Fluctuations in calcium levels can be monitored by calcium sensor proteins, including calmodulin-like (CML), calcineurin B-like protein (CBL), and calcium-dependent protein kinases (CDPK); they transmit signals and trigger downstream reactions [44]. In this study, 50 DEGs related to calcium signal transduction pathways were identified, including nine CMLs, four CDPKs, three CML binding protein, and four CBL interacting protein kinases (CIPK). Compared with the control group, they were all increased. In addition, six EF-hand proteins were identified, including five that were up-regulated. In plants, EF-hand proteins include calmodulin (CaMs), Ca^{2+} -dependent protein kinases (CDPKs), and calmodulin B-like protein (CBL); they are all Ca^{2+} receptors. Among them, CaMs and CMLs are important, and they have an important role in sensing and transmission of Ca^{2+} signals [45]. CIPK7 may be combined with the CBL, and thus participate in the low-temperature response of plants [46]. This study found that four CDPK, nine CMLs, and four CIPK protein kinases were up-regulated, indicating that Ca^{2+} -mediated signaling pathways play an important role in the response of low-temperature stress in birch.

4.2. Plant Hormone-Dependent Pathways under Low Temperature

ABA is considered to be a necessary messenger for plants to adapt to abiotic stress. Studies have shown that, under abiotic stress, genes involved in ABA synthesis and signal transduction are mostly differentially expressed, such as homologous genes of PYR/PYL and homologous genes of PP2C [47,48]. This study found that, under low-temperature stress, birch has 23 DEGs related to the ABA pathway, including 15 up-regulated and eight down-regulated. The birch *Bpev01.c0294.g0006* and *Bpev01.c0268.g0003* genes encode 9-cis-epoxy carotenoid dioxygenase (NCED), which is a key enzyme in ABA biosynthesis, up-regulated 3.66 times and 1.44 times under low-temperature stress, respectively. This indicates that ABA content may increase in birch, under low-temperature stress. Fifteen DEGs encoded protein phosphatase 2C (PP2C) were also detected, and among them, 11 were up-regulated and four down-regulated. PP2C is also a key component of the ABA signaling pathway. These DEGs indicate that ABA-mediated signal transduction is involved in the birch response to low-temperature stress.

Many researches have proved that ethylene may play a different role in different plants under low-temperature stress. For example, it plays an active role in rice, tomato and Arabidopsis, but it plays a negative regulatory role in safflower and magnolia [49]. Studies have shown that EIN3 can regulate the expression of ERF TFs [50], and EFE is a very important enzyme in the process of ethylene production [51,52]. In our study, a total of 12 DEGs related to ethylene signal transduction were identified, including 1 EFE (*Bpev01.c0299.g0032*) and 1 EIN3 (*Bpev01.c0523.g0001*) and 10 ERF1, 2, 3, 5. Interestingly, these DEGs were up-regulated and indicating that ethylene plays an positive role in the response of birch under low-temperature stress.

In addition, this study also detected 4, 5, and 11 DEGs related to the jasmonic acid signaling pathway, the gibberellin signaling pathway, and the salicylic acid signaling pathway, respectively. It indicated that these hormones may also be involved in the response of birch to low-temperature stress.

4.3. Low-Temperature Stress Signal Induces Cell Protection Process

When plants are subjected to low temperatures, plant cells will quickly accumulate compatible solutes (osmoprotectants), including sugar, amino acids, and fatty acids. These osmoprotectants can help re-establish the cell's osmotic balance and thus play a key role in protecting organelles, proteins, and membranes. Some studies have confirmed that sugars can be used as ROS scavengers to protect plants from oxidative damage caused by low temperature [53]. In this study, 30 DEGs were found in the starch and sucrose metabolic pathways, including eight genes encoded in the glycosyl hydrolases which were significantly down-regulated. It may be indicated that decomposition of sugar was reduced under low-temperature stress. Moreover, the gene encoded of trehalose-6-phosphate synthase was up-regulated, and a study in 2011 had confirmed that the accumulation of trehalose can be used for oxidative detoxification under abiotic stress [54]. In addition, a total of three genes that encoded amylase were up-regulated; we speculate that birch resists low temperature by breaking down starch into soluble sugars, under low-temperature stress.

In this study, a total of 109 DEGs related to lipid metabolism were identified in birch, under low temperature. Among them, the gene *Bpev01.c0190.g0079* encodes UDP-sulfoquinolone and is involved in the biosynthesis of sulfurized lipids, the gene *Bpev01.c1135.g0005* encodes long acyl-CoA synthetase, and the gene *Bpev01.c0364.g0019* encodes monogalactoside diacylglycerol synthase. These three genes were up-regulated and involved in the synthesis of lipids.

According to reports, amino acids are important osmotic protectants, and their accumulation is positively correlated with cold resistance [55]. In this study, genes encoded N-acetyl-L-glutamate synthase, L-lysine- α -aminotransferase, branched-chain amino acid transferase, aldolase, and cysteine synthase were up-regulated. These enzymes were involved in amino acid synthesis, and thus we speculated that the content of amino acids might increase under low-temperature stress. In addition, a total of eight DEGs which encoded phenylalanine ammonia-lyase (PAL) and peroxidase were all significantly up-regulated. PAL is one of the most relevant enzymes in the biosynthetic pathway of phenylpropane [50], peroxidase can oxidize phenols and remove reactive oxygen [51]. Based on this, we believe that the synthesis of phenylpropane and peroxidase was increased under low-temperature stress.

4.4. Transcription Factors Involved in Response to Cold Stress

The regulation of TFs is a key part of plant response to low-temperature stress, and TFs can regulate their expression through interaction with stress genes. Many TFs families are connected with adversity stress, such as AP2/EREBP, bHLH, WRKY, MYB, NAC, MYC, HSF, bZIP, and GRAS families [56–58]. In this study, a total of 362 TFs had differentially expressed and clustered into 58 TFs families, under low-temperature stress; among them, the AP2/ERF family had the most DEGs. It is worth noting that a total of 13 genes in the AP2/ERF family were up-regulated in all the samples treated at low-temperature, which was much higher than other TFs families. This indicates that the AP2/ERF family plays a more important role in birch response to low-temperature stress. In addition, we also noticed that low temperatures lead to TFs having differential expression in the C2H2, MYB-HB-like, WRKY, bHLH, bZIP, WD40-like, and GRAS families. Based on this, we speculated that these TFs families also play an indispensable role in response to birch cold stress.

4.5. Effects of Low-Temperature Stress on Photosynthesis

Chloroplasts are distinct organelles in plant cells and provide most of the energy through photosynthesis. When plants are under low-temperature stress, the expression of genes related to chlorophyll biosynthesis is suppressed, and it will cause the destruction of the thylakoid function and the inhibition of photosynthesis. In this study, we identified a total of 21 DEGs (13 up-regulated and eight down-regulated) that were related to photosynthesis; among them, genes encoded chlorophyll A/B binding protein, photosystem II reaction-center (PsbP) protein, ferredoxin 3, and diaphragm locating

protein (MinD) were all down-regulated. Chlorophyll a/b binding protein and chlorophyll–protein complexes are mainly responsible for collecting light energy and transferring it into the photochemical reaction center. Foreign protein PsbP of the photosystem II is necessary for oxygen metabolism in photosynthesis. [52]. Ferredoxin of the photosystem I regulates the direction of electron transfer in photosynthetic electron transport, to affect plant photosynthesis [59], and the tubulin FtsZ is the key protein of chloroplast division [60,61]. The genes that encoded these proteins were down-regulated, and we can speculate that low-temperature stress inhibits the processes of light energy conversion and chloroplast division in birch photosynthesis, to a certain extent. However, we noticed that the genes that encoded the ferredoxin 1, the PsaA/PsaB protein, and the phosphoenolpyruvate carboxylase (PEPC) were all significantly up-regulated. The differential expression of these genes indicated that, although the photosynthesis of birch was inhibited under low-temperature stress, it could also be promoted by increasing the expression of some genes. We identified 30 genes that were significantly up-regulated under low-temperature stress, which were related to calcium signal pathway, plant hormone, starch and sucrose metabolism, amino acids biosynthesis, and photosynthesis, respectively (Table 3).

Table 3. Thirty significantly up-regulated DEGs of birch under low-temperature stress.

Gene_ID	Time	logFC	Function
Calcium signal pathway			
<i>Bpev01.c1074.g0005</i>	2.5 h	4.66	Calmodulin-like, CML25
<i>Bpev01.c0480.g0081</i>	2 h	4.57	Calmodulin-like, CML38
<i>Bpev01.c1074.g0006</i>	3 h	4.48	Calcium-binding EF-hand family protein
<i>Bpev01.c1074.g0007</i>	2.5 h	3.94	Calcium-binding EF-hand family protein
<i>Bpev01.c0088.g0124</i>	1 h	3.79	Ca ²⁺ -dependent modulator of ICR1, CMI1
Hormone			
<i>Bpev01.c0343.g0021</i>	1 h	7.67	Pathogenesis-related gene 1
<i>Bpev01.c1161.g0016</i>	2.5 h	5.23	Ethylene responsive element binding factor 5
<i>Bpev01.c1161.g0012</i>	2.5 h	4.68	Ethylene responsive element binding factor 5
<i>Bpev01.c1161.g0014</i>	2.5 h	4.63	Ethylene responsive element binding factor 5
<i>Bpev01.c0870.g0008</i>	2.5 h	4.01	ABA 8'-hydroxylase, polypeptide 1
Starch and sucrose metabolism			
<i>Bpev01.c0162.g0017</i>	1 h	5.07	Beta-glucosidase 45
<i>Bpev01.c0080.g0100</i>	1 h	4.58	PfkB-like carbohydrate kinase family protein
<i>Bpev01.c0283.g0018</i>	1 h	3.15	Beta-amylase 5
<i>Bpev01.c0092.g0014</i>	1 h	2.77	Beta glucosidase 41
<i>Bpev01.c0275.g0002</i>	1 h	2.59	Sucrose-phosphate synthase family protein
Amino acids biosynthesis			
<i>Bpev01.c0015.g0143</i>	1 h	9.63	N-acetyl-l-glutamate synthase 2
<i>Bpev01.c0112.g0007</i>	3 h	8.44	Pyridoxal-5'-phosphate-dependent enzyme family protein
<i>Bpev01.c0511.g0007</i>	3 h	7.44	Aldolase superfamily protein
<i>Bpev01.c0148.g0010</i>	1 h	4.59	Branched-chain amino acid transaminase 2
<i>Bpev01.c0115.g0053</i>	1 h	4.03	Aldolase superfamily protein
Photosynthesis			
<i>Bpev01.c0154.g0066</i>	1.5 h	4.19	Ferritin 1
<i>Bpev01.c1891.g0002</i>	1.5 h	4.02	Photosystem II reaction center protein A
<i>Bpev01.c1891.g0006</i>	1.5 h	3.49	Photosystem II reaction center protein C
<i>Bpev01.c1891.g0007</i>	1.5 h	3.05	Photosystem I, PsaA/PsaB protein
<i>Bpev01.c0245.g0079</i>	1 h	1.76	Ferric reduction oxidase 7

5. Conclusions

In this study, the birch transcriptome dataset was generated by high-throughput sequencing and uploaded to the NCBI SRA database. In addition, the dynamic changes of gene expression under low-temperature treatment were observed, and a large number of low-temperature response genes

were discovered. These genes are connected with plant hormones, Ca^{2+} transduction pathways, phenylpropanoids, lipids, starch and sucrose, amino acids, photosynthesis, and enzymes related to the synthesis of protective metabolites. When studied we the changes in TFs under low-temperature stress, we observed that the contribution of AP2/ERF family genes was particularly prominent. Transcriptome and expression-level analysis of birch leaves provide valuable resources for the functional annotation of low-temperature-response genes.

Author Contributions: Conceptualization, S.C. (Su Chen); software, S.C. (Song Chen); validation, D.Z. and S.Y.; investigation, D.Z.; resources, S.C. (Su Chen); writing—original draft preparation, S.Y. and D.Z.; writing—review and editing, S.C. (Su Chen); supervision, S.C. (Su Chen); funding acquisition, S.C. (Su Chen). All authors have read and agreed to the published version of the manuscript.

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

Appendix A

Table A1. The genes and primers used for qRT-PCR analysis.

Gene	Primer	Primer Sequence
18S RNA	18S-R	GCAGGTTAGCGAAATGCGATAC
18S RNA	18S-F	GAGGTAGCTTCGGGCGCAACT
<i>Bpev01.c1851.g0002</i>	BP001R	CGAAGGTTGTTCTATTGGAGGT
<i>Bpev01.c1851.g0002</i>	BP001F	CATCTCCAATCTCAACCTCTGTTC
<i>Bpev01.c1851.g0001</i>	BP002R	CAGCTTCTTGTTGAGCCAATCTC
<i>Bpev01.c1851.g0001</i>	BP002F	GCGGTCTCTTCCCTTGCTTAAATG
<i>Bpev01.c1189.g0015</i>	BP003R	GAGATCCTGTTGCCTTCCAGTAC
<i>Bpev01.c1189.g0015</i>	BP003F	AGCTTGATGGGAAGAGACCAG
<i>Bpev01.c1161.g0015</i>	BP004R	GTCGGTCGATTGCGAGGTTG
<i>Bpev01.c1161.g0015</i>	BP004F	GACCTCTGCATCCCACTCTG
<i>Bpev01.c1161.g0014</i>	BP005R	GTCGGTCGATTGCGAGGTTG
<i>Bpev01.c1161.g0014</i>	BP005F	GACCTCTGCATCCCACTCTCTG
<i>Bpev01.c0455.g0003</i>	BP006R	GCTGCCATGCTTCCTGTCTC
<i>Bpev01.c0455.g0003</i>	BP006F	ACTTCCGGGATTTCGTTTCCAC
<i>Bpev01.c0390.g0007</i>	BP007R	CTTTCCAATGGGCTTGTCG
<i>Bpev01.c0390.g0007</i>	BP007F	CGGACGAGGAGTTGGTGAATC
<i>Bpev01.c0357.g0068</i>	BP008R	GCTTTCCAATACCCAGACACCG
<i>Bpev01.c0357.g0068</i>	BP008F	TCGATTCCACCCAATGATGAG
<i>Bpev01.c0210.g0037</i>	BP009R	CCATTCGGGTACTTCCGGTC
<i>Bpev01.c0210.g0037</i>	BP009F	CCTGTGTTGTCGTTGGCTTC
<i>Bpev01.c0162.g0012</i>	BP010R	GTCTAGCTCCGTTCCGGTAC
<i>Bpev01.c0162.g0012</i>	BP010F	ACTTATCCACCGGCTTGAACC
<i>Bpev01.c0073.g0013</i>	BP011R	CTCTGAATATCCTTCGCCGTCC
<i>Bpev01.c0073.g0013</i>	BP011F	CCGGGAGGACGAAGTTCAAG
<i>Bpev01.c0001.g0070</i>	BP012R	CAAGGCCAAAGATGCTTCAAGACAC
<i>Bpev01.c0001.g0070</i>	BP012F	TACTTGTCTCAAGATGCTCCTCTG
<i>Bpev01.c0001.g0069</i>	BP013R	CCTTGGTCGCCCTGTTAGTC
<i>Bpev01.c0001.g0069</i>	BP013F	GCAGGACAGGAGTGGGATAC
<i>Bpev01.c0000.g0209</i>	BP014R	GTTGCTGTTGAGTTGAATTCGG
<i>Bpev01.c0000.g0209</i>	BP014F	CCTTTGCATCTTACCCTTCTTGCTC

Table A2. Statistics of sequencing data.

Sample	Raw Date	Clean Date	Base Number	Q20%	Q30%	GC Content ¹	Mapped Ratio
CK_rep1	25,672,243	25,670,448	7.68 Gb	97.63%	93.76%	47.07%	96.07%
CK_rep2	28,604,148	28,602,122	8.56 Gb	97.69%	93.93%	47.16%	96.34%
1h_rep1	28,418,115	22,838,434	6.83 Gb	97.77%	94.08%	47.14%	96.64%
1h_rep2	27,638,049	27,255,820	8.16 Gb	97.56%	93.60%	46.90%	96.52%
1.5h_rep1	22,409,000	23,396,121	7.00 Gb	97.68%	93.92%	47.33%	96.42%
1.5h_rep2	23,397,739	23,729,617	7.10 Gb	97.58%	93.71%	47.05%	96.43%
1.5h_rep3	23,731,305	24,169,972	7.23 Gb	97.64%	93.82%	46.63%	96.46%
2h_rep1	24,171,686	22,157,427	6.63 Gb	97.58%	93.67%	47.48%	96.16%
2h_rep2	22,840,004	23,331,820	6.98 Gb	97.65%	93.85%	47.10%	96.29%
2.5h_rep1	27,257,640	24,693,732	7.39 Gb	97.56%	93.63%	47.09%	96.30%
2.5h_rep2	24,695,387	24,822,662	7.43 Gb	97.76%	94.02%	48.47%	96.33%
3h_rep1	24,824,310	28,740,756	8.60 Gb	97.67%	93.85%	47.46%	96.24%
3h_rep2	22,158,962	27,162,399	8.13 Gb	97.90%	94.35%	47.73%	96.47%
Average	25,062,968	25,120,872	7.52 Gb	97.50%	93.60%	47.28%	96.36%

¹ GC content: the ratio of guanine and cytosine among the four bases of DNA.

Table A3. Pathway enrichment analysis in DEGs (p -value ≤ 0.05).

Items	ID	DEGs	All	p -Value
Metabolic pathways	ko01100	349	2246	6.39×10^{-26}
Biosynthesis of secondary metabolites	ko01110	210	1107	1.87×10^{-24}
Ribosome	ko03010	76	364	2.58×10^{-11}
Biosynthesis of amino acids	ko01230	46	251	4.57×10^{-6}
Carbon metabolism	ko01200	43	273	0.0002
Phenylpropanoid biosynthesis	ko00940	41	166	1.89×10^{-8}
Plant hormone signal transduction	ko04075	39	273	0.00195
Plant-pathogen interaction	ko04626	30	170	0.00034
Starch and sucrose metabolism	ko00500	30	165	0.000218
Cysteine and methionine metabolism	ko00270	23	121	0.00066
Glycolysis/gluconeogenesis	ko00010	23	116	0.000396
MAPK signaling pathway-plant	ko04016	21	134	0.00812
Amino sugar and nucleotide sugar metabolism	ko00520	18	131	0.0382
Pyruvate metabolism	ko00620	18	86	0.000949
Fatty acid metabolism	ko01212	18	69	9.62×10^{-5}
Purine metabolism	ko00230	17	100	0.00828
DNA replication	ko03030	14	50	0.000304
Valine, leucine, and isoleucine degradation	ko00280	13	51	0.00104
beta-Alanine metabolism	ko00410	13	50	0.000887
Flavonoid biosynthesis	ko00941	13	22	9.57×10^{-7}
Fructose and mannose metabolism	ko00051	12	64	0.0131
Glycerolipid metabolism	ko00561	12	60	0.00858
Fatty acid biosynthesis	ko00061	12	43	0.000823
Carbon fixation in photosynthetic organisms	ko00710	11	69	0.0426
Phenylalanine metabolism	ko00360	11	32	0.000316
Phenylalanine, tyrosine and tryptophan biosynthesis	ko00400	10	56	0.0289
Fatty acid degradation	ko00071	10	47	0.0111
Propanoate metabolism	ko00640	10	43	0.0066
Tyrosine metabolism	ko00350	9	40	0.0116
Pantothenate and CoA biosynthesis	ko00770	9	28	0.00161

Table A3. Cont.

Items	ID	DEGs	All	p-Value
alpha-Linolenic acid metabolism	ko00592	8	44	0.0442
Carotenoid biosynthesis	ko00906	8	29	0.00627
Sphingolipid metabolism	ko00600	8	27	0.00439
Fatty acid elongation	ko00062	7	35	0.0398
Lysine degradation	ko00310	7	34	0.0355
Biotin metabolism	ko00780	7	16	0.0013
ABC transporters	ko02010	6	26	0.0332
Butanoate metabolism	ko00650	6	19	0.0103
Lysine biosynthesis	ko00300	6	15	0.0041
Other glycan degradation	ko00511	5	19	0.0334
Riboflavin metabolism	ko00740	5	18	0.0282
Biosynthesis of secondary metabolites-unclassified	ko00999	5	12	0.00762
Monobactam biosynthesis	ko00261	4	14	0.0449
Glycosphingolipid biosynthesis-globo and isoglobo series	ko00603	4	9	0.0143
Monoterpenoid biosynthesis	ko00902	4	8	0.0105
Stilbenoid, diarylheptanoid, and gingerol biosynthesis	ko00945	4	7	0.00746

Table A4. DEGs were annotated for calcium signal pathway under low temperature stress in birch.

Gene_ID	Time	logFC	Function
<i>Bpev01.c0480.g0081</i>	2 h	4.57	Calmodulin-like, CML38
<i>Bpev01.c1074.g0005</i>	2.5 h	4.66	Calmodulin-like, CML25
<i>Bpev01.c1074.g0006</i>	3 h	4.48	Calcium-binding EF-hand family protein
<i>Bpev01.c1074.g0007</i>	2.5 h	3.94	Calcium-binding EF-hand family protein
<i>Bpev01.c0147.g0007</i>	1 h	3.4	Calcium-dependent lipid-binding (CaLB domain) family protein
<i>Bpev01.c0274.g0041</i>	1 h	3.6	Calcium-binding EF-hand family protein
<i>Bpev01.c0445.g0003</i>	1 h	2.48	Calcium-dependent lipid-binding (CaLB domain) family protein
<i>Bpev01.c0420.g0021</i>	1 h	2.43	Calmodulin binding protein-like
<i>Bpev01.c0027.g0187</i>	2.5 h	2.94	Ca ²⁺ -dependent modulator of ICR1, CMI1
<i>Bpev01.c0088.g0124</i>	1 h	3.79	Ca ²⁺ -dependent modulator of ICR1, CMI1
<i>Bpev01.c2029.g0005</i>	1 h	3.38	Calmodulin binding protein-like
<i>Bpev01.c0180.g0001</i>	1 h	3.08	Ca ²⁺ -dependent modulator of ICR1, CMI1
<i>Bpev01.c0281.g0075</i>	2.5 h	2.7	Calmodulin-like, CML30
<i>Bpev01.c0011.g0001</i>	1 h	2.52	EF hand calcium-binding protein family
<i>Bpev01.c0328.g0033</i>	1 h	2.05	Calmodulin-like, CML46
<i>Bpev01.c0821.g0019</i>	1 h	1.96	IQ calmodulin-binding motif family protein
<i>Bpev01.c0145.g0021</i>	1 h	1.91	Calcium-dependent lipid-binding (CaLB domain) family protein
<i>Bpev01.c0088.g0130</i>	1 h	2.96	Calmodulin-like, CML30
<i>Bpev01.c2409.g0002</i>	1 h	1.68	Calcium-dependent protein kinase 1
<i>Bpev01.c0511.g0002</i>	1 h	1.56	CBL-interacting protein kinase 5
<i>Bpev01.c0022.g0034</i>	1 h	1.54	Calcium-dependent protein kinase 28
<i>Bpev01.c1700.g0004</i>	1 h	2.06	Calcium-dependent protein kinase family protein
<i>Bpev01.c0159.g0009</i>	1 h	1.44	Copine (calcium-dependent phospholipid-binding protein) family
<i>Bpev01.c0324.g0014</i>	3 h	1.49	CBL-interacting protein kinase 7
<i>Bpev01.c1519.g0006</i>	1 h	1.4	Calmodulin binding protein-like
<i>Bpev01.c0052.g0090</i>	1 h	2.12	Calmodulin-binding transcription activator protein with CG-1 and Ankyrin domains

Table A4. Cont.

Gene_ID	Time	logFC	Function
<i>Bpev01.c0570.g0019</i>	1 h	1.37	Calmodulin-like, CML30
<i>Bpev01.c0318.g0004</i>	3 h	1.36	Calmodulin-like, CML5
<i>Bpev01.c0126.g0019</i>	2.5 h	1.55	Calmodulin-like, CML5
<i>Bpev01.c0029.g0091</i>	1 h	1.35	Calcium-dependent protein kinase 19
<i>Bpev01.c0044.g0059</i>	1 h	1.32	CBL-interacting protein kinase 25
<i>Bpev01.c0147.g0003</i>	1 h	1.9	Calcium-dependent lipid-binding (CaLB domain) family protein
<i>Bpev01.c1106.g0007</i>	3 h	1.17	CBL-interacting protein kinase 20
<i>Bpev01.c2470.g0003</i>	1 h	1.17	Calcium-dependent lipid-binding (CaLB domain) family protein
<i>Bpev01.c1002.g0005</i>	1 h	1.11	Calcium-dependent lipid-binding (CaLB domain) family protein
<i>Bpev01.c0298.g0010</i>	3 h	1.4	Calcium-dependent lipid-binding (CaLB domain) family protein
<i>Bpev01.c0763.g0002</i>	2.5 h	1.74	Calmodulin-like, CML30
<i>Bpev01.c0145.g0018</i>	2.5 h	1.97	Calcium-binding EF-hand family protein
<i>Bpev01.c0022.g0125</i>	1 h	1.01	Calcium-dependent phosphotriesterase superfamily protein
<i>Bpev01.c0036.g0009</i>	1 h	1	Plant calmodulin-binding protein
<i>Bpev01.c0135.g0041</i>	1 h	-1.1	Calmodulin-binding protein
<i>Bpev01.c0523.g0005</i>	1 h	-1.1	Calcium-dependent lipid-binding (CaLB domain) plant phosphoribosyltransferase family protein
<i>Bpev01.c3512.g0001</i>	1 h	-1.14	Calcium-binding EF-hand family protein
<i>Bpev01.c1359.g0001</i>	1 h	-1.38	Na ⁺ /Ca ²⁺ exchanger, NCL
<i>Bpev01.c2062.g0001</i>	1 h	-1.44	Na ⁺ /Ca ²⁺ exchanger, NCL
<i>Bpev01.c2002.g0003</i>	1 h	-1.71	C2 calcium/lipid-binding plant phosphoribosyltransferase family protein
<i>Bpev01.c1295.g0001</i>	1 h	-1.88	C2 calcium/lipid-binding plant phosphoribosyltransferase family protein
<i>Bpev01.c2062.g0002</i>	1 h	-1.94	Na ⁺ /Ca ²⁺ exchanger, NCL
<i>Bpev01.c0442.g0045</i>	1 h	-1.99	C2 calcium/lipid-binding plant phosphoribosyltransferase family protein
<i>Bpev01.c0127.g0096</i>	2.5 h	-3.34	Calmodulin binding protein

Table A5. Hormone-related DEGs under low temperature stress in birch.

Gene_ID	Time	logFC	Function
ABA			
<i>Bpev01.c0870.g0008</i>	2.5 h	4.01	ABA 8'-hydroxylase, polypeptide 1
<i>Bpev01.c0352.g0006</i>	1 h	3.75	Protein phosphatase 2C family protein
<i>Bpev01.c0294.g0006</i>	2.5 h	3.66	Nine-cis-epoxycarotenoid dioxygenase 3
<i>Bpev01.c0115.g0107</i>	1 h	3.92	Protein phosphatase 2C family protein
<i>Bpev01.c0129.g0031</i>	2.5 h	2.04	Protein phosphatase 2C family protein
<i>Bpev01.c1183.g0023</i>	1 h	1.9	Protein phosphatase 2C family protein
<i>Bpev01.c0455.g0029</i>	1 h	1.81	Protein phosphatase 2C family protein, PP2CG1
<i>Bpev01.c0015.g0093</i>	3 h	1.57	Protein phosphatase 2C family protein, APD5
<i>Bpev01.c0268.g0003</i>	1 h	2.42	Nine-cis-epoxycarotenoid dioxygenase 4
<i>Bpev01.c0279.g0011</i>	3 h	1.26	Protein phosphatase 2C family protein, AP2C1
<i>Bpev01.c0015.g0010</i>	1 h	1.25	ABA deficient 1, ABA1
<i>Bpev01.c0500.g0004</i>	1 h	1.24	Protein phosphatase 2C family protein
<i>Bpev01.c0265.g0015</i>	1 h	1.18	Protein phosphatase 2C family protein, CIPP1

Table A5. Cont.

Gene_ID	Time	logFC	Function
<i>Bpev01.c0280.g0015</i>	2.5 h	1.67	Protein phosphatase 2C family protein
<i>Bpev01.c1081.g0001</i>	1 h	1.03	Protein phosphatase 2C family protein
<i>Bpev01.c0298.g0028</i>	1 h	−1.26	Beta-hydroxylase 1
<i>Bpev01.c0142.g0059</i>	1 h	−1.4	Protein phosphatase 2C family protein
<i>Bpev01.c0467.g0019</i>	1 h	−1.61	Protein phosphatase 2C family protein, PP2C.D3
<i>Bpev01.c0328.g0025</i>	2.5 h	−2.79	ABA 8'-hydroxylase, polypeptide 4
<i>Bpev01.c1475.g0010</i>	3 h	−1.91	Protein phosphatase 2C family protein, PP2C62
<i>Bpev01.c0245.g0042</i>	1 h	−1.91	ABA 8'-hydroxylase, polypeptide 2
<i>Bpev01.c0038.g0101</i>	1 h	−2.39	Protein phosphatase 2C family protein
<i>Bpev01.c1272.g0023</i>	2.5 h	−3.53	ABA deficient 2, ABA2
Ethylene			
<i>Bpev01.c1161.g0016</i>	2.5 h	5.23	Ethylene responsive element binding factor 5
<i>Bpev01.c1161.g0018</i>	2.5 h	3.67	Ethylene responsive element binding factor 1
<i>Bpev01.c1161.g0014</i>	2.5 h	4.63	Ethylene responsive element binding factor 5
<i>Bpev01.c1161.g0017</i>	2.5 h	3.12	Ethylene responsive element binding factor 1
<i>Bpev01.c1161.g0012</i>	2.5 h	4.68	Ethylene responsive element binding factor 5
<i>Bpev01.c1161.g0013</i>	2.5 h	4.62	Ethylene responsive element binding factor 5
<i>Bpev01.c0764.g0013</i>	1 h	2.86	Ethylene response factor 1
<i>Bpev01.c0343.g0032</i>	1 h	2.78	Ethylene response factor 1
<i>Bpev01.c1851.g0001</i>	2.5 h	3.7	Ethylene responsive element binding factor 2
<i>Bpev01.c1161.g0015</i>	2.5 h	4.43	Ethylene responsive element binding factor 5
<i>Bpev01.c0299.g0032</i>	2.5 h	1.95	Ethylene-forming enzyme
<i>Bpev01.c0523.g0001</i>	2.5 h	1.41	Ethylene-insensitive3-like 3
JA			
<i>Bpev01.c0052.g0052</i>	2.5 h	−2.92	Jasmonate-zim-domain protein 10, Jaz 10
<i>Bpev01.c0136.g0009</i>	1 h	−1.62	N-MYC down-regulated-like 1
<i>Bpev01.c0423.g0007</i>	1 h	−1.98	4-coumarate: CoA ligase 3
<i>Bpev01.c0161.g0057</i>	2.5 h	−2.5	Jasmonic acid carboxyl methyltransferase
GA			
<i>Bpev01.c0118.g0033</i>	2.5 h	3.05	Gibberellin 3-oxidase 1
<i>Bpev01.c0094.g0034</i>	1 h	−1.09	Gibberellin-regulated family protein
<i>Bpev01.c1673.g0004</i>	1 h	−1.25	Gibberellin-regulated family protein
<i>Bpev01.c0411.g0004</i>	1 h	−2.18	Gibberellin-regulated family protein
<i>Bpev01.c1170.g0013</i>	1 h	−5	Gibberellin-regulated family protein
SA			
<i>Bpev01.c0343.g0021</i>	1 h	7.67	Pathogenesis-related gene 1
<i>Bpev01.c0154.g0058</i>	1 h	2.58	Pathogenesis-related thaumatin superfamily protein
<i>Bpev01.c1477.g0007</i>	1 h	1.97	Pathogenesis-related family protein
<i>Bpev01.c0889.g0015</i>	1 h	1.46	Pathogenesis-related thaumatin superfamily protein
<i>Bpev01.c0022.g0043</i>	1 h	1.89	Pathogenesis-related thaumatin superfamily protein
<i>Bpev01.c0082.g0060</i>	1 h	−2.32	Pathogenesis-related thaumatin superfamily protein
<i>Bpev01.c1688.g0003</i>	1.5 h	−2.26	Pathogenesis-related family protein
<i>Bpev01.c1525.g0004</i>	2.5 h	−2.88	Pathogenesis-related thaumatin superfamily protein
<i>Bpev01.c0889.g0016</i>	1 h	−2.42	Pathogenesis-related thaumatin superfamily protein
<i>Bpev01.c1688.g0002</i>	3 h	−3.46	Pathogenesis-related family protein
<i>Bpev01.c0030.g0023</i>	1 h	−2.64	Pathogenesis-related thaumatin superfamily protein

Table A6. Starch and sucrose metabolism-related DEGs under low-temperature stress in birch.

Gene_ID	Time	logFC	Function
<i>Bpev01.c0162.g0017</i>	1 h	5.07	Beta-glucosidase 45
<i>Bpev01.c0080.g0100</i>	1 h	4.58	PfkB-like carbohydrate kinase family protein
<i>Bpev01.c0283.g0018</i>	1 h	3.15	Beta-amylase 5
<i>Bpev01.c0092.g0014</i>	1 h	2.77	Beta glucosidase 41
<i>Bpev01.c0275.g0002</i>	1 h	2.59	Sucrose-phosphate synthase family protein
<i>Bpev01.c1949.g0001</i>	1 h	2.07	Chloroplast beta-amylase
<i>Bpev01.c0000.g0208</i>	1 h	1.76	Haloacid dehalogenase-like hydrolase (HAD) superfamily protein
<i>Bpev01.c0842.g0013</i>	1 h	1.54	Trehalose-6-phosphate synthase
<i>Bpev01.c0053.g0012</i>	1 h	1.42	Beta glucosidase 11
<i>Bpev01.c0016.g0107</i>	1 h	1.58	Sucrose phosphate synthase 3F
<i>Bpev01.c0264.g0007</i>	1 h	1.26	Glycosyl hydrolases family 31 protein
<i>Bpev01.c1918.g0005</i>	1 h	1.32	Glycosyl hydrolase family protein
<i>Bpev01.c2037.g0001</i>	3 h	1.14	Haloacid dehalogenase-like hydrolase (HAD) superfamily protein
<i>Bpev01.c0294.g0013</i>	1 h	1.19	Sucrose synthase 3
<i>Bpev01.c0478.g0015</i>	1 h	1.01	Starch branching enzyme 2.2
<i>Bpev01.c0727.g0009</i>	1 h	-1.24	Sucrose synthase 6
<i>Bpev01.c0762.g0008</i>	1 h	-1.29	PfkB-like carbohydrate kinase family protein
<i>Bpev01.c0145.g0042</i>	1 h	-1.95	O-Glycosyl hydrolases family 17 protein
<i>Bpev01.c0051.g0185</i>	1 h	-1.51	Sucrose synthase 4
<i>Bpev01.c0127.g0110</i>	1 h	-1.53	Beta-glucosidase 47
<i>Bpev01.c2368.g0004</i>	1 h	-1.57	O-Glycosyl hydrolases family 17 protein
<i>Bpev01.c0141.g0019</i>	1 h	-1.58	Glycosyl hydrolase 9C2
<i>Bpev01.c0263.g0003</i>	1 h	-2.55	O-Glycosyl hydrolases family 17 protein
<i>Bpev01.c0717.g0033</i>	1 h	-1.77	Nudix hydrolase homolog 14
<i>Bpev01.c1136.g0002</i>	1 h	-1.84	Glycosyl hydrolase 9B18
<i>Bpev01.c0127.g0064</i>	1 h	-1.91	O-Glycosyl hydrolases family 17 protein
<i>Bpev01.c1526.g0006</i>	1 h	-2.06	Glycosyl hydrolase 9B1
<i>Bpev01.c1187.g0006</i>	1 h	-2.68	Glycosyl hydrolases family 32 protein
<i>Bpev01.c0470.g0030</i>	2.5 h	-4.96	Beta glucosidase 17

Table A7. DEGs related to lipid metabolism pathway under low-temperature stress in birch.

Gene_ID	1 h	1.5 h	2 h	2.5 h	3 h
<i>Bpev01.c0552.g0011</i>	8.94	-	-	-	8.66
<i>Bpev01.c0062.g0066</i>	5.1	-	-	-	-
<i>Bpev01.c0870.g0008</i>	-	-	-	4.01	3.62
<i>Bpev01.c0294.g0006</i>	-	-	-	3.66	3.02
<i>Bpev01.c1114.g0003</i>	3.53	-	-	-	-
<i>Bpev01.c1092.g0004</i>	5.29	3.41	-	3.17	3.55
<i>Bpev01.c1153.g0001</i>	3.32	-	-	-	-
<i>Bpev01.c0118.g0033</i>	-	-	-	3.05	-
<i>Bpev01.c0517.g0001</i>	2.94	-	-	-	-
<i>Bpev01.c0574.g0036</i>	2.39	-	-	-	-
<i>Bpev01.c0094.g0036</i>	2.77	2.19	-	-	-
<i>Bpev01.c1335.g0003</i>	2.08	-	-	-	-
<i>Bpev01.c0940.g0001</i>	3.97	1.97	-	3.03	-
<i>Bpev01.c0364.g0019</i>	1.95	-	-	-	-
<i>Bpev01.c0050.g0029</i>	2.82	1.86	-	1.83	-
<i>Bpev01.c0261.g0045</i>	1.83	-	-	-	-
<i>Bpev01.c0020.g0005</i>	1.72	-	-	-	-
<i>Bpev01.c1100.g0005</i>	-	-	-	1.69	-
<i>Bpev01.c0374.g0017</i>	1.64	-	-	-	-
<i>Bpev01.c0569.g0008</i>	1.53	-	-	1.32	1.24

Table A7. Cont.

Gene_ID	1 h	1.5 h	2 h	2.5 h	3 h
<i>Bpev01.c0901.g0021</i>	1.99	1.48	-	2.03	1.41
<i>Bpev01.c0015.g0012</i>	1.41	1.42	-	-	-
<i>Bpev01.c0480.g0025</i>	1.4	-	-	-	-
<i>Bpev01.c0841.g0011</i>	1.34	-	-	-	-
<i>Bpev01.c0401.g0013</i>	1.33	-	-	-	-
<i>Bpev01.c0063.g0018</i>	1.29	-	-	-	-
<i>Bpev01.c1135.g0005</i>	1.26	-	-	-	-
<i>Bpev01.c0015.g0010</i>	1.25	-	-	-	-
<i>Bpev01.c0018.g0112</i>	1.22	-	-	-	-
<i>Bpev01.c0275.g0035</i>	1.81	1.21	-	-	-
<i>Bpev01.c0473.g0025</i>	1.1	-	-	-	-
<i>Bpev01.c0190.g0079</i>	1.04	-	-	-	-
<i>Bpev01.c1060.g0006</i>	-1	-	-	-	-
<i>Bpev01.c0575.g0024</i>	-1.01	-	-	-	-
<i>Bpev01.c0038.g0134</i>	-1.03	-	-	-	-
<i>Bpev01.c1828.g0001</i>	-1.04	-	-	-	-
<i>Bpev01.c0850.g0003</i>	-2.16	-1.04	-	-	-
<i>Bpev01.c0821.g0002</i>	-1.04	-	-	-	-
<i>Bpev01.c0892.g0002</i>	-1.06	-	-	-	-
<i>Bpev01.c0496.g0022</i>	-1.08	-	-	-	-
<i>Bpev01.c0147.g0001</i>	-1.96	-1.09	-	-2.91	-2
<i>Bpev01.c0052.g0167</i>	-1.11	-	-	-	-
<i>Bpev01.c0717.g0003</i>	-1.19	-1.11	-	-	-
<i>Bpev01.c0425.g0035</i>	-1.19	-1.16	-	-1.55	-
<i>Bpev01.c0038.g0045</i>	-1.43	-1.17	-	-	-
<i>Bpev01.c0854.g0013</i>	-1.22	-	-	-	-
<i>Bpev01.c0555.g0007</i>	-1.25	-	-	-	-
<i>Bpev01.c0166.g0013</i>	-1.26	-	-	-	-
<i>Bpev01.c0298.g0028</i>	-1.26	-	-	-	-
<i>Bpev01.c0027.g0082</i>	-1.26	-	-	-	-
<i>Bpev01.c2636.g0002</i>	-1.29	-	-	-	-
<i>Bpev01.c0652.g0023</i>	-1.31	-	-	-	-
<i>Bpev01.c0449.g0049</i>	-1.33	-	-	-	-
<i>Bpev01.c1534.g0010</i>	-2.15	-1.34	-	-	-1.38
<i>Bpev01.c1475.g0006</i>	-2.23	-1.36	-	-	-
<i>Bpev01.c0045.g0058</i>	-1.37	-	-	-	-
<i>Bpev01.c1170.g0009</i>	-1.39	-	-	-	-
<i>Bpev01.c0506.g0024</i>	-2.39	-1.43	-	-	-
<i>Bpev01.c1484.g0012</i>	-	-1.44	-	-	-
<i>Bpev01.c0645.g0001</i>	-1.44	-	-	-	-
<i>Bpev01.c0275.g0066</i>	-1.44	-	-	-	-
<i>Bpev01.c0842.g0018</i>	-1.5	-	-	-	-
<i>Bpev01.c0163.g0009</i>	-1.78	-1.52	-	-1.43	-
<i>Bpev01.c0052.g0192</i>	-1.53	-	-	-	-
<i>Bpev01.c0531.g0014</i>	-1.54	-	-	-	-
<i>Bpev01.c1714.g0001</i>	-1.54	-	-	-	-
<i>Bpev01.c0327.g0073</i>	-1.55	-	-	-	-
<i>Bpev01.c1382.g0026</i>	-1.58	-	-	-	-
<i>Bpev01.c1080.g0002</i>	-1.52	-1.58	-	-1.79	-
<i>Bpev01.c0458.g0015</i>	-2.9	-1.6	-	-	-
<i>Bpev01.c0190.g0038</i>	-1.6	-	-	-	-
<i>Bpev01.c0114.g0058</i>	-2.25	-1.64	-	-1.76	-

Table A7. Cont.

Gene_ID	1 h	1.5 h	2 h	2.5 h	3 h
<i>Bpev01.c0052.g0046</i>	−1.67	-	-	-	-
<i>Bpev01.c0213.g0061</i>	-	−1.67	-	-	-
<i>Bpev01.c1044.g0004</i>	−1.68	-	-	-	-
<i>Bpev01.c0135.g0028</i>	−1.69	-	-	-	-
<i>Bpev01.c1627.g0007</i>	−2.7	−1.73	-	−3.79	-
<i>Bpev01.c0052.g0017</i>	−1.78	-	-	-	-
<i>Bpev01.c0237.g0054</i>	−1.8	-	-	-	-
<i>Bpev01.c0169.g0048</i>	−2.9	−1.81	-	-	-
<i>Bpev01.c1414.g0002</i>	−2.4	−1.82	-	-	-
<i>Bpev01.c1006.g0017</i>	−1.88	-	-	-	-
<i>Bpev01.c0473.g0007</i>	−1.9	-	-	-	-
<i>Bpev01.c0245.g0042</i>	−1.91	-	-	-	-
<i>Bpev01.c0253.g0008</i>	−1.95	-	-	-	-
<i>Bpev01.c0027.g0080</i>	−2.11	-	-	-	-
<i>Bpev01.c0053.g0009</i>	−2.12	-	-	-	-
<i>Bpev01.c1312.g0005</i>	−2.53	−2.15	-	-	-
<i>Bpev01.c0523.g0003</i>	−2.2	-	-	-	-
<i>Bpev01.c2716.g0001</i>	−2.28	-	-	−3.31	-
<i>Bpev01.c0000.g0055</i>	−2.49	−2.28	-	−2.21	−1.75
<i>Bpev01.c0457.g0037</i>	−2.3	-	-	-	-
<i>Bpev01.c0680.g0004</i>	−2.33	-	-	-	-
<i>Bpev01.c0224.g0002</i>	−2.49	-	-	-	−1.19
<i>Bpev01.c0161.g0057</i>	-	-	-	−2.5	-
<i>Bpev01.c0038.g0153</i>	−2.26	−2.56	-	-	-
<i>Bpev01.c1529.g0009</i>	−2.64	-	-	-	-
<i>Bpev01.c1272.g0023</i>	−2.8	-	-	−3.53	-
<i>Bpev01.c0275.g0067</i>	−2.82	-	-	-	-
<i>Bpev01.c1836.g0006</i>	−2.87	-	-	-	-
<i>Bpev01.c0939.g0009</i>	−3.05	-	-	-	-
<i>Bpev01.c0046.g0006</i>	−2.76	−3.16	−1.95	−3.68	-
<i>Bpev01.c0217.g0001</i>	−3.17	-	-	−2.85	-
<i>Bpev01.c1574.g0002</i>	−3.49	-	-	−6.21	-
<i>Bpev01.c0312.g0012</i>	-	-	-	−3.66	-
<i>Bpev01.c0261.g0078</i>	-	−3.81	-	−5.58	-
<i>Bpev01.c0565.g0007</i>	-	−3.92	-	−4.55	-
<i>Bpev01.c0594.g0020</i>	-	-	-	−4.92	-
<i>Bpev01.c0227.g0002</i>	−2.41	−8.16	-	−4.96	-

Table A8. Amino acids biosynthesis-related DEGs under low-temperature stress in birch.

Gene_ID	Time	logFC	Function
<i>Bpev01.c0015.g0143</i>	1 h	9.63	N-acetyl-l-glutamate synthase 2
<i>Bpev01.c0112.g0007</i>	3 h	8.44	Pyridoxal-5'-phosphate-dependent enzyme family protein
<i>Bpev01.c0511.g0007</i>	3 h	7.44	Aldolase superfamily protein
<i>Bpev01.c0115.g0053</i>	1 h	4.03	Aldolase superfamily protein
<i>Bpev01.c0274.g0001</i>	1 h	2.69	ATP phosphoribosyl transferase 2
<i>Bpev01.c0148.g0010</i>	1 h	4.59	Branched-chain amino acid transaminase 2
<i>Bpev01.c0462.g0003</i>	1 h	2.08	3-deoxy-d-arabino-heptulosonate 7-phosphate synthase
<i>Bpev01.c0082.g0091</i>	2.5 h	2.37	HOPW1-1-interacting 1
<i>Bpev01.c0083.g0021</i>	1 h	1.85	Phosphofructokinase 2
<i>Bpev01.c0137.g0017</i>	1 h	2.58	Cysteine synthase D1
<i>Bpev01.c0425.g0050</i>	1 h	1.31	Aldolase superfamily protein
<i>Bpev01.c2707.g0004</i>	1 h	1.26	Cytosolic NADP ⁺ -dependent isocitrate dehydrogenase

Table A8. Cont.

Gene_ID	Time	logFC	Function
<i>Bpev01.c0929.g0016</i>	1 h	1.2	O-acetylserine (thiol) lyase (OAS-TL) isoform A1
<i>Bpev01.c0146.g0019</i>	1 h	1.16	D-aminoacid aminotransferase-like PLP-dependent enzymes superfamily protein
<i>Bpev01.c0145.g0054</i>	1 h	1.57	Glutamine synthase clone R1
<i>Bpev01.c0517.g0019</i>	1 h	1.04	D-ribulose-5-phosphate-3-epimerase
<i>Bpev01.c0112.g0020</i>	1 h	1	Serine acetyltransferase 2;2
<i>Bpev01.c0000.g0226</i>	1.5 h	−1.02	Aspartate aminotransferase
<i>Bpev01.c0198.g0001</i>	1 h	−1.59	Enolase 1
<i>Bpev01.c0169.g0035</i>	1 h	−1.1	Pyruvate kinase family protein
<i>Bpev01.c0192.g0016</i>	1 h	−1.24	Arginase
<i>Bpev01.c1518.g0002</i>	1 h	−1.12	Ketol-acid reductoisomerase
<i>Bpev01.c0120.g0017</i>	1 h	−1.12	D-3-phosphoglycerate dehydrogenase
<i>Bpev01.c0874.g0032</i>	1 h	−1.13	Dihydrodipicolinate synthase
<i>Bpev01.c0146.g0013</i>	1 h	−1.14	Pyridoxal phosphate (PLP)-dependent transferases superfamily protein
<i>Bpev01.c2230.g0005</i>	1 h	−1.15	Pyruvate kinase family protein
<i>Bpev01.c0192.g0009</i>	1 h	−1.24	RNA 3'-terminal phosphate cyclase/enolpyruvate transferase, alpha/beta
<i>Bpev01.c1356.g0002</i>	1 h	−1.28	Dehydroquinase dehydratase, putative / shikimate dehydrogenase, putative
<i>Bpev01.c2160.g0012</i>	2.5 h	−1.31	Glyceraldehyde-3-phosphate dehydrogenase of plastid 2
<i>Bpev01.c1534.g0010</i>	1 h	−2.15	Plastidic pyruvate kinase beta subunit 1
<i>Bpev01.c0275.g0025</i>	1 h	−1.35	Aspartate kinase-homoserine dehydrogenase ii
<i>Bpev01.c0683.g0005</i>	1 h	−1.49	Methionine adenosyltransferase 3
<i>Bpev01.c1040.g0016</i>	1 h	−1.5	Glyceraldehyde-3-phosphate dehydrogenase C2
<i>Bpev01.c1115.g0006</i>	1 h	−1.78	Pyridoxal-5'-phosphate-dependent enzyme family protein
<i>Bpev01.c0000.g0150</i>	1 h	−2.03	3-deoxy-D-arabino-heptulosonate 7-phosphate synthase 1
<i>Bpev01.c0449.g0055</i>	1 h	−1.83	Semialdehyde dehydrogenase family protein
<i>Bpev01.c3196.g0001</i>	1 h	−2.32	Dihydrodipicolinate reductase
<i>Bpev01.c0652.g0026</i>	1 h	−2.34	Shikimate kinase 1
<i>Bpev01.c0279.g0010</i>	1 h	−2.53	Glutamine-dependent asparagine synthase 1
<i>Bpev01.c0167.g0024</i>	1.5 h	−2.65	Tyrosine transaminase family protein

Table A9. Photosynthesis-related DEGs under low-temperature stress in birch.

Gene_ID	Time	logFC	Function
<i>Bpev01.c0154.g0066</i>	1.5 h	4.19	Ferretin 1
<i>Bpev01.c1891.g0002</i>	1.5 h	4.02	Photosystem II reaction center protein A
<i>Bpev01.c1891.g0006</i>	1.5 h	3.49	Photosystem II reaction center protein C
<i>Bpev01.c1891.g0007</i>	1.5 h	3.05	Photosystem I, PsaA/PsaB protein
<i>Bpev01.c1286.g0001</i>	2.5 h	1.7	Phosphoenolpyruvate carboxykinase 1
<i>Bpev01.c0088.g0023</i>	1 h	1.43	STT7 homolog STN7
<i>Bpev01.c0299.g0030</i>	1 h	1.37	Fibrillin
<i>Bpev01.c0245.g0079</i>	1 h	1.76	Ferric reduction oxidase 7
<i>Bpev01.c1657.g0013</i>	1 h	1.16	J-domain protein required for chloroplast accumulation response 1
<i>Bpev01.c0088.g0011</i>	1 h	1.46	Hydroxyproline-rich glycoprotein family protein
<i>Bpev01.c1275.g0002</i>	1 h	1.05	Photosystem II subunit R
<i>Bpev01.c0062.g0077</i>	1 h	1.04	DegP protease 1
<i>Bpev01.c0038.g0100</i>	1 h	1.01	Chloroplast sensor kinase
<i>Bpev01.c0536.g0012</i>	1.5 h	−1.05	Ferredoxin 3
<i>Bpev01.c0142.g0012</i>	1 h	−1.14	Photosystem II reaction center PsbP family protein

Table A9. Cont.

Gene_ID	Time	logFC	Function
<i>Bpev01.c0118.g0021</i>	1 h	−1.23	Mog1/PsbP/DUF1795-like photosystem II reaction center PsbP family protein
<i>Bpev01.c0005.g0002</i>	1 h	−1.24	Translocon at the outer envelope membrane of chloroplasts 75-III
<i>Bpev01.c1024.g0015</i>	1 h	−1.34	S-adenosylmethionine carrier 1
<i>Bpev01.c0298.g0026</i>	1 h	−1.39	Septum site-determining protein (MIND)
<i>Bpev01.c0327.g0054</i>	1 h	−1.52	Tubulin/FtsZ family protein
<i>Bpev01.c0615.g0010</i>	1 h	−2.68	Chlorophyll A-B binding family protein

Table A10. Differentially expressed TFs under different low-temperature stress time points.

TF Family	Total	1 h	1.5 h	2 h	2.5 h	3 h
C2H2	58	53	11	6	16	14
AP2-EREBP	42	29	25	14	24	24
MYB-HB-like	27	21	7	0	7	6
NAM	20	18	5	0	6	3
WD40-like	20	16	5	0	4	0
WRKY	16	15	2	0	5	5
bHLH	15	12	1	0	2	1
Hap3/NF-YB	15	1	13	0	10	0
PHD	14	10	2	1	0	0
Homobox-WOX	10	10	3	0	1	2
bZIP	8	8	0	0	0	0
GRAS	8	7	4	0	5	3
Znf-B	7	5	6	0	4	4
C2C2-Dof	6	4	1	0	2	2
C2C2-GATA	6	4	1	0	2	2
BTB-POZ	5	4	1	0	2	0
C3H	5	4	2	1	3	2
C3H-WRC/GRF	5	2	3	0	3	0
HSF-type-DNA-binding	5	4	3	0	3	2
A20-like	4	4	1	0	2	0
HD-ZIP	4	2	2	0	2	1

Figure A1. Biological process pathway.

Figure A3. Molecular function pathway.

References

1. Thomashow, M.F. PLANT COLD ACCLIMATION: Freezing Tolerance Genes and Regulatory Mechanisms. *Annu. Rev. Plant Biol.* **1999**, *50*, 571–599. [[CrossRef](#)]
2. Wang, X.; Zhao, Q.; Ma, C.-L.; Zhang, Z.H.; Cao, H.-L.; Kong, Y.-M.; Yue, C.; Hao, X.-Y.; Chen, L.; Ma, J.-Q.; et al. Global transcriptome profiles of *Camellia sinensis* during cold acclimation. *BMC Genom.* **2013**, *14*, 1–15. [[CrossRef](#)]
3. Mittler, R. Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.* **2002**, *7*, 405–410. [[CrossRef](#)]
4. Uemura, M.; Yoshida, S. Studies on Freezing Injury in Plant Cells. *Plant Physiol.* **1986**, *80*, 187–195. [[CrossRef](#)]
5. Dodd, A.N.; Jakobsen, M.K.; Baker, A.J.; Telzerow, A.; Hou, S.-W.; Laplaze, L.; Barrot, L.; Poethig, R.S.; Haseloff, J.; Webb, A.A. Time of day modulates low-temperature Ca²⁺ signals in *Arabidopsis*. *Plant J.* **2006**, *48*, 962–973. [[CrossRef](#)]
6. Qi, J.; Song, C.P.; Wang, B.; Zhou, J.; Kangasjärvi, J.; Zhu, J.K.; Gong, Z. Reactive oxygen species signaling and stomatal movement in plant responses to drought stress and pathogen attack. *J. Integr. Plant Biol.* **2018**, *60*, 805–826. [[CrossRef](#)]
7. Mathur, S.; Agrawal, D.; Jajoo, A. Photosynthesis: Response to high temperature stress. *J. Photochem. Photobiol. B Biol.* **2014**, *137*, 116–126. [[CrossRef](#)]
8. Wu, G.; Xianlong, T.; Deguang, Y.; Crops, X.J.J. Research Progress on Physiology of Plant Cold Hardiness. *Crops* **2008**, *3*, S311.
9. Jeong, S.W.; Choi, S.M.; Lee, D.S.; Ahn, S.N.; Hur, Y.; Chow, W.S.; Park, Y.-I. Differential susceptibility of photosynthesis to light-chilling stress in rice (*Oryza sativa* L.) depends on the capacity for photochemical dissipation of light. *Mol. Cells* **2002**, *13*, 419–428.
10. Jan, W.; András, O.; Ravi, A.; Wu, C.J.N.A.R. The C-Terminal Region of Drosophila Heat Shock Factor (HSF) Contains a Constitutively Functional Transactivation Domain. *Nucl. Acids Res.* **1996**, *24*, 367–374.
11. Krause, E.; Dathe, M.; Wieprecht, T.; Bienert, M. Noncovalent immobilized artificial membrane chromatography, an improved method for describing peptide-lipid bilayer interactions. *J. Chromatogr. A* **1999**, *849*, 125–133. [[CrossRef](#)]
12. Medina, J.; Catala, R.; Salinas, J. The CBFs: Three *Arabidopsis* transcription factors to cold acclimate. *Plant Sci.* **2011**, *180*, 3–11. [[CrossRef](#)]
13. Nakashima, K.; Ito, Y.; Yamaguchi-Shinozaki, K. Transcriptional Regulatory Networks in Response to Abiotic Stresses in *Arabidopsis* and Grasses. *Plant Physiol.* **2009**, *149*, 88–95. [[CrossRef](#)]
14. Zhu, J.K.; Chinnusamy, V.; Ohta, M.; Kanrar, S.; Lee, B.H.; Agarwal, M. ICE1, a regulator of cold induced transcriptome and freezing tolerance in plants. *Genes Dev.* **2003**, *17*, 1043–1054.
15. Zhu, J.K.; Agarwal, M.; Kapoor, A. Snow1: Interacts with Ice1 and regulates CBF expression and freezing tolerance in *Arabidopsis*. U.S. Patent No. 7,378,573, 27 May 2008.
16. Agarwal, M.; Hao, Y.; Kapoor, A.; Dong, C.-H.; Fujii, H.; Zheng, X.; Zhu, J.-K. A R2R3 Type MYB Transcription Factor Is Involved in the Cold Regulation of CBF Genes and in Acquired Freezing Tolerance. *J. Biol. Chem.* **2006**, *281*, 37636–37645. [[CrossRef](#)]
17. Shi, Y.; Tian, S.; Hou, L.; Huang, X.; Zhang, X.; Guo, H.; Yang, S. Ethylene Signaling Negatively Regulates Freezing Tolerance by Repressing Expression of CBF and Type-A ARR Genes in *Arabidopsis*. *Plant Cell* **2012**, *24*, 2578–2595. [[CrossRef](#)]
18. Sharma, R.; Singh, G.; Bhattacharya, S.; Singh, A. Comparative transcriptome meta-analysis of *Arabidopsis thaliana* under drought and cold stress. *PLoS ONE* **2018**, *13*, e203266. [[CrossRef](#)]
19. Yun, M.; Dai, X.; Xu, Y.; Wei, L.; Zheng, X.; Zeng, D.; Pan, Y.; Lin, X.; Liu, H.; Zhang, D.J.C. COLD1 confers chilling tolerance in rice. *Cell* **2015**, *6*, 1209–1221.
20. Gu, H.; Hagberg, P.; Zhou, W. Cold pretreatment enhances microspore embryogenesis in oilseed rape (*Brassica napus* L.). *Plant Growth Regul.* **2004**, *42*, 137–143. [[CrossRef](#)]
21. Ito, Y.; Katsura, K.; Maruyama, K.; Taji, T.; Kobayashi, M.; Seki, M. Functional analysis of rice *dreb1/cbf*-type transcription factors involved in cold-responsive gene expression in transgenic rice. *Plant Cell Physiol.* **2006**, *47*, 141–153. [[CrossRef](#)]
22. Winfield, M.O.; Lu, C.; Wilson, I.D.; Coghill, J.A.; Edwards, K.J. Plant responses to cold: Transcriptome analysis of wheat. *Plant Biotechnol. J.* **2010**, *8*, 749–771. [[CrossRef](#)] [[PubMed](#)]

23. Lu, J.; Du, Z.-X.; Kong, J.; Chen, L.-N.; Qiu, Y.-H.; Li, G.-F.; Meng, X.-H.; Zhu, S.-F. Transcriptome Analysis of *Nicotiana tabacum* Infected by Cucumber mosaic virus during Systemic Symptom Development. *PLoS ONE* **2012**, *7*, e43447. [[CrossRef](#)]
24. Hu, H.; You, J.; Fang, Y.; Zhu, X.; Qi, Z.; Xiong, L.J.P.M.B. Erratum to: Characterization of transcription factor geneSNAC2 conferring cold and salt tolerance in rice. *Plant Mol. Biol.* **2010**, *72*, 567–568. [[CrossRef](#)]
25. Huang, G.-T.; Ma, S.-L.; Bai, L.-P.; Zhang, L.; Ma, H.; Jia, P.; Liu, J.; Zhong, M.; Guo, Z.-F. Signal transduction during cold, salt, and drought stresses in plants. *Mol. Boil. Rep.* **2011**, *39*, 969–987. [[CrossRef](#)] [[PubMed](#)]
26. Kurepin, L.V.; Dahal, K.P.; Savitch, L.V.; Singh, J.; Bode, R.; Ivanov, A.G.; Hurry, V.M.; Hüner, N.P.A. Role of CBFs as Integrators of Chloroplast Redox, Phytochrome and Plant Hormone Signaling during Cold Acclimation. *Int. J. Mol. Sci.* **2013**, *14*, 12729–12763. [[CrossRef](#)]
27. Dong, C.-J.; Li, L.; Cao, N.; Shang, Q.M.; Zhang, Z.-G. Roles of phenylalanine ammonia-lyase in low temperature tolerance in cucumber seedlings. *Chin. J. Appl. Ecol.* **2015**, *26*, 2041–2049.
28. Savitch, L.V.; Harney, T.; Hüner, N.P.A. Sucrose metabolism in spring and winter wheat in response to high irradiance, cold stress and cold acclimation. *Physiol. Plant.* **2000**, *108*, 270–278. [[CrossRef](#)]
29. Christenhusz, M.J.; Byng, J.W. The number of known plants species in the world and its annual increase. *Phytotaxa* **2016**, *261*, 201. [[CrossRef](#)]
30. Doyle, J. CTAB Total DNA Isolation. *Mol. Tech. Taxon.* **1991**, *57*, 283–293.
31. Chen, S.; Zhou, Y.; Chen, Y.; Gu, J. Fastp: An ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* **2018**, *34*, i884–i890. [[CrossRef](#)]
32. Salojärvi, J.; Smolander, O.-P.; Nieminen, K.; Rajaraman, S.; Safronov, O.; Safdari, P.; Lamminmaki, A.; Immanen, J.; Lan, T.; Tanskanen, J.; et al. Genome sequencing and population genomic analyses provide insights into the adaptive landscape of silver birch. *Nat. Genet.* **2017**, *49*, 904–912. [[CrossRef](#)] [[PubMed](#)]
33. Kim, D.; Langmead, B.; Salzberg, S.L. HISAT: A fast spliced aligner with low memory requirements. *Nat. Methods* **2015**, *12*, 357–360. [[CrossRef](#)] [[PubMed](#)]
34. Rau, A.; Gallopin, M.; Celeux, G.; Jaffrézic, F. Data-based filtering for replicated high-throughput transcriptome sequencing experiments. *Bioinformatics* **2013**, *29*, 2146–2152. [[CrossRef](#)]
35. Robinson, M.D.; McCarthy, D.J.; Smyth, G.K. edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* **2009**, *26*, 139–140. [[CrossRef](#)] [[PubMed](#)]
36. Xie, C.; Mao, X.; Huang, J.; Ding, Y.; Wu, J.; Dong, S.; Kong, L.; Gao, G.; Li, C.; Wei, L. KOBAS 2.0: A web server for annotation and identification of enriched pathways and diseases. *Nucleic Acids Res.* **2011**, *39*, W316–W322. [[CrossRef](#)]
37. Altschul, S.F.; Madden, T.L.; Schäffer, A.A.; Zhang, J.; Zhang, Z.; 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](#)]
38. Pruitt, K.D.; Tatusova, T.; Maglott, N.R. NCBI Reference Sequence (RefSeq): A curated non-redundant sequence database of genomes, transcripts and proteins. *Nucleic Acids Res.* **2004**, *33*, D501–D504. [[CrossRef](#)]
39. Amos, B.; Rolf, A. The SWISS-PROT protein sequence data bank and its supplement TrEMBL in 1999. *Nucleic Acids Res.* **1999**, *27*, 49–54.
40. Minoru, K.; Susumu, G. KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Res.* **2000**, *28*, 27–30.
41. Chen, J.; Xia, X.; Yin, W. A poplar DRE-binding protein gene, PeDREB2L, is involved in regulation of defense response against abiotic stress. *Gene* **2011**, *483*, 36–42. [[CrossRef](#)]
42. Dai, Z.; Sheridan, J.M.; Gearing, L.J.; Moore, D.L.; Su, S.; Wormald, S.; Wilcox, S.; O'Connor, L.; Dickins, R.A.; Blewitt, M.E.; et al. edgeR: A versatile tool for the analysis of shRNA-seq and CRISPR-Cas9 genetic screens. *F1000Research* **2014**, *3*. [[CrossRef](#)] [[PubMed](#)]
43. Huang, L.M.; Lai, C.P.; Chen, L.F.O.; Chan, M.T.; Shaw, J.F. Arabidopsis SFAR4 is a novel GDSL-type esterase involved in fatty acid degradation and glucose tolerance. *Bot. Stud.* **2015**, *56*, 33. [[CrossRef](#)] [[PubMed](#)]
44. Valmonte, G.R.; Arthur, K.; Higgins, C.M.; MacDiarmid, R.M. Calcium-Dependent Protein Kinases in Plants: Evolution, Expression and Function. *Plant Cell Physiol.* **2014**, *55*, 551–569. [[CrossRef](#)]
45. Ma, W.; Berkowitz, G.A. Ca²⁺ conduction by plant cyclic nucleotide gated channels and associated signaling components in pathogen defense signal transduction cascades. *New Phytol.* **2010**, *190*, 566–572. [[CrossRef](#)]
46. Huang, C.; Ding, S.; Zhang, H.; Du, H.; An, L. CIPK7 is involved in cold response by interacting with CBL1 in *Arabidopsis thaliana*. *Plant Sci.* **2011**, *181*, 57–64. [[CrossRef](#)] [[PubMed](#)]

47. Fu, J.; Miao, Y.; Shao, L.; Hu, T.; Yang, P. De novo transcriptome sequencing and gene expression profiling of *Elymus nutans* under cold stress. *BMC Genom.* **2016**, *17*, 1–19. [\[CrossRef\]](#)
48. Chen, C.; Zhang, Y.; Xu, Z.; Luan, A.; Mao, Q.; Feng, J.; Xie, T.; Gong, X.; Wang, X.; Chen, H.; et al. Transcriptome Profiling of the Pineapple under Low Temperature to Facilitate Its Breeding for Cold Tolerance. *PLoS ONE* **2016**, *11*, e163315. [\[CrossRef\]](#)
49. Wang, Z.; Gerstein, M.; Snyder, M. RNA-Seq: A revolutionary tool for transcriptomics. *Nat. Rev. Genet.* **2009**, *10*, 57–63. [\[CrossRef\]](#)
50. Rivero, R.M.; Ruiz, J.M.; García, P.C.; Lopez-Lefebvre, L.R.; Sánchez, E.; Romero, L. Resistance to cold and heat stress: Accumulation of phenolic compounds in tomato and watermelon plants. *Plant Sci.* **2001**, *160*, 315–321. [\[CrossRef\]](#)
51. Michalak, A. Phenolic Compounds and Their Antioxidant Activity in Plants Growing under Heavy Metal Stress. *J. Environ. Stud.* **2006**, *15*, 523–530.
52. Asada, M.; Nishimura, T.; Ifuku, K.; Mino, H. Location of the extrinsic subunit PsbP in photosystem II studied by pulsed electron-electron double resonance. *Biochim. Biophys. Acta (BBA) Bioenerg.* **2018**, *1859*, 394–399. [\[CrossRef\]](#) [\[PubMed\]](#)
53. Abeynayake, S.W.; Etzerodt, T.P.; Jonavičienė, K.; Byrne, S.; Asp, T.; Boelt, B. Fructan metabolism and changes in fructan composition during cold acclimation in perennial ryegrass. *J. Front. Plant Sci.* **2015**, *6*, 329. [\[CrossRef\]](#) [\[PubMed\]](#)
54. Li, H.-W.; Zang, B.-S.; Deng, X.-W.; Wang, X.-P. Overexpression of the trehalose-6-phosphate synthase gene OsTPS1 enhances abiotic stress tolerance in rice. *Planta* **2011**, *234*, 1007–1018. [\[CrossRef\]](#) [\[PubMed\]](#)
55. Dionne, J.; Rochefort, S.; Huff, D.R.; Desjardins, Y.; Bertrand, A.; Castonguay, Y. Variability for Freezing Tolerance among 42 Ecotypes of Green-Type Annual Bluegrass. *Crop. Sci.* **2010**, *50*, 321–336. [\[CrossRef\]](#)
56. Chen, S.; Lin, X.; Zhang, D.; Li, Q.; Zhao, X.; Chen, S. Genome-Wide Analysis of NAC Gene Family in *Betula pendula*. *Forests* **2019**, *10*, 741. [\[CrossRef\]](#)
57. Ritonga, F.N.; Chen, S. Physiological and Molecular Mechanism Involved in Cold Stress Tolerance in Plants. *Plants* **2020**, *9*, 560. [\[CrossRef\]](#)
58. Wang, F.; Chen, S.; Liang, D.; Qu, G.-Z.; Chen, S.; Zhao, X.-Y. Transcriptomic analyses of *Pinus koraiensis* under different cold stresses. *BMC Genom.* **2020**, *21*, 10–14. [\[CrossRef\]](#)
59. Caspy, I.; Nelson, N. Structure of the plant photosystem I. *Biochem. Soc. Trans.* **2018**, *46*, 285–294. [\[CrossRef\]](#)
60. Pyke, K. The genetic control of plastid division in higher plants. *Am. J. Bot.* **1997**, *84*, 1017–1027. [\[CrossRef\]](#)
61. Ishikita, H.; Stehlik, D.; Golbeck, J.H.; Knapp, E.-W. Electrostatic Influence of PsaC Protein Binding to the PsaA/PsaB Heterodimer in Photosystem I. *Biophys. J.* **2005**, *90*, 1081–1089. [\[CrossRef\]](#)

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