

Diurnal dynamics of different circadian transcription modules in Chinese Pine needles and roots during dormancy introduction

Junhe Yang

Beijing Forestry University

Kai Qu

Beijing Forestry University

Huili Wang

Beijing Forestry University

Liming Bian

Nanjing Forestry University

Yousry A. El-Kassaby

University of British Columbia

Wei Li (✉ bjfuliwei@bjfu.edu.cn)

Beijing Forestry University

Research Article

Keywords: Chinese pine (*Pinus tabuliformis* Carr.), dormancy, circadian rhythm, needles, roots

Posted Date: November 3rd, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-3531931/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Winter dormancy ensures temperate zone's trees proper response to environmental changes and enhances their adaptability. In northern hemisphere, conifers' dormancy is induced by short-day and cold temperature. Previous studies have revealed that *TFL2* is a key regulator involved in conifers' bud set and growth cessation during the dormancy-induced phase. Based on the annual expression profile analysis of *PtTFL2* in Chinese pine (*Pinus tabulaeformis* Carr.), we identified key time nodes for dormancy initiation in autumn. To provide insight of the diurnal transcriptome dynamic in needles and roots during dormancy introduction, RNA-seq was performed at 12 consecutive time points in 24 hours under natural environment in Chinese pine. Interestingly, we found that both needles and roots have rhythmic oscillatory genes, even though the roots could not receive light signals directly. We constructed the transcription factor (TF) co-expression networks of needles and roots and in the core TF modules, we identified 10 hub genes in each. Revealed a network associated with the putative core clock genes (*PtCCA1*, *PtPRR4*, *PtGI*, and *PtTCP42*), which were also isolated, suggesting that they are important in the circadian regulation of the transcriptome. Regulatory network analysis identified key TFs associated with dormancy and cold acclimation in two tissues. Our results provide new insights into the molecular regulatory mechanisms involved in pine dormancy.

1. Introduction

Dormancy is an important adaptive feature for the survival and growth of tree species distributed in temperate and sub-frigid climatic zones of the Northern Hemisphere. Conifers, as the main body of the northern hemisphere forests, require short-day and low temperature to complete their winter dormancy (Zhang et al. 2022). Trees survive adverse conditions by entering a dormant period during the winter (Fadón et al. 2020). This dormancy plays an important role in adapting to climate seasonal changes and it is not a single state but a series of states; however, valid molecular or nondestructive physiological markers to distinguish dormancy are not fully developed. At present, most studies assess the state of quiescence by visual observation, such as completion of bud set, percent bud burst, and time to 50% bud burst. Plants initiate quiescence by sensing light and temperature.

Plant circadian clock systems regulate long-term developmental processes, such as transition from vegetative to reproductive development and from growing to dormant stages, in response to long-period circannual changes in environmental factors (Bohlenius et al. 2006; Hecht et al. 2007). Previous studies have shown that the function of *LHY* (*LATE ELONGATED HYPOCOTYL*)/*CCA1* (*CIRCADIAN CLOCK ASSOCIATED 1*) genes in the circadian clock system is highly conserved in angiosperms (Takata et al. 2009). The circadian system consists of three parts: input, clock (oscillator), and output. Clock genes and their functional mechanisms have been elucidated in *Arabidopsis* and *poplar* (Singh et al. 2021). In *Arabidopsis*, the circadian clock is controlled by a suite of photoreceptors. The central oscillators of the clock senses light signals by receiving photoreceptor phytochrome and cryptochrome signals (Somers 1998; Millar et al. 2003). These results indicated that the expression dynamics of some transcripts were regulated by circadian clocks. It has been reported that 2 to 11% of the *Arabidopsis* genes are regulated

by circadian rhythm (Robert et al. 2001; Harmer et al. 2000). In *Arabidopsis*, during the day, the circadian clock-controlled genes *FLAVIN BINDING KELCH REPEAT F-box1 (FKF1)* and *GIGANTEA (GI)* form protein complexes, *CONSTANS (CO)* expression is promoted by degrading cycling DOF factor, a transcriptional repressor (Sawa et al. 2007). Then, *CO* activates the expression of *FLOWERING LOCUS T (FT)* that promotes flowering (Samach et al. 2000). Flowering is inhibited under short-day conditions (Roden et al. 2002; Eldar et al. 2002). Both *CO* and *FT* have been implicated in seasonal regulation of growth cessation and bud formation. This indicates that these genes, influenced by the seasonal environment, have a significant circadian rhythm and play an important role in growth.

Several physiological and biological processes in conifers are related to environmental circadian rhythms (Nose et al. 2014). To date, patterns of cyclic gene expression have been examined in a limited number of coniferous species to identify the 'core clock' genes e.g., *Norway spruce (Picea abie)* (Gyllenstrand et al. 2014), Japanese cedar (*Cryptomeria japonica*), and Douglas-fir (*Pseudotsuga menziesii*) (Cronn et al. 2017)). In gymnosperms, the cycling of clock genes in Norway spruce is rapidly attenuated under long days (Roden et al. 2002). This is contrary to the results of clock gene expression in other angiosperms, and these data suggest genetic conservation between gymnosperms and other plant groups, and that most of the core circadian clock components present in angiosperms are already present in lycophytes and gymnosperms, but there are fundamental differences in the circadian clock function (Gyllenstrand et al. 2014). So far, research has revealed that the existence of core clock genes and diurnal rhythms of the transcriptome in summer in Japanese cedar (Nose et al. 2014). Due to seasonal changes, the circadian rhythm of related genes is weakened in winter. The data also revealed genes that showed different expression patterns compared to angiosperms, suggesting a unique gene regulatory network in conifers. This indicates that gymnosperms may have genes that show different expression patterns under different climatic conditions as compared to angiosperms, suggesting the existence of unique gene regulatory networks in conifers.

Evidence suggests that *CO/FT* regulatory modules and circadian clocks play important roles in controlling plant growth and dormancy in angiosperms (Bohlenius et al. 2006). *Populus trichocarpa FT (PtFT1)* is the poplar ortholog of *Arabidopsis FT*. *PtFT1* is involved both in controlling the multi-year delay in flowering time, and participating in growth cessation and bud set occurring in the fall (Bohlenius et al. 2006). It is possible that the way in which dormancy signals are perceived and integrated during dormancy may differ between coniferous and deciduous tree species (Cronn et al. 2017). In conifers, *FT* orthologs do not exist, yet only undifferentiated *FT/ TFL1-like* subgene families were present (Yang et al. 2018), where *TFL2* may play an important role in growth rhythm regulation (Nystedt et al. 2013), bud set, and dormancy (Asante et al. 2011; Klintenas et al. 2012). Although *FT/TFL1-like* in pine has been implicated in dormancy, the underlying molecular mechanisms involved in needle dormancy remain largely unknown. To this end, based on the annual expression profile analysis of *PtTFL2* in Chinese pine, we identified key time nodes for autumn dormancy initiation.

Leaves, shoots, and roots are vital organs responsible for sensing and integrating external stimuli. It has been shown that in order to achieve coherent and synchronized circadian oscillations in plants, plant

circadian clocks communicate circadian information between cells and tissues (Uemoto et al. 2023). Most of the identified long-distance signals are from bud cells, and the existence, role, and pathways of circadian clock signals originating from roots and needles are unknown. To better understand the complexity of the circadian cycle of gene expression in resting conifers, we compared Chinese pine transcriptome profiles of needles and roots on a day when *PtTFL2* expression was most characteristic, and analyzed needles and roots of a total of 36 trees from the same site. Pine trees do not defoliate even during the resting stage, and their needles are more sensitive to light and temperature than roots. Therefore, we investigated whether needles and roots response to photoperiod and temperature were the same or different in the circadian rhythm related aspects of dormancy period. By comparing the transcriptome of the two samples, we further investigated whether the circadian oscillations were shared by both needles and roots or whether the roots did not have circadian oscillations due to their inability to receive direct light signals.

Due to the complexity of circadian processes regulated by multiple genes, thousands of genes are differentially expressed between needles and roots in different biological pathways. Including transcription factors (TFS) and transcriptional regulators, naturally act as master regulators during a variety of abiotic stresses and developmental processes by regulating a large number of downstream genes. Here, we focused on the main mechanisms by which TFs regulate circadian rhythms in needles and roots during the resting period. Based on the co-expression network analysis, we revealed Chinese pine core clock genes and circadian rhythms in needles and roots. We further elucidated the expression differences and relationships between related genes in needles and roots during dormancy. This study is expected to provide a necessary basis information for revealing the growth mechanism of gymnosperms during the dormancy diurnal cycle, and also to provide a new idea for studying dormancy mechanism in gymnosperms in response to cold.

2. Materials and Methods

2.1 Plant material and sample collection

Chinese pine tissue samples for the annual transcriptome sequencing cycle were collected from the Green Forest located on Beijing Forestry University campus. Current year needles were collected every 15 days from July 1, 2017 to July 1, 2019. Samples were collected at around 12 P.M., a total of 147 samples (49 time points × 3 biological replicates) were analyzed to determine the expression patterns throughout the annual cycle. The daily cycle samples were collected at 8, 12, 16, 20, 24 and 4 o'clock on March 25, June 25, July 25, August 25, September 25 and December 25, 2020, respectively. The samples of temperature treatment were set at 5 temperature gradients of 4°C, 10°C, 20°C, 30°C and 40°C under the condition of long sunshine (14h/10h) for 1-month-old seedlings. Needles were collected 8 hours later, 3 plants were collected from each group, and 2g was collected from each plant.

The samples of daily cycle samples of needles and roots were selected from 36 15-year-old Chinese pine in an artificial forest at the Beijing Hot Spring Nursery (116°33.91160E, 40°00.08610 N and 44 m above

sea level)during dormancy. The sampling time is set at October 16, 2021. From 10 am on October 16 to 8 am on October 17, every two hours, three trees were randomly selected to collect 2g of needles and roots. Sampled trees were closely planted and experienced similar light time conditions. The needles are obtained from recently developed mature shoots. Actively growing fine roots with a diameter of 1mm were sampled, rinsed with distilled water, and blotted dry. Each sampling time point was replicated using the needles from five seedlings, three of which also had their roots sampled. The samples were collected on dry ice and stored at -80°C for further processing.

2.2 RNA Extraction and Sequencing

After the tissues were frozen in liquid nitrogen and ground into powder, total RNA was extracted from the tissues according to the standard procedure of RNeasy Pure polysaccharide polyphenol plant total RNA extraction kit of Qiagen Biochemical Technology Co., LTD. (Beijing, China). For the total RNA obtained, the RNA purity was determined by Nano Photometer (CA, USA), and the RNA concentration was accurately determined by Qubit 2.0 Fluorometer (CA, USA). The integrity of RNA was detected by Agilent 2100 bioanalyzer. The total amount of each RNA sample is required to be 1µg, RIN ≥ 7.0, and qualified RNA samples are detected and continued to build the library and sequencing experiments.

The mRNA was fragmented into small pieces using divalent cations under increased temperatures. The cleaved RNA fragments were then reverse-transcribed to create the final complementary DNA (cDNA) library according to the protocol provided in the mRNASeq sample preparation kit (Illumina, Inc., San Diego, CA, USA). The average insert size for the paired-end libraries was 200–300 base pairs (bp). The pooled libraries were sequenced on the Illumina HiSeq×Ten platform (2×150 bp) using the paired-end module. The clean reads were mapped to the Chinese pine genome (Niu et al. 2022) and the transcript abundances were estimated using the Kallisto software application (Bray et al. 2016). Differential expression analysis was performed using Sleuth (Pimentel et al. 2017). Differences between the gene expression patterns of the samples under each condition were calculated and normalized using Z-score transformation(Chris Cheadle 2003).

2.3 Differential Gene Expression Analysis and Functional Analysis

Differential analysis was performed using the DESeq2 R package(Jeong et al. 2020). The differentially expressed genes (DEGs) were determined using the threshold of |fold change (FC)| > 2 and adjusted p-value (Padj) < 0.01, which was adjusted using the Benjamini-Hochberg Procedure to control the false discovery rate (FDR). Differentially expressed genes were heat mapped using TM4:MeV software (Saeed et al. 2003). Data were homogenized by the Log Transformations module of TM4:MeV software, and differential genes were clustered by HCL method. The candidate response genes were queried against the RefSeq non-redundant proteins (NR) database, Swiss-Prot database, Gene Ontology (GO) database, and Kyoto Encyclopedia of Genes and Genomes (KEGG). Information on candidate response genes was obtained through Blast comparison in the KEG Dynamic GO enrichment analysis were performed using

Omicshare online tool (<https://www.omicshare.com/tools/>) library to predict the functional annotations and pathways associated with these candidate response genes.

2.4 Construction of co-expression network

The co-expression relationship between genes is determined based on the similarity distance of the expression abundance (RPKM) of each unigene in all samples. The expression abundance between biological replicates is not combined with the average value, but is calculated separately. The similarity distance is represented by Person correlation coefficient (Pcc) (Zhao 2013; Insuk Lee 2022), and the Pcc domain value is set to -0.8/0.8 (generally considered that the absolute value > 0.8 is a strong correlation between samples) for loop iterative calculation. Cytoscape software (Kohl et al. 2011) was used to visualize the correlation coefficient matrix between genes to construct a co-expressed gene network. CytoHubba was used by degree analysis to predict the hub genes. The top 20 high-degree genes were identified by using the CytoHubba plugin (Chin et al. 2014). Prediction genes in molecular complex detection of the five top genes ranked in every module were considered as core hub genes.

3. Result

3.1 Expression of *PtTFL2* is tightly correlated with the dormancy establishment in Chinese pine.

Chinese pine needles cycle transcriptome indicated that the sunshine period changed from long to short day at the end of September, and then needles entered dormancy. Over the two consecutive years, *PtTFL2* was significantly activated under short day starting from the beginning of July through early January of the following year which was also associated with temperature decreases (Fig. 1A). The expression of *PtTFL2* was relatively flat in March, June, July and December. From August, *PtTFL2* was induced during the day. Notably, at the end of September, the sunshine period changed from long to short, and Chinese pine entered the resting period, when *PtTFL2* was significantly activated. In the diurnal cycle, *PtTFL2* first increased and then decreased during the day, and its expression was low at night, indicating that *PtTFL2* has a certain circadian rhythm. According to the changes of annual cycle expression, *PtTFL2* expression reached its peak on October 15 for the two consecutive years and then decreased (Fig. 1B), showing obvious seasonal characteristics. *PtTFL2* may be a key factor regulating dormancy and is activated only in the autumn and is responding to diurnal changes. It is speculated that *PtTFL2* in Chinese pine under short day conditions responds to low temperature and light, and exhibits a certain circadian rhythm in the dormant period and we determined that October 15th of each year is a key day for the response of dormancy genes to the circadian rhythm.

3.2 Diurnal dynamics of transcriptomes in the Chinese pine natural environment

When the Chinese pine enters dormancy under short day conditions, a total of 2,579 and 2,107 genes with rhythm were screened in needles and roots, respectively ($P_{\text{mean}} < 0.01$) (Fig. 2A). With shorter daylight hours, Chinese pine entered a dormancy and was gradually exposed to low temperature conditions on needles and roots, and significant transcriptional changes occurred in both tissues. The increase of DEG expression in needles at night was increased than that in daytime, DEG expression in needles at night increased with the decrease of temperature (Fig. 3A), possibly the expression of related cold resistance genes increased with the decrease of temperature. Although the increase and decrease of DEG expression in roots decreased during both day and night, the change in DEG expression was not as significant as in needles, which may also be due to the influence of soil as a temperature buffer (Fig. 2B).

Using a principal component analysis (PCA)(Fig. 2C). Three principal components (PC) explained 12.2% 10.2% 9.1% of the total variance, respectively, classifying the 36 samples into 4 groups. Respectively are group ND (Needle samples at 6 AM, 8 AM, 10 AM, 12 AM, 14 PM, and 16 PM) group NN (Needle samples at 18 PM, 20 PM, 22 PM, 24 PM, 2 AM, and 4 AM) group RD (Root samples at 6 AM, 8 AM, 10 AM, 12 AM, 2 PM, and 4 PM) and group RN (Root samples at 18 PM, 20 PM, 22 PM, 24 PM, 2 AM, and 4 AM). The four groups were sampled in two phases, day and night, corresponding to needles and roots respectively. Interestingly, we found roots also have rhythmic oscillatory genes, even though the roots could not receive light signals directly. PC1 clearly distinguished the needle samples taken during the day and night from the root samples. In both day and night samples, PC1 clearly distinguished coniferous samples from root samples. On PC2 and PC3, all four groups are basically on the same side. The overall group separation pattern indicated that there were significant differences in the transcriptomic profiles of needles and roots. This suggests that Chinese pine needles and roots use different specific transcriptional mechanisms.

It can be observed from the temperature chart that the air and soil temperatures gradually increase throughout the day, from 6 AM to 4 PM., from 6 p.m. to 4 a.m., as the night progresses, there is a gradual drop in both air and soil temperatures (Fig. 3A). The expression patterns of four groups of genes with circadian rhythm and corresponding low temperature were analyzed (Fig. 3B-E). The results showed that the expression levels of 77 genes in the needles decreased with the increase of temperature during the day, while the expression levels of 245 genes increased with the decrease of temperature at night. With the decrease of temperature, the expression of 131 genes in the root gradually decreased during the day, but gradually increased at night. During the day, the expression trend of 5 genes was the same in needles and roots. At night, however, this consistency spread across 107 genes in both tissues. This is in stark contrast to the five DECs observed within the needles alone. At night, significant induction of rhythmical genes occurred in both needles and roots, suggesting that the regulatory mechanisms controlling the dormant period are influenced not only by light, but also by temperature.

3.3 Diurnal rhythms in transcription of clock-related genes

In order to explore the circadian rhythm of needles and roots during the dormancy, we found that the transcriptional expression patterns of *PtCCA1*, *PtPRR4*, *PtGI*, *PtTCP42* and *PtLOB94* related to circadian rhythm and photoreceptors were significantly oscillated in both needles (Fig. 4A) and roots (Fig. 4B). The

expression pattern of *PtGI* in needles and roots was different. The expression level of *PtGI* in needles was lower during the day, but increased abruptly with the fall of night. On the contrary, in the root, the expression of *PtGI* was higher during the day, and decreased sharply with the onset of night. Although there are genes of circadian rhythm in needles and roots, they have different regulatory mechanisms.

The daily cycle expression profiles of six time points in March, June, July, August, September and December were monitored by RNA-seq for one year (Fig. 4C). The results showed that except December 25, the expression of *PtTCP42* and *PtLOB94* on March 25, June 25, July 25, August 25 and September 25 had significant circadian rhythm. *PtCCA1*, *PtPRR4* and *PtGI* showed significant circadian rhythms on March 25, June 25, July 25, August 25, September 25 and December 25. The transcriptional level of *PtCCA1* peaked at 8:00 on March 25, and then decreased. Transcription gradually increased on June 25, July 25, August 25, and September 25, and gradually decreased towards dawn, bottoming out at 12:00. Except December 25, the expression of *PtPRR4* increased first and then decreased on March 25, June 25, July 25, August 25 and September 25, and this trend was particularly obvious on June 25, July 25, August 25 and September 25, and all reached the bottom at 2AM. On March 25, June 25, July 25, August 25 and September 25, the expression of *PtGI* first increased and then decreased, and all reached a peak at 4 PM and then decreased. On December 25, the expression of *PtGI* was almost the same as *PtPRR4*, which reached a bottom at 20PM and then increased. *PtTCP42* has been identified as a diurnal expression pattern, showing a significant circadian rhythm (Nie et al. 2022). *PtLOB94* bottomed out at 20PM on August 25 and September 25, and then rose, showing a significant circadian rhythm. However, on December 25, the expression of *PtLOB94* did not show a significant circadian rhythm, indicating that low temperature may affect its circadian rhythm. This further verified that *PtCCA1*, *PtPRR4*, *PtGI*, *PtTCP42* and *PtLOB94* play an important role in the regulation of the biological clock of Chinese pine.

3.4 Characterizing responses in needles and roots to dormancy

The rhythmic regulation of transcript levels is an important characteristic of the rhythm system. During the dormancy introduction of needles and roots, genes with circadian rhythms play a unique and complementary role in coordination. In order to compare the function of rhythm genes in the needles and roots of Chinese pine in the four groups of ND, NN, RD and RN in more detail, we performed DEG-enrichment analysis using GO categories (Fig. 5A). Gene ontology (GO) categorizes 'biological process', 'molecular function' and 'cellular component'. The conservation and specificity of the transcriptional reaction between needles and roots of Chinese pine were elucidated. The results from GO enrichment analysis revealed that while most circadian genes were common between needles and roots, there were significantly more enriched classes in needles (Fig. 5A). For instance, genes related to were found to be abundant in both differentially expressed genes (DEG) as well as needle-specific genes across both tissues that regulating solute and ion transport across membranes is an important response to cold stress in both tissues, particularly at night when roots are exposed to colder soil temperatures. the relatively higher temperature experienced by needles, they exhibit greater responsiveness and diversity towards cold stress during night.

This stronger response by needles to exposure to cold is further demonstrated by the finding that 'membrane part', and 'binding' categories were only enriched in needle specific DEGs. Unsurprisingly, at night, the needles had significantly more genes involved in responding to circadian rhythms and 'nucleic acid-binding transcription factor activity' than the other three groups. It reflects the increased TFs expression of bHLH, bZIP, MYB, NAC and AP2/ERF superfamily in needles exposed to freezing temperature (Fig. 5H), which can resist low temperature.

Interestingly, the genes in the root that respond to circadian rhythms are mostly enriched during the day, these DEGs were enriched in 'cellular process' 'developmental process' 'single-organism process' 'metabolic process' 'multicellular organismal process' 'multi-organism process' 'response to stimulus' 'regulation of biological process' 'transporter activity'. These results indicate that genes in root play an important role in responding to stress and environmental stimuli. Showing that genes in roots that respond to circadian rhythms function more strongly when exposed to light, even though the roots could not receive light signals directly.

Finally, the KEGG was used to analyze the biological functions of genes that have circadian rhythms in needles and roots during day and night (Fig. 5B-G). Both day and night, the transport and catabolism of cellular equal number of genes in needles and roots. In 'environmental information processing', there is a relatively high abundance of genes involved in 'plant hormone signal transduction' pathway, with most being expressed during nighttime. The 'genetic information processing' pathway contains a relatively small number of genes, primarily distributed in 'folding, sorting and degradation' as well as 'translation'. The Metabolism module encompasses 74% of all genes, 'metabolic pathways' and 'biosynthesis of secondary metabolites' accounting for nearly 50%. Within the environmental adaptation of 'organismal systems', 'circadian rhythm plants' exhibit enriched gene expression across all groups. PtPRR4 is present in both needles (ND) and roots (RD), indicating its involvement throughout the day. Despite lacking light perception capabilities, roots may possess certain mechanisms to regulate their function.

3.5 Consistent and different diurnally oscillating genes between needles and roots in oscillation phases

We identified and analyzed cooperative genes in needles and roots separately, and set the similarity distance value characterized by Pearson correlation coefficient (Pcc) to 0.8 (usually absolute value > 0.8 is considered to be the strong correlation between samples). From the key genes with significant correlations, 470 genes were screened, 143 in needles and 186 in roots, affected development and cell activity, respectively. Co-expression networks were constructed for genes with significant circadian rhythms in needles and roots, respectively (Fig. 6A-B). In total, 329 TFs were recorded in all samples, and they may have a critical role in the dormancy of Chinese pine. CytoHubba was used to analyze the degree of the co-expression network of needles and roots, and to complete the prediction of core genes (Shannon et al. 2003). The key hub genes were chosen with a centrality degree > 8. A higher k-core score means a more topological central location. Subnetworks in the TFs were explored by k-core scoring at k-

core > 6 using the 'CytoHubba' package in Cytoscape software. Then, further subnetworks were constructed for the top ten core genes in needles and roots (Fig. 6C-D).

In this study, we identified connected core TFs associated with dormancy circadian rhythms in Chinese pine. These results demonstrated that the dormancy regulatory network in Chinese pine is highly interconnected, that most of the dormancy response circuits were common to both tissues. Among them, the number of genes related to AP2/ERF, NAC, MYB, ZFP, bZIP, bHLH, C2C2 and WRKY families were the largest, which have previously been associated with plant cold stress.

Among ten core genes of roots, four belong to AP2/ERF family protein homologous to (*PtERF1* *PtERF21* and *PtERF56* *PtDREB1*), one MYB-related transcription factor *PtCCA1*, it's a clock gene that regulates the rhythm of growth, one C2C2-CO-like zinc finger transcription factor, it participates in the circadian rhythm and is highly expressed at dusk, one C2C2-Dof transcription factor *PtDof2*, one is the *GRAS* transcription factor *PAL11* involved in lignin biosynthesis by phenylalanine ammonolyase (PAL), one NF-YA transcription factor *PtNF_YA5*, and one NAC domain transcriptional regulator superfamily protein (*PtNAC67*). Among ten core genes of needles, four belong to AP2/ERF transcription factor family member (*PtERF61* *PtERF62* and *PtERF63* *PtERF64*), C2H2 zinc finger transcription family member (*PtZFP1* *PtZFP17* and *PtZFP15* *PtZFP30*), one GeBP transcription factor *PtGeBP25*, and *PtCesA11* encodes a xylose-specific cellulose synthetase phosphorylated on one or more serine residues and is a homologue of *AtCesA7*.

These key core genes not only have a certain circadian rhythm, but also most of them are related to abiotic stress. Interestingly, although the core regulatory factors of needles and roots are different, but most of the transcription factor families of dormancy response factors are common in both tissues. The *AP2/ERF* family plays an important role in plant growth and development, which can directly or indirectly play a role in the process of biotic and abiotic stress. The protein encoded by *COL* gene is a photoperiodic transcription factor involved in the regulation of plant growth and development and response to stress.

4. Discussion

Woody plants that grow in temperate or cold regions endure stressful environmental conditions every winter, a survival mechanism known as winter dormancy (Allona et al. 2008). Conifers are the main source of wood in the world, and in this long-lived forest species, the length of dormancy limits the growing season, thus affecting wood production and quality. Consequently, the time of dormancy onset is the key ecological variable. In fact, a thorough understanding of the core regulatory factors of conifers' dormant period is of great significance for their genetic improvement. In this study, the expression of *PtTFL2*, a key regulator of germination and growth arrest in conifers during the dormancy induction stage, was analyzed for two consecutive years, and the key time node of dormancy initiation in autumn was determined. Systematic analysis of the diurnal dynamics of different circadian transcription modules in Chinese pine needles and roots during dormancy introduction.

The establishment of winter dormancy cannot be strictly considered as a photoperiodic effect, and the relevant data suggest that low temperature controls growth arrest and dormancy induction in these species, independent of photoperiodic conditions. (Heide et al. 2005; Cook et al. 2005) Expression of *P. trichocarpa FT1* gene, a putative ortholog of *AtFT*, is a critical determinant of the timing of growth cessation and bud set in the SD response. *PtTFL2* as a homologue of *ATFT* (Liu et al. 2018), the sunshine period changed from long to short at the end of September, and Chinese pine entered the dormant period, at which time *PtTFL2* was significantly activated. In addition, the expression level increased abruptly on October 15 for two consecutive years, with obvious seasonal characteristics. *PtTFL2* may be a key factor in regulating dormancy, activating only in autumn and responding to diurnal changes. Therefore, this study took October 15 as the key date for the response of dormant genes to the circadian rhythm. Compared with the root, the metabolic activities of needles were more active. Therefore, on March 25, June 25, July 25, August 25, September 25 and December 25, a total of six time points covering the whole year, daily cycle samples of Chinese pine needles were collected to screen potential regulators with significant circadian rhythm during the resting period. For future targeted breeding to produce high-yield cold-stress tolerant trees, the cold-tolerance of these genes during the dormant period was also analyzed.

The path to winter dormancy is a continuous process of plants going through different physiological states. The rate at which dormancy develops and the depth of dormancy varies according to the environmental signals that induce the dormancy process, in particular the duration of sunlight and temperature. This is clearly a complex process, probably the result of several core genes interacting with some signaling pathways. Covington et al. investigated circadian microarray data, revealing that plant hormone and multiple stress response pathways are influenced by the circadian clock (Covington et al. 2007). It has been proposed that light and ABA signal transduction pathways continuously control cold adaptation and dormancy of poplar through a series of complex processes (Rohde et al. 2007). The plant hormones abscisic acid (ABA) are believed to be important components in the crosstalk between stress signaling pathways. The results suggest that ABA is more directly involved in photoperiodic control of cold acclimation (Arora et al. 2003). This study further analyzed the expression patterns of hormone-related genes representing hormone biosynthesis and signaling pathways through the hormone signal-related genes listed in the KEGG database, and found that out of the 4003 circulating genes, 23 genes that may be related to growth hormone showed significant circadian rhythms. Among them, the expression levels of *Pt5G04180*, *Pt1G09210*, *Pt1G09200* and *Pt2G47630* gradually increased in needles at night, and suddenly increased from the same time (22PM), indicating that these four genes may be related in the resting period of Chinese pine (Fig. 7). And these four genes tended to be expressed only in night needles, The homolog of *Pt5G04180* in *Arabidopsis* is *AT5G53160*, it is a regulatory component of ABA receptor. Interacts with protein phosphatase 2Cs ABI1 and ABI2. Stimulates ABA signaling. *Pt2G47630* homologue in *Arabidopsis* is *AT4G01026*, it is an Encodes a member of the *PYR* (pyrabactin resistance) /*PYL* (PYR1-like)/*RCAR* (regulatory components of ABA receptor) family proteins with 14 members. *PYR*/*PYL*/*RCAR* family proteins function as abscisic acid sensors. Mediate ABA-dependent regulation of protein phosphatase 2Cs ABI1 and ABI2. Low temperature stress induced the increase of plant hormone ABA content (Allonaet et al. 2008). It was further indicated that ABA, as an important 'anti-

stress hormone' in plants, can also promote plant dormancy and promote plant adaptation to stressed environment.

Through screening analysis of DEG, we found that both tissues produced a progressive transcriptional response to cold, showing the most common cold adaptation response. To identify potentially key conserved transcription factors mediating cold acclimation during the dormancy phase of Chinese pine. Gene Ontology (GO) analysis of all deg induced in Chinese pine (Fig. 3) showed that, regardless of the tissue, In core stress categories such as 'transporter activity', 'response to stimulus' and 'nucleic acid binding transcription factor activity', Common genes that induce cold responses are overrepresented. Among them, there are relatively many genes in the 'response to stimulus', and the response of the two tissues is indeed different (GO diagram). Gene Ontology (GO) analysis shows that, Root-specific induction genes were enriched in 'cell', 'cell part', 'binding', 'cellular process', 'cellular process' and 'metabolic process'. When compared with roots, needle-specific induced deg was enriched in the 'single-organism process', 'organelle', 'membrane' and 'response to stimulus'. These contrast responses suggest that during the dormant period, needles are exposed to extremely cold and dry environments, which require membrane recombination and protection, and needles also need to maintain a more active metabolism during the winter. Relevant studies have shown that for herbs, the accumulation of carbohydrate and lipid metabolism plays a key role in the survival of perennial evergreen needles at extremely low temperatures (Kjellsen et al. 2013; Angelcheva et al. 2014; Strimbeck et al. 2015), measured by electrolyte leakage, both needles and roots increased cold adaptability when exposed to low temperatures (Flint et al. 1967; Ferguson et al. 2011). Xyloglucosyltransferase/hydrolase (XTH) is a cell wall modifying enzyme, which can regulate plant growth and development and stress response, and plays an important role in cell growth. According to 'organelle', the number of differential genes in night needles is twice that in roots. It shows that needles and roots have different functions in organelle's function. Among them, the expression of *Pt3G27160* in the roots of Chinese pine increased with the decrease of temperature at night (histogram green), indicating that *Pt3G27160* may respond to low temperature stress during dormancy and participate in the regulation of root growth and development (Fig. 7). According to the two-year annual cycle, the expression levels of these four genes began to rise in October every year, all of them played an important role in inducing dormancy in response to short daylight (Fig. 8A), and under different temperature treatment environments, the expression levels increased significantly only at low temperatures (Fig. 8B).

In this study, we established a regulatory network in needles and roots by observing the expression of transcription factors in needles and roots continuously for 24 hours, respectively, and found that genes in the two co-expression networks overlaps, indicating that their expression is closely coordinated during the resting period. For more reliable results, we extracted edges of the network that showed more than 0.8 bootstrap probability and their related targets from the network entire gene network. Although further research is needed to resolve this network, it provides many hints for selecting genes that play an important role in the regulation of circadian transcriptome dynamics during conifer dormancy. In the coexpression network of needles and roots, The presence of putative core clock genes shows their importance in the circadian regulation of the transcriptome. The presence of putative core clock genes

shows their importance in the circadian regulation of the transcriptome. *PtCCA1*, *PtPRR4*, *PtGI* and *PtTCP42* four homologous genes with significant circadian rhythms, which are predicted to be the core clock genes of *Arabidopsis*, are also in the coexpression network of needles and roots and are in key regulatory positions.

The *AP2/ERF* gene family was the most studied gene related to cold stress in dormant period. In this study, the gene family with the most significant circadian rhythm was also *AP2/ERF* gene family. ERF corresponds to an AP2/ERF protein and are involved in many signal transduction pathways, and some family members operate in the adversity signaling crossover pathway(Xing et al. 2019). The *ERF* subfamily plays an important role in regulating plant responses to stress factors such as hormones, pathogens, low temperature, drought and high salt, and is also an important regulatory factor in abscisic acid (ABA) and other signal transduction pathways (Yan et al. 2013). Related studies have shown that the product of chrysanthemum CmERF110 can act as a flower activator, mainly through the photoperiodic pathway. The transcription of CmERF110 follows a similar circadian rhythm, indicating that CmERF110 is a clock gene that mainly regulates flowering through the photoperiodic pathway(Xing et al. 2019). Relevant studies have shown that the high expression levels of several Ppc *AP2/ERF* genes in dormant flower buds in cherry appear in different dormancy stages, indicating that these genes are involved in the dormancy transition of flower buds and may be involved in cold mediated dormancy transition (Zhu et al. 2021). In the dormant period, *PtERF7*, *PtERF64*, *PtERF63*, *PtERF62*, *PtERF61*, *PtERF22*, *PtERF21*, *PtERF2* of *AP2/ERF* gene family in needles and *PtERF56* in roots all decreased with the decrease of night temperature. The expression level gradually increased (Fig. 7). These results suggest that *AP2/ERF* gene is also involved in cold-mediated dormancy transition during the dormancy of Chinese pine.

In this study, we found that Most of these TFs identified transcription factors were sensitive to temperature and rhythm changes, which is driven by the circadian clock, and some studies of the reaction to cold reactions have confirmed this (Bieniawska et al. 2008). The increased tolerance of plants to abiotic stress is associated with increased nutrient uptake, altered hormonal balance, increased activity of reactive oxygen scavenging systems, and synthesis of osmoregulatory substances. In contrast, the circadian clock controls plant nutrient homeostasis, hormone synthesis and signaling, redox reactions, and changes in the concentration of some major osmoregulatory substances (Covington et al. 2007; Greenham et al. 2015; Zhou et al. 2015; Atamian et al. 2016; Loudet et al. 2017). This shows that the circadian rhythm system of plants plays an important role in the face of abiotic adversity.

our study provides a comprehensive overview of the transcriptome associated with the dormancy of Chinese pine. This study reveals the core clock genes and potentially key conserved transcription factors mediating cold acclimation with significant circadian rhythm during the dormant period of Chinese pine. In this study, we constructed co-expression networks of core TFs in needles and roots to explore the unique gene regulatory network during the resting period of conifers, which provided basic data for understanding the circadian regulation mechanism during the resting period of conifers. Thus, it can be used for directional breeding, efficient production of cold-stress resistant trees, and help the forest industry to meet the challenges brought by future climate change.

5. Conclusion

Most studies of diurnal and diurnal mechanisms in plants focus on the model species *Arabidopsis*, however, dormancy does not exist in *Arabidopsis*. Based on the annual expression profile of PtTFL2, this study determined the key time node for the onset of autumn dormancy of Chinese pine. Microarray analysis showed some differences in transcriptome dynamics between needles and roots. Interestingly, we found that both needles and roots have rhythmic oscillatory genes, even though the roots could not receive light signals directly. By constructing transcription factor (TF) co-expression networks of needles and roots, Some transcriptional factors with functional similarities and synergistic effects were found to play a role in the regulatory pathway, which provided some data support for mining gene functions and analyzing related regulatory pathways. Regulatory network analysis also identified key TFs associated with dormancy and cold acclimation in both tissues. Our results provide new insights into the molecular regulatory mechanisms involved in pine dormancy.

Declarations

Data availability statement

All data supporting the findings of this study are available within the paper and within its supplementary materials published online.

Author contributions JHY collected samples, analyzed the data and wrote the manuscript. KQ and HLW collected samples in the field. WL, YAE and LMB conceived the study and revised the manuscript. All authors have read and approved the manuscript.

Declaration of Competing Interest The authors declare that they have no conflicts of interest.

Funding This work was supported by “National Key R&D Program of China (2022 YFD2200304)”.

References

1. Allona I, Ramos A, C Ibáñez, et al. (2008). Review. Molecular control of winter dormancy establishment in trees. Spanish Journal of Agricultural Research 6(S1). <http://doi.org/10.5424/sjar/200806S1-389>.
2. Angelcheva L, Mishra Y, Antti H, Kjellsen TD, Funk C, Strimbeck RG, Schroder WP. (2014). Metabolomic analysis of extreme freezing tolerance in Siberian spruce (*Picea obovata*). New Phytol 204(3):545–555. <http://doi.org/10.1111/nph.12950>.
3. Arora R, Rowland LJ, Tanino K. (2003). Induction and Release of Bud Dormancy in Woody Perennials: A Science Comes of Age. HortScience 38(5):911–921. <http://doi.org/10.1007/s00122-003-1334-9>.

4. Asante DK, Yakovlev IA, Fossdal CG, Holefors A, Opseth L, Olsen JE, Junttila O, Johnsen O. (2011). Gene expression changes during short day induced terminal bud formation in Norway spruce. *Plant Cell Environ* 34(2):332–346. <http://doi.org/10.1111/j.1365-3040.2010.02247.x>.
5. Atamian HS, Harmer SL. (2016). Circadian regulation of hormone signaling and plant physiology. *Plant Mol Biol* 91(6):691–702. <http://doi.org/10.1007/s11103-016-0477-4>.
6. Bieniawska Z, Espinoza C, Schlereth A, Sulpice R, Hinch DK, Hannah MA. (2008). Disruption of the *Arabidopsis* circadian clock is responsible for extensive variation in the cold-responsive transcriptome. *Plant Physiol* 147(1):263–279. <http://doi.org/10.1104/pp.108.118059>.
7. Bohlenius H, Huang T, Charbonnel-Campaa L et al. (2006). CO/FT regulatory module controls timing of flowering and seasonal growth cessation in trees. *Science* (5776):312. <http://doi.org/10.1126/science.1126038>.
8. Bray NL, Pimentel H, Melsted P, Pachter L. (2016). Near-optimal probabilistic RNA-seq quantification. *Nat Biotechnol.* 34(5):525–527. <http://doi.org/10.1038/nbt.3519>
9. Chin CH, Chen SH, Wu HH, Ho CW, Ko MT, Lin CY. (2014). CytoHubba: identifying hub objects and sub-networks from complex interactome. *BMC systems biology* 8 (Suppl 4)S11. <http://doi.org/10.1186/1752-0509-8-S4-S11>.
10. Cook NC, Bellen A, Cronjé PJR, Wit ID, Keulemans W, Van den Putte A, Steyn W. (2005). Freezing temperature treatment induces bud dormancy in ‘Granny Smith’ apple shoots. *Scientia Horticulturae*. 106(2):170–176. <http://doi.org/10.1016/j.scienta.2005.03.009>.
11. Cooke JE, Eriksson ME, Junttila O. (2012). The dynamic nature of bud dormancy in trees: environmental control and molecular mechanisms. *Plant Cell Environ.* 35(10):1707–1728. <http://doi.org/10.1111/j.1365-3040.2012.02552.x>
12. Corbesier L, Vincent C, Jang S et al. (2007). FT Protein Movement Contributes to Long-Distance Signaling in Floral Induction of *Arabidopsis*. *Science* 2007 316(5827):1030–1033. <http://doi.org/10.1126/science.1141752>.
13. Covington MF, Harmer SL. (2007). The circadian clock regulates auxin signaling and responses in *Arabidopsis*. *PLoS Biol* 5(8):e222. <http://doi.org/10.1371/journal.pbio.0050222>
14. Cronn R, Dolan PC, Jogdeo S, Wegrzyn JL, Neale DB, St Clair JB, Denver DR. (2017). Transcription through the eye of a needle: daily and annual cyclic gene expression variation in Douglas-fir needles. *BMC Genomics* 18(1):558. <http://doi.org/10.1186/s12864-017-3916-y>
15. Devlin PF. (2000). Cryptochromes Are Required for Phytochrome Signaling to the Circadian Clock but Not for Rhythmicity. *Plant Cell Online* 12(12):2499–2510. <http://doi.org/10.2307/3871244>.
16. Dle C, Vawter MP, Freed WJ et al. (2013). Analysis of microarray data using Z score transformation. *Journal of Molecular Diagnostics* 5(2):73–81. [http://doi.org/10.1016/S1525-1578\(10\)60455-2](http://doi.org/10.1016/S1525-1578(10)60455-2)
17. Edwards KD, Anderson PE, Hall A, Salathia NS, Locke JC, Lynn JR, Straume M, Smith JQ, Millar AJ. (2006). FLOWERING LOCUS C mediates natural variation in the high-temperature response of the *Arabidopsis* circadian clock. *Plant Cell* 18(3):639–650. <http://doi.org/10.1105/tpc.105.038315>

18. Eldar A, Dorfman R, Weiss D, Ashe H, Shilo BZ, Barkai N. (2002). Robustness of the BMP morphogen gradient in *Drosophila* embryonic patterning. *Nature* 419(6904):304–308.
<http://doi.org/10.1038/nature01061>
19. Fadón E, Fernandez E, Behn H, Luedeling E. (2020). A Conceptual Framework for Winter Dormancy in Deciduous Trees. *Agronomy* 10(2). <http://doi.org/10.3390/agronomy10020241>
20. Ferguson JC, Tarara JM, Mills LJ, Grove GG, Keller M. (2011). Dynamic thermal time model of cold hardiness for dormant grapevine buds. *Ann Bot* 107(3):389–396.<http://doi.org/10.1093/aob/mcq263>
21. Flint HL, Boyce BR, Beattie DJ. (1967). Index of injury-a useful expression of freezing injury to plant tissues as determined by the electrolytic method. *Canadian Journal of Plant Science* 47(2):229–230. <http://doi.org/10.4141/cjps67-043>.
22. Fowler S, Lee K, Onouchi H et al. (1999). GIGANTEA: a circadian clock-controlled gene that regulates photoperiodic flowering in *Arabidopsis* and encodes a protein with several possible membrane-spanning domains. *EMBO Journal* (17):18. <http://doi.org/10.1093/emboj/18.17.4679>
23. G, Richard, Strimbeck et al. (2015). Extreme low temperature tolerance in woody plants[J]. *Frontiers in Plant Science* 00884. <http://doi.org/10.3389/fpls>
24. Greenham K, McClung CR et al. (2015). Integrating circadian dynamics with physiological processes in plants. *Nat Rev Genet* 16(10):598. <http://doi.org/10.1038/nrg3976>
25. Gyllenstrand N, Karlgren A, Clapham D, Holm K, Hall A, Gould PD, Kallman T, Lagercrantz U. (2014). No time for spruce: rapid dampening of circadian rhythms in *Picea abies* (L. Karst). *Plant Cell Physiol* 55(3):535–550. <http://doi.org/10.1093/pcp/pct199>
26. Harmer SL, Hogenesch JB, Straume M, Chang HS, Han B, Zhu T, Wang X, Kreps JA, Kay SA. (2000). Orchestrated Transcription of Key Pathways in *Arabidopsis* by the Circadian Clock. *Science*, 290(5499):2110–2113. <http://doi.org/10.1126/science.290.5499.2110>
27. Hecht V, Knowles CL, Vander Schoor JK, Liew LC, Jones SE, Lambert MJ, Weller JL. (2007). Pea LATE BLOOMER1 is a GIGANTEA ortholog with roles in photoperiodic flowering, deetiolation, and transcriptional regulation of circadian clock gene homologs. *Plant Physiol* 144(2):648–661. <http://doi.org/10.1104/pp.107.096818>
28. Heide OM, Prestrud AK. (2005). Low temperature, but not photoperiod, controls growth cessation and dormancy induction and release in apple and pear. *Tree Physiology* (1):109–114. <http://doi.org/10.1093/treephys/25.1.109>.
29. Imaizumi T, Tran H G, Swartz T E et al. (2003). FKF1 is essential for photoperiodic-specific light signalling in *Arabidopsis*. *Nature* 426.6964(2003):302–6. <http://doi.org/10.1038/nature02090>
30. Jeong S, Lim CW, Lee SC. (2020). The Pepper MAP Kinase CaAIMK1 Positively Regulates ABA and Drought Stress Responses. *Frontiers in Plant Science*, 2020, 11:720. <http://doi.org/10.3389/fpls.2020.00720>.
31. Kjellsen TD, Yakovlev IA, Fossdal CG, Strimbeck GR. (2013). Dehydrin accumulation and extreme low-temperature tolerance in Siberian spruce (*Picea obovata*). *Tree Physiol* 33(12):1354–1366.

<http://doi.org/10.1093/treephys/tpt105>

32. Klintenas M, Pin PA, Benlloch R, Ingvarsson PK, Nilsson O. (2012). Analysis of conifer FLOWERING LOCUS T/TERMINAL FLOWER1-like genes provides evidence for dramatic biochemical evolution in the angiosperm FT lineage. *New Phytol* 196(4):1260–1273. <http://doi.org/10.1111/j.1469-8137.2012.04332.x>
33. Kohl M, Wiese S, Warscheid B. (2011). Cytoscape: software for visualization and analysis of biological networks. *Methods Mol Biol* 696:291–303. http://doi.org/10.1007/978-1-60761-987-1_18
34. Lee I, Date SV, Adai AT et al. (2004). A Probabilistic Functional Network of Yeast Genes[J]. *Science* 2004, 306(5701):1555–1558. <http://doi.org/10.1126/science.1099511>.
35. Loudet O, Hasegawa PM. (2017). Abiotic stress, stress combinations and crop improvement potential. *Plant J.* 90(5):837–838. <http://doi.org/10.1111/tpj.13604>
36. Marques, Jorge, Dong et al. (2015). Redox rhythm reinforces the circadian clock to gate immune response. *Nature* 523 (7561):472-U221. <http://doi.org/10.1038/nature14449>
37. Millar, Andrew J. (2003). A suite of photoreceptors entrains the plant circadian clock. *J Biol Rhythms.* 18(3):217–226. <http://doi.org/10.1177/0748730403018003004>
38. Nelson DC, Lasswell J, Rogg LE et al. (2000). FKF1, a Clock-Controlled Gene that Regulates the Transition to Flowering in Arabidopsis. *Cell* 101(3):331–340. [http://doi.org/10.1016/S0092-8674\(00\)80842-9](http://doi.org/10.1016/S0092-8674(00)80842-9).
39. Nie YM, Han FX, Ma JJ, Chen X, Song YT, Niu SH, Wu HX. (2022). Genome-wide TCP transcription factors analysis provides insight into their new functions in seasonal and diurnal growth rhythm in *Pinus tabuliformis*. *BMC Plant Biol* 22(1):167. <http://doi.org/10.1186/s12870-022-03554-4>.
40. Niu S, Li J, Bo W, Yang W, Zuccolo A, Giacomello S, Chen X, Han F, Yang J, Song Y, et al. (2022). The Chinese pine genome and methylome unveil key features of conifer evolution. *Cell* 185(1):204–217 e214. <http://doi.org/10.1016/j.cell.2021.12.006>
41. Nose M, Watanabe A. (2014). Clock genes and diurnal transcriptome dynamics in summer and winter in the gymnosperm japanese cedar (*cryptomeria japonica* (L.f.) d.don). *Bmc Plant Biology* 14(1):308. <http://doi.org/10.1177/0748730403018003004>
42. Nystedt B, Street NR, Wetterbom A, Zuccolo A, Lin YC, Scofield DG, Vezzi F, Delhomme N, Giacomello S, Alexeyenko A, et al. (2013). The Norway spruce genome sequence and conifer genome evolution. *Nature* 497(7451):579–584. <http://doi.org/10.1038/nature12211>
43. P Suárez-López, Wheatley K, Robson F, et al. (2001). CONSTANS mediates between the circadian clock and the control of flowering in Arabidopsis. *Nature* 410(6832):1116–20. <http://doi.org/10.1038/35074138>.
44. Pimentel H, Bray NL, Puente S, Melsted P, Pachter L. (2017). Differential analysis of RNA-seq incorporating quantification uncertainty. *Nat Methods.* 14(7):687–690. <http://doi.org/10.1101/058164>
45. Putterill J, Robson F, Lee K et al. (1995). The CONSTANS gene of arabidopsis promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. *Cell* 80(6):847 – 57.

[http://doi.org/10.1016/0092-8674\(95\)90288-0](http://doi.org/10.1016/0092-8674(95)90288-0).

46. Roden L C, Carre I A, Morris K et al. (2023). Floral responses to photoperiod are correlated with the timing of rhythmic expression relative to dawn and dusk in Arabidopsis. PNAS 99.20:p.13313–13318. <http://doi.org/10.1073/pnas.192365599>
47. Rohde A, Ruttink T, Hostyn V, Sterck L, Van Driessche K, Boerjan W. (2007). Gene expression during the induction, maintenance, and release of dormancy in apical buds of poplar. J Exp Bot. 58(15–16):4047–4060. <http://doi.org/10.1093/jxb/erm261>
48. Saeed AI, Sharov V, White J et al. (2003). TM4: a free, open-source system for microarray data management and analysis. Biotechniques 34(2):374–378. <http://doi.org/10.1089/154065803762851360>.
49. Samach A, Onouchi H, Gold SE, Ditta GS, Schwarz-Sommer Z, Yanofsky MF, Coupland G. (2000). Distinct Roles of CONSTANS Target Genes in Reproductive Development of Arabidopsis. Science 288(5471), 1613–1616. <http://doi.org/10.1126/science.288.5471.1613>
50. SAUNDERS, David S. (2010). Circadian rhythms and the evolution of photoperiodic timing in insects. Physiological Entomology 34(4):301–308. <http://doi.org/10.1111/j.1365-3032.2009.00699.x>.
51. Sawa M, Nusinow DA, Kay SA, Imaizumi T. (2007). FKF1 and GIGANTEA complex formation is required for day-length measurement in Arabidopsis. Science 318(5848):261–265. <http://doi.org/10.1126/science.1146994>
52. Schaffer R, Landgraf J, Accerbi M et al. (2001). Microarray Analysis of Diurnal and Circadian-Regulated Genes in Arabidopsis. The Plant Cell 13(1):113–123. <http://doi.org/10.2307/3871157>
53. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. (2003). Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 13(11):2498–2504. <http://doi.org/10.1101/gr.1239303>
54. Singh RK, Bhalerao RP, Eriksson ME. (2021). Growing in time: exploring the molecular mechanisms of tree growth. Tree Physiol 41(4):657–678. <http://doi.org/10.1093/treephys/tpaa065>
55. Somers DE.(1998). Phytochromes and Cryptochromes in the Entrainment of the Arabidopsis Circadian Clock. Science 282(5393):1488–1490. <http://doi.org/10.1126/science.282.5393.1488>.
56. Takata N, Saito S, Saito CT, et al. (2010). Molecular phylogeny and expression of poplar circadian clock genes, LHY1 and LHY2. New Phytologist 181(4):808–819. <http://doi.org/10.1111/j.1469-8137.2008.02714.x>.
57. Turck F, Fornara F, Coupland G. (2008). Regulation and identity of florigen: FLOWERING LOCUS T moves center stage. Annu Rev Plant Biol 59:573–594. <http://doi.org/10.1109/TASC.2007.898014>.
58. Uemoto K, Mori F, Yamauchi S, Kubota A, Takahashi N, Egashira H, Kunimoto Y, Araki T, Takemiya A, Ito H, et al. (2023). Root PRR7 improves the accuracy of the shoot circadian clock through nutrient transport. Plant Cell Physiol 64 (3):352–362. <http://doi.org/10.1146/annurev.arplant.59.0326>
59. 092755

60. Xing X, Jiang J, Huang Y, et al. (2019). The Constitutive Expression of a Chrysanthemum ERF Transcription Factor Influences Flowering Time in *Arabidopsis thaliana*. *Molecular biotechnology* 61(1):20–31. <http://doi.org/10.1007/s12033-018-0134-z>
61. Yan HW, Hong L, Zhou YQ, et al. (2013). A genome-wide analysis of the ERF gene family in sorghum. *Genetics & Molecular Research* 12(2):2038–2055. <http://doi.org/10.4238/2013.May.13.1>.
62. Yang L, Bangtong L, Guihua D, et al. (2018). Expression profiles and regulation of FT/TFL1-like genes in *Pinus tabuliformis* *Journal of Beijing Forestry University* 40(10):60–66. <http://doi.org/10.13332/j.1000-1522.20180040>
63. Zhang JX, Liu HM, Yang BN, Wang HL, Niu SH, El-Kassaby YA, Li W. (2022). Phytohormone profiles and related gene expressions after endodormancy release in developing *Pinus tabuliformis* male strobili. *Plant Sci* 316:111167. <http://doi.org/10.1016/j.plantsci.2021.111167>.
64. Zheng ZL, Zhao Y. (2013). Transcriptome comparison and gene coexpression network analysis provide a systems view of citrus response to ‘*Candidatus Liberibacter asiaticus*’ infection. *BMC Genomics* 14(1):1–21. <http://doi.org/10.1186/1471-2164-14-27>
65. Zhu Y, Liu X, Gao Y et al. (2021). Transcriptome-based identification of AP2/ERF family genes and their cold-regulated expression during the dormancy phase transition of Chinese cherry flower buds. *Scientia Horticulturae* 275:109666. <http://doi.org/10.1016/j.scienta.2020.109666>.

Figures

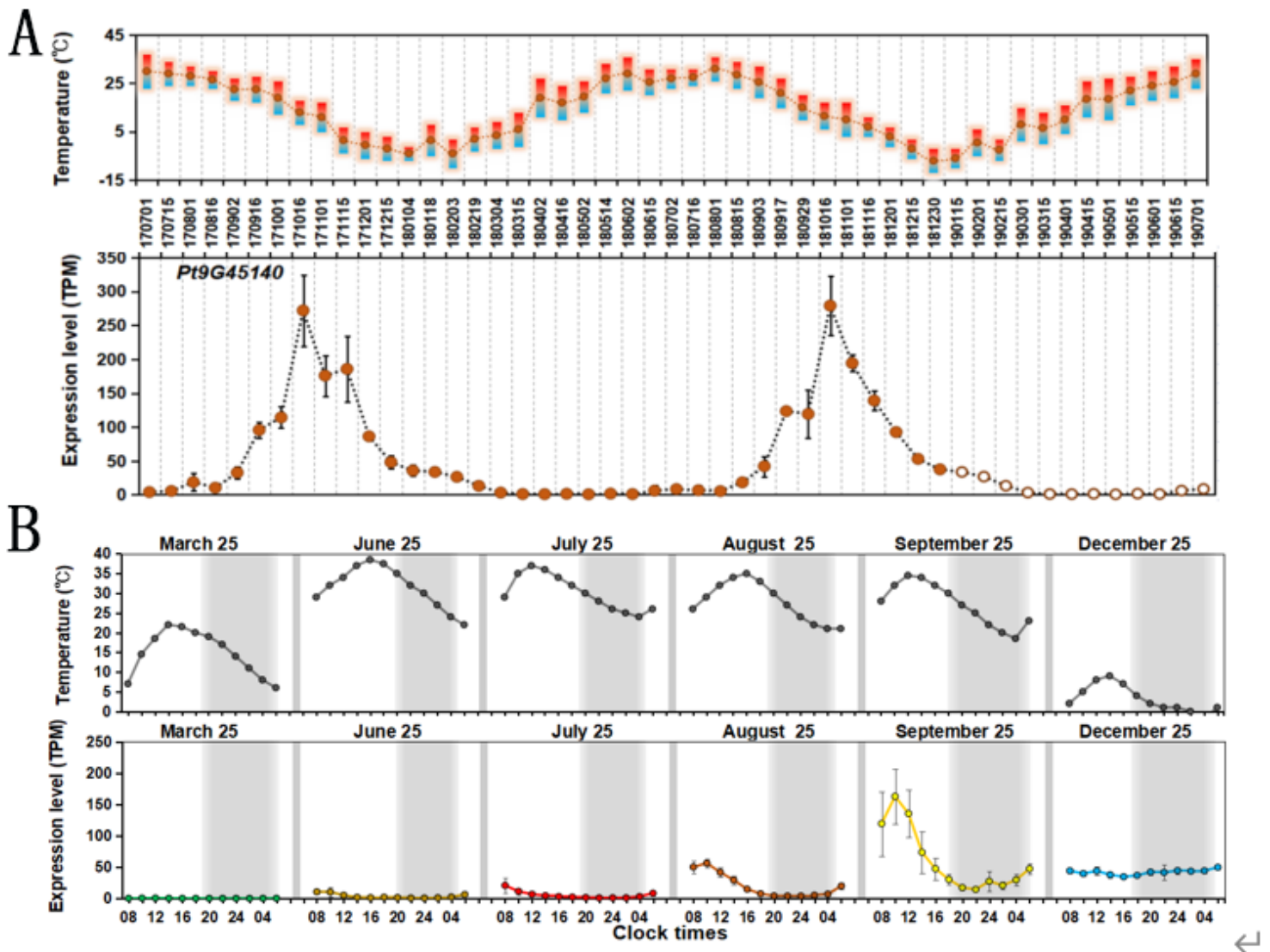


Figure 1

The expression of *PtTFL2* during the annual and diurnal cycles of Chinese pine. (A) Annual sampling time and temperature (orange rectangle represents day length, red circle represents sampling time of the day, blue square represents the highest temperature of the day, blue square represents the lowest temperature of the day and the annual expression of *PtTFL2*) (B) The expression of *PtTFL2* at 8:00, 12:00, 16:00, 20:00, 24:00 and 4:00 in March 25, June 25, July 25, August 25, September 25, and December 25. Error bars represented variability of three independent replicates.

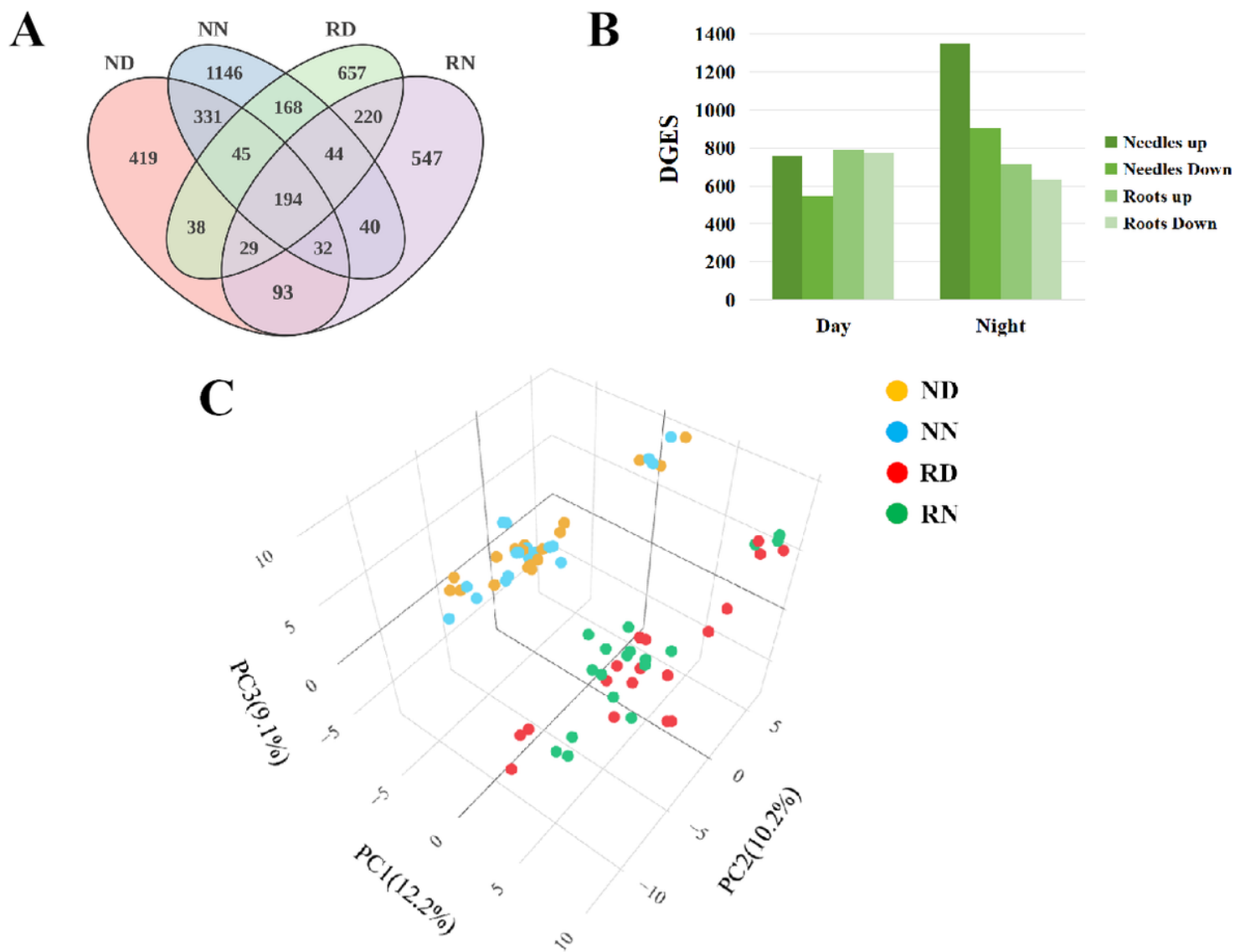


Figure 2

Transcriptome analysis of needles and roots during dormancy. (A) Venn diagram of DEG distribution for each set of sampling times. (B) The numbers of DEGs ($|\text{Log}_2 \text{ fold change}| > 1$, $\text{FDR} < 0.05$) arising from four set of sampling. (C) Principal component analysis (PCA) of transcriptomic profile of four set of sampling.

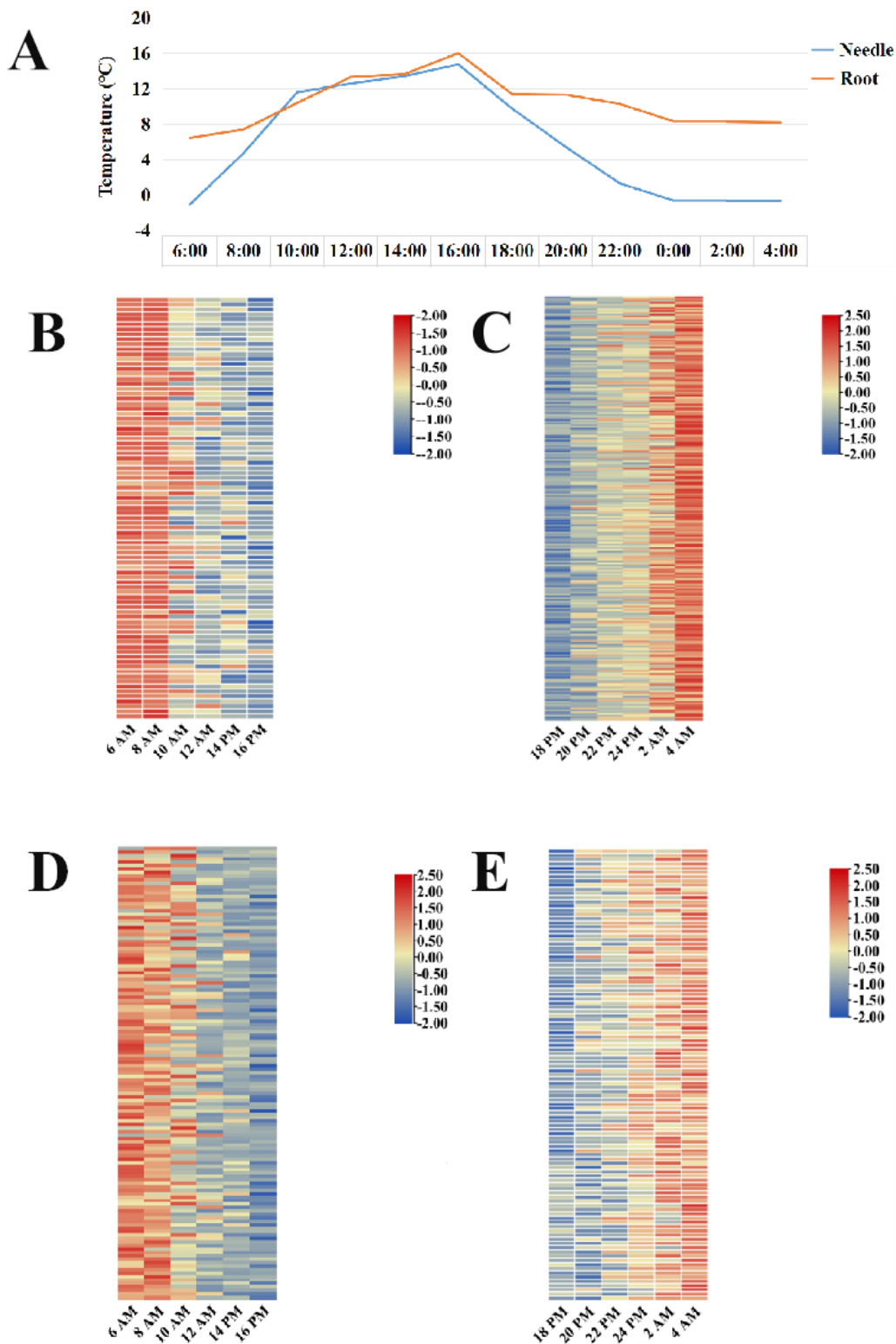


Figure 3

Hierarchical clustering analysis of four groups genes with circadian rhythm and corresponding low temperature. (A) The corresponding ambient temperature of needles and root samples collected during the dormancy. (B-E) Hierarchical clustering analysis of decreased expression of circadian rhythm genes with increasing temperature in ND NN RD and RN group. Each datapoint consists of the expressions

(FPKM) of three biological replicates. Red indicates high relative gene expression and blue indicates low relative gene expression.

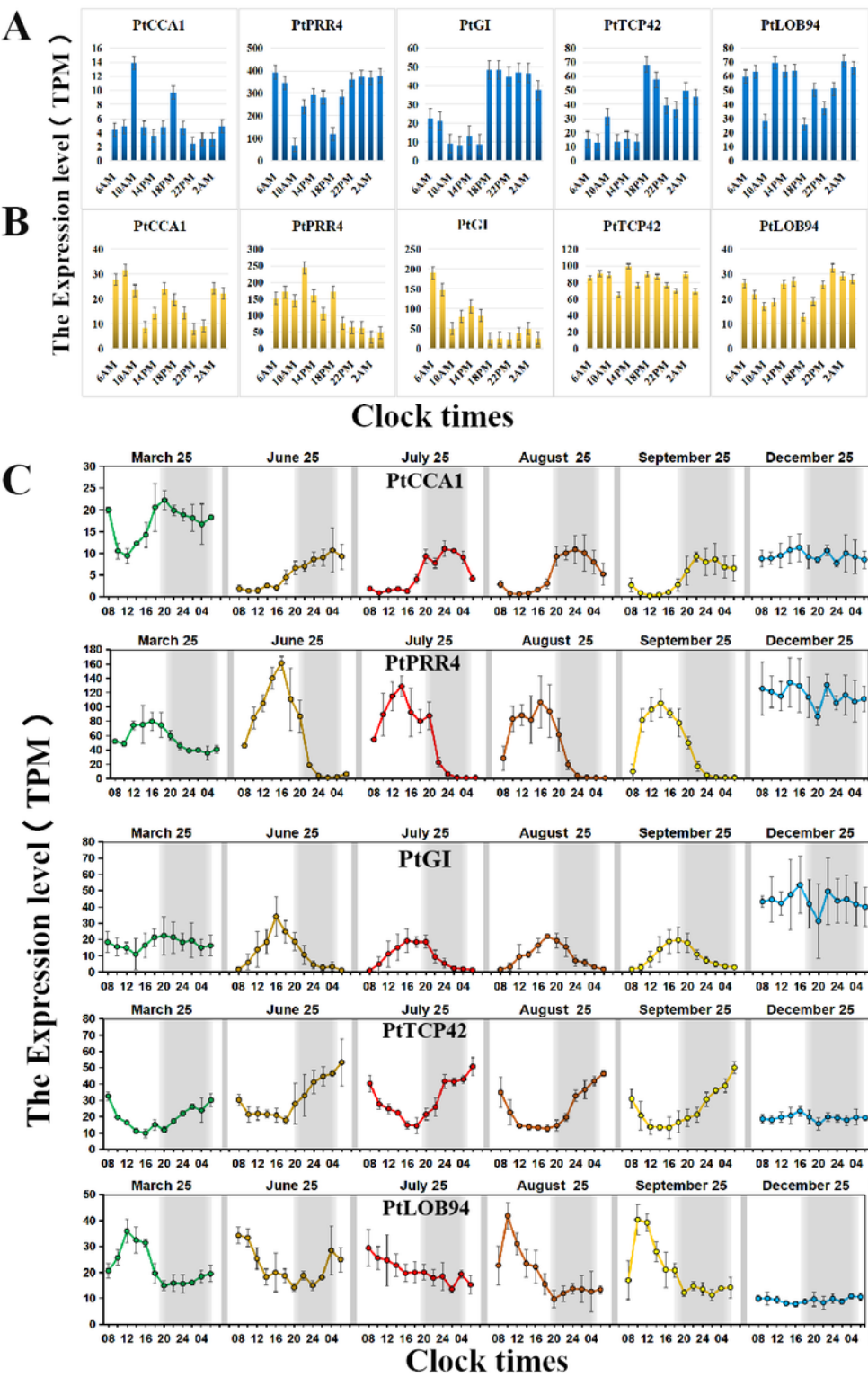


Figure 4

Diurnal rhythms in transcription of clock-related genes. (A-B) Expression trend of clock-related genes in needles and roots of Chinese pine at dormancy stage. (C) The expression of clock-related genes at 8:00,

12:00, 16:00, 20:00, 24:00 and 4:00 in March 25, June 25, July 25, August 25, September 25 and December 25. Error bars represented variability of three independent replicates.

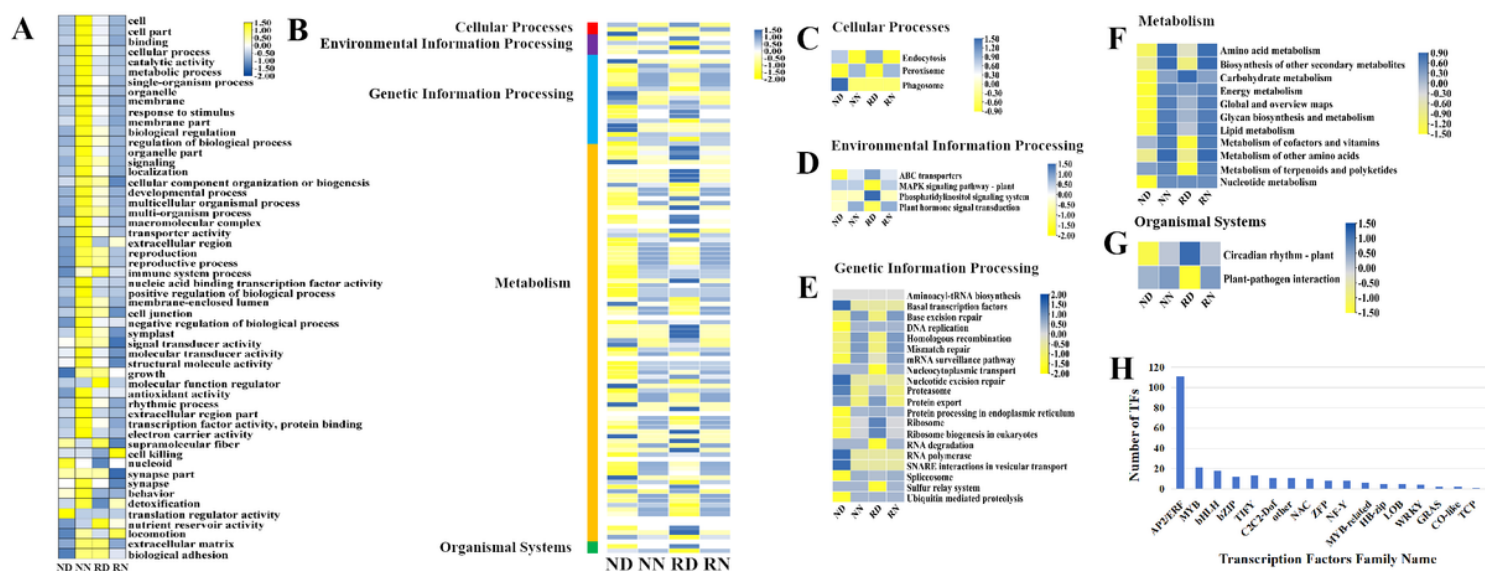


Figure 5

Enrichment analysis and KEGG analysis of four group genes with circadian rhythm. (A) Enrichment analysis of four group genes with circadian rhythm.(B) KEGG pathways of four group genes with circadian rhythm.(C-G) Four groups of genes with circadian rhythms are involved in cellular processes environmental information processing genetic information processing metabolism and organismal systems. (H) Four groups of transcription factors with circadian rhythms.

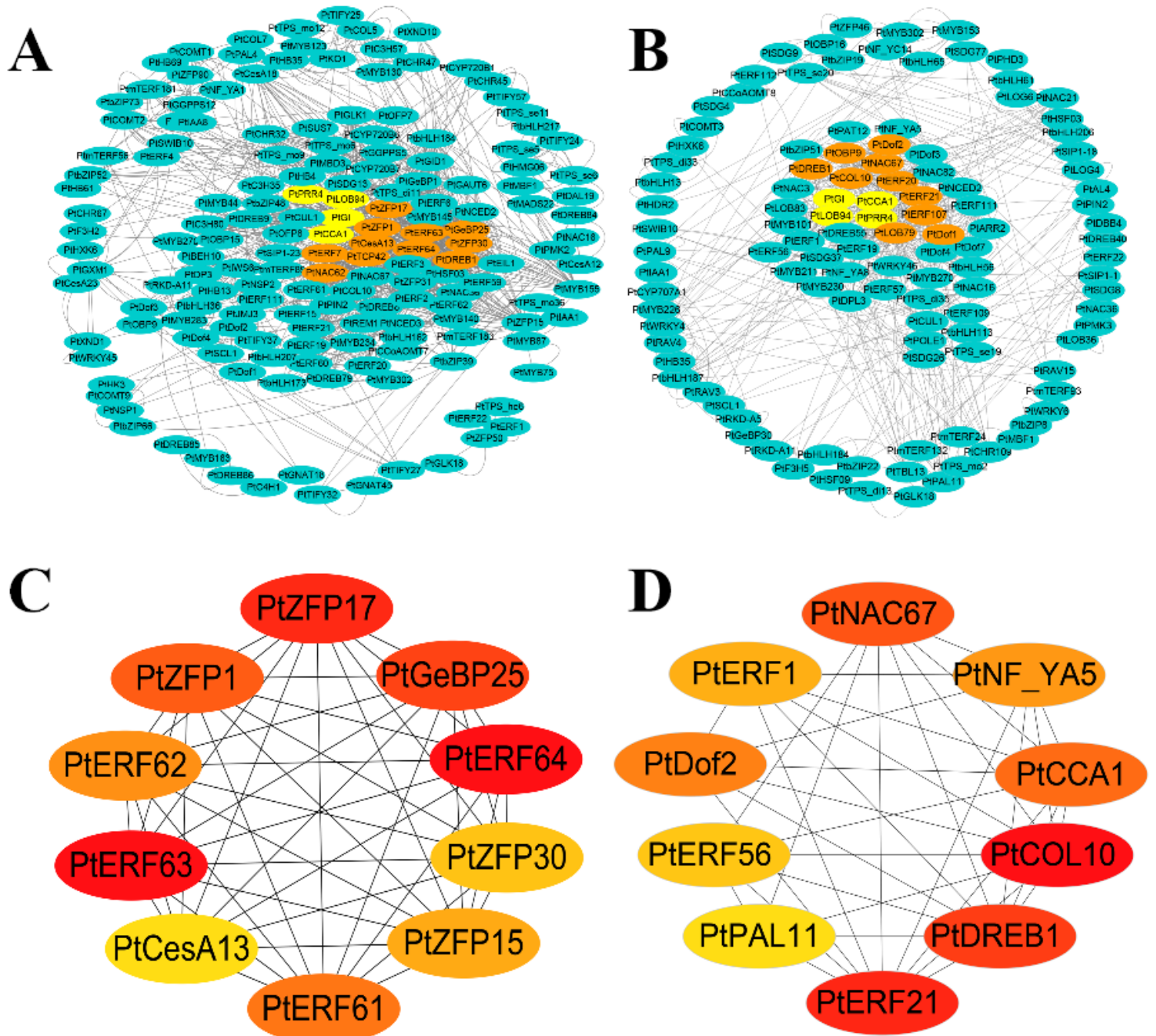
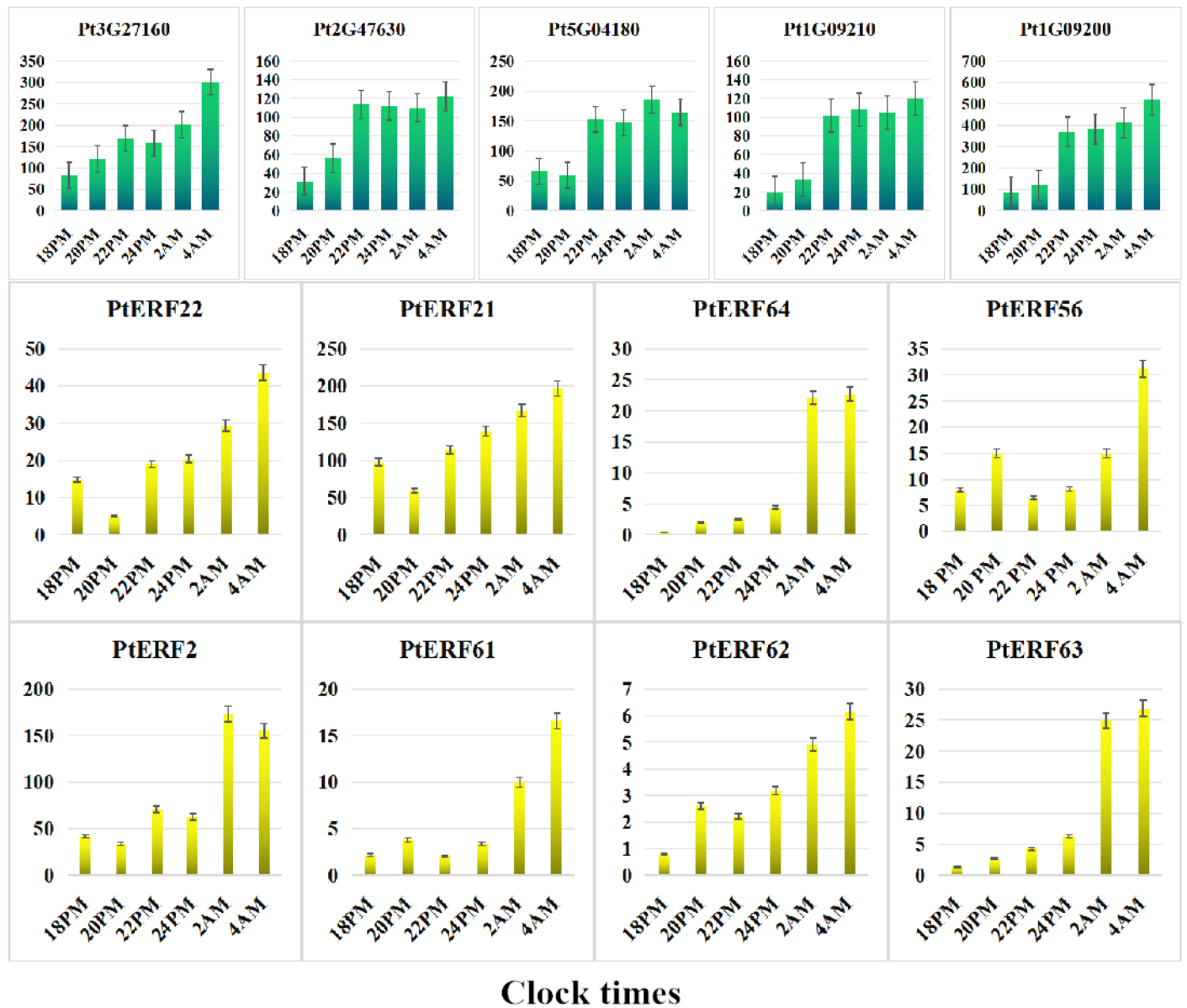


Figure 6

Transcription factor collaborative networks with significant circadian rhythms in needles and roots of Chinese pine. (A-B) The networks were constructed based on interactions, co-expression in needles and roots. Yellow is the clock gene and orange is the core gene. (C-D) In the respective modules of needles and roots, the top 10 ranked genes were hub genes, analysis with cytoHubba by degree centrality with a degree score (size and color change of the nodes denoted the level of degree score).

The Expression level (TPM)



Clock times

Figure 7

The expression of circadian rhythm genes in different time periods. Error bars represented variability of three independent replicates.

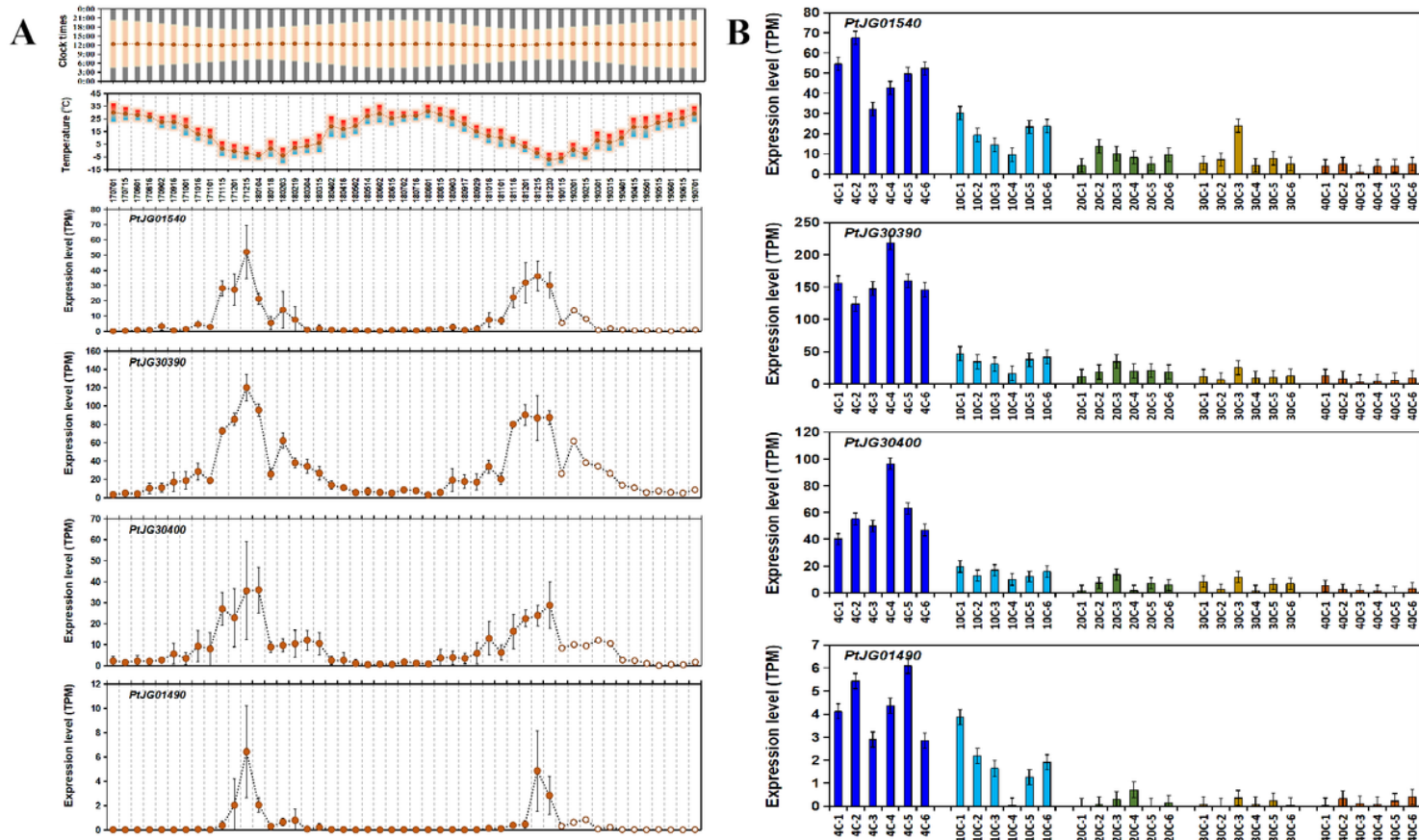


Figure 8

Expression levels of *Pt5G04180*, *Pt1G09210*, *Pt1G09200* and *Pt2G47630* in different environments. (A) Annual cycle expression of *Pt5G04180*, *Pt1G09210*, *Pt1G09200* and *Pt2G47630*. Monitoring lasts for two years, from July 1, 2017 to July 1, 2019. (B) Expression levels of *Pt5G04180*, *Pt1G09210*, *Pt1G09200* and *Pt2G47630* at 4°C, 10°C, 20°C, 30°C and 40°C. Error bars represented variability of three independent replicates.