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Comparative RNA-Seq analysis of *Betula platyphylla* under low and high temperature stresses

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SILVICULTURE

ABSTRACT

Background: *Betula platyphylla* Sukaczev is one of important tree species due to its ecological and economic value. It is a cold-tolerant tree species which also faces heat stress during summer. In the current study, RNA-Seq profiling of two-month-old *B. platyphylla* seedlings were conducted utilizing the MGISEQ-2000 platform.

Results: In total, 856,347,961 clean reads were obtained from 26 RNA-Seq libraries. Totally, 822,552,820 reads were successfully mapped to *B. platyphylla* reference genome. Further, a total of 360 and 264 DEGs were discovered under cold and heat exposure, respectively, while a total of 104 DEGs were identified under both cold and heat exposure. It was found that several pathways including response to cold, response to heat, response to temperature stimulus, response to stress, lipid metabolic, jamonic acid and ethylene, even developmental processes were significantly enriched in GO enrichment analysis of cold and heat stress in biological process term. Several transcription factors (TFs), including MYB66, CBF2, bHLH96and bZIP7 take a pivotal role in response to temperature stresses. Furthermore, heat shock proteins were identified under cold and heat stress, respectively, suggesting these genes participate in reducing cold and heat stress detrimental effect by interacting with TFs or other genes related to abiotic stresses, chlorophyll and photosynthesis, osmoprotectants, and phytohormone as well.

Conclusion: This study not only underlying *B. platyphylla's* molecular mechanism in response to temperature stresses but also provides candidate genes involved in response to temperature stresses.

Keywords: Cold, Heat, Transcriptome, White birch

HIGHLIGHTS

26 RNA-Seq libraries were evaluated to figure out molecular changes under. Cold and heat stress in wild type *Betula platyphylla* seedlings.
A total of 104 DEGs were identified under both cold and heat stress.
MYB66, bZIP7, bHLH96, and HSP21 play crucial role in cold stress.
CBF2, bHLH96, and HSP21 play crucial role in heat stress.

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INTRODUCTION

Betula platyphylla Sukaczev is known as white birch, a deciduous tree species with a valuable economic value from Northern areas (Ritonga et al., 2021a). It belongs to the Betulaceae family that later treated as a synonym of *B. pendula* subsp. *mandshurica* (Shaw et al., 2015;Ritonga et al., 2021a). *B. platyphylla* is also known as cold-tolerant tree species, but this species faces heat in summer. Temperature is an important parameter that caused detrimental effects on plant growth and development (Akhter et al., 2019), consequently causing economic losses and a huge risk in global food security (Fahad et al., 2017). Despite of the fact plants could not escape various stresses during their life cycle, the plant has unique and complex adaptation strategies to cope with various stresses.

In the past ten years, research into the molecular response of genes to stress and their adaptive mechanisms has increased significantly in tree species (Yang et al., 2019;Lv et al., 2020a;Lv et al., 2020b). Transcriptome analysis is one of the most effective sequencing technologies that has been extensively used to identify the response of plants under stress conditions (Yan et al., 2020). As we mentioned above, the RNA-Seq analysis of several tree species, such as *Nothofagus pumilio* (Estravis-Barcala et al., 2021), *Hevea brasiliensis* (Deng et al., 2018), and *Pinus koraiensis* (Wang et al., 2020a) in response to temperature stresses have been identified. RNA-Seq analysis of cold and heat stress in plants species have revealed that differentially expressed gene (DEGs) significantly enriched in metabolic pathways, biosynthesis of secondary metabolites, RNA and DNA binding, enzyme activity, chloroplast and photosynthesis, and transcription factors (TFs) (Mangelsen et al., 2011;Jayakodi et al., 2019;Gao et al., 2021). With significant progress in transcriptomic analysis using next-generation sequencing (NGS), several important genes related to cold or heat stress have been confirmed, and their roles were identified. Nevertheless, it is still limited in tree species. Since *B. platyphylla* is a cold-tolerant tree species which also experiences heat stress during summer, we consider its potential in plant breeding improvement, particularly about temperature stresses mechanisms (Ritonga et al., 2021a).

As we mentioned previously, different genes involved in photosynthesis, hormonal activity, sugar and sucrose, antioxidant, amino acid, lipid, and TFs regulation under cold and heat stress (Singh et al., 2020;Zhou et al., 2020). It was revealed that some trees have a high cold/heat tolerance by regulating and modifying the expression of specific genes (Wang et al., 2020a;Estravis-Barcala et al., 2021). However, in the present study, we focused on identifying candidate genes that might be related to cold, heat, and both cold and heat stress in *B. platyphylla*. Using high-throughput NGS to analyze gene expression, spliced, assembled, and annotated the sequence. We have also undertaken signal pathway enrichment analysis with a focus on genes related to cold and heat stress. The obtained results will advance our insight and understanding of cold and heat stress mechanisms in *B. platyphylla*, and provide basic pieces of information that will be helpful to plant breeding programs in the future works.

MATERIAL AND METHODS

Plant materials and temperature treatments

The wild-type (WT) seedling of White birch was used in this study. The seedlings were grown on solid agar medium with woody plant medium (WPM) complemented by 0.8 m L^{-1} 6-benzylaminopurine (BA) and 0.02 mg L^{-1} naphthalene- acetic acid (NAA) in tissue culture bottles. Then, when the adventitious buds grew up, seedlings were cut and grown on 0.2 mg L^{-1} NAA 0.5 Murashige and Skoog (0.5 MS) medium with 1% sucrose and 0.75% agar (pH 6) (16-h light and 8-h dark photoperiod). After 1 month in ½ MS medium, the WT seedling was transplanted into a 45- plug tray (3 cm in diameter by 3 cm in height) which contained black soil (v/v): perlite: vermiculite = 4:2:2 and maintained in a growth room at 24 ± 1 °C with a 16-h light and 8-h dark photoperiod. After one month, seedlings were treated in an artificial climate box based on the treatment time and temperature points. Plants were divided into three groups, one group was under 24 ± 1 °C as control and the other two groups were transferred to cold (6 °C) and heat (35 °C) treatment for 6 hours, 24 hours, 2 days, 4 days, 7 days, and 14 days, and each group had the stress repeated three times. All treated plants were placed in the same light and photoperiodic conditions. The first to the fourth leaves were sampled after treatment finished for the next measurement. Leaf samples were immediately frozen in liquid nitrogen and stored at −80 °C until use. Three independent biological samples for each treatment were harvested, and each replicate contained ten plants.

RNA extraction, cDNA library construction, and Illumina sequencing

We used Cetyltrimethylammonium Bromide (CTAB) method to obtain the extraction of total RNAs (Chang et al., 1993), and referred to previously documented studies (Yan et al., 2020;Liu et al., 2021). cDNA libraries construction were provided from RNA samples and the integrity of RNA was estimated utilizing the Qubit Fluorometer and the Agilent 2100 Bioanalyzer. The cDNA libraries were constructed according to the user manual of MGIEasy RNA Library Prep Set and sequenced using the MGISEQ-2000 platform (BGI, Wuhan, China). Then, each raw reads data were submitted to the National Center for Biotechnology Information (NCBI) Sequencing Read Archive (SRA) database under the accession number PRJNA811313.

Identification of differentially expressed genes (DEGs)

RNA-Seq by expectation-maximization (RSEM) pipeline was used for transcript quantification from RNA-Seq data (Li and Dewey, 2011). As an aligner,

Spliced Transcripts Alignments to a Reference (STAR) (Dobin et al., 2013) was used to map the reads of sequencing to the reference genome with parameters which is suggested by RSEM (Liu et al., 2021). Then, the levels of gene expression were confirmed by using STAR mapping results. When gene expressions of all samples were calculated, the results were merged using TMM normalization to remove the effect on the calculated genes expression. The edgeR software was used in the differential gene calculation phase (Robinson et al., 2010) England. Further, the comparison between all samples with control was utilized to count the differential genes with threshold was set to FDR < 0.05 and absolute value of log_2FC <-5. The fold changes of DEGs were the TMM average of cold stress treatment in different time points divided by the control TMM average. The minimal value of count per million was 1 to screen low expression.

Gene Ontology (GO) enrichment analysis

To understand the biological functions of DEGs, we obtain the reference genome by downloading the *B. platyphylla* genome. Complete annotation result was obtained from Gene Ontology (GO) functional annotation software (http://pantherdb.org/) (Mi and Thomas, 2009). For enrichment analysis, AnnotationForge was used to pact the results of *B. platyphylla* annotation into a package of OrgDb (Carlson and Pages, 2019). Then, the ClusterProfiles program was utilized to perform the significant differentially expressed RNA obtained from enrichment analysis (Yu et al., 2012). The utilization of hypergeometric test is helpful for GO enrichment analysis and we assessed each enriched GO term significance. To correct the *p*-value of GO terms, we utilized the Bonferroni method with an adjusted p-value less than 0.1 was regarded significantly enriched by DEGs.

The PlantTFcat was used to identify TFs identified in *B. platyphylla* (Dai et al., 2013). To determine whether the TFs were correctly annotated, we used National Centre for Biotechnology Information (NCBI) (Marchler-Bauer et al., 2015). The relevant gene families of all identified pathways were collected using TAIR and selected genes that have expression significantly elevated (Log_2FC <-5). The E-value threshold for all steps was arranged to 1e-5. The co-expressed networks of selected genes pathways were observed by utilizing WGCNA (Weighted Correlation Network Analysis) analysis (Langfelder and Horvath, 2008;Botía et al., 2017) and the correlations were shown by Cytoscape in http://www.ehbio.com/ online software (Shannon et al., 2003). The correlation coefficient is calculated by the "Pearson" algorithm. The similarity matrix was transformed into an adjacency matrix, and then transformed into a TOM (topological overlap measure) matrix with the signed network type. The "deepSplit" value was set to 2 during clustering, the "minModuleSize" value was set to 30, the "merge_CutHeight" value was set to 0.15 and the "R Square Cut" value was adjusted to 0.85.

RESULTS

The summary of statistical RNA-Seq data

In total, 856,347,961 clean reads were obtained from 26 RNA-Seq libraries with an average of 32,936,460. The summary of RNA-Seq data statistical analysis is represented in Table 1. As presented in Table 1, a total of 822,552,820 reads were successfully mapped to *B. platyphylla* reference genome using STAR (Dobin et al., 2013), of which more than 93.11% reads were mapped in each sample. It was clearly presented that total clean or mapped reads under heat stress were higher compared to cold stress. However, the percentage of mapped reads in response to cold stress (96.31%) is higher than heat stress (95.77%). The results indicated that the obtained sequencing reads are qualified for subsequent analysis.

Table 1. Statistical summary of RNA-Seq data in *B. platyphylla* under temperature stresses.

Type of treatment	sample ID	Clean reads	Mapped reads	Mapped reads (%)	Type of treatment	sample ID	Clean reads	Mapped reads	Mapped reads (%)
Control	**Ck_rep 1**	**30,910,157**	**29,700,540**	**96.09**	**Control**	**Ck_rep 2**	**29,677,699**	**28,574,852**	**96.28**
6 °C	6 hours_rep 1	29,022,648	27,863,919	96.01	35 °C	6 hours_rep 1	32,367,458	30,968,005	95.68
	6 hours_rep 2	32,451,187	31,383,313	96.71		6 hours_rep 2	32,219,968	30,834,931	95.70
	24 hours_rep 1	30,235,523	29,207,979	96.60		24 hours_rep 1	30,834,931	28,710,276	93.11
	24 hours_rep 2	34,737,471	33,511,127	96.47		24 hours_rep 2	27,765,502	26,500,489	95.44
	2 days_rep 1	36,519,078	35,227,291	96.46		2 days_rep 1	40,610,883	38,715,051	95.33
	2 days_rep 2	36,125,564	34,861,176	96.50		2 days_rep 2	36,403,430	34,822,347	95.66
	4 days_rep 1	32,805,042	31,665,283	96.53		4 days_rep 1	36,049,499	34,674,643	96.19
	4 days_rep 2	34,804,068	33,748,669	96.97		4 days_rep 2	38,746,095	37,175,076	95.95
	7 days_rep 1	30,657,043	29,646,750	96.70		7 days_rep 1	33,911,684	32,610,018	96.16
	7 days_rep 2	29,797,476	28,688,469	96.28		7 days_rep 2	31,039,463	30,129,266	97.07
	14 days_rep 1	32,328,230	30,467,486	94.24		14 days_rep 1	33,954,506	32,693,960	96.29
	14 days_rep 2	34,515,820	33,225,231	96.26		14 days_rep 2	27,857,536	26,946,673	96.73

Principal Component Analysis (PCA)

As shown in Figure 1a, results confirmed that 65.9% of the total identified variability was represented by PC1 (43%), while PC2 represented 22.9% under cold and heat stress. According to biplot, half of the variables are loaded in PC1 and others are loaded in PC2. PC1 separated among all time points of cold exposure which positioned on the positive side (upper and lower direction). While the PC2 variability was clearly presented control, 6 hours, 2 days, 4 days, 7 days, and 14 days of heat exposure which were located towards the upper side, and 24 hours of heat exposure treatment identified lower side. Additionally, PCA also showed distinct differences between cold and heat treatment.

The differentially expressed genes (DEGs)

Venn diagram was constructed using InteractiVenn Software (Heberle et al., 2015). The illumina reads from 26 samples under temperature and control stress were also aligned, and the calculation of FPKMs have been obtained. A total of 360 DEGs were discovered under cold exposure compared to control group after removing duplicate gene ID at each time point (Figure 1b). While, a total of 264 DEGs were discovered under heat exposure and also after removing duplicate gene ID at various exposed time points. A total of 104 DEGs was identified under cold and heat exposure.

The summary of up-regulated and down-regulated DEGs at each time point under cold and heat stress was clearly presented in Table 2 and Figure 1c. It was clearly shown that total DEGs at 6 hours, 2 days, and 4 days of heat stress were lower than that at 24 hours, 7 days, and 14 days. Interestingly, total DEGs at 24 hours of heat stress were higher than others, illustrating that *B. platyphylla* significantly responds to heat exposure at 24 hours and activates numerous genes as a protective response. Further, the DEGs reduce at 2 days of heat exposure and increase again at 4 days of stress. This phenomenon occurs until the sixth time point. However, the up-regulated and down-regulated DEGs in response to cold stress were gradually increased from 6 hours until 14 days of cold stress. This finding demonstrates that cold exposure duration significantly increased the number of DEGs.

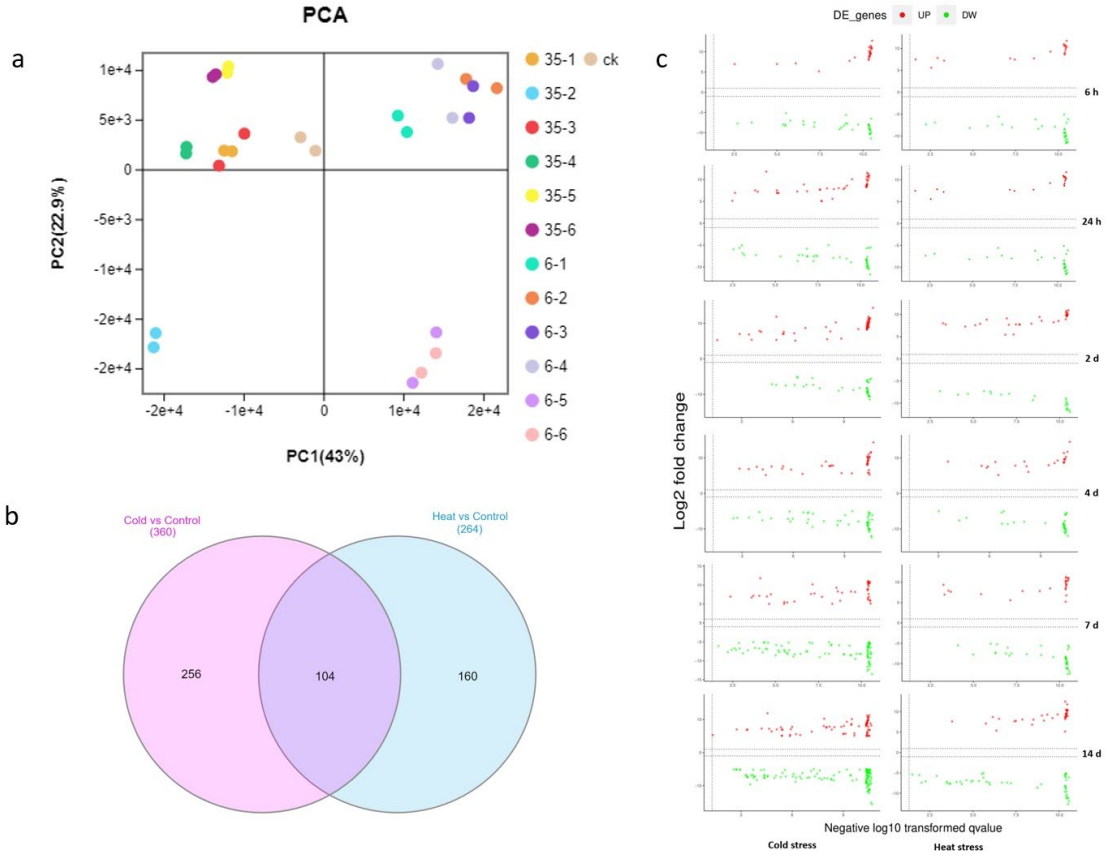

Figure 1. (a) The PCA of a different time and temperature points. Ck: control; 6-1: 6 °C for 6 hours; 6-2: 6 °C for 24 hours; 6-3: 6 °C for 2 days; 6-4: 6 °C for 4 days; 6-5: 6 °C for 7 days; 6-6: 6 °C for 14 days; 35-1: 35 °C for 6 hours; 35-2: 35 °C for 24 hours; 35-3: 35 °C for 2 days; 35-4: 35 °C for 4 days; 35-5: 35 °C for 7 days; 35-6: 35 °C for 14 days.
(b) Venn diagram presented the DEGs under cold and heat stresses in *B. platyphylla*. (c) Volcano plot of DEGs for each temperature stress under different time points. The numbers of up-, down-regulated, and no differentially expressed genes are represented in red, blue, and black in each plot, respectively.

Comparative Gene Ontology (GO) enrichment analysis of DEGs

The current study represented stress-responsive GO enrichments analysis to figure out the mechanism underlying *B. platyphylla* responses to temperature stresses. We compared the representative pathway in the biological process (BP). It was clearly shown that unique pathways including response to cold, response to heat, response to temperature stimulus, cell growth, response to stress, lipid metabolic process, even developmental processes were significantly enriched in GO enrichment analysis of cold and heat stress (Figure 2a). These findings demonstrate that the most important pathways of *B. platyphylla* are similar to respond to cold and heat stress. The distinctive and similar pathway that is identified in GO enrichments illustrates that these data would be beneficial to compare molecular mechanisms for other abiotic stresses.

The expression pattern of Transcription Factors (TFs) and related genes

The similar and distinctive TF families identified under cold and heat stresses were represented in Table 3. As confirmed in previous studies, Myeloblastosis (MYB) TF family was the dominant TF identified in under cold stress and HSP. However, several important TF families that play significant roles to increase cold and heat tolerance in plants were also found such as APETALA2/Ethylene Responsive Element Binding Factor (AP2/ERF), bZIP, Heat Shock Protein (HSP), and so on.

Table 2. Total up and down-regulated genes in a different time and temperature points.

Treatment	Up	Down	Total	Up	Down	Total
		6 °C			35 °C	
ck vs 6 h	36	27	63	31	19	50
ck vs 24 h	43	43	86	66	53	119
ck vs 2 d	38	54	92	21	32	53
ck vs 4 d	51	41	92	34	23	57
ck vs 7 d	90	43	133	38	25	63
ck vs 14 d	127	80	207	50	36	86

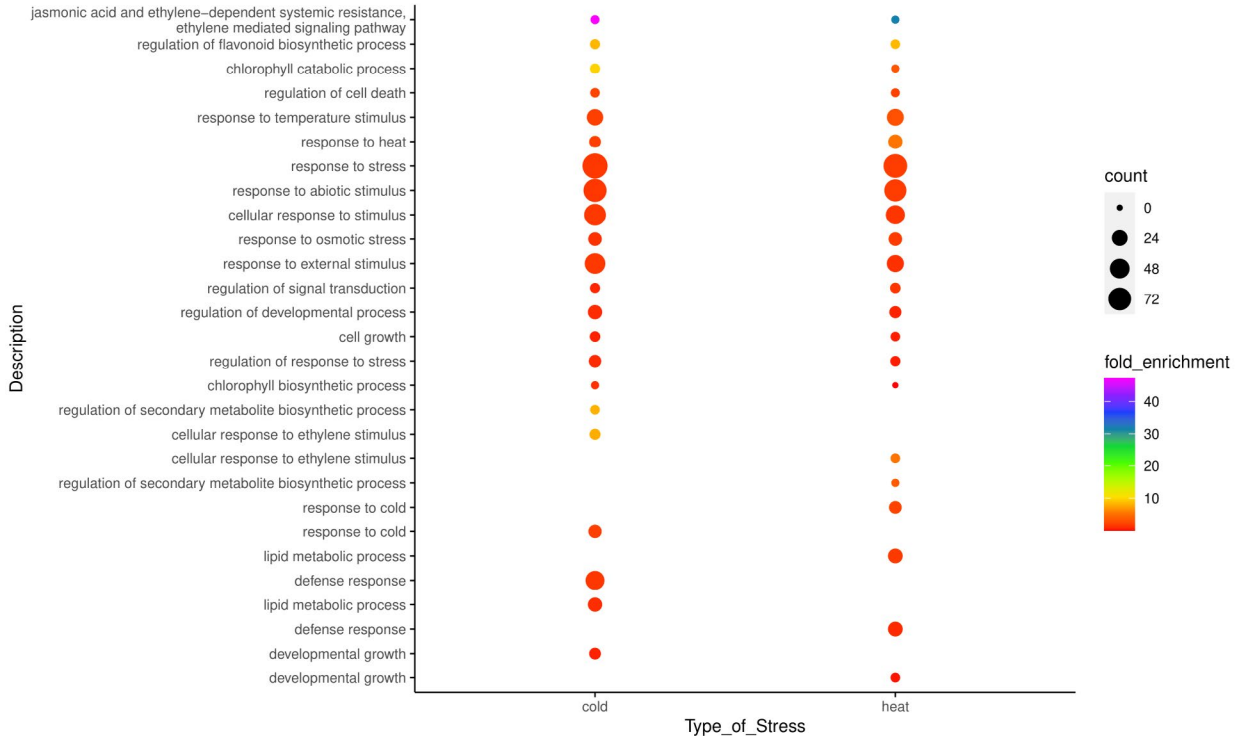

Figure 2. Representative pathways related GO terms in biological processes. Bubble color indicates fold enrichment; size indicates gene numbers of the DEGs in GO terms under cold and heat stress.

Table 3. Total up and down-regulated TFs in a different time and temperature points.

Type of stress	Expression	TF gene ID	Log FC							Function
			Control	6 h	24 h	2 d	4 d	7 d	14 d	
Cold	Up	BPChr04G21825	0.00	8.74	10.27	-	-	16.69	-	Serine/threonine-protein phosphatase PP1 isozyme 4, TOPP4
	Down	BPChr08G10784	4.28	-	200.22	-	224.93	479.61	1117.28	Early light-induced protein 1, ELIP1
		BPChr08G16217	6.34	-	-	0.11	-	-	-	AtBZIP7
		BPChr06G13544	16.04	-	-	-	-	-	0.34	MYB66
		BPChr08G02982	31.16	-	-	0.69	0.25	-	-	Heat Shock Protein 21, HSP21
		BPChr06G31071	73.96	0.00	-	0.00	-	-	-	Sedoheptulose-1,7-bisphosphatase
Heat	Up	BPChr02G23413	0.02	4.31	4.15	-	-	-	-	AtCBF2
		BPChr08G02982	31.17	1458.00	3097.18	-	1447.07	1234.67	-	Heat Shock Protein 21, HSP21
	Down	BPChr06G30704	75.84	-	0.00	-	-	-	-	bHLH096
		BPChr06G30940	1.24	0.00	-	-	-	-	-	Plasma Membrane Intrinsic Protein 1;4, PIP1;4

The current study discovered 5 TF involved to respond to cold stress in *B. platyphylla*. Totally, 2 of which were up-regulated and 3 of which were down-regulated. Then, 3 putative TFs were identified in response to heat stress, 1 of which was up-regulated and 2 of which were down-regulated. Furthermore, the involvement of HSP also found to respond to cold and heat stress, HSP21 was down-regulated, suggesting that this gene might be obviously correlated with cold stress response and contribute to cold resistance enhancement. Contrastingly, HSP21 was up-regulated in response to heat stress, illustrating this gene has specific function under temperature stresses. Additionally, the number of other TF families, such as Serine/threonine-protein phosphatase PP1 isozyme 4 (TOPP4), Early light-induced protein 1, Sedoheptulose-1,7-bisphosphatase, and Plasma Membrane Intrinsic Protein (PIP) were also assumed involved in cold and heat tolerance in *B. platyphylla*. Interestingly, we also identified the involvement of CBF2 to heat stress. The involvement of CBFs was not found in cold stress response. We speculated that each species, as well as the type of stresses, might influence this phenomenon.

DISCUSSION

Birch is a cold-tolerant tree species, that has strong resistance to another abiotic stress. A genome sequence of *B. platyphylla* has been released by Chen et al. (2021) and it was assumed as a basic foundation of our understanding. Among published reports in *B. platyphylla*, the functions of several genes related to environmental stresses have been confirmed, such as *BplMYB46* under osmotic stress (Wang et al., 2019), *BpARF* under drought stress (Li et al., 2020), and *BpHSP* under heat stress (Liu et al., 2018). Recent research in *B. platyphylla* has shown that TFs, and DEGs related to abiotic stresses, plant hormones, lipid, photosynthesis and chlorophyll, and signal transduction might be involved in the cold tolerance mechanism (Yan et al., 2020) . The principal results of this study are the changes during response under cold and stress. In short, our results are in accordance with previous reports related to cold and heat stress (Bashir et al., 2019; Yan et al., 2020).

GO enrichment results showed that several pathways were enriched (Figure 2). For instance, the "response to stress", "cellular response to stimulus", and "response to abiotic stimulus" were significantly enriched in BP terms under both cold and heat stresses, which indicated an implication of response to stress, cellular response to stimulus, and response to abiotic stimulus to respond cold and heat stress. Developmental growth, such as seed dormancy and germination, stomatal disclosure, and flowering were regulated by phytohormones, which was also found to be enriched in *B. platyphylla* under cold and heat stress. Jasmonic acid (JA) and ethylene plays a significant role in seed development and early stages of development (Corbineau et al., 2014;Bhavanam and Stout, 2021). We speculated the enrichment of response to JA and ethylene was correlated to developmental process enrichment and temperature stresses. In a variety of temperature stress studies, JA and ethylene involves in altering temperature stress related genes to enhance cold or heat resistance (Sharma and Laxmi, 2016;Pérez-Llorca et al., 2023). Moreover, it has been revealed that temperature stresses dramatically affect tree developmental stage (Teskey et al., 2015). Temperature stimulus, as a detector of environmental change, plays a prominent role in plants coping with temperature stresses. Low or high temperatures stimulate important pathways as a result of which the increase of cold or heat tolerance in *B. platyphylla* (Yan et al., 2020).

Membrane lipid remodeling is the most effective adaptation strategy to defend against temperature stresses and commonly used as biomarker (Liu et al., 2019;Yan et al., 2019). A recent study reported that the duration of heat

stress positively correlated to lipid content (Shiva et al., 2020). It is also revealed by Su et al. (2009) that higher heat tolerance was associated with the content of glycolipid digalactosyldiacylglycerol (DGDG) and the ratio of DGDG in turfgrasses. Similar results were identified in Arabidopsis (Higashi et al., 2015).In summary, the GO terms belonging to "lipid metabolic process" are significantly enriched under cold and heat stresses, illustrating that lipid metabolism plays an important function in *B. platyphylla* against cold and heat stress. Similarly, an increase in lipid content under temperature stress was revealed in *B. platyphylla* (Ritonga et al., 2021b).

A total of 160 DEGs were only expressed under heat stress and 256 DEGs were only expressed under cold stress. This result demonstrates that several DEGs might be not involved in a specific stress and species. For example, DEGs related to regulation of flavonoid biosynthetic process (GO:0009813) and response to starvation (GO:0009267) were differently enriched under cold and heat stress. However, in heat-tolerant rice, flavonoids biosynthetic pathways were confirmed to be involved in response to heat stress during meiosis. A high amount of flavonoids accumulation demonstrating that flavonoid biosynthetic pathways likely act a significant function in the heat resistance of rice during reproductive stage (Cai et al., 2020). Similarly, *Camellia sinensis* showed the abundant flavonoid glycosides under heat stress (Su et al., 2018). In a typical Mediterranean tree species, *Quercus ilex*, it has been reported that flavonoid contents increased in response to cold stress (Brossa et al., 2009), suggesting the presence of flavonoid biosynthetic process in response to cold and heat stress might enhance cold and heat tolerance in *B. platyphylla*. Under long-term heat stress, plants evolved to stimulate their antioxidant machinery, like antioxidant enzymes and flavonoids to alleviate the cytotoxicity of ROS (Chandran et al., 2019). Moreover, in *Malus domestica*, *HSFA8a* associated with *HSP90* to regulate the transcription of flavonoid biosynthetic pathway genes like *MdDFR*, *MdFLS*, and *MdANS* speculated that flavonoid biosynthesis required *MdHSFA8a* and *HSP90* in regulating the flavonoids biosynthetic genes transcription (Wang et al., 2020b). We implied that the enriched pathway of flavonoid biosynthesis in this study might also be related to the up-regulated HSPs and HSFs TF.

A general strategy to impart or improve environmental stress tolerance in plants is modifying plants genetically like manipulating the TFs expression. Various TF families were found to contribute to signal transduction under environmental stresses (An et al., 2018). In this study, MYB, AP2/ERF, bHLH, bZIP, TFs families were identified to be significantly enriched under cold and heat stress (Table 3). The MYB TF is an active player in abiotic stress signaling and is widely present in all eukaryotes (Li et al., 2015). MYB TF has been studied extensively and found to be involved in regulating secondary wall deposition, as well as transcriptional regulation under environmental stresses (Guo et al., 2017). Our result was also similar to an early report about MYB TFs potentially involved in response to cold stress by regulating bHLH, resulting in reducing the detrimental effect of cold stress (An et al., 2020). Our result was also similar to an early report about MYB TFs potentially regulating flavonoid biosynthesis in *Erigeron breviscapus* (Zhao et al., 2022). We assumed that the enriched flavonoid biosynthesis pathway has positive correlation with down-regulated MYB TF under cold stress.

Another TFs family, AP2/ERF, has been stated to contribute to cold and heat stress tolerance (Lv et al., 2019), which is consistent with our results. An AP2/ERF TF, CBF2 was activated in response to heat stress, while no AP2/ERF TFs were activated in response to cold stress. This result demonstrates AP2/ERF plays a crucial role in *B. platyphylla* to cope with heat stress. AP2/ERF TF acts as a key regulator of abiotic stress response, especially temperature stress (Mizoi et al., 2012). Similarly, in sunflower, AP2/ERF TFs were also confirmed to effectively resist cold and heat stress (Najafi et al., 2018).

In response to heat stress, Heat Shock Factor (HSF) is also considered as a pivotal TF family that contributes to heat stress tolerance. HSF together with heat shock proteins (HSPs) modulates the expression of downstream genes (Kumar et al., 2018). HSFs bind to HSP promoters and acts as a mediator of bound histones dissociation, consequently activating HSPs transcription (Bourgine and Guihur, 2021) consequently increase HSP genes transcription level (Zhang et al., 2011). Indeed, the positive correlation between HSPs and HSFs has been revealed in previous studies (Guo et al., 2016). At the same time, HSFs also associate with other TFs, including AP2/ERF, bZIP and bHLH under high temperature and other abiotic stresses (Huang et al., 2016;Agarwal et al., 2019). All of the TFs families that were already mentioned above were enriched under cold and heat stresses, indicating that these TFs families might play a crucial role in response to cold and heat stress.

CONCLUSION

In summary, the utilization of RNA-Seq technology to analyze transcriptome profiling of *B. platyphylla* clearly shows the molecular mechanism of this species to cope with cold and heat stress. However, molecular mechanisms of *B. platyphylla* in response to temperature stresses are complex. We prioritize discussion into the field of cold stress and heat stress TFs, candidate genes, and major pathways which are believed to be the important key to obtaining cold and heat tolerance. A total of 360 and 264 DEGs were identified under cold and heat stress, respectively, and 104 DEGs were overlapped between cold and heat stress. A total of 5 and 3 TFs were involved in response to cold and heat stresses, respectively. Dominant TFs that are identified both under cold and heat stress (MYB and AP2/ERF) and HSP might play a crucial role in *B. platyphylla* responses to temperature stresses. Our findings provide essential information for improving plants quality, especially crops and other important tree species. These findings provide new insights and understandings about cold/ heat mechanisms involved in *B. platyphylla*. Overall, the combination molecular and genetic engineering techniques directly contribute to plant breeding.

AUTHORSHIP CONTRIBUTION

Project Idea: XL; SC

Funding: SC

Database: FNR;

Processing: FNR;

Analysis: FNR; SC; FI; RS;

Writing: FNR;

Review: FNR; RS; XZ; XL; SC

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