



## Effect of the photoperiod on bud dormancy in *Liriodendron chinense*

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### ABSTRACT

Bud dormancy and its release are complex physiological phenomena in plants. The molecular mechanisms of bud dormancy in *Liriodendron chinense* are mainly unknown. Here, we studied bud dormancy and the related physiological and molecular phenomena in *Liriodendron* under long-day (LD) and short-day (SD). Bud burst was released faster under LD than under SD. Abscisic acid (ABA), superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GR) activities were increased significantly under LD in *Liriodendron* buds. In contrast, the contents of gibberellic acid (GA3), ascorbic acid (AsA), glutathione (GSH), malondialdehyde (MDA), and ascorbate peroxidase (APX) activity decreased under LD but increased under SD. Differentially expressed genes (DEGs) were up-regulated under LD and down-regulated under SD and these changes correspondingly promoted (LD) or repressed (SD) cell division and the number and/or size of cells in the bud. Transcriptomic analysis of *Liriodendron* buds under different photoperiods identified 187 DEGs enriched in several pathways such as flavonoid biosynthesis and phenylpropanoid biosynthesis, plant hormone and signal transduction, etc. that are associated with antioxidant enzymes, non-enzymatic antioxidants, and subsequently promote the growth of the buds. Our findings provide novel insights into regulating bud dormancy via flavonoid and phenylpropanoid biosynthesis, plant hormone and signal transduction pathways, and ABA content. These physiological and biochemical traits would help detect bud dormancy in plants.

### 1. Introduction

Bud dormancy is an important trait that permits temperate woody perennials to endure harsh winter conditions. Plants enter dormancy when their growth is slowed and their metabolic activities are reduced (Arora et al., 2003; Liu and Sherif, 2019). Bud dormancy is an essential step in the phenology cycle because it impacts the quality of bud break, flowering, and fruiting in the spring. Dormancy is a temporary suspension of visible growth (Beauvieux et al., 2018). Bud dormancy, a complicated physiological phenomenon that helps plants grow, survive, and develop, influences the time of bud break (Yang et al., 2021). In all species, including cotton (Shi et al., 2021), poplar (Gómez-soto et al., 2021), aspen (Tylewicz et al., 2018), *Rosa* sp. (Henry et al., 2011), grapevine (Meitha et al., 2015), bud dormancy involves cell division and expansion, new organogenesis, and elongation (Girault et al., 2008) and

is controlled by a genetic program and environmental cues. In *Rosa* sp., abundant evidence has shown that light modulates bud dormancy and ultimately plant architecture. Light triggers a high bud source through sugar accumulation, concomitantly increasing transcript levels and acid vacuolar invertase enzymatic activity in buds (Girault et al., 2010). Plants are exposed to a daily light/dark cycle. Many biochemical, physiological, and developmental processes in plants, such as flowering and hypocotyl growth, as well as abiotic and biotic stress responses, are affected by changes in this rhythm caused by changes in the photoperiod (Greenham and McClung, 2015; Shim and Imaizumi, 2015; Abuelsoud et al., 2020). However, photoperiod and coordination among regulatory factors in response to bud dormancy remain elucidated.

Bud dormancy release is regulated by photoperiod in subtropical trees such as *Liriodendron chinense*, *Torreya grandis*, *Metasequoia glyptostroboides*, *Cinnamomum chekiangense*, and *Phoebe chekiangensis*. In

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comparison between SD with LD, the SD decreased the bud burst percentage and delayed the timing of the bud burst (Zhang et al., 2021). For a wide range of forest tree species, the timing of bud burst exhibits a continuous phenotypic variation typical of a quantitative trait (Derory et al., 2001). According to new research, just a few woody species are photoperiod sensitive (Zhang et al., 2021; Zohner et al., 2016). However, bud development in temperate (Pletsers et al., 2015) and subtropical (Zhang et al., 2021; Jewaria et al., 2021) trees is controlled by a combination of photoperiod, chilling (temperature and duration), and spring or forcing temperatures. The mechanisms underlying dormancy release and bud break are intimately linked, but the underlying molecular mechanisms are not well understood. Endogenous ABA levels have been reported to increase as woody plant buds enter dormancy and drop as dormancy is released over the winter months (Or et al., 2000; DÜRING and BACHMANN, 1975; Koussa et al., 1994). Plant hormones are essential in regulating bud dormancy (Ionescu et al., 2017; Horvath et al., 2003). Abscisic acid and bioactive gibberellins are known to play antagonistic roles in bud dormancy, with a high GAs content linked to dormancy release (DR) and a high ABA content related to dormancy depth (Cooke et al., 2012; Hernández et al., 2021). Changes in the expression of ABA-signaling and GAs-related genes have been identified at the transcriptional level as DR advances (Tong et al., 2009; Hernández et al., 2021). Still, the participation of phytohormones in regulating bud dormancy under varying photoperiods remains to be illustrated.

Antioxidant metabolism plays a crucial part in the development of plants. POD and SOD activities have traditionally been physiological indices for plant growth in biotic and abiotic stressors (Liu et al., 2016). During dormancy release, changes in antioxidant enzyme activities, proline, and free poly-amine contents accelerate bud break (Ben Mohamed et al., 2012). The stress-relieving enzymatic activity of polyphenol oxidase and peroxidase increase during the early phases of bud sprouting and diminish at full bloom (Somkuwar et al., 2021). Plants' stress tolerance is linked to antioxidant metabolism, as is widely known. Peroxidase (POD) and superoxide dismutase (SOD) are two antioxidants induced in wizened and normal flower buds in Chinese pear plants (Liu et al., 2016). It has long been assumed that POD activity is linked to plant stress tolerance (Liu et al., 2016). Flavonoid biosynthetic pathways are essential mechanisms in plants to respond against light induction, temperature, drought, and salt stress (Nabavi et al., 2020; Shi et al., 2021). However, there are no previous results on genes/transcription factors encoding enzymes or plant secondary metabolites during bud dormancy in *Liriodendron chinense* under different photoperiods.

Previous studies under controlled conditions have suggested that specific genes are involved in bud dormancy in different species; for example, PICKLE (*PKL*) or plasmodesmata-located protein1 (*PDLP1*) in the aspen targets cell-cell communication during bud dormancy in photoperiod (Tylewicz et al., 2018). Chalcone synthase (*CHS*) and hydroxylcinnamoyl transferase (*HCT*) in cotton control bud growth (Shi et al., 2021). Early bud-break 1 (*EBB1*) gene causes bud burst in poplars (Busov et al., 2016); (Yordanov et al., 2014). *Populus centrono-dialis* 1 (*CENL1*) expression levels, which are sequentially downregulated during the dormancy cycle (Rinne et al., 2011); late elongated hypocotyl1 (*PttLHY1*) and *PttLHY2* in hybrid aspen results in delayed budburst (Ibáñez et al., 2010), short vegetative protein-like (*SVL*), acts as a central regulator of dormancy release and bud burst in poplar (Singh et al., 2018). The regulatory network of bud dormancy by antioxidant enzymes, flavonoid biosynthesis, phenylpropanoid biosynthesis, plant hormone and signal transduction pathway in *Liriodendron chinense* has not been reported previously under photoperiod variations.

In the present study, we aim to identify the key genes underlying the effects of photoperiod in *Liriodendron chinense* through systematic research at multiple levels: including observation of bud budburst, the contents of the abscisic acid (ABA), and gibberellin (GA3), main antioxidant enzymes (SOD, POD, CAT, APX, GR) activities, non-enzymatic antioxidants (AsA and GSH) contents, malondialdehyde (MDA), and hydrogen peroxide ( $H_2O_2$ ) levels in *Liriodendron* buds were examined to

see how these factors interacted with bud dormancy maintenance and release.

## 2. Material and methods

### 2.1. Experimental site and procedure

The experiment was conducted at the Zhejiang Agricultural and Forestry University (Hangzhou, China) to understand the role of different photoperiods during bud dormancy. This location is situated in subtropical southeastern China at latitude 30°14'N and longitude 119°42'E having a monsoonal climate. The hydrometeorological parameters of this region in January with an average annual rainfall of 1614 mm, an average annual air temperature of +15.6 °C, and a mean monthly air temperature of +4.5 °C in January of +28.9 °C in July (Zhang et al., 2021). On December 17, 2020, we chose 200 *Liriodendron chinense* seedlings from the Tianmushan National Forest Station nursery (Hangzhou, China) for experimental purposes and transferred them to our university growing chamber. We executed this plan by inducing photoperiod variations after chilling in natural conditions outdoors.

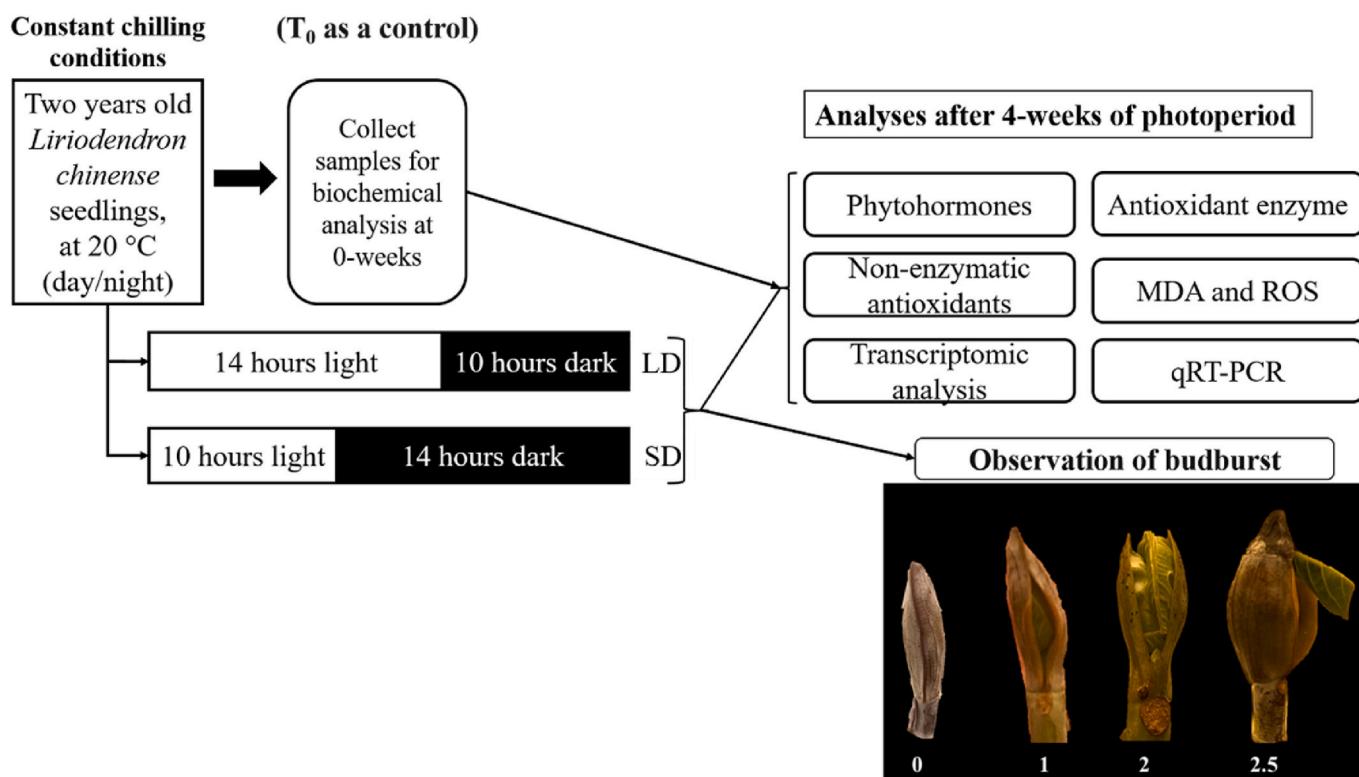
The seedlings were kept at 20 °C (day/night) with a light intensity of 200  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and relative humidity of 50% after being transferred from the nursery to the growth chamber. The seedlings were divided into two groups long day (LD), having 14 h (h) light period and 10 h dark period, and a short day (SD), having 10 h light period and 14 h dark period (Fig. 1).

### 2.2. Quantification of ABA and GA3 contents

We quantified the contents of two different types of phytohormone (ABA and GA3) at two stages, 0 weeks ( $T_0$  as a control) and after weeks (4 weeks) of photoperiod (LD and SD).

The contents of endogenous ABA and GA3 hormones were extracted and purified from the frozen samples stored at -80 °C. Approximately 0.4 g of the samples were firstly grounded in liquid nitrogen. The samples were extracted with 1.5 mL of pre-cooled 80% methanol aqueous solution. After grinding, the samples were extracted overnight at 4 °C. The samples were centrifuged at 8000 g for 10 min, and the supernatant was collected. The remaining residues were extracted with 0.5 mL of 80% methanol aqueous solution for 2 h. After centrifugation, the supernatant was again collected, and finally, the two collected supernatants were combined. The remaining organic phase (methanol aqueous solution) was evaporated to dryness with nitrogen ( $N_2$ ) at 40 °C until no organic phase was left. After that, 2 mL petroleum ether was added to the samples for further extraction and decolorization. This step was repeated three times. A proper amount of 1 mol  $\text{L}^{-1}$  citric acid aqueous solution was added to the lower aqueous phase to adjust their pH to 2–3. After adding 2 mL of ethyl acetate for extraction twice, the upper organic phase was transferred to a new EP tube and blown-dried with  $N_2$ .

Additionally, 0.2 mL of mobile phase was added to dissolve and mix well. Finally, the samples were filtered with a syringe filter and used to determine ABA and GA<sub>3</sub>. The separation and determination of ABA and GA<sub>3</sub> were performed using high-performance liquid chromatography (HPLC) system (Waters 2695, Milford, USA). The HPLC system comprised a C18 reversed-phase column (250 mm\*4.6 mm, 5  $\mu\text{m}$ ) through which separation was carried out. This process was maintained at 35 °C with a flow rate of 1  $\text{mL min}^{-1}$  and 10  $\mu\text{L}$  injection volume. The mobile phase for ABA was 1% acetic acid aqueous solution: methanol (60:40 v/v) and was quantified by monitoring the detection at 254 nm wavelength (Qi et al., 2021). Similarly, the mobile phase for GA<sub>3</sub> was 0.1% phosphoric acid in water: acetonitrile (85:15 v/v) and was quantified by monitoring the detection at 210 nm wavelength (Sun et al., 2019).



**Fig. 1.** Schematic overview of the experimental setup and stages of bud burst as observed in *Liriodendron chinense* underlying different photoperiods. (0) Dormant bud, (1) The buds are enlarged, and the bud scales are cracked, (2) Completely cracked, not unfolded, (2.5) New leaves break through bud scales.

### 2.3. Determination of antioxidant enzyme activities

The determination of antioxidant enzyme activities (SOD, POD, CAT, APX, and GR) were carried out in the collected samples at 0-week ( $T_0$  as a control) and after 4-weeks of photoperiodic treatments (LD and SD), which were stored at  $-80^{\circ}\text{C}$ . To determine the aforementioned antioxidant enzymes, the commercial kits were purchased from Suzhou Comin Biotechnology Co., Ltd., Ltd., Jiangsu, China. The tissue samples were prepared by weighing 0.1 g frozen bud sample and homogenized on an ice bath in 1 mL phosphate buffer solution (100 mM, pH 7.8). After grinding, the homogenate was centrifuged at  $4^{\circ}\text{C}$  for 10 min using 8000 rpm. The supernatant was collected and put on ice for further analysis. Next was to prepare the working solution by mixing sodium acetate, glacial acetic acid, guaiacol, and hydrogen peroxide with a 2.6:1.5:1 (uL) ratio. The working solution was kept at room temperature for 10 min. Finally, the POD activity was determined by mixing the working solution and sample supernatant, and the change in absorbance (OD) was recorded at 470 nm at 1 min and after 2 min.

A 0.1 g tissue sample was ground and homogenized in a 1 mL phosphate buffer solution in an ice bath. The homogenates were centrifuged at 8000 rpm for 10 min at  $4^{\circ}\text{C}$ . The supernatant was collected for further testing. The working solution (hydrogen peroxide and phosphate buffer) was kept at room temperature ( $25^{\circ}\text{C}$ ) for 10 min. Finally, the CAT activity was determined by mixing the sample supernatant and the working solution. The initial OD was quickly recorded at 240 nm, and the final OD was recorded after 1 min.

The APX activity was determined by grinding 0.1 g of bud samples in 1 mL of reagent 1 (potassium hydrogen phosphate trihydrate, monopotassium phosphate, and polyvinylpyrrolidone (PVP) solution). After grinding the samples, the homogenates were centrifuged at  $4^{\circ}\text{C}$  for 20 min at 13,000 rpm. The supernatant was collected and put on ice for further analysis. Next was to prepare the working solution by mixing reagent 1 (potassium hydrogen phosphate trihydrate, monopotassium phosphate, and PVP solution), reagent 2 (ascorbic acid), and reagent 3

( $\text{H}_2\text{O}_2$ ). The working solution was then kept at room temperature for 30 min. The samples supernatant and working solution were mixed, and OD was measured at 290 nm for 10 s and 130 s.

The GR activity was measured by extracting 0.1 g bud samples in 1 mL of reagent 1 (potassium hydrogen phosphate trihydrate, monopotassium phosphate, and bovine serum albumin (BSA) solution). After the samples were ground, the homogenates were centrifuged for 15 min at 8000 rpm at  $4^{\circ}\text{C}$ . The supernatant was collected and put on ice for further analysis. The reagent 1 was kept at room temperature for 10 min. After this, we took a blank tube and a sample tube. In the blank tube, we added reagents 1, 2 (NADPH), and 3 (GSSG solution), while in the sample tube, we added reagent 1, reagent 2, reagent 3, and supernatant. The OD was measured at 340 nm for 10 s and 190 s.

The SOD activity was also measured by extracting 0.1 g bud samples in a 1 mL phosphate buffer solution. The bud samples were homogenized and centrifuged at 8000 rpm at  $4^{\circ}\text{C}$  for 10 min after centrifugation, the supernatant was collected for further analysis and kept in an ice bath. The next step was to prepare the working solution by adding reagent 2 (water-soluble tetrazolium salt (WST-8) solution) to reagent 1 (disodium phosphate, monosodium phosphate, and diethylenetriaminepentaacetic acid (DTPA) solution) and mixed thoroughly. We took a sample tube and a control tube. In the sample tube, we added sample supernatant, reagent 3 (xanthine oxidase solution), working solution, and reagent 4 (hypoxanthine), while in the control tube, we added distilled water instead of sample supernatant. The tubes were well mixed and stood at room temperature for 30 min. Finally, the OD was measured at 450 nm.

### 2.4. Quantification of non-enzymatic antioxidants

To quantify non-enzymatic antioxidants, we collect samples at the same two stages, 0-weeks ( $T_0$  as a control) and after 4-weeks of photoperiod (LD and SD). In brief, for AsA content quantification, the tissue samples from LD and SD were prepared by weighing about 1 g of buds and homogenising in 1 mL acetic acid solution. After proper grinding

and homogenization, the final volume was 10 mL. The samples supernatant was collected after centrifugation at 8000 rpm at 4 °C for 20 min. We took a sample tube and a control tube. In the sample tube, we added sample supernatant, reagent 1 (ethylenediaminetetraacetic acid (EDTA) solution), reagent 2 (acetic acid solution), and reagent 3 (Fast Blue B Salt), while in the control tube, we added distilled water instead of sample supernatant. The OD was noted at 420 nm after keeping the tubes at room temperature for 20.

The GSH content was quantified by grinding 0.1 g of tissue in 1 mL of reagent 1 (metaphosphoric acid solution). After proper grinding and homogenization, the extracted samples were centrifuged at 8000 rpm for 10 min at 4 °C. The supernatant of the samples was collected for further testing. Furthermore, reagent 2 (potassium hydrogen phosphate trihydrate, monopotassium phosphate, and EDTA solution) was incubated in a water bath for 30 min at 25 °C. Finally, we added sample supernatant, reagent 2, and reagent 3 (DTNB, sodium citrate solution) in the sample tube, whereas distilled water was added instead of supernatant in the blank tube reagents were the same. The tubes were mixed well and let stand for 2 min. After 2 min, the OD was noted at 412 nm.

## 2.5. Determination of MDA and H<sub>2</sub>O<sub>2</sub>

The MDA content was determined by grinding a 0.1 g bud sample in a 1 mL phosphate buffer solution. After homogenization, the homogenate was centrifuged at 4 °C for 10 min using 8000 rpm. The supernatant was collected and put on ice for further analysis. Next was adding and mixing reagent 1 (trichloroacetic acid (TCA), thiobarbituric acid (TBA) solution with sample supernatant and incubating for 30 min at 95 °C in a water bath (cover tightly to prevent water loss). After incubation, it cooled to room temperature and centrifuged at 10,000 rpm for 10 min at 25 °C. Finally, the OD was noted at 532 and 600 nm.

The H<sub>2</sub>O<sub>2</sub> content was determined on an ice bath by grinding 0.1 g of bud samples in 1 mL of reagent 1 (acetone). The homogenate was centrifuged at 8000 rpm for 10 min at 4 °C, and the supernatant was collected. Reagents 2 (titanium sulfate) and Reagent 3 (concentrated Ammonia Water) should be in a water bath at 25 °C for more than 10 min. In the sample tube, we added supernatant, reagent 2, and reagent 3, while reagent 1 was used in place of the supernatant, and the other reagents were the same in the control tube. Again centrifuge was carried out at 4000 rpm for 10 min at 25 °C. The supernatant was discarded, and the pellet remained in both tubes. Finally, reagent 4 (sulfuric acid solution) was added to both tubes to dissolve the pellet (precipitate). Let the tubes stand at room temperature for 5 min, and the OD was measured at 415 nm.

## 2.6. Determination of bud burst

For the bud burst determination, we identified four physiological stages in developing the buds, as shown in Fig. 1 in LD and SD, followed by the Zhang et al. (2021) method.

## 2.7. RNA extraction, cDNA synthesis, and transcriptome sequencing

Six samples were prepared for transcriptome sequencing that was taken from buds of seedlings treated with two different photoperiods (after 4 weeks), such as long day (LD) and short-day (SD), with three biological repeats (2 treatments × 3 biological repeats = 6 samples). First, the RNA was extracted from these samples, and its integrity was evaluated following the RNA Nano 6000 assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, CA, USA). After RNA extraction, it was an input material for RNA sample preparation. Poly-T oligo-attached magnetic beads were used to isolate the mRNA from the total RNA. The first-strand cDNA was obtained from the mRNA using a random hexamer primer, M-MuLV reverse transcriptase enzyme, and RNaseH to degrade the RNA. DNA polymerase I and dNTP was used in the following step to yield the second-strand cDNA.

Moreover, AMPure XP System (Beckman Coulter, Beverly, USA) was utilized to purify library fragments and select suitable cDNA fragments (preferentially 370–420 bp in length). A PCR was performed on the library fragments, and the PCR product was again purified using the AMPure XP System. The quality of the final libraries was assessed using Qubit2.0 Fluorometer (Carlsbad, CA, USA) and qRT-PCR. Finally, the final libraries were sequenced on an Illumina Novaseq platform of the Novogene Company (Tianjin, China). One hundred fifty (150 bp) paired-end reads were generated after sequencing.

The clean reads were obtained from the raw reads by removing low-quality reads and reads containing adapters and N bases using in-house Perl scripts. It was further assessed for obtaining high-quality clean reads by measuring Q20, Q30, and GC contents. The subsequent analysis was performed on the obtained high-quality clean reads. Trinity with default parameters settings was used for transcriptome assembly (Grabherr et al., 2011; Liu et al., 2019).

## 2.8. Gene annotation, differential expression, GO, and KEGG analyses

Multiple databases annotated the gene function, including Nr, Nt, Pfam, KOG, Swiss-Prot, KO, and GO. DESeq2 R package (1.20.0) was used to differentiate genes between the two groups (treatments), which provided a statistical procedure for the differential expression. A P-value < 0.05 in DESeq2 of a gene was considered differentially expressed. Finally, the DEGs were functionally classified into different GO terms and pathways using the cluster profile R package in Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases by setting the P-value < 0.05.

## 2.9. qRT-PCR validation of DEGs

Quantitative real-time PCR (qRT-PCR) was used to validate the expression levels of differentially expressed genes (DEGs). The qRT-PCR was performed, taking the same RNA samples for sequencing as a template from the buds of the *Liriodendron chinense* from LD and SD conditions (after 4-weeks) by using three technical and three biological replications. According to the manufacturer's protocol, total RNA was extracted using a Total RNAPlant Extraction Kit (Tiangen, Beijing, China). According to the manufacturer's instructions, the first-strand cDNA was synthesized using the TaKaRa PrimeScript 1st-strand cDNA Synthesis Kit (TaKaRa, Dalian, China). Quantitative real-time polymerase chain reaction (qRT-PCR) was conducted using the SYBR Green Real-time PCR Master Mix (TOYOBO, Osaka, Japan) on an ABI7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) (Hussain et al., 2021; Jin et al., 2013). The actin gene *LtActin97* of *Liriodendron* was used as a reference gene (forward primer: TTCCCGTTAG-CAGTGGTCG, reverse primer: TGGTCGCACAATGGTATCG), and the sequence provided by (Liu et al., 2019), for calculating the expression levels of target genes were used the 2<sup>-ΔΔCT</sup> method (Shi et al. 2019, 2021). All primers for RT-qPCR were designed by Primer Premier 5.0 software and are listed in the Additional file Table S1.

## 2.10. Regulatory cis-elements

Putative cis-element regulatory regions were identified within the promoter region using PlantCARE (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>) to analyze the gene promoters and identify their cis-elements (Lescot et al., 2002; Rombauts et al., 1999).

## 2.11. Statistical analysis

GraphPad Prism 9 statistical software was used for the statistical analyses. At P ≤ 0.05, the student's t-test tested for significant differences between the means.

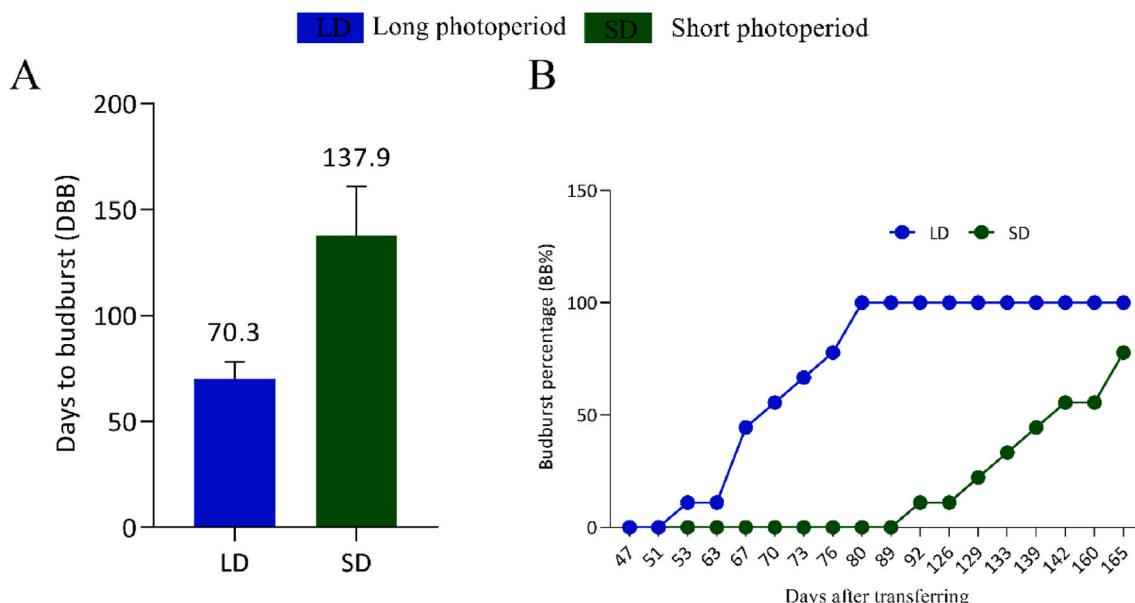
### 3. Results

#### 3.1. Effect of photoperiod on the days and percentage of bud burst

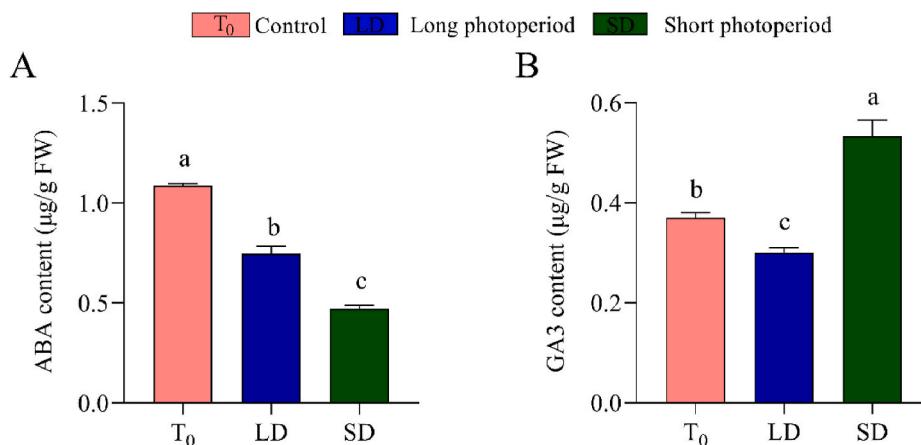
Our results displayed the different phenological stages in the bud burst in *Liriodendron* and the effect of photoperiod (LD and SD) on days to bud burst (DBB) and bud burst percentage (BB%). In *Liriodendron* seedling, the DBB was significantly affected by photoperiod, and higher values of DBB were observed under SD than under LD (Fig. 2A). A significant difference of BB% was observed between LD and SD in *Liriodendron* bud. So the BB% was generally higher under LD than under SD (Fig. 2B). Comparing the effect of LD and SD, we observed that the DBB and BB% ratio were higher in SD than in LD. These outcomes above provide a solid foundation to unveil the underlying regulatory mechanism or interplay between biochemical changes and transcript adjustments.

#### 3.2. Phytohormone contents in bud response to photoperiod

The contents of the two (ABA and GA3) phytohormones were observed in the *Liriodendron* bud and were highly significantly affected ( $p < 0.05$ ) between control ( $T_0$ ) and different photoperiod (LD and SD). ABA content decreased by 31% in LD and 57% in SD compared to control ( $T_0$ ). Similarly, the level of ABA was higher (59%) in LD compared to SD (Fig. 3A); otherwise, the content of GA3 decreased by 19% in LD but increased by 44% in SD, compared with  $T_0$  (Fig. 3B). Likewise, the level of GA3 was lower (44%) in LD compared to SD. Please see the supplementary file (Table S2), which presents the raw data used for the biochemical analysis.



**Fig. 2.** Effects of photoperiod on the bud burst in seedlings of *Liriodendron chinense*. Effect of photoperiod on the timing/days of bud burst (DBB) and percentage of bud burst (BB%) (A) days to bud burst and (B) bud burst percentage.



**Fig. 3.** Effects of photoperiod on contents (mean  $\pm$  standard deviation) of phytohormones (A) ABA and (B) GA3 in *Liriodendron chinensis* seedlings. The contents were measured at the beginning of the experiment on December 17, 2020 ( $T_0$  as a control) and after 4-weeks of exposure to long day (LD, 14 h) and short day (SD, 10 h) treatments under +20 °C. The different lowercase letters indicate statistical differences at  $P \leq 0.05$ .

### 3.3. Effect of photoperiods on antioxidant enzyme activities

The effect of different photoperiods on the antioxidant enzyme activities during bud dormancy is portrayed in Fig. 4A–E. Different photoperiods significantly affected the antioxidant enzyme activities ( $P < 0.05$ ). In addition, a non-significant effect of photoperiod (LD vs SD) was recorded regarding POD activity, while the POD activity showed significant differences between control and short day ( $T_0$  vs SD). Compared with control, the POD activity decreased by 8% in LD and 11% in SD while showing non-significant differences between control and LD ( $T_0$  vs LD). Similarly, POD activity was higher in LD (3%) than in SD. The activities of SOD, CAT, APX and GR showed significant differences between photoperiods (LD vs SD), while compared with control also observed significant differences ( $T_0$  vs LD, and  $T_0$  vs SD). During photoperiod conditions, SOD, CAT, and GR activities increased by 16%, 60%, and 32% in LD than in SD, while the activity of APX was lower by 17% in LD while increasing in SD. Compared to the control, the SOD and APX activities decreased by 15% and 51% in LD, 27%, and 40% in SD, respectively. Compared with the control ( $T_0$ ), an increment of 133% in LD and 77% in SD is observed in the GR activity. Likewise, the CAT activity was six times higher in LD and three times higher in SD than in  $T_0$ . The different photoperiods have diverse effects on the antioxidant enzyme activities; SOD and APX activities are decreased while CAT and GR are increased during bud dormancy of *Liriodendron*. Please see the supplementary file (Table S2), which presents the raw data used for the biochemical analysis.

### 3.4. Effect of photoperiods on non-enzymatic antioxidants contents

The contents of AsA and GSH observed significant differences between different photoperiods (LD vs SD). In contrast, compared with control, differences observed in AsA between  $T_0$  and LD are insignificant, and the same for GSH between  $T_0$  and SD are statistically non-significant (Fig. 5A and B). During LD and SD conditions, the contents of AsA and GSH increased by 93% and 64% in SD than in LD. Compared with the control, the AsA content was shown an increment of 6% in LD and 105% in SD (Fig. 5A). Notably, the GSH content dropped by 37% in LD; on the contrary, a 3% rise was observed in SD treatment compared to control (Fig. 5B). Please see the supplementary file (Table S2), which presents the raw data used for the biochemical analysis.

### 3.5. Effect of photoperiods on MDA and hydrogen peroxide

The malondialdehyde (MDA) exhibited significant ( $P < 0.05$ ) differences for the different photoperiods (LD vs SD), while a non-significant increase was found in the  $H_2O_2$  content when subjected to photoperiod conditions (LD vs SD) (Fig. 6A and B). Similarly, the content of MDA was higher (18%) in SD than in LD. Otherwise, the content of  $H_2O_2$  was higher (7%) in LD than in SD. Compared with control, the content of MDA was observed with non-significant differences between control and long-day ( $T_0$  vs LD), while significant differences were shown between control and short-day ( $T_0$  vs SD). The results show that the LD reduced the MDA content by 9%, while an 18% increase was noted in the SD-treated buds relative to  $T_0$ . Similarly, the  $H_2O_2$  content are 14% and 20% less in LD and SD, respectively, compared to the control. Please see the supplementary file (Table S2), which presents the raw data used for the biochemical analysis (Fig. 6A, B).

## 4. Comparative transcriptome between long- and short-day light

### 4.1. Identification of DEGs in bud dormancy release in response to light

To achieve more information about the transcriptome analysis from *Liriodendron chinense* buds' long and short days. RNA-Seq generated 23080231 (buds from the long-day light) and 21905861 (buds from the short-day light) clean reads after filtering. The Q30 percentage for each

sample ranged from 93.09% to 94.81%, and the guanine-cytosine (GC) content was 46.52% (Table S3). The FPKM violin diagram (Fig. S1) and FPKM density distribution (Fig. S2) suggested that the density of the noticed genes followed a standard normal distribution. As shown in (Figs. S3A and B, Table S4), we identified 114,549 genes, but 187 DEGs were obtained significantly between long and short daylight of bud, including 103 up-regulated and 84 down-regulated genes. To validate the result of identified DEGs, we determined the expression profiles of the 10 candidate genes by qRT-PCR, and gene-specific primers (Table S1) were designed using *Liriodendron chinense* nucleotide sequences. After the amplification of genes, the amplified products were confirmed by sequencing.

A total of 187 DEGs were found, and the DEGs belong to the different pathways, which were classified into several subclasses: including chalcone synthase, E3 ubiquitin-protein ligase, auxin response factor, basic helix-loop-helix, B-box zinc finger, cellulose synthase activity, cytochrome P450, leucine-rich repeat, homeobox, heavy-metal-associated domain, protein kinase domain, ribosomal protein, WRKY transcription factor, YABBY protein, and zinc finger etc. (Table S5).

### 4.2. GO and KEGG enrichment analyses of bud transcriptome

A total of 187 DEGs, including 103 up-regulated and 84 down-regulated genes, were annotated into GO entries (including molecular function). Iron ion binding, heme binding, and tetrapyrrole binding were the most enriched GO terms in the molecular function (Figs. S3C). After identifying DEGs in the bud from the long- and short-daylight, we performed a KEGG enrichment analysis to observe the functional enriched DEGs. We identified 20 significantly enriched pathways (Fig. 7) involving flavonoid biosynthesis, phenylpropanoid biosynthesis, nitrogen metabolism, cyanoamino acid metabolism, plant hormone signal transduction, starch, and sucrose metabolism cutin, suberine and wax biosynthesis, circadian rhythm – plant etc.

### 4.3. Flavonoid biosynthesis pathway analysis

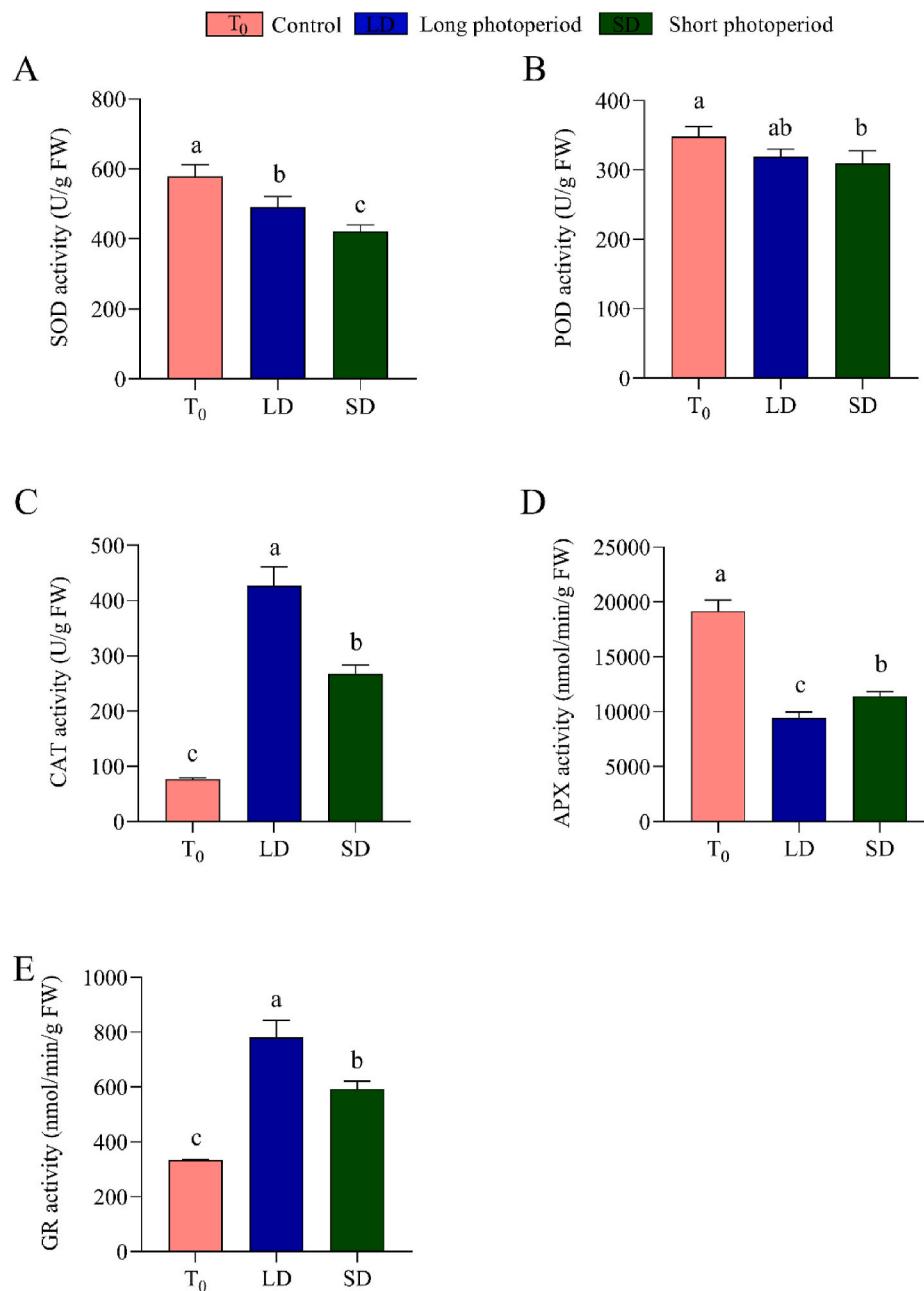
Our results demonstrated that photoperiod induces various DEGs expressions associated with flavonoid biosynthesis during the bud dormancy based on GO and KEGG analyses. *CHS* (Cluster-13653.15502), *CHS2* (Cluster-13653.48358), *CHS3* (Cluster-13653.28590), and *HCT* (Cluster-23216.0) genes were up-regulated (3.14, 2.47, 1.61, and 6.22 log2-fold change) during LD in the bud dormancy release (Fig. 8A and B). Only two genes (*CHS* and *HCT*) were selected for the qRT-PCR, and the validation by qRT-PCR confirmed the higher transcription levels in the *Liriodendron* bud. Because these genes were up-regulated in LD and down-regulated in SD, the results showed our qRT-PCR (Figs. S4A and B).

### 4.4. Phenylpropanoid biosynthesis pathway analysis

The novel genes found which are involved in phenylpropanoid biosynthesis pathways, including *HCT* (Cluster-23216.0), *E1.11.1.7* (Cluster-12136.0), *BGLU46* (Cluster-13653.37090), *COMT* (Cluster-13653.64504), and *BGLU18* (Cluster-13653.36774) genes, were up-regulated (6.22, 3.85, 2.18, 2.06, and 2.45 log2-fold change) in LD during bud dormancy release (Fig. 9A and B). These five genes associated with the phenylpropanoid biosynthesis pathways were selected for the qRT-PCR, and the validation by qRT-PCR confirmed the higher transcription levels in the *Liriodendron* bud. The expression level of five genes significantly increased under LD compared to SD in our qRT-PCR result (Figs. S4B-F).

### 4.5. Plant hormone signal transduction in response to light

More than 25 DEGs were associated with the plant hormone and signal transduction pathway response to bud dormancy in LD and SD based on



**Fig. 4.** Effect of photoperiods concentrations (mean ± standard deviation) of antioxidant enzyme (A) SOD, (B) POD, (C) CAT, (D) APX, and (E) GR in *Liriodendron Chinensis* seedlings. The contents were measured at the beginning of the experiment on December 17, 2020 (T<sub>0</sub> as a control) and after 4-weeks of exposure to long day (LD, 14 h) and short day (SD, 10 h) treatments under +20 °C. The different lowercase letters indicate statistical differences at P ≤ 0.05.

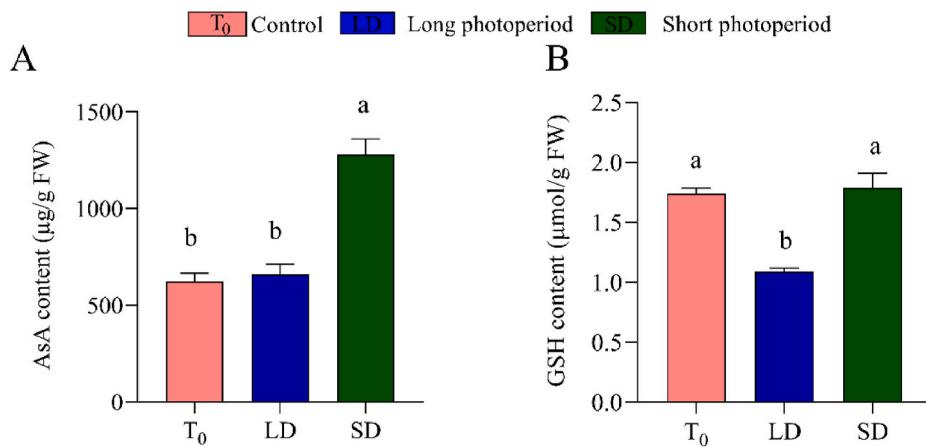
the transcriptomic analyses. These genes were found in the abscisic acid (ABA) and gibberellin (GA) signaling pathways. Under LD, two genes (*PYL4* and *PYL8*) encoding the ABA receptor PYL/PYR were upregulated. In response to LD, two genes of ZEP, NCED, ABF, three genes of PP2C, and three other genes were up-regulated and linked with SnRK2 (Fig. 10B). In the gibberellin signaling system, one DEG expressing GID1 protein was up-regulated in LD, while two GID2 genes were up-regulated in LD and SD. DEELA consists of three genes, two expressed in SD and one up-regulated in LD (Fig. 10B). *A-ARR* (Cluster 13653.4569) and *CYCD3* (Cluster-13653.58550) genes were down-regulated (-6.49 and -1.61 log2-fold change) in SD during bud dormancy. The above two genes were selected for the qRT-PCR, and the validation by qRT-PCR confirmed the lower transcription levels in the *Liriodendron* bud. Because these genes were down-regulated in LD and up-regulated in SD, the results showed in the qRT-PCR (Figs. S4G and H).

#### 4.6. Cutin, suberin and wax biosynthesis

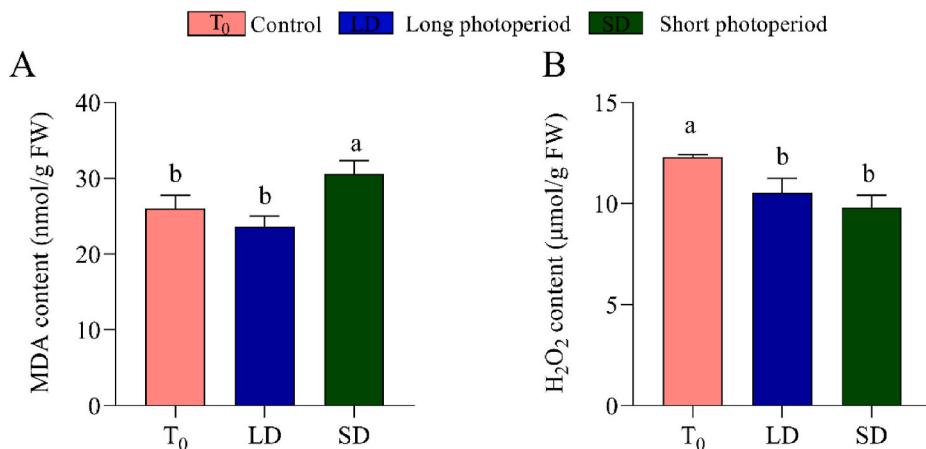
The regulation of structural genes involved in cutin, suberin and wax biosynthesis pathways, including *HHT1* (Cluster-13653.62712) and *CYP86A1* (Cluster-37749.0) genes, were up-regulated (3.98 and 7.74 log2-fold change) in LD during bud dormancy (Figs. S5A and B). These two genes were selected for the qRT-PCR, and the validation by qRT-PCR confirmed the higher transcription levels in the *Liriodendron* bud. Because these genes were up-regulated in LD compared to SD, the results showed in our qRT-PCR (Figs. S4I and J).

#### 4.7. Identification of regulatory elements in light

Analysis of cis-regulatory elements of 26 DEGs during light revealed the presence of 22 different motifs. We analyzed 22 motifs commonly



**Fig. 5.** Effect of photoperiods concentrations (mean  $\pm$  standard deviation) of non-enzymatic antioxidants (A) Ascorbic acid (AsA) and (B) Glutathione (GSH) in *Liriodendron Chinensis* seedlings. The contents were measured at the beginning of the experiment on December 17, 2020 (T<sub>0</sub> as a control) and after 4-weeks of exposure to long day (LD, 14 h) and short day (SD, 10 h) treatments under +20 °C. The different lowercase letters indicate statistical differences at P  $\leq$  0.05.



**Fig. 6.** Effect of photoperiods concentrations (mean  $\pm$  standard deviation) of oxidative stress biomarkers (A) Malondialdehyde (MDA), and (B) Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in *Liriodendron Chinensis* seedlings. The contents were measured at the beginning of the experiment on December 17, 2020 (T<sub>0</sub> as a control) and after 4-weeks of exposure to long day (LD, 14 h) and short day (SD, 10 h) treatments under +20 °C. The different lowercase letters indicate statistical differences at P  $\leq$  0.05.

differentially expressed in light. Cis-regulatory motifs are involved in light responsiveness present in the promoter region of genes. Similarly, we identified the TCCC-motif, TCT-motif, I-box, GT1-motif, GATA-motif, Box 4, AE-box, ATCT-motif, etc., cis-acting regulatory element involved in light responsiveness (Table S5).

## 5. Discussion

We aimed to examine the effect of different photoperiods (LD and SD) on bud dormancy in *Liriodendron chinense*. Plant hormones are essential in inducing and breaking bud dormancy (Horvath et al., 2003). ABA is a phytohormone that helps plant growth, development, and stress resistance (Hussain et al., 2021, 2022). Although there is some indication that ABA has a role in maintaining bud dormancy in woody plants, there is little molecular data to support this (Arora et al., 2003). In numerous temperate experiments, endogenous ABA levels rose as grape buds entered dormancy and fell as dormancy was liberated over the winter (Or et al., 2000; DÜRING and BACHMANN, 1975; Koussa et al., 1994). According to Rohde et al. (2002), ABA concentrations in poplar apical buds increased significantly after 3–4 weeks of short days. For example, in *Prunus mume*, ABA levels were high at the start of endodormancy and gradually declined until dormancy release (Rohde et al., 2002). Peach (Wang et al., 2016) and other woody species like pear (Li et al., 2018) have shown similar results. According to other

studies, ABA is only necessary for bud development and maintenance, not dormancy release (Ionescu et al., 2017). Photoperiod significantly impacted the timing/days of bud burst (DBB) and the percentage of bud burst (BB%). This conclusion is consistent with the previous finding that a long photoperiod control budburst, DBB, and BB% were generally higher under LD conditions than SD conditions in the *Liriodendron* species (Zhang et al., 2021). The early release of bud burst and BB% in LD compared to SD confirms that LD is a requirement for budburst (Zhang et al., 2021).

Endogenous ABA levels increased as grape buds entered dormancy in various temperate trials and dropped during the winter as dormancy was released (Or et al., 2000; DÜRING and BACHMANN, 1975; Koussa et al., 1994). Rohde et al. (2002) found that after 3–4 weeks of short days, ABA concentrations in apical buds increased considerably in poplar. Because both promoting and inhibitory effects of GAs on bud break have been reported, the influence of GAs on bud break remains a matter of debate (Ionescu et al., 2017; Zheng et al., 2018). According to Finch-Savage and Leubner-Metzger (2006), the ratio of ABA/GAs decreases during seed dormancy release. However, as described in dormant seeds (Finch-Savage and Leubner-Metzger, 2006), sweet cherry floral buds, and *Prunus mume* (Finch-Savage and Leubner-Metzger, 2006), it appears that the ratio of ABA/GAs, rather than absolute hormone contents, maybe more important in the control of dormancy release (Zheng et al., 2018). ABA has been shown to mediate photoperiodic control of bud

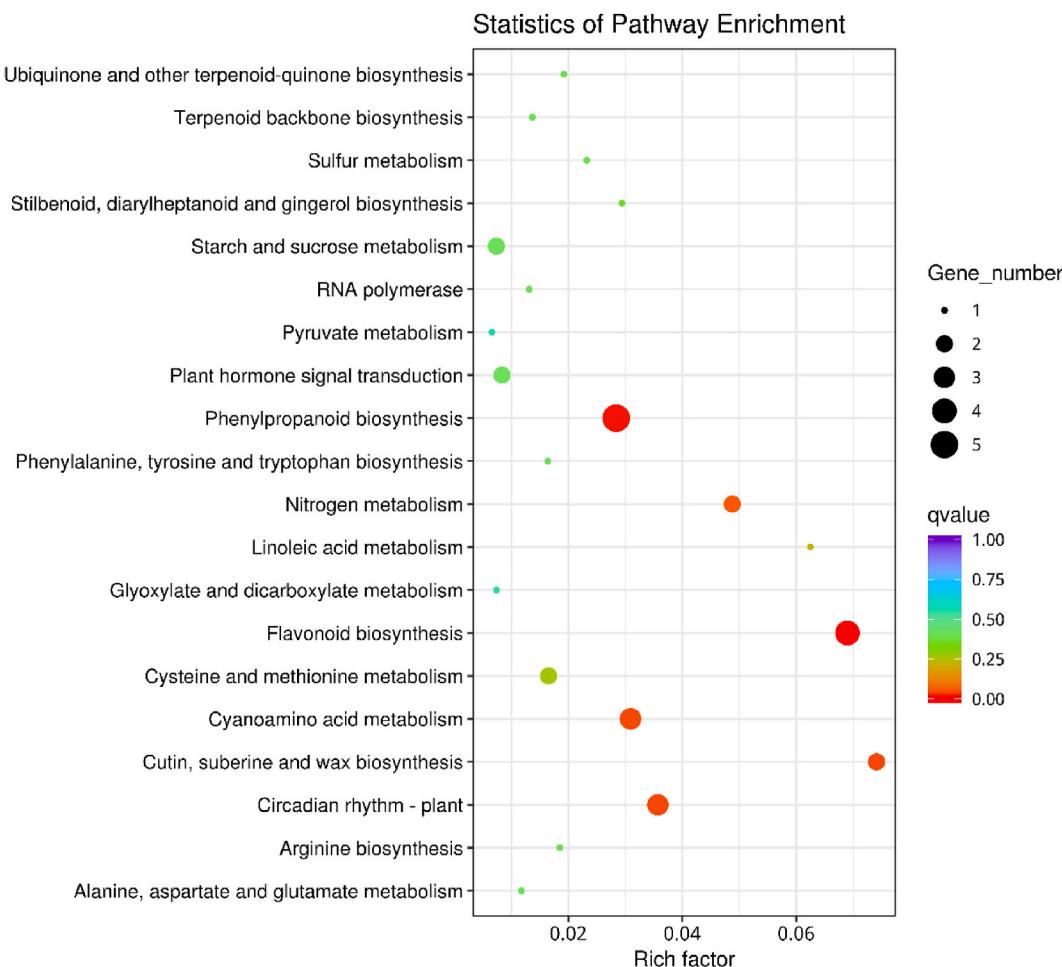


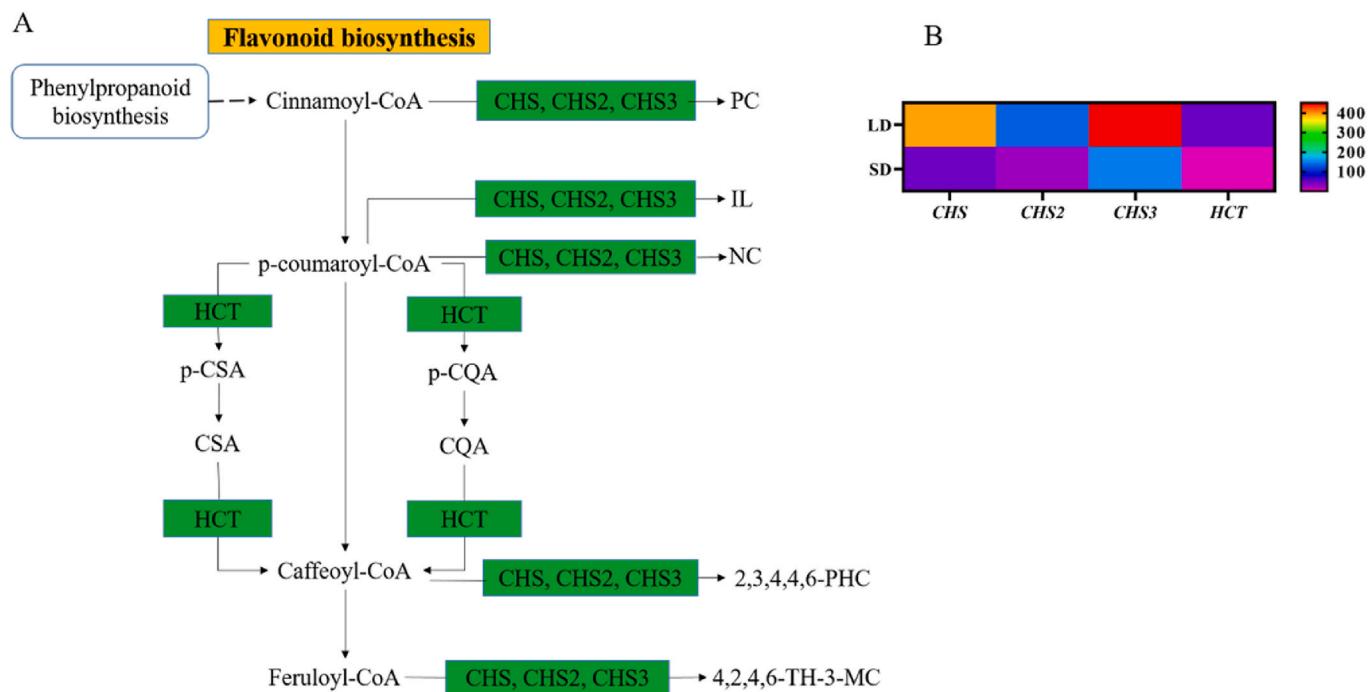
Fig. 7. KEGG enrichment analyses of annotated DEGs between a long day and short daylight.

dormancy (Singh et al., 2018; Tylewicz et al., 2018), and our results indicate a relationship between ABA concentration and bud dormancy. Therefore, we probed ABA's role in the photoperiodic control of bud dormancy. Levels of ABA are high during the induction and development of dormancy but decrease during dormancy release and bud break (Singh et al., 2020). At the dormancy stage, we also discovered a significant concentration of ABA in LD compared to SD and control. As a result, our findings are similar to those of earlier researchers. Sweet cherry flower buds had a higher endogenous ABA/GA3 ratio during natural dormancy induction and a lower endogenous ABA/GA3 ratio when dormancy was released (Hernandez et al., 2021). Furthermore, the respective functions of GA3 and GA4 applications are to promote peach bud burst and dormancy release in Japanese apricot flower buds (Zhuang et al., 2015). In another study, the GA3 application promotes kiwi bud break under chilling (Lionakis and Schwabe, 1984). Consistent with this, in the present study, the maximum concentration of GA3 was found in SD compared to LD and T0 (Junttila and Jensen, 1988). In earlier studies, the researchers suggested that reduced GA levels are a prerequisite for initiating growth cessation under SD conditions (Maurya and Bhalerao, 2017).

Antioxidant defense and detoxification pathways, including CAT, GR, SOD, APX, and peroxidase superfamily proteins, are elevated during dormancy release, implying that oxidative stress is a significant part of the process (Beauvieux et al., 2018). SOD, POD, CAT, and APX are enzyme scavengers found in plants that help keep ROS under control (Garbero et al., 2011). Our results showed that SOD and POD activities decreased during bud dormancy, whereas Abassi et al. (1998) discovered that SOD and POD activities were insufficient in apple buds during

dormancy and increased upon bud break and that dormant tissues had low levels of O<sub>2</sub>-free radicals. During dormancy, CAT activity increased while SOD, POD, and APX activity decreased. Therefore, changes in gene expression, enzyme activity, and H<sub>2</sub>O<sub>2</sub> levels in dormant floral buds of pear cultivars may contribute to antioxidant defense mechanisms (Hussain et al., 2015). Like SOD, CAT is an antioxidant enzyme. It captures hydrogen peroxide and transforms it into water and oxygen. Our findings revealed that CAT activity was higher in LD than SD during bud dormancy. CAT activity in pear flower buds was high during the dormant stage, according to Shao and Ma (2004), which validates our findings. Additionally, APX enzymes cooperate with SOD to scavenge H<sub>2</sub>O<sub>2</sub> generated by SOD, such as superoxide anion radical (O<sub>2</sub>) dismutation to H<sub>2</sub>O<sub>2</sub>. APX enzymes also work with SOD to scavenge H<sub>2</sub>O<sub>2</sub> produced by SOD, such as superoxide anion radical (O<sub>2</sub>) dismutation to H<sub>2</sub>O<sub>2</sub> (Hussain et al., 2015). APX regulates intracellular ROS levels by converting H<sub>2</sub>O<sub>2</sub> to water with the help of ascorbate as an electron donor, resulting in the creation of dehydroascorbate (Hussain et al., 2015). APX activity was lower during bud dormancy in the current investigation. The current findings on APX activity in pear flower buds align with those of Shao and Ma (2004), who found that APX activity reduced throughout the dormancy phase and increased upon bud break. Similarly, the activity of APX was not affected by photoperiod (Abuelsoud et al., 2020).

According to our results, the activity of GR increased in LD and SD compared to control in dormant buds or during bud dormancy. These results agree with a recent work by Hernandez et al. (2021) showed that the activity of GR increased in the dormant bud of a peach. Our findings are also supported by Wei et al. (2019); the photoperiod significantly



**Fig. 8.** Differentially expressed genes (DEGs) are involved in Flavonoid biosynthesis pathways. (A) The proposed pathways show Flavonoid biosynthesis. This figure shows only the genes associated with the flavonoid biosynthesis pathway. p-CSA indicates-p-Coumaroyl shikimic acid; p-CQA-p-Coumaroyl quinic acid; CSA-Caffeoyl shikimic acid; CQA-Caffeoyl quinic acid; PC- Pinocembrin chalcone; IL- Isoliquiritigenin; NC- Naringenin chalcone; PHC-Pentahydroxychalcone; TH- Tetrahydroxy; MC- methoxychalcone; CHS- Chalcone synthase; THT2- Tryptamine hydroxycinnamoyltransferase 2; and HCT- Hydroxycinnamoyltransferase. (B) The heat map of selected DEGs shows their expression behaviour corresponding to “A”. LD and SD indicate long and short days. Pink and red indicate lower and higher expression values, respectively.

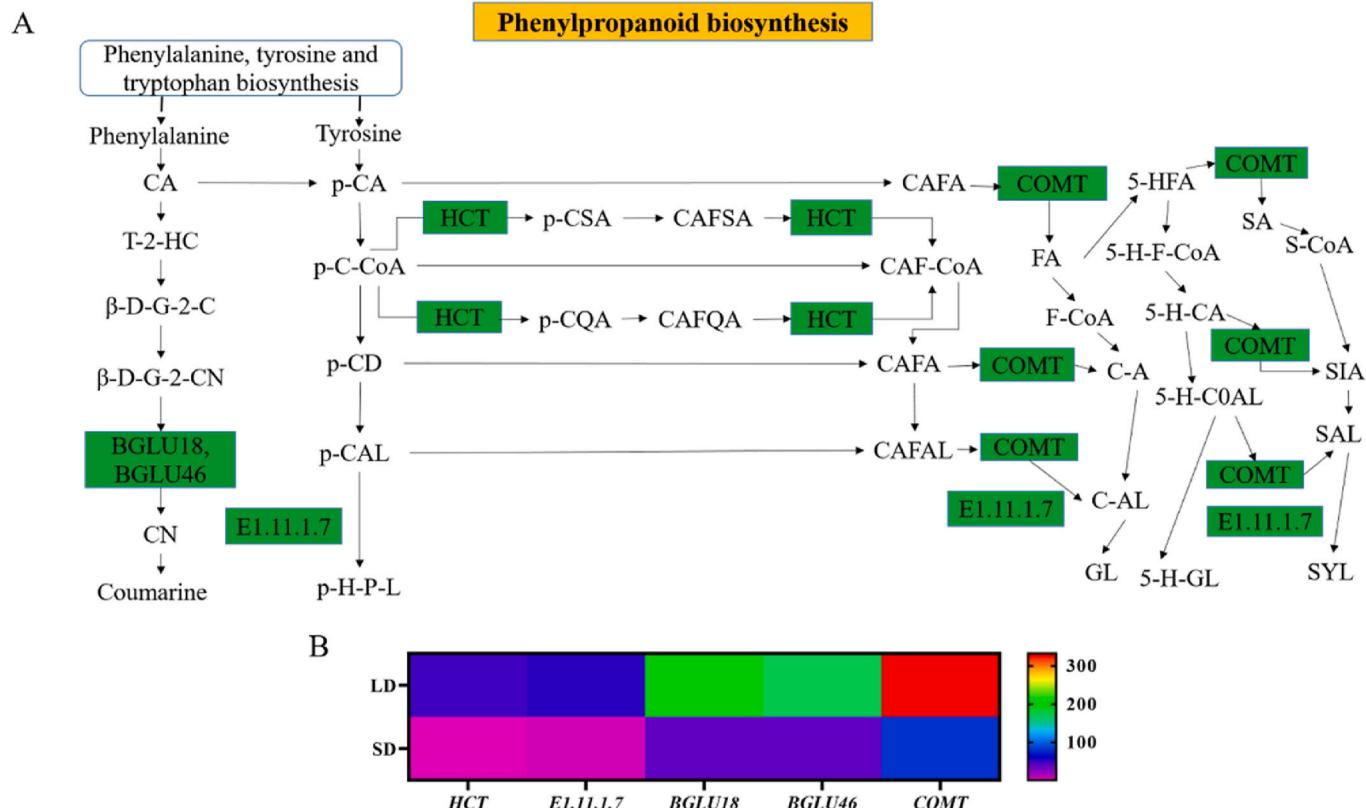
impacted the antioxidant enzyme activities; therefore, GR was significantly higher in LD than in SD. In earlier studies, the activities of the non-enzymatic antioxidant enzymes of the AsA-GSH scavenging system were not affected by photoperiod (Abuelsoud et al., 2020). GSH is one of the key components of the non-enzymatic defense system that is crucial for plant survival and performs various functions under stressful conditions (Khan et al., 2019). Our findings are supported by Abuelsoud et al. (2020), who found that the AsA and GSH contents were maximum in SD compared to LD, and AsA and GSH have no decisive role in the stress response in *Arabidopsis thaliana*. Higher GSH contents facilitate protein synthesis via controlled monosome formation, which activates and deactivates redox-dependent enzymes during bud break to scavenge free radicals (Bartolini and Zanol, 2006).

Biochemical analysis of MDA and H<sub>2</sub>O<sub>2</sub> showed non-significant differences between control and LD and H<sub>2</sub>O<sub>2</sub> between LD and SD. MDA decreased during control and LD compared to SD, while H<sub>2</sub>O<sub>2</sub> content decreased in LD and SD compared with control. According to Monteiro et al. (2022), the content/level of MDA decreased during the dormancy stage and increased in time of budburst. The presence of H<sub>2</sub>O<sub>2</sub>-sensitive antioxidant enzymes, such as Fe-SOD and Cu, Zn-SOD, and APX and POX, may aid in producing a scenario of regulated oxidative stress (i.e., increased O<sub>2</sub> buildup), which may be involved in dormancy release (Hernandez et al., 2021). Takemura et al. (2015) reported a non-significant increase in H<sub>2</sub>O<sub>2</sub> concentration in pear floral buds, which coincided with a considerable reduction in CAT activity.

The transcriptome investigation shed light on the events at the molecular levels and offered new information on the gene expression networks in the bud dormancy and development of buds (Tylewicz et al., 2018; Shi et al., 2021; Tuttle et al., 2015; and Wang et al., 2017). Of the total 114,549 annotated genes in the *Liriodendron chinense* reference, we obtained 187 significant DEGs between LD and SD buds, including 103 up-regulated and 84 down-regulated genes. Interestingly, these DEGs are expressed in several pathways: flavonoid biosynthesis,

phenylpropanoid biosynthesis, cutin, suberin, wax biosynthesis, starch and sucrose metabolism, nitrogen metabolism cyanoamino acid metabolism, plant hormone signal transduction, circadian rhythm–plant etc. Flavonoid and phenylpropanoid biosynthesis pathways were highly enriched in RNA-seq analyses during the bud's development, which aligns with the results (Shi et al., 2021). The flavonoid and phenylpropanoid biosynthesis pathway-related genes *CHS*, *CHS2*, *CHS3*, *HCT*, *E1.11.1.7*, *BGLU46*, *COMT*, and *BGLU18* are up-regulated during photoperiod (LD), which is consistent with the increased concentration of phytohormone (ABA), and activities antioxidant enzymes (SOD, CAT, GR). In previous studies, the downregulation of *CHS* genes and *HCT* genes during axillary bud growth suggests that the regulation of the phenylpropanoid and flavonoid biosynthesis pathways is a significant factor in promoting axillary bud outgrowth (Shi et al., 2021). Shi et al. (2021) highlighted that the phenylpropanoid and flavonoid biosynthesis pathways regulate axillary bud growth by promoting auxin transport in upland cotton, which is in harmony with our findings. *CHS* catalyzes the initial step in the flavonoid biosynthetic pathway, and the effect of light on *CHS* expression is principally transcriptional, as initially demonstrated in parsley cells (Jenkins et al., 2001).

The physiological activities in flower buds, including flower bud dormancy, are regulated by plant hormones (Zhu et al., 2015; Bai et al., 2013; and Zhong et al., 2013). Genes involved in hormone production were improperly expressed during flower bud germination, resulting in a significant buildup of ABA and a minor accumulation of GA and flower buds (Liu et al., 2016). Our findings showed that the ABA biosynthesis (ZEP, NCED, and ABA2) and ABA signaling (PYR/PYL, PP2C, SnRK2, and ABF) were mainly up-regulated during bud dormancy, while the ABA content is also increased. According to previous studies, the over-expression of ABA biosynthesis genes increases seed ABA content, enhances seed dormancy, or delays germination (Finch-Savage and Leubner-Metzger, 2006; Finkelstein et al., 2002; Kushiro et al., 2004). Other plant hormones such as cytokinin (A-ARR), brassinosteroid



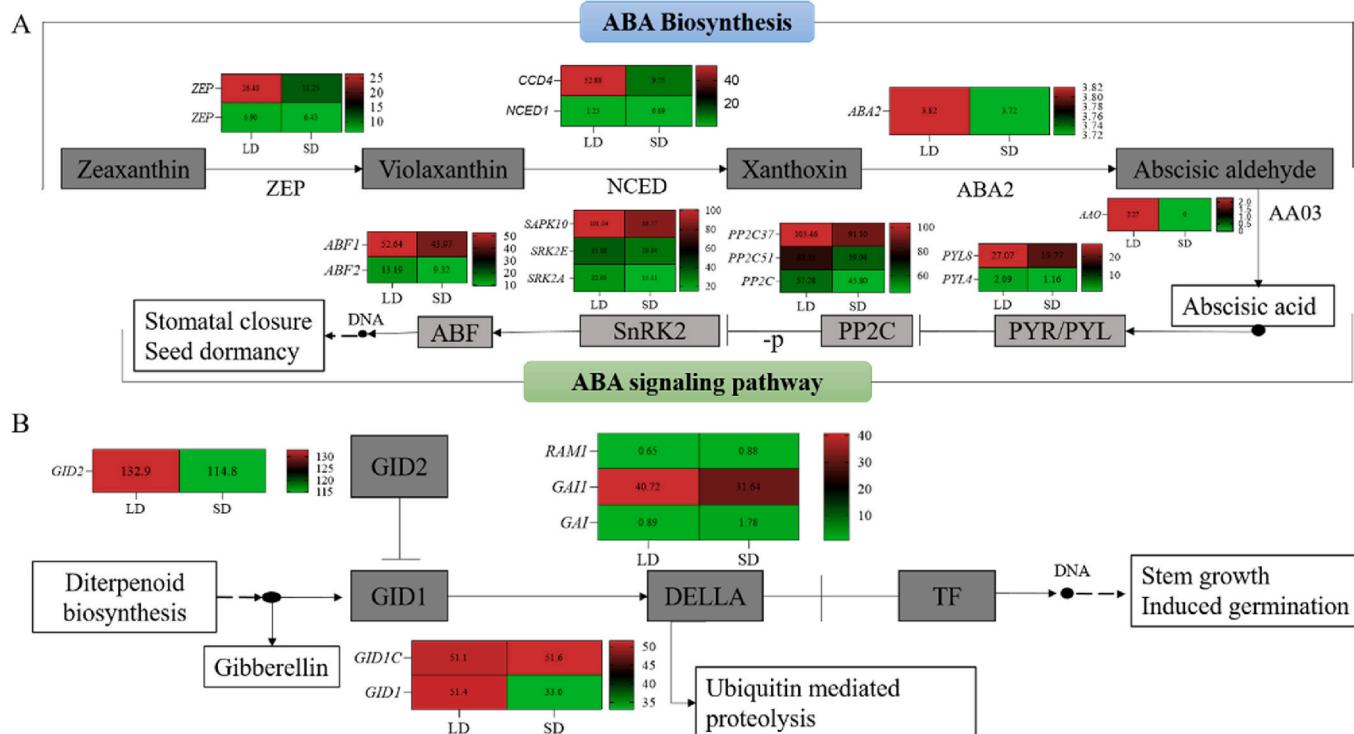
**Fig. 9.** Differentially expressed genes (DEGs) are involved in Phenylpropanoid biosynthesis pathways. (A) The proposed pathways show Phenylpropanoid biosynthesis. This figure shows only those genes associated with the Phenylpropanoid biosynthesis pathway from RNA-seq analysis. CA-indicates Cinnamic acid; T-2-HC-Trans-2-Hydroxycinnamate; β-D-G-2-C-β-D-Glucosyl-2-coumarate; β-D-G-2-CN-β-D-Glucosyl-2-coumarinate; CN-Coumarinate; p-CA-p-Coumaric acid; p-C-CoA-p-Coumaroyl-CoA; p-CD-p-Coumaraldehyde; p-CAL-p-Coumaryl alcohol; p-H-P-L- p-Hydroxy-phenyl lignin; CAFA-Caffeic acid; p-CSA-p-Coumaroyl shikimic acid; p-CQA-p-Coumaroyl quinic acid; CAFSA-Caffeoyl shikimic acid; CAFQA- Caffeoylquinic acid; CAF-CoA- Caffeoyl-CoA; CAFA-Caffeoyl-aldehyde; CAFAL-Caffeoyl alcohol; FA-Ferulic acid; F-CoA-Feruloyl-CoA; C-A-Coniferyl-aldehyde; C-AL-Coniferyl-alcohol; GL-Guaiacyl lignin; 5-HFA-5-Hydroxyferulic acid; SA-Sinapic acid; 5-H-F-CoA- 5-Hydroxy-feruloyl-CoA; 5-H-CA- 5-Hydroxy-coniferaldehyde; 5-H-COAL- 5-Hydroxy-coniferyl alcohol; 5-H-GL- Hydroxy-guaiacyl lignin; S-CoA- Sinapoyl-CoA; SIA- Sinapaldehyde; SAL- Sinapyl alcohol; SYL- Syringyl lignin. (B) The heat map of selected DEGs shows their expression behavior corresponding to “A”. LD and SD indicate long and short days. Pink and red indicate lower and higher expression values, respectively.

(*CYCD3*), and auxin (*ARF2*, *SAUR67*) genes were down-regulated during bud dormancy in SD buds. Down-regulation of *CYCD3* expression, according to Dewitte et al. (2003) and Ascencio-Ibanez et al. (2008), favours the buildup of the endocycle 4C population and facilitates infection. Cell division is a complete occurrence during the plant life cycle influenced by many factors such as light, temperature, nutrients, and phytohormones (Hu et al., 2000; Hussain et al., 2020). Brassinosteroid (BR) promotes plant cell division through CycD3 induction, which was suggested by recent studies on cell division activated by cytokinins. It is well established that BR and cytokinin promote cell division through CycD3 induction and might play a similar role in cell division. BR is involved in cell division via the induction of CycD3 transcription in proliferating tissues such as shoot meristem and axillary buds (Hu et al., 2000). In our study, the cytokinin receptor CRE and A-ARR gene showed downregulation in bud dormancy release in response to photoperiod. Type-A ARR genes are key components in the cytokinin signaling pathway and are involved in light signal transduction (To et al., 2004). Cytokinin helps promote bud growth, whereas auxin has an inhibitory effect (Tantikanjana et al., 2001). Our results suggested that the phytohormone-related genes such as auxin, cytokinin, and BR were down-regulated and spontaneously expressed under SD instead of LD conditions, which is necessary for cell elongation/division bud break/dormancy occurrence late in SD compared to LD. Our findings showed that Cytochrome P450 (*CYP86A1*, *CYP93A3*, *CYP92A6*, *CYP82C4*,

*CYP76B10*, *CYP71A*) genes were up-regulated during bud dormancy in long-day. Several studies signify the role and involvement of the cytochrome P450 family genes in lignin, flavonoids, BR, and GR biosynthesis pathways (Tantikanjana et al., 2001; Chapple, 1998).

## 6. Conclusions

The present study investigated the molecular events in bud dormancy release in *Liriodendron chinense* by ecophysiological (DBB and BB%) biochemical (Phytohormone contents, antioxidant enzyme activities, non-enzymatic antioxidant, MDA, and ROS contents) and transcriptomic analyses of bud in response to different photoperiods (LD and SD). Based on these results, the high content of ABA and SOD, CAT, and GR activities were mainly increased under LD in *Liriodendron* buds. In contrast, the contents of GA3, AsA, GSH, MDA, and APX activity were increased in the SD. New cis-acting regulatory elements are involved in light responsiveness, such as the G-box motif, TCCC-motif, TCT-motif etc. This study provides new information about the molecular mechanisms of flavonoid, phenylpropanoid, hormone signal transduction, cutin, suberin and wax biosynthesis, and starch and sucrose metabolism pathways related to bud dormancy underlying photoperiod. The above findings provide novel insights into regulating the hormone signal transduction, flavonoid, phenylpropanoid, and biosynthesis pathway in the bud dormancy of *Liriodendron chinense* under different photoperiods.



**Fig. 10.** Differentially expressed genes (DEGs) are involved in the plant hormone signal transduction pathway. (A) The proposed pathways show plant hormone signal transduction. This figure only shows genes associated with the plant hormone signal transduction from RNA-seq analysis. ZEP (Zeaxanthin epoxidase), NCED (9-cis-epoxycarotenoid dioxygenase), ABA2 (Xanthoxin dehydrogenase) (AAO3) Abscisic aldehyde oxidase, PYR (Pyrabactin resistance-ABA receptor), PP2C (Protein phosphatase 2C), -p (indicates de-phosphorylation process), SnRK2 (Serine/threonine-protein kinase), and ABF3 (ABA-responsive element-binding factor 3). (B) This figure only shows genes associated with the gibberellin signaling pathway from RNA-seq analysis. GID1 (Gibberellin insensitive dwarf1), GID2 (Gibberellin insensitive dwarf2), and TF (Transcription factor). LD and SD indicate long and short days. Green and red indicate lower and higher expression values, respectively.

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## CRediT authorship contribution statement

**Quaid Hussain:** Conceptualization, Methodology, Writing – original draft, preparation, All authors have read and agreed to the published version of the manuscript. **Manjia Zheng:** Methodology, Writing – original draft, preparation, All authors have read and agreed to the published version of the manuscript. **Heikki Hänninen:** Writing – review & editing, All authors have read and agreed to the published version of the manuscript. **Rishikesh P. Bhalerao:** Writing – review & editing, All authors have read and agreed to the published version of the manuscript. **Muhammad Waheed Riaz:** Formal analysis, All authors have read and agreed to the published version of the manuscript. **Muhammad Sajjad:** Formal analysis, All authors have read and agreed to the published version of the manuscript. **Rui Zhang:** Conceptualization, Supervision, Funding acquisition, All authors have read and agreed to the published version of the manuscript. **Jiasheng Wu:** Supervision, Funding acquisition, All authors have read and agreed to the published version of the manuscript.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jplph.2022.153835>.

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