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Research Article

Genome-wide identification, characterization and expression analysis of the ABA receptor PYL gene family in response to ABA, photoperiod, and chilling in vegetative buds of *Liriodendron chinense*

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ABSTRACT

Abscisic acid (ABA) is a signaling phytohormone in plants to improve stress adaption under harsh environments. ABA is sensed by the pyrabactin resistance1/PYR1-like/regulatory components of the ABA receptor (PYR1/PYL/RCAR) (named PYLs) and is the core regulator of ABA stress signaling. ABA receptor PYL gene family have been well studied in rubber tree, apple, *Theobroma cacao, Ricinus communis*, and *Vitis vinifera*. We first revealed the genome-wide comprehensive analysis to classify the PYL genes in the *L. chinense*. This study highlighted the seven *LchiPYL* genes in the *L. chinense* genome. The detailed investigations about gene structure variations, chromosomal distributions, phylogenetic tree, 3D structure, motif analysis, cis-regulatory elements, subcellular location, and expression profiles in buds and stress responses were carried out in this article. Phylogenetic tree analysis exploited that the seven *LchiPYL* genes were divided into four groups (Group I–IV) from L. *chinense,* and three were highly linked with other species. By analyzing the cis-elements in the promoters, we identified five hormones-, six stress-, three growth and biological process, and two metabolic-related responsive elements. The expression analysis showed that all seven genes were up-and down-regulated against response to ABA, photoperiod, chilling, and chilling + photoperiod treatments. Our findings opened up new future research directions and provided insight into the PYL family genes in *Liriodendron chinense*.

1. Introduction

Abscisic acid (ABA) is the central player in regulating several aspects of plant improvements during growth and development (i.e., cell elongation and division, seed germination, maturation and dormancy, participating in the root growth, and seedling growth, leaf senescence, embryo maturation, and fruit ripening) and strengthening the plants against harsh conditions by synchronizing many functions (Hussain et al., 2021a, 2020, 2021b; Cutler et al., 2010; Nambara and Mario-n-Poll, 2005; Zhu, 2016; Sah et al., 2016). ABA controls stomatal movement and aperture/opening during plant physiological regulation (Hussain et al., 2020). In addition, it helps plant adaptation under abiotic stresses like light and temperature alterations, drought, waterlogging chilling, salinity, etc., and biotic stresses as pathogen attacks (Hussain et al., 2021a; Zhao et al., 2017; Chan, 2012). Furthermore, a complex interaction among ABA and different phytohormones has also been exploited (Aleman et al., 2016; Finkelstein et al., 2008). Mainly ABA is known as a signaling molecule and triggers the signal transduction mechanisms against stressors in plants (Aleman et al., 2016; Finkelstein et al., 2008; Miyakawa et al., 2013; Xing et al., 2016; Lumba et al., 2010; Wang et al., 2018a).

ABA regulatory pathways reveal that it participates in the bud break mechanism by acting as a negative regulator, e.g., overexpressing plants harboring TCP18/BRC1 AND RCAR/PYL1 constructs delayed the bud

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break function (Singh et al., 2018). ABA biosynthesis is a vital process to maintain ABA concentration that controls the bud break to suppress or promote the bud dormancy release function in plants. For example, reducing endogenous ABA amount in birch, grapevine, and potatoes (Koussa et al., 1994; Or et al., 2000; Li et al., 2004; Destefano-Beltrán et al., 2006) results in bud dormancy release and contrast ABA treatment delayed the bud break in birch tree (Rinne et al., 1994), hybrid aspen (Singh et al., 2018), grape (Zheng et al., 2015), pear (Li et al., 2018), and grapevine (Rubio et al., 2019). Similarly, the chilling stress condition displayed the bud break dormancy release function in birch trees by altering the ABA production and confirming the involvement of ABA (Li et al., 2004). Previous studies have been highlighted that the freezing condition is non-significant in altering the ABA concentration in birch (Rinne et al., 1994). However, the chilling effect on apple and cherry buds was exploited. The outcomes indicated a decrease in ABA amount in apple buds (non-chilled and chilled); the dormancy release function was observed in chilling treated buds. Nevertheless, chilled cherry buds displayed a higher ABA level than non-chilled ones. These results showed that ABA plays a key player in bud break or dormancy release functions in plants (Saure, 1985). ABA receptors have been involved in the ABA signalling mechanism in plants. Recently, deciphering the potential targets or ABA receptors has been of great interest to explore plant adaption against environmental stressors.

Significant progress has been achieved in unraveling the ABA mechanism and mining the ABA receptors last year's (Bai et al., 2019). PYLs are the most widely used plant hormone receptors (Zhang et al., 2017a). Plants' receptors (ABA) can bind ABA and trigger the ABA signaling cascade. Through genetic and biochemical screening, the Pyrabactin-Resistance 1 (PYR1) or PYR1-Like (PYL) regulatory factors are known as ABA receptors in *A. thaliana* (Bai et al., 2019; Yadav et al., 2020). ABA contents increase under abiotic stresses, activating a signalling mechanism to trigger plant adaption machinery (PAM) (Duarte et al., 2019). ABA alterations during activation of PAM explored the novel plant regulatory networks operated by plant ABA receptors (Cutler et al., 2010; Zhu, 2016). ABA receptors (PYLs) are the breakthroughs to improve understanding of stress signalling in plants in the last years (Cutler et al., 2010; Zhu, 2016).

Several PYLs have been found and functionally described in Arabidopsis, owing to their importance as essential regulators of ABA signaling in various plants (Yang et al., 2016; Ma et al., 2009; Santiago et al., 2009; Park et al., 2015; Zhao et al., 2014), e.g., rice (Yadav et al., 2020; Kim et al., 2012), tomato (Gonzalez-Guzman et al., 2014), soybean (Bai et al., 2013), wheat (Gordon et al., 2016), maize (Fan et al., 2016; Wang et al., 2018b), poplar (Yu et al., 2016), rubber tree (Guo et al., 2017), strawberry (Chai et al., 2011), cotton (Zhang et al., 2017b), Brachypodium distachyon (Palareti et al., 2016; Zhang et al., 2017a), apple (Hou et al., 2020), *Theobroma cacao* (Hou et al., 2020), *Ricinus communis* (Hou et al., 2020), and *Vitis vinifera* (Hou et al., 2020). Highly similar signalling modules among dicotyledonous and monocotyledonous models plants (Arabidopsis and rice) demonstrated and displayed that the ABA regulatory pathways are conserved across plant species (Yadav et al., 2020). For example, AtPYR1 belongs to the subfamily of cyclase named START (star-responsive lipid transfer) domain, and all AtPYLs (AtPYL1 to 13) have high similarity with AtPYR1 in Arabidopsis. Fourteen receptors belonging to the PYL family are currently well-defined in Arabidopsis containing the START domain and recognized as AtPYR1 to AtPYR13. Several subfamilies have been identified among the 14 *AtPYLs* (Bai et al., 2019; Ma et al., 2009; Park et al., 2009). Furthermore, genome mining of *Hevea brasiliensis* (rubber tree) displayed 14 ABA receptor genes, and protein sequence analysis (PSA) revealed that these HbPYLs may be subclassified among three sub-families (Guo et al., 2017). Eight SlPYLs were identified from tomato (*Solanum Lycopersicum*) and clustered into three subfamilies based on their evolutionary connection (Sun et al., 2011). Similarly, genomic sequences of *H. brasiliensis* and *Brachypodium distachyon* PYLs can be classified into two clades: without introns and with introns (Palareti et al., 2016; Zhang et al., 2017a).

Budburst is an ecologically and economically important trait in trees (Busov et al., 2016), which shapes plant architecture's structure (Shi et al., 2021). The timing of bud-break is affected by bud endodormancy, a complex physiological phenomenon that helps plants grow, survive, and develop (Yang et al., 2021a). Until the Late Tertiary, the genus Liriodendron, which belongs to the Magnoliaceae subfamily Liriodendroideae, consisted of multiple species spread throughout the Northern Hemisphere, but currently only consists of two sister species with a characteristic intercontinental disjunction distribution: one in East Asia (L. *chinense*) and the other in eastern North America (L. *tulipifera*) (Chen et al., 2019; Long et al., 2019). Liriodendron plants are often planted as ornamental trees because of their unusual leaves, conical crown, straight trunk, tulip-shaped blooms, and distinctive leaf shapes with colourful petals (Yang et al., 2021a; Sheng et al., 2021). Furniture and farming equipment are among the many things made from liriodendron wood (Long et al., 2019; Williams and Feist, 2004). Extracts from Liriodendron leaves have been found in numerous studies to have substantial cytotoxic effects on tumor cell lines (Chen et al., 2015, 2013), as well as inhibitory activities against farnesyl protein transferase (FPTase) and tumor cell proliferation (Mi et al., 2007).

Herein, we used comparative proteomic and genomic methods and experimental verification to characterize and identify ABA PYLs receptors that could be the essential signaling regulators in *Liriodendron chinense* to cope with environmental stressors. The *Liriodendron chinense* genome contained a total of seven *LchiPYL* genes. This study also looked at the evolutionary variations, chromosomal positions, gene structures, and conserved sequence motifs among other agriculturally robust plants with PYL genes. Furthermore, the expression profiles of *LchiPYLs* within the bud have been studied under various stages of development and ABA treatment, photoperiod, and chilling conditions. We also conducted extensive research into the function of cis-regulatory components found within ABA-receptors' promoter sequences to show hormone and stress-response cis-elements, growth and biological processes, and metabolic responses. Transcript expression of *LchiPYL* genes was validated through the qRT-PCR analysis using the *Liriodendron chinense* vegetative buds treated (ABA foliar sprays, photoperiod, and chilling). Study outcomes may provide valuable knowledge to enhance understanding of ABA receptors' function in *Liriodendron chinense* and perform molecular characterization of *LchiPYL* genes after developing genetic material that can improve plant adaptation against stressors.

2. Materials and methods

2.1. Identification and characterization of LchiPYL genes in Liriodendron chinense

The genome sequences for *Liriodendron chinense* were retrieved using NCBI database (https://www.ncbi.nlm.nih.gov/ accessed on 25 January 2022, PRJNA418360), as well as the *L. chinense* protein database (https://hardwoodgenomics.org/Genome-assembly/2630420, accessed on 25 January 2022) (Chen et al., 2019). The Arabidopsis PYL protein sequences were acquired from phytozome v13 (https://phytozome-next.jgi.doe.gov/, accessed on 25 January 2022) to discover the PYL genes family in L. *chinense*. Well-defined 14 members of PYL family in A. *thaliana* are *AT1G73000/ PYR1-like 3, AT5G46790/ PYR1-like 1, AT5G53160/, AT2G38310/ PYR1-like 4, AT1G01360, AT4G27920/ PYR1-like 10, AT5G05440, AT2G40330/ PYR1-like 6, AT4G01026/ PYR1-like 7, AT5G45870 - PYR1-like 12, AT5G45860/PYR1-like 11, AT2G26040/ PYR1-like 2, AT4G18620/ PYR1-like 13, AT4G17870,* and their protein sequences were collected using TAIR database (http://www.arabidopsis.org/, accessed on 25 January 2022). The protein sequences of the PYL family members of *L. chinense* were identified through a specific genome database (https://hardwoodgenomics.org/Genome-assembly/2630420, accessed on 25 January 2022) using protein sequences of *A. thaliana* PYLs as a reference. Protein sequence

analysis (PSA) of PYL associated with domain profile (PF10604.11) was carried out by the Pfam database (http://pfam.xfam.org/ accessed on 25 January 2022).

Finally, seven PYL family genes (*Lchi16997, Lchi13641, Lchi00864, Lchi01385, Lchi11622, Lchi23679,* and *Lchi01227*) were recognized and confirm using the databases, i.e., *L. chinense* genome database (https://hardwoodgenomics.org/Genome-assembly/2630420) and NCBI database (https://www.ncbi.nlm.nih.gov/) (Chen et al., 2019; Yang et al., 2021b; Hu et al., 2021).

2.2. Chromosomal/scaffold distribution of PYL genes in Liriodendron chinense

We determined the genome position and protein sequences of all PYL genes of *L. chinense* using https://hardwoodgenomics.org/Genome-assembly/2630420), accessed on 25 January 2022 and estimated the distribution positions of PYL genes on scaffold or chromosome. PYL genes were represented on the *Liriodendron chinense* chromosomes using MapGene2Chromosome (MG2C, http://mg2c.iask.in/mg2c_v2.0/, accessed on 25 January 2022) (Chen et al., 2019; Yang et al., 2021b; Hu et al., 2021).

2.3. Phylogenetic tree construction

Protein sequences of PYL genes from *Liriodendron chinense*, apple, *Theobroma cacao, Ricinus communis, Vitis vinifera, A. thaliana*, rice, foxtail millet, barley, sorghum and Brachypodium were used to compute the phylogenetic analysis, and to display the *Liriodendron chinense* PYL gene family evolution relating to other species. MEGA11 (V 6.06) software was used to align the protein sequences numerous times (www.megasoftware.net). With 1000 bootstrap replicates, the phylogenetic tree was created using the neighbour-joining (NJ) function. The phylogenetic tree was viewed and edited using the software Fig Tree V1.4.4, accessed on 25 January 2022) (Yadav et al., 2020; Tamura et al., 2021).

2.4. PYL family members' structure and important motif analysis

There are PYL family's seven genes identified from L. *chinense* genome. Structural analysis of seven PYL genes was determined by web program (http://gsds.cbi.pku.edu.cn/ accessed on 25 January 2022) [77] and showed the exon/intron arrangements of PYL genes. More conserved strings or regions among protein sequences of the seven PYL proteins were displayed through the online tool (MEME v5.4.1, https://meme-suite.org/meme/tools/glam2scan, accessed on 25 January 2022 (Raza et al., 2021). Program setting was: Site distribution zero or one occurrence per sequence (zoops); motif finding mode classic mode; sequence alphabet DNA, RNA, or protein; and a total of ten motifs. TBtools program was used to visualize the MEME results by downloading the accompanying mast file (Su et al., 2021; Chen et al., 2020).

2.5. Promoter sequence's analysis of PYL family members in L. chinense

Two thousand five hundred (2500 bp) upstream sequences of PYL family members were collected using database (https://hardwoodgenomics.org/Genome assembly/2630420) of L. *chinense* and retrieved sequences were analysed to find CREs by PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/). On the 25 January 2022, I was able to get a hold of some information. For PYL genes, the number of occurrences of each CRE motif was counted, and the most frequently occurring CREs were utilized to generate Fig. 4 in TBtools (Chen et al., 2020).

2.6. Subcellular localization, and 3D structure

Cell-PLoc 2.0 (http://www.csbio.sjtu.edu.cn/bioinf/Cell-PLoc-2/, accessed on 25 January 2022) to predict the subcellular localization of the PYL family genes. SWISS-MODEL (https://swissmodel.expasy.org/interactive, accessed on 25 January 2022) to estimate the three-dimensional (3D) structure (Li et al., 2021; Hu et al., 2021).

2.7. Plant material and stress conditions

The experiments were conducted in the Zhejiang Agricultural and Forestry University (Hangzhou, China) to understand the role of different concentrations of abscisic acid (ABA-10uM, and ABA-100uM) photoperiods (P-LD and P-SD), chilling, chilling + photoperiod (C+P-LD, C+P-SD) during bud endodormancy. We selected two years old, *Liriodendron chinense* seedlings from the Tianmushan National Forest Station nursery (Hangzhou, China) and transferred them to our university growth chamber for experimental purposes. After transferring the seedlings from the nursery to the growth chamber, for photoperiod and chilling, the seedlings were kept at 20 °C (day/night) with a light intensity of 200 μmol m^{-2} s^{-1} a relative humidity of 50% (Fig. 1) (Zhang et al., 2021).

2.8. RNA extraction and qRT-PCR analysis

We used the Total RNAPlant Extraction Kit (Tiangen, Beijing, China) and followed the manufacturer's procedure. The cDNA was prepared thru the TaKaRa PrimeScript 1st Strand cDNA Synthesis Kit (TaKaRa, Dalian, China) and followed the manufacturer's procedure. The qRT-PCR was carried out using the SYBR Green Real-time PCR Master Mix (TOYOBO, Osaka, Japan) through an ABI7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) (Hussain et al., 2021b).

The qRT-PCR analysis was used to validate the transcript profile of *Lchi01385, Lchi01227, Lchi16997, Lchi13641, Lchi00864, Lchi11622*, and *Lchi23679* in the bud treated by several stress conditions (Fig. 1). The qRT-PCR was carried out using the three technical repeats. The actin gene *LtActin97* of *Liriodendron* was used as a reference gene (forward primer: TTCCCGTTCAGCAGTGGTCG, reverse primer: TGGTCGCACAACTGGTATCG), and the sequence provided by Zong et al. (2021) and Liu et al. (2021), for calculating the expression levels of target genes were used the $2^{-\Delta\Delta CT}$ method. In this study all used primers were targeted using CDS sequences of PYL thru Primer Premier 5.0 software and details are provided in supplementary Table S1. Final results of the qRT-PCR analysis were presented through GraphPad Prism 9.0.0 software.

3. Results

3.1. Identification of PYL gene family in Liriodendron chinense

We explored seven PYL genes in the *Liriodendron chinense* genome (Tables 1 and S2) using queries of the well-defined protein sequences of 14 PYLs from *A. thaliana* genome. Compared to the previously described PYL genes in other species, e.g., the *A. thaliana*, rice, foxtail millet, barley, sorghum and Brachypodium, we found relatively lower number of genes (Table S3). Two proteins (Lchi16997 and Lchi11622) were determined to have only one Polyketide cyc2 domain, according to domain analysis (PF10604.11), two proteins (*Lchi13641* and *Lchi23679*) were found to have two Polyketide_cyc2 and Bet_v_1 domain (PF10604.11 and PF00407.21), and two proteins (*Lchi00864* and *Lchi01385*) were found to have two Polyketide_cyc2 domain (PF03364.22), and one protein (*Lchi01227*) were found to have one Bet_v_1 domain (PF00407.21), respectively (Table S4).

Table 1 contains comprehensive statistics for seven PYL genes. Out of seven *PYLs* only two genes (*Lchi01385* and *Lchi01227*) were located on the Scaffold432, and the remaining genes (*Lchi16997, Lchi13641, Lchi00864, Lchi11622*, and *Lchi23679*) were located on the Scaffold3097, Scaffold1315, Scaffold723, Scaffold37, and Scaffold78 (Fig. 2; Table 1), respectively. The genomic sequence length, coding sequence length, protein length, gene strand, and the number of exons were all

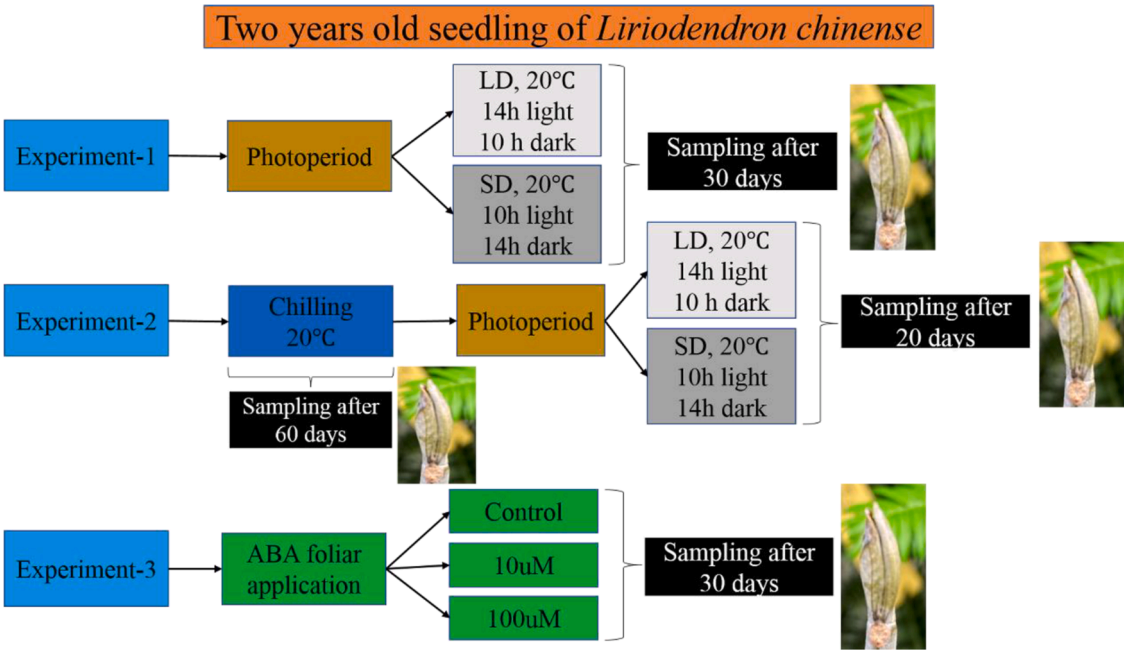

Fig. 1. The experiment's setup is shown in this schematic flow.

Table 1
The seven PYL genes found in *Liriodendron chinense* are described.

Gene ID	Chromosome	GS	GSL	CDSL	PL	Subcellular localization	Number of Exon					
							E1		E2		E3	
							S	E	S	E	S	E
Lchi16997	Scaffold3097	+	3001	594	197	Nucleus	1	158	773	988	2781	3001
Lchi13641	Scaffold1315	+	5036	687	228	Cytoplasm, Mitochondrion	1	93	4443	5036		
Lchi00864	Scaffold723	-	648	648	215	Cytoplasm	1	648				
Lchi01385	Scaffold432	-	1178	792	263	Cytoplasm	1	710	1097	1178		
Lchi11622	Scaffold37	-	3597	636	210	Cytoplasm, Vacuole	1	564	3526	3597		
Lchi23679	Scaffold78	-	1348	453	150	Vacuole	1	270	1165	1348		
Lchi01227	Scaffold432	-	3143	483	160	Cytoplasm	1	297	2958	3143		

Note: GS-Gene Strand, GSL-Genomic sequence length, CDSL-Coding sequence length, PL-Protein length, S-start, and E-End.

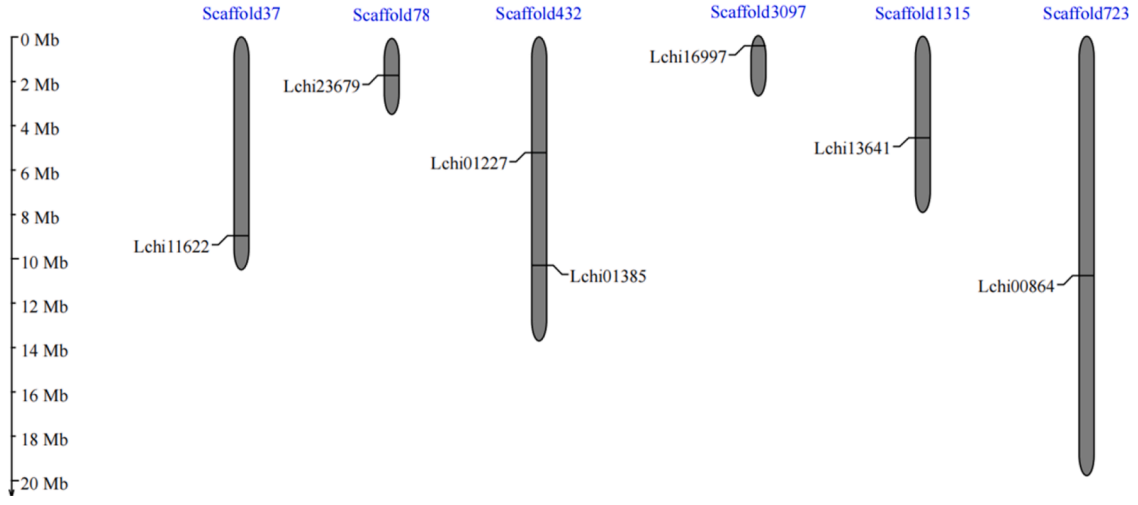

Fig. 2. PYL gene distribution on *Liriodendron chinense* chromosomes as a scaffold. The chromosomal/scaffold numbers are located at the top of each chromosome. On the left and right sides of each chromosome, the names of each *LcPYL* gene are displayed. The PYL genes are shown by the bars on the chromosomes/scaffold.

mentioned in detail, respectively (Table 1). The results of subcellular localization predicted that three proteins would be found in the cytoplasm, one protein was located on the nucleus, one protein was located on the vacuole, one protein was located on the cytoplasm and mitochondrion, and the remaining one protein was located on the cytoplasm and vacuole (Table 1).

3.2. Phylogenetic relationships of PYL genes

A multiple-sequence-alignment (MSA) analysis of the predicted PYL proteins of *Liriodendron chinense* (Fig. S1), apple, *Theobroma cacao, Ricinus communis*, and *Vitis vinifera*, Brachypodium, maize, sorghum, foxtail millet, barley, rice, wheat, and *A. thaliana* (Table S3) was demonstrated to show a phylogenetic tree that indicated four key groups (Group I–IV) (Fig. 3). Group I consisted of 53 PYL members, according to the findings (2 *LchiPYLs*, 12 TaPYLs, 4 HvPYLs, 5 ZmPYLs, 3 SbPYLs, 4 SiPYLs, 3 OsPYLs, 4 BdPYLs, 3 TcPYRL, 2 RcPYRL, 5 MdPYL, and 6 ATGPYLs); Group II was included 43 *PYL* members (2 *LchiPYLs*, 8 TaPYLs, 3 HvPYLs, 3 ZmPYLs, 3 SbPYLs, 2 SiPYLs, 3 OsPYLs, 3 BdPYLs, 3 TcPYRL, 2 RcPYRL, 3 VvPYRL, 4 MdPYL, and 4 ATGPYLs); Group III contained 43 *PYL* members (1 *LchiPYLs*, 6 TaPYLs, 4 HvPYLs, 5 ZmPYLs, 2 SbPYLs, 2 SiPYLs, 7 OsPYLs, 2 BdPYLs, 3 TcPYRL, 3 RcPYRL, 2 VvPYRL, 3 MdPYL,and 4 *ATGPYLs*); and Group IV was comprised of three PYL members (2 *LchiPYLs* and 1 HvPYLs) (Fig. 3). PYLs that cluster into the same sub-group may have comparable roles in general. It's worth noting that homologs of *LchiPYLs* genes from other plant species were found in each group, with Group I, II, and IV having more members than Group III (Fig. 3). In addition, the *LchiPYLs* have a stronger evolutionary relationship with the other species in each group.

3.3. PYL genes structures and conserved motifs investigation in Liriodendron chinense

The PYL genes' gene structures (exon-intron arrangements) (Tables S5 and S6) were studied to learn more about the *Liriodendron chinense* family's gene expansion. The exon-intron structures and conserved motifs (Fig. 4A–C) of PYL genes were studied to understand their structural properties better. The variations among introns and exons of the PYLs were 1–2 and 1–3 (Fig. 3B), respectively. Gene structure study found that the PYL gene family has a wide range of gene structures, with the majority of PYL genes having one–two introns; nevertheless, some PYL gene family members are intron less *Lchi00864*. In *Lchi16997*, there were a maximum of two introns detected. These findings demonstrated that the gene structures of PYL members within a group were extremely similar to those of their evolutionary relatives.

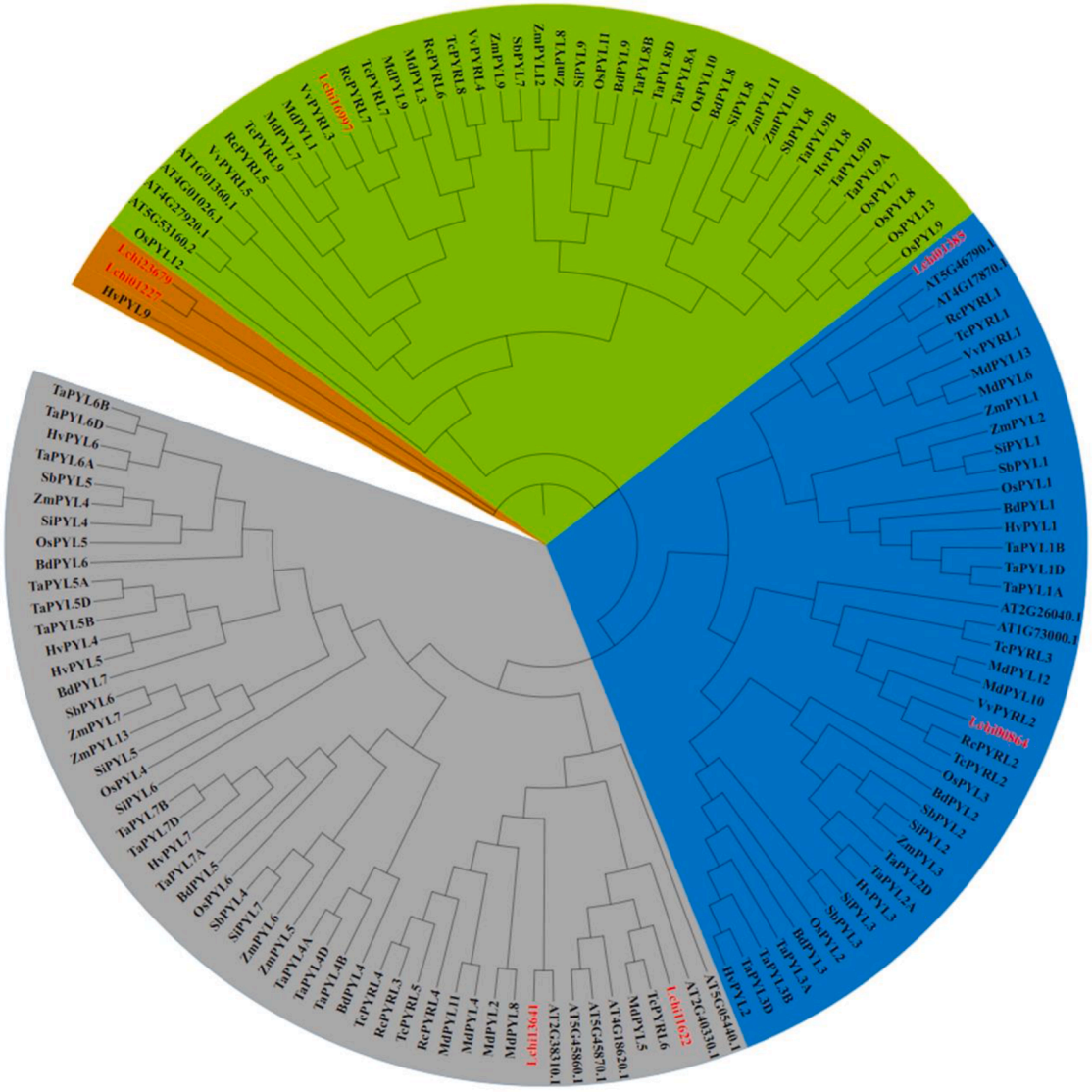

Fig. 3. The maximum likelihood technique was utilized to perform phylogenetic analysis of PYL proteins from *Liriodendron chinense* (7 Lchi), apple (12 MdPYL), *Theobroma cacao* (9 TcPYRL), *Ricinus communis* (7 RcPYRL), and *Vitis vinifera* (4 VvPYRL), Wheat (26 TaPYL), Arabidopsis (14 ATGPYLs), Maize (13 ZmPYL), Rice (13 OsPYL), Brachpodium (9 BdPYL), Barley (12 HvPYL), Foxtail millet (8 SiPYL), and Sorghum (8 SbPYL) PYL proteins are divided into four groups: I, II, III, and IV, which are represented by the colours black, blue, green, and orange, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

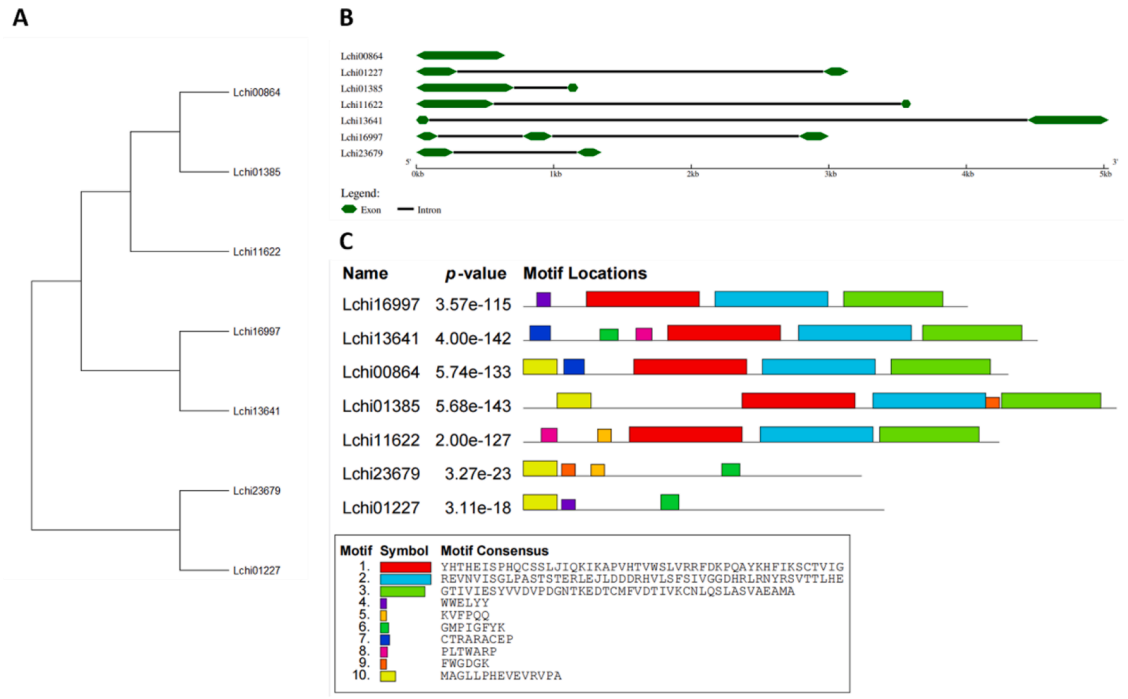

Fig. 4. The PYL family gene structure and motif analysis in *Liriodendron chinense*. (a) The PYLs were divided into four categories based on phylogenetic relationships and domain identification. PYL gene structure (b). Exon regions are shown by the green horizontal line, whereas the black horizontal line represents introns. (c) In *Liriodendron chinense* PYLs, conserved motif compositions were discovered. Different color boxes represent various motifs. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

The three-dimensional (3D) structure was estimated using SWISS-MODEL, and the predicted 3D structures revealed similar conserved *LchiPYL* protein structures. Fig. 5 shows the detailed information on the projected 3D structures.

Further, we also elucidated the conserved motifs of PYL genes using the MEME online servers. Finally, 10 conserved motifs were identified in seven PYL genes (Figs. 4C and S2). The motifs 1, 2, 3, and 10 were found in most PYL proteins due to the prediction. Motifs 1, 2, and 3 only recognized in *Lchi16997, Lchi13641, Lchi00864, Lchi01385*, and *Lchi11622* and motif 10 has existed in *Lchi00864, Lchi01385, Lchi23679*, and *Lchi01227*. Motifs 6 was identified to *Lchi13641, Lchi23679*, and *Lchi01227,* respectively. Interestingly, motif four was identified in *Lchi16997, Lchi01227,* and motif five was identified on *Lchi11622* and *Lchi23679*. Furthermore, motif seven was recognized in *Lchi13641* and *Lchi008648*, while motif nine was identified in *Lchi01385, Lchi23679.* PYL proteins exhibited four highly conserved motifs with 50, 50, 44, and

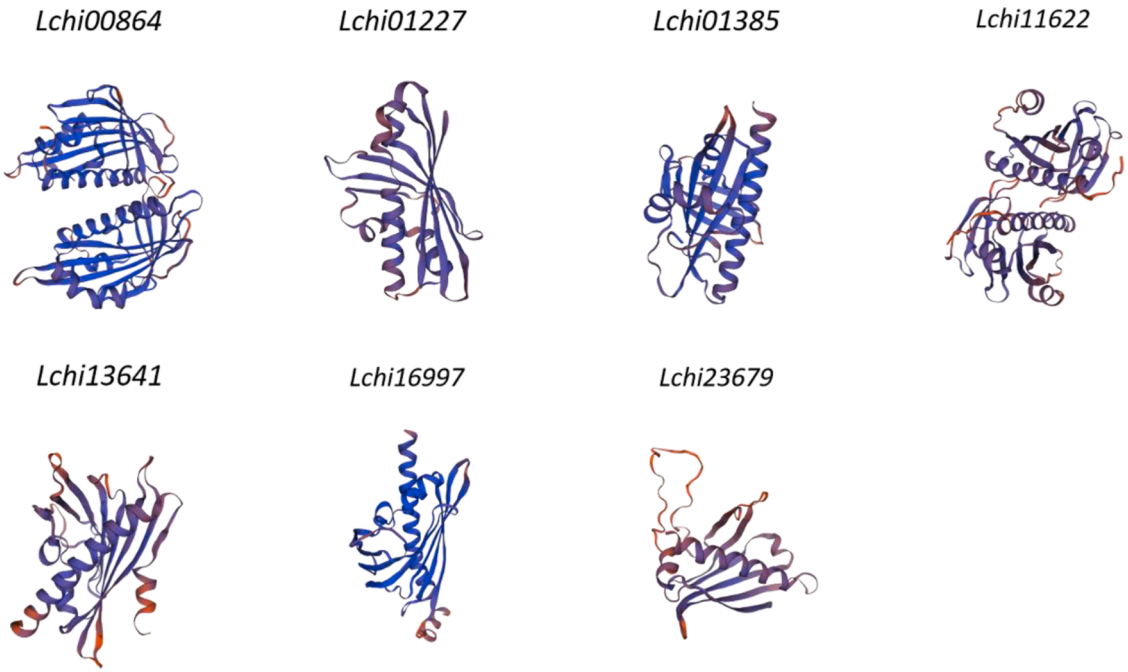

Fig. 5. 3D structures of PYL family showing functional sites.

15 amino acids: motif 1, motif 2, motif 3, and motif 10 (Fig. S2).

3.4. Determination of cis-regulatory elements in the promoters of seven PYL genes

Screening a 2500 bp portion from each gene's TAS (transcriptional-activation-site) against the PlantCARE database to determine gene functions and regulatory patterns. We determined the potential cis-elements among the slected region of promoters of the PYL genes in *Liriodendron chinense* (Table S7). Several cis-regulatory elements in PYLs were shown in (Fig. 6A,B). Table S8 contains detailed information on the discovered items. The PYLs gene family has 242 unique CREs, including hormone-responsive (71), stress-responsive (134), growth and biological process responsive (12), and metabolic responsive (25) elements (Fig. 6B). Overall, five hormone-related responsive elements were discovered, including ABA, auxin, GA, MeJA, and SA, signifying and suggesting as the desirable targets to explore the role of hormones under stress condition's (Fig. 6; Table S8). As demonstrated in Fig. 5, most hormone-related responsive regions are unique to a few genes. The auxin (7%), ABA (34%), SA (7%), MeJA (39%), and GA (13%) responsive-elements are extensively dispersed and frequently found in seven genes, demonstrating their vital roles in phytohormone-related responses in plants (Fig. 6A–D). Six stress-related response elements were also predicted (anoxic specific inducibility, circadian regulation, defense and stress, drought, light, and low-temperature), demonstrating that these PLYs genes may respond to stress-related stimuli (Fig. 7A–D; Table S8). Furthermore, 80% of the light-responsive components were found, implying that PYLs play a crucial role under light stress response. Aside from that, there's one defensive and stress-response element (5%), anoxic specific inducibility (2%), circadian control (3%), drought (6%), and low temperature (4%) were identified in all PYL genes (Fig. 7; Table S5). CREs associated with growth and biological process responsive, including meristem expression (42%), cell cycle regulation (25%), and seed-specific regulation (33%) elements, were identified in most of the PYL genes promoters. Furthermore, two metabolic responsive (anaerobic induction (88%) and zein metabolism regulation (12%) elements were identified (Fig. 7A–D; Table S8).

3.5. Real-time qRT-PCR expression profiling of PYL genes in responses to bud endodormancy release in Liriodendron chinense

To investigate the *LchiPYL* genes' expression profiles under different concentrations of abscisic acid foliar application (ABA-10uM, and ABA-100uM), photoperiods (P-LD and P-SD), chilling, chilling + photoperiod (C+P-LD, C+P-SD) conditions, the qRT-PCR-based expression profiling of seven *LchiPYL* genes was performed (Fig. 8).

According to the RT-qPCR results, the *Lchi00864* transcript expression was moderately more at the P-LD and C+P-SD conditions and followed by P-SD and ABA-10 relatively low at the other conditions. In response to all of these conditions, *Lchi01227* was up-regulated under P-LD, P-SD, C+P-LD, and C+P-SD relatively low expression levels at the other conditions. Similarly, the *Lchi01385* gene expression was increased at the P-SD, chilling, and C+P-SD conditions compared to other conditions. The expression level of *Lchi01385* was significantly upregulated in P-SD, ABA-10, C+P-SD compared to different stress conditions. Similarly, the *Lchi13641* transcript expression was increased at the (ABA-10, CK, P-LD, and C+P-LD), *Lchi23679* (chilling, and P-SD), and *Lchi16997* was up-regulated in P-SD, chilling, P-LD, and ABA-100 (Fig. 8).

4. Discussion

ABA is a vital phytohormone that supports plants' growth, development, and response against stressors (Hussain et al., 2021a; Cutler et al., 2010; Nambara and Marion-Poll, 2005). Budburst is an ecologically and economically valuable trait in trees (Busov et al., 2016), altering the structure of plant architecture (Shi et al., 2021). Bud endodormancy, a complicated physiological phenomenon that aids

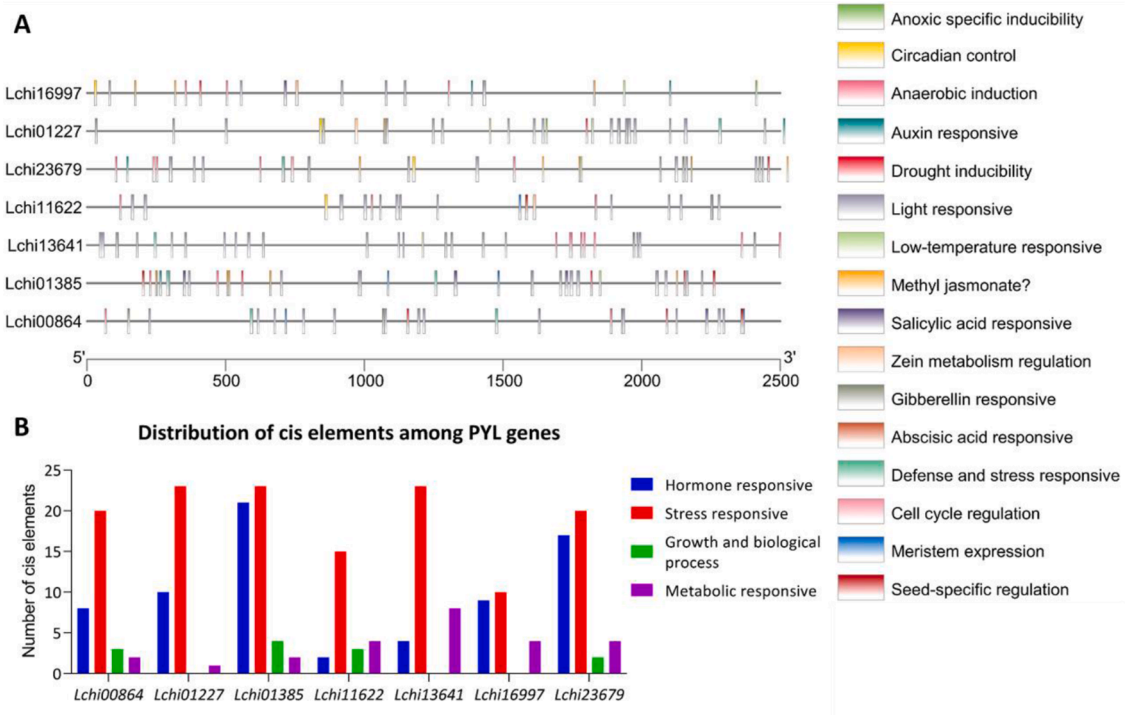

Fig. 6. In the promoters of PYL genes, there are cis-regulatory Elements (CREs). Vertical bars depict the positional distribution of projected CREs on PYL promoters. PlantCARE was used to examine the promoter sequences (2500 bp) of seven PYL genes. The color of individual cis-elements is depicted in this legend. (B) The distribution of cis-elements in the promoters of PYL genes is associated with hormones, stress, growth and biological process, and sensitive metabolic elements. Colour-coded boxes show cis-elements that have been identified.

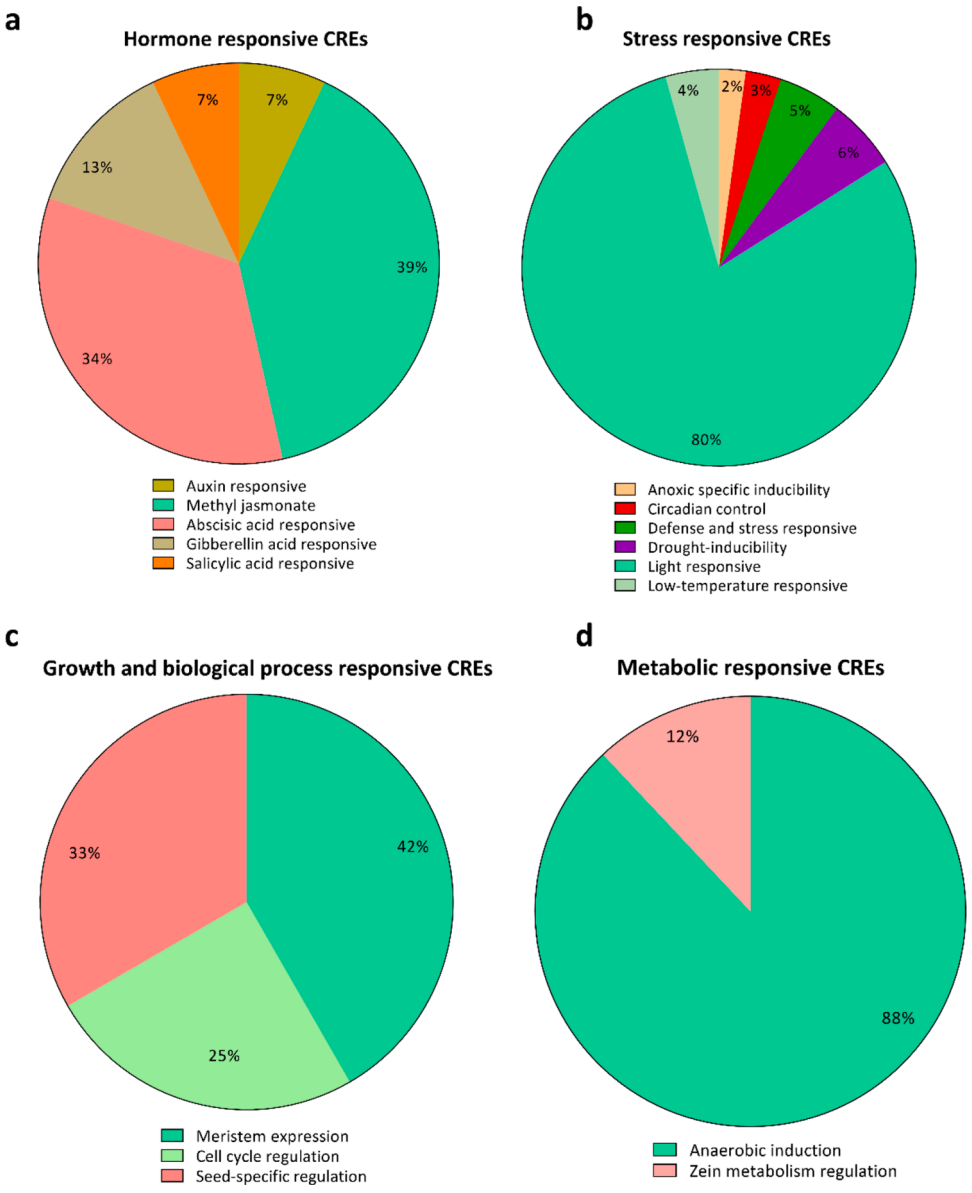

Fig. 7. Based on the proposed functions, percentage-wise distribution of cis-regulatory elements (CREs) in the promoters of PYL genes. (a) Hormone-responsive CREs, (b) stress-responsive CREs, (c) growth and biological process-responsive CREs, and (d) metabolic-responsive CREs are the four types of CREs.

plant growth, survival, and development, influences the timing of bud rupture (Yang et al., 2021a). Reports revealed that RCAR/PYL1 and TCP18/BRC1 overexpressing plants delayed bud break in hybrid aspen compared to wild-type control plant life. These results highlight that both genes (*RCAR/PYL1* and *TCP18/BRC1*) act as repressive regulators during the bud break mechanism (Singh et al., 2018). Similarly, silenced SVLRNAi and RCAR/PYL overexpressing plants displayed the increased activity of oxidase (GA2) to lead suppression of early and delayed bud break function, respectively (Singh et al., 2018). AtPYLs are recognized as ABA receptors in *A. thaliana*; further, biochemical and genetic studies (Yang et al., 2016; Ma et al., 2009; Santiago et al., 2009; Park et al., 2015; Zhao et al., 2014) suggested that ABA receptors (PYLs) are the key regulators to establish the ABA signaling mechanism activation (Cutler et al., 2010; Zhu, 2016; Yang et al., 2016; Ma et al., 2009; Santiago et al., 2009; Park et al., 2015; Zhao et al., 2014). ABA-responsive PYLs participate in plant response to ABA stimuli reception under various environmental stressors. PYLs have been characterized in many species, which include Arabidopsis (Yang et al., 2016; Ma et al., 2009; Santiago et al., 2009; Park et al., 2015; Zhao et al., 2014), rice (Yadav et al., 2020; Kim et al., 2012), tomato (Gonzalez-Guzman et al., 2014), soybean (Bai et al., 2013), wheat (Gordon et al., 2016), maize (Fan et al., 2016; Wang et al., 2018b), poplar (Yu et al., 2016), rubber tree (Guo et al., 2017), strawberry (Chai et al., 2011), cotton (Zhang et al., 2017a), and *Brachypodium distachyon* (Palareti et al., 2016; Zhang et al., 2017a), *Setaria viridis* (Duarte et al., 2019), *Nicotiana tabacum* (Bai et al., 2019). Still, *Liriodendron chinense* PYL family's members are not identified and characterized under various environmental conditions. In *L. chinense*, we investigated the PYL own family's genome-wide identity and expression.

In the genome of L. *chinense*, there are seven PYL genes. The PYL family was studied for gene identities, gene structure, chromosomal location, phylogenetic relationships, conserved motifs, cis-regulatory elements, and expression profiles (Chen et al., 2019; Yang et al., 2021b; Hu et al., 2021). We explored that the proteins encoding PYLs in L. *chinense* possess the polyketide cyclase 2 (PF10604) domain and belong to Bet v 1-like (PF00407.21) superfamily, and this domain has a hydrophobic cavity, which is highly preferable binding site for the ABA phytohormone. Our results discovered that *L. chinense* has the seven *LchiPYL* genes; however, we compared the number of the gene with

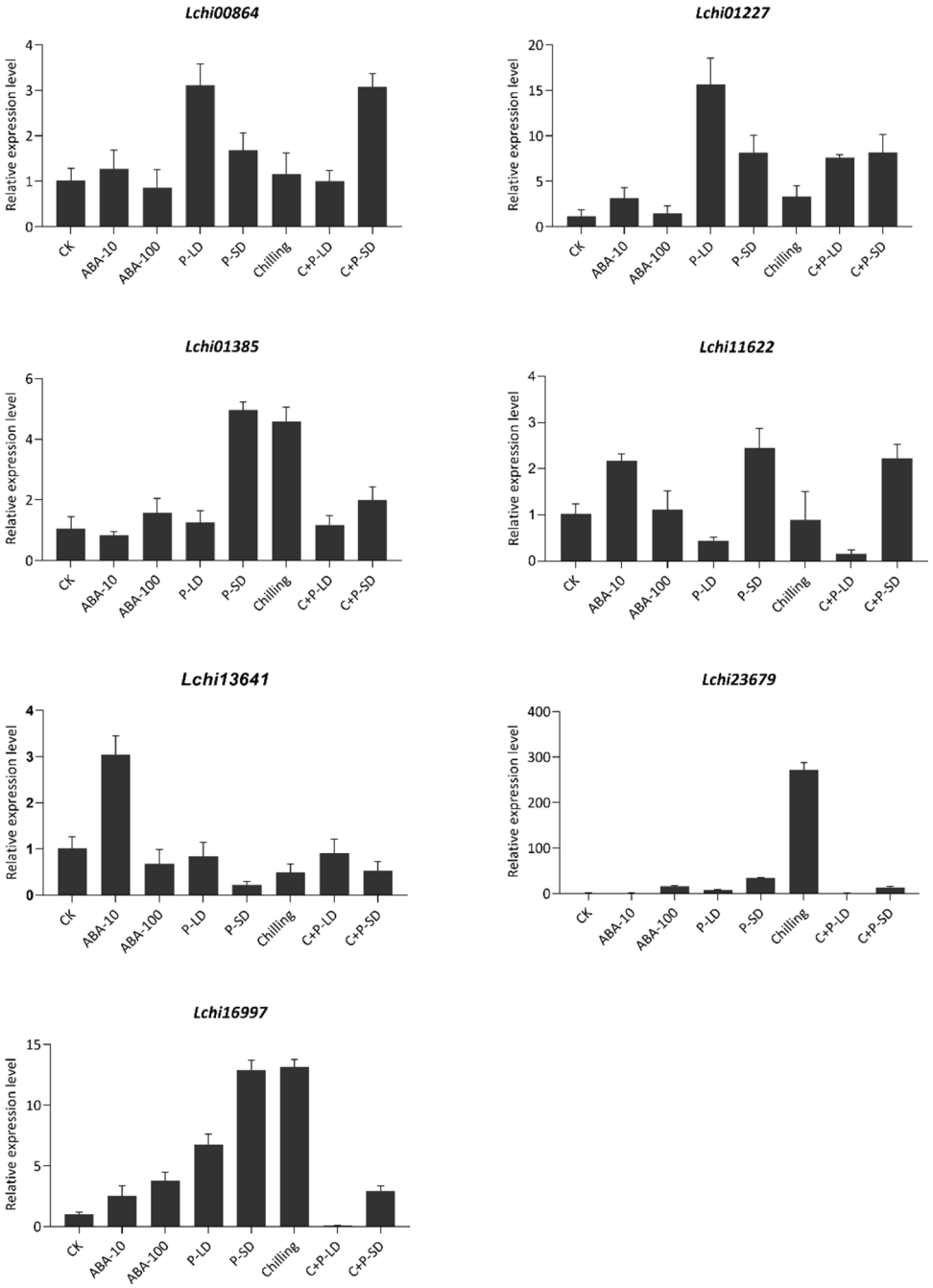

Fig. 8. The qRT-PCR expression analysis of *LchiPYLs* under different concentrations of abscisic acid (ABA-10uM, and ABA-100uM) photoperiods (P-LD and P-SD), chilling, chilling + photoperiod (C+P-LD, C+P-SD) during bud endodormancy at seedling stage in *Liriodendron chinense*.

S. viridis, sorghum and maize, which incorporates eight, eight, and 11 PYL genes, and to the more distant *A. thaliana*, and rice which has 14 and 13 PYL receptors gene. As compared with the number of PYLs in the reported plants including an Arabidopsis (14), rice (13), maize (11), a tomato (15), a *Brachypodium distachyon* (12), a soybean (23), a poplar (14), rubber tree (14), and Gossypium (27), apple (13), *Theobroma cacao* (9), *Ricinus communis* (7), and *Vitis viniferab* (5) (Yang et al., 2016; Ma et al., 2009; Santiago et al., 2009; Park et al., 2015; Zhao et al., 2014; Yadav et al., 2020; Kim et al., 2012; Gonzalez-Guzman et al., 2014; Bai et al., 2013; Fan et al., 2016; Wang et al., 2018b; Yu et al., 2016; Guo et al., 2017; Zhang et al., 2017a, 2017b; Palareti et al., 2016).

We analyzed the seven finalized members, the phylogenetic examination of PYL proteins from *Liriodendron chinense, Brachypodium*, wheat, sorghum, foxtail millet, barley, maize, and *A. thaliana* confirmed that these PYLs proteins could be extensively categorized among four distinct subfamilies. Group I is the largest group of PYL genes (39), accompanied by Group IV and Group II (33 and 32 PYL genes), and Group III has the least individuals (four PYL genes). The same or associated outcomes have been observed in A. *thaliana* and rice (Zhang et al., 2017a; Yadav et al., 2020; Kim et al., 2012). Additionally, structural analysis of genes determined that the maximum of the *LchiPYL* genes owned, the 1 to 3 and 1 to 2 exons and introns, respectively, from group (Fig. 4). Comparable findings were also suggested in rice, cotton, and *Setaria viridis*, where all PYL genes had accompanied the number of introns exons (Yadav et al., 2020; Kim et al., 2012; Zhang et al., 2017a; Hou et al., 2020)

Promoter sequences consist of the cis-regulatory motifs/elements prophesied to understand better the *LchiPYL* genes' role against several

environmental stressors. Our results suggested that four kinds of cis-elements existed, i.e., hormone-responsive, stress-responsive, growth and biological process responsive, and metabolic responsive (Figs. 6 and 7; Table S8). Extreme of the known cis-elements were light, methyl jasmonate, ABA, anaerobic induction, gibberellin, drought, defenses and stress, low temperature, auxin, salicylic acid, meristem, and so on. In keeping with preceding reviews, cis-elements subsidize plant stress responses (Zhang et al., 2017b). Moreover, other researchers (Yadav et al., 2020; Kim et al., 2012; Zhang et al., 2017a; Hou et al., 2020) reported similar findings in specific agricultural plants in which PYL genes were discovered to play a substantial role under diverse stress circumstances. These findings could help us better understand *LchiPYL* genes in various situations and conditions.

The PYL genes' expression profiles have been tested using different tissues in many plant species stressed by abiotic stresses, e.g., the *GhPYLs* were discovered to be expressed in tissues of cotton like roots, stems, leaves, flowers, and fibers (Zhang et al., 2017a). Only *OsPYL6* transcript was increased in 14 DOS (day-old-seedlings) of japonica cv. Nipponbare, while 200Mm of ABA downregulated the expression of *OsPYL2, OsPYL3, OsPYL4*, and *OsPYL10* under previous studies (Tian et al., 2015). Mostly *OsPYLs* displayed the expression among all tissues in rice, although *OsPYL3* and *OsPYL5* were primarily articulated in leaves, and the *OsPYL1* was mainly expressed in roots tissues (Tian et al., 2015). our results demonstrated that 10μM ABA strongly increased *Lchi13641, Lchi13641, Lchi00864*, and *Lchi01227* had a virtually identical expression pattern *Lchi16997* in the *Liriodendron chinense* vegetative bud. At 100 M ABA in buds, ABA also downregulated *Lchi00864, Lchi01227*, and all other genes, as reported by Tian et al. (2015). The PYL*11* was elevated in maize leaves, while PYL*6* and PYL*10* were upregulated in maize roots (Fan et al., 2016). In the rubber tree, five PYLs were discovered to be expressed in all tissues investigated, with four genes being expressed favourably in leaves, four and one in roots and flowers, respectively (Guo et al., 2017). These findings corroborated our *Liriodendron chinense*, demonstrating that different PYLs in plants have varied biological activities.

5. Conclusion

This study demonstrates the genome-wide identification and characterization of PYLs genes in *L. chinense* to help researchers better understand how ABA receptors work in *Liriodendron chinense* and perform molecular characterization of *LchiPYL* genes after developing genetic material that can help plants adapt to stresses. Finally, the *Liriodendron chinense* genome included seven *LchiPYL* genes divided into four groups (Group I–IV). PYLs (ABA receptors) play a critical ABA signaling role in plants' response to various stressors. We comprehensively examined the structural variations among genes, phylogenetic analysis, highly conserved sequences (motifs), cis-regulatory elements, transcript expression profile in various untreated tissues, and abiotically stressed buds using several hormones to acquire further information. The *LchiPYL* genes were expressed differently in buds, indicating that they may have specific budburst/endodormancy release roles. These findings will be used to investigate the vital *LchiPYL* genes in *L. chinense*'s changing processes and stress responses utilizing various functional confirmation methods, such as deletion via the CRISPR/Cas9 system, overexpression, and so on.

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CRediT authorship contribution statement

Quaid Hussain: Conceptualization, Methodology, Software, Formal analysis, Writing – original draft, Writing – review & editing. **Manjia Zheng:** Methodology, Formal analysis, Writing – review & editing. **Muhammad Furqan Ashraf:** Software, Writing – review & editing. **Rayyan Khan:** Writing – review & editing. **Muhammad Yasir:** Software. **Saqib Farooq:** Writing – review & editing. **Rui Zhang:** Writing – review & editing, Supervision, Funding acquisition. **Jiasheng Wu:** Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare no conflict of interest.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2022.111200.

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