Identification of the lateral organ boundary domain gene family

and its preservation by exogenous salicylic acid in Cerasus humilis

Abstract：

{#StartQA#812}Introduction: Lateral organ boundary domain (LBD) genes encode transcription factors (TFs) unique to plants and regulate various biological functions, including plant growth and development.

Methods: Cerasus humilis LBD (ChLBD) genes in the Cerasus humilis genome were analyzed, and their structure, motif compositions, cis-acting elements, chromosomal distribution, collinearity, physical and chemical properties of encoded proteins, amino acid sequences, and phylogeny were identified.

Results: The Cerasus humilis genome contained 41 ChLBD genes. These can be categorized into two distinct subfamilies, Class I and Class II. LBD genes belonging to the same class have relatively conserved gene structures and sequences, encoding similar amino acids. Analyzing predictions based on cis-acting elements and specific genes indicates that ChLBD could control numerous genes associated with growth, metabolism, hormonal functions, and stress reactions. Analysis of transcriptome data revealed a majority of ChLBD genes to be expressed in specific tissues. The expression patterns of six ChLBD genes were examined using quantitative real-time polymerase chain reaction (qRT-PCR) during fruit storage following treatment with low-concentration salicylic acid (SA). The findings indicated varied gene expression patterns.

Discussion: The findings of this study lay the groundwork for future research into the biological role of LBD genes in Cerasus humilis.

Keywords: Cerasus humilis; gene family; LBD transcription factor; fruit storage; gene expression

1 Introduction

The lateral organ boundary domain (LBD) gene family, a unique set of transcription factors (TFs), plays a vital role in regulating plant growth and development in mature plants. The initial discovery of LBD genes in Arabidopsis thaliana seedlings was achieved by analyzing gene trap expression patterns at their stem ends. Recent studies suggest that LBD proteins are involved in multiple aspects of plant tissue or organ growth and development, including the formation of plant collaterals, leaves, roots, inflorescences, embryo sacs, and flowers, which are potentially under the regulation of LBD proteins [1-3]. Concurrently, LBD proteins influence various physiological and biochemical functions, including nitrogen metabolism, penicillin synthesis, and collateral organ development [4-7]. In Arabidopsis thaliana, cytokinin-regulated AtLBD3/AtASL9 is involved in plant developmental regulation [8]. In Oryza sativa L., OsLBD37 and OsLBD38 are highly expressed in headings and can increase yield [9]. Additionally, the persistently high expression of STLBD2-6 in Solanum tuberosum stems during drought conditions implies a potential role in stem defense mechanisms upon exposure to such conditions [10].{#BreakQA#376}

LBD TFs are distinguished by three unique conserved areas stretching from the N-terminus to the C-terminus: the zinc-finger C-block (CX2CX6CX3C), Gly-Ala-Ser-block (GASblock), and the leucine-like zipping-block module (LX6LX3LX6L) [11]. Within these, the C-block houses four crucial cysteine motifs indispensable for DNA attachment. Situated at the heart of the LOB domain, the preserved proline residues in GASblock are crucial for the biological activity of LBD proteins in Arabidopsis thaliana [6]. Structural characteristics of LBD gene family members classify them into two separate subfamilies: Class I and Class II [2, 11]. Proteins in Class I are distinguished by their zinc finger pattern, gas cluster GASblock, and leucine ziplike helix design, which fall into five classifications (IA, IB, IC, ID, and IE). In contrast, Class II LBD proteins, which do not possess a full leucine ziplike domain, are classified into two distinct types: IIA and IIB [12, 13]. Research on plants such as Arabidopsis thaliana and Oryza sativa L. indicates Class I LBD genes are mainly involved in plant growth, encompassing the formation of lateral roots, leaves, and flowers [1, 11]. Conversely, Class II LBD genes might participate in metabolic activities such as anthocyanin production and the excess nitrogen response [1, 5].

The Cerasus humilis plant is extensively utilized in North China, which is attributable to its significant ecological and economic importance. Cerasus humilis, akin to other Rosaceae species, is abundant in calcium and has earned the nickname "calcium fruit" because of its elevated calcium levels. The distinct composition of Cerasus humilis contributes significantly to its nutritional content, rendering it ideal for those needing a nutritious diet. However, the transportation and storage of Cerasus humilis can be affected by abiotic stresses such as temperature. Therefore, it is particularly important to adopt appropriate means to ensure the quality of fruit. As an endogenous plant hormone, salicylic acid (SA) is an important signal molecule widely present in plants and is related to defense responses. It participates in the regulation of many physiological processes in plants [14]. In recent years, the role of SA in the storage and preservation of fruits and vegetables has been extensively evaluated. Exogenous SA can hinder the biosynthesis of ethylene in fruits, delaying their ripening and aging process, and as a signal molecule, SA can improve plant tolerance to abiotic stress [15]. Therefore, we treated Cerasus humilis with 1 mmol L-1 SA and observed the deterioration of fruit at different treatment times, which provides new insights into the storage and transportation of Cerasus humilis. In addition, there is growing evidence that the LBD gene family also plays a role in abiotic stress responses [16], and phytohormones also affect the expression of LBD genes through various mechanisms [17]. However, there are no reports on the relationship between LBD genes in Cerasus humilis and SA treatment. Therefore, we identified the Cerasus humilis LBD (ChLBD) gene family and further explored the expression changes in calcium fruit LBD genes in response to SA treatment at low temperatures.

Swift advancement in genome sequencing techniques has led to an extensive and methodical examination of the LBD gene family across the entire genome in key agroforestry models and plants such as Arabidopsis thaliana [18], Oryza sativa L. [13], Vitis vinifera L. [19], Passiflora edulis[16], Ginkgo biloba[20], and Rosa rugosaThunb [21]. Unveiling the Cerasus humilis genome lays the groundwork for the identification of its LBD gene family at the genome-wide level. Therefore, in this study, the phylogenetic relationships, evolutionary patterns, chromosomal localization, collinearity relationships, and expression patterns of 41 LBD gene family members in Cerasus humilis were analyzed using bioinformatics methods, and 16 forecasted cis-regulatory components were linked to hormones, plant growth, development, and stress reactions. This lays a theoretical groundwork for upcoming research into the functional aspects of the LBD gene family in Cerasus humilis.

2 Materials and Methods

2.1 Data acquisition and preparation of plant material

Cerasus humilis genome sequencing and gene annotation files from the national genome database (https:// NGDC. CNCB. Ac. Cn/databases) to download [22]. Arabidopsis thaliana LBD protein sequences were downloaded from the TAIR database (https://www.arabidopsis.org/). The s were downloaded from the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov).

2.2 Genome-wide identification of the LBD gene family members in Cerasus humilis

First, possible sequences were obtained by BLASTP scanning all Cerasus humilis sequences using TBtools using Arabidopsis thaliana LBD (AtLBD) protein sequences as queries [23]. Additionally, the Pfam database (http://pfam.xfam.org/) provided a hidden Markov model for the standard LBD family protein structure (PF03195) [24]. The HM-MER3.0 tool was employed to identify distinct proteins with the PF03195 protein structure in calcaria [25], and any overlapping sequences between HMMsearch and BLASTP findings were eliminated [23]. Then, the conservative structure domain database (CDD, https://www.ncbi.nlm.nih.gov/) was used to detect structural domains. Domain deletion and incomplete sequences were manually eliminated, and for the presence of multiple transcripts for the same gene, the longest transcript was selected as the representative sequence, thereby identifying ChLBD gene family members. In addition, the protein molecular weight and isoelectric point of LBD genes in Cerasus humilis were predicted using the ExPASy Proteomics server (http://expasy.org), an online proteomics and sequence analysis tool [26].

2.3 Phylogenetic analysis

The selected LBD amino acid sequences were aligned multiple times using Clustal X [27]. The phylogenetic trees of Arabidopsis thaliana and Cerasus humilis were constructed using the neighborhood join (NJ) method and the bootstrap node support with 1000.0 replicates [28]. The evolutionary tree was also created using the online software Evolview.

2.4 Collinearity and evolutionary gene analysis

ChLBD protein positions were derived based on the Cerasus humilis annotation GFF file, utilizing the Circos of TBtools for charting the gene chromosome. Utilizing MCScanX, the collinearity links among LBD proteins in Arabidopsis thaliana, Malus pumila Mill., Vitis vinifera L., and Oryza sativa L. were discerned. These findings were depicted through the Dual Synteny Plot with TBtools (v2.0697)[29].

2.5 Analysis of gene structure, conserved motifs, and cis-regulatory elements

The ChLBD gene's intron–exon distribution was determined using the Cerasus humilis genome's GFF annotation file. Utilizing the MEME online tool (https://meme-suite.org/meme/) for protein sequencing [30], the conserved motifs found in ChLBD proteins were pinpointed. The fine-tuned parameters included the distribution of loci, assigning either 0 or 1 to each sequence, a total of 10 patterns, and preset values for additional parameters. The TBtools program facilitated the identification of the 1500 bp promoter area preceding each gene's transcription initiation point for all ChLBD genes [31]. Subsequently, predictions of cis-acting components in the assumed promoter regions of ChLBD genes were performed using the PlantCare website [32]. Ultimately, the TBtools program was employed to visualize the aforementioned outcomes [31].

2.6 ChLBD gene expression analysis

For examining distinct LBD gene expression patterns in various tissues, the previously published transcriptome data of Cerasus humilis were utilized, encompassing the expression of diverse tissues (root, stem, leaf, flower, and fruit) and various fruit development phases (young fruit, green fruit, slightly red fruits, red fruit). Utilizing the Log2 (FPKM+1) algorithm, transcriptome data were transformed to determine gene expression levels, followed by the application of TBtools software for the visualization of LBD gene expression outcomes.

2.7 RNA extraction and real-time PCR analysis

This research study utilized healthy Cerasus humilis, aged 5, cultivated in a Heilongjiang province orchard, as the experimental materials. A total of 18 calcium fruits underwent a cleansing process using distilled water, followed by drying. Cerasus humilis was submerged in a SA solution of 1 mmol L-1 for 8 m [33], followed by storage in a chilled setting of (4±1)°C [34]. Fruits were gathered from every group at intervals of 6 h, 12 h, 24 h, 3 d, and 5 d post-storage. The fruits were immediately frozen in liquid nitrogen and stored at −80°C. Using a Plant Total RNA Extraction Kit (Simgen Biotechnology Co., Ltd.), the total RNA was isolated from Cerasus humilis pulp. Following the guidelines provided by the manufacturer, the total RNA mass was measured using electrophoresis on a 1% agarose gel and a Nanodrop 1000 spectrophotometer. Subsequently, the total RNA underwent reverse transcription using a SureScriptTM first-strand cDNA Synthesis kit (GeneCopoeia, Rockville, MD, USA), and the resultant cDNA concentration was measured using a nanodroplet spectrophotometer. The creation of the primer was executed via the Primer 3 website (https://primer3.org/) (Supplementary Table). LBD gene expression was measured using qRT-PCR, employing the BlazeTaqTM SYBR®Green QPCR Mix 2.0 QPCR kit. The procedure for PCR involved heating the plates at 94 °C for 30 s, followed by 45 cycles of 94°C for 12 s, 58°C for 30 s, 72°C for 45 s, and finally heating them at 79°C for 1 s for reading. Following the final PCR cycle, the temperature was increased from 55°C to 99°C at a speed of 0.5°C/s to produce sample melting curves [35]. Actin served as the benchmark gene, and the comparative gene expression was determined using the 2-DDCt technique [36]. Every reaction was conducted three times, with the outcomes presented as the average of three separate biological duplicates.

3 Results

3.1 ChLBD gene identification

Initially, Cerasus humilis' entire genome protein sequence underwent a hidden Markov model search (HMMsearch), utilizing the hidden Markov model for the LOB domain (PF03195). Subsequently, Cerasus humilis' entire genome protein sequence underwent a local BLASTP analysis, utilizing the AtLBD protein as the search sequence. At the outset, we preserved shared protein sequences that were recognized by both techniques. For additional confirmation of the candidate LBD genes' dependability, the Pfam and NCBI–CDD databases served to verify the integrity of the candidate proteins' LOB domains. Ultimately, 41 ChLBD genes were pinpointed, each encompassing LBD domains. The designation ChLBD1-ChLBD41 for ChLBD was derived from its distribution across chromosomes. The complete coding sequences (CDSs) of ChLBD genes varied between 327 bp and 1737 bp. Forty-one ChLBD genes were categorized into two segments based on whether the LX6LX3LX6L leucine zipper-like domain of LBD proteins was present or absent, with 35 genes in Class I and 6 in Class II. Furthermore, our analysis extended to the physicochemical characteristics of the 41 ChLBD proteins. The length of the sequence ranged from 109 (ChLBD30) to 579 (ChLBD35) amino acids, with a molecular mass between 12.068 (ChLBD30) and 62.748 (ChLBD35) kDa, and the isoelectric point measured between 4.74 (ChLBD20) and 9.19 (ChLBD3). Fundamental information about each ChLBD family member is concisely presented in Supplementary Table 1.

 3.2 ChLBD gene phylogenetic analysis

The use of phylogenetic trees is extensive in illustrating the evolutionary connections among gene families. A phylogenetic tree was developed utilizing 43 AtLBD proteins and 41 recognized ChLBD proteins (FIG. 1). Following the earlier reported LBD protein categorizations, they were segregated into two primary classifications: Class I and Class II. Among them, Class I comprises 73 individuals, while Class II includes 11 members. Class I is divided into five subcategories (Ia-Ie), while Class II is further divided into IIa and IIb. Subclass Ie boasts the highest member count, in contrast to IIa, which has the fewest. Alignments of various sequences for the 55 PeLBD proteins were conducted to explore the existence and locations of preserved protein domains. Every LBD family member possesses a well-preserved LOB area at its N-terminus, encompassing roughly 100 amino acids. A comparative analysis of various sequences reveals the presence of zinc finger domains in all LBD proteins. Conversely, the presence of the leucine zipper domain is exclusive to Class I ChLBD proteins, akin to findings in various other plant species [16, 23, 35].

3.3 Chromosomal localization analysis

To delve deeper into the chromosomal distribution of ChLBD genes, we charted every ChLBD gene family member within the calcium fruit genome. The findings indicated an irregular distribution of 41 ChLBD genes across eight chromosomes in the Cerasus humilis genome. Among them, Chr3 contains the most ChLBD genes (10 members), followed by Chr6 and Chr2, with seven and eight ChLBD members, respectively. Chr8 contains the fewest ChLBD genes (2 members). Additionally, our findings indicated the absence of a direct relationship between chromosome length and LBD gene quantity, and no chromosomal prejudice was observed in the allocation of these two ChLBD varieties, akin to the gene distribution in Passiflora edulis for LBD [24].

3.4 Collinearity and evolutionary analysis of genes

Current research suggests that the growth of gene families is propelled by varied gene replication patterns, which are believed to be a key motivator in species' evolution [37]. In this study, using the MCScanX method, three tandem repeat events (ChLBD10/11, ChLBD15/16, and ChLBD37/38/39) were found in the Cerasus humilis genome, which were located on Chr3 and Chr7, respectively. In addition, there are 24 ChLBD genes on repeated segments in the calcium fruit genome. They were matched to 12 fragment repeat gene pairs (ChLBD2 and ChLBD23, ChLBD3 and ChLBD32, ChLBD2 and ChLBD29, ChLBD1 and ChLBD36, ChLBD7 and ChLBD13, ChLBD1 and ChLBD18, ChLBD6 and ChLBD17, CHLBD2 and CHLBD23, ChLBD8 and ChLBD36, ChLBD23 and ChLBD29, ChLBD24 and ChLBD31, ChLBD33 and ChLBD40, ChLBD34 and ChLBD41). These results suggest that part of ChLBD may be produced through gene replication and that fragment replication events are the main drivers of ChLBD evolution. Furthermore, the vast majority of duplicated gene pairs fit into Class I, representing roughly 79% of all ChLBD genes, suggesting that these chromosomal segments might not have undergone complete differentiation throughout evolution and could be functionally superfluous or redundant [24].

To delve deeper into the genesis and development of LBD family genes, we performed a collinear examination of various species, encompassing dicotyledonous plants such as Arabidopsis thaliana, Malus pumila Mill., Vitis vinifera L., and monocotyledonous plants such as Oryza sativa L., pinpointing 27, 75, 47, and 21 collinear LBD gene duos. Dicotyledonous plants exhibited a higher collinear gene count compared with monocotyledonous plants, and Malus pumila Mill had more collinear gene pairs. The rose family topped the list. The findings offer crucial insights into the genetic roles of rose family cash crops.

3.5 Gene structure and conserved motif analysis

To identify the genetic structure and evolutionary trajectory of LBD genes in Cerasus humilis, we studied the intron–exon composition of 41 ChLBD genes. The number of introns in ChLBD genes ranges from 0 to 10, among which, most genes contain only one intron, while the ChLBD35 and ChLBD20 genes contain multiple introns, indicating that some introns in ChLBD genes may be lost during evolution. In addition, genes clustered into subclasses may exhibit different structural features, such as ChLBD3 in IIa containing no untranslated (UTR) regions, while ChLBD32 has two CDS and two UTR regions, and it is speculated that some members of this subclass may have undergone gene splicing or gene fragment insertion during evolution [23, 38].

The MEME instrument was employed to forecast the conserved motif of ChLBD proteins. Our research identified and named 10 preserved motifs as Motif1-10. Consequently, the quantity and variety of preserved components in ChLBD protein sequences remain largely unchanged. The majority of members grouped within the same subbranch exhibit comparable motif patterns, indicating that proteins derived from the same subbranch might serve analogous roles. The fundamental areas in LOB domains, Motif 1 and Motif 2, are identifiable across all ChLBD gene family members. Members of Class I possess Motif 1 (CX2CX6CX3C), Motif 2(GAS block), and Motif 3 (LX6LX3LX6L), in contrast with Class II members which do not have Motif 3 (LX6LX3LX6L). The findings lend additional credence to the idea of categorizing the ChLBD gene family into two distinct clusters [23].

3.6 Cis-regulatory element analysis

Cis-acting elements are non-coding DNA sequences in gene promoter regions that regulate the transcription of their associated genes. In this instance, a segment of 1500 base pairs before the transcription initiation point of ChLBD genes was chosen as the benchmark hypothetical promoter region [24]. Subsequently, ChLBD gene promoter sequences were isolated and entered into the PlantCARE database to identify cis-acting elements. The findings revealed that in Cerasus humilis, LBD gene promoter regions comprise hormone-related components such as SA, ababolic acid, and methyl jasmonate. Furthermore, our research identified cis-acting components associated with stress, including light, defense mechanisms, stress, and low temperatures. Similarly, LBD gene family promoters encompassed numerous elements associated with growth and development, including meristem expression. Sixteen significant cis-acting elements were chosen for additional examination. The figure illustrates the varied distribution patterns of cis-acting elements within ChLBD genes' promoter regions. Within this group, the methyl jasmonate response elements stand out as the most abundant cis-acting elements, extensively dispersed across Class 1 and Class 2 ChLBD promoter areas. The findings indicate that the operational expression of LBD genes in Cerasus humilis is controlled by multiple cis-acting factors associated with hormones, plant growth and development, and stress reactions.

3.7 Analysis of ChLBD gene expression

{#ResumeQA#}To investigate the potential function of ChLBD genes further, we analyzed ChLBD gene expressions in different plant tissues and at different growth stages. Figure 6(a) shows the expression profile of ChLBD gene in different tissues, in which, It has 18 members (ChLBD34, ChLBD35, ChLBD7, ChLBD1, ChLBD6, ChLBD39, ChLBD17, ChLBD23, ChLBD38, ChLBD36, ChLBD5, ChLBD14, ChLBD8, ChLBD37, and ChLBD26, ChLBD27, ChLBD25, ChLBD32) are highly expressed in the root, and 7 members (ChLBD4, ChLBD21, ChLBD2, ChLBD29, ChLBD31, ChLBD11, ChLBD41) are highly expressed in the flower. Six members (ChLBD13, ChLBD40, ChLBD18, ChLBD9, ChLBD16, ChLBD15) were highly expressed in stems, ChLBD12 was highly expressed in leaves, ChLBD3 and ChLBD28 were highly expressed in fruits, and ChLBD10 was highly expressed in roots and fruits. Six members (ChLBD30, ChLBD33, ChLBD19, ChLBD22, ChLBD20, and ChLBD24) are not expressed in all tissues and may be expressed in other tissues or during growth and development cycles.{#BreakQA#146}

Figure 6(b) shows the expression profiles of ChLBD genes at different developmental stages of Cerasus humilis, with three repeats in each stage, among which 18 members (ChLBD11, ChLBD8, ChLBD16, ChLBD15, ChLBD1, ChLBD21, ChLBD32, ChLBD1, ChLBD3, ChLBD10, ChLBD12, ChLBD9, ChLBD2, ChLBD4, ChLBD6, ChLBD5, ChLBD19, and ChLBD23) have higher expression levels in young fruit than the other three stages. Seven members (ChLBD38, ChLBD24, ChLBD41, ChLBD13, ChLBD36, ChLBD33, and ChLBD39) were highly expressed in green fruits but hardly expressed in red fruits and slightly red fruits, which may be mainly involved in regulating fruit growth. Five members (ChLBD25, ChLBD26, ChLBD27, ChLBD40, and ChLBD22) are expressed in red and slightly red fruits but not in young and green fruits, which may be mainly involved in the regulation of the fruit ripening process. Six members (ChLBD30, ChLBD35, ChLBD14, ChLBD17, ChLBD20, and ChLBD29) were not expressed in any of the four developmental stages and may be expressed in other growth stages or tissues. It is not difficult to find that genes in the same subclass may have different expression profiles. These results suggest that these genes play an extremely important role in the growth and development of calcium fruit and have evolved different regulatory modes.

3.8 RNA extraction and real-time fluorescence quantitative PCR analysis

The comparison of Cerasus humilis treated with SA at 0 h, 6 h, 12 h, 24 h, 3 d, and 5 d showed that the surface folding degree increased with an increase in storage time, and the fruit softening degree became more serious. In order to further understand the function of ChLBD genes and analyze the influence of the exogenous hormone SA on ChLBD gene expression, six representative ChLBD genes were screened according to heat maps and analyzed using qRT-PCR technology. The results were shown as follows: After the fruits were treated with SA, the storage time of the fruit was prolonged. The expression levels of ChLBD40 and ChLBD33 decreased first and then increased on the fifth day of storage, and the expression levels of ChLBD26 and ChLBD34 decreased on the sixth hour of storage and then increased again on the fifth day. The expression levels of ChLBD27 and ChLBD7 were the highest at 0 h after the SA treatment. In summary, the results of qRT-PCR validation supported the results of transcriptome data analysis and also suggested that ChLBD genes showed different expression patterns in response to SA, which may balance the relationship between developmental and environmental responses.

4. Discussion

Cerasus humilis is nutritionally rich and, together with Hippophae rhamnoides L., Vaccinium uliginosum L., etc., is known as the third-generation fruit with high nutritional and healthcare value. Hence, an increasing number of researchers pay attention to it. Its shape is similar to Prunus pseudocerasus, but the size and nutritional value are greater than Prunus pseudocerasus, with high yield, high fruit calcium content, strong edibility, significant economic benefits, and other characteristics with applications as a food and medicine, which is currently being developed for other applications [39]. The storage conditions after harvest significantly impact the quality of fruits. Consequently, the examination and analysis of TFs linked to fruit quality hold immense importance for fruit storage [35]. LBD genes, unique to plants, produce a preserved LOB (lateral organ boundary) domain and are involved in numerous biological functions, such as responding to abiotic stress, metabolizing nitrogen, and developing lateral organs. For instance, in Arabidopsis thaliana., AtLBD16, AtLBD17, AtLBD18, and AtLBD29 are crucial in the initiation of the callus associated with plant regeneration. The proteins AtLBD16, AtLBD18, and AtLBD29 play roles in the development of lateral roots, while AtLBD29 also participates in the auxin signaling mechanism that controls the synthesis of fiber walls [18]. Currently, studies indicate a connection between LBD genes and fruit development, playing a role in controlling fruit softening [40]. Due to the important role of LBD genes in fruit development, it has been identified in different plant fruits, such as Cucumis melo L. [38], Passiflora edulis [24], and Pyrus bretschneideri Rehd. [23]. Yet, there is still no published research concerning the ChLBD gene family. Our research entailed an in-depth examination of the ChLBD gene family, centering on the alterations in their expression when calcium fruits are stored and treated with SA. This study's findings aid in deepening our comprehension of ChLBD genes' operational processes and improving the storage of Cerasus humilis.

This research study identified 41 ChLBD genes in Cerasus humilis, revealing their irregular distribution across eight chromosomes through chromosomal localization analysis. These genes were categorized into Class I (88%) or Class II(12%), depending on whether the LX6LX3LX6L leucine zipper domain at the C-terminal was present or absent. Echoing earlier research findings, Class I's membership exceeds Class II [19, 20, 23]. Phylogenetic categorization reveals that 83 LBD genes in Cerasus humilis and Arabidopsis thaliana are segmented into seven distinct groups: Ia, Ib, Ic, Id, Ie, IIa, and IIBa. Although there are variances, the phylogenetic tree largely aligns with findings from earlier research studies [41]. Genes with analogous functions tend to group within the same subclade, offering a crucial benchmark for forecasting gene functionality [23].

Gene replication fosters the creation of novel genes and their functions, significantly influencing evolutionary processes. Gene replication in evolution primarily follows three methods: fragment replication, tandem replication, and translocation events. In the expansion of plant gene families, segment replication and tandem replication are predominantly observed [42, 43]. A total of 12 fragment repeats were identified, while only three tandem repeats (ChLBD10/11, ChLBD15/16, and ChLBD37/38/39) dominated the amplification of the ChLBD gene family. Similar to other species in different taxonomic groups [10, 16, 24, 44]. A comparative study of the Cerasus humilis genome against four sequenced plant genomes revealed notable collinearity between Cerasus humilis and dicotyledonous plants within the LBD family. Merely a handful of LBD members are collinear with monocotyledonous plants. This finding aligns with the evolutionary link observed between dicotyledonous and monocotyledonous plants [16].

The structure and motif distribution of genes provides valuable information for studying phylogenetic relationships between members of the same gene family, as well as strong evidence for studying evolutionary relationships between species or genes [35]. As with other plants such as Arabidopsis thaliana, Passiflora edulis, Cucumis melo L., etc., highly related gene members tend to exhibit similar motif structures and intron–exon structures [24, 38, 45]. The resemblance in motif and intron–exon configurations in Class I and II ChLBD in Cerasus humilis suggests a largely preserved evolutionary structure for this gene family. Nonetheless, structural variances might exist among certain subclass members, and it is theorized that these members have experienced gene splicing or gene segment integration during evolutionary history [16, 46, 47]. Variations in motif composition and gene structure across various subpopulations could result in functional diversity in the LBD gene family [24]. Moreover, Motif 3 (leucine ziplock motif) is present in all Class I ChLBDs, in contrast to Class II members, underscoring the dependability of the Class I and II classifications in this research study.

The cis-acting elements in the promoter region play an important role in gene regulation and expression [48]. For example, the promoter of the BpBEE1 gene in Betula platyphylla Suk. contains elements related to various hormone responses, and after treatment with MeJA, SA, BL, and ABA, GUS activity in the veins and roots of Betula platyphylla Suk. increased, and promoter activity is also significantly enhanced [49]. Elements acting on cis located in genes' upstream promoter regions can attach to TFs, significantly influencing biological signal transmission, a crucial element in regulating gene expression [50]. This research study identified numerous regulatory MOBS within the assumed promoter region of ChLBD genes linked to hormone control, growth, development, and stress reactions. Among them, the methyl jasmonate response element is the most abundant cis-acting element, widely distributed in most ChLBD genes. It has been documented that the LBD gene family plays a role in the abiotic stress response [51], and MYB binding sites are also present in the promoters of numerous ChLBD genes. Most ChLBD's copious methyl jasmonate response components might play a role in the control network governing plant growth and stress reactions. In addition, it has been reported that exogenous SA can hinder ethylene biosynthesis in fruits, delay fruit ripening and aging processes, and be used for fruit preservation. We also found that ChLBD gene promoters contain 36 SA reactive cis-acting elements. Therefore, the addition of SA in low-temperature environments can regulate the expression of the ChDof gene to ensure fruit quality.

Increasing proof suggests that LBD genes have a distinct function in plant development. Typically, gene expression patterns serve as crucial indicators for forecasting gene functionality [51]. In this study, we downloaded transcriptome data of Cerasus humilis to analyze the expression of ChLBD genes in different plant tissues and at different growth stages. The results showed that the ChLBD gene family exhibited multiple expression patterns in five different tissues, even within a subclass. Some valuable ChLBD genes may play roles in specific physiological processes. For example, ChLBD3 and ChLBD28 are mainly expressed in fruit. At the same time, we found that the expression of ChLBD also differs in different fruit growth stages, which indicates that ChLBD genes may be involved in a variety of biological processes. Interestingly, the expression levels of ChLBD27 and ChLBD40 increased during fruit ripening. For Vitis vinifera L., the expression of two LBD genes (GSVIVT01008284001 and GSVIVT01024592001) showed a decrease prior to the fruit's discoloration phase and a steady rise as the fruit ripened [52]. During fruit ripening in Musa acuminata, there is an increase in the expression of MaLBD1, MaLBD2, and MdLBD3. This trio of genes plays a role in controlling fruit ripening by initiating the transcription of MaEXP, a gene linked to cell wall loosening [53]. Similarly, it is hypothesized that ChLBD27 and ChLBD40 play a role in controlling fruit ripening and associated color alterations. Nonetheless, the role of LBD genes within fruits remains ambiguous and warrants additional exploration in subsequent research studies.

{#ResumeQA#}Investigating the impact of SA on ChLBD gene activity and deepening our comprehension of ChLBD genes' roles, we examined six ChLBD genes using RT-qPCR. Post-treatment with SA, there was a temporal shift in the expression patterns of numerous ChLBD genes, suggesting SA influences ChLBD gene expression. Earlier research indicated that Class I and Class II LBD genes might be involved in distinct biological functions [2, 11]. Yet, this investigation revealed no notable disparities in how these LBD gene types react to hormonal stimuli.

5. Conclusion

In this study, for the first time, the LBD TF gene family members in Cerasus humilis were systematically identified and analyzed. A total of 41 ChLBD genes were identified and distributed on eight chromosomes, which could be divided into two classes and seven subclasses. Class I members contain Motif 1 (CX2CX6CX3C), Motif 2 (GAS block), and Motif 3 (LX6LX3LX6L), while Class II members lack Motif 3 (LX6LX3LX6L). Fragment duplication was the main reason for LBD gene family expansion in Cerasus humilis. In addition, the expression of ChLBD genes was specific to different tissues and growth stages, and ChLBD genes in the same subgroup also showed different expression patterns. qRTPCR analysis showed that some ChLBD genes were responsive to SA. The results indicated that SA may regulate the quality of fruit after picking by influencing the expression of some genes. The results of this study provide a solid basis for further study of the function of ChLBD genes, especially their role in the regulation of fruit quality during postharvest storage. Currently, investigations into LBD TFs primarily concentrate on uncovering novel members and examining biological roles. Yet, there is a need for a more in-depth exploration into their genesis, development, and the traits of each subclass [54].{#StopQA#290}