Introduction

Lateral organ boundary Domain (LBD), also known as AS2/LOB gene family, are plant-specific transcription factors that regulate lateral organ development, morphogenesis, and metabolism (Iwakawa et al. 2002; Majer and Hochholdinger 2011). The LBD gene was first identified at the base of the lateral organs of Arabidopsis through the insertion of enhancer traps (Iwakawa et al. 2002; Shuai et al. 2002). LBD transcription factors have three specific conserved structural domains arranged from the N to the C terminus, including the zinc finger domain (C-block, CX2CX6CX3C), the Gly-Ala-Ser block (GAS-block), and the leucine zipper-like module (LX6LX3LX6L). The C-block is essential for binding to DNA, the GAS-block affects DNA binding activity, and the leucine zipper-like module is associated with protein dimerization (Lee et al. 2009; Matsumura et al. 2009; Majer and Hochholdinger 2011). The LBD gene family can be classified into two subfamilies based on the characteristics of its structural domain: class I and class II. Class I members contain a conserved C-block, a GAS-block, and relatively complete leucine zipper-like motifs, while Class II members contain an incomplete leucine zipper-like domain. Most LBD family members belong to class I and play a key role in plant organ development and signal transduction (Majer and Hochholdinger 2011; Yu et al. 2020a). Class II LBD proteins are mainly involved in synthesizing secondary plant metabolites, such as anthocyanins (Zhang et al. 2020).

The LBD gene family members have been identified in many plants, including 43 in Arabidopsis, 44 in maize, 35 in rice, 57 in poplar, 46 in tomato, and 90 in soybean (Yordanov et al. 2010; Wang et al. 2013; Zhang et al. 2014; Guo et al. 2016; Yang et al. 2017, 2006). Previous studies have shown that the LBD gene family plays a crucial role in plant organ boundaries, especially in early morphogenesis of plant embryos, roots, leaves and flowers, tissue regeneration, and stress responses (Semiarti et al. 2001; Iwakawa et al. 2007; Goh et al. 2019). In Arabidopsis, AtLBD20/AtASL4 is an auxin response factor in adaxially-rolled leaves in Arabidopsis (Iwakawa et al. 2007; Goh et al. 2019). In rice, OsLBD3-7 induces adaxially-rolled leaves in rice. Moreover, the overexpression of OsLBD3-7 induces adaxially-rolled leaves in rice. In Arabidopsis, OsLBD3-7 induces adaxially-rolled leaves in rice. In addition, LBD gene family members have been
implicated in stress responses in many plant species. GmLBD12 is involved in the response of soybean to abiotic stresses (drought, salt, cold) and hormones treatment indole acetic acid (IAA), abscisic acid (ABA), and salicylic acid (SA) treatment (Yang et al. 2017a). MaLBD5 mediates transcriptional activation of jasmonate biosynthesis gene MaAOCC2 in regulating cold tolerance of banana fruit (Ba et al. 2016). All 30 LBD gene family members in grape have been implicated in stress response (He et al. 2018).

Common bean (Phaseolus vulgaris L.) is an important leguminous plant that is rich in nutrients, such as protein, fat, carbohydrate, and dietary fiber. The planting area of common bean has been expanding worldwide in recent years. However, abiotic stresses, such as saline-alkali stress, significantly affect the growth and quality of common bean (Talaat 2014). Therefore, research into the molecular mechanisms of stress response in common bean is an important topic in agricultural science. The structure and function of LBD family genes have been explored and analyzed in some plant species, and their roles in regulating plant growth and development and stress response have been verified. However, the molecular characteristics and functions of LBD family members in common bean have not been fully elucidated. In this study, we first identified the LBD gene family in the common bean using an in silico genome-wide screening analysis. Then, the phylogenetic relationship, gene structure, chromosome distribution, evolutionary relationship, cis-regulatory elements, gene replication, collinearity, and spatio-temporal expression patterns of the putative common LBD genes (PvLBDs) were systematically analyzed. This study lays a foundation for further analysis of the function of the PvLBD gene family in common bean and other crops and provides insights into the breeding of common bean varieties with high-stress resistance.

**Materials and methods**

**Identification of PvLBD genes and chromosomal mapping**

The reference sequence of the common bean genome and its annotated proteins were obtained from the Ensembl plants (http://plants.ensembl.org/index). HMM software (http://hmmer.janelia.org/) was used to obtain putative LBD proteins containing LOB domain (PF03195) from Pfam database (http://pfam.xfam.org). The InterPro (http://www.ebi.ac.uk/interpro/) (Finn et al. 2017) and SMART (http://smart.embl-heidelberg.de/) (Letunic et al. 2015; Han et al. 2019) were used to further confirm the reliability of the LOB domain prediction. WoLF PSORT (http://wolfsort.hgc.jp/), P3DB (http://www.p3db.org/), Interpro (http://prosite.expasy.org/), and ExPaSy Proteomics Server (http://prosite.expasy.org/) were used to verify the integrity of the LOB domains of candidate LBD genes. Maphart software was used to map the PvLBD genes on chromosomes. The PvLBD genes were sequentially named based on their precise location on the chromosome.

**Phylogenetic, gene structure, and conserved motifs, and promoter predictions of PvLBD genes**

The LBD proteins of Arabidopsis, soybean, and maize were obtained from Ensembl plants using the LOB domain. All the LBD proteins, including PvLBDs, were used for multiple sequence alignment via ClustalX (Larkin et al. 2007) with default parameters. A phylogenetic tree with 1,000 bootstrap replications was modified and constructed using the Maximum Likelihood method of MEGA X based on the JTT + G model. The structure of PvLBD genes was obtained from the GSDS platform (http://gsds.cbi.pku.edu.cn/) (Guo et al. 2007). The correspondence between DNA and protein sequences was determined using GeneWise (Binney et al. 2004). An in-house Perl script was used to convert the coordinates in the LOB domain of the protein sequence to those in the nucleotide sequence. The MEME tool (http://meme.nbcr.net/meme/) (Bailey et al. 2009) was used to predict the conserved motifs with the following parameters: the width of characteristic motifs was between 10 and 50 residues, and the E value was less than $1e^{-20}$. The upstream 1.5 kilobases (kb) genomic DNA sequences of the PvLBDs were retrieved from the common bean genome. The sequences were then submitted to the PlantCare database (http://bioinformatics.psb.ugent.be/webtools/plantcare/) to identify the putative cis-regulatory elements in the promoter regions.

**Collinearity analysis**

Multiple Collinearity Scan toolkit (MCScanX) was used to examine the syntenic and collinearity of LBD genes (Wang et al. 2012). A visualization tool Circos (Version 0.69) (Krzywinski et al. 2009), was then used to express the collinearity of the duplicated genes.

**Growth conditions and treatments**

Common bean seeds (Longjiang Ziyun) were obtained from the National Coarse Cereals Engineering Research Center (Daqing, Heilongjiang, China). The seeds were placed on Petri dishes, then 11 mL of distilled water was added to each petri dish and incubated at 28 °C in darkness to germinate. After germination, the seedlings were separately exposed to different stress treatments on the sixth day. Seedlings cultured with distilled water were used as controls (CK). Salt stress was induced by treating the seedlings with 70 mmol/L (NaCl) (Zhang et al. 2021), while heavy metal stress was simulated by exposing the seedlings to 0.5 mg/L (CdCl$_2$) (Zhao et al. 2020) and 60 mg/L (HgCl$_2$) (Mohammadi et al. 2021) for 24 h.

**RNA extraction, complementary DNA synthesis, and gene expression analysis**

RNA isolator Total RNA Extraction Reagent (Vazyme Bio-tech, China) was used to isolate total RNA from the samples. RNA quantity and integrity were determined by measuring the optical density of the extracted RNA samples at 260 nm and via 1.0% agar gel electrophoresis, respectively. Single-stranded cDNA was synthesized from the RNA samples using Evo M-MLV RT Premix for qPCR (AG11706, Accurate Biology, Hunan, China). The Light Cycler system (Roche 480II, Roche, Switzerland) and TransStart® Top Green qPCR SuperMix (AQ131-04, TransGen Biotech, Beijing) were used for qRT-PCR reactions. The related expressions were determined using the 2$^{-\Delta \Delta C_{T}}$ method. The reference gene ACTIN-11 was used as the
control (Zhang et al. 2021). The primers used in this study are listed in Table S1. Each treatment had three biological replicates. Each sample also had three technical replicates.

**Statistical analysis**
Office Excel 2013 and SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) were used for all data analyses (Li et al. 2018).

**Results**
**Identification of LBD genes in common bean.**

HMM profile, InterPro, and SMART analyses identified 47 *PvLBD* genes in the common bean (*Phaseolus vulgaris* L.) genome. The genes were named *PvLBD1* – *PvLBD47* based on their chromosomal position (Table 1 and Figure 1). The *PvLBD* genes encode proteins with lengths ranging from 60 amino acids (aa) (*PvLBD42*) to 328 aa (*PvLBD41*) (average length of 206 aa), predicted molecular weight (MW) of 6572.64 (*PvLBD42*) – 36748.78 (*PvLBD41*) D, and pI (isoelectric point) of 5.05 (*PvLBD43*) – 9.82 (*PvLBD20*) (Table 1).

The 47 *PvLBD* genes are distributed on 11 chromosomes (Figure 1). There are 9 *PvLBDs* (*PvLBD28*-36) on Chromosome 8, while chromosomes 2, 6, and 10 have two *PvLBDs* each. The chromosomal localization showed that the distribution and density of the *PvLBDs* are uneven and clustered. Most *PvLBD* genes are distributed at both ends of the chromosome, with a few in the middle.

**Phylogenetic analysis and conserved sequence alignment**

To clarify the evolutionary relationships between common bean *PvLBD* proteins and *LBD* proteins of other plant species, we constructed a phylogenetic tree based on multiple protein sequence alignment of 47 *PvLBD* proteins, 90 *LBD* proteins of leguminous crops (soybean, *GmLBDs*), 47 *LBD* proteins of the dicotyledonous model plant (*Arabidopsis*, *AtLBDs*), and 44 *LBD* proteins of monocotyledon plants (maize, *ZmLBDs*) (Figure 2).

Phylogenetic analysis suggested that the *LBDs* could be classified into two major clades (class I and II). Class I had 216 members: 43, 85, 46 and 42 in common bean (91.5%),
soybean (94.5%), Arabidopsis (97.9%), and maize (95.5)% respectively. Class II had 12 members: 4, 5, 1, and 2 in common bean (8.5%), soybean (5.5%), Arabidopsis (2.1%), and maize (4.5)% respectively. Moreover, class I could be subdivided into eleven sub-classes (Ia–II), and sub-classes (II), respectively (Figure 2 and Figure S1). Phylogenetic relationships indicated that the LBD proteins of common bean are more homologous to soybean LBD proteins than those of Arabidopsis or maize.

Multiple sequence alignments were created for the 47 PvLBD proteins to investigate the presence of conserved protein domains (Figure 3). The results indicated that all the...
PvLBDs have a conserved cysteine-rich C-motif (CX2CX6CX3C). 43 PvLBDs had complete cysteine-rich C-Motif, GAS-block, and leucine zipper-like structure and were classified as class I, while the remaining 4 PvLBDs had incomplete leucine-zipper structures and belonged to Class II.

**Gene structure and conserved motif analysis**

To further investigate the evolutionary relationships among the 47 PvLBDs, we utilized full-length PeLBD protein sequences to construct a second phylogenetic tree. The results showed that the 47 PvLBDs were classified into two major groups, class I and class II. Furthermore, class I was divided into 11 subclasses, including Ia, Ib, Ic, Id, Ie, If, Ig, Ih, II, Ij, Ik, and II with 3, 4, 2, 5, 1, 2, 9, 2, 4, 5, 2, and 4 members, respectively. While Class II had 4 members (Figure 4A). The MEME program identified 10 motifs according to PvLBDs sequence characteristics (Figure 4B). All LBDS contained motifs 1 and 2, constituting a highly conserved part of the LOB structural domain. The relative positions of motifs in most sequences were similar. However, each subgroup had specific motifs, indicating that the PvLBD gene family members have different evolutionary processes.

The exon-intron structures of the PvLBDs were analyzed to reveal their structural and evolutionary features (Figure 4C). Each common bean LBD gene has about 1–2 exons, while some PvLBDs lack intron. 29 genes contained one intron, 4 genes (PvLBD34/38/41/46) contained two introns, and 14 genes (PvLBD4/7/10/12/13/14/15/20/30/33/39/44/47) were intron lack. PvLBD genes in the same phylogenetic clade had similar structures.

**Collinearity analysis and the tandem replication of PvLBDs**

Gene duplication events are common in all species and can generate new functional genes and drive evolution. Herein, nine gene pairs were identified as duplicated gene pairs in common bean PvLBD gene family, including PvLBD36/PvLBD27, PvLBD37/PvLBD19, PvLBD30/PvLBD20, etc.
PvLBD30/PvLBD5, PvLBD31/PvLBD20, PvLBD25/PvLBD2, PvLBD20/PvLBD5, PvLBD18/PvLBD33, PvLBD7/PvLBD11. Notably, the nine gene pairs belong to fragment replication (Figure 5A).

The orthologous relationship between PvLBD genes in common bean and Arabidopsis, soybean, and maize genomes was assessed to clarify the evolutionary differences between LBD genes. The LBD family genes in common bean were colinear with 24 genes in Arabidopsis, 51 in soybean, and four in maize (Figure 5B). Therefore, there was higher collinearity between the common bean and soybean genomes than between the common bean and monocotyledon genomes. Furthermore, the most soybean LBD genes had corresponding orthologs in moso bamboo common bean, and most of them had more than two orthologs, suggesting that moso bamboo has undergone additional WGD event(s) during its evolution.

Selection pressure analysis of coding sequences was expressed using the non-synonymous mutation rate (Ka) to synonymous mutation rate (Ks) ratio. Ka/Ks < 1 represents purification selection or negative selection, while Ka/Ks > 1 represents Darwinian selection or positive selection. PvLBD30/PvLBD5 and PvLBD30/PvLBD20 underwent purification selection with the Ka/Ks < 1, while PvLBD20/PvLBD5 and PvLBD18/PvLBD33 underwent positive selection with the Ka/Ks > 1 (Table S3).

Analysis of Cis-regulatory element in PvLBD promoters

Cis-acting elements are non-coding DNA sequences in gene promoter sequences that regulate gene transcription. Genomic sequences (1.5-kb) genomic sequences (1.5-kb) upstream from 5′-UTR of 47 PvLBDs were extracted from common bean genome sequences to investigate the cis-acting elements of PvLBDs. Fifteen cis-regulatory elements belonging to hormone-, resistance-, and development-related elements, were identified using Plant CARE software (Figure 5 and Table S2). Several plant hormone response elements were found in the promoter of PvLBDs. Particularly, multiple copies of abscisic acid elements (ABRE) were found in most PvLBD promoter regions. Additionally, three resistance-related elements (MBS: drought stress inducibility element, LTR: low-temperature responsiveness, and ARE: anaerobic responsiveness) were identified. MBS was identified in 13 PvLBD genes, LTR in seven PvLBD genes, and ARE in 36 PvLBD genes. Development-related elements were also identified, including CAT-box (meristem expression element) and RY-element (seed-specific regulation element). The CAT-box was found in 14 PvLBD genes and RY-element in three PvLBD genes. These results suggest that PvLBD family may be involved in hormone response, defense response, and growth regulation.

Tissue-specific expression of PvLBD genes

The transcriptional profiles from the Phytozome database were used to explore the expression patterns of PvLBD genes in nine different tissues, including flower buds, flowers, mature green pods, leaves, nodules, root, stem, young pods, and young trifoliate. The 47 PvLBD genes had different expression patterns among the tissues (Figure 7). Seventeen PvLBD genes were detected in all tissues, suggesting their involvement in the development or physiology of multiple tissues. However, four PvLBD genes (PvLBD1/9/31/36) were undetectable in the tissues. Three genes (PvLBD6/11/28) were highly expressed in flower buds, flowers, mature green pods, and pods, suggesting that they potentially participate in the development and function of common bean reproductive organs. Additionally, two genes (PvLBD37/47) were highly abundant in young trifoliate, one gene (PvLBD13) was highly abundant in leaves, five genes (PvLBD3/21/23/26/44) were highly abundant in...
roots, five genes (PvLBD16/20/22/31/32) were highly abundant in root nodules, and two genes (PvLBD10/45) were highly expressed in stems.

There was a significant difference in PvLBD gene expression abundance between different developmental stages of the same tissue. The expression abundances of
PvLBD1/17/41 in flower buds were significantly higher than in flowers, while the expression abundances of PvLBD40/15/35 were significantly lower than in flowers. The expression abundances of PvLBD6/11/18/28 in mature green pods were significantly higher than in young pods, while the expression abundances of PvLBD 8/13/14/17/46 were significantly lower than in young pods. The expression abundances of PvLBD 37/47 in young trifoliate were higher than in leaves, while the expression abundances of PvLBD 13/20 were lower than in leaves.

Stress-induced expression patterns of PvLBD genes

The expression abundances of the 47 PvLBDs in cotyledon, hypocotyl, and radical of common bean under NaCl, CdCl₂, and HgCl₂ stress were detected using qRT-PCR to evaluate the roles of PvLBDs in response to abiotic stress (Figure 8). The PvLBD1/6/11/20/31/34/38/40/41 genes in common bean cotyledon, hypocotyl, and radical were significantly up-regulated under NaCl, CdCl₂, and HgCl₂ treatments. In contrast, the other 36 PvLBDs were down-regulated under the abiotic stress. Notably, the PvLBD6/11 gene expression in common bean cotyledon, hypocotyl, and radical was higher under NaCl stress than CdCl₂ and HgCl₂ stresses. Moreover, the expression abundances of PvLBD1/40 genes in common bean cotyledon, hypocotyl, and radical were higher under CdCl₂ stress than under NaCl and HgCl₂ treatments. The expression abundances of PvLBD20/31/34/38/41 genes in common bean cotyledon, hypocotyl, and radical were higher under HgCl₂ stress than under other stress treatments. It’s worth noting that the expression levels of PvLBD1/6/11/20/31/34/38/40/41 genes were up-regulated by all the three stress treatments.

Discussion

Herein, 47 PvLBD genes were identified in the common bean genome and classified into two classes, class I (40, 85%) and class II (7, 15%). In addition, class I genes were further divided into 13 subclasses (Figure 2). Previous studies have shown 86% of AtLBD genes in Arabidopsis, 82% of GmLBD genes in soybeans, and 84% of ZmLBD genes in maize belong to class I (Shuai et al. 2002; Zhang et al. 2014; Yang et al. 2017). Similarly, in this study, the number of LBD members was significantly higher in class I than in class II. A total of 223 LBD genes from the 4 species were further divided into 13 subclasses (Ia – II and Ii), and their
phylogenetic relationships were consistent with those reported in previous studies (Shuai et al. 2002; Zhang et al. 2014; Yang et al. 2017). Furthermore, the expression abundance of homologous \( \text{PvLBD} \) gene pairs in the same tissue or under the same stress was similar (Figures 7 and 8), suggesting that the \( \text{PvLBD} \) gene pairs in common bean may have mainly led to functional redundancy.

Gene structure analysis showed that the conserved domains differed between class I and II (Figures 3 and 4). Moreover, some members of the same subclass had structural differences. This difference may be because members of different subclasses underwent splicing or insertion of gene fragments during evolution (Staiger and Brown 2013; Li et al. 2016b). Similar conserved sequences and gene structures in the LBD subclass often imply that these genes have similar biological functions, an indication that the motifs of the LBD family have been widely conserved throughout the evolutionary process.

The LBD gene family in common bean is larger than in \( \text{Arabidopsis} \) (Figure 2), possibly due to the expansion of \( \text{PvLBD} \) genes, like in soybean (Yang et al. 2017) and maize (Zhang et al. 2014). Gene replication produces differentiation in gene function, a critical factor in environmental adaptation and speciation (Conant and Wolfe 2008). Functional differentiation of duplicated gene pairs may occur during evolution, resulting in neo-functionalization, sub-functionalization, or non-functionalization (Prince and Pickett 2002). Gene duplication is the most common evaluation mechanism for gene family expansion. Gene family expansion in plants mainly occurs through tandem and fragment duplications (Cannon et al. 2004). Tandem replication mainly occurs in the recombination region of chromosomes. The gene family members formed by tandem replication are usually closely arranged on the same chromosome, forming a gene cluster with similar sequence and similar function. In contrast, genes formed via fragment duplication are usually

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**Figure 7.** Expression heatmap of \( \text{PvLBDs} \) in nine tissues, including flower buds, flowers, green mature pods, leaves, nodules, root, stem, young pods, and young trifoliate based on Phytozome database. Red and blue indicate high and low expressions, respectively.
far apart or even located on different chromosomes (Holub 2001). The duplicated genes remain in the plant genome and play an essential role in adaptation to the environment (Hanada et al. 2008; Jiang et al. 2010). In this study, 9 pairs of \( \text{PvLBD} \) genes were formed through fragment duplication and thus located on different chromosomes (Figure 5A). Furthermore, 24, 51, and 4 common bean \( \text{PvLBD} \) genes homologous to \( \text{Arabidopsis} \), soybean, and \( \text{Arabidopsis LBD} \) genes, respectively, were detected using a phylogenetic tree (Figure 5B). Gene duplication analysis did not detect any tandem duplication in \( \text{PvLBD} \) gene family, indicating that tandem duplication does not significantly affect the evolution of common bean \( \text{LBD} \) gene family, similar to other plant species (Lu et al. 2018; Chen et al. 2020). Therefore, fragment repetition may play a dominant role in the expansion of the \( \text{PvLBD} \) gene family. Gene replication and collinear analysis confirmed that although \( \text{PvLBD} \) genes did not undergo positive selection, they underwent strong purification selection, which was consistent with \( \text{LBD} \) genes in soybean (Yang et al. 2017) and maize (Zhang et al. 2014).

Cis-acting elements located in gene promoters play a crucial role in signal transduction and gene expression regulation. In this study, we found that the \( \text{PvLBD} \) promoters contain numerous motifs related to hormone response pathways, including those of gibberellin, abscisic acid, salicylic acid, and auxin (Figure 6). Thus, we concluded that \( \text{PvLBD} \) genes might participate in the plant growth regulation and stress response. In particular, the response elements of abiotic stress such as drought stress, low-temperature, anaerobic were found on \( \text{PvLBD} \) genes. Nonetheless, further molecular analysis should be performed to verify the specific expression patterns of different LBD genes.

LBD proteins regulate the development of plant branches and the formation of flowers, stems, leaves, and roots (Semiarti et al. 2001; Iwakawa et al. 2002; Majer et al. 2012; Cabrera et al. 2014; Yu et al. 2020a, 2020b). Tissue-specific expression of genes can provide insights into their functions in growth and development (Xiao et al. 2019). In this study, \( \text{PvLBD6/11/28} \) genes were highly expressed in the flowers and pods of common bean, implying involvement in the development of flowers and pods.

Figure 8. The expression abundances of 47 \( \text{PvLBDs} \) in common bean cotyledon (C), hypocotyl (H), and radical (R) under abiotic stresses. Gene expression abundances of common bean cotyledons, hypocotyls, and radicals grown under control conditions were set to 1 as the normalization in qRT-PCR analysis. The red and blue colors indicate up-regulation and down-regulation, respectively.
of reproductive organs (Figure 7). Furthermore, *PvLBD10* and *PvLBD45* were specifically expressed in the stem of common bean, suggesting that they may regulate the growth and development of common bean. *PvLBD3/21/23/26/44* genes were specifically expressed in roots, suggesting that these genes participate in root development. Further analysis showed that *PvLBD* genes in class I were highly expressed in specific tissues. This is similar to the findings of previous that showed that different LBD genes regulate plant growth in different periods and different tissues. For example, *PcLBD20/38* genes were up-regulated in bamboo shoots during rapid growth and development period, indicating that they are involved in the rapid growth and development of bamboo shoots (Huang et al. 2021). *AtLBD13* and *AtLBD16* are highly expressed in *A. thaliana* roots and regulate lateral root development (Cannon et al. 2004; Goh et al. 2019; Lee et al. 2019). RA2, an ortholog of LOR, can regulate reproductive growth and participate in maize florescence morphogenesis (Vollbrecht et al. 2005; Bortiri et al. 2006).

The accumulation of Cd, Ni, Hg, and other heavy metal ions due to climate change and modern industrial development has led to soil heavy metal pollution and significantly affected plant growth and production. In this study, Na⁺, Cd²⁺, and Hg²⁺ stresses regulated all *PvLBD* genes, of which 17% of the genes were up-regulated while 83% were down-regulated (Figure 8). These results indicate that *PvLBD* genes are extensively involved in various abiotic stress responses in common bean, similar to previous studies that showed that LBD genes regulate resistance to stress (Kong et al. 2017; Zhang et al. 2019; Wang et al. 2021). Notably, the expression levels of *PvLBD1/6/11/20/34/38/40/41* genes were up-regulated by all the three stress treatments, indicating that these genes were participate in response to multiple heavy metal stresses. Previous studies have also shown that LBD genes were differentially expressed under different abiotic stresses. For instance, *StLBD2-6* and *StLBD3-5* are up-regulated in potato under drought stress (Liu et al. 2019). In wheat, *Ta-6B-LBD81*, *Ta-4B-LBD51*, and *Ta-U-LBD90* are up-regulated under salt stress, *Ta-A-LBD40* and *Ta-4D-LBD62* are up-regulated under cold stress, while *Ta-2A-LBD13*, *Ta-2B-LBD15*, and *Ta-2D-LBD18* are up-regulated under drought stress (Wang et al. 2021). In this study, *PvLBD6* and *PvLBD11* were highly up-regulated under NaCl stress; *PvLBD1* and *PvLBD40* were highly up-regulated under CdCl₂ stress; *PvLBD20/31/34/38/41* were highly up-regulated under HgCl₂ stress (Figure 8). These results indicate that *PvLBD* genes play different roles in regulating different metal stress responses in the common bean.

**Conclusions**

In this study, 47 putative *PvLBDs* were identified in common bean and classified into two classes and thirteen subclasses. Gene structure and phylogenetic analysis showed that each subclass has a similar gene structure and motif composition, suggesting that *PvLBD* genes have remained conserved throughout evolution. Furthermore, the *PvLBD* gene family probably completed the genome expansion through the fragment repetition. The promoter of *PvLBD* contains a large number of cis-acting elements related to plant development, hormone and abiotic stress response. Tissue-specific expression pattern revealed that LBD genes have diverse functions and are involved in multiple tissue and developmental processes of common bean. Most LBD gene family members had significant responses to NaCl, CdCl₂, and HgCl₂ stresses. This study provides candidate genes for further assessing the function of *PvLBD* gene family in common bean, and insights for exploring the regulatory role of LBD genes on growth and stress resistance in common bean.

**Author contributions**

QZ, YZ and JD conceived and designed the experiments. YD, QZ, YG and WL performed the experiments; WL, JG, JZ and SL validated the data; QZ, XY, and SL performed the formal analysis; YD and QZ participated in original draft preparation; YD, QZ, YZ and JD revised the manuscript. All authors have read and agreed to the published version of the manuscript.

**Disclosure statement**

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.

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References

Ba J, Kuang J, Chen J, Lu W. 2016. MaJAZ1 attenuates the MaLBD5-mediated transcriptional activation of jasmonate biosynthesis gene MaAOC2 in regulating cold tolerance of banana fruit. J Agric Food Chem. 64(4):738–745.

Bailey TL, Boden M, Buske FA, Frith M, Grant CE, Clementi L, Ren J, Li WW, Noble WS. 2009. MEME SUITE: tools for motif discovery and searching. Nucleic Acids Res. 37:W202–W208.

Binney E, Clamp M, Durbin R. 2004. GeneWise and genomewise. Genome Res. 14:988–995.

Bortiri E, Chuck G, Vollbrecht E, Rocheford T, Martienssen R, Hake S. 2008. Turning a hobby into a job: how duplication of Arabidopsis Meloidogyne spp. provides a molecular link between lateral root and root-knot nematode feeding site development. New Phyol. 203:632–645.

Cabrera J, Díaz-Manzano FE, Sanchez M, Rosso MN, Melillo T, Goh T, Fukaki H, Cabo S, Hofmann S, Jenell F, Escobar C. 2014. For lateral organ boundaries-DOMAIN 16 during the interaction Arabidopsis-Meloidogyne spp. provides a molecular link between lateral root and root-knot nematode feeding site development. New Phytol. 13:1694–1708.

Conant GC, Wolfe KH. 2000. Turning a hobby into a job: how duplicated genes find new functions. Nat Rev Genet. 9:938–950.

Finn RD, Attwood TK, Babbitt PC, Bateman A, Bork P, Bridge AJ, Cerutti L, Chenna R, Clementi L, Cuff J, Cusack B, Deretic V, Eberhardt R, Eddy SR, Eufroid M, Fannon C, Feil R, Fink A, Finlayson B, Frost S, Frist U, Gremont JP, Guschanski K, Haft D, Heger A, Hettich R, Haussler D, Heideman J, Holm L, Hunter C, Iandolo J, Jiang HH, Joshi P, Kato H, Kjeldgaard M, Koonin E, Kritsman D, Kulp D, Lamesch P, Letunic I, Lepeniotis G, Letunic I, Li M, Li S, Liu P, Longin C, Lu S, Lu C, Lyche J, Ma A, McNally S, Megハウス D, McIndoo J, Maycox K, Mazumder S, McTigue DJ, Mitchell R, Miyata T, Modrek B, Montoya-Levina L, Murphy P, O’Donovan J, Ovchinenko S, Pauly B, Panopoulous-Afarellou G, Park M, Park Y, Perlmann T, Perroux-Franklin M, Philippakis AA, Pfeiffer T, Poon R, Prasad K, Pugin A, Reif J, Rice P, Raj B, Ranganathan R, Ribeiro A, Risso AG, Roelants M, Rosen N, Sahm H, Salzberg S, Simon C, Soding J, Sugasawa K, Suyama M, Thul R, Tate MG, Tatusova T, Triendl V, Vahed P, Vezzi F, Wang Z, Wilms A, Wolf A, Wootton JC, Xu D, Zhang P, Zhang J, Zhang K, Zhimulev I, Zhou Y, Zhu Z, Zunino V. 2013. Pfam: the protein families database. Nucleic Acids Res. 41:D222–D230.

Huang B, Huang Z, Ma R, Ramakrishnan M, Chen J, Zhang Z, Yrijäli K. 2021. Genome-wide identification and expression analysis of LBD transcription factor genes in moso bamboo (Phyllostachys edulis). BMC Plant Biol. 21:296.

Iwakawa H, Iwasaki M, Kojima S, Ueno Y, Soma T, Tanaka H, Semiarti E, Machida Y, Machida C. 2007. Expression of the ASYMMETRIC LEAVES2 gene in the adaxial domain of Arabidopsis leaves represses cell proliferation in this domain and is critical for the development of properly expanded leaves. Plant J. 51:173–184.

Iwakawa H, Ueno Y, Semiarti E, Onouchi K, Kojima S, Tsukaya H, Hasebe M, Soma T, Ikezaki M, Machida C, Machida Y. 2002. The ASYMMETRIC LEAVES2 gene of Arabidopsis thaliana, required for formation of a symmetric flat leaf lamina, encodes a member of a novel family of proteins characterized by cysteine repeats and a leucine zipper. Plant Cell Physiol. 43:467–478.

Jiang SY, Ma Z, Ramachandran S. 2010. Evolutionary history and stress regulation of the lectin superfamily in higher plants. BMC Evol Biol. 10:79.

Kong Y, Xu P, Jing X, Chen L, Li L, Li X. 2017. Decipher the ancestry of the plant-specific LBD gene family. BMC Genomics. 18(1):1–10.

Krzywinski M, Schein J, Biroli I, Connors J, Gascogne R, Horsman D, Jones SJ, Marra MA. 2009. Circos: an information aesthetic for comparative genomics. Genome Res. 19:1639–1645.

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin T, Wallace IM, Wilm A, Lopez R, et al. 2007. Clustal w and Clustal X version 2. Bioinformatics. 23:2947–2948.

Lee HW, Cho C, Pandey SK, Park Y, Kim MJ, Kim J. 2019. LBD16 and LBD18 acting downstream of ARF7 and ARF19 are involved in adventitious root formation in arabidopsis. BMC Plant Biol. 19(1):1–11.

Lee HW, Kim NY, Lee DJ, Kim J. 2009. LBD18/ASL20 regulates lateral root formation in combination with LBD16/ASL18 downstream of ARF7 and ARF19 in arabidopsis. Plant Physiol. 151:1377–1389.

Letunic I, Doerks T, Bork P. 2015. SMART: recent updates, new developments and status in 2015. Nucleic Acids Res. 43:D257–D260.

Li C, Zou X, Zhang C, Shao Q, Liu J, Liu B, Li H, Zhao T. 2016a. OsLBD3-7 overexpression induced adaxially rolled leaves in rice. Plant & Soil. 4118:1–11.

Li J, Meng X, Zong Y, Chen K, Zhang H, Liu J, Li J, Gao C. 2016b. Gene replacements and insertions in rice by intron targeting using CRISPR-Cas9. Nat Plants. 2:16139.

Li M, Zhang K, Sun Y, Cui H, Cao S, Yan L, Xu M. 2018. Growth, physiology, and transcriptional analysis of two contrasting carex rigescens genotypes under salt stress reveals salt-tolerance mechanisms. J Plant Physiol. 229:77–88.

Liu H, Cao M, Chen X, Ye M, Zhao P, Nan Y, Li W, Zhang C, Kong L, Kong N, et al. 2019. Genome-wide analysis of the lateral organ boundaries domain (LBD) gene family in Solanum tuberosum. Int J Mol Sci. 20. doi:10.3390/ijms20135630.

Liu H, Wang S, Yu X, Yu J, He X, Zhang S, Shou H, Wu P. 2005. ARL1, a LBD-domain protein required for adventitious root formation in rice. Plant J. 43:47–56.

Lu Q, Shao F, Macmillan C, Wilson IW, van der Merwe K, Hussey SG, Myburg AA, Dong X, Qiu D. 2018. Genomewide analysis of the lateral organ boundaries domain gene family in Eucalyptus grandis reveals members that differentially impact secondary growth. Plant Biotechnol J. 16:124–136.

Majer C, Hochholdinger F. 2011. Defining the boundaries: structure and function of LBD domain proteins. Trends Plant Sci. 16:47–52.

Majer C, Xu C, Berendzen KW, Hochholdinger F. 2012. Molecular interactions of ROOTLESS CONCERNING CROWN AND SEMINAL ROOTS, a LBD domain protein regulating shoot-borne root initiation in maize (Zea mays L.). Philosophical Transactions of the Royal Society of London 367(1595):1551–1565.

Matsumura Y, Iwakawa H, Machida Y, Machida C. 2009. Characterization of genes in the ASYMMETRIC LEAVES2/LATERAL ORGAN BOUNDARIES (AS2/LOB) family in Arabidopsis thaliana, and functional and molecular comparisons between AS2 and other family members. PLoS ONE. 5(8):525–537.

Mohammadi S, Pourakbar L, Siavash Moghaddam S, Popovic-Djordjevic J. 2021. The effect of EDTA and citric acid on biochemical processes and changes in phenolic compounds profile of okra ( Abelmoschus esculentus L.) under mercury stress. Ecotoxicol Environ Saf. 208:111607.

Naito T, Yang J, Kita T, Koizumi N, Mizuno T. 2007. A link between cytokinin and ASL9 (ASYMMETRIC LEAVES 2 LIKE 9) that belongs to the AS2/LOB (LATERAL ORGAN BOUNDARIES)
