Plant Science 301 (2020) 110683

Contents lists available at ScienceDirect

Plant Science

journal homepage: www.elsevier.com/locate/plantsci

CRISPR/Cas9 targeted mutagenesis of *SlLBD40*, a lateral organ boundaries domain transcription factor, enhances drought tolerance in tomato

Lun Liu [a], Jialong Zhang [a], Jiayi Xu [a], Yafei Li [a], Luqin Guo [a], Zhirong Wang [a], Xichun Zhang [b], Bing Zhao [a], Yang-Dong Guo [a,*], Na Zhang [a,*]

[a] *College of Horticulture, China Agricultural University, Beijing, 100193, China*
[b] *College of Plant Science & Technology, Beijing University of Agriculture, Beijing, 102206, China*

ARTICLE INFO

Keywords:
CRISPR/Cas9
Drought tolerance
Jasmonic acid
SlLBD40
Tomato

ABSTRACT

The LATERAL ORGAN BOUNDARIES DOMAIN (LBD)-containing genes are plant-specific genes that play important roles in lateral organ development. In this study, we identified *LBD40* (Solyc02g085910), which belongs to subfamily II of the LBD family of genes in tomato. *LBD40* was highly expressed in roots and fruit. *LBD40* expression was significantly induced by PEG and salt. Moreover, *SlLBD40* expression was induced by methyl jasmonate treatment, while *SlLBD40* expression could not be induced in the *jasmonic acid-insensitive1* (*jai1*) mutant or *MYC2*-silenced plants, in which jasmonic acid (JA) signaling was disrupted. These findings demonstrate that *SlLBD40* expression was dependent on JA signaling and that it might be downstream of *SlMYC2*, which is the master transcription factor in the JA signal transduction pathway. Overexpressing and CRISPR/Cas9 mediated knockout transgenic tomato plants were generated to explore *SlLBD40* function. The drought tolerance test showed that two *SlLBD40* knockout lines wilted slightly, while *SlLBD40* overexpressing plants suffered severe wilting. The statistical water loss rate and midday leaf water potential also confirmed that knockout of *SlLBD40* improved the water-holding ability of tomato under drought conditions. Taken together, our study demonstrates that *SlLBD40*, involved in JA signaling, was a negative regulator of drought tolerance and that knockout of *SlLBD40* enhanced drought tolerance in tomato. This study also provides a novel function of *SlLBD40*, which belongs to subfamily II of LBD genes.

1. Introduction

Water scarcity is one of the most destructive abiotic stressors in agriculture, as it seriously reduces crop productivity [1]. Drought greatly affects plant growth, weakens photosynthesis, accelerates the accumulation of reactive oxygen species, disturbs cellular homeostasis, and even causes death [1–3]. Roots are the primary organ responsible for drought stress, as they soak up water from soil. Photosynthetic production decreases in plants suffering from drought and is preferentially allocated to the roots, which improves the root/shoot ratio and helps roots absorb water from the soil [4]. Lateral root growth is inhibited to promote development of the primary root [5].

As a widely grown vegetable crop, tomato has gained popularity for its good nutrition and taste. However, the yield and quality of tomato are severely influenced by a myriad of abiotic stressors. As tomato is sensitive to water supply, drought is the primary growth limiting factor, particularly at the seed germination and seedling stages.

The LATERAL ORGAN BOUNDARIES DOMAIN (LBD) protein family encodes a conserved and plant-specific lateral organ boundaries (LOB) domain [6,7]. There are 42 LBD genes in the *Arabidopsis* genome and 46 in tomato, which have been assigned to two subfamilies [8,9]. In tomato, subfamily I is comprised of 40 genes consisting of a four-Cys motifs (C-motif), a Gly-Ala-Ser block (GAS-block), and a Leu zipper-like motif (L-motif), while subfamily II includes six genes only containing the C-motif [7,8]. It has been reported that the C-motif is required for the capacity to bind to the promoter region of downstream genes. The GAS-block appears to assist the C-motif in binding to the promoter region, and the L-motif is involved in protein-protein interactions [6,10,11].

LBD genes were initially found to be expressed in cells of the lateral organs, including the shoot apical meristem and lateral roots of *Arabidopsis* [7]. Subsequent studies reported that a number of LBD factors participate in the formation of lateral roots. For instance, the transcription factor AtLBD16, an auxin-inducible protein, targets *PUCHI* for

---

* Corresponding author at: College of Horticulture, China Agricultural University, 2# Yuanmingyuan West Road, Beijing 100193, China.
E-mail addresses: yaguo@cau.edu.cn (Y.-D. Guo), zhangna_cau@163.com (N. Zhang).

https://doi.org/10.1016/j.plantsci.2020.110683
Received 2 June 2020; Received in revised form 10 September 2020; Accepted 13 September 2020

lateral root initiation in *Arabidopsis* [12]. The LBD gene *OsARL1*, which is an auxin responsive gene, is required to initiate the formation of adventitious root primordia in rice [13]. All pollen is aborted in the *lbd10* and *lbd27 Arabidopsis* double mutants, indicating that *AtLBD10* and *AtLBD27* may play a critical role in pollen development [14]. Moreover, recent studies show that the LBD gene family also participates in the stress response. Expression of *VvLBD01*, *VvLBD02*, *VvLBD04*, *VvLBD08*, and *VvLBD18* in grape is involved in the responses to NaCl, mannitol, heat stress, and low temperature treatments [15]. In soybean, ninety LBD homologous genes were identified, among which the *GmLBD12* was induced by various stresses, including drought stress [16]. Coincidentally, proteomic analysis showed that, in rice, LBD proteins were downregulated in *Semi-rolled leaf1, 2* (*SRL1* and *SRL2*) mutant with increased drought tolerance during drought stress, indicating that LBD proteins may be involved in drought response in rice [17]. *AtLBD20* is a negative regulator that responds to Fusarium Wilt in *Arabidopsis*. Knockout of *AtLBD20* enhances tolerance to *Fusarium* infection, while overexpression of *AtLBD20* makes plants susceptible [18]. Nevertheless, the direct relationship between LBD factors and drought stress is unknown, and the effect of the LBD transcription factor family on abiotic resistance in tomato remains unknown as well.

The *jasmonic acid-insensitive1* (*jai1*) is a JA-insensitive mutant in tomato, which has lost the function of the tomato orthologue of CORONATINE-INSENSITIVE1 due to a 6.2-kb deletion and fails to express JA-responsive genes [19]. Therefore, many studies have been conducted using the mutant to explore the mechanisms involved in plant growth, development, and defense related to the JA signaling pathway [20–23]. In this study, the mutant was used to determine whether *SlLBD40* is involved in JA signaling in tomato. MYC2, a basic helix-loop-helix transcription factor, mediates various JA responses in the JA signaling pathway, including inhibition of root growth, apical hook formation in the dark, leaf senescence, and defense against herbivores and pathogenic fungi [24]. In this study, we detected *SlLBD40* expression in *SlMYC2*-silenced plants, which were obtained by virus-induced gene silencing (VIGS), to explore whether *SlLBD40* expression was affected by *SlMYC2*.

VIGS is an RNA-mediated post-transcriptional gene silencing method. It functions as an antivirus defense mechanism to downregulate gene expression in plants [25]. It is a widely used reverse and forward-genetics method to explore gene function because of its ability to rapidly degrade mRNA of the target gene, and is simple to manipulate. VIGS has been successfully used in many species, including eggplant [26], pepper [27], strawberry [28], sweet cherry [29], cotton [30], barley [31], and potato [32]. In our previous studies, VIGS was used to verify the function of *SlHSP40* in tomato [33]. VIGS has been employed to verify the heat tolerance function of *SoHSC70*, since it is difficult to study gene function using the transgenic method in spinach [34]. VIGS makes it possible to study gene function *in situ*. Furthermore, a VIGS cDNA library has been applied during fast-forward genetic screens for genes of various physiological responses. This high-throughput approach provides a large-scale phenotypic analysis and simple, fast identification of the gene responsible for the phenotype of interest [35, 36]. In this study, we silenced *SlMYC2* in tomato plants by VIGS and detected *SlLBD40* expression in *SlMYC2*-silenced plants to explore the upstream and downstream relationships between *SlLBD40* and *SlMYC2*.

Here, we showed that *SlLBD40*, which belongs to subfamily II of the LBD family, was highly expressed in tomato roots and notably induced by PEG, salt, and methyl jasmonate (MeJA) treatments. Moreover, *SlLBD40* was dependent on JA signaling and it might be downstream of *SlMYC2*. An overexpression and gene editing study showed that *SlLBD40* functions as a negative regulator of drought tolerance. Knocking out *SlLBD40* by CRISPR/Cas9 improved water-holding ability and enhanced drought tolerance in tomato.

2. Materials and methods

2.1. SlLBD40 cloning and sequence analysis

We obtained the full-length open reading frame of *SlLBD40* (Solyc02g085910) in the Sol Genomic Network database (https://solgenomics.net). The reference genome used for the sequence is Heinz1706 (version SL4.0). Then, we cloned it via the real time-polymerase chain reaction (RT-PCR). The primer pair was designed by Primer Premier 5 according to the *SlLBD40* cDNA sequence. The phylogenetic tree was constructed by the neighbor-joining (NJ) algorithm using the MEGA program (ver. 5.0).

2.2. Plant materials and stress treatments

All tomato seedlings (*Solanum lycopersicum* L. cv. Micro Tom) including the wild-type (WT) and all transgenic lines were cultivated in growth chambers under 16 h light and 8 h dark conditions at 25℃/18℃, 70 % relative humidity, and 200 μmol m^{-2} s^{-1} light intensity.

We detected the expression of the LBD subfamily II genes in various tissues. The tomato seedlings were grown hydroponically. The roots, flower buds, leaves, flowers, mature green fruits, and red fruits were sampled from the 10-week-old seedlings. Three biological replicates were performed for each tissue.

We applied Yamazaki nutrient solution with 10 % PEG or 150 mM NaCl to 4-week-old WT seedlings for the dehydration and salt stress treatments in a hydroponic system, respectively. The roots of these plants were collected at specific times (0, 1, 3, and 6 h for the PEG treatment and 0, 1, 3, 6, 9, and 12 h for the NaCl treatment).

The *jai1* mutants used in this study were homozygous plants. As homozygous *jai1* plants are sterile, we obtained homozygous *jai1* mutant plants by identifying the heterozygous progeny. Homozygous *jai1* plants were identified as described by Li et al. [19] (Fig. S1).

For MeJA and drought treatment, tomato seedlings were grown in pots 12 cm in diameter with soilless media (PINDSTRUP, Denmark). MeJA (100 μM) was sprayed on the leaves of 3-week-old tomato seedlings and the leaves were collected at specific times (0, 0.5, 1, 3, 6, and 12 h). Each sample was collected with three biological replicates.

Three-week-old tomato plants from the *SlLBD40* knockout line, as well as *SlLBD40*-OE-3 and WT plants were used for the drought stress tolerance experiments. The water supply to the tomato plants in the experimental groups was stopped for 10 days. Then, the degree of wilting in the different lines was observed and recorded with photographs.

The 17-day-seedlings of tomato were grown in soil and were divided into two groups. One group as the control was under well-watered conditions. The water supply to the other group was stopped. After 7 days, when the difference was showed in various lines, the seedlings were used to measure the stomatal conductance, MDA content, Fv/Fm ratios and midday leaf water potential.

2.3. Total RNA isolation and qRT-PCR analysis

Total RNA was extracted using TRIzol Reagent (Ambion, Austin, TX, USA) according to the manufacturer's instructions, and DNase I (Ambion) was used to remove the genomic DNA residue. Next, 1 μg total RNA was used to synthesize cDNA using the Prime Script™ RT Reagent Kit (Takara, Kyoto, Japan). We examined relative gene expression levels by qRT-PCR on a LightCycler480 machine (Roche Diagnostics, Rotkreuz, Switzerland) using SYBR Premix Ex Taq polymerase (Takara), and we employed the tomato *SlEFα* gene (*SlEFα,* Solyc06g005060) as the internal reference gene. The $2^{-\Delta\Delta CT}$ method was used to analyze the results [37,38]. Three biological replicates were performed for each sample. All primers used for qRT-PCR are listed in Table S1.

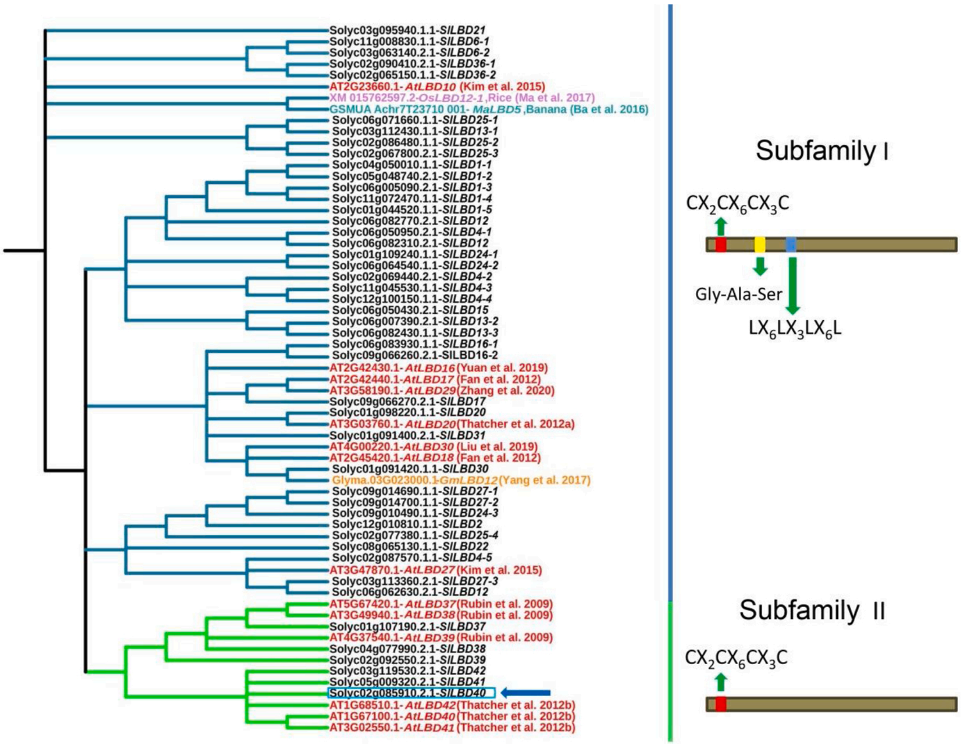

Fig. 1. Phylogenetic tree of LBD family genes in tomato and reported LBD genes in the other species, and the conserved domain of LBD family genes. The blue arrow is pointing towards *SlLBD40*.

2.4. Subcellular localization of the SlLBD40 protein

The coding sequence (CDS) of *SlLBD40* without a stop codon was amplified and cloned into pCAMBIA-1302 to form the 35S::LBD40-GFP fusion construct. The construct and the control vector were transformed in *Agrobacterium tumefaciens* strain GV3101 and injected into 4-week-old tobacco leaves [39]. Green fluorescent protein (GFP) was observed under a universal fluorescence microscope after 2 days cultivation of these transformed tobacco plants.

2.5. VIGS assay for tomato

The tobacco rattle virus (TRV) based vectors pTRV1 and pTRV2 were used to silence the *SlMYC2* tomato gene. A 283 bp fragment of *SlMYC2* CDS was amplified using PCR and was cloned into pTRV2 to generate pTRV2-*MYC2*. Then, pTRV1, pTRV2, and pTRV2-*MYC2* were transformed into GV3101, respectively. The mixture of pTRV1 and pTRV2-*MYC2* was carried out on the Micro Tom germline following a protocol described previously [40]. A mixture of pTRV1 and pTRV2 empty was used as control.

2.6. Construction of the overexpression vector and plant transformation

The CDS of *SlLBD40* was amplified via PCR and was cloned into pCAMBIA1305 (Invitrogen, Carlsbad, CA, USA) to construct the 35S::*SlLBD40*-3×FLAG fusion protein vectors in the overexpressing lines. The constructs were inserted into *A. tumefaciens* strain GV3101 and transformed into the Micro Tom cotyledon to obtain regeneration plants. T_2 generation plants showing no segregation were used in further experiments, and the WT lines were used as control.

2.7. Generation of SlLBD40 knockout lines using CRISPR/Cas9 technology

Single-guide RNA (sgRNA) target of the *SlLBD40* coding region was designed and synthesized as shown in Fig. 6 and Table S1. The vectors used for CRISPR were constructed according to the methods described by Yan et al. [41]. We inserted the construct into *A. tumefaciens* strain GV3101 and transferred it to the Micro Tom cotyledon to obtain regeneration plants. Finally, we performed PCR and Sanger sequencing to screen the target knockout lines of the T_2 generation.

2.8. Protein extraction and western blot

Proteins were extracted from tomato leaves of the WT and *SlLBD40*-OE-3. The leaves were ground to a powder in liquid nitrogen using a protein extraction buffer (50 mM HEPES at pH 7.5, 150 mM KCl, 1 mM EDTA, 0.5 % Triton X-100, 1 mM DTT, and 0.2 mM PMSF). The mixture was chilled on ice for 30 min and then centrifuged at 12,000 × g for 30 min at 4℃. Then, the supernatant and loading buffer were transferred to a new tube and boiled for 10 min. The proteins were separated by 10 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a polyvinylidene fluoride membrane (Millipore, Billerica, MA, USA). The membrane was blocked in 5 % nonfat milk in TBST buffer containing 20 mM Tris−HCl (pH 8.0), 150 mM NaCl, and 0.1 % Tween 20 at room temperature for 1 h. The membrane was incubated with primary antibody overnight at 4℃, and washed five times in 1 × TBST (5 min each). The corresponding secondary antibody (CWBIO, Beijing, China) was incubated at a 1:10,000 dilution for 2 h at room temperature. After five washes with 1 × TBST (5 min each), the Immobilon Western Chemiluminescent HRP Substrate (CWBIO) was used to visualize the proteins. A Tanon 5500 chemiluminescence imaging system was used to detect the chemiluminescence signals.

2.9. Analysis of water loss rate, stomatal conductance, MDA content, Fv/Fm ratios and midday leaf water potential

Leaves were obtained from the WT, *SlLBD40*-OE-3, CR-*LBD40*-2, and CR-*LBD40*-8 plant lines and dried at 55℃. The weight of every sample was recorded every half hour for 3 h. The water loss rate was measured

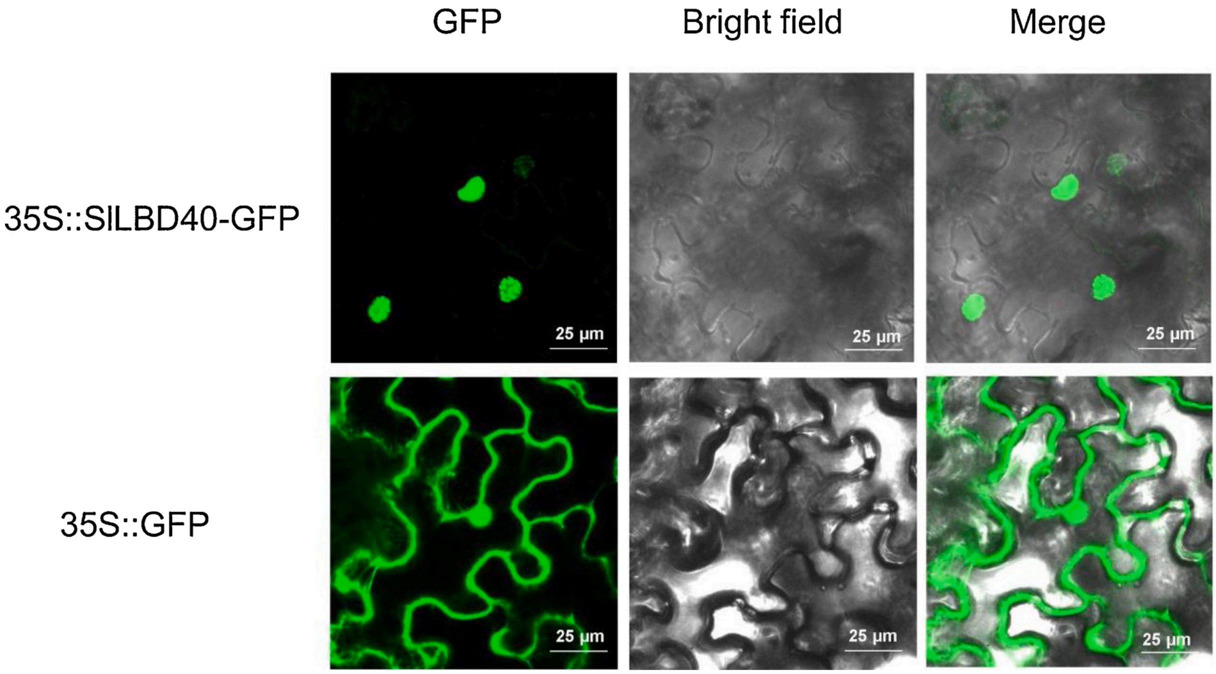

Fig. 2. Subcellular localization of SlLBD40 in cell nucleus. Tobacco leaves were used for subcellular localization. Green fluorescence images were taken in a dark field, while the outline of the cell was photographed in a bright field. 35S: *SlLBD40*-GFP represents SlLBD40 and GFP fusion protein. 35S:GFP represents the control. Bars =25 μm.

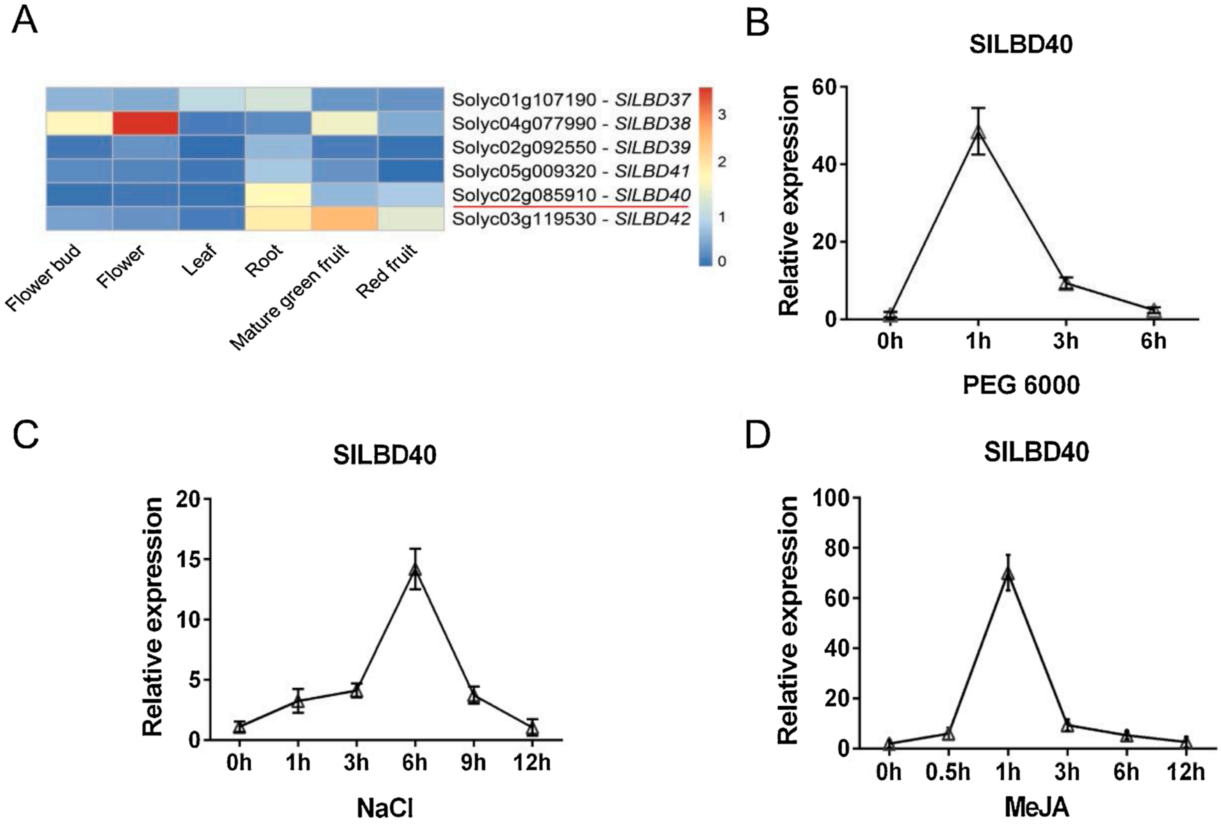

Fig. 3. The expression patterns of LBD genes in subfamily II in various tomato tissues and SlLBD40 was induced by PEG 6000 and NaCl. (A) Expression analysis of LBD genes in subfamily II in various tomato tissues. These plants were grown in a hydroponic system. (B) and (C) Responses of *SlLBD40* in root at different point to PEG 6000 and NaCl. (D) Responses of *SlLBD40* in leaves at different time point of MeJA treatment. *SlEFα* gene was used as the internal control. Bars represent ± SD of three biological replicates.

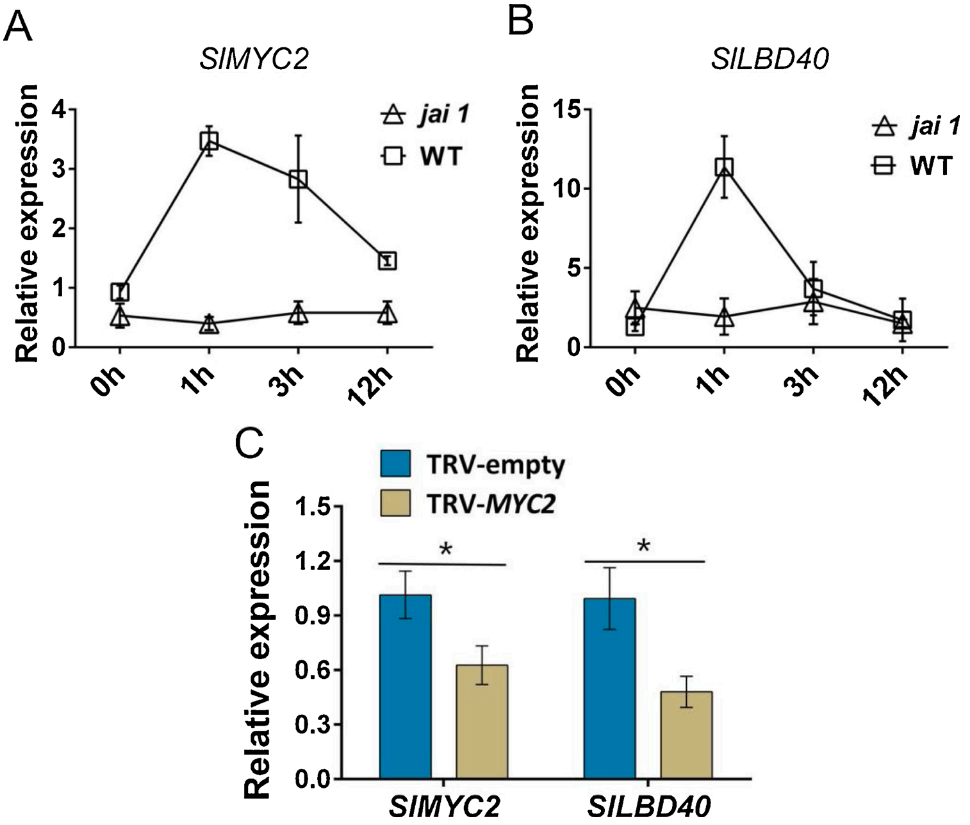

Fig. 4. *SlLBD40* is dependent on JA signaling. (A, B) The expression of *SlMYC2* and *SlLBD40* in WT and *jai1* mutants. (C) The expression of *SlLBD40* was repressed in *SlMYC2*-silenced plants. The expression of *SlLBD40* in TRV- *SlMYC2* and TRV-empty tomato plants was analysis by qRT-PCR. *SlEFα* gene was used as the internal control. Values are means ± SD of three independent replicates. Asterisks indicate $p < 0.05$.

according to the method of Mao et al. [42]. The method used to analyze MDA was described by Zhang et al. [43]. Leaf stomatal conductance was measured on sunny days at 10:00–13:00 on three leaves each plant using LI-6400 XT infrared gas analyzer (LI-Cor Biosciences, USA) [44]. The method used to analyze the Fv/Fm values was described by Qi et al. [45]. Three leaves from per plant were measured and three biological replicates were performed for each line. The midday leaf water potential was measured by Psypro Dewpoint Potentia Meter (USA) according to the method of Marchin et al. [46] with some modification.

3. Results

3.1. SlLBD40 gene sequence analysis

The sequence of the *SlLBD40* (Solyc02g085910) clones from tomato had an ORF of 816 nucleotides encoding a polypeptide containing 272 amino acid residues. The *SlLBD40* protein belonged to the LOB domain-containing protein family with a highly conserved N-terminal domain. *SlLBD40* only contained a typical LOB consisting of four conserved Cys motifs of $CX_2CX_6CX_3C$ and belonged to subfamily II. In Fig. 1, we aligned the tomato LBD family amino sequences with the reported LBDs in *Arabidopsis* [14,18,47–52], soybean [16], banana [53] and rice [54]. The result showed that *SlLBD40* had a closer relationship with *AtLBD40*, *AtLBD41* and *AtLBD42* [52] in *Arabidopsis* (Fig. 1). *AtLBD40* and *AtLBD41* were reported to respond to multiple pathogens [52].

3.2. Subcellular localization of the SlLBD40 protein

Transient expression assays in *Nicotiana benthamiana* leaves and fluorescence microscopy were used to investigate the subcellular localization of SlLBD40. We constructed a *SlLBD40*-GFP fusion protein vector driven by the CaMV35S promoter. The fusion vector and GFP control were introduced into *N. benthamiana* leaf epidermal cells via *Agrobacterium*-mediated infiltration. The results showed that the fluorescent signals from *SlLBD40*-GFP were detected in the nucleus, while the GFP control signals were distributed in plasma, cytoplasm and nucleus (Fig. 2), indicating that SlLBD40 was localized in the nucleus.

3.3. SlLBD40 expression pattern

To investigate the physiological significance of *SlLBD40*, we examined the expression pattern of all genes in LBD subfamily II in various tomato tissues by qRT-PCR. The results showed that *SlLBD40* mainly expressed in root, while Solyc04g077990 mainly expressed in flower. Solyc03g119530 expressed highly in both root and fruit (Fig. 3A). Tissue expression patterns suggest differences in the function of these genes.

Plant roots are closely associated with water absorption. We speculated that the present gene may be involved in plant resistance to water stress. Thus, we investigated whether *SlLBD40* expression was induced by abiotic stressors and some stress-related hormones. As shown in Fig. 3B, C, the relative expression of *SlLBD40* increased 46-fold after 1 h of PEG treatment and 14-fold after 6 h of salt treatment. *SlLBD40* expression was significantly upregulated at 0.5 h by the MeJA treatment (Fig. 3D). These results suggest that *SlLBD40* is PEG, salt, and MeJA inducible, and may be involved in the responses to abiotic stress and JA signaling in tomato.

3.4. SlLBD40 expression is dependent on JA signaling

To assess whether the role of *SlLBD40* is dependent on JA signaling in tomato, WT plants and the *jai1* mutant, which harbors a mutation in the tomato homolog of *Arabidopsis COI1* [19], were treated with MeJA. We investigated the expression of *SlMYC2* and *SlLBD40* before treatment and at different times after treatment. The results showed that *SlMYC2*

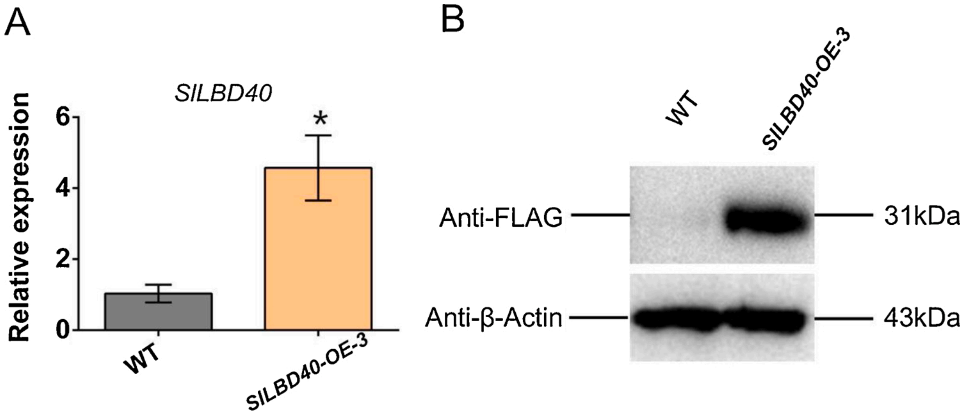

Fig. 5. The overexpression lines of *SlLBD40*. (A) The expression of *SlLBD40* in *SlLBD40*-OE-3 was significantly higher than that of WT. Values are means ± SD of three independent replicates. Asterisks indicate $p < 0.05$. (B) The FLAG protein was investigated in *SlLBD40*-OE-3 plants but not in WT. The β-Actin was detected in *SlLBD40*-OE-3 plants and WT.

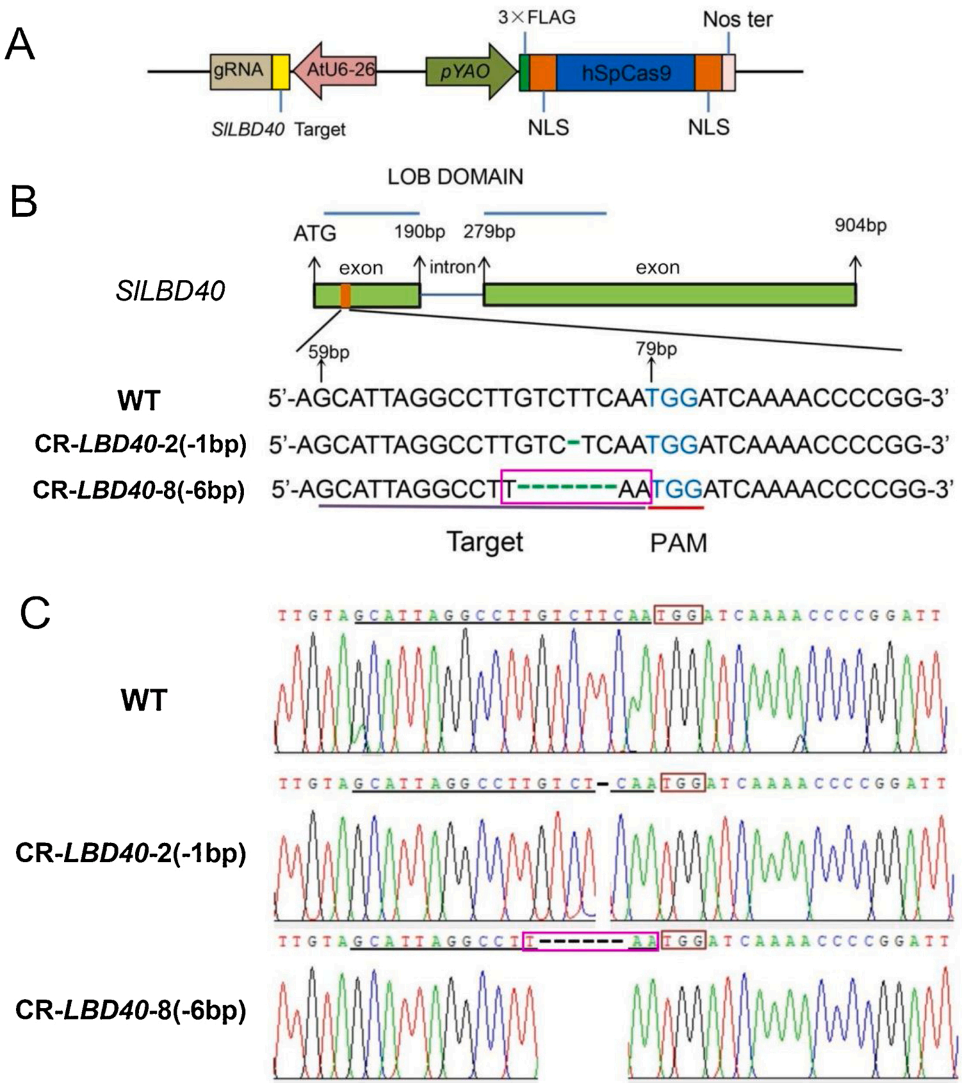

Fig. 6. The gene editing construct and editing detail of two sllbd40-mutants. (A) The construct used in CRISPR/Cas9 technology. (B) Gene editing analysis of CR-*LBD40*-2 and CR-*LBD40*-8. Target site and editing detail were showed. Blue letters represent the protospacer adjacent motif (PAM). Blue lines represent the conserved LOB domain. (C) The analysis of the sequences reveals 2 types of mutations, either 1 or 6 bp deletions. The 6 bp deletion in CR-*LBD40*-8 caused a termination codon, which was marked with pink box in B, C.

and *SlLBD40* expression was induced by MeJA in WT plants. However, *SlLBD40* expression was not induced by JA in *jai1* mutant plants (Fig. 4A, B). This result demonstrates that *SlLBD40* is dependent on JA signaling.

Moreover, we obtained *SlMYC2*-silenced plants via the VIGS system. We examined *SlLBD40* expression in *MYC2*-silenced plants and WT plants to determine whether *LBD40* expression was affected by *MYC2*. The plants infected with TRV-empty were the control group. *SlMYC2*

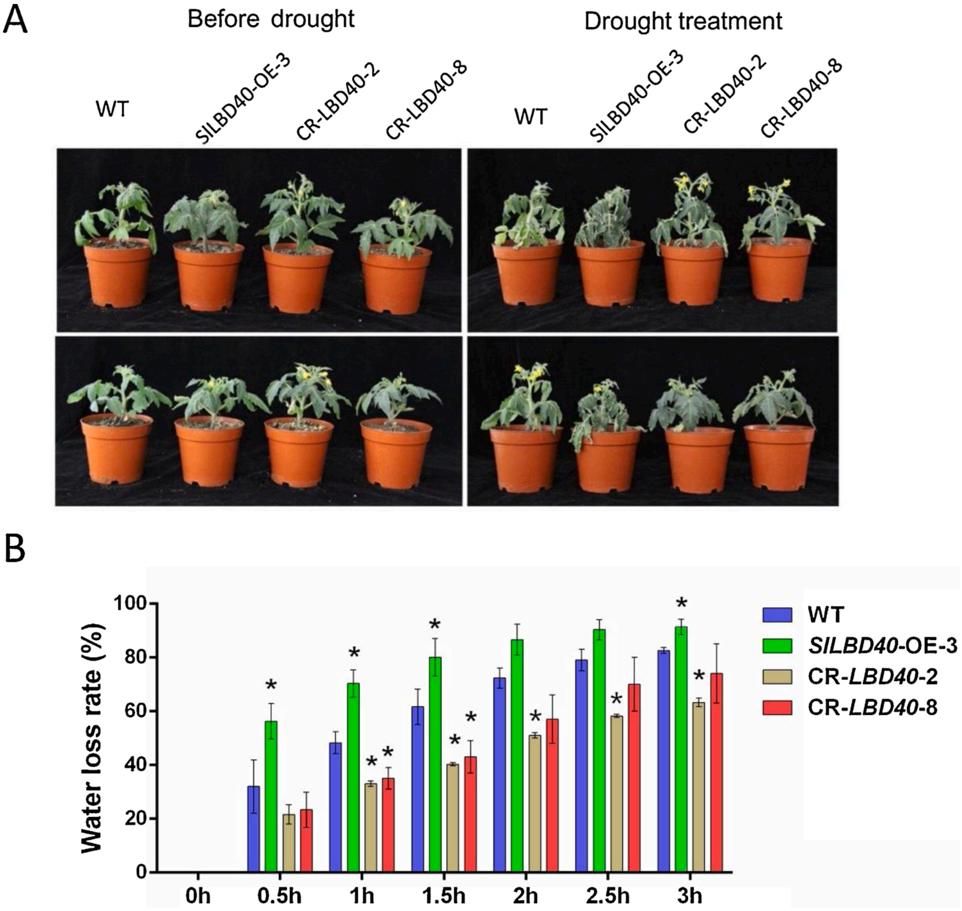

Fig. 7. Effects of CR-*LBD40*-2, CR-*LBD40*-8 and *SlLBD40*-OE-3 on drought resistance. (A) Phenotype of wild-type (WT), CR-*LBD40*-2, CR-*LBD40*-8 and *SlLBD40*-OE-3 plants under control and drought treatment. (B) Water loss rate of all lines. Values are means ± SD of three independent replicates. Asterisks indicate $p < 0.05$.

expression decreased significantly in plants injected with TRV-*MYC2*. *SlLBD40* expression was also downregulated in *MYC2*-silenced plants than that injected with TRV-empty plants (Fig. 4C). This result suggests that *SlLBD40* is involved in *MYC2* transcriptional regulation and possibly as a downstream gene of *SlMYC2*.

3.5. Overexpressing and knockout lines were obtained via stable Agrobacterium-mediated transformation

To further explore whether *SlLBD40* contributes to abiotic stress resistance in tomato, three *SlLBD40*-FLAG overexpressing lines were generated under control of the 35S promoter via *Agrobacterium*-mediated transformation. As shown in Fig. 5A, *SlLBD40* expression in line OE-3 was significantly higher than that in the WT. To further confirm *SlLBD40* protein translation in the overexpressing lines, we examined the SlLBD40-FLAG fusion protein expression level in OE-3 and WT plants by western blot using the FLAG-antibody. The FLAG protein was used in the OE-3 line but not in the WT (Fig. 5B). These results suggest that *SlLBD40* was overexpressed in the OE-3 line. Thus, we selected the OE-3 line for further study.

Moreover, we generated *SlLBD40* knockout mutants in the tomato cultivar Micro Tom using CRISPR/Cas9 gene editing technology with Cas9 endonuclease expression controlled by the *YAO* promoter [41] (Fig. 6A). As shown in Fig. 6B, sgRNA was designed to specifically target the *SlLBD40* CDS regions. Two T_1 transgenic lines (CR-*LBD40*-2 and CR-*LBD40*-8) were genome edited and the editing details of all target sites are shown in Fig. 6B, C. We found one-nucleotide deletion at position 74 (or 75) in the CR-*LBD40*-2 coding region and this deletion caused frame shifts, resulting in premature termination of *SlLBD40* translation. There is a six-nucleotide deletion from 71th bp to the 76th bp in the CR-*LBD40*-8 line, causing an amino acid deletion and a new codon “TAA” formation, which is exactly a termination codon. The detail is showed in the following Fig. 6B, C. Therefore, the translation of LBD40 protein in the *CR-LBD40−8* line is premature terminated. CR-*LBD40*-2 and CR-*LBD40*-8 were selected for further evaluation of abiotic stress tolerance.

3.6. Overexpressing lines showed decreased tolerance to drought stress and the knockout lines showed increased tolerance to drought

SlLBD40-OE-3, CR-*LBD40*-2, CR-*LBD40*-8, and the WT lines were used for the drought tolerance evaluation. Under normal growth conditions in soil, no visibly different growth performance was observed between the WT and *SlLBD40*-OE-3, CR-*LBD40*-2, or CR-*LBD40*-8 lines. However, water was withheld for 10 d from all of these 3-week-old plants. The CR-*LBD40*-2 and CR-*LBD40*-8 plants exhibited slight wilting, and the OE-3 line exhibited severe wilting and leaf rolling (Fig. 7A).

Furthermore, the water loss rates of the WT, OE-3, CR-*LBD4*0-2, and CR-*LBD40*-8 plants were measured to evaluate drought response. The average water loss rate of WT plants was significantly higher than that of the CR-*LBD40*-2 and CR-*LBD40*-8 plants, but significantly lower than that of *SlLBD40*-OE-3 plants. For example, at 1 h point, the average water loss rate of WT plants was 49 %, while SlLBD40-OE-3 plants lost 70 % water, which was significantly higher than WT. Meanwhile CR-*LBD40*-2 and CR-*LBD40*-8 plants only showed 33 % and 35 % water loss respectively.

The data of leaf stomatal conductance, MDA content, Fv/Fm ratios, and midday leaf water potential did not show significant differences

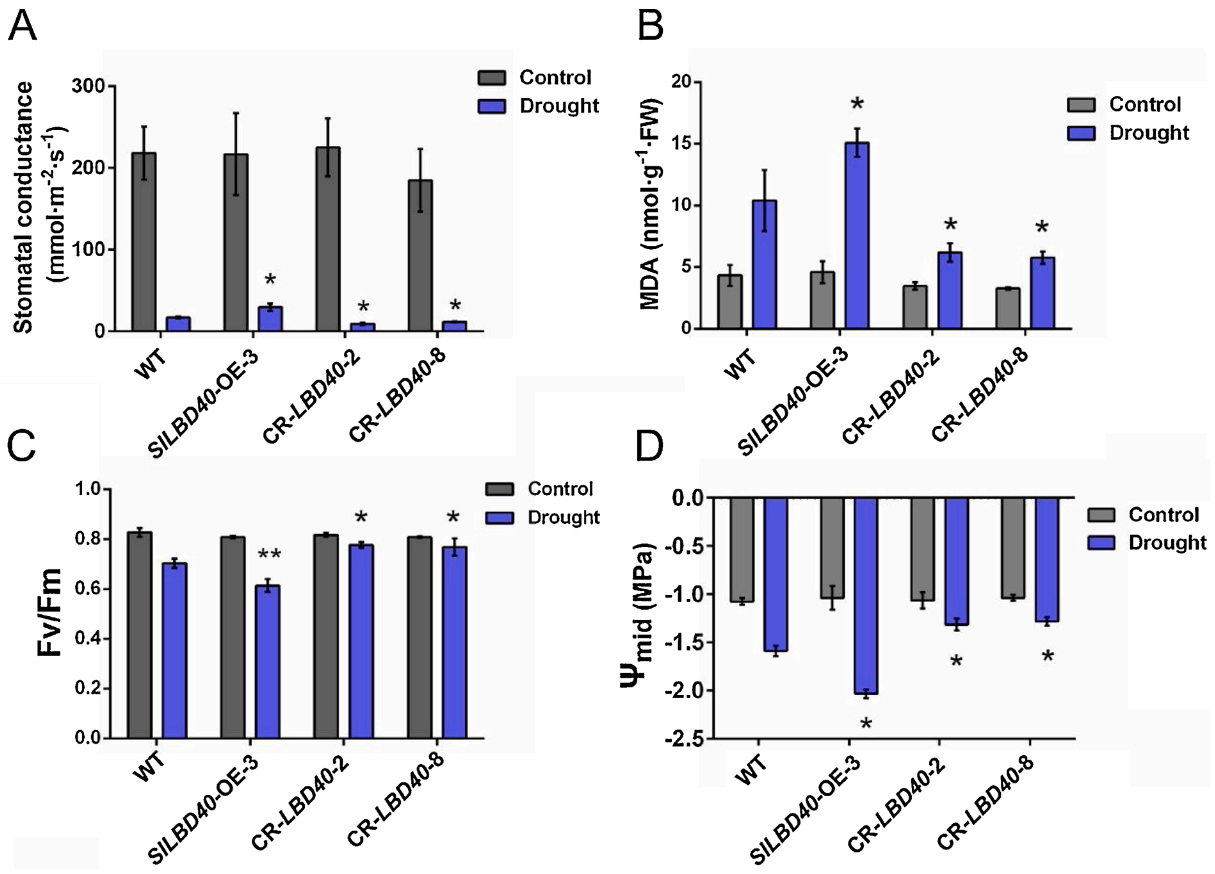

Fig. 8. Differences in stomatal conductance (mmol·m^{-2}·s^{-1}) (A), MDA content (B), Fv/Fm (C) and midday leaf water potential (Ψ_{mid}, MPa) (D) between control and drought treatment of wild-type (WT), *SlLBD40*-OE-3, CR-*LBD40*-2 and CR-*LBD40*-8 plants. Values are means ± SD of three independent plants as replicates. Asterisks indicate $p < 0.05$ and double asterisks indicate $p < 0.01$.

among the WT, OE-3, CR-*LBD40*-2, and CR-*LBD40*-8 plants under well-watered condition. However, after drought treatment for 5 days, leaf stomatal conductance showed significantly difference among WT, CR-*LBD40*-2, CR-*LBD40*-8 and *SlLBD40*-OE-3 plants. The average leaf stomatal conductance of *SlLBD40*-OE-3 plants was 70 % higher than that of WT plants. The leaf stomatal conductance of CR-*LBD40*-2 and CR-*LBD40*-8 plants were 45 %, 31 % lower than WT plants, respectively (Fig. 8A). MDA content usually reflects the damage degree of plant cell membrane. The higher MDA content reflects the more serious damage of plant cell membrane. MDA content in *SlLBD40*-OE-3 plants was significantly higher than that of WT plants. MDA content of CR-*LBD40*-2, CR-*LBD40*-8 plants were significantly lower than that of WT plants (Fig. 8B). Fv/Fm ratios of *SlLBD40*-OE-3 plants were significantly lower than that of WT plants. Fv/Fm ratios of CR-*LBD40*-2 and CR-*LBD40*-8 plants were significantly higher than that of WT plants (Fig. 8C), which indicated that knockout of *SlLBD40* alleviated the damage of the photosynthetic system of tomato seedling under drought stress. The midday leaf water potential of CR-*LBD40*-2 and CR-*LBD40*-8 plants was significantly higher than that of WT plants. And, midday leaf water potential of *SlLBD40*-OE-3 plants was significantly lower than that of WT plants (Fig. 8D), which indicated that overexpression of *SlLBD40* caused more water loss than WT plants. These results showed that knockout of *SlLBD40* enhanced drought tolerance in transgenic tomatoes.

4. Discussion

JA signaling plays key roles in plant defense responses to abiotic stress. In *Arabidopsis*, Hu et al. [55] reported that jasmonate significantly enhances plant freezing tolerance by regulating *CBF/DREB1* expression. It has also been reported that JA plays an important role in the tolerance to a combination of high light and heat stress [56]. In tomato, *SlMYC2* functions in MeJA-induced chilling tolerance [57]. The response to salt stress by the accumulation of protease inhibitors is dependent on JA [58]. In our study, *SlLBD40* was highly induced by the MeJA treatment. Moreover, transgenic plants overexpressing *SlLBD40* had weakened drought tolerance. These results suggest that *SlLBD40* may participate in drought resistance, which is associated with JA signaling in tomato.

LBD genes participate in JA-mediated responses. In *Arabidopsis*, *AtLBD20* is regulated by JA signaling and acts as a negative regulator of the JA-regulated genes *VEGETATIVE STORAGE PROTEIN2* and *THIONIN2.1* [18]. In banana, *MaLBD5* is induced by MeJA and cold treatment. The interaction between *MaLBD5* and *MaJAZ1* attenuates transcriptional activation of *MaLBD5* to JA biosynthetic genes. These results show that *MaLBD5* is involved in MeJA-mediated cold tolerance in banana fruit [53]. In this study, *SlLBD40* may act downstream of *SlMYC2* and be involved in JA signaling according to the *jai1* mutant and VIGS results. The *jai1* mutant was used to verify that *SlMYC2* was induced by MeJA, which depends on Jai1/COI1 [59]. The *coi1−1* mutants have been used in *Arabidopsis* to demonstrate that *AtCBF/DREB1* and their target genes are regulated by jasmonate [55]. These studies suggest that *jai1/coi1* mutants can be widely used to verify whether a gene is in the JA signaling pathway. VIGS has been used to determine that *MeHsf3* regulates the transcripts of *Enhanced Disease Susceptibility 1* and *pathogen-related gene 4* in cassava [60]. The loss of function of *CabZIP53* by VIGS significantly hampers the expression of immunity and thermotolerance-associated genes in pepper [61]. These results suggest that the VIGS system is very effective for verifying upstream or downstream relationships and gene functions.

As plant-specific transcription factors, LBD proteins play key roles in diverse biological processes, including lateral root growth, auxin-induced callus formation, pollen development, and the pathogen response [12,14,18,62]. Ariel et al. [63,64] reported that *MtLBD1* is repressed by salt stress and abscisic acid, and induced by auxin during lateral root formation. Several LBD genes in *Sorghum bicolor* are significantly induced by salt and drought [65]. Yang et al. [16] reported that LBD subfamily II genes in soybean, including *GmLBD5*, *GmLBD23*, *GmLBD45*, *GmLBD51*, *GmLBD70*, and *GmLBD77*, responded to PEG, NaCl, and cold treatment. *GmLBD12* and *GmLBD74*, belonging to subfamily I, also responded to these three treatments. In rice, *OsLBD12−1* expression was induced by salt stress. *lbd12−1* loss-of-function mutants exhibited larger shoot apical meristem size than that of WT under salt stress [54]. Liao et al. [17] reported that the rolled leaf rice mutant exhibited enhanced tolerance to drought. Proteomic analysis showed that four LBD protein, Q8L3S3 (OsLBD-like), Q7XGL4 (OsLBD31), Q852M3 (OsLBD4) and Q8L4M5 (OsLBD), were significantly down-regulated in the rolled leaf mutant plants. These LBD genes may function as negative regulators of drought tolerance in rice.

We noticed that an RNA-seq experiment result showed that the expression of *SlLBD40* did not show a significant change after drought treatment in tomato [66]. In that research, the RNA samples were extracted from leaves of tomato in Dr1 (16 d of irrigation withholding), RW (7 days of irrigation), and Dr2 (6 d of irrigation withholding). The drought treatment used in that study was a moderate and long-time process which is different from the method in our study. In our study, PEG was used to simulate drought treatment. We tested *SlLBD40* expression in roots in the shorter time course evaluation. The expression of *SlLBD40* increased significantly at 1 h after PEG treatment, suggesting that *SlLBD40* should be an early response gene to drought.

The function of genes in subfamily I of the LBD family has been widely reported. Most of these studies focused on development of plant organs and tissues. *AtLBD16, AtLBD18,* and *AtLBD29* regulate later root development by regulating expression of the *AtPR-1* homolog *AtPRH1* [47]. Also, *AtLBD16* and *AtbZIP59* constitute a complex that regulates changes in auxin-induced cell fate during callus formation [62]. However, only two studies have reported the functions of genes in subfamily II. *AtLBD37, AtLBD38*, and *AtLBD39* negatively regulate anthocyanin biosynthesis and nitrogen availability in *Arabidopsis* [50]. Overexpression of *OsLBD37* and *OsLBD38* delays the heading date and increases yield [67]. The function of drought tolerance by *SlLBD40* determined in our study provides a novel role for genes in LBD subfamily II.

Author contributions

L L, B Z, N Z and Y-D G conceived this project and designed the research. L L, J-L Z and J-Y X performed most of the experiments. Y-F L, L-Q G and Z-R W participated in this work. X-C Z and L L analyzed the data. N Z and L L wrote the article. All authors discussed the manuscript.

Declaration of Competing Interest

The authors declare that they do not have a conflict of interest.

Acknowledgements

We thank Professor Chuanyou Li (Institute of Genetics and Developmental Biology, Chinese Academy of Sciences) for sharing the seeds of *jai1* mutants. This work was supported by the grants to Zhao B (The National Key Research and Development Program of China, 2019YFD1000300) and to Guo Y-D (BAIC07, CEFF-PXM2019-014207-000032). We also thank the support from Engineering Research Center of Breeding and Propagation of Horticultural Crops and Beijing Key Laboratory of Growth and Developmental Regulation for Protected Vegetable Crops.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.plantsci.2020.110683.

References

[1] A. Gupta, A. Rico-Medina, A.I. Caño-Delgado, The physiology of plant responses to drought, Science 368 (2020) 266–269.
[2] J. Zhu, Abiotic stress signaling and responses in plants, Cell 167 (2016) 313–324.
[3] D.W. Lawlor, G. Cornic, Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants, Plant Cell Environ. 25 (2002) 275–294.
[4] M. Yamaguchi, R.E. Sharp, Complexity and coordination of root growth at low water potential: recent advances from transcriptomic and proteomic analyses, Plant Cell Environ. 33 (2010) 590–603.
[5] L. Xiong, R. Wang, G. Mao, J.M. Koczan, Identification of drought tolerance determinants by genetic analysis of root response to drought stress and abscisic acid, Plant Physiol. 142 (2006) 1065–1074.
[6] A. Husbands, E.M. Bell, B. Shuai, H.M.S. Smith, P.S. Springer, LATERAL ORGAN BOUNDARIES defines a new family of DNA-binding transcription factors and can interact with specific bHLH proteins, Nucleic Acids Res. 35 (2007) 6663–6671.
[7] B. Shuai, C.G. Reynaga-Peña, P.S. Springer, The lateral organ boundaries gene defines a novel, plant-specific gene family, Plant Physiol. 129 (2002) 747–761.
[8] H.W. Lee, N.Y. Kim, D.J. Lee, J. Kim, LBD18/ASL20 regulates lateral root formation in combination with LBD16/ASL18 downstream of ARF7 and ARF19 in Arabidopsis, Plant Physiol. 151 (2009) 1377–1389.
[9] X. Wang, X. Liu, L. Su, Y. Sun, S. Zhang, Y. Hao, C. You, Identification, evolution and expression analysis of the LBD gene family in tomato (In chinese), Scientia Agricultura Sinica 46 (2013) 2501–2513.
[10] H.W. Lee, M. Kim, N.Y. Kim, S.H. Lee, J. Kim, LBD18 acts as a transcriptional activator that directly binds to the *EXPANSIN14* promoter in promoting lateral root emergence of Arabidopsis, Plant J. 73 (2013) 212–224.
[11] H.W. Lee, M. Kim, M.Y. Park, K. Han, J. Kim, The conserved proline residue in the LOB domain of LBD18 is critical for DNA-Binding and biological function, Mol. Plant 6 (2013) 1722–1725.
[12] T. Goh, K. Toyokura, N. Yamaguchi, Y. Okamoto, T. Uehara, S. Kaneko, Y. Takebayashi, H. Kasahara, Y. Ikeyama, Y. Okushima, K. Nakajima, T. Mimura, M. Tasaka, H. Fukaki, Lateral root initiation requires the sequential induction of transcription factors LBD16 and PUCHI in *Arabidopsis thaliana*, New Phytol. 224 (2019) 749–760.
[13] H. Liu, S. Wang, X. Yu, J. Yu, X. He, S. Zhang, H. Shou, P. Wu, ARL1, a LOB-domain protein required for adventitious root formation in rice, Plant J. 43 (2005) 47–56.
[14] M. Kim, M. Kim, M.R. Lee, S.K. Park, J. Kim, *LATERAL ORGAN BOUNDARIES DOMAIN (LBD)10* interacts with *SIDECAR POLLEN/LBD27* to control pollen development in Arabidopsis, Plant J. 81 (2015) 794–809.
[15] H. Cao, C. Liu, C. Liu, Y. Zhao, R. Xu, Genomewide analysis of the lateral organ boundaries domain gene family in *Vitis vinifera*, J. Genet. 95 (2016) 515–526.
[16] H. Yang, G. Shi, H. Du, H. Wang, Z. Zhang, D. Hu, J. Wang, F. Huang, D. Yu, Genome-wide analysis of soybean LATERAL ORGAN BOUNDARIES domain-containing genes: a functional investigation of GmLBD12, Plant Genome 10 (2017).
[17] S. Liao, X. Qin, L. Luo, Y. Han, X. Wang, B. Usman, G. Nawaz, N. Zhao, Y. Liu, R. Li, CRISPR/Cas9-induced mutagenesis of semi-rolled Leaf1,2 confers curled leaf phenotype and drought tolerance by influencing protein expression patterns and ROS scavenging in rice (Oryza sativa L.), Agronomy 9 (2019) 9728.
[18] L.F. Thatcher, J.J. Powell, E.A.B. Aitken, K. Kazan, J.M. Manners, The lateral organ boundaries domain transcription factor LBD20 functions in fusarium wilt susceptibility and jasmonate signaling in Arabidopsis, Plant Physiol. 160 (2012) 407–418.
[19] L. Li, Y. Zhao, B.C. McCaig, B.A. Wingerd, J. Wang, M.E. Whalon, E. Pichersky, G. A. Howe, The tomato homolog of CORONATINE-INSENSITIVE1 is required for the maternal control of seed maturation, jasmonate-signaled defense responses, and glandular trichome development, Plant Cell 16 (2004) 126–143.
[20] A.N. Poudel, R.E. Holtsclaw, A. Kimberlin, S. Sen, S. Zeng, T. Joshi, Z. Lei, L. W. Sumner, K. Singh, H. Matsuura, A.J. Koo, 12-hydroxy-Jasmonoyl-l-Isoleucine is an active jasmonate that signals through CORONATINE INSENSITIVE 1 and contributes to the wound response in Arabidopsis, Plant Cell Physiol. 60 (2019) 2152–2166.
[21] S. Hase, S. Takahashi, S. Takenaka, K. Nakaho, T. Arie, S. Seo, Y. Ohashi, H. Takahashi, Involvement of jasmonic acid signalling in bacterial wilt disease resistance induced by biocontrol agent Pythium oligandrum in tomato, Plant Pathol. 57 (2008) 870–876.
[22] J.B. Runyon, M.C. Mescher, G.W. Felton, C.M. De Moraes, Parasitism by Cuscuta pentagona sequentially induces JA and SA defence pathways in tomato, Plant Cell Environ. 33 (2010) 290–303.
[23] R. Schubert, S. Dobritzsch, C. Gruber, G. Hause, B. Athmer, T. Schreiber, S. Marillonnet, Y. Okabe, H. Ezura, I.F. Acosta, D. Tarkowska, B. Hause, Tomato MYB21 acts in ovules to mediate jasmonate-regulated fertility, Plant Cell 31 (2019) 1043–1062.
[24] K. Kazan, J.M. Manners, MYC2: the master in action, Mol. Plant 6 (2013) 686–703.
[25] A.J. Hamilton, D.C. Baulcombe, A species of small antisense RNA in posttranscriptional gene silencing in plants, Science 286 (1999) 950–952.

[26] C. Wang, D. Fu, Virus-induced gene silencing of the eggplant chalcone synthase gene during fruit ripening modifies epidermal cells and gravitropism, J. Agr. Food Chem. 66 (2018) 2623–2629.

[27] C.W. Lim, W. Baek, S.C. Lee, Roles of pepper bZIP protein CaDILZ1 and its interacting partner RING-type E3 ligase CaDSR1 in modulation of drought tolerance, Plant J. 96 (2018) 452–467.

[28] C. Li, N. Yamagishi, I. Kasajima, N. Yoshikawa, Virus-induced gene silencing and virus-induced flowering in strawberry (*Fragaria* × *ananassa*) using apple latent spherical virus vectors, Hortic. Res. 6 (2019) 18.

[29] X. Shen, K. Zhao, L. Liu, K. Zhang, H. Yuan, X. Liao, Q. Wang, X. Guo, F. Li, T. Li, A Role for PacMYBA in ABA-Regulated Anthocyanin Biosynthesis in Red-Colored Sweet Cherry cv. Hong Deng (*Prunus avium* L.), Plant Cell Physiol. 55 (2014) 862–880.

[30] X. Gao, F. Li, M. Li, A.S. Kianinejad, J.K. Dever, T.A. Wheeler, Z. Li, P. He, L. Shan, Cotton *GhBAK1* mediates *Verticillium* wilt resistance and cell death, J. Inter Plant Biol. 55 (2013) 586–596.

[31] I. Hein, M. Barciszewska-Pacak, K. Hrubikova, S. Williamson, M. Dinesen, I. E. Soenderby, S. Sundar, A. Jarmolowski, K. Shirasu, C. Lacomme, Virus-induced gene silencing-based functional characterization of genes associated with powdery mildew resistance in barley, Plant Physiol. 138 (2005) 2155–2164.

[32] G. Brigneti, A.M. Martín-Hernández, H. Jin, J. Chen, D.C. Baulcombe, B. Baker, J. D.G. Jones, Virus-induced gene silencing in Solanum species, Plant J. 39 (2004) 264–272.

[33] X. Wang, H. Zhang, Q. Xie, Y. Liu, H. Lv, R. Bai, R. Ma, X. Li, X. Zhang, Y. Guo, N. Zhang, SlSNAT interacts with HSP40, a molecular chaperone, to regulate melatonin biosynthesis and promote thermotolerance in tomato, Plant Cell Physiol. 61 (2020) 909–921.

[34] C. Qi, X. Lin, S. Li, L. Liu, Z. Wang, Y. Li, R. Bai, Q. Xie, N. Zhang, S. Ren, B. Zhao, X. Li, S. Fan, Y. Guo, SoHSC70 positively regulates thermotolerance by alleviating cell membrane damage, reducing ROS accumulation, and improving activities of antioxidant enzymes, Plant Sci. 283 (2019) 385–395.

[35] M. Li, F. Li, P. He, Construction of a cotton VIGS library for functional genomics study, Methods Mol. Biol. 1287 (2015) 267–279.

[36] H. Yan, D. Fu, B. Zhu, H. Liu, X. Shen, Y. Luo, Sprout vacuum-infiltration: a simple and efficient agroinoculation method for virus-induced gene silencing in diverse solanaceous species, Plant Cell Rep. 31 (2012) 1713–1722.

[37] J. Wang, L. Zhang, Y. Cao, C. Qi, S. Li, L. Liu, G. Wang, A. Mao, S. Ren, Y. Guo, CsATAF1 positively regulates drought stress tolerance by an ABA-Dependent pathway and by promoting ROS scavenging in cucumber, Plant Cell Physiol. 59 (2018) 930–945.

[38] H. Zhang, N. Zhang, R. Yang, L. Wang, Q. Sun, D. Li, Y. Cao, S. Weeda, B. Zhao, S. Ren, Y. Guo, Melatonin promotes seed germination under high salinity by regulating antioxidant systems, ABA and GA4 interaction in cucumber (*Cucumis sativus L.*), J. Pineal Res. 57 (2014) 269–279.

[39] D. Luo, L. Ba, W. Shan, J. Kuang, W. Lu, J. Chen, Involvement of WRKY transcription factors in abscisic-acid-Induced cold tolerance of banana fruit, J. Agi. Food Chem. 65 (2017) 3627–3635.

[40] D. Fu, B. Zhu, H. Zhu, W. Jiang, Y. Luo, Virus-induced gene silencing in tomato fruit, Plant J. 43 (2005) 299–308.

[41] L. Yan, S. Wei, Y. Wu, R. Hu, H. Li, W. Yang, Q. Xie, High-efficiency genome editing in *Arabidopsis* Using *YAO* promoter-driven CRISPR/Cas9 system, Mol. Plant 8 (2015) 1820–1823.

[42] X. Mao, H. Zhang, X. Qian, A. Li, G. Zhao, R. Jing, TaNAC2, a NAC-type wheat transcription factor conferring enhanced multiple abiotic stress tolerances in Arabidopsis, J. Exp. Bot. 63 (2012) 2933–2946.

[43] L. Zhang, L. Tian, J. Zhao, Y. Song, C. Zhang, Y. Guo, Identification of an apoplastic protein involved in the initial phase of salt stress response in rice root by two-dimensional electrophoresis, Plant Physiol. 149 (2009) 916–928.

[44] S. Li, J. Zhang, L. Liu, Z. Wang, Y. Li, L. Guo, Y. Li, X. Zhang, S. Ren, B. Zhao, N. Zhang, Y. Guo, *SlTLFP8* reduces water loss to improve water-use efficiency by modulating cell size and stomatal density via size and stomatal density via endoreduplicaion, Plant Cell Environ. (2020), https://doi.org/10.1111/pce.13867 (in press).

[45] C. Qi, H. Zhang, Y. Liu, X. Wang, D. Dong, X. Yuan, X. Li, X. Zhang, X. Li, N. Zhang, Y. Guo, CsSNAT positively regulates salt tolerance and growth of cucumber by promoting melatonin biosynthesis, Environ. Exp. Bot. 175 (2020).

[46] R.M. Marchin, A. Ossola, M.R. Leishman, D.S. Ellsworth, A simple method for simulating drought effects on plants, Front. Plant Sci. 10 (2019) 1715.

[47] F. Zhang, W. Tao, R. Sun, J. Wang, C. Li, X. Kong, H. Tian, Z. Ding, PRH1 mediates ARF7-LBD dependent auxin signaling to regulate lateral root development in *Arabidopsis thaliana*, PLoS Genet. 16 (2020), e1008044.

[48] M. Fan, C. Xu, K. Xu, Y. Hu, LATERAL ORGAN BOUNDARIES DOMAIN transcription factors direct callus formation in *Arabidopsis* regeneration, Cell Res. 22 (2012) 1169–1180.

[49] C. Liu, H. Yu, L. Li, SUMO modification of LBD30 by SIZ1 regulates secondary cell wall formation in *Arabidopsis thaliana*, PLoS Genet. 15 (2019), e1007928.

[50] G. Rubin, T. Tohge, F. Matsuda, K. Saito, W. Scheible, Members of the LBD family of transcription factors repress anthocyanin synthesis and affect additional nitrogen responses in *Arabidopsis*, Plant Cell 21 (2009) 3567–3584.

[51] T. Yuan, H. Xu, J. Li, Y. Lu, Auxin abolishes SHI-RELATED SEQUENCE5-mediated inhibition of lateral root development in *Arabidopsis*, New Phytol. 225 (2020) 297–309.

[52] L.F. Thatcher, K. Kazan, J.M. Manners, Lateral organ boundaries domain transcription factors new roles in plant defense, Plant Signal. Behav. 7 (2012) 1702–1704.

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

[54] W. Ma, F. Wu, P. Sheng, X. Wang, Z. Zhang, K. Zhou, H. Zhang, J. Hu, Q. Lin, Z. Cheng, J. Wang, S. Zhu, X. Zhang, X. Guo, H. Wang, C. Wu, H. Zhai, J. Wan, The LBD12-1 transcription factor suppresses apical meristem size by repressing argonaute 10 expression, Plant Physiol. 173 (2017) 801–811.

[55] Y. Hu, L. Jiang, F. Wang, D. Yu, Jasmonate regulates the INDUCER OF CBF EXPRESSION–C-REPEAT BINDING FACTOR/DRE BINDING FACTOR1 cascade and freezing tolerance in *Arabidopsis*, Plant Cell 25 (2013) 2907–2924.

[56] D. Balfagón, S. Sengupta, A. Gómez-Cadenas, F.B. Fritschi, R.K. Azad, R. Mittler, S. I. Zandalinas, Jasmonic acid is required for plant acclimation to a combination of high light and heat stress, Plant Physiol. 181 (2019) 1668–1682.

[57] D. Min, F. Li, X. Zhang, X. Cui, P. Shu, L. Dong, C. Ren, *SlMYC2* involved in methyl jasmonate-induced tomato fruit chilling tolerance, J. Agric. Food Chem. 66 (2018) 3110–3117.

[58] J.E. Dombrowski, Salt stress activation of wound-related genes in tomato plants, Plant Physiol. 132 (2003) 2098–2107.

[59] M. Du, J. Zhao, D.T.W. Tzeng, Y. Liu, L. Deng, T. Yang, Q. Zhai, F. Wu, Z. Huang, M. Zhou, Q. Wang, Q. Chen, S. Zhong, C. Li, C. Li, MYC2 orchestrates a hierarchical transcriptional cascade that regulates jasmonate-mediated plant immunity in tomato, Plant Cell 29 (2017) 1883–1906.

[60] Y. Wei, G. Liu, Y. Chang, C. He, H. Shi, Heat shock transcription factor 3 regulates plant immune response through modulation of salicylic acid accumulation and signaling in cassava, Mol. Plant Pathol. 19 (2018) 2209–2220.

[61] A. Noman, A. Hussain, M.F. Ashraf, M. Ifnan Khan, Z. Liu, S. He, *CabZIP53* is targeted by *CaWRKY40* and act as positive regulator in pepper defense against *Ralstonia solanacearum* and thermotolerance, Environ. Exp. Bot. 159 (2019) 138–148.

[62] C. Xu, H. Cao, Q. Zhang, H. Wang, W. Xin, E. Xu, S. Zhang, R. Yu, D. Yu, Y. Hu, Control of auxin-induced callus formation by bZIP59-LBD complex in *Arabidopsis* regeneration, Nat. Plants 4 (2018) 108–115.

[63] F. Ariel, A. Diet, M. Verdenaud, V. Gruber, F. Frugier, R. Chan, M. Crespi, Environmental regulation of lateral root emergence in *Medicago truncatula* requires the HD-Zip I transcription factor HB1, Plant Cell 22 (2010) 2171–2183.

[64] F.D. Ariel, A. Diet, M. Crespi, R.L. Chan, The LOB-like transcription factor MtLBD1 controls *Medicago truncatula* root architecture under salt stress, Plant Signal. Behav. 5 (2010) 1666–1668.

[65] S. Wang, Y. Bai, C. Shen, Y. Wu, S. Zhang, D. Jiang, T.J. Guilfoyle, M. Chen, Y. Qi, Auxin-related gene families in abiotic stress response in *Sorghum bicolor*, Funct Integr Genomic. 10 (2010) 533–546.

[66] P. Iovieno, P. Punzo, G. Guida, C. Mistretta, M.J. Van Oosten, R. Nurcato, H. Bostan, C. Colantuono, A. Costa, P. Bagnaresi, M.L. Chiusano, R. Albrizio, P. Giorio, G. Batelli, S. Grillo, Transcriptomic changes drive physiological responses to progressive drought stress and rehydration in tomato, Front. Plant Sci. 7 (2016) 371.

[67] C. Li, S. Zhu, H. Zhang, L. Chen, M. Cai, J. Wang, J. Chai, F. Wu, Z. Cheng, X. Guo, X. Zhang, J. Wan, OsLBD37 and OsLBD38, two class II type LBD proteins, are involved in the regulation of heading date by controlling the expression of *Ehd1* in rice, Biochem. Bioph. Res. Co. 486 (2017) 720–725.