

Article

Identification of *Picea mongolica* LEA Gene Family Implicates *PmLEA25* in Drought Resistance

Yaping Wang ¹ , Jiaqi Li ² and Yu'e Bai ^{1,*}

¹ College of Forestry, Inner Mongolia Agricultural University, Huhhot 010018, China; yapingwang113@gmail.com

² Inner Mongolia Autonomous Region Forestry and Grassland Seedling Station, Huhhot 010052, China; 18447053040@163.com

* Correspondence: baiyue@imau.edu.cn

Abstract: *Picea mongolica* is a rare and valuable tree species in China, having high tolerance for drought, cold, and sand burial. The late embryogenesis abundant protein (LEA protein) is a crucial transcription factor that plays a key role in both plant embryonic development and stress response. LEA genes have, however, not yet been reported in *P. mongolica*. In this study, through the analysis of genome data from *Picea abies* and transcriptome data from *P. mongolica*, a total of 49 *PmLEAs* were discovered and categorized into eight subfamilies based on their Pfam domain and phylogenetic relationship. RNA-Seq research revealed that 37 *PmLEAs* were differentially expressed at various stages of embryonic development. Using qRT-PCR, we found that most *PmLEAs* responded strongly to drought stress, with genes in the same subfamily exhibiting identical expression patterns. In particular, *PmLEA25* is the most highly induced by drought treatment. Furthermore, we heterologously transformed *PmLEA25* into *Arabidopsis*. The overexpression of *PmLEA25* remarkably increased the germination rate, root length, and antioxidant capacity in *Arabidopsis* under drought treatment, compared with WT. The results serve as a point of reference for gaining a deeper comprehension of the function of *PmLEA25* in the molecular process of stress resistance in *P. mongolica*. Additionally, they offer significant genetic materials for the purpose of breeding stress-resistant spruce species.

Keywords: LEA gene family; functional analysis; drought stress; *Picea mongolica*



Citation: Wang, Y.; Li, J.; Bai, Y. Identification of *Picea mongolica* LEA Gene Family Implicates *PmLEA25* in Drought Resistance. *Forests* **2024**, *15*, 1988. <https://doi.org/10.3390/f15111988>

Academic Editor: John E. Carlson

Received: 1 October 2024

Revised: 6 November 2024

Accepted: 8 November 2024

Published: 11 November 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Picea mongolica, an evergreen tree in the family *Pinaceae*, is a rare and endangered tree species in China, as well as an outstanding windbreak and sand-fixing tree in northern China. It grows naturally in the Hunsandak sands of Inner Mongolia and is highly resistant to drought, cold, and sand burial. The wood is mostly utilized for papermaking, furniture, and musical instrument manufacturing, while the bark is used to extract turpentine [1]. Spruce forests, as a natural protective barrier, contribute significantly to local agricultural production and have a vital impact on the ecological protection of the capital city of Beijing, which is only 300 km away, as well as the ecological environment of northern China. As a result, many scholars have been drawn to study its distribution [2], seedling [3], physiological biochemistry [4], somatic embryogenesis [5], and endophytic diversity [6], but its molecular biology is rarely studied, particularly the limited research on resistance gene mining and the resistance improvement research process.

Transcription factors (TFs) regulate gene expression in a variety of important plant developmental processes, including cell morphogenesis, signal transduction, and stress responses [7]. LEA proteins are a type of protein that plays an important function in plant stress responses [8]. With low concentrations of cysteine (Cys), tryptophan (Trp), and hydrophilic amino acid residues like glycine (Gly) and lysine (Lys), the majority of LEA proteins are extremely hydrophilic [9]. Plant LEA proteins can be categorized into

distinct subfamilies using various classification criteria, such as conserved motifs and evolutionary relationships of LEA proteins [10]. Dure et al. [11] classified LEA proteins into three subfamilies based on the comparison of their amino acid sequences: the first subfamily includes proteins derived from the Em gene, the second comprises proteins from the RAB and dehydrin genes, and the third encompasses proteins from other LEA genes. As more LEA proteins are identified, the classification criteria for LEA proteins are evolving. Currently, LEA protein families are typically categorized into eight subfamilies based on their original sequence: LEA_1, LEA_2, LEA_3, LEA_4, LEA_5, LEA_6/PvLEA18, DHN, SMP, and AtM [10].

LEA proteins were initially identified in cotton (*Gossypium* spp.) and accumulate in considerable amounts during late embryonic development [12]. In recent years, the continuous release of plant genome and transcriptome data have shown the presence of 51, 34, 32, 27, 88, and 23 LEAs in *Arabidopsis thaliana* [13], *Oryza sativa* [14], *Zea mays* [15], *Solanum lycopersicum* [16], *Populus tremula* [17], and *Pinus tabulaeformis* [18], respectively. The role of LEA protein in biological processes like drought resistance, salt tolerance, and low-temperature tolerance in plants has been shown by a number of studies. Drought, cold, and salt stress may all considerably boost the expression of 12 LEAs in *Arabidopsis* [19,20], with *AtLEA14* overexpression greatly increasing salt resistance [21]. *SiLEA4* and *SiLEA5* can enhance tomato resilience to low temperatures and dehydration via modulating antioxidants [22,23]. In soybean, the heterologous expression of *GmLEA2-1* in *Arabidopsis* enhanced drought and salt tolerance, whereas *GmLEA4* interacted with *GmCaM1* to significantly improve *Arabidopsis* seed vigor under conditions of an elevated temperature and humidity stress [24,25]. The *MsLEA3-1* gene, located in the nucleus, exhibits high expression levels when induced by salt and ABA, and its overexpression in tobacco significantly enhances salt tolerance [26]. The *LEA4-4* gene augments antioxidant enzyme activity in transgenic *Arabidopsis* and is involved in the ABA signaling system, providing tolerance to salt and drought stress in transgenic *Arabidopsis* [27]. In alfalfa, *MsABF2* modulates gene expression by binding to the promoter region of *MsLEA-D34*, thereby augmenting the plant's resilience to drought and salinity stress [28]. *MsDIUP1* improves the plant's salt tolerance by regulating stress signaling, antioxidant defense, ion homeostasis, osmotic regulation, and photosynthesis [29]. Additionally, poplar *PtrDHN-3* transgenic yeast and *Arabidopsis thaliana* improved their salt tolerance [30].

In previous studies, the LEA gene family has been identified and studied across various species, but no reports have been published on *P. mongolica*. In our study, we identified all *PmLEA* gene family members in *P. mongolica* and investigated their evolutionary relationships and expression patterns at various stages of embryonic development and during drought stress. Simultaneously, *PmLEA25* was evaluated for its role in drought stress, enhancing understanding of the LEA family and providing essential genetic resources for future breeding of stress-resistant plants.

2. Materials and Methods

2.1. Plant Materials and Drought Stress Treatment

Viable seeds of *P. mongolica* from one single tree were soaked in water overnight before being sown in sandy soil under conditions of 25 °C, 65% relative humidity, and a 16 h light/8 h dark cycle. The one-year-old potted seedlings exhibiting constant growth and vitality were subjected to 20% PEG 6000 drought stress. Following treatment durations of 0, 6, 12, and 24 h, the plants were rapidly frozen in liquid nitrogen and subsequently stored at −80 °C in a refrigerator.

The *Arabidopsis* wild-type (WT) seeds utilized in this investigation were derived from the Columbia (*Col-0*) ecotype. The seeds first underwent sterilization with 75% ethanol for 5 min, followed by three washes with sterile water, and subsequently underwent sterilization with 5% NaClO for 10 min, concluding with 5 to 6 washes with sterile water. For drought treatment, wild-type and transgenic *Arabidopsis* seeds were placed in a 1/2 MS medium supplemented with 200/300 mM Mannitol. Three-week-old potted seedlings

were subjected to drought treatment using a 20% PEG 6000 solution for one week prior to phenotypic and physiological investigation. The cultivation parameters for *A. thaliana* were established at 16 h of illumination and 8 h of darkness, 75% humidity, 22 °C, and 5000 Lux.

2.2. Identification of PmLEAs and Sequence Analysis

The amino acid sequences of 51 LEAs from the *Arabidopsis* genome (<https://www.arabidopsis.org/>, accessed on 6 January 2024) were used as probes to identify LEA proteins in *P. mongolica*. To identify LEA transcription factor candidate genes, a local BlastP search of *P. mongolica* transcriptome data (PRJNA649217) was performed using BioEdit 7.2.0 software [31], with both BLASTP and HMM alignment, using default parameters and E values less than 0.001. The conserved domain of the potential LEA protein was then evaluated using SMART, and only LEA sequences with complete conserved domains were retained. The LEAs were further aligned using the *Picea abies* genome (<https://plantgenie.org/>, accessed on 10 January 2024), and the members of the LEAs were eventually identified and named after *Arabidopsis*-comparable genes. Furthermore, the sequence length, molecular weight (MW), isoelectric point (PI), and GRAVY of LEA proteins were ascertained utilizing ExPASy (<https://www.expasy.org/>, accessed on 16 January 2024). Additionally, we used the MEME (<https://meme-suite.org/meme/>, accessed on 23 January 2024) web platform to examine protein conserved motifs in all LEA proteins from *P. mongolica* and *Arabidopsis*, and 20 motifs were selected for prediction.

2.3. Phylogenetic and Evolutionary Tree Construction

Multiple sequence alignments of all LEA proteins in *P. mongolica* and *Arabidopsis* were performed using Clustal X2.1 software. The phylogenetic and evolutionary tree was generated using MEGA X with the neighborhood connection (NJ) method, utilizing a bootstrap option set to 1000 iterations while retaining default settings for all parameters.

2.4. Expression Analysis of PmLEAs

The LEA gene family was analyzed by hierarchical clustering and an expression heat map by TBtools-II v2.003 software based on FPKM in the transcriptome database (PRJNA649217) during the different developmental stages of somatic embryos of *P. mongolica*. The Plant Total RNA Extraction Kit (TAKARA) was used to extract total RNA from the various samples, and its integrity and concentration were determined using 1.0% agarose gel electrophoresis and Nanodrop 2000. The synthesized cDNA products were stored at −20 °C and diluted five times before use as templates. The qRT-PCR primers were designed using Primer3 software (Table S1). The qRT-PCR was carried out using iCycler iQ equipment (Bio-Rad, Hercules, CA, USA) using SYBR qPCR Master Mix (Vazyme, Nanjing, China) according to the manufacturer's instructions. *EF1α* (MA_505653g0010) was utilized as the reference gene. Relative gene expression levels were estimated using the $2^{-\Delta\Delta C_t}$ technique. A significant difference analysis and histograms were obtained using GraphPad Prism 10 software. Each treatment included three biological replicates and three technical replicates per sample.

2.5. Characterization of Cis-Elements in Promoter Region of PmLEA25

PlantCARE suggested that the *PmLEA25* genes' promoter region (2.0 kb upstream of genomic DNA sequences) contains putative cis-elements.

2.6. Construction of PmLEA25 Overexpression Vector and Genetic Transformation of *Arabidopsis thaliana*

The coding sequence (CDS) of the *PmLEA25* gene was cloned from *P. mongolica* cDNA, and the cloning primers are specified in Table S1. The CDS was inserted into the pDONR222 vector using Gateway technology. The resulting pDONR222-*PmLEA25* plasmid was freeze-thawed into *Agrobacterium tumefaciens* GV3101 before being heterologously transformed into *Arabidopsis* using the floral dip method [32].

2.7. Screening of Positive Plants

Genomic DNA was extracted from WT and positive *Arabidopsis thaliana* leaves. PCR was conducted using primers pMDC32-F and *PmLEA25*-R (Table S1) to verify the effective integration of the target gene into the genome, based on the size of the positive plasmid band. Samples with the correct band size were further analyzed for *PmLEA25* expression levels in *Arabidopsis*, using *ACT3* as the internal reference gene.

2.8. Phenotypic Observation and Physiological Assays

The germination rates of normal and stress-treated WT and OE-*PmLEA25 Arabidopsis* seedlings were measured over one week, and root length was assessed after 14 d. The growth phenotypes of *Arabidopsis* potted seedlings were assessed under normal and drought stress treatments for 7 d, comparing WT and OE-*PmLEA25*. Standard methods were used to determine the activity of antioxidant enzymes, including malondialdehyde (MDA), superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), as per established protocols (jiubang Biotechnology Co., Ltd., Quanzhou, China).

3. Results

3.1. Identification and Phylogenetic Study of LEA Gene Family in *P. mongolica*

A total of 49 *PmLEAs* were identified in the *P. abies* entire genome and the *P. mongolica* transcriptome database using HMM Search. The 49 *PmLEAs* were systematically designated according to their resemblance to *Arabidopsis* homologs, representing the inaugural instance of systematic nomenclature (Table 1). The physicochemical study revealed considerable heterogeneity in the amino acid sequence lengths of the 49 *PmLEAs* (Table 1). The longest sequence, *PmLEA31*, consisted of 698 amino acid residues, while the shortest sequence, *PmLEA1.2b*, consisted of only 60 amino acid residues. The theoretical molecular weights of these sequences varied from 6.7194 kDa to 22.872 kDa. The theoretical isoelectric points (pIs) of *PmLEAs* ranged from 4.07 kDa to 9.82 kDa, with 22 (45%) having a pI greater than 7 and 27 (55%) having a pI less than 7. Furthermore, the majority of the *PmLEAs* encode hydrophilic proteins; 46 of them have a GRAVY value less than zero. The most hydrophilic are from the LEA_1 subfamily, with average GRAVY values of −1.472 and −1.461, respectively. In contrast, three genes have GRAVY values greater than zero, and these are entirely from the LEA_2 and DHN subfamilies.

Table 1. The sequence features of 49 *PmLEAs* in *P. mongolica*.

Gene Name	Gene ID	Length of CDS (bp)	Protein (aa)	Mw (Da)	PI	GRAVY
<i>PmLEA1.2a</i>	MA_185382g0010	252	83	9555.36	5.14	−1.461
<i>PmLEA1.2b</i>	MA_68242g0010	183	60	6719.4	4.92	−1.187
<i>PmLEA1.2c</i>	MA_135240g0030	252	83	9499.34	5.14	−1.261
<i>PmLEA2</i>	MA_10436352g0020	234	77	8747.75	6.54	−0.816
<i>PmLEA3.3</i>	MA_17049g0010	315	104	11,318.64	9.45	−0.641
<i>PmLEA4.2a</i>	MA_858786g0010	261	86	9425.61	9.82	−0.834
<i>PmLEA4.2b</i>	MA_186086g0010	264	87	9547.77	8.98	−1.108
<i>PmLEA4.3a</i>	MA_235g0020	237	78	8653.65	6.59	−1.133
<i>PmLEA5</i>	MA_49897g0020	594	197	21,261.5	4.6	−0.568
<i>PmLEA6</i>	MA_33768g0010	258	85	9119.17	8.82	−0.906
<i>PmLEA7</i>	MA_487045g0010	279	92	10,216.44	9.68	−0.636
<i>PmLEA8</i>	MA_40361g0020	258	85	9247.29	7.99	−1.048
<i>PmLEA9</i>	MA_1400g0010	855	284	30,111.9	5.35	−0.432
<i>PmLEA10</i>	MA_41069g0010	228	75	8480.41	7.11	−1.483
<i>PmLEA11</i>	MA_121405g0010	459	152	16,652.2	5.47	0.101
<i>PmLEA12</i>	MA_6165882g0010	327	108	11,317.4	5.46	−1.041
<i>PmLEA13</i>	MA_9339426g0010	444	147	15,429.68	8.69	−1.3
<i>PmLEA14</i>	MA_10069912g0010	327	108	11,235.26	5.36	−0.995

Table 1. Cont.

Gene Name	Gene ID	Length of CDS (bp)	Protein (aa)	Mw (Da)	PI	GRAVY
PmLEA15	MA_2988g0020	339	112	12,307.49	7.93	−1.472
PmLEA16	MA_183489g0030	267	88	9669.63	6.55	−1.097
PmLEA17	MA_10427927g0010	576	191	19,696.26	4.98	−0.176
PmLEA18	MA_10367411g0010	327	108	11,221.27	6.22	−0.999
PmLEA19	MA_717888g0010	618	205	22,872.86	4.07	−0.664
PmLEA20	MA_295448g0010	297	98	10,065.36	7.88	0.077
PmLEA21	MA_6771381g0010	615	204	22,078.02	6.51	−0.108
PmLEA22	MA_3108g0010	519	172	18,936.52	6.06	−0.998
PmLEA23	MA_116141g0010	195	64	7691.76	9.46	−1.188
PmLEA24	MA_9957327g0010	288	95	9578.78	7.78	0.211
PmLEA25	MA_10435107g0010	354	117	13,180.96	9.3	−0.831
PmLEA26	MA_722400g0010	357	118	13,335.19	9.3	−0.892
PmLEA27	MA_17597g0010	429	142	15,786.39	6.63	−0.904
PmLEA28	MA_8427244g0010	504	167	17,801.05	6.05	−0.086
PmLEA29	MA_432438g0020	420	139	15,036.16	9.12	−0.347
PmLEA30	MA_21240g0010	300	99	10,253.2	6.15	−1.004
PmLEA31	MA_111913g0010	2097	698	77,148.18	6.28	−1.091
PmLEA32	MA_590086g0010	327	108	11,317.4	5.46	−1.041
PmLEA33	MA_99592g0010	330	109	11,703.22	6.57	−0.317
PmLEA34	MA_10428902g0010	345	114	12,130.63	6.82	−0.254
PmLEA35	MA_403263g0010	318	105	11,438.77	6.27	−0.525
PmLEA36	MA_175705g0010	365	79	8798.91	9.86	−0.644
PmLEA37	MA_15415g0010	438	145	15,866.77	9.34	−0.569
PmLEA38	MA_9170359g0010	330	109	11,878.32	8.05	−0.627
PmLEA39	MA_198577g0010	330	109	11,836.38	9	−0.483
PmLEA40	MA_90896g0010	333	110	11,886.34	9.51	−0.501
PmLEA41	MA_132211g0010	294	97	10,697.91	6.02	−0.427
PmLEA42	MA_42290g0010	306	101	10,939.48	6.4	−0.372
PmLEA43	MA_326206g0010	474	157	16,742.6	5.59	−0.693
PmLEA44	MA_4752780g0010	195	64	6690.4	8.1	−0.87
PmLEA45	MA_41035g0010	680	190	20,880.29	9.17	−1.168

A phylogenetic tree was generated utilizing the protein sequences of 49 PmLEAs and 51 AtLEAs. The tree demonstrated that all LEAs grouped together based on the class of Pfam structural domains (Figure 1), confirming the alignment of the *P. mongolica* subfamily classification with the *Arabidopsis* LEA gene subfamily. The analysis of Pfam structural domains indicated their widespread occurrence in the majority of PmLEAs, with numerous instances exhibiting multiple LEA structural domains. The 49 PmLEAs were categorized into eight subfamilies according to their structural domains (Figure 1): 5 members of PmLEA_1, 1 member of PmLEA_2, 12 members of PmLEA_3, 16 members of PmLEA_4, 2 members of PmLEA_5, 6 members of DHN, 5 members of ATM, and 2 members of SMP. Notably, no genes from the PmLEA_6 subfamily were found in *P. mongolica*.

3.2. Protein Conserved Motif Analysis of LEAs in *P. mongolica*

The study revealed the discovery of 20 conserved motifs (Figure 2), each with an average length ranging from 14 to 50 amino acids. The results showed that all subfamilies had unique conserved patterns in their genes, except for PmLEA19, PmLEA22, PmLEA37, and PmLEA4.3. There is considerable variation in the number of motifs present in PmLEAs. Among them, PmLEA31 stands out with the highest number of motifs, totaling 10, surpassing all other PmLEA protein sequences. Motif 2 is present in all LEA_3 subfamily proteins, whereas Motif 3 is observed in most LEA_4 subfamily proteins. Generally, the content of motifs varies greatly within subfamilies, and most members within a subfamily display similar motifs. This implies that genes belonging to the same subfamily carry out comparable functions. However, the conserved patterns of different PmLEAs within

specific subfamilies also differ. For example, the dehydrin subfamily contains PmLEA20 and PmLEA24, which have a higher prevalence of Motif 9. This suggests that PmLEA proteins belonging to the same subfamily may have different functions. Furthermore, they contain K, S, and Y segments (Table S2).

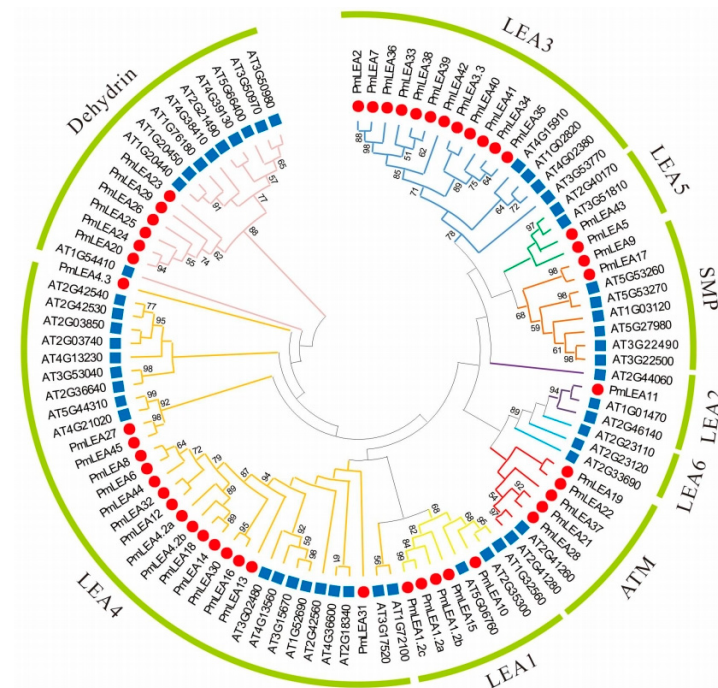


Figure 1. Phylogenetic relationship between 49 LEAs of *P. mongolica* and 51 LEAs of *A. thaliana*. Red circles and blue squares represent *P. mongolica* and *A. thaliana*.



Figure 2. Analysis of conserved motifs of 49 LEAs in *P. mongolica* and 51 LEAs in *Arabidopsis*.

3.3. Transcript Pattern of PmLEAs During Somatic Embryo Development by RNA-Seq

Based on the heatmap analysis (Figure 3), we found that out of the 49 PmLEAs, 37 were expressed in six different stages. Most of these genes exhibited high levels of transcripts during the early (Megagametophy) and late (cotyledon embryos and mature embryos) stages of somatic embryo development. Notably, *PmLEA1.2a/11/38/41*, *PmLEA14/11/18/26*, and *PmLEA7/9/17/22* displayed exceptionally high transcript abundance in Megagametophy, cotyledon embryos, and mature embryos, respectively. This indicates that these genes are essential for somatic embryo beginning and subsequent developmental events. Furthermore, it is important to mention that two genes (*PmLEA1.2b/24*) exhibited high expression levels in cleavage polyembryony, two genes (*PmLEA21/29*) showed high expression levels in dominant embryos, and two genes (*PmLEA1.2c/19*) displayed high expression levels in pre-cotyledon embryos. These genes exhibited significant expression levels in pre-cotyledon embryos. In summary, a total of five genes (*PmLEA5/10/13/31/43*) were consistently up-regulated, while three genes (*PmLEA11/38/41*) were consistently down-regulated during somatic embryo development. The aforementioned findings indicate that various PmLEAs may have distinct roles in the process of somatic embryo development in *P. mongolica*.

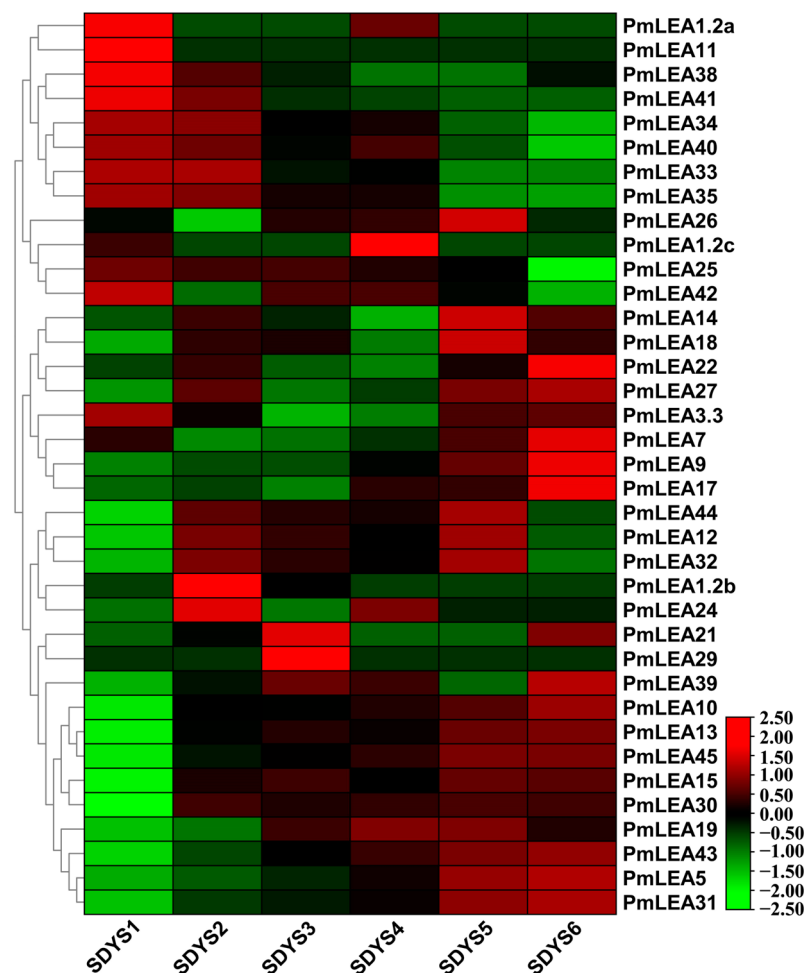


Figure 3. Transcription expression profiles of PmLEAs during embryonic development. SDYS1: Megagametophy; SDYS2: cleavage polyembryony; SDYS3: dominant embryos; SDYS4: pre-cotyledon embryos; SDYS5: cotyledon embryos; SDYS6: mature embryos. Red indicates high expression, and green indicates low expression.

3.4. Expression Analysis of *PmLEAs* Under Drought Stress

To further examine the response of the *PmLEAs* to abiotic stress, we analyzed the expression profiles of 49 *PmLEAs* under drought conditions (Figure 4). The findings showed that, with extended stress treatment duration, most *PmLEAs* show significant differential expression under drought stress conditions. Specifically, 11 genes (*PmLEA8/14/18/20/25/30/31/34/35/40/41*) were up-regulated by over 5-fold under drought treatment. In contrast, *PmLEA22* was continuously down-regulated during drought therapy. Additionally, we observed that *PmLEA8* showed a more than 7-fold increase in expression after 6 h of drought stress. Furthermore, we noted that the expression of *PmLEA25/35/41* increased by more than 18-fold after 6 h of drought stress, the expression of *PmLEA20/25* increased by more than 70-fold after 12 h of drought stress, and the expression of *PmLEA25/41* increased by more than 20-fold after 24 h of drought stress. In general, under drought stress, most *PmLEAs* reached peak expression at 24 h. The results indicate that the *PmLEA* gene family may have various functions in the context of abiotic stress development. Notably, *PmLEA25* exhibited up-regulation by over 80-fold during drought stress, suggesting its possible role as a candidate gene in the control of abiotic stress responses in *P. mongolica*.

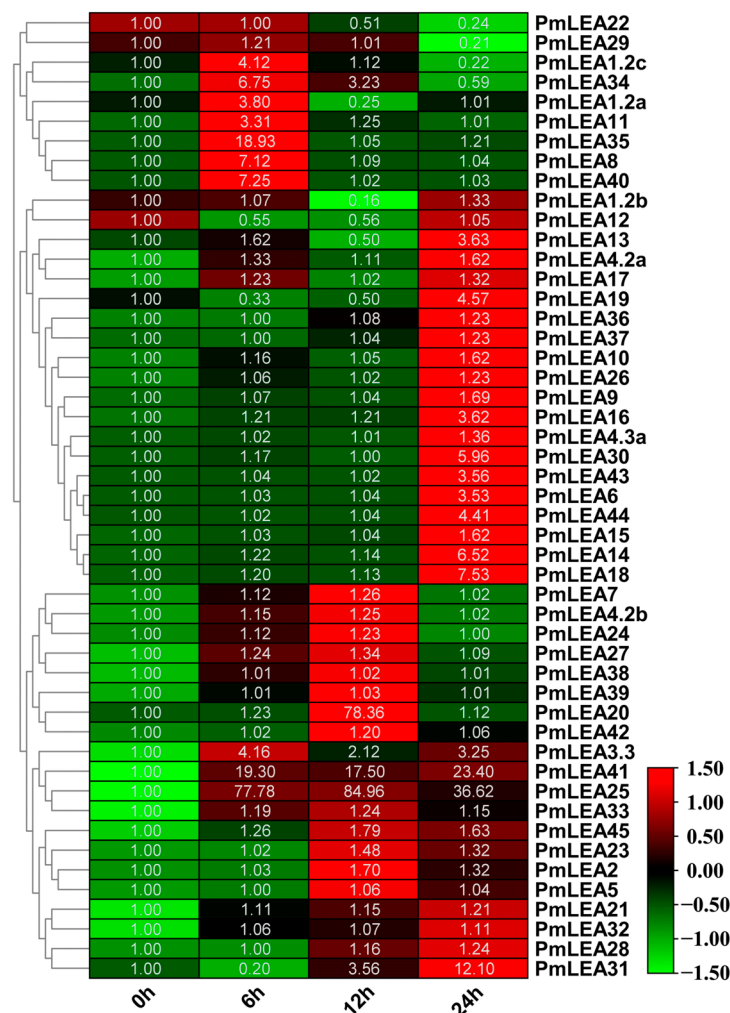


Figure 4. Expression analysis of 49 *PmLEAs* identified from *P. mongolica* under drought stress. Red indicates high expression, and green indicates low expression.

There were multiple regulatory elements associated with stress and hormone responses within the promoter of *PmLEA25* (Figure S1). The results revealed an abundance of regulatory elements associated with stress and hormone responses within the promoter of *PmLEA25*. These include STRE elements related to stress response, anaerobic induction-

regulating elements such as ARE, damage and pathogen response elements like the W box, and MeJA-responsive cis-regulatory elements. Additionally, motifs such as the TGACG motif associated with the jasmonic acid signaling pathway and TGA element linked to salicylic acid response were identified (Table S3). These cis-elements collectively indicate the significant involvement of this gene in stress response, potentially subject to complex regulatory networks involving jasmonic acid and salicylic acid signaling pathways.

3.5. Overexpression of *PmLEA25* Enhances Drought Resistance in Transgenic *Arabidopsis*

Using the primers pMDC32-F and *PmLEA25*-R, the DNA from *Arabidopsis* leaves was extracted and confirmed by PCR (Figure 5B). In positive plants, gel electrophoresis showed a single band of the anticipated size (500 bp), indicating that the *PmLEA25* gene had been integrated into the *Arabidopsis* genome. Additionally, using the RT-PCR analysis, three stable overexpressing *PmLEA25* lines were chosen for treatments against drought stress (Figure 5C).

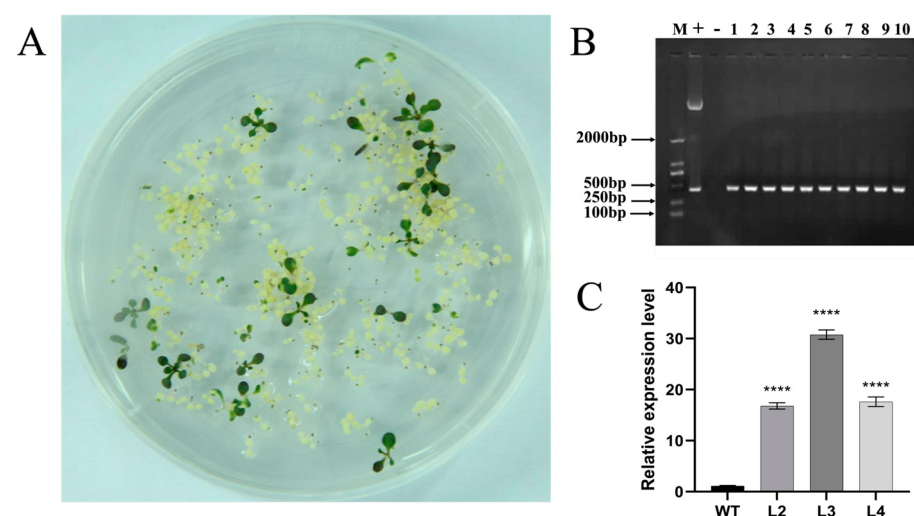


Figure 5. Screening and identification of *PmLEA25* transgenic *Arabidopsis*. (A) Hygromycin screening of transgenic plants. (B) PCR results of *PmLEA25*. M: 2000 bp DNA Marker. +: Positive control. -: Negative control. 1–10: Different transgenic lines. (C) Expression levels of *PmLEA25* in WT and three overexpressing *PmLEA25* lines analyzed by RT-PCR. **** p value < 0.0001.

Under drought conditions, the germination rate of *Arabidopsis thaliana* diminished as Mannitol concentration increased, and the germination rate of WT *Arabidopsis* was markedly lower than that of the overexpressed *PmLEA25* lines. Under 200 mM Mannitol stress conditions, the germination rates of the L2, L3, and L4 strains began to be approximately twice that of the WT after the first day. On day 4, the germination rate of the WT was only 30.23%, while the germination rates of the L2, L3, and L4 lines were 77.85%, 80.60%, and 81.34%, respectively. Moreover, compared to the WT, the overexpressed *PmLEA25* lines exhibited a faster growth rate. Under 300 mM Mannitol stress, similar to the trend observed with 200 mM Mannitol, the germination rates of the L2, L3, and L4 lines continuously surpassed those of the WT plants beginning on day 3. On day 6, the peak values were seen, with germination rates of 72.36%, 73.06%, and 68.41% for the L2, L3, and L4 lines, respectively, whereas the WT exhibited a germination rate of merely 42.65% (Figure 6).

In the normal 1/2 MS medium, there was no notable variation in the primary roots and lateral roots of the L2, L3, and L4 lines compared with the WT. In the 1/2 MS medium supplemented with 200 mM Mannitol, the cotyledons of all plants were shrunk and curled to a certain extent, and the primary root lengths of the L2, L3, and L4 lines were significantly greater than those of the WT. In the 1/2 MS medium with 300 mM Mannitol, the primary root lengths of the L2, L3, and L4 lines were much greater than that of the WT, and the number of lateral roots also exhibited a significant increase (Figure 7).

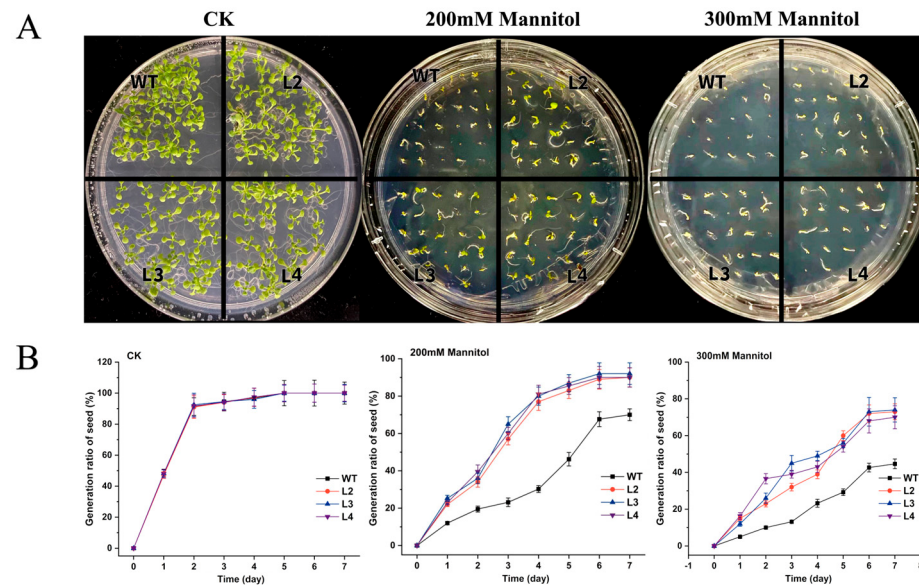


Figure 6. Effects of drought stress on the seed germination rate of WT and overexpressed *PmLEA25* lines. (A) Germination phenotypes of WT and overexpressed *PmLEA25* lines at varying doses of Mannitol. (B) Germination rates of WT and overexpressed *PmLEA25* lines under varying amounts of Mannitol.

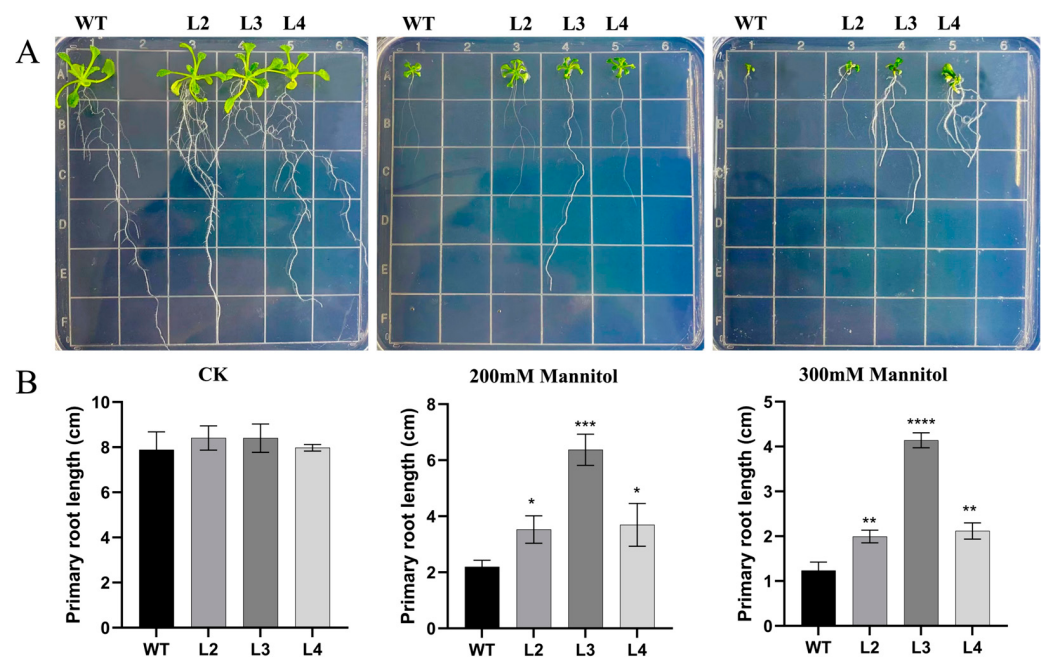


Figure 7. Effects of drought stress on the root length of WT and overexpressed *PmLEA25* lines. (A) Root phenotypes of WT and overexpressed *PmLEA25* lines at varying amounts of Mannitol. (B) Root length statistics of WT and overexpressed *PmLEA25* lines under varying amounts of Mannitol (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$).

Through subjecting 20-day-old WT and overexpressed *PmLEA25* plants to one week of drought stress treatments, we observed chlorosis and wilting in leaves of both WT and overexpressed *PmLEA25* lines. However, the WT exhibited more severe wilting symptoms (Figure 8A). Abiotic stress enhances the generation of reactive oxygen species (ROS), resulting in oxidative damage to the cellular membrane. Antioxidant enzymes are crucial for ROS scavenging and plant defense mechanisms. To further investigate the correlation between the enhanced drought tolerance of overexpressed *PmLEA25* lines and antioxidant

enzyme activity, we measured the activity of antioxidant enzymes in plants grown subjected to identical drought stress conditions (Figure 8B). The findings indicated that in *Arabidopsis*, the overexpressed *PmLEA25* lines displayed markedly reduced malondialdehyde (MDA) levels relative to the WT, although the activities of peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT) demonstrated an inverse pattern (Figure 6B). The above results indicate that the expression of the *PmLEA25* reduces membrane damage in plants due to drought and enhances the detoxification of active oxygen free radicals, thereby increasing its drought resistance.

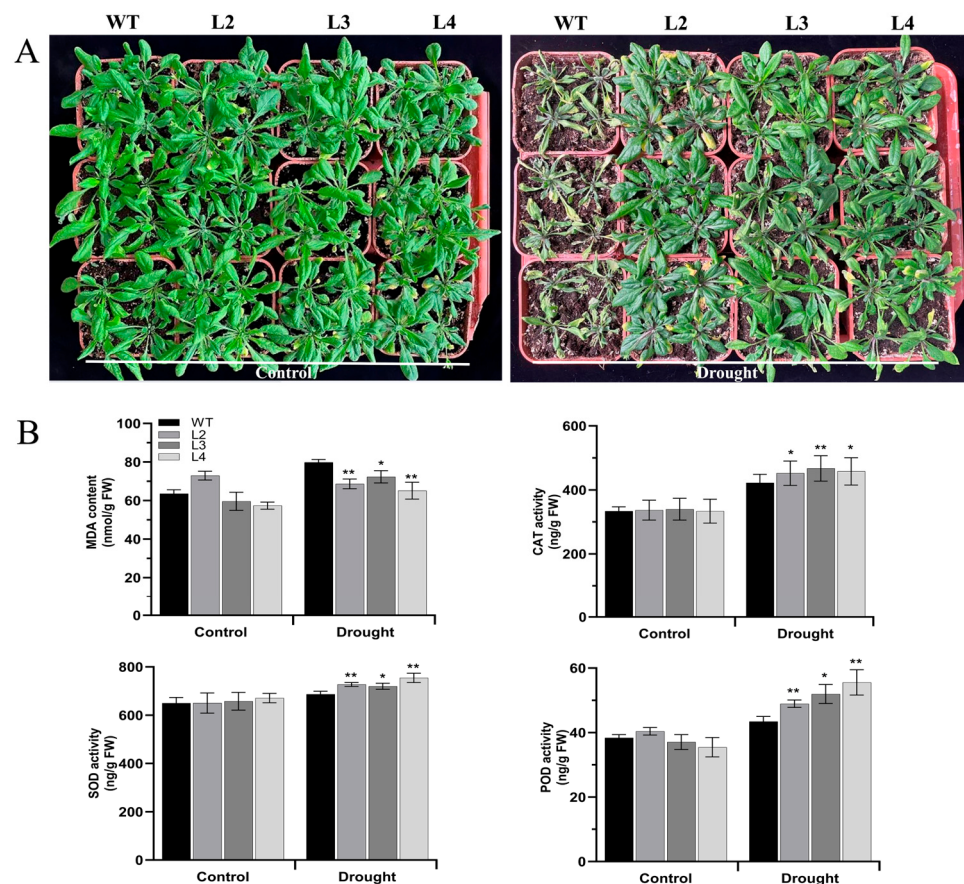


Figure 8. Phenotypic observation and physiological changes in WT and overexpressed *PmLEA25* lines. (A) Phenotype variation in WT and overexpressed *PmLEA25* lines after one-week drought treatment. (B) Antioxidant enzyme activities including MDA, SOD, POD, and CAT (*, $p < 0.05$; **, $p < 0.01$).

4. Discussion

P. mongolica, one of Inner Mongolia's three main sand management tree species, is widely distributed in the Hunsandak Desert and has excellent cold and drought tolerance, making it an important resource for resistance gene mining. LEA proteins assist plants in responding to various abiotic stress, such as salt stress, drought stress, high-temperature stress, and low-temperature stress [33–35]. LEA-related genes have recently been discovered in a range of plant species [15,17,36–39]; however, they have not been detected in *P. mongolica*.

Research showed that various plants differ in the number of members and distribution of subfamilies within the LEA gene family. For instance, Jin et al. [40] identified 48 *CsLEAs* in the tea tree, which were divided into the LEA_1 to LEA_5, DHN, and SMP subfamilies, but lacked the LEA_6 and ATM subfamilies. Similarly, Yu et al. [41] identified 26 *CkLEAs* in *Caragana korshinskii*, classified into eight subfamilies but lacking the LEA_5 subfamily. In soybean, 36 *GmLEAs* were identified, which can be divided into eight subfamilies, among

which the ATM subfamily is missing [42]. In poplar, a total of 87 LEAs were identified, divided into eight subfamilies, with the LEA_2 subfamily including the largest number of members [43]. In our study, we discovered 49 *PmLEAs* in *P. mongolica* (Table 1). The LEA_4 subfamily comprises the highest number of members, while the LEA_6 subfamily is absent (Figure 1). Members of the *LEA* gene family have been found in several species, each exhibiting a characteristic structural domain, but the distribution of members across different subfamilies shows significant variation, frequently accompanied by common subfamily deletions. In addition, in the tea tree, due to different identification methods and database ranges, 33 *CsLEAs* were identified by Wang et al. [44] while 48 *CsLEAs* were identified by Jin et al. [40]. Therefore, we hypothesize that there are more than 49 *PmLEAs* in *P. mongolica*, and there may be other members that have not been identified. The abundance of members in the LEA_4 subfamily suggests that it has evolved more rapidly and may have more complex physiological functions (Figure 1). Additionally, based on the physicochemical property analysis, most of the *PmLEAs* are small, hydrophilic molecules with highly conserved motifs within the same subfamily but widely varying motifs among subfamilies, such as the K, S, and Y fragments in all DHN subfamily members (Figure 2, Table S2), consistent with the findings of Jin et al. [40], Yu et al. [41], and Liang et al. [36], implying subfamily-specific functional roles for LEAs.

The mRNA of LEAs remains at elevated levels in desiccation-tolerant embryos, whereas the transcripts of storage proteins are entirely degraded during the final stages of embryonic development [45]. Our research findings indicate that most *PmLEAs* exhibit high levels of transcripts during the late phases of somatic embryonic development, especially notable in mature embryos (Figure 3). Similar results have been reported in wheat [46], and soybeans [47], indicating that during the development of somatic embryos in *P. mongolica*, *PmLEAs* may have different functions at various stages. *LEAs* are abundantly expressed in response to stressors such as drought, low temperature, high temperature, and high salinity [48]. In *Caragana korshinskii*, 19 *CkLEAs* were up-regulated over 2-fold in response to drought and salt stress and were influenced by stresses including ABA, high temperature, and low temperature [41]. In wheat, 46 *TaLEAs* exhibited up-regulation in expression due to low-temperature stress, whereas 177 *TaLEAs* were induced under drought stress, with 50 *TaLEAs* up-regulated during drought stress and 25 consistently up-regulated under both high-salt and low-temperature stress [49]. In the tea tree, 23 *CsLEAs* were up-regulated by low-temperature stress, 44 by high-temperature stress, 33 by drought stress, and 28 by ABA stress, of which 14 responded to all stresses [40]. Following drought treatment in *P. mongolica*, it was demonstrated that the expression of numerous *LEA* genes was up-regulated due to drought stress, with *PmLEA25* being the most notable (Figure 4), suggesting that *PmLEA25* plays a crucial role in the response to drought stress. The promoter was further predicted and analyzed to contain elements responsive to ARE, MEJA, and STRE (Figure S1), which were speculated to play a protective role in relevant adversities and may augment the resistance of *P. mongolica* to diverse abiotic adversities.

PmLEA25 proteins are classified as members of the DHN subfamily, while dehydrin proteins are classified as members of the second set of family proteins, also referred to as LEB proteins or water stress proteins (WSPs) [50]. Dehydrins, which are proteins found in plants, have significant functions in the way plants react to various abiotic stressors [51,52]. Various stress conditions lead to the accumulation of different types of dehydrins. The overexpression of the wheat *LEA* gene *DHN-5* in *Arabidopsis* dramatically enhances the plant's ability to withstand osmotic stress [53]. The introduction of the barley *LEA* gene *HVA7* into rice resulted in a substantial improvement in the tolerance of transgenic rice lines to drought and salt treatments [54]. An increased expression of the wheat *LEA* gene (*WCOR410*) in strawberry plants can greatly enhance the ability of leaves to withstand cold temperatures [55]. Furthermore, studies have verified that DHNS can greatly enhance the ability of pepper, peanut, and cotton plants to withstand or respond to abiotic stress [56–58]. The overexpression of *PmLEA25* enhanced drought tolerance, with transgenic plants showing better growth and less damage than wild-type plants under

stress conditions (Figures 6 and 7), suggesting that *PmLEA25* has a positive regulatory role in abiotic stress tolerance.

In response to abiotic stress, plants generate significant quantities of ROS and membrane lipid peroxidation products, such as MDA, which compromise cell membrane stability and cause cellular injury [59]. To mitigate this damage, plants synthesize antioxidant enzymes to neutralize ROS, as well as osmoregulatory compounds, such as proline, to maintain osmotic balance within the cell membrane [60]. The LEA protein has the ability to directly decrease the amounts of intracellular ROS and strengthen the plant's antioxidant system, hence diminishing oxidation [35]. The DHN gene *HbDHN2* was discovered in *Hevea brasiliensis*, and its overexpression in *Arabidopsis* greatly significantly enhanced tolerance to salt and drought. Under drought and salt stress, the transgenic lines exhibited longer root systems, reduced electrolyte leakage, and enhanced SOD, APX, and CAT activity, as well as elevated proline levels compared to the WT [61]. Furthermore, overexpressing the lemon (*Leguminosae*) group II LEA gene, *CkLEA2-3*, in *Arabidopsis* resulted in overexpression lines that accumulated less malondialdehyde and maintained higher SOD activity and GSH content under drought stress, thereby improving drought tolerance [41]. After converting the alfalfa *MfLEA3* gene into tobacco, the transgenic lines collected less ROS and had considerably greater SOD, CAT, and APX activity after a low temperature, drought, and strong light irradiation [62]. Under drought stress, we discovered that the overexpression of *PmLEA25* in *Arabidopsis* plants efficiently enhanced SOD, POD, and CAT enzyme activities; reduced MDA production; and improved antioxidant capacity (Figure 8). To summarize, the activation of the *PmLEA25* gene in plants positively regulates the plants' ability to withstand stress. This study offers significant stress resistance gene resources for *P. mongolica* and serves as a reference for further investigation into the plant's stress resistance mechanism.

Drought, salinity, and cold temperatures can induce water deficits in plants, leading to the accumulation of various protective proteins, with LEA proteins being the most prevalent [63]. The LEA gene family has a crucial role in drought-tolerant plants like *P. mongolica*, particularly in contexts of escalating drought and desertification. Enhancing the expression of this gene will be essential for the growth and survival of *P. mongolica*. Our research indicates that the overexpression of the *PmLEA25* gene can markedly enhance the drought resistance of transgenic *Arabidopsis* plants. While the precise molecular mechanisms underlying this enhancement warrant further investigation, the findings suggest a potential avenue for improving drought tolerance in *P. mongolica*. The lengthy and unstable traditional breeding cycle allows for the utilization of the LEA gene to enhance the drought tolerance of *P. mongolica* by advanced molecular breeding, hence augmenting its adaptability and survival rate in desertified regions. This not only mitigates ecological issues arising from drought but also establishes a scientific foundation for desertification prevention and ecological restoration.

5. Conclusions

In summary, this study offers a comprehensive and systematic analysis of the LEA family in *P. mongolica*. We found 49 *PmLEA* genes, categorized into eight subfamilies. The transcriptional profiling and gene expression pattern analysis revealed that several *PmLEA* genes have unique functions during somatic embryo development or drought stress treatment, with the *PmLEA25* gene exhibiting considerable up-regulation under drought conditions. Subsequent functional verification demonstrated that *Arabidopsis* plants overexpressing the *PmLEA25* gene enhanced their drought resistance by modulating antioxidant enzyme activity. Consequently, *PmLEA25* may serve as a candidate gene for drought resistance and offer theoretical support for the molecular breeding of *P. mongolica* and other plant species. Moreover, our findings enhance the comprehension of the function of LEA under drought stress and establish a basis for further investigation into the molecular mechanisms of drought resistance in *P. mongolica*.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/f15111988/s1>, Figure S1. Analysis of cis-elements in the promoter of *PmLEA25* gene. Table S1. Primer sequences used in this study. Table S2. Conserved motifs in different subfamilies of the *LEA* gene of *Picea mongolica*. Table S3. *PmLEA25* promoter cis-acting element.

Author Contributions: Y.W. and J.L. devised the experiment. Y.W. and Y.B. conducted the experiment. Y.W. and J.L. analyzed bioinformatics, phenotypic, and physiological data. Y.W. wrote and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the National Natural Science Foundation of China (32360402) and the Natural Science Foundation of Inner Mongolia Autonomous Region (No. 2022MS03037).

Data Availability Statement: The raw RNA-Seq data used in this study have been deposited in the Sequence Read Archive (SRA) database of the National Center for Biotechnology Information (NCBI) with the access number PRJNA649217, “<https://www.ncbi.nlm.nih.gov/sra/?term=PRJNA649217>” (accessed on 10 November 2024). ”.

Conflicts of Interest: The authors affirm that there are no conflicts of interest regarding the publication of this paper.

References

- Ha, B.R.; Bai, Y.E.; Hou, W.F.; Qing, S. Research progress and prospect of *Picea mongolica* of species endemic to sandy land. *North. Hortic.* **2021**, *14*, 152–157. (In Chinese)
- Zou, C.J.; Han, S.J.; Xu, W.D. Formation, distribution, and nature of *Picea mongolica* in China. *J. For. Res.* **2001**, *12*, 187–191.
- Huang, S.X.; Li, X.B.; Lin, T.M.; Quan, C.M.; Luo, J.C.; Wang, L.X. Breeding techniques and annual growth rhythm of *Picea mongolica* seedlings. *J. Beijing For. Univ.* **2003**, *25*, 11–14. (In Chinese)
- Li, X.Y. Analysis on physiological and biochemical indexes of leaves of 8 *Picea* Plants. *For. Sci. Tech.* **2020**, *4*, 26–30. (In Chinese)
- Yan, J.; Peng, P.; Duan, G.Z.; Lin, T.; Bai, Y.E. Multiple analyses of various factors affecting the plantlet regeneration of *Picea mongolica* (H. Q. Wu) W.D. Xu from somatic embryos. *Sci. Rep.* **2021**, *11*, 6694. [CrossRef]
- Shi, X.; Wang, C.E.; Liu, Y.; Jiang, H.Y.; Jin, S.T. Distribution of Endophytic Fungi in *Picea mongolica*. *J. Anhui Agric. Sci.* **2016**, *44*, 20–22. (In Chinese)
- Latchman, D.S. Transcription Factors: An Overview. *Int. J. Biochem. Cell Biol.* **1997**, *29*, 1305–1312. [CrossRef]
- Artur, M.A.S.; Zhao, T.; Ligterink, W.; Schranz, E.; Hilhorst, H.W.M. Dissecting the Genomic Diversification of Late Embryogenesis Abundant (LEA) Protein Gene Families in Plants. *Genome Biol. Evol.* **2019**, *11*, 459–471. [CrossRef]
- Garay-Arroyo, A.; Colmenero-Flores, J.M.; Garciarrubio, A.; Covarrubias, A.A. Highly hydrophilic proteins in prokaryotes and eukaryotes are common during conditions of water deficit. *J. Biol. Chem.* **2000**, *275*, 5668–5674. [CrossRef]
- Hunault, G.; Jaspard, E. LEAPdb: A database for the late embryogenesis abundant proteins. *BMC Genom.* **2010**, *11*, 221. [CrossRef]
- Dure, L.R.; Crouch, M.; Harada, J.; Ho, T.H.; Mundy, J.; Quatrano, R.; Thomas, T.; Sung, Z.R. Common amino acid sequence domains among the LEA proteins of higher plants. *Plant Mol. Biol.* **1989**, *12*, 475–486. [CrossRef] [PubMed]
- Dure, L.; Greenway, S.C.; Galau, G.A. Developmental biochemistry of cottonseed embryogenesis and germination: Changing messenger ribonucleic acid populations as shown by in vitro and in vivo protein synthesis. *Biochemistry* **1981**, *20*, 4162–4168. [CrossRef] [PubMed]
- Hundertmark, M.; Hinch, D.K. LEA (late embryogenesis abundant) proteins and their encoding genes in *Arabidopsis thaliana*. *BMC Genom.* **2008**, *9*, 118. [CrossRef] [PubMed]
- Wang, X.S.; Zhu, H.B.; Jin, G.L.; Liu, H.L.; Wu, W.R.; Zhu, J. Genome-scale identification and analysis of LEA genes in rice (*Oryza sativa* L.). *Plants* **2007**, *172*, 414–420. [CrossRef]
- Li, X.; Cao, J. Late embryogenesis abundant (LEA) gene family in Maize: Identification, evolution, and expression profiles. *Plant Mol. Biol. Rep.* **2016**, *34*, 15–28. [CrossRef]
- Cao, J.; Li, X. Identification and phylogenetic analysis of late embryogenesis abundant proteins family in tomato (*Solanum lycopersicum*). *Planta* **2015**, *241*, 757–772. [CrossRef]
- Cheng, Z.H.; Zhang, X.M.; Yao, W.J.; Zhao, K.; Liu, L.; Fan, G.F.; Zhou, B.; Jiang, T.B. Genome-wide search and structural and functional analyses for late embryogenesis-abundant (LEA) gene family in poplar. *BMC Plant Biol.* **2021**, *21*, 110. [CrossRef]
- Gao, J.; Lan, T. Functional characterization of the late embryogenesis abundant (LEA) protein gene family from *Pinus tabulaeformis* (Pinaceae) in *Escherichia coli*. *Sci. Rep.* **2016**, *6*, 19467. [CrossRef]
- Wilhelm, K.S.; Thomashow, M.F. *Arabidopsis thaliana* *cor15a*, an apparent homologue of *cor15a*, is strongly responsive to cold and ABA, but not drought. *Plant Mol. Biol.* **1993**, *23*, 1073–1077. [CrossRef]
- Welin, B.V.; Olson, A.; Nylander, M.; Palva, E.T. Characterization and differential expression of *dhn/lea/rab*-like genes during cold acclimation and drought stress in *Arabidopsis thaliana*. *Plant Mol. Biol.* **1994**, *26*, 131–144. [CrossRef]
- Jia, F.J.; Qi, S.D.; Li, H.; Liu, P.; Li, P.C.; Wu, C.G.; Zheng, C.C.; Huang, J.G. Overexpression of Late Embryogenesis Abundant 14 enhances *Arabidopsis* salt stress tolerance. *Biochem. Biophys. Res. Commun.* **2014**, *454*, 505–511. [CrossRef] [PubMed]

22. Kong, H.; Xia, W.W.; Hou, M.J.; Ruan, N.; Li, J.; Zhu, J.B. Cloning and function analysis of a *Saussurea involucrata* LEA4 gene. *Front. Plant Sci.* **2022**, *13*, 957133. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Liu, X.Y.; Xia, W.W.; Zhang, X.L.; Li, A.W.; Qin, J.W.; Sun, H.L.; Li, J.; Zhu, J.B. Overexpression of the *SiLEA5* gene in *Saussurea involucrata* increases the Low-Temperature tolerance of transgenic tomatoes. *Horticulturae* **2022**, *8*, 1023. [\[CrossRef\]](#)
24. Wang, Z.K.; Yang, Q.; Shao, Y.P.; Zhang, B.B.; Feng, A.Y.; Meng, F.L.; Li, W.B. *GmLEA2-1*, a late embryogenesis abundant protein gene isolated from soybean (*Glycine max* (L.) Merr.), confers tolerance to abiotic stress. *Acta Biol. Hung.* **2018**, *69*, 270–282. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Shen, Y.Z.; Wei, J.P.; Zhou, Y.L.; Zhu, Y.J.; Liu, S.S.; Wang, Y.L.; Ma, H. Soybean late embryogenesis abundant protein GmLEA4 interacts with GmCaM1, enhancing seed vigor in transgenic *Arabidopsis* under high temperature and humidity stress. *Plant Growth Regul.* **2022**, *99*, 583–595. [\[CrossRef\]](#)
26. Bai, Y.Q.; Yang, Q.C.; Kang, J.M.; Sun, Y.; Gruber, M.; Chao, Y.H. Isolation and functional characterization of a *Medicago sativa* L. gene, *MsLEA3-1*. *Mol. Biol. Rep.* **2012**, *39*, 2883–2892. [\[CrossRef\]](#)
27. Jia, H.L.; Wang, X.M.; Shi, Y.H.; Wu, X.M.; Wang, Y.Q.; Liu, J.N.; Fang, Z.H.; Li, C.Y.; Dong, K.H. Overexpression of *Medicago sativa* *LEA4-4* can improve the salt, drought, and oxidation resistance of transgenic *Arabidopsis*. *PLoS ONE* **2020**, *15*, e0234085. [\[CrossRef\]](#)
28. Lv, A.M.; Su, L.T.; Wen, W.W.; Fan, N.N.; Zhou, P.; An, Y. Analysis of the function of the Alfalfa *Mslea-D34* gene in abiotic stress responses and flowering time. *Plant Cell Physiol.* **2021**, *62*, 28–42. [\[CrossRef\]](#)
29. Luo, D.; Zhang, X.; Li, Y.L.; Wu, Y.G.; Li, P.Z.; Jia, C.L.; Bao, Q.Y.; Zhou, Q.; Fu, C.X.; Liu, W.X.; et al. *MsDIUP1* encoding a putative novel LEA protein positively modulates salt tolerance in alfalfa (*Medicago sativa* L.). *Plant Soil* **2023**, *487*, 547–566. [\[CrossRef\]](#)
30. Zhou, M.Q.; Peng, N.F.; Yang, C.P.; Wang, C. The Poplar (*Populus trichocarpa*) dehydrin gene *PtrDHN-3* enhances tolerance to salt stress in *Arabidopsis*. *Plants* **2022**, *11*, 2700. [\[CrossRef\]](#)
31. Hall, T.A. BioEdit: A user-friendly biological sequence alignment program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* **1999**, *41*, 95–98.
32. Zhang, X.; Henriques, R.; Lin, S.S.; Niu, Q.W.; Chua, N.H. Agrobacterium-mediated transformation of *Arabidopsis thaliana* using the floral dip method. *Nat. Protoc.* **2006**, *1*, 641–646. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Tunncliffe, A.; Wise, M.J. The continuing conundrum of the LEA proteins. *Naturwissenschaften* **2007**, *94*, 791–812. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Bhardwaj, R.; Sharma, I.; Kanwar, M.; Sharma, R.; Handa, N.; Kaur, H.; Poonam, D.K. LEA proteins in salt stress tolerance. In *Salt Stress Plants*; Springer: New York, NY, USA, 2013; pp. 79–112.
35. Mohanty, S.; Hembram, P. An overview of LEA genes and their importance in combating abiotic stress in Rice. *Plant Mol. Biol. Rep.* **2024**, 1–15. [\[CrossRef\]](#)
36. Liang, Y.; Xiong, Z.; Zheng, J.; Xu, D.; Zhu, Z.; Xiang, J.; Gan, J.; Raboanatahiry, N.; Yin, Y.; Li, M. Genome-wide identification, structural analysis and new insights into late embryogenesis abundant (LEA) gene family formation pattern in *Brassica napus*. *Sci. Rep.* **2016**, *6*, 24265. [\[CrossRef\]](#)
37. İbrahim, M.; Kibar, U.; Kazan, K.; Özmen, C.Y.; Mutaf, F.; Aşçı, S.D.; Aydemir, B.C.; Ergül, A. Genome-wide identification of the LEA protein gene family in grapevine (*Vitis vinifera* L.). *Tree Genet. Genomes* **2019**, *15*, 55. [\[CrossRef\]](#)
38. Jia, C.; Guo, B.; Wang, B.; Li, X.; Yang, T.; Li, N.; Wang, J.; Yu, Q. The LEA gene family in tomato and its wild relatives: Genome-wide identification, structural characterization, expression profiling, and role of *SILEA6* in drought stress. *BMC Plant Biol.* **2022**, *22*, 596. [\[CrossRef\]](#)
39. Huang, R.L.; Xiao, D.; Wang, X.; Zhan, J.; Wang, A.Q.; He, L.F. Genome-wide identification, evolutionary and expression analyses of LEA gene family in peanut (*Arachis hypogaea* L.). *BMC Plant Biol.* **2022**, *22*, 155. [\[CrossRef\]](#)
40. Jin, X.; Cao, D.; Wang, Z.; Ma, L.; Tian, K.; Liu, Y.; Gong, Z.; Zhu, X.; Jiang, C.; Li, Y. Genome-wide identification and expression analyses of the LEA protein gene family in tea plant reveal their involvement in seed development and abiotic stress responses. *Sci. Rep.* **2019**, *9*, 14123. [\[CrossRef\]](#)
41. Yu, X.M.; Yue, W.R.; Yang, Q.; Zhang, Y.N.; Han, X.M.; Yang, F.Y.; Wang, R.G.; Li, G.J. Identification of the LEA family members from *Caragana korshinskii* (Fabaceae) and functional characterization of *CkLEA2-3* in response to abiotic stress in *Arabidopsis*. *Braz. J. Bot.* **2019**, *42*, 227–238. [\[CrossRef\]](#)
42. Li, L.; Xu, H.L.; Yang, X.L.; Li, Y.X.; Hu, Y.K. Genome-wide identification, classification and expression analysis of LEA gene family in Soybean. *Sci. Agric. Sin.* **2011**, *44*, 3945–3954. (In Chinese)
43. Li, J.; Zhao, A.C.; Wang, X.L.; Liu, X.Q.; Yu, M.D. On genome-scale identification and expression onanalysis of LEA gene in Poplar. *J. South China Norm. Univ.* **2016**, *41*, 48–56. (In Chinese)
44. Wang, W.D.; Gao, T.; Chen, J.F.; Yang, J.K.; Huang, H.Y.; Yu, Y.B. The late embryogenesis abundant gene family in tea plant (*Camellia sinensis*): Genome-wide characterization and expression analysis in response to cold and dehydration stress. *Plant Physiol. Bioch.* **2019**, *135*, 277–286. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Goldberg, R.B.; Barker, S.J.; Perez-Grau, L. Regulation of gene expression during plant embryogenesis. *Cell* **1989**, *56*, 149–160. [\[CrossRef\]](#) [\[PubMed\]](#)
46. Zan, T.; Li, L.Q.; Li, J.T.; Zhang, L.; Li, X.J. Genome-wide identification and characterization of late embryogenesis abundant protein-encoding gene family in wheat: Evolution and expression profiles during development and stress. *Gene* **2020**, *736*, 144422. [\[CrossRef\]](#)

47. Liu, Y.; Wang, L.; Xing, X.; Sun, L.; Pan, J.; Kong, X.; Zhang, M.; Li, D. ZmLEA3, a multifunctional group 3 LEA protein from maize (*Zea mays* L.), is involved in biotic and abiotic stresses. *Plant Cell Physiol.* **2013**, *54*, 944–959. [\[CrossRef\]](#)
48. Abdul Aziz, M.; Sabeem, M.; Mullath, S.K.; Brini, F.; Masmoudi, K. Plant Group II LEA Proteins: Intrinsically Disordered Structure for Multiple Functions in Response to Environmental Stresses. *Biomolecules* **2021**, *11*, 1662. [\[CrossRef\]](#)
49. Liu, H.; Xing, M.; Yang, W.; Mu, X.; Wang, X.; Lu, F.; Wang, Y.; Zhang, L. Genome-wide identification of and functional insights into the late embryogenesis abundant (LEA) gene family in bread wheat (*Triticum aestivum*). *Sci. Rep.* **2019**, *9*, 13375. [\[CrossRef\]](#)
50. Pan, X.X.; Hu, H.F.; Chen, N.; Zhang, H.F.; Chen, R.G. Research progress on the role of dehydrin in plant abiotic stress. *J. Agric. Biotech.* **2022**, *30*, 594–605. (In Chinese)
51. Maszkowska, J.; Dębski, J.; Kulik, A.; Kistowski, M.; Bucholc, M.; Lichocka, M.; Klimecka, M.; Sztatelman, O.; Szymańska, K.P.; Dadlez, M.; et al. Phosphoproteomic analysis reveals that dehydrins ERD10 and ERD14 are phosphorylated by SNF1-related protein kinase 2.10 in response to osmotic stress. *Plant Cell Environ.* **2019**, *42*, 931–946. [\[CrossRef\]](#)
52. Mittler, R.; Kim, Y.; Song, L.; Coutu, J.; Coutu, A.; Ciftci-Yilmaz, S.; Lee, H.; Stevenson, B.; Zhu, J.K. Gain-and loss-of-function mutations in *Zat10* enhance the tolerance of plants to abiotic stress. *FEBS Lett.* **2006**, *580*, 6537–6542. [\[CrossRef\]](#) [\[PubMed\]](#)
53. Brini, F.; Yamamoto, A.; Jlaiel, L.; Takeda, S.; Hobo, T.; Dinh, H.Q.; Hattori, T.; Masmoudi, K.; Hanin, M. Pleiotropic effects of the wheat dehydrin DHN-5 on stress responses in *Arabidopsis*. *Plant Cell Physiol.* **2011**, *52*, 676–688. [\[CrossRef\]](#) [\[PubMed\]](#)
54. Xu, D.; Duan, X.; Wang, B.; Hong, B.; Ho, T.H.D.; Wu, R. Expression of a late embryogenesis abundant protein gene, HVA1, from barley confers tolerance to water deficit and salt stress in transgenic rice. *Plant Physiol.* **1996**, *110*, 249–257. [\[CrossRef\]](#) [\[PubMed\]](#)
55. Houde, M.; Dallaire, S.; N'Dong, D.; Sarhan, F. Overexpression of the acidic dehydrin WCOR410 improves freezing tolerance in transgenic strawberry leaves. *Plant Biotechnol. J.* **2004**, *2*, 381–387. [\[CrossRef\]](#) [\[PubMed\]](#)
56. Jing, H.; Li, C.; Ma, F.; Ma, J.H.; Khan, A.; Wang, X.; Zhao, L.Y.; Gong, Z.H.; Chen, R.G. Genome-wide identification, expression diversification of dehydrin gene family and characterization of *CaDHN3* in pepper (*Capsicum annuum* L.). *PLoS ONE* **2016**, *11*, e0161073. [\[CrossRef\]](#)
57. Williams, T.C.R.; Costa, M.M.D.C.; Araujo, A.C.G.; Danchin, E.G.J.; Grossi-de-Sá, M.F.; Guimaraes, P.M.; Brasileiro, A.C.M. Contrasting effects of wild *Arachis* dehydrin under abiotic and biotic stresses. *Front. Plant Sci.* **2019**, *10*, 497.
58. Kirungu, J.N.; Magwanga, R.O.; Pu, L.; Cai, X.Y.; Xu, Y.C.; Hou, Y.Q.; Zhou, Y.; Cai, Y.F.; Hao, F.S.; Zhou, Z.L.; et al. Knockdown of *Gh_A05G1554* (*GhDHN_03*) and *Gh_D05G1729* (*GhDHN_04*) dehydrin genes, reveals their potential role in enhancing osmotic and salt tolerance in cotton. *Genomics* **2020**, *112*, 1902–1915. [\[CrossRef\]](#)
59. Soliman, W.S.; Fujimori, M.; Tase, K.; Sugiyama, S. Heat tolerance and suppression of oxidative stress: Comparative analysis of 25 cultivars of the C3 grass *Lolium perenne*. *Environ. Exp. Bot.* **2012**, *78*, 10–17. [\[CrossRef\]](#)
60. Ge, S.B.; Jiang, X.C.; Wang, L.Y.; Yu, J.Q.; Zhou, Y.H. Recent advances in the role and mechanism of arbuscular mycorrhizainduced improvement of abiotic stress tolerance in horticultural plan. *Acta Hort. Sin.* **2020**, *47*, 1752–1776. (In Chinese)
61. Cao, Y.; Xiang, X.; Geng, M.; You, Q.; Huang, X. Effect of *HbDHN1* and *HbDHN2* genes on abiotic stress responses in *Arabidopsis*. *Front. Plant Sci.* **2017**, *8*, 470. [\[CrossRef\]](#)
62. Shi, H.F.; He, X.Y.; Zhao, Y.J.; Lu, S.Y.; Guo, Z.F. Constitutive expression of a group 3 LEA protein from *Medicago falcata* (*MfLEA3*) increases cold and drought tolerance in transgenic tobacco. *Plant Cell Rep.* **2020**, *39*, 851–860. [\[CrossRef\]](#) [\[PubMed\]](#)
63. Li, N.; Zhao, Y.j.; Zhao, Q.; Huang, J.; Zhang, S.H. Research on barley HVA1 gene and Lea protein with plant drought tolerance. *Biotechnol. Bull.* **2006**, *4*, 25–29. (In Chinese)

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.