Spatiotemporal Expression and Bioinformatic Analyses of the HD-Zip Transcription Factor Family in *Larix olgensis*

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Received: 24 February 2020 / Accepted: 13 August 2020 / Published online: 10 September 2020
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Abstract

Larix olgensis is one of the main coniferous tree species in northeastern China and has excellent timber properties and strong tolerance to stress. Thirteen HD-Zip family genes with a complete CDS region were identified on the basis of cambium transcriptome data from *Larix olgensis*. All 13 genes were analyzed via bioinformatics by their conserved domain protein sequence and amino acid composition, including their physicochemical properties and protein structure. The spatiotemporal expression and abiotic stress responses of these genes were analyzed by real-time quantitative PCR. The results showed that the 13 HD-Zip genes of *Larix olgensis* were expressed in the roots, stems, and leaves at different stages. The expression of three of these genes (*LoHDZ2*, *LoHDZ11*, *LoHDZ13*) was highest in nonlignified roots, indicating that they might be related to the secondary growth of *Larix olgensis*; in addition, three genes (*LoHDZ5*, *LoHDZ9*, *LoHDZ10*) were highly expressed in partially and completely lignified stems and leaves. These 13 genes were expressed specifically under drought stress. The expression of two of them (*LoHDZ1*, *LoHDZ5*) was obviously upregulated, and the expression of 6 genes (*LoHDZ2*, *LoHDZ3*, *LoHDZ4*, *LoHDZ8*, *LoHDZ10*, *LoHDZ13*) was significantly downregulated. The expression trends indicate that these genes could be involved in drought stress. The expression of all 13 genes was downregulated when the plants were treated with 0.2 M NaCl for 96 h, indicating that these genes are inhibited by salt stress. Overall, the results have significant implications for the study of the gene function of members of the *LoHD-Zip* transcription factor family.

Keywords *Larix olgensis* · Growth and development · Bioinformatics · Spatiotemporal expression analysis · HD-Zip family

Introduction

Larix olgensis is a deciduous tree species in the genus *Larix* and is the main timber and afforestation species in Northeast

Key Message

In this study, we screened and identified thirteen HD-Zip family genes with complete CDS regions based on the sequencing results of *Larix olgensis*. According to the characteristics, we divided these genes into four subfamilies: HD-ZipI, HD-ZipII, HD-ZipIII, and HD-ZipIV. We used bioinformatics-related software and online websites to analyze the physiological and biochemical indexes and protein structure of HD-Zip family genes. And we performed real-time fluorescence quantitative analysis of these genes in different tissues at different periods and in response to abiotic stress.

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China (Yu and Bao 2010). High salinity, drought, and low temperature constitute the main abiotic factors affecting the growth of larch and other tree species in Northeast China (Jinhua et al. 2003). Studies on the molecular mechanisms involving these genes can unravel the mystery of plant growth under adverse conditions, and genetic engineering can be used to improve the ability of plants to resist various abiotic stresses. The present research on gene function mainly focuses on functional genes and transcription factors. Transcription factors, which are also known as *trans*-acting factors, are DNA-binding proteins that can specifically interact with *cis*-acting elements within the promoter region of eukaryotic genes and activate or inhibit transcription via interactions between them and with other related proteins (Liu et al. 2008). Studies have shown that many transcription factors are involved in the regulation of plant growth and abiotic stress, such as HD-Zip WRKY, AP2/ERF, and MYB (Schna and Davis 1992).

Homeodomain-leucine zippers (HD-Zip) are a kind of plant-specific transcription factor. HD-Zip belongs to the homeobox protein family and has a highly conserved homeodomain (HD). Consisting of 61 amino acids (Schna

and Davis 1992), this domain can be folded into a typical spiral-ring-helix-turn-helix structure that can specifically bind to DNA (Bharathan et al. 1997). Its end is closely linked to the leucine zipper (LZ) domain, and the leucine zipper domain mainly mediates the formation of protein dimers (Jain et al. 2008). This indicates that HD-Zip genes play an important role in certain developmental processes of higher plants (Ariel et al. 2007). Based on sequence conservation, gene structure and physiological functions, HD-Zip transcription factors can be divided into four subclasses: HD-ZipI, HD-ZipII, HD-ZipIII, and HD-ZipIV (Mukherjee et al. 2009) (Fig. 1). Due to the differences in gene composition and the structure of their encoded proteins in different subfamilies, they are involved in different plant development processes and regulate different metabolic processes. Current research on the HD-Zip transcription factor family in *Arabidopsis*, rice, apple, alfalfa, and other plant species has revealed several conclusions: HD-ZipI proteins may be involved in light signal transduction (Henriksson et al. 2005), regulation of leaf and seed development (Henriksson et al. 2005; Kim et al. 2007), and responses to stress (Harris et al. 2016; Zhao et al. 2014a, b); the conserved domain unique to the HD-Zip class II subfamily comprises CPSCE (Ciarbelli et al. 2008) and plays an important role in mediating responses to light quality changes (Franklin et al. 2003), shade (Carabelli et al. 2013), and abiotic stress (Park et al. 2013); the HD-ZipIII subfamily genes have a complex structure that allows them to inhibit the transcription of genes mainly involved in plant embryo development (Prigge et al. 2005), meristem formation (Zhu et al. 2013), and vascular development (Robischon et al. 2011); and the HD-Zip class IV subfamily genes play an important role in plant epidermal cell development (Ingram et al. 2000), material accumulation and transport (Ito et al. 2002), and resistance to both biotic stress and abiotic stress (Nakamura et al. 2006).

In this study, BLAST alignments were performed via the NCBI website (<https://blast.ncbi.nlm.nih.gov>) by analyzing the results of the existing transcriptome data from the *Larix*

olgensis cambium. All 13 HD-Zip transcription factor family genes (named *LoHDZ1~LoHDZ13*) with complete CDS regions were obtained. To understand the function of the members of the HD-Zip family of *Larix olgensis*, bioinformatic analysis and gene expression analysis were performed on these 13 HD-Zip genes. Because there are few studies on the larch HD-Zip transcription factor family, this study provides a partial reference for future studies on the functions of larch HD-Zip transcription factor family genes.

Materials and Methods

Materials

Seeds of *Larix olgensis* used in this experiment were obtained from Jixi, Heilongjiang Province. Full, lustrous *Larix olgensis* seeds were selected and soaked in deionized water for 4–5 days, which was replaced 3–4 times during this period. The plants were then planted in a soil to vermiculite to perlite (5:3:2 v/v/v) nutrient soil matrix (the substrates used in this experiment were nutrient-enriched components arranged at that ratio). Using plastic wrap to maintain sufficient moisture, the plant growth conditions consisted of a 16-h light/8-h dark photoperiod, a humidity of 75%, and a constant temperature of 22 °C.

After 25–30 days, when the needles of larch were fully extended, the seedlings exhibiting good growth were selected and transplanted to a seedling basin for further cultivation in the nutrient-enriched soil matrix. Seedlings grown for 2 months were treated with 7% (w/v), 14%, 21%, and 28% PEG₆₀₀₀ for 0 h (control), 24 h, 48 h, 72 h, and 96 h, at which times samples were collected (Lian et al. 2019). After processing, extracted RNA of complete seedlings for reverse transcription; other larch seedlings were sprayed with 0.2 M NaCl solution (Ma et al. 2012), after which samples were collected after 0 h (control), 12 h, 24 h, 48 h, and 96 h. After

Fig. 1 The structure of HD-Zip subfamily

treatment, extracted RNA of complete seedlings for reverse transcription and real-time PCR was performed.

The roots, stems, and leaves (organ parts were taken from the same plant during the same period) of *Larix olgensis* plants that had grown for 3 months (no lignification period), 6 months (partial lignification period), and 9 months (complete lignification period) were collected. The organs were collected from the same plant as from which material was collected for the expression analysis of the genes in different tissues at different periods.

Methods

Bioinformatic Analysis

The study was based on the existing transcriptome data of *Larix olgensis* and compared 13 HD-Zip family genes with complete CDS regions using the NCBI website. Thirteen HD-Zip transcription factor family genes were classified according to their conserved domain characteristics by using BioEdit bioanalytical software. Thirteen genes were translated into their corresponding amino acid sequences by using BIOXM software. ExPASy online software was then used to analyze the physical and chemical properties of the *LoHD-Zip* protein and the molecular weight and isoelectric point of the 13 HD-Zip genes; moreover, the physicochemical properties, such as the fat coefficient and stability coefficient (Zhang et al. 2017), were obtained. The secondary structure of the protein was analyzed by SOPMA online software. And their subcellular localization was predicted by SUBA4 online software MEGA 5.0 which was used to construct an evolutionary tree of the 13 *LoHD-Zip* and 50 *Arabidopsis* HD-Zip family genes. The bioinformatics website is shown in Table 1.

Real-Time PCR

Fluorescence quantitative PCR was used to analyze the spatiotemporal expression pattern of the genes in larch and their patterns of involvement in the abiotic stress response.

In this study, the RNA of *Larix olgensis* was extracted by using a PureLink™ Plant RNA Reagent Kit, and the extraction procedure was carried out according to the manufacturer's instructions; the RNA of *Larix olgensis* was then reverse transcribed using a kit (ReverseScript^{RT} reagent Kit, TaKaRa) to obtain cDNA. Primer Premier 5 software (Xin-Yu and Yan-Ning 2004) was used to design the primers (Table 2), and the fluorescence quantification reagent was TaKaRa's SYBR Premix Ex TaqII (Tli RnaseH Plus) Kit. The test procedure was carried out according to the instructions of the kit (Table 3), and an ABI 7500 real-time PCR instrument (made by American ABI company) was used. After PCR, the dissociation curve was analyzed. The dissolution curve was constructed according to the manufacturer's information provided with the ABI 7500 instrument, the relevant data were derived, and the difference between the three CT values obtained was less than 1. Data analysis was performed using Microsoft Excel 2016, and the data were calculated using the $-\Delta\Delta C_t$ method and plotted with GraphPad Prism 5 software. A-Tubulin was selected as a reference gene, in which the accession was *MF278617.1* in the NCBI.

The real-time fluorescent quantitative PCR conditions were as follows: 94 °C for 30 s, 94 °C for 5 s, 60 °C for 15 s, and 72 °C for 10 s. Steps 2–5 required 40 to 45 cycles.

Results

Identification of the HD-Zip Gene Family Members in *Larix olgensis* and Analysis of the Physicochemical Properties of the Encoded Proteins

The thirteen genes were divided into four subcategories: HD-ZipI, HD-ZipII, HD-ZipIII, and HD-ZipIV. Among these genes, 5 belonged to the HD-ZipI subfamily (*LoHDZ2*, *LoHDZ3*, *LoHDZ4*, *LoHDZ7*, *LoHDZ11*), 2 belonged to the HD-ZipII subfamily (*LoHDZ1*, *LoHDZ10*), 4 belonged to the HD-ZipIII subfamily (*LoHDZ6*, *LoHDZ8*, *LoHDZ12*, *LoHDZ13*), and 2 belonged to the HD-ZipIV subfamily (*LoHDZ5*, *LoHDZ9*). Analysis of each subfamily revealed

Table 1 Bioinformatics analysis software and website used in this paper

Phylogenetic tree	MEGA 5.0
Physical and chemical properties of protein	ExPASy (http://web.expasy.org/protparam/)
Protein function domain	SMART (http://smart.embl-heidelberg.de/smart/set_mode.cgi?NORMAL=1)
Signal peptide	SignalP (http://www.cbs.dtu.dk/services/SignalP/)
Secondary structure	SOPMA (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_sopma.html)
Tertiary structure	SWISS MODEL (https://www.swissmodel.expasy.org/)
Subcellular localization	SUBA4 (http://suba.live/index.html)

Table 2 The primers used in real-time RT-PCR

Gene name	Forward and reverse primers (5'-3')	
<i>LoHDZ1</i>	TCCATATCCATACCCATCTGTTTC	TTTTTTATTGTGAACCAGTAGGGG
<i>LoHDZ2</i>	CTTGGCGTTGGTGTGTCTATG	TGGGCATGAACCAAGAAAC
<i>LoHDZ3</i>	ACAGACAGCAGTGGGGTCC	TGCTTCAAAAAGTTGGGAGG
<i>LoHDZ4</i>	TTGACCTATAGCACTGAAGACCC	AGAATGTCCGCTATCAATGTGAG
<i>LoHDZ5</i>	CTCGTCATGCTAAAGCTGGC	TATTGTCACTACCCGATTGCTC
<i>LoHDZ6</i>	TGGTTGGTCTTTGTTGGGTAG	TCAATGTTACAATCAGCCCACTC
<i>LoHDZ7</i>	GAGGACAACAGTTCTGCTAATGC	AGATCGAGTACCTTGAAACGTTG
<i>LoHDZ8</i>	TTCTTGCGAGAGCATCGATC	GAGAATGACCTGGCCACCTC
<i>LoHDZ9</i>	TGGTAGCGGTGCTGAAGATG	CAGGAAGCCAGAAGGACGT
<i>LoHDZ10</i>	TCATATGTGCCCTTCCTGC	GCCTTGGTGTGTTGTCTGG
<i>LoHDZ11</i>	TTACAGCCAAAAGCGAAGAAAG	TTCTTCATCGGTCTTGTAGGC
<i>LoHDZ12</i>	AACTCTTGGTGGTGGCATCC	GACTCCCTGAAAATCCCCCT
<i>LoHDZ13</i>	GATGATGGCTGGACTTTGATG	AGCCCTTCAGCAGATGAAAAC
<i>LoB80280</i>	GCCGTGCTGCTGGATAATGAGG	TGTCTGGAACCTCAGTCACATCAACG

that the transcription factors of both *LoHD-ZipI* and *LoHD-ZipII* contained an HD region and a Zip region. The *LoHD-ZipII* transcription factor also contained a unique CPSCE motif consisting of 5 amino acids; in addition to the HD region and the Zip region, the *LoHD-ZipIII* transcription factor included a SAD region and a START region with unknown functions and a MEKHLA C-terminal domain. The *LoHD-ZipIV* transcription factors included an HD region, Zip region, SAD region, and START region. Furthermore, it can be seen (Fig. 2) that the conservation of the *LoHD-ZipIV* and *LoHD-ZipIII* subfamilies is higher than that of the *LoHD-ZipI* and *LoHD-ZipII* subfamilies.

The physicochemical properties of the HD-Zip transcription factor family genes in *Larix olgensis* showed that the length of the 13 genes is between 800 and 2500 bp and that the molecular weight of the 13 genes of the transcription factor family ranges between 31.29 and 92.72 kDa. The isoelectric point of the protein is between 4 and 7, except for that of *LoHDZ1*, which is within the acidic range, and the fat coefficient is higher than 50.59 (Table 4). These results indicate that the proteins of the HD-Zip transcription factor family have a relatively high thermal stability. All the average hydrophobicity coefficients are negative, which indicates that all the HD-

Zip proteins are hydrophilic. According to the signal peptide analysis, the *LoHD-Zip* protein has no signal peptide, indicating that it is a non-secreted protein. Protein transmembrane analysis showed that all the HD-Zip proteins are non-transmembrane proteins. The subcellular localization of the 13 HD-Zip genes was predicted by SUBA4 online software. The preliminary analysis focused on the nucleus, and the results indicated that *LoHD-Zip* plays a role in the nucleus.

The phylogenetic tree of the HD-Zip family genes of *Larix olgensis* and *Arabidopsis thaliana* (Fig. 3) shows that HD-Zip genes have a high degree of homology between *Larix olgensis* and *Arabidopsis*. The genetic distance between *LoHDZ9* and *Arabidopsis AT2G032370.1* is the closest, indicating that the kinship is the closest; in addition, the genetic distance between *LoHDZ11* and *Arabidopsis AT3G01470.1* is close, indicating a close relationship. The results of the phylogenetic tree provides a theoretical reference for future exploration of the function of HD-Zip family genes in *Larix olgensis*.

The secondary structural characteristics of the 13 HD-Zip transcription factor family genes in *Larix olgensis* showed that the *LoHD-Zip* protein has α -helix (Hh), extended fold (Ee), and random coil (Cc) structures (Fig. 4, Table 5). α -Helix and random coil are the major components of the members of this family. The results showed that the secondary structures of most *LoHD-ZipI*, *LoHD-ZipII*, and *LoHD-ZipIV* subfamily members were mainly irregular curls and that all *LoHD-ZipIII* secondary structures were mainly α -helix. *LoHDZ11* had the largest proportion of α -helix, while *LoHDZ10* had the smallest proportion. *LoHDZ10* had the largest proportion of random curl (Cc), while *LoHDZ11* had the smallest proportion. *LoHDZ9* had the largest proportion of extended fold (Ee), while *LoHDZ3* had the smallest proportion. The protein secondary structure largely determines the protein formation configuration and is important for the function of the protein (Table 6).

Table 3 Real-time fluorescence quantitative PCR reaction system

Component	Volume
Template	1 μ l
Forward primer	1 μ l
Reverse primer	1 μ l
2 $\times$ TransStart Top Green qPCR SuperMix	10 μ l
Passive reference dye (50 $\times$) (optional)	1 μ l
ddH ₂ O	6 μ l
Total volume	20 μ l

Fig. 2 HD-Zip conserved domain analysis of *LoHD-ZipI*–IV protein from *Larix olgensis*. **a** HD-Zip conserved domain analysis of *LoHD-ZipI* protein from *Larix olgensis*. **b** HD-Zip conserved domain analysis of *LoHD-ZipII* protein from *Larix olgensis*. **c** HD-Zip conserved domain analysis of *LoHD-ZipIII* protein from *Larix olgensis*. **d** HD-Zip conserved domain analysis of *LoHD-ZipIV* protein from *Larix olgensis*

Table 4 Physicochemical properties of HD-Zip transcription factor family genes in *Larix olgensis*

Gene	Size (bp)	Molecular weight (Da)	Aliphatic	Grand average of	Instability index	Signal peptide	Transmembrane	Subcellular localization
<i>LoHDZ1</i>	915	33,912.34	68.72	−0.682	72.34	Non-signal peptide	Non-transmembrane protein	Nuclear
<i>LoHDZ2</i>	945	36,893.37	69.39	−0.71	57.57			
<i>LoHDZ3</i>	996	37,507.45	66.53	−0.824	66.31			
<i>LoHDZ4</i>	930	34,652.61	69.74	−0.769	62.47			
<i>LoHDZ5</i>	2538	92,722.76	86.91	−0.148	45.7			
<i>LoHDZ6</i>	981	35,218.12	72.13	−0.609	66.21			
<i>LoHDZ7</i>	1077	39,510.80	64.64	−0.823	53.39			
<i>LoHDZ8</i>	825	31,292.52	62.66	−0.932	64.75			
<i>LoHDZ9</i>	2292	83,839.95	80.88	−0.336	46.02			
<i>LoHDZ10</i>	2523	92,255.59	91.52	−0.075	44.5			
<i>LoHDZ11</i>	2304	84,858.18	50.59	−0.356	48.48			
<i>LoHDZ12</i>	2529	92,072.81	87.24	−0.143	44.79			
<i>LoHDZ13</i>	2487	91,479.97	89.14	−0.084	42.21			

Gene Expression Analysis

Expression of Genes in Different Tissues and Organs at Different Stages

Roots grown for 3 months were used as controls. The quantitative results showed that the 13 genes were expressed

differently at different stages in the larch roots, stems, and leaves (Fig. 5).

LoHDZ2, *LoHDZ4*, *LoHDZ11*, and *LoHDZ13* showed different degrees of downregulation in different tissues and different developmental stages compared with the control group. These results indicated that the four genes had the highest expression in non-lignified roots. The expression level of

Fig. 3 Phylogenetic trees of *LoHD-Zip* protein and *Arabidopsis* HD-Zip protein

Table 5 Secondary structure characteristics of *LoHD-Zip* transcription factor family gene proteins

Gene name	Subfamily	Alpha helix (Hh) (%)	Extended strand (Ee) (%)	Random coil (Cc) (%)
<i>LoHDZ1</i>	HD-ZipII	30.26	9.87	56.58
<i>LoHDZ2</i>	HD-ZipI	38.96	11.35	48.16
<i>LoHDZ3</i>	HD-ZipI	34.14	7.86	54.98
<i>LoHDZ4</i>	HD-ZipI	34.86	14.42	46.53
<i>LoHDZ5</i>	HD-ZipIV	39.49	11.78	45.22
<i>LoHDZ6</i>	HD-ZipIII	40.36	14.88	39.17
<i>LoHDZ7</i>	HD-ZipI	34.74	13.64	49.03
<i>LoHDZ8</i>	HD-ZipIII	40.59	13.49	41.42
<i>LoHDZ9</i>	HD-ZipIV	34.68	14.99	46.54
<i>LoHDZ10</i>	HD-ZipII	25.70	14.80	56.98
<i>LoHDZ11</i>	HD-ZipI	48.18	10.58	32.85
<i>LoHDZ12</i>	HD-ZipIII	41.69	13.90	40.14
<i>LoHDZ13</i>	HD-ZipIII	43.24	12.56	39.49

LoHDZ11 in partially lignified stems was approximately 7 times lower than that in the control group.

The expression of *LoHDZ5*, *LoHDZ9*, and *LoHDZ10* showed different degrees of upregulation in the treatment groups compared with the control group. *LoHDZ5* was highly expressed in partially lignified and fully lignified leaves. The expression level of *LoHDZ9* in fully lignified roots and non-lignified leaves was approximately 6 times higher than that in the control group, and *LoHDZ10* had a relatively high expression level in the leaves.

LoHDZ8 had the highest expression level in the partially lignified roots, and the expression level was approximately 10 times higher than that in other organs.

Gene Expression Under Drought Stress

All *LoHD-Zip* genes were significantly differentially regulated under different concentrations of PEG₆₀₀₀ (Fig. 6).

The expression of *LoHDZ1* and *LoHDZ5* was upregulated in response to various concentrations of PEG₆₀₀₀ for different times. The expression level was the highest after treatment with 28% PEG₆₀₀₀ for 48 h, which was approximately 10 times higher than that of the control group.

LoHDZ3, *LoHDZ4*, and *LoHDZ13* were downregulated in response to various concentrations of PEG₆₀₀₀ treatment. The expression level of *LoHDZ3* was downregulated after treatment with 21% PEG₆₀₀₀ for 24 h and 28% PEG₆₀₀₀ for both 72 h and 96 h, the latter of which was approximately 10 times higher than that of the control group. The expression level of *LoHDZ4* was the lowest after treatment with 7% and 14% PEG₆₀₀₀ for 96 h. After treatment with 21% PEG₆₀₀₀, the expression level of *LoHDZ4* was the lowest but was not significantly different after 72 h or 96 h, and the expression level was the lowest after treatment with 28% PEG₆₀₀₀ for 72 h. *LoHDZ13* had the lowest expression level after treatment with 14% PEG₆₀₀₀ for 48 h and 21% PEG₆₀₀₀ for 24 h.

Table 6 Main structure and function of genes in different subfamilies

Subfamily	Protein secondary structure	Functions	References
HD-ZipI	Mainly irregular curls	Mainly involved in light signal transduction; abiotic stress and leaf development processes	Wei et al. 2018
HD-ZipII	Mainly irregular curls	Mainly involved in environmental responses; the light quality response; the shade avoidance response and abiotic stress responses	Wei et al. 2018; Xiuming 2017
HD-ZipIII	Mainly α -helix	Control the establishment of the various polarities, paraxial cells and structure, and leaf and vascular system formation; regulate the meristematic growth of stem tips; and promote the formation of meristems and both the proliferation and differentiation of stem cells	Henriksson 2005
HD-ZipIV	Mainly irregular curls	Mainly participate in the differentiation and material accumulation within epidermal and subepithelial cells	Baima et al. 2001

Fig. 4 Predicted secondary structure of *LoHD-Zip* transcription factor

The expression levels of *LoHDZ2* and *LoHDZ7* after 21% and 28% PEG₆₀₀₀ treatments (except the 21% PEG₆₀₀₀ treatment for 24 h) were lower than those in the control group. In response to treatment with 21% PEG₆₀₀₀ for 96 h, the expression level was especially the lowest; *LoHDZ10* was downregulated in all time periods under the 14%, 21%, and 28% PEG₆₀₀₀ treatments. The expression level after 14% PEG₆₀₀₀ treatment for 72 h was approximately 13 times lower than that of the control group.

Gene Expression Under Salt Stress

The expression levels of the 13 *LoHD-Zip* genes in larch seedlings treated with 0.2 M NaCl solution showed that each gene was downregulated after salt stress treatment, and the expression of the 13 genes was the lowest after 96 h of treatment (Fig. 7). The expression level of 5 genes (*LoHDZ1*, *LoHDZ2*, *LoHDZ7*, *LoHDZ8*, *LoHDZ11*) was approximately 5 times lower than that of the control group after 96 h of treatment. In addition, the expression levels of 6 genes (*LoHDZ3*, *LoHDZ4*, *LoHDZ5*, *LoHDZ6*, *LoHDZ9*, *LoHDZ13*) after treatment for 96 h were approximately 8 times lower than those of the control group.

Discussion

Identification and Analysis of the Physicochemical Properties of the HD-Zip Family Genes in Larch

In recent years, genome-wide identification of HD-Zip family genes in various plant species has been performed. For example, researchers have identified 50, 33, and 63 HD-Zip genes in the model plant species *Arabidopsis* (Ciarbelli et al. 2008), rice (Agalou et al. 2008), and poplar (Hu et al. 2012), respectively. In addition, the functions of HD-Zip family genes at the whole-genome level have been studied in plant species such as soybean (Belamkar et al. 2014), wheat (Yue et al. 2018), corn (Zhao et al. 2011), and cassava (Ding et al. 2017), including their expression patterns under abiotic stresses such as high salt, drought, chilling injury, and ABA. These functions serve as a certain reference basis for HD-Zip family genes in terms of their regulation of plant abiotic stress responses. However, larch HD-Zip family genes have not yet been investigated in this respect.

Based on the results of the transcriptome sequencing of *Larix olgensis*, 32 candidate sequences with HD domains were identified (Table 7). When the *Arabidopsis* HD-Zip family sequences were used as a basis for comparison, 13 HD-Zip genes with an intact CDS domain were identified; this number was smaller than that of other investigated species. The isoelectric points of the four subfamilies of the larch HD-Zip family genes are mostly acidic, mainly because the proteins

Fig. 5 Expression of genes in different tissues at different times

contain many acidic amino acids, including serine. The fat coefficient is greater than 50.59, and most of the proteins are highly hydrophilic. The secondary structure prediction results show that α -helix and random coil account for the largest proportion, and members of the same subfamily exhibit similar trends, as in a study in birch (Prigge and Clark 2006). Therefore, these findings provided further evidence for identifying HD-Zip family genes in *Larix olgensis*. Although the secondary structure of each subfamily of genes is similar, their tertiary structure is different. The differences and similarities in the spatial structure of these proteins may lead to functional similarities and differences. The results of the signal peptide analysis showed that HD-Zip proteins did not have a signal peptide, indicating that they are non-secreted proteins, and the results of the protein transmembrane analysis revealed that the HD-Zip proteins were non-transmembrane proteins. It is speculated that the properties of these proteins may be related to the functions of these protein families. The results of this study therefore provide a reference for future studies of the functions of this family in larch.

Tissue-Specific Expression of LoHD-Zip Genes

HD-Zip transcription factors are present in low-lying bryophytes from ferns and gymnosperms to more evolved

angiosperms. These transcription factor family genes play an important regulatory role in the growth and development of plants (Floyd 2006). In *Arabidopsis*, rice, and *Populus euphratica*, HD-ZipIII genes are strongly expressed in the vascular bundles of roots, stems, and leaves. Localization revealed that HD-ZipIII genes in *Arabidopsis thaliana* are specifically expressed in protoplasts and xylem cells (Kangj and Denglar 2002). This study preliminarily targeted the expression patterns of members of the HD-Zip family in the roots, stems, and leaves of *Larix olgensis* during the periods of complete lignification, partial lignification, and unlignification. Real-time fluorescent quantitative PCR was performed on these 13 genes. The results showed that 8 of them (*LoHDZ2*, *LoHDZ4*, *LoHDZ5*, *LoHDZ8*, *LoHDZ9*, *LoHDZ10*, *LoHDZ11*, *LoHDZ13*) were expressed specifically in different tissues at different stages.

According to previous studies, members of the HD-ZipI subfamily are mainly involved in light signal transduction, abiotic stress, and leaf development processes (Wei et al. 2018). Members of the HD-ZipI subfamily *ATHB1* in *Arabidopsis* are a transcriptional activator involved in leaf development (Aoyama et al. 1995). *LoHDZ2*, *LoHDZ4*, and *LoHDZ11* belong to the HD-ZipI subfamily. The results showed that these three genes had the highest expression in non-lignified roots. However, the *LoHD-ZipI* subfamily gene

Fig. 6 Gene expression levels at different concentrations of PEG₄₀₀₀ treatment at different times

expression level in the non-lignified roots was higher than that in the leaves, suggesting that the members of this subfamily may be involved in root development, which is the same conclusion as that from Xiuming's research on tomato HD-Zip family genes (Xiuming 2017).

In *Arabidopsis*, HD-ZipII subfamily genes were mainly involved in environmental responses, the light quality response, the shade avoidance response, and abiotic stress responses (Wei et al. 2018; Xiuming 2017). Light of different qualities or wavelengths has distinct biological effects, including different effects on plant morphological structure and chemical composition, photosynthesis, and organ growth and development. According to a previous study, the *Arabidopsis* HD-ZipII subfamily gene *ATHB17* played a role in regulating leaf morphology, chlorophyll content, chloroplast number, and photosynthesis (Hymus et al. 2013). *LoHDZ10* belongs to the HD-ZipII subfamily. According to the evolutionary phylogenetic tree in this study, *LoHDZ10* clustered with two genes of the *Arabidopsis* HD-ZipII subfamily. The expression level of *LoHDZ10* in the leaves was significantly higher than that in the stems and roots, indicating that it may be involved

in leaf development, which is consistent with results of *Arabidopsis* research.

In *Arabidopsis* studies, the HD-ZipIII protein was shown to control the establishment of the various polarities (including the polarity of leaves and stem axes), paraxial cells, paraxial structure, and leaf and vascular system formation; regulate the meristematic growth of stem tips; and promote the formation of meristems and both the proliferation and differentiation of stem cells (Henriksson 2005). Baima et al. (2001) found that the overexpression of the HD-ZipIII subfamily gene *ATHB-8* in *Arabidopsis* increased xylem tissue production and overexpression in leaf and root protoplast cells. *LoHDZ8* and *LoHDZ13* belong to the HD-ZipIII subfamily. The expression of *LoHDZ8* in partially lignified roots was relatively high, but the expression of *LoHDZ13* in non-lignified roots was higher, indicating that these two genes may be involved in root development and that their function may be similar to that of *Arabidopsis* HD-ZipIII subfamily genes.

The HD-ZipIV subfamily genes mainly participate in the differentiation and material accumulation within epidermal and subepithelial cells (Baima et al. 2001). According to previous studies, the *Arabidopsis* HD-ZipIV subfamily gene

Fig. 7 Expression of genes after treatment with different concentrations of NaCl solution

ANL2 was involved in the regulation of leaf epidermal anthocyanin accumulation and root cell differentiation (Harris et al. 2011). The expression level of *LoHDZ5* in partially lignified and fully lignified leaves was relatively high; moreover, *LoHDZ9* was highly expressed in fully lignified roots and non-lignified leaves. *LoHDZ5* and *LoHDZ9* belong to the HD-ZipIV subfamily, and it was speculated that *LoHD-ZipIV* subfamily genes also may be involved in the development of roots and leaves of *Larix olgensis*.

Analysis of Differentially Expressed *LoHD-Zip* Genes Under Abiotic Stress

Studies had shown that the expression of HD-Zip transcription factor I and II subfamily genes was induced by drought, high salt, ABA, and chilling. These two subfamily genes are involved in the hormone signaling pathway and regulate plant cell expansion, division, and differentiation to enhance plant tolerance by interacting with hormone pathway genes and other

downstream genes (Harris et al. 2011). Current studies on HD-Zip transcription factor I and II subfamilies had focused on *Arabidopsis*, rice, and sunflower (Agalou et al. 2008). Studies on the *Arabidopsis* HD-ZipI subfamily suggest that *ATHB7* played a role in signaling pathways that mediate the drought responses (Soderman et al. 1996); *ATHB12* and *ATHB7* had similar molecular mechanisms, as these genes played a role in the regulation of water-deficient growth responses (Olsson et al. 2004). In this study, two-month-old *Larix olgensis* seedlings were treated with solutions of PEG₆₀₀₀ at concentrations of 7%, 14%, 21%, and 28%. The results showed that *LoHDZ2*, *LoHDZ3*, *LoHDZ4*, and *LoHDZ7* of the HD-ZipI subfamily showed obvious downregulated expression. Genes within the same subfamily had the same conserved domain and similar protein secondary and tertiary structures, so they may have similar functions. Accordingly, it is speculated that the HD-ZipI subfamily of *Larix olgensis* may also be involved in the response to drought stress expression patterns; the expression of the HD-ZipII subfamily gene *LoHDZ1* was upregulated under drought stress. Similarly, the *Arabidopsis* HD-ZipII subfamily gene *ATHB17* also showed hypersensitivity to drought stress in young seedlings (Park et al. 2013). Moreover, it can be seen from the phylogenetic tree that *LoHDZ1* and *LoHDZ2* clustered together, indicating that their homology is high, and it is speculated that they may have similar functions. Therefore, *LoHDZ1* may also be involved in drought stress responses. Additionally, the expression of the *LoHDZ5* of the HD-ZipIV subfamily was upregulated at various time points in the different treatments. Yu et al. (2008) showed that the three genes of the *Arabidopsis* HD-ZipIV subfamily could improve drought tolerance, and Fu et al. (2013) found that the overexpression of *HDG11* was positively correlated with the drought tolerance of plants and the activity level of SOD. It is speculated that *LoHDZ5* also played an important role in the response to drought stress.

According to previous studies, Nakamura et al. (2006) significantly induced the expression of the *Arabidopsis* HD-ZipI genes *AtHB7* and *AtHB12* with 100 mmol/L NaCl, and the expression levels were upregulated by 12 and 25 times; however, the expression levels of the *MH381401* and *MH381603* genes in *Betula platyphylla* were slightly downregulated after salt stress treatment (Wang et al. 2018). *AtHDG1* from *Arabidopsis* can also improve the salt tolerance of cotton, poplar, and perennial grass species (Cao et al. 2009). *Oshox22* in rice affects ABA biosynthesis and regulates drought and salt responses, which indicates involvement of this gene in ABA-mediated signal transduction pathways (Zhang et al. 2012); similarly, the

Table 7 Gene annotation of HD-Zip family of *Larix olgensis*

GeneID	Gene name	Gene annotation	Reference species
Unigene0000951	NO	Homeobox-leucine zipper-like protein	<i>Picea glauca</i>
Unigene0002048	NO	Class IV HD-Zip protein HDZ41	<i>Physcomitrella patens</i>
Unigene0004956	<i>LoHDZ1</i>	Homeobox-leucine zipper protein HAT22-like	<i>Juglans regia</i>
Unigene0005129	NO	CRHB10, partial	<i>Ceratopteris richardii</i>
Unigene0006537	<i>LoHDZ2</i>	CRHB10, partial	<i>Ceratopteris richardii</i>
Unigene0008901	NO	Homeobox-leucine zipper protein ATHB-6	<i>Sesamum indicum</i>
Unigene0010092	<i>LoHDZ3</i>	Homeobox-leucine zipper protein HAT5	<i>Nelumbo nucifera</i>
Unigene0010231	<i>LoHDZ4</i>	Homeobox-leucine zipper protein HAT5	<i>Nelumbo nucifera</i>
Unigene0010772	<i>LoHDZ5</i>	Homeobox 1	<i>Picea abies</i>
Unigene0011650	<i>LoHDZ6</i>	Homeodomain-leucine zipper protein 33	<i>Larix kaempferi</i>
Unigene0012596	NO	Homeobox-leucine zipper-like protein	<i>Picea glauca</i>
Unigene0012503	NO	Homeobox-leucine zipper-like protein	<i>Picea glauca</i>
Unigene0012939	<i>LoHDZ7</i>	Homeobox-leucine zipper-like protein	<i>Picea glauca</i>
Unigene0014219	<i>LoHDZ8</i>	Homeodomain-leucine zipper protein 32	<i>Larix kaempferi</i>
Unigene0014220	NO	HB3	<i>Pinus tabulaeformis</i>
Unigene0014222	NO	HB3	<i>Pinus tabulaeformis</i>
Unigene0014359	<i>LoHDZ9</i>	Homeobox 1	<i>Picea abies</i>
Unigene0016152	NO	Homeobox-leucine zipper protein ATHB-6	<i>Sesamum indicum</i>
Unigene0018088	NO	Homeobox-leucine zipper protein ANTHOCYANINLESS 2	<i>Jatropha curcas</i>
Unigene0023890	NO	Homeobox-leucine zipper-like protein	<i>Picea glauca</i>
Unigene0024415	<i>LoHDZ10</i>	Homeobox-leucine zipper protein HOX11	<i>Amborella trichopoda</i>
Unigene0025326	NO	Homeodomain protein HB2	<i>Picea abies</i>
Unigene0026237	NO	Homeobox-leucine zipper-like protein	<i>Picea glauca</i>
Unigene0026503	NO	Homeobox-leucine zipper protein HOX11-like	<i>Juglans regia</i>
Unigene0029271	<i>LoHDZ11</i>	Homeobox-leucine zipper protein HOX21-like	<i>Asparagus officinalis</i>
Unigene0029906	NO	Homeobox-leucine zipper protein HOX11	<i>Arachis duranensis</i>
Unigene0030012	NO	GL2-type homeodomain protein, partial	<i>Oryza sativa Japonica Group</i>
Unigene0030904	NO	Homeobox-leucine zipper protein HAT14-like	<i>Gossypium raimondii</i>
Unigene0031482	<i>LoHDZ12</i>	Homeodomain-leucine zipper protein C3HDZ1	<i>Pseudotsuga menziesii</i>
Unigene0032774	NO	Homeobox-leucine zipper protein HAT14-like	<i>Gossypium arboreum</i>
Unigene0032843	<i>LoHDZ13</i>	Homeodomain-leucine zipper protein 34	<i>Larix kaempferi</i>
Unigene0032930	NO	Homeobox-leucine zipper protein HOX11-like	<i>Cicer arietinum</i>

Zmhdz10 gene in maize can actively regulate the drought tolerance and salt tolerance of plants via ABA-dependent signaling pathways (Zhao et al. 2014a, b), and *Hahb4* in sunflower responded to high salt, drought, and abiotic stresses such as ABA (Dezar et al. 2005). In this study, the expression of 13 genes showed significant downregulation at 96 h after treatment with 0.2 M NaCl solution. Consistent with the conclusions in the above studies, it is speculated that the expression of the 13 genes of the family in the present study is induced by salt, and the specific regulatory mechanism remains to be studied. Based on the existing transcriptome data of *Larix olgensis*, this study

identified and analyzed the larch HD-Zip family and provided a basis for future studies of these gene family members and their function in *Larix olgensis*.

Authors' Contributions Peiqi An and Lei Zhang conceived and designed the study. Peiqi An, Qing Cao, and Chen Wang performed the experiments. Peiqi An wrote the paper. Junhun Wang, Hanguo Zhang, and Lei Zhang reviewed and edited the manuscript. All authors read and approved the manuscript.

Funding This project was supported by the National Science and Technology Major Project (2018ZX08020003-001-001), the National Natural Science Foundation of China (Grant No.31700595), and the

Fundamental Research Funds for the Central Universities (2572019BA13), Heilongjiang Touyan Innovation Team Program.

Availability of Data and Material Reliable and transparent data.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

Ethics Approval Not applicable.

Code Availability Not applicable.

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References

- Agalou A, Purwantomo S, Overmas E et al (2008) A genome-wide survey of HD-Zip genes in rice and analysis of drought-responsive family members. *Plant Mol Biol* 66(1–2):87–103
- Aoyama T, Dong CH, Wu Y et al (1995) Ectopic expression of the Arabidopsis transcriptional activator Athb-1 alters leaf cell fate in tobacco. *Plant Cell* 7(11):1773–1785
- Ariel FD, Manavella PA, Dezar CA, Chan RL (2007) The true story of the HD-Zip family. *Trends Plant Sci* 12(9):419–426
- Baima S, Possenti M, Matteucci A, Wisman E, Altamura MM, Ruberti I, Morelli G (2001) The Arabidopsis ATHB-8 HD-zip protein acts as a differentiation-promoting transcription factor of the vascular meristems. *Plant Physiol* 126(2):643–655
- Belamkar V, Weeks NT, Bharti AK, Farmer AD, Graham MA, Cannon SB (2014) Comprehensive characterization and RNA-Seq profiling of the HD-Zip transcription factor family in soybean (*Glycine max*) during dehydration and salt stress. *BMC Genomics* 15:950
- Bharathan G, Janssen BJ, Kellogg EA, Sinha N (1997) Did homeodomain proteins duplicate before the origin of angiosperms fungi and metazoa? *Proc Natl Acad Sci U S A* 94(25):13749–13753
- Cao YJ, Wei Q, Liao Y, Song HL, Li X, Xiang CB, Kuai BK (2009) Ectopic overexpression of AtHDG11 in tall fescue resulted in enhanced tolerance to drought and salt stress. *Plant Cell Rep* 28(4):579–588
- Carabelli M, Turchi L, Ruzza V et al (2013) Homeodomain-Leucine zipper II family of transcription factors to the limelight: central regulators of plant development
- Ciarbelli AR, Cioffi A, Salvucci S, Ruzza V, Possenti M, Carabelli M, Fruscalzo A, Sessa G, Morelli G, Ruberti I (2008) The Arabidopsis Homeodomain-leucine Zipper II gene family: diversity and redundancy. *Plant Mol Biol* 68(4–5):465–478
- Dezar CA, Fedrigo GV, Chan RL (2005) The promoter of the sunflower HD-zip protein gene Habb4 directs tissue-specific expression and is inducible by water stress, high salt concentrations and ABA. *Plant Sci* 169(2):0–456
- Ding ZH, Fu LL, Yan Y et al (2017) Genome-wide characterization and expression profiling of HD-Zip gene family related to abiotic stress in cassava. *PLoS One* 12(3):e0173043
- Floyd SK (2006) Evolution of class III homeodomain-leucine zipper genes in streptophytes. *Genetics* 173(1):373–388
- Franklin KA, Prækel U, Stoddart WM, Billingham OE, Halliday KJ, Whitlam GC (2003) Phytohormones B₂D and E act redundantly to control multiple physiological responses in Arabidopsis. *Plant Physiol* 131(3):1340–1346
- Fu R, Liu W, Li Q et al (2013) Comprehensive analysis of the homeodomain-leucine zipper IV transcript. *Genome* 56(7):395–405
- Harris JC, Hrmova M, Lopato S, Langridge P (2011) Modulation of plant growth by HD-Zip class I and II transcription factors in response to environmental stimuli. *New Phytol* 190(4):823–837
- Harris JC, Somaraj P, Taylor M, Bazanova N, Baumann U, Lovell B, Langridge P, Lopato S, Hrmova M (2016) Molecular interactions of the γ-clade homeodomain-leucine zipper class I transcription factors during the wheat response to water deficit. *Plant Mol Biol* 90(4–5):435–452
- Henriksson E (2005) Homeodomain leucine zipper class I genes in Arabidopsis. Expression patterns and phylogenetic relationships. *Plant Physiol* 139(1):509–518
- Henriksson E, Olsson ASB, Johannesson H, Johannesson H, Hanson J, Engström P, Söderman E (2005) Homeodomain leucine zipper class I genes in Arabidopsis expression patterns and phylogenetic relationships. *Plant Physiol* 139(1):509–518
- Hu RB, Chi XY, Chai GH et al (2012) Genome-wide identification, evolutionary expansion and expression profile of homeodomain-leucine zipper gene family in poplar (*Populus trichocarpa*). *PLoS One* 7(2):e31149
- Hymus GJ, Cai S et al (2013) Application of HB17, an Arabidopsis class II homeodomain-leucine zipper transcription factor to regulate chloroplast number and photosynthetic capacity. *J Exp Bot* 64(14):4479–4490
- Ingram GC, Boissard-Lorig C, Dumas C, Rogowsky PM (2000) Expression patterns of genes encoding HD-Zip homeo domain proteins define specific domains in maize embryos and meristems. *Plant J* 22(5):401–414
- Ito M, Sentoku N, Nishimura A, Hong SK, Sato Y, Matsuoka M (2002) Position dependent expression of GL2-type homeobox gene Rocl: significance for protoderm differentiation and radial pattern formation in early rice embryogenesis. *Plant J* 29(4):497–507
- Jain M, Tyagi AK, Khurana JP (2008) Genome-wide identification, classification, evolutionary expansion and expression analyses of homeobox genes in rice. *FEBS J* 275(11):2845–2861
- Jinhua L et al (2003) Current status and development strategies of wood fiber raw materials for pulp and papermaking in China. *CSIR* 16(6):32–35
- Kang, Denglar N (2002) Cell cycling frequency and expression of the homeobox gene ATHB-8 during leaf vein development in Arabidopsis. *Planta* 216(2):212–219
- Kim YK, Son O, Kim MR, Nam KH, Kim GT, Lee MS, Choi SY, Cheon CI (2007) ATHB2, an Arabidopsis class I homeodomain-leucine zipper gene, is expressed in the adaxial region of young leaves. *Plant Cell Rep* 26(8):1179–1185
- Lian L, Xu H et al (2019) Expression of antioxidant enzyme genes in rice under PEG-simulated drought-stress. *FJAS* 34(3):255–263
- Liu L, Hai DU, Tang XF et al (2008) The roles of MYB transcription factors on plant defense responses and its molecular mechanism. *Hereditas* 30(10):1265–1271
- Ma Q, Kang J, Long R, Zhang T, Xiong J, Zhang K, et al. (2017). Comparative proteomic analysis of alfalfa revealed new salt and drought stress-related factors involved in seed germination. *Molecular Biology Reports* 44:261–170

- Mukherjee K, Brocchieri L, Burglin TR (2009) A comprehensive classification and evolutionary analysis of plant homeobox genes. *Mol Biol Evol* 26(12):2775–2794
- Nakamura M, Katsumata H, Abe M, Yabe N, Komeda Y, Yamamoto KT, Takahashi T (2006) Characterization of the class IV homeodomain-leucine zipper gene family in *Arabidopsis*. *Plant Physiol* 141(4):1363–1375
- Olsson A, Engström P, Söderman E (2004) The homeobox genes ATHB12 and ATHB7 encode potential regulators of growth in response to water deficit in *Arabidopsis*. *Plant Mol Biol* 55(5):663–677
- Park MY, Kim SA, Lee SJ, Kim SY (2013) ATHB17 is a positive regulator of abscisic acid response during early seedling growth. *Mol Cell* 35(2):125–133
- Prigge MJ, Clark SE (2006) Evolution of the class III HD-Zip gene family in land plants. *Evol Dev* 8(4):350–361
- Prigge MJ, Otsuga D, Alonso JM, Ecker JR, Drews GN, Clark SE (2005) Class III homeodomain-leucine zipper gene family members have overlapping, antagonistic, and distinct roles in *Arabidopsis* development. *Plant Cell* 17(1):61–76
- Robischon M, Du J, Miura E et al (2011) The *Populus* classIII HD-ZIP popREVOLUTA influences cambium initiation and patterning of woody stems. *Plant Physiol* 55(3):1214–1225
- Schena M, Davis RW (1992) HD-Zip proteins: members of an *Arabidopsis* homeodomain protein superfamily. *Proc Natl Acad Sci U S A* 89(9):3894–3898
- Soderman E, Mattsson J, Engström P (1996) The *Arabidopsis* homeobox gene ATHB-7 is induced by water deficit and by abscisic acid. *Plant J* 10(2):375–381
- Wang J, Zhang W, Li L (2018) Bioinformatics and response salt stress analysis of HD-Zip gene family in *Betula platyphylla*. *Plant Res* 38(06):931–938
- Wei L, Yi W, Wang K, Zhao M et al (2018) The structure, function and expression pattern of HD-Zip gene family. *Bulletin of Botanical Research* 16(03):781–790
- Xin-Yu Z, Yan-Ning G (2004) To design PCR primers with Oligo 6 and Primer Premier 5. *Bioinformatics*
- Xiuming J (2017) Bioinformatics analysis of tomato HD-Zip transcription factor family and identification of HD-ZipI subfamily stress-resistance related genes. Northeast Agricultural University
- Yu X, Bao F (2010) Study on the sharpness equation of larch plantation. Northeast Forestry University
- Yu H, Chen X, Yuan-Yuan et al (2008) Activated expression of an *Arabidopsis* HD-START protein confers drought tolerance with improved root system and reduced stomatal density. *Plant Cell* 20(4):1134–1151
- Yue H, Shu DT, Wang M et al (2018) Genome-wide identification and expression analysis of the HD-Zip gene family in wheat (*Triticum aestivum* L.). *Genes* 9(2):70
- Zhang S, Haider I, Kohlen W, Jiang L, Bouwmeester H, Meijer AH, Schluepmann H, Liu CM, Ouwerkerk PBF (2012) Function of the HD-Zip I gene Oshox22 in ABA-mediated drought and salt tolerances in rice. *Plant Mol Biol* 80(6):571–585
- Zhang X, Yao W, Zhao K, Jiang T, Zhou B (2017) Analysis of bioinformatics and response salt stress in HD-Zip transcription factor family of *Populus nigra*. *Plant Res* 37(05):715–721
- Zhao Y, Zhou YQ, Jiang HY et al (2011) Systematic analysis of sequences and expression patterns of drought-responsive members of the HD-Zip gene family in maize. *PLoS One* 6(12):e28488
- Zhao Y, Ma Q, Xiaolei et al (2014a) A novel maize homeodomain-leucine zipper (HD-Zip) I gene, Zmhdz10, positively regulates drought and salt tolerance in both rice and *Arabidopsis*. *Plant Cell Physiol* 55(6):1142–1156
- Zhao Y, Ma Q, Jin XL et al (2014b) A novel maize homeodomain-leucine zipper HD-Zip I gene Zmhdz10, positively regulates drought and salt tolerance in both rice and *Arabidopsis*. *Plant Cell Physiol* 55(6):1142–1156
- Zhu YY, Song DL, Sun JY et al (2013) PtrHB7, a classIII HD-Zip gene, plays a critical role in regulation of vascular cambium differentiation in *Populus*. *Mol Plant* 6(4):1331–1334

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