Pak. J. Bot., 56(1): 27-34, 2024. DOI: http://dx.doi.org/10.30848/PJB2024-1(10)

EXPRESSION PROFILE OF *PDPAPHB12* GENE IN RESPONSE TO STRESS FOR *POPULUS DAVIDANA* × *P. ALBA* VAR. *PYRAMIDLIS*

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Abstract

In this study, biotic and abiotic stress response gene was investigated in poplar. *PdPapHB12*, the specific response gene in Shanxin poplar, was cloned by real-time quantitative polymerase chain reaction. *PdPapHB12* encoded a non-transmembrane and hydrophilic protein, and also was a transcription factor of HD-Zip I subfamily. The tissue-specific expression profile of *PdPapHB12* in poplar was achieved with and without various stresses and inductions. It was verified that *PdPapHB12* was expressed in the shoot tip (ST), leaves (L), stems (S), and roots (R) for poplar plantlets, and the highest expression was obtained in L3. The effects of abiotic stress (NaCl, Na_2CO_3, and polyethylene glycol 6000), biotic stress (inoculating the roots of poplar by pathogenic fungus), and phytohormone induction on the *PdPapHB12* expression was further investigated. It was found that the *PdPapHB12* expression was the most evident changes by polyethylene glycol 6000 in all tissues. Moreover, the expression was obviously increased by *F. oxysporum* ($p<0.05$), and significantly decreased by *C. chrysosperma* induction in all tissues. The expression was significantly increased by inducting jasmonic acid and abscisic acid in all component, while the increased expression was obtained only in L2 and R under salicylic acid induction. However, the *PdPapHB12* expression was down-regulated under the SA induction in ST.

Key words: Gene expression; *PdPapHB12*; Abiotic stress; Biotic stress; Phytohormone induction.

Introduction

Poplar is the tree species with extreme adaptability to different climate conditions. Due to quick growth and big biomass production, poplars are widely cultivated. Traditionally, the poplars were employed as a source of fibre, timber and fuel (Fernàndez-Martínez *et al*., 2013). The poplar (*Populus davidiana* × *P. alba* var. *Pyramidalis,* cv "Shanxin"), an improved landscape bred cultivar, derives from a cross-breeding between *P. davidiana* and *P. alba* var. *pyramidalis*. Due to the rapid growth, narrow crown, and cold tolerant characteristics, Shanxin poplar, one of excellent species, was selected for urban landscaping, sandstorm prevention, and wood production in the plain regions of China (Zhang *et al*., 2018; Zhai *et al*., 2019).

The HD-Zip family included a group of transcription factors reported, for example, *Arabidopsis* (Henriksson *et al*., 2005), rice (Jain *et al*., 2008), maize (Zhao *et al*., 2011), grapes (Li *et al*., 2017), apples (Zhang *et al*., 2022), poplars (Hu *et al*., 2012). The transcription factors of HD-Zip played a regulatory roles in plant growth and development, for example, flower and fruit development (Lin *et al*., 2008; Wei *et al*., 2012; Lü *et al*., 2014), stress responses (Agalou *et al*., 2008; Valdés *et al*., 2012; Wang *et al*., 2019), phytohormone synthesis (Ohgishi *et al*., 2001; Morelli *et al*., 2002; Ré *et al*., 2011), and photomorphogenesis (Ciarbelli *et al*., 2008). HD-Zip transcription factors included two main structural domains: a homeodomain and a leucine zipper. The HD-Zip family was also divided into four subfamilies, based on structure and function (Ariel *et al*., 2007). A plenty of studies on HD-Zip I subfamily members in respond to abiotic stress have been widely paid attention. *ATHB7* in *Arabidopsis* was known as a negative regulator of abscisic acid (ABA) signal in response to drought stress (Ré *et al*., 2014). Additionally, overexpression of *SlHZ24*, a gene in the same subfamily, improved salt stress tolerance in tomatoes (Hu *et al*., 2016). Recently, the hormone-mediated regulatory mechanisms of HD-Zip I and II subfamilies in biotic stress have been widely investigated. For example, Hu *et al*., (2012) identified poplar genome, and reported 63 HD-Zip family genes. Moreover, *PtrHox52* and *PtrHox14* (*POPTR_0002s17680.1 v2.1*), which were closely related to *ATHB7* (*POPTR_0014s09860.1 v2.1*), were up-regulated under drought and salt stresses. However, the mechanism underlying *PtrHB12* gene stress and hormone induction remains unknown in the poplar HD-Zip family.

According to the databases of Shanxin poplar constructed previously in our lab (Yin *et al*., 2023), we selected *Potri.014G103000.1 v3.1*, a specifical expressed gene in full-length cDNA sequence. It was annotated as similar to homeobox leucine zipper protein [co-ortholog (1 of 4) of AAD38144, At2g46680, At3g61890] in *P. trichocarpa v3.0* in *Phytozome 13*. In *Arabidopsis thaliana* TAIR10, *At2g46680,* called as ATHB7, ATHB-7, and HB-7, was defined as homeobox-leucine zipper protein ATHB-12-related, while *At3g61890,* called as ATHB12, ATHB-12 and HB-12, was named as homeobox-leucine zipper protein ATHB-12-related. This indicated that ATHB7 and ATHB12 might have the same function. In addition, *Potri.014G103000.1 v3.1* was defined as homeobox-leucine zipper protein ATHB-12-related in both the *v3.1* and *v4.1* genomic databases in *Phytozome 13*; therefore, the gene *Potri.014G103000.1 v3.1* was named *PdPapHB12*. Recently, the rereference was not reported on the *PdPapHB12* in the Shanxin poplar. Therefore, the tissue-specific expression of *PdPapHB12* was studied, and the differential regulation profile in all components was discussed under various stresses and inductions.

Material and Methods

Plant material and pathogen strains: 4-week-old tissue-cultured plantlets were grown in woody plant medium (WPM), containing 1-naphthlcetic acid (NAA, 100 μg/L) and 6-Benzylaminopurine (6-BA, 500 μg/L). The plantlets were cut and cultured in the WPM loading indole-3-butyric acid (IBA, 100 μg/L) for 2 weeks until rooting. Then, the 6-week-old plantlets were dealt with and without stresses, as following 26ºC and a 16/8 h light/dark cycle. The plantlets with the height (ca. 10-12 cm) were selected and collected as samples in our experiments.

Pathogenic fungi strains, including *Alternaria alternata* NECCFP002 (Aa), *Cytospora chrysosperma* C29 (Cc), *Sclerotinia sclerotiorum* NECC20005 (Ss), *Rhizoctonia solani* NECC20006 (Rs), and *Fusarium oxysporum* NECC20007 (Fo), were provided for the following experiments.

PCR amplification and sequencing of the *PdPapHB12* gene: A transcriptome database of the interactions in Shanxin poplar, the plant beneficial fungus *T. asperellum*, and/or the pathogenic fungus *A. alternata* were constructed in our previous study (Yin *et al*., 2023). The differentially expressed genes (DEGs) were selected, based on the reported literature (Gang *et al*., 2019). The full-length cDNA sequence of the specifical gene, *Potri.014G103000.1 v3.1*, was obtained after screening. Based on *Potri.014G103000.1 v3.1*, the primers of full-length sequence were constructed with Primer *v*6.0 software (Table 1). Total RNA was obtained from the plantlets by cetyltrimethylammonium bromide method (Yin *et al*., 2023), and DNA was removed by digestion with DNase I. cDNA reverse transcription and the amplified PCR of *PdPapHB12* (Table 1) were showed in our previous study (Yin *et al*., 2023). The amplification of Real-time fluorescent quantitative PCR (RT-PCR) was conducted with cDNA and Takara Primer STAR®Max DNA Polymerase as template and reaction reagent. The fragments were amplified, purified, and then sequenced (Rui Biotech, Beijing, China).

Bioinformatics analysis of *PdPapHB12:* The open reading frames, the conserved domain, and homologous protein sequences of PdPapHB12 were obtained with NCBI on-line softs. Psort II, TMHMM Server *v2.0*, ExPASy-Prot, and SOPM were employed to evaluate the characteristics, the transmembrane domain, the subcellular localization, and the secondary structure of PdPapHB12. Multiple sequence alignment and the phylogenetic tree were analyzed employing DNAMAN *v9.0.* and MEGA *v6.0*, respectively.

Collecting of poplar plant samples in different tissues: In untreated poplar plantlets all components were collected. In the shoot tip, the buds, unexpanded leaves and stems were used and named as ST; then the 3^{rd}-5^{th} (young stem), the 7^{th}-9^{th} (developing stem), and the 11^{th}-13^{th} internodes (lignified stem) were used and named S1, S2, and S3, respectively; finally, the root (R) was used. The relative leaves on the internode of "S samples" were also used, and named L1, L2, and L3, respectively. All samples of poplars in our experiments were described in our previous study, in order to obtain total RNA and analyze tissue-specific expression of *PdPapHB12* (Yin *et al*., 2023).

Plantlet treatments: All experiments were performed in a sterile condition using 6-week-old plantlets, which were cultured, and the roots were immersed in liquid WPM, according to our previous study (Yin *et al*., 2023). Ten seedlings were set up in each experimental group with three replicates per treatment. The untreated plantles were used as a control.

Under abiotic stress, salt stress was conducted in liquid WPM including NaCl (the concentration of 200 mM), drought stress was tested in liquid WPM with polyethylene glycol 6000 (PEG6000, the concentration of 30%), and alkali stress was performed in the liquid WPM adjusted to pH 10 by Na_2CO_3. After treatment for 48 h, the samples of poplar plantlets were collected from S, L2, and R.

Under biotic stress, Fungus stains were cultured separately on potato dextrose agar (PDA) medium in an incubator under aseptic conditions at 26°C for 10 days, to obtain enough conidia or mycelia. For the inoculum, conidia of Aa, Fo, Cc, and Ss were harvested, and loaded into the liquid WPM, and kept in the concentration of 1×10^5 cfu/mL; total mycelia of Rs with 1×4 cm^2 square-shaped PDA was added into the liquid WPM. After the inoculation for 48 h, the samples of poplars were collected from S, L2, and R.

Under hormone induction, the plantlets were placed severally in liquid WPM containing ABA, jasmonic acid (JA), and salicylic acid (SA) at the concentration of 100 μM under aseptic conditions for 48 h. Samples were collected from S, L2, and R. Plant material collected were kept in liquid nitrogen for the assays of the differential regulation profile of *PdPapHB12*.

Table 1. The primers for PCR.

Gene	Primer	GC%	T_m/ºC	Product size/bp
PdPapHB12	F---CGAAGGCAGGTGAAGATCC	57.9	60.8	717
	R---TCAAGCCCAGAAATCCCAC	52.6	61.0	
	*q*F---CGAAGGCAGGTGAAGATC	55.6	56.8	172
	*q*R---GATTCGAACATAGTTTCCAATG	36.4	56.3	
PdPapACT7F	F---TCACTCATTGGAATGGAAGC	45.0	58.6	173
	R---GGAGCAAGAGCTGTGATCTC	55.0	57.7	
PdPapEF1-a2	F---GGAAGTGCAGGCTGAGTTG	57.9	59.6	176
	R---CACTAAGAAAGAGTATCTGGCCC	47.8	58.5	
PdPapTub-a	F---TCAGCCACCTACTGTAGTACCTG	52.2	58.5	173
	R---CTTCCATGCCTTCACCAAC	52.6	59.1	

RT-qPCR analysis of *PdPapHB12*: RT-qPCR amplification was analyzed in TransStart Top Green qPCR SuperMix kit. Three internal reference genes (IRGs) and the GenBank accession numbers were *ACT7F* (MN196665), *EF1-a2F* (MN196666), and *Tub-aF* (MN196667). Forward and reverse primes of genes were listed in (Table 1). The reaction condition was described by the literature (Yin *et al*., 2023). RT-qPCR experiments were conducted with biological tri-replicates, each with technical tri-replicates.

Data analysis: The $2^{-\Delta Ct}$ method was used to calculate *PdPapHB12* expression in different tissues of Shanxin plantlets under the treated conditions, and was compared with the average expression of the three IRGs (Riedel *et al*., 2014). ANOVA was calculated, to compare with the significant difference between a treated group (T) and a control (CK) in all tissues ($p < 0.05$).

Results and Analysis

Serial analysis of *PdPapHB12*: PCR amplification of *PdPapHB12* resulted in a 767 bp gene fragment (Fig. 1A). In open reading frame of *PdPapHB12*, the start codon was the 51st bp, and a stop codon was the 767th bp, including 238 amino acids, with homeodomain superfamily (accession is smart00389, cd00086 and pfam00046, respectively) and homeobox associated leucine zipper (HALZ, accession is pfam02183) domains of PdPapHB12 (Fig. 1B). The molecular formula of PdPapHB12 protein is $C_{1137}H_{1833}N_{327}O_{390}S_{10}$, with a molecular weight of 60.5436 kD, a pI value of 5.18, a non-transmembrane structure, and an instability index of 30.99. PdPapHB12 could be a non-transmembrane, unstable and hydrophilic protein.

Subcellular localization results showed that the PdPapHB12 was distributed into nucleus (87.0%), cytoplasm (4.3%), and mitochondria (8.7%), respectively. PdPapHB12, a transcription factor, could take part in transcriptional regulation in the nucleus. In PdPapHB12 protein, the secondary structure was composed of irregular coils for 50.00%, α-helices for 42.86%, β-turns for 2.52%, and extended chains for 4.62%, respectively.

Sequence alignments and phylogenetic analysis: Sequence search in the GenBank database obtained ten HD-Zip homologous protein sequences (from seven plant species) with the highest identity to PdPapHB12 (Total score > 397, Query cover > 93%, E value < 5e-149, Ident > 91.60%). Multiple sequence alignment of PdPapHB12 with ten HD-Zip protein sequences revealed that ten protein sequences had conserved homeobox and HALZ domains located near the N-terminal; homeobox and HALZ were attributed to the HD-Zip I subfamily (Fig. 1B and Fig. 2). The amino acid, which was component of the homeobox conserved domain of the PdPapHB12, was totally identical to that of XP_002320889.1 from *Populus trichocarpa*, KAH8488871.1 from *P. deltoides*, QEQ92614.1 from *P. ussuriensis*, KAG6748255.1 from *P. tomentosa*, and XP_034904328.1 from *P. alba* (Fig. 2). The conserved domain of HALZ was located downstream of the homeobox, and had an alpha helix structure, where there was a leucine at the 7th position (Landschulz *et al*., 1988). The obtained ten-protein sequences were similar to the HALZ of PdPapHB12 (Fig. 2). The repeated 4th leucine in the KAF9669113.1 protein was taken place by methionine (M). Moreover, the repeated 6th leucine in the KAG6784901.1, XP_034933448.1, and KAG6786903.1 proteins, was substitute for isoleucine (I). These results mean that the four proteins could have slightly different structure and functions with PdPapHB12.

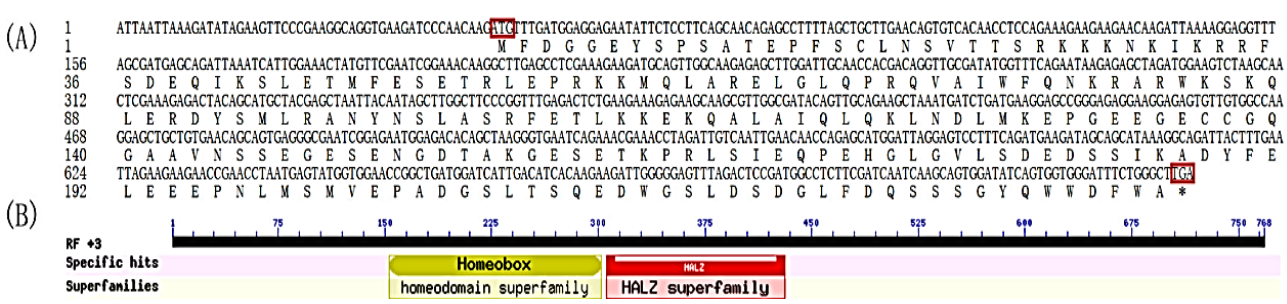

Fig. 1. *PdPapHB12* sequences of nucleotide and amino acid (A) and the conserved domain (B).

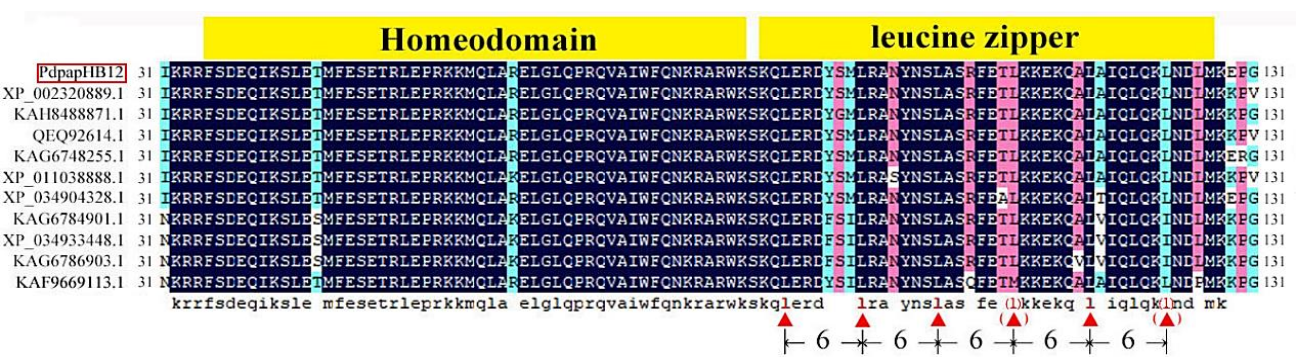

Fig. 2. Multiple sequence alignment of homeodomain and HALZ in HD-Zip I subfamily.

(PdPapHB12 is marked with red frame; leucines in the conserved domain of HALZ are marked with red triangles and the 4th and 6th repeated leucines, which are not absolutely conserved, are marked with red triangles in brackets).

Phylogenetic tree included ten HD-Zip I family homologous sequences, and was grouped into a. and b. Group a contained XP_034933448.1 (*P. alba*), KAG6784901.1, KAG6786903.1, XP_011038888.1 (*P. euphratica*), XP_002320889.1, QEQ92614.1, KAH8488871.1, and *PdPapHB12* for a total of eight proteins; PdPapHB12 was phylogenetically closest to KAH8488871.1. Group b contained KAF9669113.1 (*Salix dunnii*), KAG6748255.1 (*P. tomentosa*), and XP_034904328.1 (*P. alba*); XP_034904328.1 was phylogenetically furthest related to PdPapHB12 (Fig. 3).

Tissue-specific expression of *PdPapHB12*: The *PdPapHB12* expression was obtained in all tissues (ST, S, L, and R) in the plantlets, and the highest expression was shown in L3, while the lowest one was in ST (Fig. 4). The expression in L1, L2 and L3 were 6.52, 30.94 and 97.13 times of ST (p<0.05), respectively. The expression of *PdPapHB12* in leaf tissues tended to increase in a gradient as the leaves changed from young to old (L1< L2< L3). However, the *PdPapHB12* expression in stem tissues was highest in the middle (S2> S3> S1), and the relative expressions of S2/S1 and S2/S3 were 5.82 and 1.47, respectively.

(X-axis was each component of the plantlets, including ST, L1, L2, L3, S1, S2, S3, and R. Y-axis was the relative expression of *PdPapHB12* standardized by average expression of three IRGs. Among different samples, a and b showed significant difference in different tissues (p<0.05), and data were analyzed by mean ± SD.)

Tissue-specific regulation of *PdPapHB12* under abiotic stress: Liquid WPM containing separately NaCl, Na_2CO_3, and PEG6000 was used to simulate the expression changes of *PdPapHB12* in differential tissue of Shanxin poplar. In ST, the *PdPapHB12* expression was obviously increased (p<0.05), which was 4046.31, 842.17, and 13428.07 times of CK, for NaCl, Na_2CO_3, and PEG6000 treatment, respectively. In L2, the *PdPapHB12* expression was significantly up-regulated to 345.53, 423.62, and 2431.43 times of CK (p<0.05) under the same stresses. In R, the expression was obviously increased to 905.71 and 5.44 times of CK in high osmotic and alkaline stresses, respectively (p<0.05). However, it was not obvious in salt stress (p>0.05), with the expression in the treated groups being 5.56 times of CK (Fig. 5).

(X-axis was each component of plantlets, including ST, L2, and R. Y-axis was the relative *PdPapHB12* expression standardized by mean expression of three IRGs. Among different samples, a and b showed significant difference in different tissues (p<0.05), and data were analyzed by mean ± SD.)

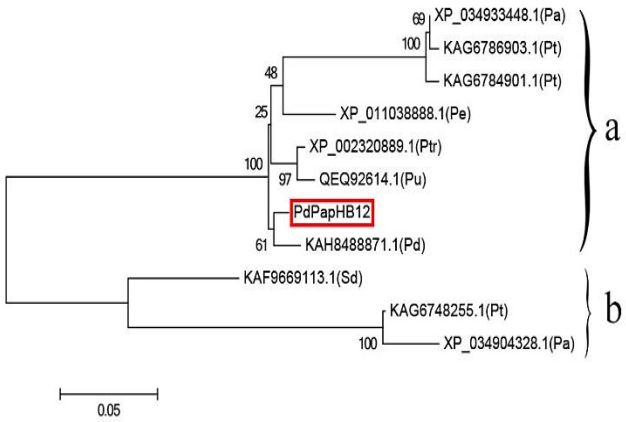

Fig. 3. Phylogenetic tree.

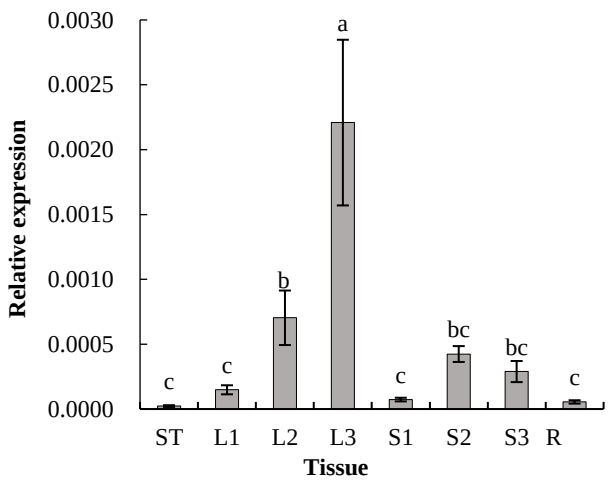

Fig. 4. Tissue-specific expression of *PdPapHB12*.

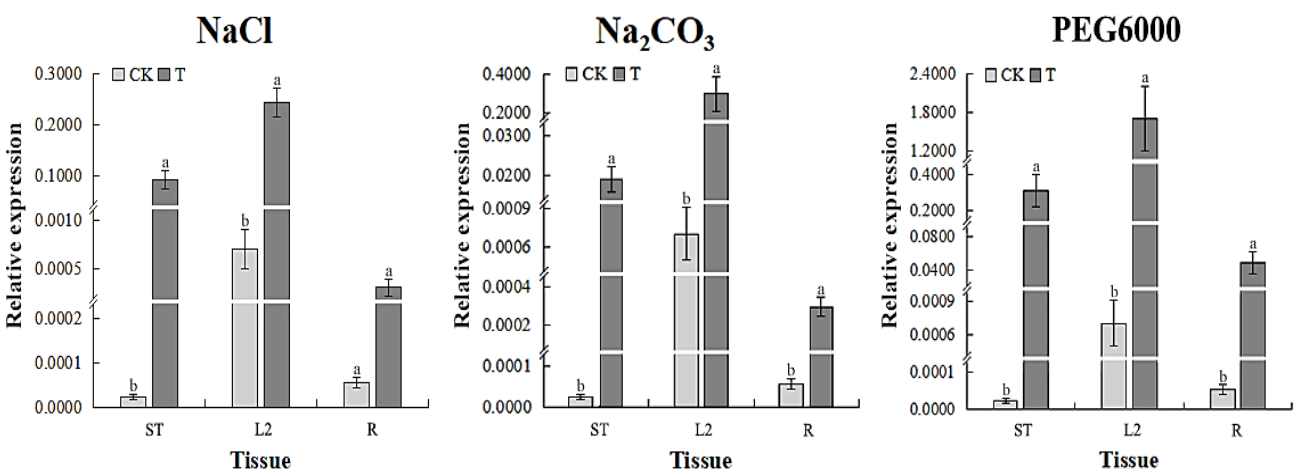

Fig. 5. *PdPapHB12* expression in ST, L2 and R under abiotic stress.

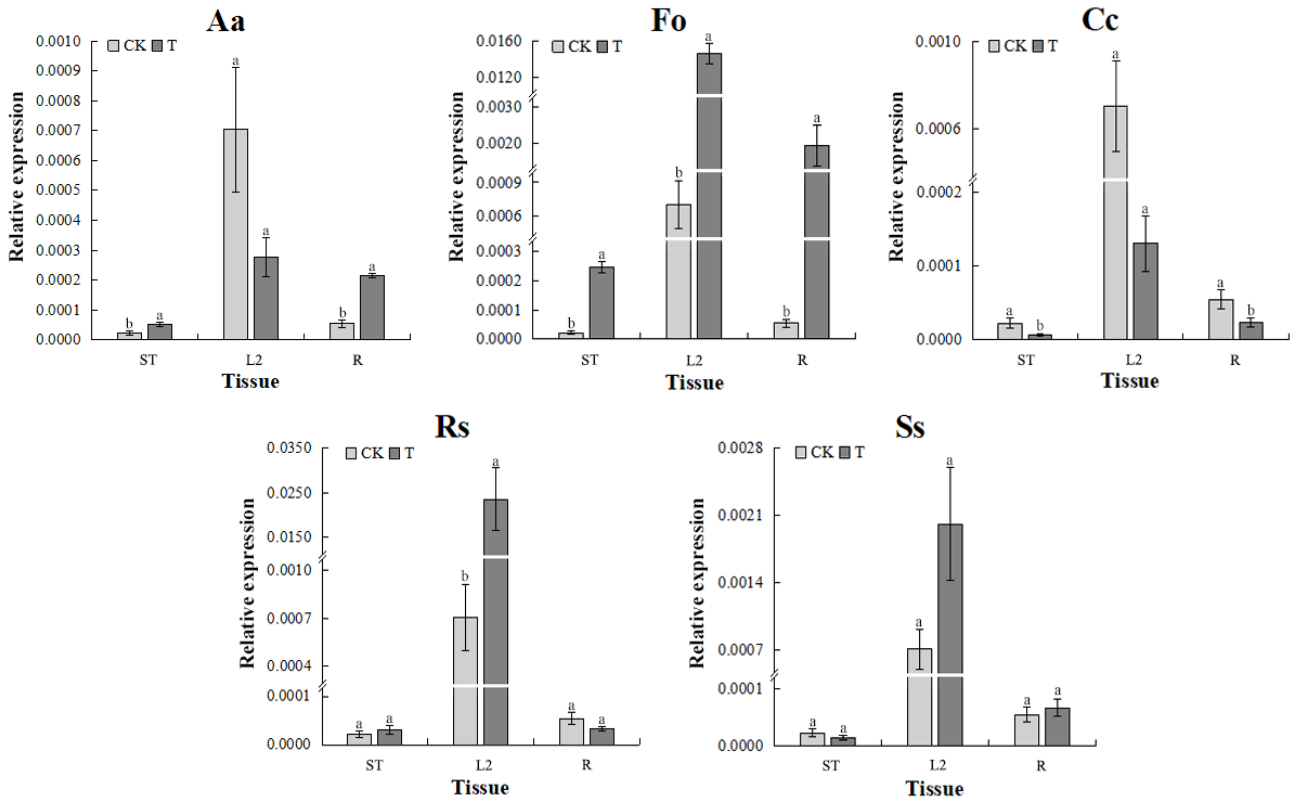

Fig. 6. *PdPapHB12* expression in ST, L2, and R under biotic stress.

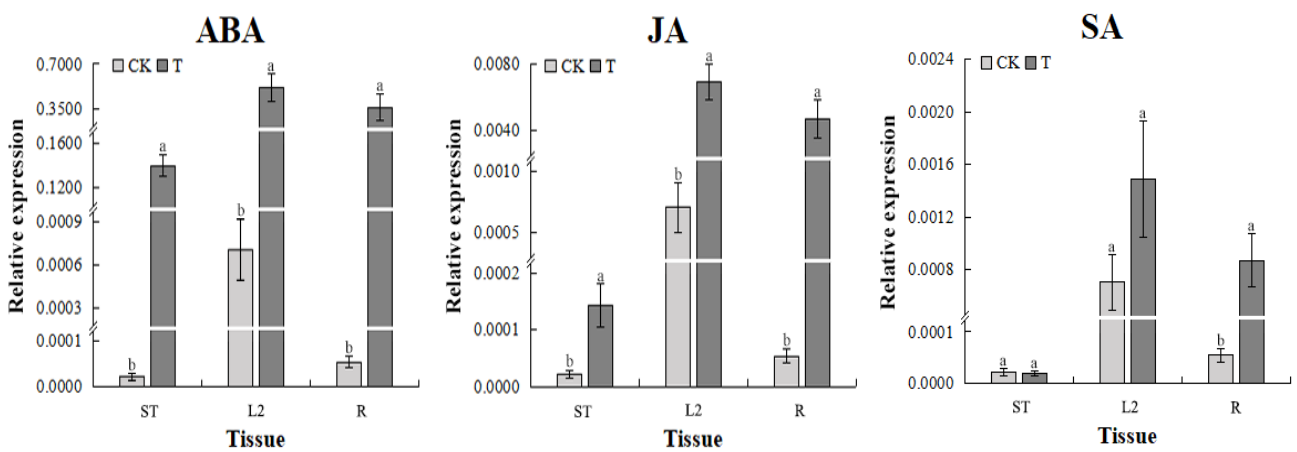

Fig. 7. *PdPapHB12* expression in ST, L2, and R under hormone induction.

Tissue-specific regulation of *PdPapHB12* under biotic stress: In ST, the expression of *PdPapHB12* was significantly up-regulated by Aa and Fo (p<0.05; 2.28 and 10.78 times of CK, respectively) and down-regulated by Cc (p<0.05, 0.30 time of CK) for 48 h after inoculation of the roots in the plant soil-borne pathogenic fungi (Fig. 6). The expression did not obviously alter under Rs and Ss stresses (p>0.05). Compared with CK, the expression was mildly up-regulated in the treated Rs, while it was slightly down-regulated under the induced Ss.

In L2, root inoculation with Aa and Cc for 48 h resulted in the down-regulation of *PdPapHB12*, while the expression alteration was not significant (p>0.05). During the Rs and Fo inductions for 48 h, the expression was increased obviously, and was 33.42 and 20.72 times of CK, respectively (p<0.05). In contrast, the *PdPapHB12* expression was non-significantly up-regulated (p>0.05) with Ss inoculation.

In R, the *PdPapHB12* expression was increased during inoculating Aa and Fo for 48 h (p<0.05; 3.96 and 35.29 times of CK, respectively). Compared to CK, Cc inoculation resulted in a significant down-regulation of the expression, which was 0.43 time of CK (p<0.05); while Ss and Rs inoculation had a slight effect on the *PdPapHB12* expression (p>0.05).

(X-axis was each component of plantlets, including ST, L2 and R. Y-axis was the relative *PdPapHB12* expression standardized by mean expression of three IRGs. Among different samples, a and b showed significant difference in different tissues (p < 0.05), and data were analyzed by mean ± SD.)

Tissue-specific regulation of *PdPapHB12* under phytohormone induction (SA, JA, and ABA): The expression in ST, L2, and R of poplar plantlets altered after three phytohormone induction for 48 h (Fig. 7). In ABA induction, the *PdPapHB12* expression was obviously increased (p<0.05), and were 6114.76, 734.82, and 6565.95 times of CK in ST, L2 and R. In JA induction, the expression was 6.32, 9.78, and 85.48 times of CK in ST, L2 and R, respectively. In SA induction, it was found that the expression change was not significantly observed in ST and L2 (p>0.05); the expression was down-regulated to 0.85 times, and up-regulated to 2.11 times of CK, respectively. However, the expression was up-regulated significantly to 15.94 time of CK in R (p<0.05).

(X-axis was each component of plantlets, including ST, L2 and R. Y-axis was the relative *PdPapHB12* expression standardized by mean expression of three IRGs. Among different samples, a and b showed significant difference in different tissues (p<0.05), and data were analyzed by mean ± SD.).

Discussion

In HD-Zip family, the expression of *PtrHB5* and *PtrHB12* was increased in the leaves and roots of *P. trichocarpa* under abiotic stress (Hou *et al*., 2021). Among 63 HD-Zip family genes in *P. simonii* × *P. nigra,* 48 ones responded to salt stress (Guo *et al*., 2021). The literature suggested that HD-Zip family genes had important regulatory roles to poplars in response to stress. *PdPapHB12* was cloned, and assigned to the HD-Zip I subfamily (Ariel *et al*., 2007). Bioinformatic analysis identified PdPapHB12 as a non-transmembrane and hydrophilic protein, and predicted localization in the nucleus, cytoplasm, and mitochondria, accounting for 87.0%, 4.3%, and 8.7%, respectively. Phylogenetic analysis of homologous proteins obtained that PdPapHB12 had the closest relationship with *P. deltoides* KAH8488871.1 sequence. Thus, these proteins could have similar biological functions.

The profile of tissue-specific *PdPapHB12* regulation under different stresses was verified, and the spatial expression characteristics of *PdPapHB12* were investigated firstly using 6-week-old poplar seedlings. Through RT-qPCR analysis, the *PdPapHB12* expression was observed in ST, L, S, and R, while the highest expression was shown in L3 and the lowest expression was presented in ST. The expression in L3 was 96.04 times of that in ST. The results showed that HD-Zip I-like proteins possibly participate the regulation of organ maturation and senescence.

Silencing *PhHD-Zip* in *Petunia hybrida*, which belonged to the same cluster as the *Arabidopsis ATHB7* and *ATHB12*, resulted in a significant suppression of ethylene synthesis genes (*ACO* and *ACS*) and ethylene production. Overexpression of *PhHD-Zip* led to premature petal failure (Chang *et al*., 2014). Our results hypothesized that *PdPapHB12* possibly involved in the aging process of plant organs. The same results were also found that methyl jasmonate (MeJA) content in senescent leaves were more 4 times higher than in non-senescent leaves (He *et al*., 2002). Exogenous MeJA inducted *Arabidopsis* plants, and led to maturation, senescence, and abscission in leaves. In our study, exogenous JA was used to induce Shanxin poplar. In JA induction, the *PdPapHB12* expression was significantly up-regulated in all tissues. Therefore, we speculated that *PdPapHB12* inducted by JA could take part in organ aging process, while the mechanism was not deeply investigated.

In previous reports, HD-Zip subfamily I played a significant role in plant subjected to abiotic stress (Guan *et al*., 2022). For example, transgenic poplar *(Populus simonii* × *P. nigra*) plants by overexpressing *PsnHDZ63* exhibited the enhanced salt tolerance (Guo *et al*., 2021). Zhao *et al*., (Zhao *et al*., 2014) demonstrated that maize (*Zea mays* L.) *Zmhdz10* positively regulated drought and salt tolerance in plants through an ABA-dependent signaling pathway, in consistent with our experimental results. It was found that the expression of *PdPapHB12* was significantly up-regulated in all tissues under salt, alkaline, and high osmotic stresses, and drought stress exhibited the greatest effect on *PdPapHB12* expression. In previous studies, *ATHB6*, *ATHB7*, and *ATHB12* in *Arabidopsis* were induced under drought and ABA stresses (Söderman *et al*., 1999; Ré *et al*., 2014; Romani *et al*., 2016). *ATHB7* and *ATHB12* acted as the positive transcriptional regulators of protein phosphatase 2C (*PP2C)* genes, which repressed *PYL5* and *PYL8* encoding gene of ABA receptors, resulting in negatively regulating ABA (Söderman *et al*., 1996; Valdés *et al*., 2012). We used exogenous ABA to induce *PdPapHB12* in Shanxin poplar. The expression of *PdPapHB12* was significantly up-regulated in all tissues, indicating that the process responding to drought stress was positively related to the ABA signaling pathway, in good agreement with the literature (Henriksson *et al*., 2005). Na^+, CO_3^{2-}, HCO_3^-, and high pH value had negative effect on plant growth, because osmotic stress disrupted cellular activities. For instance, excessive reactive oxygen species (ROS) were produced under salt and alkali stresses in plants, which resulted in membrane lipid peroxidation, enzyme inactivation, and DNA damage (Bai *et al*., 2018; González *et al*., 2019). However, this result suggested that *PdPapHB12* played a positive regulatory role in the response to salt, alkaline, and drought stresses in Shanxin poplar.

In this study, five soil-borne plant pathogenic fungus were found to alter the spatial expression of *PdPapHB12* in Shanxin poplar. The expression of *PdPapHB12* was obviously up-regulated in ST and R infested with Aa, Rs, and Fo, in L2 infested with Rs and Fo (p<0.05), and in R infested with Aa, Fo, and Ss (p<0.05). As it is known, Aa can cause a variety of plant diseases. 20 selected *HD-Zip* genes participated the response of Aa infestation in the pear (*Pyrus* spp.) fruit (Wang *et al*., 2015). Fo is a soil-borne pathogenic fungus capable of causing plants to wilt and roots to rot. It secreted toxins, degraded enzymes, destroyed plant vascular bundles, and impeded plant nutrient and moisture transport, leading to leaf etiolation, root rot, and plant death (Ding *et al*., 2018; Li & Simigocki, 2019). It was hypothesized that Fo infestation caused physiological drought in the Shanxin poplar seedlings, and significantly increased the *PdPapHB12* expression in all tissues. Notably, Cc was one of the

pathogens of poplar rots, and Cc infestation decreased the *PdPapHB12* expression in all sites. The mechanism, affecting biotic stress on the *PdPapHB12* expression, will be investigated in the future.

In this study, the *PdPapHB12* expression was significantly increased in all tissues of Shanxin poplar by exogenous JA and ABA inductions (p<0.05). In SA induction, the expression was slightly decreased in ST (p<0.05), and did not significantly up-regulated in L2 (p>0.05), while SA induction resulted in significant up-regulation of *PdPapHB12* in R (p<0.05). SA and JA productions were affected by ABA, and were helpful to synthesize related proteins and improve plant resistance to salt, heat, drought, and disease (Yasuda *et al*., 2008; Nakata *et al*., 2013; Li *et al*., 2017), in consistence with our result.

Conclusions

In this study, the expression of *PdPapHB12* were achieved in Shanxin poplar in ST, L, S and R. Under abiotic stress, the expression was obviously affected by PEG6000 in all tissues. Under biotic stress, the expression was significantly up-regulated with Fo induction (p<0.05) and down-regulated with Cc induction in ST, L2, and R. Under ABA and JA induction, the expression was significantly up-regulated in all tissues (p<0.05). The results indicated that the *PdpapHB12* expression in different components and its tissue-specific regulation profile have response to various stresses and inductions. Thus, this study provides a basis for constructing the highly-resistant Shanxin poplar species.

Acknowledgments

This work was supported by the National Undergraduate Innovation Training Program (DC-2023131), the Joint Guidance (Lian-He-Yin-Dao) project of the Natural Science Foundation of Heilongjiang Province (LH2019C015), and the National Natural Science Foundation of China (NSFC: 31370642).

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(Received for publication 28 November 2022)