Pak. J. Bot., 55(2): 469-482, 2023. DOI: http://dx.doi.org/10.30848/PJB2023-2(20)

BIOINFORMATICS ANALYSIS AND GENE EXPRESSION PATTERNS OF *YABBY* GENE IN POPLAR UNDER HEAT, OSMOTIC, AND SALT STRESS

SHENMENG WANG, QINGXIN DONG AND CHENGJUN YANG*

Northeast Asia Biodiversity Research Center, Northeast Forestry University, Harbin 150040, China
**Corresponding author's e-mail: nxyycj@163.com*

Abstract

YABBY is a plant-specific and widely distributed transcription factor involved in the regulation of plant growth and development and plays an important role in plant response to environmental stress. In this study, 33 genes encoding YABBY family transcription factors were identified from *Populus trichocarpa*, and these *YABBY* genes were divided into 4 subfamilies. The physicochemical properties showed that the YABBY protein family members were between 133aa-244aa in length, rich in acidic amino acids, and had high thermal stability and localization in the nucleus. The genes are unevenly distributed on 9 chromosomes, and there is a phenomenon of aggregate distribution on the chromosomes. During the evolution of genes, homologous recombination and tandem replication events occurred, including three significant tandem repeat regions and four defined chromosome homologous recombinations. Expression of *YABBY* gene in mature leaves, young leaves, roots, xylem, female flowers, male flowers, and seeds during germination of *P. trichocarpa*. Most of the *YABBY* gene is expressed in young leaves and seedlings. It is speculated that these genes mainly function in these two parts, and there are some differences in the expression levels of each member in each tissue and growth process. The expression of three abiotic stresses (heat, salt, and infiltration) in 10 selected genes of *Populus simonii×P. nigra* was further studied by real-time quantitative PCR to better understand the function and regulation mechanism of YABBY family transcription factors.

Key words: YABBY transcription factor; Bioinformatics; Heat stress; Salt stress; Osmotic stress.

Introduction

Transcription factor (TF), also known as a trans-acting factor, has the function of activating and inhibiting transcription (Liu *et al*., 1999) and enters the nucleus at a specific time to interact with functional regions of cis-elements or other transcription factors to regulate gene expression. Studies have shown that it plays an important role in plant growth and development (Kizis *et al*., 2001). Typical transcription factors include the DNA-binding domain, the activation domain, the oligomerization site, and the nuclear localization signal (Yamada & Sato, 2013). According to the DNA binding domain, transcription factors can be divided into more than 50 families such as MIKC, WRKY, AP2/EREB, and YABBY. In addition, some transcription factors can be divided according to the number and position of amino acid residues in the DNA domain. For example, transcription factors with a zinc finger domain are classified into subclasses such as C2H2, C2HC, C2C2, C2HCC2C2, and C2C2C2C2 according to the number of cysteine and histidine (Duo, 2013). Previous studies have shown that mainly related to plant stress resistance are bZIP, WRKY, AP2/EREBP, MYB, and zinc finger protein transcription factors (Zhu *et al*., 2013).

The *YABBY* gene family is a family of small proteins (Bowman, 2000) unique to plants that consist of a zinc finger structure and a "helix-loop-helix" (also known as YABBY domain) structure that is very similar to the HMG box (Golz *et al*., 2004). The amino acid residues in these two domains are highly conserved (Sawa *et al*., 1999; Siegfried *et al*., 1999), while the regions outside this domain have little sequence homology. The C-terminal YABBY domain is very similar to the first two helical sequences of the HMG-box (Golz *et al*., 2004; Sieber *et al*., 2004). In the process of establishing the dorsal-ventral axis of the lateral organs of plants, this family of genes mainly determines the growth of the distal axis cells and affects the development of plant lateral organs. It is a kind of transcription factor related to plant morphogenesis (Bowman, 2000). It plays a key role in regulating the polarity of plant leaves, the formation, and development of reproductive organs. In addition, studies have shown that the *YABBY* gene family not only plays a key role in plant growth and development but plays an important role in plant stress (Navarro *et al*., 2004; Liu *et al*., 2007; Yamaguchi *et al*., 2004; Schluttenhofer & Yuan L, 2015). Therefore, the research on the function of this family member has important application value for forest breeding and agricultural production.

Drought is a long-standing worldwide problem that seriously affects the normal growth of plants. Woody plants under drought may produce holes and embolism, reduce water conductivity and cause plants to die. Drought-resistance research has always been the focus of plant research. This year, many remarkable achievements have been made in the research work on forest drought resistance. *OsYABBY1* gene may be involved in leaf curl during osmotic stress (Dai *et al*., 2007). *GmYABBY3*, *GmYABBY10*, and *GmYABBY16* in soybean are very sensitive to the regulation of osmotic stress (Zhao *et al*., 2017).

In recent years, the greenhouse effect has become more serious, and the increase in temperature has had a serious impact on plants. The cotton *YABBY* gene showed a down-regulation trend under heat stress conditions, including *Ghyabby1_Dt*, *Ghyabby2_At / DT*, and *Ghyabby4_At /Dt* (Yang *et al*., 2018). Welker believes that although the wax density and structure of cabbage leaves are related to its reflection of light, it has no effect on heat resistance (Welker & Furuya S, 2010), but in their subsequent studies, it was found that the wax powder on the surface of the heat-resistant varieties is dense, so it is considered that the heat resistance may be related to the structure and quantity of the wax grains (Welker & Furuya S, 1995).

Salt stress and ionic toxicity can cause disturbances in many physiological and biochemical processes such as water, gas exchange, and ion balance of crops, which in turn leads to reduced or even reduced crop yields (Friedman *et al*., 1989). The cations of salt stress mainly include Mg^{2+}, Na^{+}, and K^{+} plasmas, and the anions mainly include CO_3^{2-}, Cl^{-} and SO_4^{2-} Under salt stress, the intracellular ROS increase, leading to metabolic disorders of reactive oxygen species, causing a series of toxic effects on cells and affecting plant growth (Salin, 1988). Under salt stress, leaf chlorophyll content decreased and photosynthesis decreased (Rao G G & Rao G R, 1981). GmYABBY10 plays an important role in Arabidopsis salt stress (Zhao *et al*., 2017).

In this study, the bioinformatics analysis of the *PtYABBY* gene family was carried out, and the experiment of thermal, osmotic, and salt stress simulation of natural stress was carried out with *Populus simonii×P. nigra* as experimental material. RT-PCR was used to analyze the relative expression of the *PnYABBY* gene under heat stress, osmotic stress, and salt stress. It lays a foundation for the in-depth study of the function of the PnYABBY transcription factor in stress, and clarifies the response and biological function of the *YABBY* gene to stress, which is of great significance for improving the stress resistance of woody plants such as *P. simonii×P. nigra*.

Materials and Methods

Plant material and treatment: *P. simonii×P. nigra* were grown at Northeast Forestry University Forest Farm, Harbin, China. Cuttings of *P. simonii×P. nigra* were cultivated in pots containing a sterile mixture of turfy soil, vermiculite, and perlite (3:2:1) in the greenhouse under long-day conditions (16 h light/8 h dark) at 25℃, and supplied with water every 3 days. The seedlings were cut when they were 10 cm tall and then rooted in water that was aerated constantly by aquarium pumps. After 20 days of cultivation, high temperature, infiltration, and salt treatment were carried out. The hydroponic seedlings with the same growth state were pretreated in the light incubator at 25℃ for 1 day, and then the pretreated hydroponic seedlings were treated at 25℃, 35℃, 43℃ for 2 h. The tissue culture seedlings were pretreated in water for one day, then transferred to hydroponic culture solution for 3 days, and then transferred to hydroponic culture medium supplemented with 20% (m/v) PEG6000 for treatment for 0h, 12h, 24h, 48h, 96h (the hydroponic culture solution is changed once every 3 days during the stress). The tissue culture seedlings with good growth and consistency were pretreated for 1 d and then transferred to hydroponic culture tanks with a concentration of 0mM, 75mM, 150mM, and 250mM for 0.25 d, 1 d, 2 d, 4 d. Three times for each experiment and each treatment. Leaves, stems, and roots were collected at the indicated times after these treatments and stored at -80°C for further analysis.

Identification of YABBY family genes in *P. trichocarpa*: The data required for this article, including the nucleotide sequence, genomic sequence, and protein sequence of the rice, Arabidopsis, and *P. simonii* YABBY genes, are at phytozome11 (https://phytozome.jgi.doe.gov.pz/portal.htmL). The website and the PlantTFDB (http://planttfdb.cbi.pku.edu.cn/) website are available (Tuskan, 2006; Jin *et al*., 2014). Using the online tool ProtParam (http://web.expasy.org/protparam/) (Artimo *et al*., 2012) provided by ExPASy, the amino acid number, molecular weight, theoretical isoelectric point, positive and negative charge base, aliphatic of the YAMABY transcription factor family members. The physicochemical properties such as amino acid index and hydrophobicity, protein instability coefficient were analyzed. The subcellular localization information of the YABBY protein was predicted using the online software WoLF PSORT (http://www.genscript.com/psort/wolf_psort.htmL) (Horton *et al*., 2014).

Conservative motif analysis of PtYABBY proteins: Using the searched 33 YABBY family member protein sequences, upload them to the conservative motif analysis software MEME (http://meme-suite.org/tools/meme) to analyze their conserved motifs. The maximum output is set to 10, conservative. The base length is set to 6-100, the motif position is set to 2-120, and other parameters are set as default parameters.

Construction of the phylogenetic tree of *PtYABBY*: Collect *YABBY* gene family members from *P. trichocarpa*, rice, and Arabidopsis, obtain their accession numbers, and download their protein sequences from the Phytozome v12 website, perform multiple alignments of the clustered W sequence using MEGA5 software, and set the bootstrap test value, establish the Neighbor-Joining system evolution tree.

Chromosome Localization of the *PtYABBY* Gene: According to the Transcript Name of the *PtYABBY* gene of the Phytozome 11 in the plant genome database, the chromosome map of the *PtYABBY* gene was constructed by MapInspect software, and the distribution of *PtYABBY* gene on the chromosome was analyzed.

Analysis of the expression patterns of each member of *PtYABBY* gene family: The NCBI accession number of the poplar *PtYABBY* gene was uploaded to the Affymetrix website using the NetAffx software to obtain the probe number (Hossain *et al*., 2012). The BAR online software was used to obtain the expression information of each gene in different parts, and the heat map of each gene expression pattern was made by the online heat map creation software BAR HeatMapper Plus（http://bar.utoronto.ca/ntools/cgi-bin/ntools_heatmapper_plus.cgi).

Adaptive evolution analysis of *PtYABBY* gene: The entire nucleotide coding sequence of the *PtYABBY* gene was submitted to the Selecton program (https://selecton.tau.ac.il/) (Stern *et al*., 2007), and the model was set to a mechanical-empirical model (MEC). The selection pressure of each protein site was calculated using the Selecton program. The selection pressure of each member of the *PtYABBY* gene family was then obtained.

QRT-PCR analysis: Fluorescence quantification kit uses Kangwei Century Biotechnology fluorescence quantitative kit, the reaction system is SYBR Mixture 10 μL, template 2 μL, water 6.4 μL, upstream primer 0.4 μL, downstream primer 0.4 μL; reaction procedure is 95°C 10min; 95°C 15s, 60°C 1min, 95°C 15 s,40 cycles; 60°C 1min; 95°C 15s; 60°C 15s. Quantitative primers are shown in the attached table. According to each reaction, the corresponding CT value was obtained, and the data was analyzed by the $2^{-\triangle\triangle CT}$ method to determine the relative expression level of the YABBY gene family gene. Three biological replicates were performed three times each time.

Results

Identification of YABBY genes in *P. trichocarpa*: A total of 33 members of the *PtYABBY* gene family were identified based on the plant genome database phytochrome 11 and the plant transcription factor database PlantTFDB 3.0. According to the position of the gene on the chromosome, the YABBY gene of *P. trichocarpa* was named *PnYABBY1 - 11.6*.

The online tool Prot Param was used to analyze the physicochemical properties of the YABBY transcription factor family proteins (Table 1). It was found that the amino acid number of different proteins was not much different, and the amino acid length of more members was between 133 and 244 aa. The isoelectric point of most amino acid sequences is distributed in the acidic range. The aliphatic amino acid index is an indicator of the thermal stability of the protein. In general, the YABBY family of proteins has high thermal stability. The analysis results show that most members are hydrophilic; other GARVY values are distributed between -0.839 (*PtYABBY*2.5) and -0.082 (*PtYABBY*10.4). The results of protein subcellular localization analysis are shown in (Table 2). It can be seen from the table that the YABBY protein is distributed in the nucleus, chloroplast, endoplasmic reticulum, cytoplasm, mitochondria, and vacuoles, and mainly distributed in the nucleus.

Chromosome localization of *PtYABBY* in *P. trichocarpa*: Based on the accession number of the gene, the position information of the poplar YABBY gene on the corresponding chromosome was downloaded from the Phytozome website, and then the chromosome map was drawn based on the chromosome homologous recombination event map in the poplar genome sequencing data published in 2006. The chromosomal map shows that during the evolution of the YABBY gene, homologous recombination and tandem replication events occurred, including three significant tandem repeat regions and four identified chromosome homologous recombinations (Fig. 1).

Table 1. Parameters for the 33 identified *YABBY* genes and deduced polypeptides present in the *P. trichocarpa* genome.

Gene name	Locus name	Amino acid no.	Molecular weight (Da)	Isoelectric points	Aliphatic index	GRAVY	Instability coefficient	Cellular localization
PtYABBY1	Potri.001G120200.1.	214	23889.13	7.19	78.32	-0.337	47.24	Nucleus
PtYABBY1.1	Potri.001G120200.2	212	23693.92	7.73	78.06	-0.325	45.86	Nucleus
PtYABBY1.2	Potri.001G120200.3	172	19432.46	6.48	100.17	0.158	53.36	Nucleus
PtYABBY2	Potri.001G214700.1	208	23189.27	9.30	75.53	-0.453	50.60	Chloroplast
PtYABBY2.1	Potri.001G214700.2	198	22393.42	9.48	70.96	-0.602	53.64	Chloroplast
PtYABBY2.2	Potri.001G214700.3	191	21121.99	8.54	80.68	-0.412	53.94	Nucleus
PtYABBY2.3	Potri.001G214700.4	189	20906.78	8.54	81.53	-0.393	51.06	Nucleus
PtYABBY2.4	Potri.001G214700.5	140	15687.65	9.30	67.71	-0.820	50.57	Nucleus
PtYABBY2.5	Potri.001G214700.6	142	15902.86	9.30	66.76	-0.839	54.45	Nucleus
PtYABBY2.6	Potri.001G214700.7	206	22974.06	9.30	76.26	-0.436	47.93	Chloroplast
PtYABBY3	Potri.002G145100.1	210	23339.60	7.17	75.24	-0.313	49.85	Cytosol
PtYABBY4	Potri.003G112800.1	213	23744.12	7.71	78.69	-0.285	45.92	Nucleus
PtYABBY4.1	Potri.003G112800.2	140	15580.94	8.29	89.00	-0.109	54.71	Nucleus
PtYABBY4.2	Potri.003G112800.3	158	17607.38	6.20	105.38	0.319	54.92	Cytosol
PtYABBY5	Potri.006G067800.1	188	21233.98	8.44	73.67	-0.438	47.68	Extra
PtYABBY6	Potri.008G097800.1	180	20043.86	9.25	65.00	-0.585	47.43	Nucleus
PtYABBY7	Potri.008G189000.1	219	25062.27	6.32	66.76	-0.580	43.69	Extra
PtYABBY7.1	Potri.008G189000.2	215	24548.72	6.31	68.00	-0.524	42.53	Extra
PtYABBY8	Potri.010G042400.1	216	24252.30	5.84	74.91	-0.465	53.47	Extra
PtYABBY8.1	Potri.010G042400.2	153	17035.43	7.72	85.36	-0.329	47.33	Nucleus
PtYABBY9	Potri.014G066700.1	212	23477.84	8.56	71.27	-0.291	48.68	Nucleus
PtYABBY9.1	Potri.014G066700.2	197	21785.97	8.99	78.22	-0.156	43.32	Nucleus
PtYABBY9.2	Potri.014G066700.3	161	17852.25	7.01	64.22	-0.523	49.34	Nucleus
PtYABBY9.3	Potri.014G066700.4	165	18305.83	7.01	69.76	-0.454	52.21	Cytosol
PtYABBY9.4	Potri.014G066700.5	153	17203.69	7.03	78.95	-0.082	54.49	Nucleus
PtYABBY10	Potri.016G067300.1	244	27269.07	9.20	83.52	-0.344	52.64	Nucleus
PtYABBY11	Potri.018G129800.1	188	21283.09	8.19	76.76	-0.389	44.42	Nucleus
PtYABBY11.1	Potri.018G129800.2	182	20734.54	8.50	75.00	-0.355	39.16	Cytosol
PtYABBY11.2	Potri.018G129800.3	145	16445.80	8.46	83.24	-0.093	46.30	Nucleus
PtYABBY11.3	Potri.018G129800.4	133	14930.83	6.93	73.91	-0.321	50.62	Cytosol
PtYABBY11.4	Potri.018G129800.5	133	14930.83	6.93	73.91	-0.321	50.62	Cytosol
PtYABBY11.5	Potri.018G129800.6	168	19114.54	9.33	71.43	-0.610	40.15	Chloroplast
PtYABBY11.6	Potri.018G129800.7	136	15556.38	9.63	66.10	-0.818	45.07	Nucleus

Table 2. Subcellular localization of *YABBY* protein in poplar poplar.

Genetic entry number	Subcellular localization
Potri.001G120200.1	nucl: 11, cysk: 2
Potri.001G120200.2	nucl: 6, cyto: 4, extr: 2, chlo: 1
Potri.001G120200.3	nucl: 7, cyto: 3, extr: 2, chlo: 1
Potri.001G214700.1	chlo: 9, nucl: 5
Potri.001G214700.2	chlo: 8, nucl: 5
Potri.001G214700.3	nucl: 11.5, cyto_nucl: 6.5, plas: 1
Potri.001G214700.4	nucl: 8, cyto: 2, mito: 1, plas: 1, extr: 1
Potri.001G214700.5	nucl: 12, cyto: 2
Potri.001G214700.6	nucl: 13
Potri.001G214700.7	chlo: 9, nucl: 5
Potri.002G145100.1	cyto: 9, nucl: 2, cysk: 2
Potri.003G112800.1	nucl: 11, cysk: 2
Potri.003G112800.2	nucl: 6, cyto: 4, chlo: 3
Potri.003G112800.3	cyto: 5, golg: 4, chlo: 1, nucl: 1, mito: 1, plas: 1
Potri.006G067800.1	extr: 4, nucl: 2, cyto: 2, E.R.: 2, chlo: 1, mito: 1, plas: 1
Potri.008G097800.1	nucl: 13
Potri.008G189000.1	extr: 4, nucl: 2.5, E.R.: 2, cysk_nucl: 2, chlo: 1, cyto: 1, mito: 1, plas: 1
Potri.008G189000.2	extr: 4, nucl: 3.5, cysk_nucl: 2.5, E.R.: 2, chlo: 1, mito: 1, plas: 1
Potri.010G042400.1	extr: 4, chlo: 3, E.R.: 3, nucl: 2, plas: 1
Potri.010G042400.2	nucl: 8, cyto: 3, mito: 1, plas: 1
Potri.014G066700.1	nucl: 7, cyto: 5, plas: 1
Potri.014G066700.2	nucl: 8, cyto: 4, plas: 1
Potri.014G066700.3	nucl: 7, cyto: 6
Potri.014G066700.4	cyto: 8, nucl: 4, plas: 1
Potri.014G066700.5	nucl: 5, chlo: 4, cyto: 4
Potri.016G067300.1	nucl: 5, E.R.: 3, cyto: 2, mito: 1, plas: 1, extr: 1
Potri.018G129800.1	nucl: 6, cyto: 5, chlo: 2
Potri.018G129800.2	cyto: 8, nucl: 4, extr: 1
Potri.018G129800.3	nucl: 5, cyto: 4, extr: 2, chlo: 1, plas: 1
Potri.018G129800.4	cyto: 7, nucl: 5, plas: 1
Potri.018G129800.5	cyto: 7, nucl: 5, plas: 1
Potri.018G129800.6	chlo: 8, nucl: 5
Potri.018G129800.7	nucl: 14

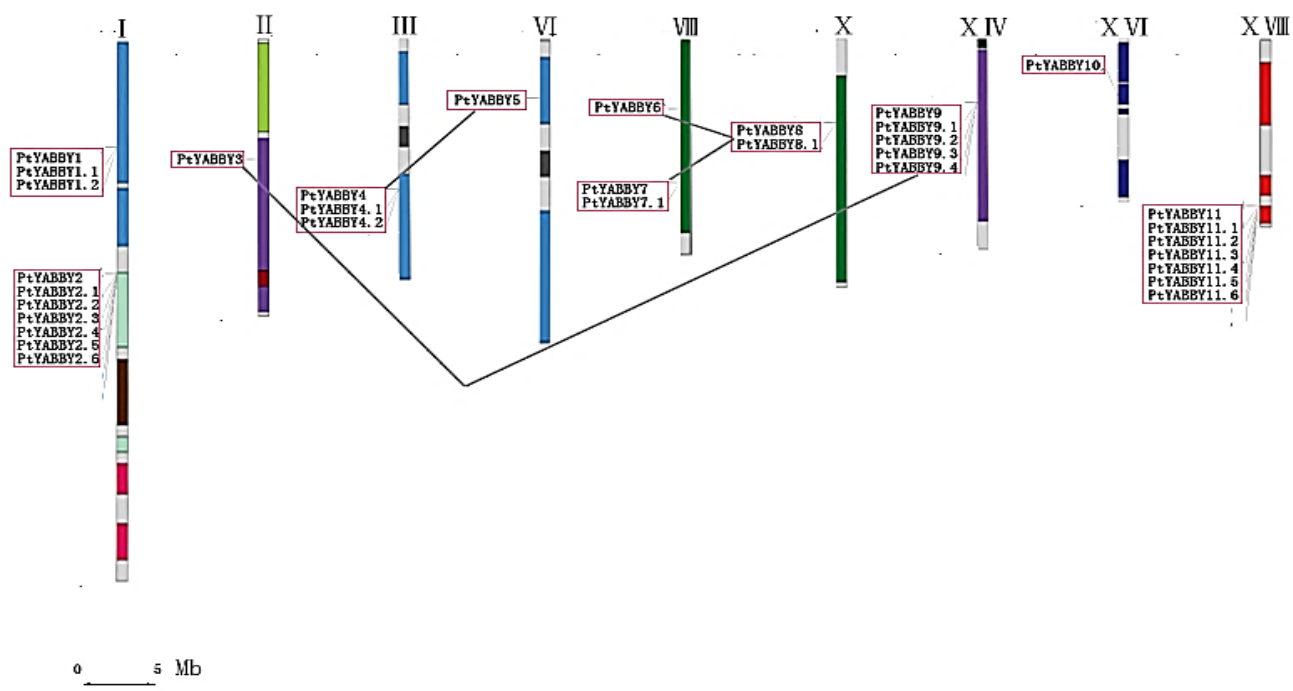

Fig. 1. Chromosome mapping of YABBY gene in Populus hairy poplar.

XVIII stands for poplar chromosome numbers from 1 to 18, the same color region represents homologous recombination events on chromosomes, and the genes linked to gray lines are homologous genes produced by the homologous recombination time of the chromosomes. The genes in the red box are homologous genes produced by the tandem replication of genes.

Phylogenetic analysis of the *PtYABBY* gene family: To further reveal the evolutionary relationship between the PtYABBY gene members, we collected YABBY protein sequences of *A. thaliana*, *Oryza sativa*, and *P. trichocarpa* to construct an orthologous phylogenetic tree (Fig. 2). The 33 YABBY transcription factors were divided into four subfamilies, namely CRC/INO, YAB1/YAB3, YAB2, and YAB5. In the phylogenetic tree, most of the members of the YABBY transcription factor family are associated with poplar itself in a small branch, and Arabidopsis and rice are mostly in their respective branches. The results indicated that the PtYABBY transcription factor is not high in affinity with Arabidopsis and rice.

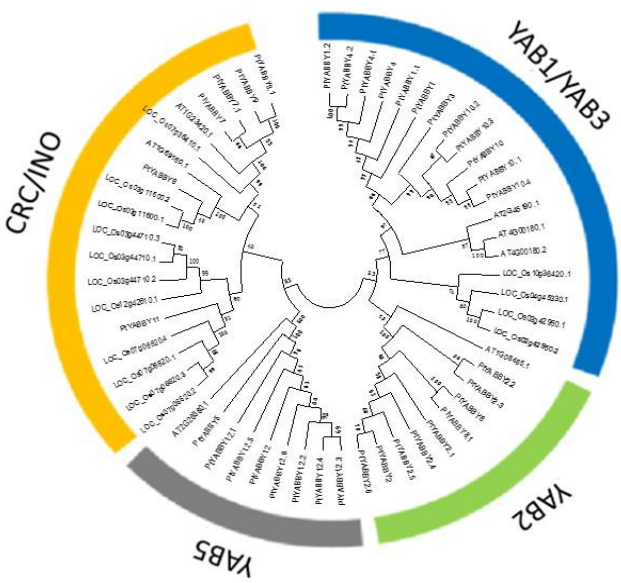

Fig. 2. Phylogenetic analysis of YABBY proteins from *P. trichocarpa*, *A. thaliana*, and *O. Sativa*. The deduced full-length amino acid sequences were used to generate the phylogenetic tree by the Neighbor-Joining (NJ) method.

Conservative motif analysis: Conserved motifs on the PtYABBY protein sequence were analyzed using the MEME Conserved Motif Analysis Site (Bailey T L & Elkan C, 1995). We found that there are three conserved motifs on the PtYABBY protein sequence: motif 1, motif 2, and motif 3 (Fig. 3). Motif 1 is a member of most family members of the *PtYABBY* gene, with a total length of 50 amino acids, and the consensus sequence is CCYCCAGAGAAGAGRCARMGAGTHCCTTCTGCAT AYAAYCGSTTCATCAA. 30 members have motif 2 that contains 50 amino acids, and the consensus sequence is YTGTKACHGTYMGATGTGGKCACTGCACCAATCT VYTSTCTGTSAACATG. 29 members have motif 3 that contains 50 amino acids, and its consensus sequence is CACAGAGAAGCWTTCAGYACAGCWGCMAARAAT TGGGCMCATTTYCCWCA.

Evolutionary stress analysis: The PtYABBY protein sequence was selected and input into the Selecton server to obtain the selection pressure map of each locus (Fig. 4). The labeling color of the protein primary structure site was between white and purple, mostly in purple and deep purple, indicating that these sites were experienced. The yellow spots were concentrated at positions 191-211, indicating that the rate of non-synonymous substitution of these sites by positive selection pressure is much lower than synonymous substitution (Fig. 4), and the rate of occurrence varies with genes which are considered to be the result of evolutionary choice.

Tissue-specific expression profiles of *PtYABBY* genes in the *Populus* genome: To explore the tissue-specific expression pattern of the *YABBY* gene in various parts of *Populus trichocarpa*, we downloaded the expression pattern of the *PtYABBY* gene in each sample on the Popgenie website and screened 8 tissue samples for heat map (Fig. 5). The expression abundance of the *PtYABBY* gene was higher in young leaves, while the expression level in continuous growth seedlings was slightly lower than that in young leaves. The expression level in roots was not high and each member in seedlings was not high. Most of the genes in male and female inflorescences were up-regulated, and the *PtYABBY1*, *PtYABBY9*, and *PtYABBY11* genes were down-regulated. The results showed that the *PtYABBY* gene was mainly expressed in young leaves.

Expression of PnYABBY gene under heat stress: The reverse transcription cDNA of RNA extracted from the leaves, stems, and roots of seedlings treated with stress at 25°C, 35°C and 43°C was used as the experimental material for fluorescence quantitative experiments (Fig. 6). The results were as follows: with increasing temperature, the expression levels of genes in various tissues vary greatly. The *PnYABBY1*, *PnYABBY2*, and *PnYABBY4* genes were up-regulated, and the *PnYABBY9* gene was unchanged. The relative expression of the other genes was down-regulated. The *PnYABBY1*, *PnYABBY2*, and *PnYABBY3* genes showed a downward trend in the stem. In the leaves *PnYABBY1*, *PnYABBY3*, *PnYABBY5*, *PnYABBY6*, *PnYABBY8*, *PnYABBY9*, *PnYABBY10*, and *PnYABBY11* genes showed an up-regulation trend, *PnYABBY2* gene increased first and then decreased, and the relative expression of the *PnYABBY4* gene showed a downward trend.

Expression of *PnYABBY* gene under osmotic stress: The reverse transcription cDNAs of the leaves, stems, and roots of the seedlings at 0h, 12h, 24h, and 48h at different time points were treated by osmotic stress as experimental materials (Fig. 7). Under the osmotic stress, the relative expression of the *PnYABBY4* gene in the roots of *P. simonii* decreased with the increase of stress time, and the other genes were up-regulated and then down-regulated. The relative expression of the *PnYABBY4* gene in the stem was up-regulated and then up-regulated, and the other genes were up-regulated and then down-regulated. The relative expression of *PnYABBY2* and *PnYABBY8* genes in the leaves was up-regulated and then down-regulated. The relative expression of the other genes was up-regulated and then down-regulated.

Fig. 3. Determination of conservative motif of PtYABBY.

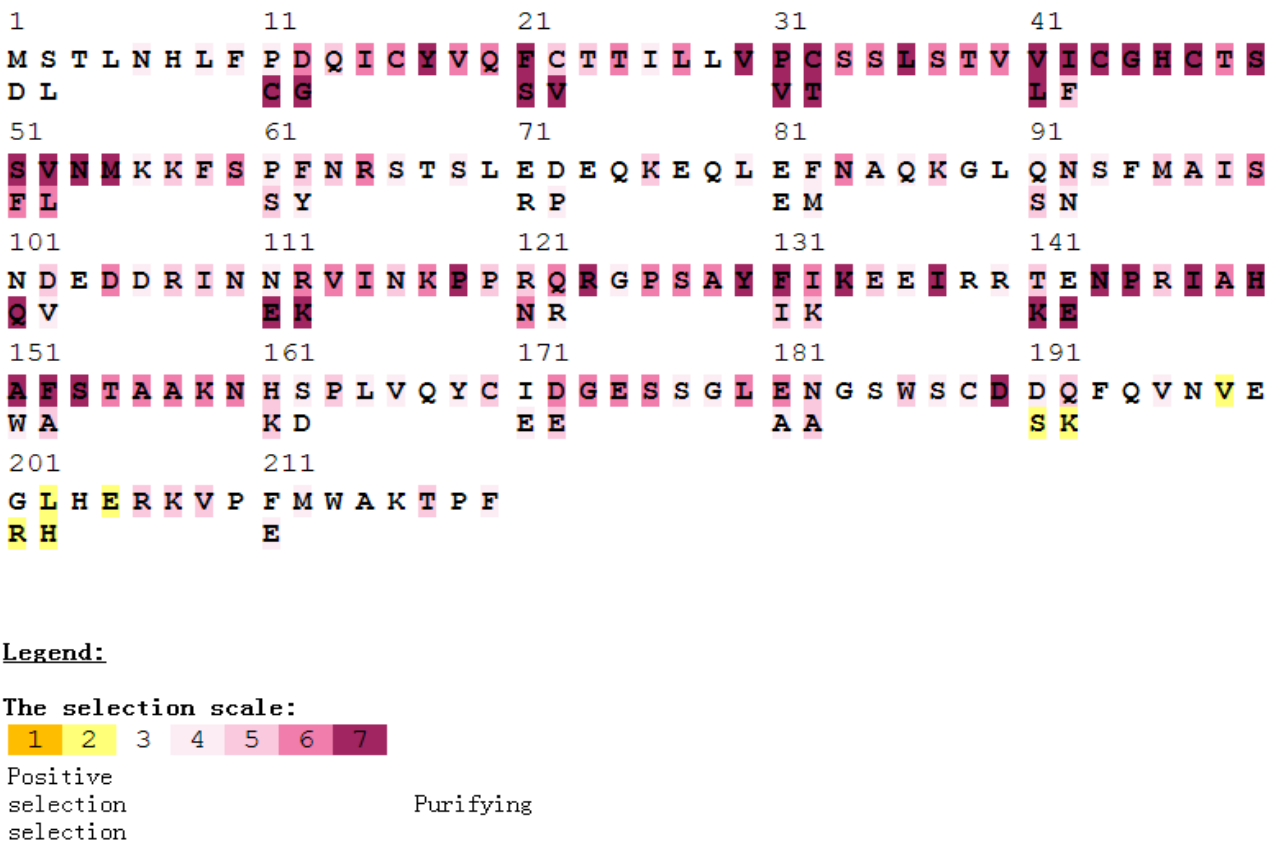

Fig. 4. Selecton pressure Analysis of PtYABBY protein sequence based on MEC Model.

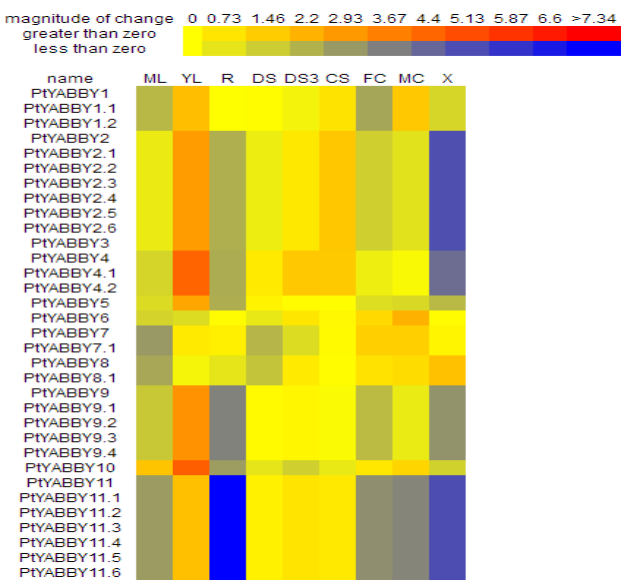

Fig. 5. Analysis of *PtYABBY* gene expression patterns YL: young leaves ML: mature leaves X: stem R: Roots DS3: germinating seeds lasting dark and then lighting for 3 hours: seedlings growing in darkness CSCS: continuous growth of seedlings MC male inflorescences: female inflorescence.

Expression of *PnYABBY* gene under salt stress

Expression of *PnYABBY* gene in roots under salt stress: Under salt stress, the relative expression of the *YABBY* gene in the roots of *P. simonii* was significantly different under different salt stress (Fig. 8). The relative expression of *PnYABBY4* gene was up-regulated with the increase of stress time at 75mM NaCl concentration, and the relative expression of the other genes was down-regulated. Under the condition of 150mM NaCl concentration, the relative expression of *PnYABBY8* gene was up-regulated, the *PnYABBY4* gene was unchanged, and the other genes were down-regulated. Under the condition of 250mM NaCl concentration, the relative expression of *PnYABBY1* and *PnYABBY4* genes were up-regulated with the increase of stress time, and the other genes were down-regulated.

Expression of *PnYABBY* gene in stems under salt stress: The relative expression of the *YABBY* gene in the stem of *P. simonii* under salt stress was low (Fig. 9). Under the condition of 75mM NaCl concentration, the relative expression of *PnYABBY4* gene was up-regulated with the increase of stress time, *PnYABBY2* gene had no significant change, and the relative expression of other genes was down-regulated. Under the condition of 150mM NaCl concentration, the relative expression of *PnYABBY1*, *PnYABBY2*, *PnYABBY5*, *PnYABBY6*, and *PnYABBY10* genes was down-regulated with the increase of stress time, and there was no significant change in *PnYABBY4*, *PnYABBY8*, and *PnYABBY9* genes. Under the condition of 250mM NaCl concentration, the relative expression of *PnYABBY2*, *PnYABBY6*, and *PnYABBY10* genes was up-regulated and then down-regulated, *PnYABBY4* and *PnYABBY5* genes were up-regulated, and *PnYABBY8* and *PnYABBY9* genes were not significantly changed.

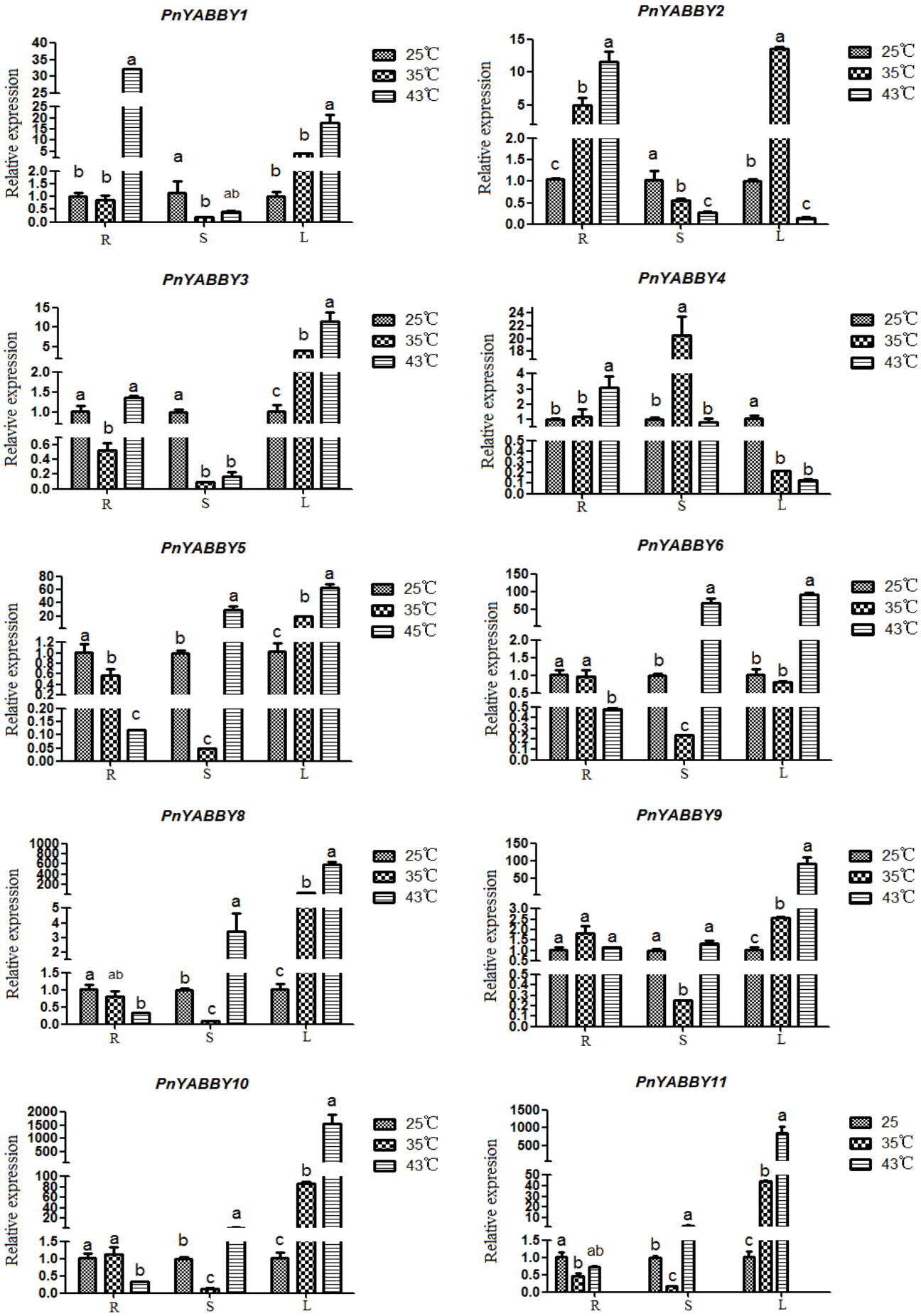

Fig. 6. Heat stress treatment of relative expression of *PnYABBY* gene.

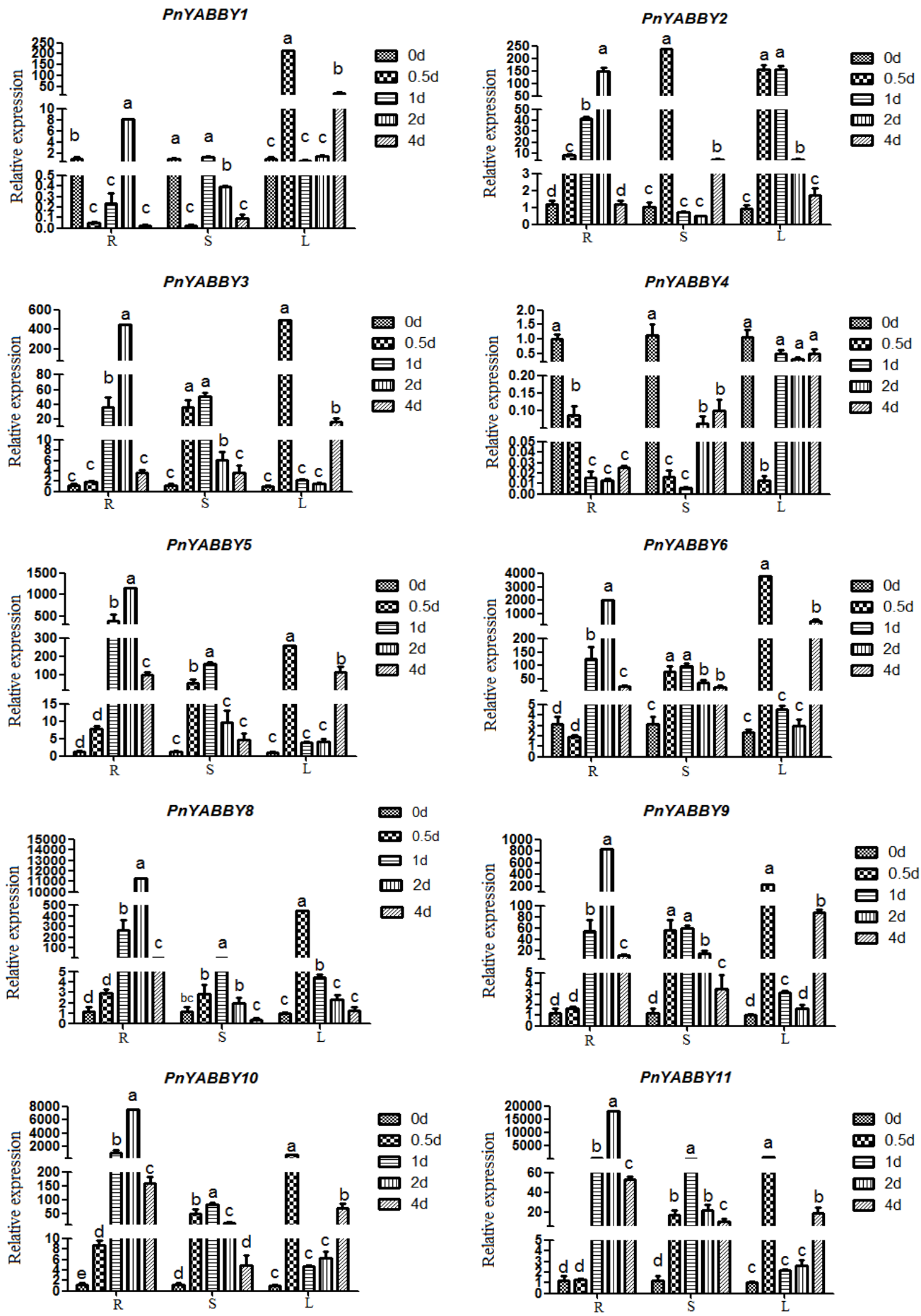

Fig. 7. Relative expression of *PnYABBY* gene at different time points under osmotic stress.

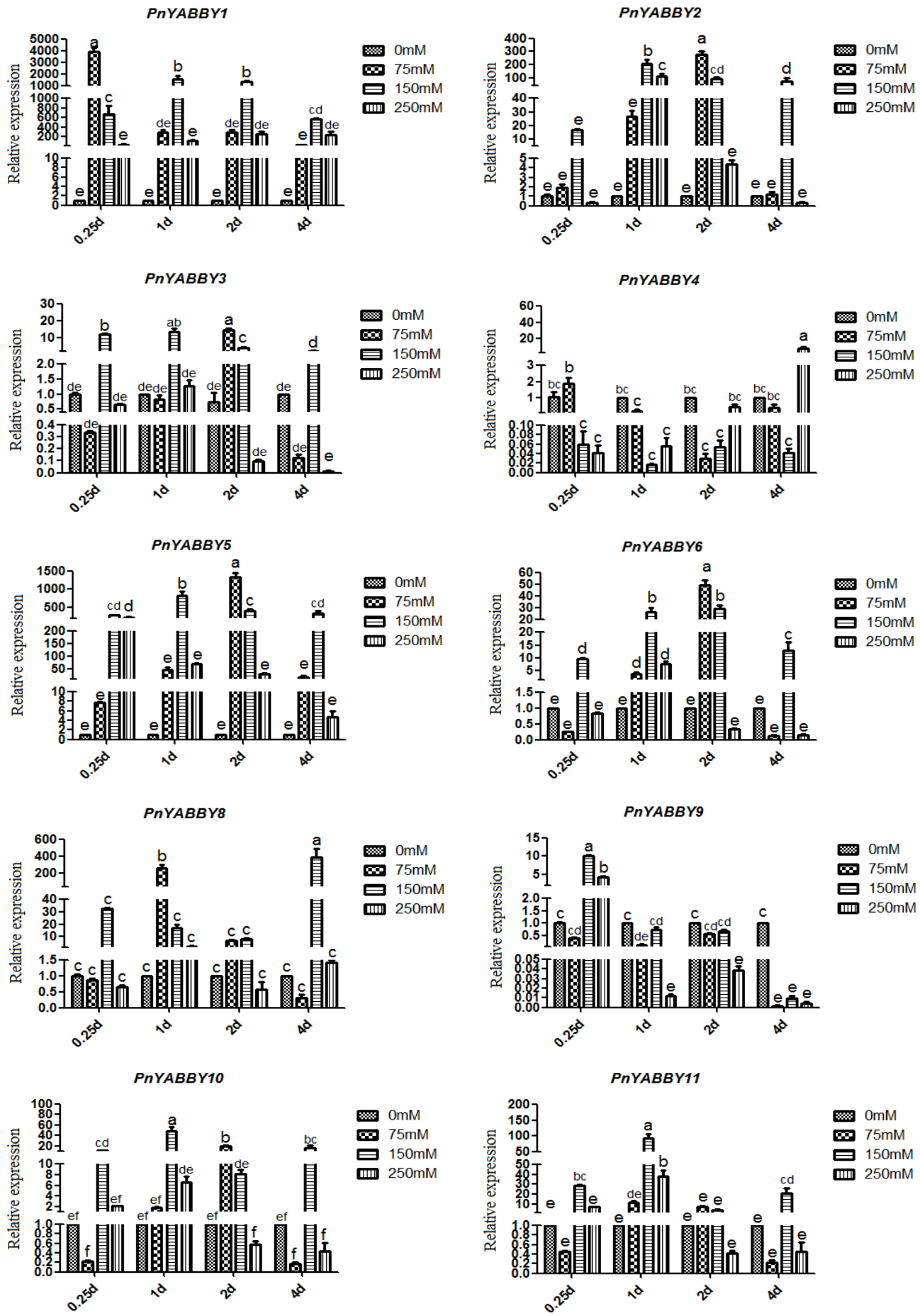

Fig. 8. Relative expression of *PnYABBY* gene in roots under salt stress.

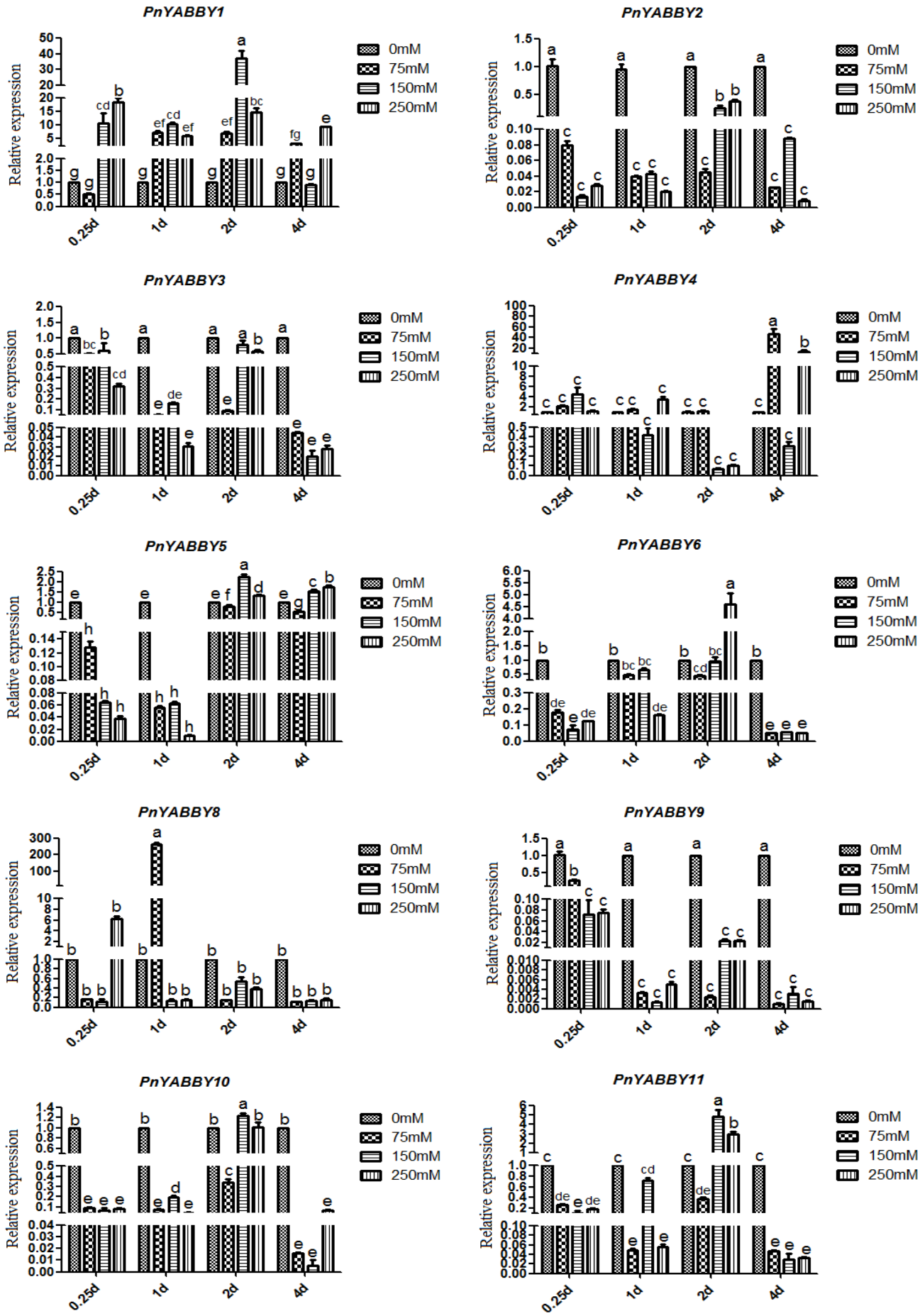

Fig. 9. Relative expression of *PnYABBY* gene in stems under salt stress.

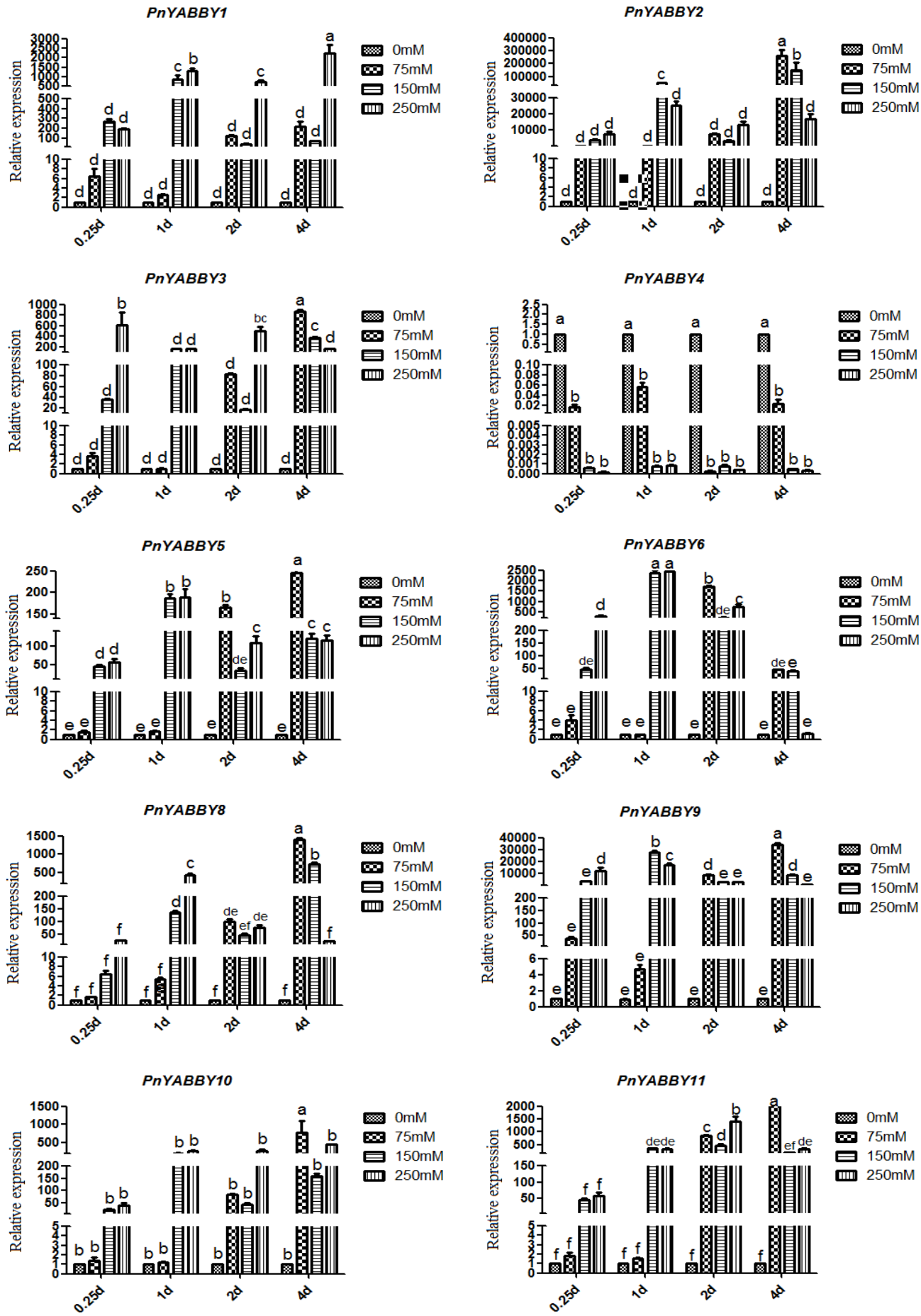

Fig. 10. Relative expression of *PnYABBY* gene in leaves under salt stress.

Expression of *PnYABBY* gene in leaves under salt stress: The YABBY gene in leaves was highly expressed under salt stress (Fig. 10). Under the condition of salt concentration of 75mM, the relative expression of *PnYABBY1*, *PnYABBY4*, and *PnYABBY8* genes did not change significantly with the increase of stress time, *PnYABBY6* gene was down-regulated, and the other genes were up-regulated. At the salt concentration of 150mM, the *PnYABBY4* and *PnYABBY10* genes did not change significantly with the increase of stress time, the *PnYABBY3* gene was up-regulated, and the other genes were down-regulated. Under the condition of salt concentration of 250mM, the relative expression of *PnYABBY2*, *PnYABBY4* and *PnYABBY10* did not change significantly with the stress time. The *PnYABBY5*, *PnYABBY6*, *PnYABBY8*, *PnYABBY9*, and *PnYABBY11* genes were up-regulated and then down-regulated.

Result and Discussion

In this study, we identified 33 *PtYABBY* genes in the *P. trichocarpa* genome (Tuskan, 2006; Jin *et al*., 2014). The physicochemical properties of the amino acid composition, molecular mass, isoelectric point, and hydrophobicity of the protein were analyzed using the ProtParam tool in expasy (Artimo *et al*., 2012). The theoretical isoelectric point is 5.84 (PtYABBY9) and the highest is 9.63 (PtYABBY11.6), and the isoelectric point of most amino acid sequences is distributed in the acidic range, indicating that the PtYABBY protein family is rich in acidic amino acids. The aliphatic amino acid index is an index to measure the thermal stability of the protein. The higher the index, the better the thermal stability of the protein (Zhao *et al*., 2015). The minimum index in the table is 64.22 (PtYABBY10.2) and the maximum is 105.38 (PtYABBY4.2), indicating the high thermal stability. The positive and negative values of GARVY represent the difference in protein sparsity/ hydrophilicity. The analysis results show that most of the members are hydrophilic. Only PtYABBY1.1 and PtYABBY4.2 are hydrophobic, and their GARVY values are 0.158 and 0.319. The results of protein subcellular localization indicated that PtYABBY protein was mainly distributed in the nucleus, which indicated that the regulation of PtYABBY transcription factor on target gene transcription mainly occurred in the nucleus (Horton *et al*., 2014).

The localization of genes on chromosomes is one of the factors influencing gene function (Cao *et al*., 2010). The results of chromosomal map analysis showed that the distribution of *PtYABBY* gene on 18 chromosomes was relatively scattered, among which the number of chromosomes was the highest, and there were 10 *PtYABBY* genes. The second was chromosome 18, and there were 7 *PtYABBY* genes. The smallest number on chromosomes 2, 6, and 16 has only one *PtYABBY* gene. The *PtYABBY* gene has a clustered distribution on the chromosome.

The analysis of the role of selection in the evolution of related genes is an important part of evolutionary biology research (Nei, 2005). The results showed that many sites of *P. trichocarpa* were under the pressure of strong evolutionary selection, and the evolution of dominant genes was evolved in the *PtYABBY* gene.

In the process of plant growth and development, it is often affected by many abiotic stress factors such as high temperature, drought, high salt, and damage. Plants adapt to the external environment to form a variety of complex signal transduction and regulation mechanisms (Guo *et al*., 2007). Zinc finger protein is a kind of transcription factor with typical finger-conserved domains. According to structural features, it mainly includes C2C2, C2HC, C2H2, and C3H4. Among them, the function of C2H2-type zinc finger protein is clear that mainly involved in plant growth and development and response to stress (Wu & Zhong, 2013). In addition to C2H2 with a typical zinc finger protein domain, GATA, DOF, YABBY, and others also have a zinc finger protein domain (Mo *et al*., 2013). According to the experimental data of heat, osmosis, and salt stress, the response of *PnYABBY* gene to different stress conditions is different. The relative expression of each gene in roots, stems, and leaves of *P. simonii* is very different. The *PnYABBY* gene is mainly expressed in leaves, followed by stems. The *PnYABBY* gene was induced by heat stress and was specifically expressed in leaves. The *PnYABBY4* gene was inhibited by osmotic stress, and other *PnYABBY* genes were induced by osmotic stress. Under salt stress, the relative expression of *PnYABBY* gene in roots, stems, and leaves of *P. simonii* was significantly different. *PnYABBY10* and *PnYABBY11* were induced by salt stress in roots. In the leaves, *PnYABBY3* and *PnYABBY5* were induced by low salt induction and high salt inhibition, and *PnYABBY8* and *PnYABBY11* were induced by low salt and high salt, and medium salt was inhibited. *PnYABBY1* in stems was induced by low salt and high salt, and medium salt inhibited expression. *PnYABBY3* was inhibited by low salt and high salt, and medium salt-induced expression. The above results indicate that the *PnYABBY* gene plays a certain role in the plant's stress resistance signaling pathway.

According to the existing research on *YABBY* gene, most of the research on *YABBY* gene is single factor stress analysis, but the stress of plants in adversity comes from many aspects. In this paper, three kinds of experiments were carried out to simulate the natural stress, and the relative expression of *PnYABBY* gene under heat stress, osmotic stress, and salt stress was studied. The experimental results showed that *PnYABBY* gene can regulate the anti-reverse effect of *P. simonii* on abiotic stress. A comprehensive analysis of the results of three stress experiments can be applied to improve the application of high temperature and salt and alkali resistance in forest trees.

Conclusion

We mainly conducted bioinformatics analysis on The YABBY gene of poplar and screened out 10 YABBY genes. The relative expression levels of these 10 genes were determined by heat, osmosis and salt stress tests. Successfully cloned PnYABBY3 and PnYABBY4 genes, studied the subcellular localization of these two genes, and analyzed the prospect of the application of Poplar

YABBY gene in forest stress. Our results provide important information about the YABBY gene and new insights into its changes in response to stress in different environments. It was determined that the YABBY gene in young black poplars could be effectively applied to improve tree stress resistance

References

Artimo, P., M. Jonnalagedda and K. Arnold. 2012. ExPASy: SIB bioinformatics resource portal. *Nucl. Acids Res.*, 40(Web Server issue): 597-603.

Bailey, T.L. and C. Elkan. 1995. The value of prior knowledge in discovering motifs with MEME, F, 1995 [C].

Bowman, J.L. 2000. The YABBY gene family and abaxial cell fate. *Curr. Opin. Plant Biol.*, 3(1): 17-22.

Cao, X., L.F.G. Shang and H. Yu. 2010. Bioinformatics analysis of grape SBP gene family. *Genom. & Appl. Biol.*, 29(4): 791-798.

Dai, M., Y. Zhao and Q Ma. 2007. The rice YABBY1 gene is involved in the feedback regulation of gibberellin metabolism. *Plant Physiology*, 144(1): 121.

Duo, L.U. 2013. C2H2 zinc-finger recognition of biomolecules. *Yao Xue Xue Bao*, 48(48): 834-41.

Friedman, R.A., A. Altman and N. Levin. 1989. The effect of salt stress on polyamine biosynthesis and content in mung bean plants and in halophytes. *Physiol. Plantarum*, 76(3): 295-302.

Golz, J.F., M.R. Roccaro and A. Hudson. 2004. GRAMINIFOLIA promotes growth and polarity of Antirrhinum leaves. *Develop.*, 131(15): 3661-70.

Guo, S., W. Huang and Y. Jiang. 2007. Cloning and expression analysis of rice C2H2 zinc finger protein gene RZF71.*Genetics*, 29(5): 607-613.

Horton, P., K. Park and T. Obayashi. 2014. Protein subcellular localization prediction with wolf psort; *proceedings of the Asia-Pacific Bioinformatics Conference 13-16 February 2006*, Taipei, Taiwan, F.

Hossain, Z., M. Hajika and S. Komatsu. 2012. Comparative proteome analysis of high and low cadmium accumulating soybeans under cadmium stress. *Amino Acids*, 43(6): 2393-416.

Jin, J., H. Zhang and L. Kong. 2014. PlantTFDB 3.0: a portal for the functional and evolutionary study of plant transcription factors. *Nucleic Acids Research*, 42(Database issue): 1182-7.

Kizis, D, V. Lumbreras and S.M. Pag. 2001. Role of AP2/EREBP transcription factors in gene regulation during abiotic stress. *Febs Letters*, 498(2-3): 187-9.

Liu, H.L., Y.Y. Xu and Z.H. Xu. 2007. A rice YABBY gene, OsYABBY4, preferentially expresses in developing vascular tissue. *Develop. Genes & Evol.*, 217(9): 629-37.

Liu, L., M.J. White and T.H. Macrae. 1999. Transcription factors and their genes in higher plants functional domains, evolution and regulation. *Europ. J. Biochem.*, 262(2): 247-57.

Mo, X.T., J. Zhao and Y.L. Fan. 2013. Advances in the structure and function of maize transcription factors. *China Agri. Sci. & Techn. Herald*, 15(3): 7-17.

Navarro, C., N. Efremova and J.F. Golz. 2004. Molecular and genetic interactions between STYLOSA and GRAMINIFOLIA in the control of Antirrhinum vegetative and reproductive development. *Develop.*, 131(15): 3649-59.

Nei, M. 2005. Selectionism and neutralism in molecular evolutio. *Molecul. Biol. & Evolut.*, 22(12): 2318.

Rao, G.G., G.R. Rao. 1981. Pigment composition and chlorophyllase activity in pigeon pea (*Cajanus indicus* Spreng) and Gingelley (*Sesamum indicum* L.) under NaCl salinity. *Ind. J. Exp. Biol*., 19(8): 768-770.

Salin, M.L. 1988. Toxic oxygen species and protective systems of the chloroplast. *Physiol. Plantarum*, 72(3): 681-9.

Sawa, S., K. Watanabe and K. Goto. 1999. FILAMENTOUS FLOWER, a meristem and organ identity gene of Arabidopsis, encodes a protein with a zinc finger and HMG-related domains. *Genes & Develop.*, 13(9): 1079-88.

Schluttenhofer, C. and L. Yuan. 2015. Regulation of specialized metabolism by WRKY transcription factors. *Plant Physiol.*, 167(2): 295-306.

Sieber, P., M. Petrascheck and A. Barberis. 2004. Organ Polarity in Arabidopsis. NOZZLE Physically Interacts with Members of the YABBY Family. *Plant Physiol.*, 135(4): 2172-85.

Siegfried, K.R., Y Eshed and S.F. Baum. 1999. Members of the YABBY gene family specify abaxial cell fate in Arabidopsis. *Develop.*, 126(18): 4117.

Stern, A., A. Doronfaigenboim and E. Erez. 2007. Selection advanced models for detecting positive and purifying selection using a Bayesian inference approach. *Nucl. Acids Res.*, 35(Web Server issue): 506-11.

Tuskan, G. 2006. The genome of black cottonwood, *P. trichocarpa* (Torr. & Gray). *Science*, 313(5793): 1596-604.

Welker, O.A. and S. Furuya. 1995. Influence of heat stress on growth and leaf epicuticular structure of cabbages. *J. Agron. & Crop Sci.*, 174(1): 53-62.

Welker, O.A. and S. Furuya. 2010. Surface structure of leaves in heat tolerant plants. *J. Agron. & Crop Sci.*, 173(3-4): 279-88.

Wu, C.Y. and S.W. Zhong. 2013. Progress in research on rice zinc finger protein gene. *Chinese Rice*, 19(6): 23-28.

Yamada, Y. and F. Sato. 2013. Chapter eight-transcription factors in alkaloid biosynthesis. *Elsevier Sci. & Techny*.

Yamaguchi, T., N. Nagasawa and S. Kawasaki. 2004. The YABBY gene DROOPING LEAF regulates carpel specification and midrib development in *Oryza sativa*. *The Plant Cell*, 16(2): 500-9.

Yang, Z., Q. Gong and L. Wang. 2018. Genome-wide study of YABBY genes in upland cotton and their expression patterns under different stress. *Frontiers in Genetics*, 9(33):

Zhao, J.N., W.J. Yao. and Z. Wang. 2015. Bioinformatics analysis of poplar AP2/ERF transcription factor family. *J. North. Forestry Univ.*, 43(10): 21-9.

Zhao, S.P., D. Lu and T.F. Yu. 2017. Genome-wide analysis of the YABBY family in soybean and functional identification of GmYABBY10 involvement in high salt and drought stresses. *Plant Physiol. & Biochem.*, 119(132):

Zhu, Z., J. Shi and W. Xu. 2013. Three ERF transcription factors from Chinese wild grapevine Vitis pseudoreticulata participate in different biotic and abiotic stress-responsive pathways. *J. Plant Physiol.*, 170(10): 923-33.

(Received for publication 29 September 2021)