Advances in Biological Sciences Research (ABSR), volume 6
2017 2nd International Conference on Biological Sciences and Technology (BST 2017)

Research Advances of AP2/ERF Transcription Factors

Lei WANG[1,2, a,*], Yao SUN[1] and Yao LI[1]

[1]Institute of Advanced Technology, Heilongjiang Academy of Sciences, Harbin, Heilongjiang, PR China

[2]College of Biological Sciences and Technology, Beijing Forestry University, Beijing, PR China

[a]wleileiyu@163.com

*Corresponding author

Keywords: AP2/ERF transcription factor, Stress resistance, Poplar

Abstract. The AP2/ERF transcription factor family is one of the largest transcription factor groups in plants and it plays very important roles in growth, development, biotic and abiotic stress response. In this study, we summarized the research advances on AP2/ERF transcription factor involved in plant growth, development, stress resistance, regulation of secondary metabolites and the current research status in poplar, in order to provide reference for plant-breeding applications.

Introduction

Transcription factor (TF), also known as trans-acting factor, is a kind of protein that can specifically bind to the cis-acting element in the promoter of eukaryotes. It plays a role as a switch in initiating downstream gene expression via interaction between transcription factor and proteins [1]. With the completion of genome sequences in plants and the development of bioinformatics analysis, a variety of transcription factors, which are associated with drought, high salinity, cold, hormone, pathogen response, growth and development, have been isolated from higher plants since Paz-Ares first reported the TF from *Zea mays* in 1987 [2].

The AP2/ERF (APETALA 2/ethylene-responsive element binding factor, AP2/ERF) family is one of the largest TF families in plants. It accounts for nearly 9% of the TFs in plants and contains a 58 or 59 amino acid conserved domain. The AP2/ERF TF is widely involved in regulation of growth, development and various stress responses in plant [3]. According to the characteristics and number of AP2/ERF domain, it can be divided into 3 families: AP2, RAV, and ERF. The AP2 family contains two repeated AP2/ERF domains, and this family plays a very important role in plant developmental processes [4]. The RAV family contains one AP2/ERF domain and a B3 domain, which plays an important role in response to ethylene, brassinolide, biotic and abiotic stress [5]. The ERF family contains one AP2/ERF domain and it can be divided into two subfamilies: CBF/DREB subfamily and ERF subfamily. The differences between the two subfamilies are the 14th and 19th amino acid. The 14th and 19th amino acids of DREB are valine and glutamic acid, while the corresponding amino acids in ERF are alanine and aspartic acid. DREB and ERF transcription factors are mainly involved in plant response to hormone [6] and environmental stresses [7].

In this review, we analyzed the functions of AP2/ERF TFs in plant growth, development, stress resistance, regulation of secondary metabolites in medicinal plant and the current research status in poplar, in order to provide reference for plant-breeding applications.

AP2/ERF in Plant Growth and Development

AP2/ERF TFs were first found from model plant *Arabidopsis thaliana* and proved that AP2/ERF TFs were involved in regulation of flower development [8]. The AP2/ERF TFs played roles in plant growth [9], flower development [10], fruit maturation [11] in further studies. The ethylene-sensitive tree peony (*Paeonia suffruticosa*) cultivar 'Luoyanghong' and ethylene-insensitive cultivar 'Xueying Taohua' were used to analyze the expression profiles of *ERF* genes in different floral tissues (petals, stamens, pistils or sepals) and in petals of different developmental stages by real-time PCR. The results suggested that, comparing to other floral tissues, transcript of *PsERF1* was the most abundant in petals, which increased during development of 'Luoyanghong' cut flowers while decreased during that of 'Xueying Taohua' cut flowers, implying an important role of *PsERF1* in ethylene response of tree peony cut flowers [12]. MaERF11 from *Musa acuminata* binds to promoters of ethylene biosynthetic gene *MaACO1* and three soften-related *MaEXP2/7/8* via electrophoretic mobility shift assay (EMSA) and chromatin immunoprecipitation (ChIP) analyses. DLR (dualluciferase reporter assay) showed that MaERF11 had transcriptional repression activity, and regulated ethylene biosynthesis and fruit soften through suppressing *MaACO1* and *MaEXP2/7/8*. Yeast two-hybrid (Y2H), bimolecular fluorescence complementation (BiFC) and Co-IP (Co-Immunoprecipitation) assays all confirmed the interaction between MaERF11 and MaHDA1. This interaction demonstrated that MaERF11 and MaHDA1 formed a transcription repression complex, co-regulating *MaACO1* and *MaEXP2/7/8* expression, and further regulating banana fruit ripening [13]. *JcDREB2* gene was cloned from *Jatropha curcas.* Transgenic *Oryza sativa*, over-expressing *JcDREB2*, exhibited dwarfed and GA (gibberellin)-deficient phenotypes with shorter shoots and roots than those of wild-type plants, which indicated that *JcDREB2* might influence GA metabolism [14].

AP2/ERF in Plant Stress Resistance

The growth and development of plant are influenced under stresses. The AP2/ERF transcription factors are related to stress response in plant under biotic and abiotic stresses. Up to now, *AP2/ERF* genes have been isolated from wheat [15], *Oryza sativa*, cotton [16], soybean [17], maize, *Arabidopsis thaliana*, *Brassica napus* [18], *Hevea brasiliensis* [19], tobacco, grape [20], poplar, and their functions have been analyzed.

The *AP2/ERF* genes are mainly induced by hormone and environmental factors, including abscisic acid (ABA) and ethylene, which are the key regulators in response to biotic and abiotic stresses. In the ABA induced signal transduction pathways, DREB which belong to the ERF family acts as an up/down regulator in downstream gene expression. In maize, both DBF1 and DBF2 bound to the *rab17* DRE cis-element. *DBF1* was induced by drought, salt stress and ABA, and it functions as an activator of transcription of *rab17* promoter. While *DBF2* was not induced by ABA treatment, its over expression not only resulted in a decrease in *rab17* promoter activity, but also had down-regulated the effect of ABA [21]. Over expression of *AtDREB2A* could enhance the expression level of downstream drought stress-responsive genes as well as drought stress tolerance in Arabidopsis, meanwhile retarding the growth of transgenic plants [22-23]. *OsDREB1A* and *OsDREB1B* in *Oryza sativa* could be induced by cold stress, while *OsDREB2A* was induced by drought and salt stress. Over expression of *OsDREB1A* could enhance transgenic plants tolerance to drought, salt and cold stress [24]. *Tsi1* gene in tobacco was induced by ethylene, NaCl, and SA (salicylic acid), and also induced under water or salt

treatment [25]. Tsi1 not only bound to GCC box, but also weakly bound to DRE cis-element. Besides, over expression of *Tsi1* conferred resistance to salt stress and pathogen attack [26]. *JcERF* isolated from *Jatropha curcas*, was a member of *ERF* subfamily. Expression of the *JcERF* gene was rapidly induced under salinity, drought, ethylene and mechanical wounding treatments. However, no significant changes were observed under ABA treatment. Meanwhile, JcERF protein could specifically bind to the GCC box. Over expression of *JcERF* cDNA in transgenic Arabidopsis could enhance the tolerance to salt and cold stress [27]. AP2/ERF played different roles under different stress conditions. ERF B-3 subgroup gene *OsBIERF3* from *Oryza sativa* was induced by ACC (l-amiocycpropane-1-carboxlate) and JA (jasmine acid). The over expression and RNAi vectors containing *OsBIERF3* were constructed and then transformed into rice. The results showed the death rates of RNAi lines were significantly lower than the over-expression lines under cold stress. Height, root length and fresh weight of the over-expression seedlings were higher than the RNAi plants under high salt condition. These results indicated that OsBIERF3 positively regulated high salinity stress response but negatively regulated cold stress response in *Oryza sativa* [28].

AP2/ERF in Regulation of Secondary Metabolites in Medicinal Plant

The secondary metabolites in medicinal plant are the major active constituents of plant drug. A variety of TFs have been found to be involved in metabolism and synthesis regulation of plant terpenes, flavonoids and alkaloids [29]. Studies on the synthesis and regulation of secondary metabolites in medicinal plants have attracted more attention.

The antiviral function of *Isatis indigotica Fort* is based on lignans compounds. TF Ii049, which was belong to AP2/ERF family and related to lignans synthesis, was obtained by screening transcriptomics and metabolomics data of *Isatis indigotica Fort*. The RNAi expression vector was constructed and analyzed. The results showed a significantly decreased content of lariciresinol after *Ii049* gene was repressed. The results of EMSA and Y1H (yeast one-hybrid) assay further confirmed that Ii049 could regulate expression of lignans synthesis pathway genes by directly binding to the promoter and then influence the synthesis of lignans [30]. TAR1, an AP2 transcription factor, controlling the development of trichomes and the biosynthesis of artemisinin, was isolated from *Artemisia annua*. The content of artemisinin increased when *TAR1* was over-expressed. TAR1 may be able to directly bind to amorpha-4, 11-diene synthase (ADS) and cytochrome P450 monooxygenase (CYP71AV1) in order to positively regulate the biosynthesis of artemisinin [31]. TcDREB, an ERF transcription factor from *Taxus cuspidata*, could bind to the promoters of taxoid 10β-hydroxylase (T10βH), Taxane 13α-hydroxylase (T13αH), taxadiene 5α-hydroxylase (T5αH) and taxadiene synthase gene (TASY) as well as methyl jasmonate (MeJA) responsive element GCC-box of TASY promoter in taxol biosynthesis pathways. These results suggested that TcDREB might be involved in taxol biosynthesis pathways of *Taxus cuspidate* [32-33]. The full-length cDNA of *LeERF-1* was cloned from *Lithospermum erythrorhizon* by rapid amplification of cDNA ends (RACE). Real-time PCR results showed that *LeERF-1* was significantly down-regulated by white, blue and red light, and the inhibitory effect of red light was the weakest compared to other light conditions. These expression patterns were consistent with the content variation of shikonin and its derivatives under different light signal conditions [34].

AP2/ERF in Poplar

As a valuable forest resource and important ecology species, poplar is also the model plant for forest physiology and genetic engineering. With the completion of genome sequencing in poplar, it has provided a genetic basis for fully understanding the molecular mechanism on stress response.

Wide genome analysis of poplar has identified 2576 TFs which can be classified into 64 different families including 200 AP2/ERF domain genes [35]. 13 expressed sequence tags (ESTs) encoding AP2/ERF domain were isolated from poplar cDNA library under drought, salt and cold stress treatment [36]. AP2/ERF gene was isolated from *Populus simonii* × *P. nigra* by cDNA-AFLP [37]. Four *ERF* genes were obtained from *Populus deltoides* × *P. euramericana* and all of them were located in the nucleus by PEG-mediated transformation of poplar protoplast. Four genes were predominantly expressed in leaves, while had lowest expression in the stem [38]. *PtDREB2A* gene was cloned from *Populus tomentosa*. Tissue differential expressions were detected by real-time PCR, and the results indicated that *PtDREB2A* transcripts had their mRNA products in roots, stems, leaves and apical shoot meristems. The *PtDREB2A* mRNA products were the most abundant in leaves, and the expression level was medium in stem bark and roots, but a weak expression was detected in apical shoot meristems, phloem, cambium, and xylem of stem. Meanwhile, the expression of *PtDREB2A* was not only induced by heat-shock, cold, drought and high-salt stress, but also by IAA (indole-3-acetic acid), NAA (1-Naphthylacetic acid), and GA3, but not by ABA. Moreover, there existed abundant SNP (single nucleotide polymorphisms) mutations in *Populus tomentosa* natural population [39]. *JERF* from *Lycopersicon esculentum* was transformed into *Populus alba×P.berolinensis,* which enhanced the salt tolerance of transgenic plants [40]. 200 *AP2/ERF* genes and 75 *DREB* genes were obtained from *Populus trichocarpa* by *in silico* cloning method, which provide gene resources for studying ERF stress tolerance mechanism [41-42]. The temporal and spatial expression pattern of *ERF76* gene was analyzed by RT-qPCR under salt stress condition. The results showed that *ERF76* gene was induced by salt in leaf, stem and root tissues, and the expression level was the highest in root which was followed by leaves and stem. It reached the peak at 12h in all the tissues. The transgenic poplar lines over expressing *ERF76* gene driven by CAMV35S promoter were obtained by *Agrobacterium*-mediated transformation method. Under salt stress condition, the plant height, root length, fresh weight, antioxidase activity, and stress responsive protection molecular content in transgenic poplar were higher than those in wild type plants. The accumulation of plant reactive oxygen species and relative electrical conductivity in transgenic poplar were lower than those in wild type. In addition, the over expression of *ERF76* could improve salt tolerance. RNA-seq results also showed that partial genes in ABA and GA signal transduction pathways might be directly or indirectly regulated by *ERF76* [43-44].

Conclusion

As one of the largest TF family in plants, AP2/ERF plays a crucial role in plant growth, development, biotic and abiotic stress response. However, the number of AP2/ERF family members is so huge that there is the problem of function interleaving and function redundancy between the members which brings challenge for further research on AP2/ERF functions. Up to now, *AP2/ERF* genes have been cloned from a variety of plant species and their functions have been analyzed. It has been confirmed that ERF is in involved in stress response and the over expression of *ERF* gene can

enhance stress tolerance of receptor plants. However, it is still unclear that which protein can interact with ERF, what the characteristics of ERF downstream genes are, how ERF is involved in GA and ABA-mediated signal transduction and cross-talk signal pathways, and especially what the stress tolerance mechanism of ERF is in woody plants. These problems still need further research. With the improvement of molecular biology experimental methods, especially the application of gene editing technique, and the innovation of research approaches, researches on AP2/ERF TFs will be clearer and more distinct.

Acknowledgement

This work was supported by National Natural Science Foundation [grant number 31400576], State Key Laboratory of Tree Genetics and Breeding Foundation of Northeast Forestry University [grant number K2013102].

References

[1] C. Schwechheimer, M. Bevan, The regulation of transcription factor activity in plants, Trends Plant Sci. 3 (1998) 278-283

[2] J. Paz-Ares, D. Ghosal, U. Wienand, P.A. Peterson, H. Saedler, The regulatory cl locus of *Zea mays* encodes a protein with homology to myb proto-oncogene products and with structural similarities to transcriptional activators, EMBO. 6 (1987) 3553-3558

[3] J.Y. Zhang, Q.J. Wang, Z.R. Guo, Progresses on plant AP2/ERF transcription factor, Hereditas. 34 (2012) 835-847

[4] K.D. Jofuku, P.K. Omidyar, Z. Gee, J.K. Okamuro, Control of seed mass and seed yield by the floral homeotic gene APETALA2, Proc. Natl. Acad. Sci. USA. 102 (2005) 3117-3122.

[5] Y.X. Hu, Y.X. Wang, X.F. Liu, J.Y. Li, Arabidopsis RAV1 is down-regulated by brassinosteroid and may act as a negative regulator during plant development, Cell Res. 14 (2004) 8–15.

[6] M.T. Sears, H. Zhang, P.J. Rushton, M. Wu, S. Han, A.J. Spano, M.P. Timko, NtERF32: a non-NIC2 locus AP2/ERF transcription factor required in jasmonate -inducible nicotine biosynthesis in tobacco, Plant Mol. Biol. 84 (2014) 49-66

[7] C.W. Lu, X.Q. Pan, B.Z. Zhu, ERF transcription factors and their function in plant tolerance to enviromental stress, Biotech. Bull. 29 (2012) 22-27.

[8] K.D. Jofuku, B.G. den Boer, M.M.Van, J.K. Okamuro, Control of arabidopsis flower and seed development by the homeotic gene APETALA2. Plant Cell. 6 (1994) 1211-1225

[9] S.Y. Lee, E.Y. Hwang, H.Y. Seok, V.N. Tarte, M.S. Jeong, S.B. Jang, Y.H. Moon, Arabidopsis AtERF71/HRE2 functions as transcriptional activator via cis-acting GCC box or DRE/CRT element and is involved in root development through regulation of root cell expansion, Plant Cell Rep. 34 (2015) 223-231.

[10] E. Huala, I.M. Sussex, LEAFY interacts with floral homeotic genes to regulate Arabidopsis floral development, Plant Cell. 4 (1992) 901-913.

[11] M.Y. Chung, J. Vrebalov, R. Alba, J. Lee, R. McQuinn, J.D. Chung, P. Klein, J. Giovannoni, A tomato (*Solanumly copersicum*) APETALA2/ERF gene, SlAP2a, is a negative regulator of fruit ripening, Plant J. 64 (2010) 936-947.

[12] F. Wu, C. Zhang, J. Guo, A. Liu, L. Dong, Isolation and expression analysis of ERF transcription factor genes in tree peony cut flowers. Acta Hortic. Sinica.43 (2016) 109-120.

[13] Y.C.Han, Histone deacetylases are involved in ERF-mediated transcriptional regulation of banana fruit ripening, South China Agricultural University, Guangzhou, 2016. (In Chinese)

[14] Y. Tang, K. Liu, J. Zhang, X. Li, K. Xu, Y. Zhang, J.Qi, D.Yu, J.Wang, C.Li. JcDREB2, a physic nut AP2/ERF gene, alters plant growth and salinity stress responses in transgenic rice, Front Plant Sci. 8 (2017) 306

[15] L. Zhang, Functional analysis of salt-responsive gene TaERF5 in wheat, Chinese Academy of Agricultural Sciences, Beijing, 2013. (In Chinese)

[16] K. Liu, Gene cloning and characterization of b3 and b1 subgroup transcription factors of erf family from *Gossypium barbadense* L, Chinese Academy of Agricultural Sciences, Beijing, 2011. (In Chinese)

[17] X. Sun, Functional identification and promoter separation of stress resistance related genes, GmDERB3 from soybean (*Glycine max* L.), Xin Jiang Agricultural University, Urumchi, 2007. (In Chinese)

[18] J. Zhuang, A. Xiong, R. Peng, J. Chen, J. Zhang, B. Zhu, F. Gao, X. Fu, Q. Yao, Isolation and function analysis of AP2/ERF transcription factor of *Brassica napus*, Chin. J. Oil Crop Sci. 31(2009) 391-400.

[19] C. Duan, X. Argout, V. Gébelin, M. Summo, J.F. Dufayard, J. Leclercq, Kuswanhadi, P. Piyatrakul, J. Pirrello, M. Rio, A. Champion, P. Montoro, Identification of the *hevea brasiliensis* AP2/ERF superfamily by RNA sequencing, BMC Genomics, 14 (2013) 30-52

[20]Zhuang J, Peng RH, Cheng ZM, J. Zhang, B. Cai, Z. Zhang, F. Gao, B. Zhu, X.Y. Fu, X.F. Jin, J.M. Chen, Y.S.Qiao, A.S. Xiong, Q.H.Yao, Genome-wide analysis of the putative AP2/ERF family genes in *Vitis vinifera*, Sci. Hortic. 123 (2009) 73-81

[21] D. Kizis, M. Pages, Maize DRE-binding proteins DBF1 and DBF2 are involved in rab17 regulation through the drought responsive element in an ABA-dependent pathway, Plant J. 30 (2002) 679-689.

[22] Y. Sakuma, K. Maruyama, Y. Osakabe, F. Qin, M. Seki, K. Shinozaki, K. Yamaguchi-Shinozaki, Functional analysis of an Arabidopsis transcription factor, DREB2A, involved in drought-responsive gene expression, Plant Cell. 18 (2006) 1292-1309

[23] Y. Sakuma, K. Maruyama, F. Qin, Y. Osakabe, K. Shinozaki, K. Yamaguchi-Shinozaki,Colloquium paper: dual function of an Arabidopsis transcription factor DREB2A in water-stress-responsive and heat-stress responsive gene expression, Proc Natl Acad Sci USA. 103(2006b) 18822-18827

[24] J.G. Dubouzet, Y. Sakuma, Y. Ito, M. Kasuga, E.G. Dubouzet, S. Miura, M. Seki, K. Shinozaki, K. Yamaguchi-Shinozaki, OsDREB genes in rice, *Oryza sativa*

L., encode transcription activators that function in drought, high salt and cold-responsive gene expression, Plant J, 33 (2003) 751-763

[25] J.M. Park, C.J. Park, S.B. Lee, B.K. Ham, R.Shin, K.H. Paek, Over expression of the tobacco Tsi1 gene encoding an EREBP/AP2-type transcription factor enhances resistance against pathogen attack and osmotic stress in tobacco, Plant Cell. 13 (2001) 1035-1046

[26] B.K. Ham, J.M. Park, S.B Lee., M. J. Kim, I.J. Lee, K.J. Kim, C.S. Kwon, K.H. Paek, Tobacco Tsip1, a DnaJ-type Zn finger protein, is recruited to and potentiates Tsi1-mediated transcriptional activation, Plant Cell.18 (2006) 2005-2020

[27] M. Tang, J. Sun, Y. Liu, F. Chen, S. Shen, Isolation and functional characterization of the JcERF gene, a putative AP2 /EREBP domain- containing transcription factor, in the woody oil plant *Jatropha curcas*, Plant Mol Biol. 63 (2006) 419-428

[28] J.B. Mo, Functional analysis of rice ERF transcription factor B-3 subgroup genes, Zhejiang University, Hangzhou, 2012. (In Chinese)

[29] S.R. Wu, K. Gao, X. Liu, C. Sui, Research progress on AP2/ERF transcription factors regulating biosynthesis of active ingredients in medicinal plants, Chin. Tradit. Herb. Drugs. 47 (2016) 1605-1612

[30] R. Ma, L. Zhang, W. Chen, Synthesis of Isatis lignan compounds regulated by AP2/ERF family transcription factors, Shanghai 10th Youth Academic Conference on plant biology, Shanghai, 2016. (In Chinese)

[31] Tan H, Xiao L, Gao S, Q. Li, J. Chen, Y. Xiao, Q. Ji, R. Chen, W. Chen, L. Zhang, Trichome and artemisinin regulator 1 is required for trichome development and artemisinin biosynthesis in *Artemisia annua* L., Mol Plant. 8 (2015) 1396-1411.

[32] Y. Dai, Q. Qin, D. Dai, L. Kong, W. Li, X. Zha, Y. Jin, K. Tang, Isolation and characterization of a novel cDNA encoding methyl jasmonate-responsive transcription factor TcAP2 from *Taxus cuspidate*, Biotechnol Lett. 31(2009) 1801-1809.

[33] Y.L. Dai, Molecular Cloning and Characterization of AP2-Type Transcription Factors Involved in Isoprenoid Biosynthetic Pathway of *Taxus cuspidata*, Fudan university, Shanghai, 2008. (In Chinese)

[34] W. Zhang, A. Zou, J. Miao, Y. Yin, R. Tian, Y. Pang, R. Yang, J. Qi, Y. Yang, *LeERF-1*, a novel AP2/ERF family gene within the B3 subcluster, is down-regulated by light signals in *Lithospermum erythrorhizon*, Plant Biology. 13 (2011) 343-348

[35] Q.H. Zhu, A.Y. Guo, G. Gao, Y.F. Zhong, M. Xu, M. Huang,J. Luo. DPTF: a database of poplar transcription factors, Bioinformatics. 23 (2007) 1307-1308

[36] T. Nanjo, N. Futamura, M. Nishiguchi, T. Igasaki, K. Shinozaki, K. Shinohara, Characterization of full-length enriched expressed sequence tags of stress treated poplar leaves, Plant Cell Physiol. 45 (2004) 1738-1748.

[37] L. Wang, B.R. Zhou, L.L. Wu, B. Guo, T. Jiang, Differentially expressed genes in *Populus simonii×Populus nigra* in response to NaCl stress using cDNA-AFLP, Plant Sci. 180 (2011) 796-801.

[38] J.L. Song, Cloning and expression pattern analysis of poplar ERF family genes, Nanjing Forestry University, Nanjing, 2013. (In Chinese)

[39] Q. Guo, B. Wang, B. Wang, B. Li, D. Zhang, Isolation, Expression and Single Nucleotide Polymorphisms Analysis of PtDREB2A in *Populus tomentosa*, Scientia Silvae Sinicae. 47 (2011) 49-56.

[40] Y. Li, X. Su, B. Zhang, Q. Huang, X. Zhang, R. Huang. Identification of salt tolerance in transgenic *JERFs Populus alba× P. berolinensis*. 9th Youth Academic Conference on Forestry, Chengdu, 2010. (In Chinese)

[41] J. Zhuang, B. Cai, R.H. Peng, B. Zhu, X.F. Jin, Y. Xue, F. Gao, X.Y. Fu, Y.S. Tian, W. Zhao, Y.S. Qiao, Z. Zhang, A.S. Xiong, Q.H. Yao, Genome-wide analysis of the AP2/ERF gene family in *Populus trichocarpa*, BBRC. 371 (2008) 468-474

[42] Y. Chen, J. Yang, Z. Wang, H. Zhang, X. Mao, C. Li. Gene structures, classification, and expression models of the DREB transcription factor subfamily in *Populus trichocarpa*, Sci. World J. 5 (2013) 954640

[43] W. Yao, X. Zhang, B. Zhou, K. Zhao, R. Li, T. Jiang, Expression pattern of ERF gene family under multiple abiotic stresses in *Populus simonii×P.nigra*, Front. Plant Sci. 8 (2017) 181

[44] W. Yao, S. Wang, B. Zhou, T. Jiang, Transgenic poplar over expressing the endogenous transcription factor *ERF76* gene improves salinity tolerance, Tree Physiol. 36 (2016)1-13