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ORIGINAL ARTICLE

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Expression of Resistance Genes in Breeding Valuable Genotypes of Pine and Fast-growing Species of Birch and Poplar under Drought

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Rationale. This article presents a study of the stress response to the effects of water deficiency in various genotypes of Scots pine, contrasting in seed yield, breeding genotypes of silver and downy birch, hybrids of black, white poplar and intersectional poplar hybrids.

Results. A contrasting response of genotypes to the effects of drought was revealed, including activation of the *pP5CS*, *pICDH*, *pNAC034*, *pDREB2*, *pCA* genes in poplars 'E.s.-38', 'POK', and 'Veduga', while in black poplar the expression of all analyzed genes was reduced. In silver birch 'Ug.-1', all analyzed genes *bPAL*, *bCA*, *bLEA8*, *bNAC19 и bDREB2* were upregulated, while in downy birch '15-1' *bPAL* and *bNAC19* were downregulated and for the rest the changing in expression was not shown. An increase in the expression of the *SAMS* gene was detected in response to drought in pine genotypes of different seed production, medium-yielding No. 4 and low-yielding No. 23. For the low-yielding genotype No. 22, no changes in the expression of the analyzed genes were recorded under drought conditions.

Conclusion. The results obtained indicate the development of a stress response and show differences in the adaptive response in different genotypes of pine, poplar and birch, which indicates the value of the tested genes in selection for drought conditions in different tree species.

Key words: drought, breeding genotypes, gene expression

In the context of global climate change, long-lived plants such as perennial tree species are facing adverse conditions. Large-scale forest mortality will have serious impacts on water and carbon cycles, species distribution and economy (Andriantelomanana *et al.*, 2024). Drought is one of the most detrimental stresses that suppress plant growth and productivity. Among the species threatened by population decline are fast-growing species such as poplar and birch, which are the main tree species in the broad-leaved forests of the middle zone of Central Russia and are widely distributed and used worldwide. Their ecological role and economic importance cannot be overstated, especially due to the possibility of their use in production for long-term carbon sequestration (Di Stefano *et al.*, 2024). Scots pine, one of the most widespread tree species worldwide, is also experiencing massive mortality in a changing climate, the rate of which has only increased over the past two years (Bose *et al.*, 2024).

Plants have developed multiple mechanisms to respond to drought stress, including physiological-biochemical and molecular-genetic, complex signaling pathways involving hormones, receptors, protein kinase cascades, transcription factors, and stress-related protein regulators (Wang *et al.*, 2017; Yao *et al.*, 2021). Transcriptomic analysis using microarray technology has identified many genes that are induced by abiotic stress, and these genes have been classified into two main groups. One group encodes products that directly protect plant cells from stress, i.e., functional compounds.

These products are likely involved in the metabolic response to stress, protecting cells from stressful influences by removing toxic elements, restoring cellular homeostasis, and possibly restoring normal growth patterns. These include water channel proteins, proline, detoxification enzymes, antifreeze proteins, and late embryogenesis abundant (LEA) proteins (Shinozaki *&* Yamaguchi-Shinozaki, 2007; Jan *et al.* 2013; Rini, 2019).

Products of another group regulate gene expression and signal transduction in response to abiotic stress. Previous studies, including genomic ones, have shown that various transcriptional regulatory systems are involved in the induction of stress-responsive genes. Several groups of *cis*- and *trans*-factors are known to be involved in transcription, which is a response to stress. Some of them are controlled by abscisic acid (ABA), while others are not, indicating the involvement of both ABA-dependent and ABA-independent regulatory systems in the regulation of stress-responsive gene expression. The same group of genes can be induced by different types of stress, such as drought and cold, indicating the existence of cross-pathways between signaling mechanisms (Shinozaki, 2003).

Transcription factors (TFs) are large families of DNA-binding proteins that regulate gene expression during ontogenesis, differentiation, and response to various environmental signals (Yanagisawa, 1998). They include families unique to plants, such as *AP2/ERF*, *bZIP*, *WRKY*, *MYB*, and *NAC* (Martin *&* Paz-Ares, 1997). One of the most important families of TFs regulating plant response to stress is APETALA2/ethylene response factors (AP2/ERF). The genes of this family are divided into subfamilies that perform different roles in ontogenesis and protection from adverse environmental conditions. TFs control the expression of key genes of secondary metabolism under stress by binding to regulatory regions in the promoters of target genes. *DREB* subfamily of transcription factors, specifically binding to *cis*-acting dehydration-responsive element/C-repeat (*DRE/CRT*), is involved in the response to cold and drought (Sakuma, 2006).

The *ERF* subfamily is involved in the response to abiotic and biotic stress, growth and development, primary and secondary metabolism, and hormonal signaling (Wang *et al.*, 2017). To date, sufficient information has been accumulated on the involvement of transcription factors in the mechanisms of plant response to various abiotic stresses. Overexpression of *AP2/ERF* family of TFs, *EFR96* (Wang *et al.*, 2017) and *HcTOE3* (Yin *et al.*, 2021) in *Arabidopsis* contributed to an increase in the expression of Δ-pyrroline-5-carboxylate synthetase (*P5CS*) and proline content, and greater resistance to drought and cold stress. It has been shown that the *AP2/ERF* family can also control lignan/lignin biosynthesis in *Isatis indigotica* through the

activation of the salicylic acid signaling pathway and the transcriptional regulation of key genes for lignin biosynthesis (Ma *et al.*, 2017), a biopolymer presented in the secondary walls of tracheal elements and wood fibers and playing an important role in both growth and development and in plant responses to various biotic and abiotic stresses (Cesarino, 2019).

The interest in studying gene transcription and its regulation under the influence of abiotic stress in woody plants lies in the need to identify specific resistance factors and select markers for molecular genetic selection of promising genotypes. In this work, the expression of genes associated with drought resistance was analyzed in birch, poplar and pine plants of breeding value.

MATERIAL AND METHODS

Characteristics of research objects

To assess the drought resistance of plants, we used the breeding genotype of downy birch (*Betula pubescens* Ehrh.) ‘15-1’ and the variety of silver birch (*B. pendula* Roth.) ‘Uglyancheskaya-1’ (‘Ug.-1’), poplars of different hybrids and varieties (*Populus* L.), belonging to different sections, as well as Scots pine (*Pinus sylvestris* L.), differing in seed yield (Table 1).

The source material for growing seedlings of silver birch of the ‘Ug.-1’ variety was prepared in the archive of clones of plus trees, established in the territory of the Tellermanovsky forestry enterprise of the Voronezh region. The source material of downy birch and poplar was taken from test crops of the Semiluksky nursery of the Voronezh region. The pine material was selected from test crops of Scots pine, bred for their productivity, established in forest seed plantation No. 68 of the Semiluk forest nursery by Efimov Yu.P.

Water Deficiency Experiment Conditions

To simulate drought conditions, the irrigation moisture capacity of birch and poplar samples was reduced to 35±5%, while in the control to 80±5%. Experimental needle samples were collected during the dry period in June 2023, and control samples were collected after the restoration of normal precipitation. The samples were fixed by instant freezing in liquid nitrogen and stored at -80°C until further analysis.

Determination of expression of resistance genes

RNA was isolated using a standard set of NucleoSpin® RNA columns (Macherey-Nagel, Germany) from poplar samples. Modified CTAB method was used to extract RNA from birch and pine samples (Grodetskaya *et al.*, 2021).

RNA concentration was determined on a Qubit 2.0 fluorimeter (Thermo Fisher Scientific, USA) using the manufacturer's standard reagents. RNA in an amount of 0.5 μg was selected for reverse transcription using the AmpliSens kit (Central Research Institute of Epidemiology of Rospotrebnadzor, Russia) in accordance with the manufacturer's recommendations.

The search for PCR primers was carried out based on literature data and using the NCBI (National Center for Biotechnology Information) nucleotide sequence database. For genes whose sequences were not available in the database for the desired object, the most conservative fragments identical in organisms from different families were searched for using the ClustalOmega program (European Bioinformatics Institute EMBL, UK). The selection of primers for poplar, birch and pine resistance genes from the NCBI database and sequence fragments selected based on the analysis through ClustalOmega was performed using the Primer3 program (Estonia). The list of primers for the analysis of drought resistance gene expression is presented in Table 2.

The specificity of primer amplification at the corresponding temperature was assessed by electrophoresis in 1% agarose gel. Expression was assessed using a Roche Light Cycler 480 II amplifier (Roche, Switzerland). A standard 5x qPCR–HS SYBR kit (Eurogen, Russia) containing SYBR Green I as an intercalating dye was used for quantitative PCR. The *18S* genes for poplar and *GAPDH* for birch and pine were used as reference genes. The results were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak *&* Schmittgen, 2001) using the Light Cycler software (Roche, Switzerland) and the MS Excel software package.

Statistical analysis was performed using the standard error of the mean (SEM) and Student's t-test in Excel 2010. Differences were considered statistically significant at $p<0.05$.

RESULTS

To analyze the adaptive capacity of promising birch genotypes to the effects of drought, a study was conducted on the expression of the resistance genes *bDREB2*, *bLEA8*, *bPAL*, *bNAC19* and *bCA*. It was found that the silver birch 'Ug.- 1' developed an adaptive response to drought for all analyzed genes. The expression of the genes of downy birch '15-1' did not increase in response to stress, the level of transcripts corresponded to the control (*bLEA8*) or was reduced (*bPAL*, *bCA*, *bNAC19* and *bDREB2*) (Figure 1)

It was revealed that the expression of the analyzed genes differed in poplar genotypes in response to drought (Figure 2). The expression of the *pP5CS* gene increased under drought conditions in poplar 'POK', 'E.s.-38' and 'Veduga' by 1.5-58.3 times. An increase of 1.5-8.4 times was also revealed for the *pCA* gene. In black poplar, the transcript level increased only for *pCA*, while the expression of the *pICDH*, *pDREB2*, *pNAC034* and *pP5CS* genes was decreased relative to the control. Expression of all analyzed resistance genes in poplar 'E.s.-38' significantly increased relative to the samples under normal conditions. For the 'POK' genotype, an increase in expression was shown for *pICDH* and *pP5CS*, while the expression of *pNAC034* decreased relative to the control. Expression of the *NAC034* transcription factor genes increased in 'Veduga' and 'E.s.-38', while *DREB2* responded positively to drought only in 'E.s.-38'. For the 'Veduga' genotype, an increase in the expression of *pP5CS* and *pCA* was also shown.

The effect of stress in Scots pine genotypes differing in yield was studied by changes in the expression of the *psPAL, psSAMS, psTAT-D* and *psDREB2* genes (Figure 3).

A 3.3-fold increase in *psSAMS gene* expression was found in the medium-yielding genotype No. 4, while *psDREB2* expression decreased 3.1 times. For the low-yielding genotype No. 23, an increase in *psSAMS, psTAT– D,* and *psDREB2* gene expression was observed by 2.1-2.8 times, while *psPAL* expression was reduced. For the low-yielding genotype No. 22, an increase in *pTAT-D* expression was detected, while the level of *psPAL, psSAMS,* and *psDREB2* transcripts did not change relative to the control.

Table 1. Characteristics of genotypes of promising fast-growing woody plants

N	Name of genotype	Invariant number	Origin	Author (Breeder)
Birch				
4	*'15-1'*	20– 17	Mother tree 20–17, hybrid (B–2 × *B. pubescens*, pollen mixture)	Isakov I.Yu.
5	*'Ug.-1'**	–	*B. pendula* Roth.	Kozmin A.V. and others
White poplars with a pyramidal crown shape				
1	*'Veduga'*	26– 07	*P. alba* L. × *P. bolleana* Laurche	Tsarev A.P.
Intersectional hybrids				
2	*'Э.с.-38'**	49	*P. deltoides* Marsh. × *P. balsamifera* L.	Veresin M.M.
Black poplars with a pyramidal crown shape				
3	*'POK' **	91	*P. piramidalis* Ros. × *P. nigra* L.	Albensky A.V.
Black poplars with a spreading crown shape				
4	*Black poplar ('osokor')*		*P. nigra* L.	species
Pine				
5	Medium-yielding No.4	4	*P. sylvestris* L.	Efimov Yu.P.
6	Low-yielding №22	22		
7	Low-yielding №23	23		

* 'Ug.-1' – 'Uglyancheskaya-1'; 'E.s.-38' – 'Elite seedling-38'; 'POK' – 'Pyramidal-osokoreviy Kamyshinsky'

Table 2. Primer sequences for stress resistance genes

Gene	Sequence (5′→3′)
	Birch (Pääkkönen *et al.*, 1998)
bDREB2	F: AGGCAGAGAACATGGGGAAA
	R: GAAAGTTGAGGCGAGCGTAA
bLEA8	F: AATGACTTTGACATGGGCGT
	R: TATCCCAAACTGCAGAGCCA
bPAL	F: CTGTGGCTGCAACGGTTT
	R: TCAATTTGAGGTCCGAGCCA
bNAC19	F: CAAGCTCAGGGTCAGACTCA
	R: ACACGTTCGAGTTCTGCCTA
bCA	F: ACATAGTCGTTGTGGTGGGA
	R: TGACAAGTTCACTGCCTCCT
bGAPDH	F: CAGCCGAAGATGTCAATGCA
	R: GGCCACTTGTTTGCTACCAA
	Poplar (Chen *et al.*,2009; Lan *et al.*, 2013; Wu *et al.*, 2015)
pICDH	F: ACTCGGCATTACAGGGTTCA
	R: GACTCCACAGCTCCGATACA
pP5CS	F: TCTATGGCCTGCACTGTTGA
	R: TGCTTATTCCGACCTCTGCA
pDREB2	F: TGTATGCTCGTATGCTCGT
	R: TCCTCATACACGCAGACCTC
pNAC034	F: GTGTATTTCGACACGTCAGATTCT
	R: ATACATGAACATGTCCTGAAGCG
pCA	F: TGCGAGAAGGAGGCTGTTAA
	R: GCCGGCTACAGTTTCACATT
p18S	F: GGCTCTGCCCGTTGCTCT
	R: CGTCACCCGTCACCACCA
	Pine (Sun *et al.*, 2022)
psPAL	F: GAG GGA ATT TCC AGG GCA CA
	R: GAT CTC GGC CCC TTT CAG TC
psSAMS	F: ACT GCA AAG TGC TGG TT
	R: ATG GGT CAG TGG CAT AAG
psTAT– D	F: TGG ATG TTC CTT AAA GAC AGT GG
	R: TCT CAC AGT ATG GAG CGT CTG
PmAP2/ERF11	F: GGT TCC TGT GCT CGT CTC AAT CTG
	R: GGT ACT AGC GGC TGA GGC ATT AAA G
psGAPDH	F: GGA CAG TGG AAG CAT CAT
	R: AAC CGA ATA CAG CAA CAG A

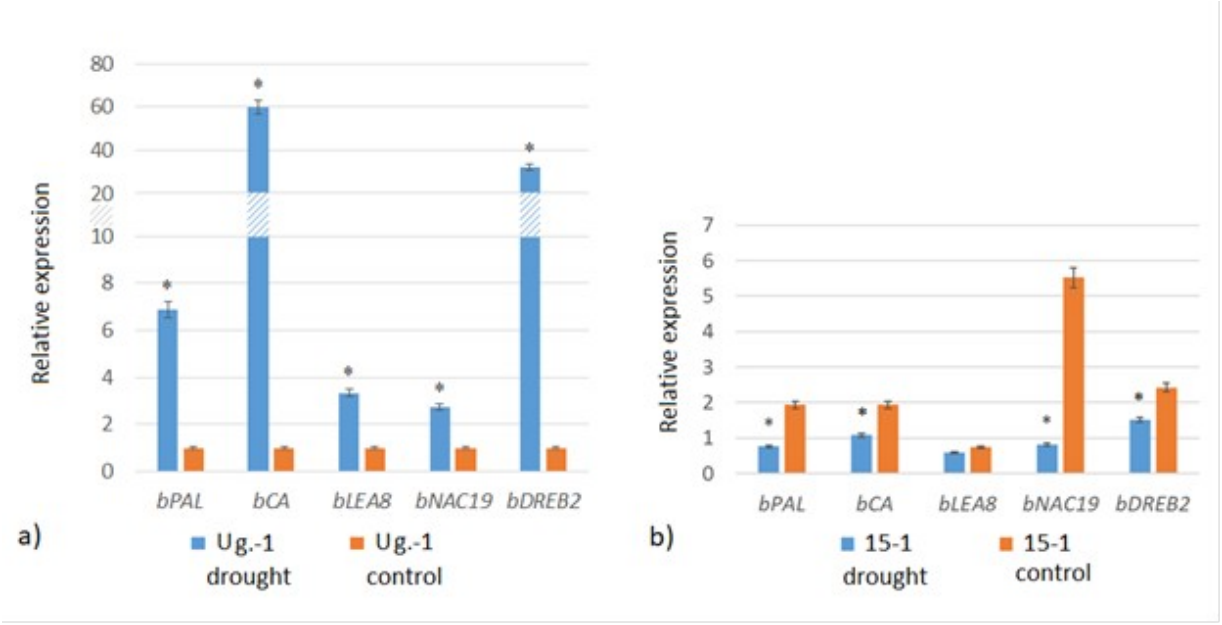

Figure 1. Expression of drought resistance genes in birch: a) 'Ug.-1' and b) '15– 1'. * significant change in expression is indicated

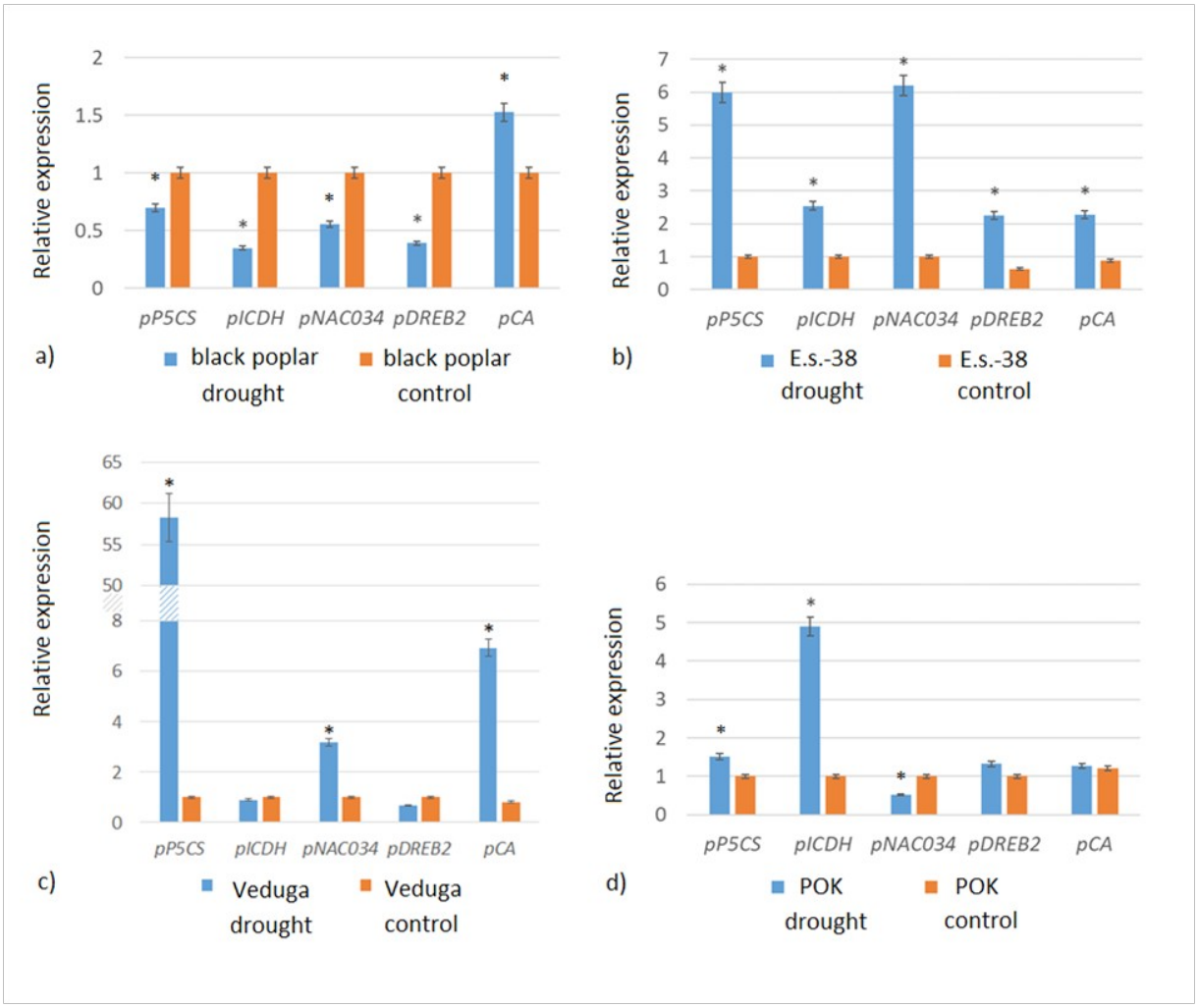

Figure 2. Expression of drought resistance genes in different poplar genotypes: a) black poplar, b) 'E.s.-38', c) 'POK', d) 'Veduga'. * significant change in expression is noted

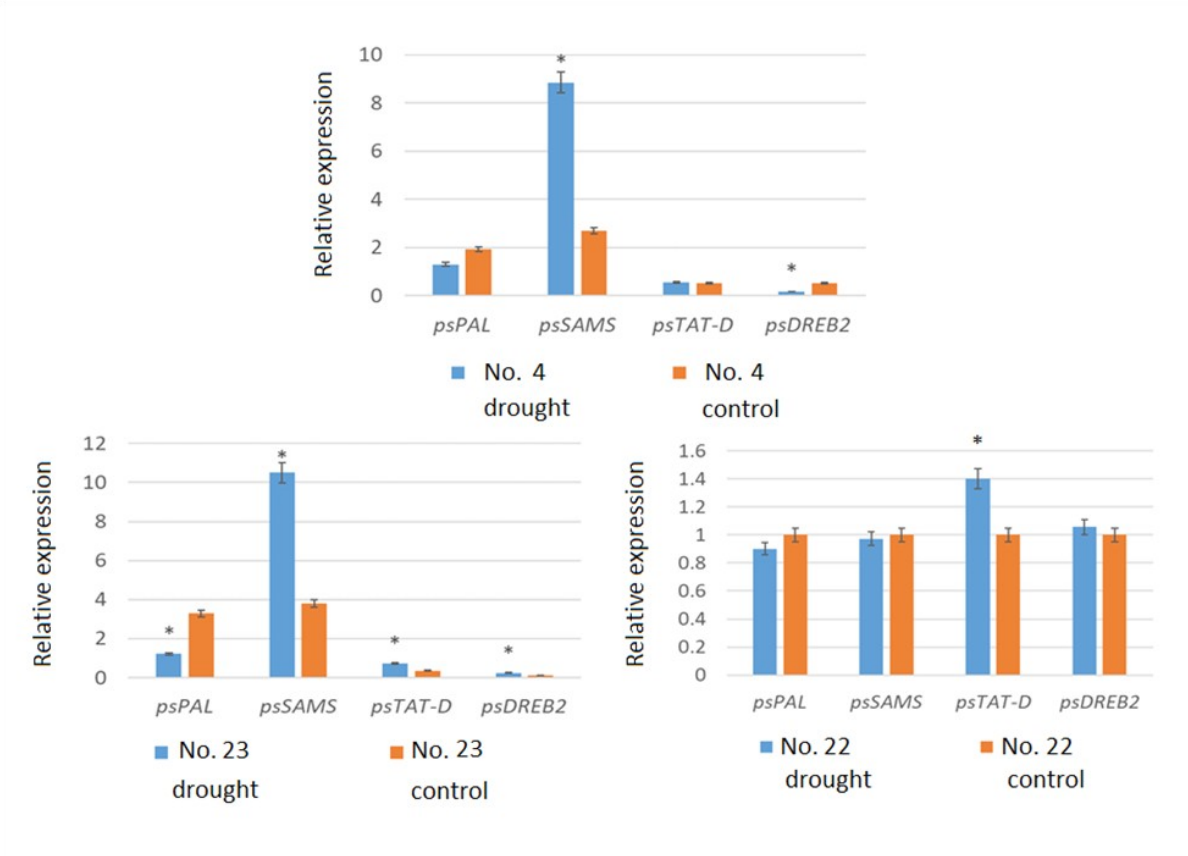

Figure 3. Expression of drought resistance genes in pine genotypes with contrasting seed productivity

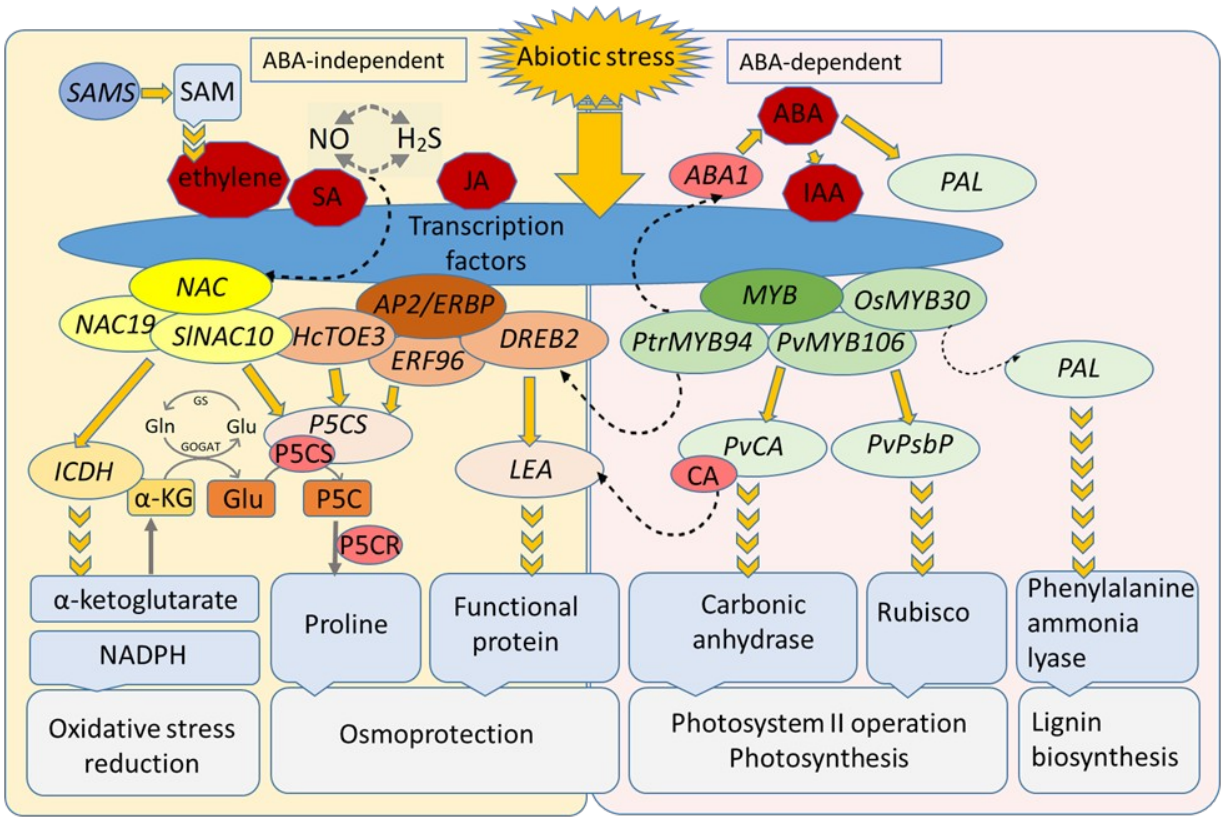

Figure 4. Plant key genes of secondary metabolism transcription regulation under abiotic stress (author's own composition).

DISCUSSION

The mechanism of adaptation to abiotic stress, such as drought, through the expression of transcription factors and the genes they control in plants can be represented as a diagram (Figure 4). Adaptation to stress involves abscisic acid (ABA)-dependent TF families, such as the ABA-responsive element/ABRE-binding factors (*ABRE/ABF*), *WRKY*, Nuclear Factor Y families, and ABA-independent *AP2/ERF* and *NAC* (*NAM*, *ATAF1/2*, and *CUC2*) families (Yao *et al.*, 2021). Other authors attribute TFs of the *AP2/ERBP* subfamily (*AP2/ERF*) and *NAC* to be regulated by ABA, as well as cytokinins and jasmonate (JA) (Liu *&* Zhang, 2017; Khan *et al*., 2019). One of the most important *AP2/ERBP* subfamilies is the *DREB* superfamily, whose members are involved in the mechanisms of response to drought, salinity, and low-temperature stress, with *DREB2* predominantly associated with drought and salinity. The expression of *DREB* and, in particular, *DREB2* genes was increased under drought stress in *Broussonetia papyrifera* (Sun *et al.*, 2014) and *Eucalyptus* sp., (Nguyen *et al.*, 2017), *Malus* sp. (Zhao *et al.*, 2012), drought-sensitive and drought-tolerant coffee genotypes (Torres *et al.*, 2019). Overexpression of *DREB2* promoted drought tolerance in *Robinia pseudoacacia* (Xiu *et al.*, 2016).

In almond, drought-, cold-, and light-related stress-responsive elements and hormone-responsive *cis*-regulatory elements have been identified within the promoter regions of the *PnaDREB* family of proteins (Qian *et al.*, 2023). The Arabidopsis *DREB2A* orthologs in euphrates poplar have been shown to be involved in drought response (Yao *et al.*, 2021). In *Pinus massoniana* Lamb., nine *PmAP2/ERF* genes were constitutively expressed upon drought stress, and other genes showed up- or down-regulation in response to drought stress, which also depended on the tissue studied (Sun *et al.*, 2022). *NAC* transcription factors are one of the largest families of transcription factors in plants and play an important role in their development and response to adverse factors. The expression of *SlNAC10* and *NAC19* genes was induced by drought, salinity and ABA (Du *et al.*, 2022; Khan *et al*., 2019). *NAC19* expression showed an almost 150-fold increase upon exposure to S-nitrosocysteine, a nitric oxide (NO) donor that plays a signaling role in stress (Khan *et al.*, 2019). In the present study, it was revealed that *NAC* and/or *DREB2* transcription factors are involved in drought adaptation of birch genotypes ‘U.g.-1’, poplar ‘E.s.-38’ and ‘Veduga’ and pine No. 23.

Transcription factors are important regulators of gene expression in ontogenesis and stress. In *B. platyphylla*, *BpERF2* of the *AP2/ERF* superfamily, which also includes *DREB2*, has been shown to regulate multiple LEA genes including *LEA8* (Tikhomirova *et al.*, 2022). It has been postulated that *SlNAC10* may play an important role in regulating genes of proline synthesis-related enzymes by binding to the promoters of *AtP5CS1*, *AtP5CS2*, and *AtP5CR* and regulating the transcription of these genes (Du *et al.*, 2022). *P5CS* expression was also regulated by *DREB* TF in rice (Xiong *et al.*, 2020). *P5CS* is a vital gene involved in proline biosynthesis from glutamine, which is required for ROS reduction in modulating ABA and drought stress responses. This gene encodes pyrroline-5-carboxylate synthase and plays a role in membrane and protein stabilization under osmotic stress. Its expression increased under drought stress in pine (Du *et al.*, 2018). The study showed that drought adaptation in poplars ‘E.s.-38’, ‘Veduga’ and ‘POK’ can occur through proline biosynthesis, and *pP5CS* is an important regulator of drought response in these genotypes. The substrate for *P5CS* is glutamate, synthesized from α-ketoglutarate via the GS-GOGAT pathway. Cytosolic $NADP^+$-isocitrate dehydrogenase (ICDH) is a Krebs cycle enzyme that catalyzes the reversible oxidative decarboxylation of isocitric acid to produce α-ketoglutarate (2-oxoglutarate, 2-OG), CO_2, and the reduced form of NADPH. Under abiotic stress, ICDH supplies NADPH, an important reducing element in oxidative stress (Corpas *&* Palma, 2023), and 2-OG to mediate nitrogen assimilation via the GS–GOGAT cycle, as demonstrated by a study of cytosolic ICDH from *Guzmania monostachia*, which transcript levels were upregulated under drought stress (Gonçalves *&* Mercier, 2021). The study revealed an increase in the expression of cytosolic *pICDH* in ‘E.s.-38’

and 'POK', which may be associated with the need to ensure the functioning of the tricarboxylic acid cycle and amino acid synthesis, as well as the supply of NADPH and 2-oxoglutarate to the GS-GOGAT cycle to maintain nitrogen metabolism and proline synthesis.

Phenylalanine ammonia lyase (PAL) is the first determined enzyme of the phenylpropane metabolic pathway, involved in phenolic metabolism and playing a crucial role in plant growth and development (Zhang *et al.*, 2022; Feduraev *et al.*, 2020). PAL encodes the first enzyme of the lignin synthesis pathway, an extremely important indicator of abiotic stress, including salinity, waterlogging, drought, temperature fluctuations and heavy metal exposure (Han *et al.*, 2022). High levels of *PAL* gene expression were found in the roots of drought-tolerant wheat genotypes compared to drought-sensitive genotypes (Rasool *et al.*, 2021). Increased *PAL* expression was observed under drought stress in peony (Li *et al.*, 2020), poplar hybrid *P. tremula* × *P. alba* (Ahmed *et al.*, 2021). In *Populus cathayana*, an increase in the expression of the *PAL* isoform was detected during an experiment on the effect of drought, while the other isoform was activated only in plants from a drought-resistant population, but not in poplar growing in a well-watered population (Xiao *et al.*, 2009). In hibiscus, the effect of drought suppressed the expression of the *PAL* gene, while the effect of salinity and abscisic acid provoked its activation (Jeong *et al.*, 2012). The conducted study shows that *bPAL* is involved in the response to drought in silver birch. In pine, *psPAL* was reduced in the low-yielding genotype No. 23 or did not differ significantly from the control in genotypes No. 4 and No. 22 under natural drought conditions, indicating the absence of a significant role of the PAL isoform in question in regulating the drought stress response in Scots pine. Plants overcome osmotic stress caused by drought through the synthesis of a wide range of metabolites and functional proteins - dehydrins and late embryogenesis proteins (LEA) (Yao *et al.*, 2021). It was revealed that the expression of the *bLEA8* gene increased significantly in silver birch in response to drought, while in downy birch it did not differ from the level of the control variant, which indicates the participation of *LEA8* in the development of the stress response in birch, on the one hand, and the presence of genotypic differences in the activity of representatives of the late embryogenesis protein family in the two birch species under drought conditions, on the other.

Carbonic anhydrase (CA) is a common protein in most photosynthetic organisms. In plants, CA isoforms belong to three different families (α, β, and γ) and mainly catalyze the reversible conversion of CO_2 to HCO^{3-}. Ensuring the equilibrium of CA in this reaction is necessary to maintain the CO_2 concentration for the function of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). CA reduces the resistance to CO_2 diffusion inside mesophyll cells by facilitating the transport of CO_2 in both gas and liquid phases. Furthermore, physical and/or biochemical interactions between CA and other membrane-bound compartments, such as aquaporins, are proposed to trigger the CO_2 sensing response through stomatal movement (Momayyezi *et al.*, 2020). According to current understanding (Çevik *et al.*, 2023], mild to moderate drought stress stimulates carbonic anhydrase activity, and the main isoform involved in the stress response is β–CA. Severe drought causes a decrease in CA activity, which may be due to low intracellular CO_2 levels (Momayyezi *et al.*, 2020). Activation of Rubisco and carbonic anhydrase, as well as other proteins associated with carbon and nitrogen metabolism, could contribute to the drought tolerance of cassava by increasing the efficiency of carbohydrate conversion and protecting the plant from oxidative stress (Chang *et al*., 2019). CA activity generally increases under mild to moderate stress severity, indicating that CA is likely to be involved in adaptation to drought, high salinity, heat, bright light, free carbon deficiency, and bicarbonate excess (Polishchuk, 2021). In the study, *bCA* expression increased almost 60 times relative to the control in silver birch, while in downy birch, *bCA* expression tended to decrease, which may be due to their genotypic differences. (Kotrade *et al.*, 2019) showed that *β–CA* expression differed in two oak species, indicating their species-specific characteristics.

S-adenosylmethionine (SAM) is widely involved in plant stress response and growth regulation, ethylene and polyamine biosynthesis, transmethylation and

transsulfuration. This coenzyme is encoded by a group of genes, some of which showed an increase in expression in response to drought stress, which was more significant in drought-tolerant cotton than in drought-sensitive cotton (Sun *et al.*, 2022). An increase in *SAMS* gene expression was also observed in tomato in response to drought and flooding (Heidari *et al.*, 2020), and in cotton under drought and salinity conditions (Kilwake et al., 2023). Under drought conditions, the expression of lignin biosynthesis-related genes such as *SAMS* can change significantly, suggesting that lignin content is an effective indicator for assessing drought tolerance (Han et al., 2022). The study found that the *psSAMS* gene may be an important indicator of drought stress in Scots pine, which adapts to drought conditions, among other pathways, through lignin synthesis.

The Tat– D nuclease gene (*TAT– D*) is associated with programmed cell death (apoptosis), which is induced by exposure to unfavorable factors, such as oxidative, salt stress and UV irradiation (Qiu *et al*., 2005). The effect of osmotic stress on the expression of the *TAT– D* gene was studied in embryogenic cell cultures of Scots pine. It was shown that the gene expression level did not change throughout the experiment (Muilu-Mäkelä *et al.*, 2015). The study revealed that the *TAT– D* gene was significantly activated in pine as a result of controlled drought exposure under greenhouse conditions, as well as in the low-yielding genotype No. 23, while the change in expression in genotype No. 22 was insignificant, and in the medium-yielding genotype No. 4 this indicator remained at the control level. The obtained results may indicate a negative impact of drought on sensitive pine genotypes, which was expressed in an increase in the expression of *TAT–D*, which is involved in the development of the programmed cell death mechanism.

CONCLUSION

Thus, the key mechanisms involved in the development of the response to drought were the biosynthesis of phenolic compounds and lignin (increased expression of *PAL* and *SAMS* in birch and pine), proline and osmoprotectant proteins (activation of *P5CS* and *LEA8* in poplar). The synthesis of proline and dehydrin proteins in different tree species can be controlled by the ERF and NAC transcription factor families, which is known from previous studies and is confirmed by the results of the present work, since the expression of the *DREB2*, *NAC034* and *NAC19* genes increased simultaneously with the increase in the level of *LEA8* and *P5CS* transcripts. Adaptation of photosynthesis also apparently plays an important role in drought in birch and poplar, which was expressed in an increase in the expression of the carbonic anhydrase gene *CA*, which probably contributed to maintaining the supply of CO_2 for the normal functioning of Rubisco under moderate stress. The negative impact of stress on the studied tree species was also revealed in the course of the work, which was expressed in a decrease in the expression of *ICDH*, which ensures the functioning of nitrogen metabolism and the supply of 2-oxoglutarate for the synthesis of amino acids. An increase in the expression of *TAT- D*, which is involved in the development of apoptosis, in sensitive pine genotypes (low-yielding pine genotype No. 23 under greenhouse experiment conditions) also indicated a negative impact of drought. In downy birch ‘15-1’ and black poplar, the expression of all analyzed genes was reduced, which indicates a negative impact of drought on these genotypes.

The studied genes are part of large gene families, the study of the expression of representatives of which during drought will help to more deeply reveal the mechanisms of adaptation in tree species. The significant increase in *pCA* expression shown in the present work indicates an important role of *CA* in the development of a response to drought and the adaptation of the photosynthetic apparatus to stress conditions, however, the specific mechanism of this process, especially in woody plants, has not been established and requires further study.

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CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

REFERENCES

Ahmed, U., Rao, M. J., Qi, C. and Xie, Q. (2021). Expression profiling of flavonoid biosynthesis genes and secondary metabolites accumulation in *Populus* under drought stress. *Molecules*, *26*(18), 5546.

Andriantelomanana, T., Améglio, T., Delzon, S., Cochard, H. and Herbette, S. (2024). Unpacking the point of no return under drought in poplar: insight from stem diameter variation. *New Phytologist*, *242*(2), 466-478.

Bose, A. K., Gessler, A., Büntgen, U. and Rigling, A. (2024). Tamm review: Drought-induced Scots pine mortality–trends, contributing factors, and mechanisms. *Forest Ecology and Management*, *561*, 121873.

Cesarino, I. (2019). Structural features and regulation of lignin deposited upon biotic and abiotic stresses. *Current opinion in biotechnology*, *56*, 209-214.

Cevik, S., Kurt, O., Güzel Değer, A. Y. Ş. İ. N., Kocacinar, F. and Binzet, R. (2023). Beta-carbonic anhydrase gene expression levels change depending on the drought severity in both the leaves and roots of *Arabidopsis thaliana*. *Turkish Journal of Botany*, *47*(6), 541-555.

Chang, L., Wang, L., Peng, C., Tong, Z. and Wang, D. (2019). The chloroplast proteome response to drought stress in cassava leaves. *Plant Physiology and Biochemistry*, *142*, 351-362.

Chen, J., Xia, X. and Yin, W. (2009). Expression profiling and functional characterization of a *DREB2*-type gene from *Populus euphratica*. *Biochemical and Biophysical Research Communications*, *378*(3), 483-487.

Corpas, F. J. and Palma, J. M. (2023). Functions of NO and H2S signal molecules against plant abiotic stress. In *Plant abiotic stress signaling* (pp. 97-109). New York, NY: Springer US.

Di Stefano, V., Di Domenico, G., Menta, M., Pontuale, E., Bianchini, L. and Colantoni, A. (2024). Comparison between Different Mechanization Systems: Economic Sustainability of Harvesting Poplar Plantations in Italy. *Forests*, *15*(3), 397.

Du, M., Ding, G. and Cai, Q. (2018). The transcriptomic responses of *Pinus massoniana* to drought stress. *Forests*, *9*(6), 326.

Du, X., Su, M., Jiao, Y., Xu, S. and Song, J. (2022). A transcription factor *SlNAC10* gene of *Suaeda liaotungensis* regulates proline synthesis and enhances salt and drought tolerance. *International Journal of Molecular Sciences*, *23*(17), 9625.

Feduraev, P., Skrypnik, L., Riabova, A., Pungin, A. and Tokupova, E. (2020). Phenylalanine and tyrosine as exogenous precursors of wheat (*Triticum aestivum* L.) secondary metabolism through *PAL*-associated pathways. *Plants*, *9*(4), 476.

Gonçalves, A. Z. and Mercier, H. (2021). Transcriptomic and biochemical analysis reveal integrative pathways between carbon and nitrogen metabolism in *Guzmania monostachia* (*Bromeliaceae*) under drought. *Frontiers in Plant Science*, *12*, 715289.

Grodetskaya, T., Fedorova, O., and Evlakov, P. (2021, October). Optimized method for RNA extraction from leaves of forest tree species. In *IOP Conference Series: Earth and Environmental Science* (Vol. 875, No. 1, p. 012008). IOP Publishing.

Han, X., Zhao, Y., Chen, Y., Xu, J. and Jiang, C. (2022). Lignin biosynthesis and accumulation in response to abiotic stresses in woody plants. *Forestry Research*, *2*(1).

Heidari, P., Mazloomi, F., Nussbaumer, T. and Barcaccia, G. (2020). Insights into the *SAM* synthetase gene family and its roles in tomato seedlings under abiotic stresses and hormone treatments. *Plants*, *9*(5), 586.

Jan, A. T., Singhal, P. and Haq, Q. M. R. (2013). Plant

abiotic stress: deciphering remedial strategies for emerging problem. *Journal of Plant Interactions*, *8*(2), 97-108.

Jeong, M. J., Choi, B. S., Bae, D. W., Shin, S. C. and Park, S. U. (2012). Differential expression of kenaf phenylalanine ammonia-lyase (*PAL*) ortholog during developmental stages and in response to abiotic stresses. *Plant Omics*, *5*(4), 392-399.

Khan, M., Imran, Q. M., Shahid, M., Mun, B. G. and Lee, S. U. (2019). Nitric oxide-induced AtAO3 differentially regulates plant defense and drought tolerance in *Arabidopsis thaliana*. *BMC Plant Biology*, *19*, 1-19.

Kilwake, J. W., Umer, M. J., Wei, Y., Mehari, T. G. and Magwanga, R. O. (2023). Genome-Wide characterization of the *SAMS* gene family in cotton unveils the putative role of *GhSAMS2* in enhancing abiotic stress tolerance. *Agronomy*, *13*(2), 612.

Kotrade, P., Sehr, E. M. and Brüggemann, W. (2019). Expression profiles of 12 drought responsive genes in two European (deciduous) oak species during a two-year drought experiment with consecutive drought periods. *Plant Gene*, *19*, 100193.

Lan, T., Gao, J. and Zeng, Q. Y. (2013). Genome-wide analysis of the *LEA* (late embryogenesis abundant) protein gene family in *Populus trichocarpa*. *Tree genetics & genomes*, *9*, 253-264.

Li, T., Wang, R., Zhao, D. and Tao, J. (2020). Effects of drought stress on physiological responses and gene expression changes in herbaceous peony (*Paeonia lactiflora* Pall.). *Plant signaling & behavior*, *15*(5), 1746034.

Liu, C. and Zhang, T. (2017). Expansion and stress responses of the *AP2/EREBP* superfamily in cotton. *BMC genomics*, *18*, 1-16.

Livak, K. J. and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *methods*, *25*(4), 402-408.

Ma, R., Xiao, Y., Lv, Z., Tan, H. and Chen, R. (2017). *AP2/ERF* transcription factor, *Ii049*, positively regulates lignan biosynthesis in Isatis indigotica through activating salicylic acid signaling and lignan/lignin pathway genes. *Frontiers in Plant Science*, *8*, 1361.

Martin, C., and Paz-Ares, J. (1997). *MYB* transcription factors in plants. *Trends in Genetics*, *13*(2), 67-73.

Momayyezi, M., McKown, A. D., Bell, S. C. and Guy, R. D. (2020). Emerging roles for carbonic anhydrase in mesophyll conductance and photosynthesis. *The Plant Journal*, *101*(4), 831-844.

Muilu-Mäkelä, R., Vuosku, J., Hamberg, L., Latva-Mäenpää, H. and Häggman, H. (2015). Osmotic stress affects polyamine homeostasis and phenolic content in proembryogenic liquid cell cultures of Scots pine. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *122*, 709-726.

Nguyen, H. C., Cao, P. B., San Clemente, H., Ployet, R. and Mounet, F. (2017). Special trends in *CBF* and *DREB2* groups in *Eucalyptus gunnii* vs *Eucalyptus grandis* suggest that *CBF* are master players in the trade-off between growth and stress resistance. *Physiologia plantarum*, *159*(4), 445-467.

Pääkkönen, E., Seppänen, S., Holopainen, T., Kokko, H. and Kärenlampi, S. (1998). Induction of genes for the stress proteins *PR-10* and *PAL* in relation to growth, visible injuries and stomatal conductance in birch (*Betula pendula*) clones exposed to ozone and/or drought. *New Phytologist*, *138*(2), 295-305.

Polishchuk, O. V. (2021). Stress-related changes in the expression and activity of plant carbonic anhydrases. *Planta*, *253*(2), 58.

Qian, C., Li, L., Guo, H., Zhu, G. and Yang, N. (2023). Genome-Wide analysis of *DREB* family genes and characterization of cold stress responses in the woody plant *Prunus nana*. *Genes*, *14*(4), 811.

Qiu, J., Yoon, J. H. and Shen, B. (2005). Search for apoptotic nucleases in yeast: role of Tat-D nuclease in apoptotic DNA degradation. *Journal of Biological Chemistry*, *280*(15), 15370-15379.

Rasool, F., Uzair, M., Naeem, M. K., Rehman, N. and Afroz, A. (2021). Phenylalanine ammonia-lyase

(*PAL*) genes family in wheat (*Triticum aestivum* L.): Genome-wide characterization and expression profiling. *Agronomy*, *11*(12), 2511.

Rini, D. (2019). Sequence variation of *DREB2* gene as a potential molecular marker for identifying resistant plants toward drought stress. *Nusantara Bioscience, 11*(1), 35-43

Sakuma, Y., Maruyama, K., Osakabe, Y., Qin, F., Seki, M., Shinozaki, K. and Yamaguchi-Shinozaki, K. (2006). Functional analysis of an Arabidopsis transcription factor, *DREB2A*, involved in drought-responsive gene expression. *The Plant Cell*, *18*(5), 1292-1309.

Shinozaki, K. and Yamaguchi-Shinozaki, K. (2007). Gene networks involved in drought stress response and tolerance. *Journal of experimental botany*, *58*(2), 221-227.

Shinozaki, K., Yamaguchi-Shinozaki, K. and Seki, M. (2003). Regulatory network of gene expression in the drought and cold stress responses. *Current opinion in plant biology*, *6*(5), 410-417.

Sun, J., Peng, X., Fan, W., Tang, M. and Liu, J. (2014). Functional analysis of *BpDREB2* gene involved in salt and drought response from a woody plant *Broussonetia papyrifera*. *Gene*, *535*(2), 140-149.

Sun, S., Liang, X., Chen, H., Hu, L. and Yang, Z. (2022). Identification of *AP2/ERF* transcription factor family genes and expression patterns in response to drought stress in Pinus massoniana. *Forests*, *13*(9), 1430.

Tikhomirova, T. S., Krutovsky, K. V., and Shestibratov, K. A. (2022). Molecular traits for adaptation to drought and salt stress in birch, oak and poplar species. *Forests*, *14*(1), 7.

Torres, L. F., Reichel, T., Déchamp, E., de Aquino, S. O. and Duarte, K. E. (2019). Expression of *DREB*-like genes in *Coffea canephora* and *C. arabica* subjected to various types of abiotic stress. *Tropical Plant Biology*, *12*, 98-116.

Wang, X., Hou, C., Zheng, K., Li, Q., Chen, S. and Wang, S. (2017). Overexpression of *ERF96*, a small ethylene response factor gene, enhances salt tolerance in Arabidopsis. *Biologia Plantarum*, *61*, 693-701.

Wang, X., Hou, C., Zheng, K., Li, Q., Chen, S. and Wang, S. (2017). Overexpression of *ERF96*, a small ethylene response factor gene, enhances salt tolerance in Arabidopsis. *Biologia Plantarum*, *61*, 693-701.

Wu, H. L., Li, L., Cheng, Z. C., Ge, W. and Gao, J. (2015). Cloning and stress response analysis of the *PeDREB2A* and *PeDREB1A* genes in moso bamboo (*Phyllostachys edulis*). *Genetics and Molecular Research*, *14*(3), 10206-10223.

Xiao, X., Yang, F., Zhang, S., Korpelainen, H. and Li, C. (2009). Physiological and proteomic responses of two contrasting *Populus cathayana* populations to drought stress. *Physiologia plantarum*, 136(2), 150-168.

Xiong, C., Zhao, S., Yu, X., Sun, Y. and Li, H. (2020). Yellowhorn drought-induced transcription factor *XsWRKY20* acts as a positive regulator in drought stress through ROS homeostasis and ABA signaling pathway. *Plant Physiology and Biochemistry*, *155*, 187-195.

Xiu, Y., Iqbal, A., Zhu, C., Wu, G. and Chang, Y. (2016). Improvement and transcriptome analysis of root architecture by overexpression of *Fraxinus pennsylvanica DREB2A* transcription factor in *Robinia pseudoacacia* L.'Idaho'. *Plant biotechnology journal*, *14*(6), 1456-1469.

Yanagisawa, S. (1998). Transcription factors in plants: physiological functions and regulation of expression. *Journal of Plant Research*, *111*, 363-371.

Yao, T., Zhang, J., Xie, M., Yuan, G., Tschaplinski, T. J., Muchero, W. and Chen, J. G. (2021). Transcriptional regulation of drought response in *Arabidopsis* and woody plants. *Frontiers in plant science*, *11*, 572137.

Yin, F., Zeng, Y., Ji, J., Wang, P., Zhang, Y. and Li, W. (2021). The halophyte *Halostachys caspica AP2/ERF* transcription factor *HcTOE3* positively regulates freezing tolerance in *Arabidopsis*. *Frontiers in Plant Science*, *12*, 638788.

Zhang, C., Yao, X., Ren, H., Wang, K. and Chang, J.

(2022). Genome-wide identification and characterization of the phenylalanine ammonia-lyase gene family in pecan (*Carya illinoinensis*). *Scientia Horticulturae*, *295*, 110800.

Zhao, T., Liang, D., Wang, P., Liu, J., and Ma, F. (2012). Genome-wide analysis and expression profiling of the *DREB* transcription factor gene family in *Malus* under abiotic stress. *Molecular genetics and genomics*, *287*, 423-436.