

# POLYMORPHISM OF SOME TRANSCRIPTION FACTOR GENES RELATED TO DROUGHT TOLERANCE IN WHEAT

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Received 17.12.2017

The aim of the research was to study polymorphism of preselected gene loci of three transcription factors (*TaNAC2a*, *TaWRKY2*, and *TaWRKY19*) and the Late Embryogenesis Abundant (*LEA*) proteins dehydrin (*Td29b*) related to wheat drought tolerance. The genes structure and chromosome location were established via bioinformatics tools. It is stated that *TaWRKY2* and *TaWRKY19* genes were comprised of 4 exons and 3 introns located on 2BS and 1DS chromosome arms, respectively; *TaNAC2a* — 2 exons and 1 intron 7AS; *Td29b* — single exon gene 3AS. Using polymerase chain reaction, no polymorphism was observed. Polymorphic bands were detected for *TaWRKY2* locus. The screening of the distribution of the revealed polymorphic loci was carried out for a set of wheat and rye varieties, old landraces and interspecific hybrids. The polymorphism of *TaWRKY2* locus indicated the presence of some other possible alleles of the gene. The obtained data are important for further investigations of wheat drought tolerance.

**Key words:** *Triticum* spp., polymerase chain reaction, transcription factors, *TaNAC2a*, *TaWRKY2*, *TaWRKY19*, *LEA*, *Td29b*, drought tolerance.

Common wheat (*Triticum aestivum* L.) is very important widely grown crop used for bread baking, food and animal feed. Wheat yield is the third largest cereal production in the world, after maize and rice [1]. In consequence of methods of modern plant breeding, numerous varieties with increased productivity were obtained. However, due to the recent undesired climate changes and global warming, the selection of drought-tolerant germplasm donors must be constantly monitored to include them in contemporary breeding programs [2]. Marker-assisted selection (MAS) based on DNA markers can be effectively applied in the process of such selection [3–6]. While different types of DNA sequences can be employed for this purpose.

First DNA marker systems to study drought tolerance in plants were based on non-coding DNA sequences — RAPD (Random Amplified Polymorphic DNA) [7, 8], SSR (Simple Sequence Repeats) [9, 10] and

ISSR (Inter Simple Sequence Repeats) [8] etc. Presently, attention mostly attracted to target encoding gene sequences, which play a great role in plant response to stress factors. These genes predominantly represented with transcriptional factors (TFs) and dehydrin genes [11].

In present work, our aim was to study DNA polymorphism of preselected gene loci of three transcription factors (*TaNAC2a*, *TaWRKY2*, *TaWRKY19*) and the *LEA* dehydrin (*Td29b*) related in their expression response to wheat drought tolerance.

*WRKY* transcription factors represent family of proteins that have *WRKY* domain (approximately 60 amino acids), involving the conserved *WRKYGQK* domain and a zinc-finger-like motif [12, 13]. These proteins are of great importance for biotic and abiotic stress responses [14, 15]. Overexpression of *TaWRKY2* as well as *TaWRKY19* increased dehydration stress tolerance in transgenic

*Arabidopsis* plants [12]. It was also found out that *TaWRKY2* overexpressing plants had enhanced *STZ* and *RD29B* gene expressions due to temperate binding to the loci from *RD29B STZ-1* and *STZ-2* locus of *Arabidopsis*. As to *TaWRKY19* transgenic plants, they had higher expression levels of *DREB2A*, *RD29B*, *Cor6.6* and *RD29A* genes [12].

Another *TF* family that highly introduced in common wheat is represented with proteins containing a highly conserved *NAC* domain at the N-terminus and a variable transcriptional regulation domain at the C-terminus [16, 17]. Overexpression of different *TaNAC* responded to enhanced biotic and abiotic tolerance [18, 19]. It was postulated in the [16], that *TaNAC2a* transgenic plants of tobacco had extremely increased drought tolerance.

Special role in response to dehydration stress relates to dehydrin proteins, which help plant cell cope with osmotic changes. The number of dehydrins were described in wheat [20, 21]. Late Embryogenesis Abundant (*LEA*) proteins belong to above mentioned group of proteins and can be candidate for wheat improvement [22]. It was reported [23] that *LEA* proteins accumulation enhanced stress tolerance protecting plant cells against dehydration. It was also described the importance of *Td29b* dehydrin in common wheat, which synthesis was highly induced by dehydration.

## Materials and Methods

The subject of the study was a set of wheat cultivars of Ukrainian and foreign origin (25 and 36, consequently), a set of 52 old wheat species, distant and interspecific hybrids, 4 varieties of rye.

BLAST searches and sequence analyses were implemented by BLASTn on the *Triticum*

*aestivum* genome (<https://blast.ncbi.nlm.nih.gov/> and <https://wheat-urgi.versailles.inra.fr>). The schemes of exon-intron structures were obtained by employing the online Gene Structure Display Server bioinformatic tools (<http://gsds.cbi.pku.edu.cn/>) from both coding sequence (CDS) and genomic sequences [24].

Total DNA was isolated from one kernel with the modified CTAB method [25]. Polymerase chain reaction (PCR) of 20 µl included 0.5 µM of forward and reverse primers each (Metabion, Germany), 1× Reaction Buffer B (Solis BioDyne, Estonia), 2 mM MgCl<sub>2</sub>, 0.2 µM of each deoxyribonucleoside triphosphate (Thermo Fisher Scientific, USA), 1 unit of FIREPol<sup>®</sup> DNA Polymerase (Solis BioDyne, Estonia), 30 ng of total plant DNA. Primer sequences for loci *TaNAC2a*, *TaWRKY2*, *TaWRKY19* and *Td29b* used in the study and PCR conditions are indicated in the Table 1. The CDS accessions in the GenBank are HM027575.2 (*TaNAC2a*), EU665425.1 (*TaWRKY2*), EU665430.1 (*TaWRKY19*) and AJ890139.1 (*Td29b*).

The PCR products were separated by means of electrophoresis in 2% agarose gels in lithium borate buffer, 0.1 µg/ml ethidium bromide [26]. Gels were visualized in UV-light with a photosystem Canon EOS 600D. GelAnalyzer 2010 software was applied to identify the size of amplified fragments (<http://www.gelanalyzer.com>). Frequencies for each combination of amplified fragments were calculated according to [27].

## Results and Discussion

As it was denoted above, data on CDS only are available for those three studied transcriptional factors (*TaNAC2a*, *TaWRKY2*, and *TaWRKY19*) and the dehydrin (*Td29b*). Thus, we managed to predict the exon-intron

Table 1. Primer sequences and PCR conditions

TF gene	Primer sequences 5'→3'	PCR conditions
<i>TaNAC2a</i>	F: GGTAGTGCGGTGCTTCCAAT R: TGAATGTTGTTGCTCGTCCC [16]	94 °C — 30 s; 58 °C — 30 s 72 °C — 30 s; 35 cycles
<i>TaWRKY2</i>	F: GGCGCTGCCGACGTCATCTT R: AGCAGAGGAGCGACTCGACGA [12]	94 °C — 30 s; 58 °C — 30 s 72 °C — 30 s; 35 cycles
<i>TaWRKY19</i>	F: AGGGAAGCATACGCATGACGTGC R: GGCGAGATCGTTCAGAATGGCTGT [12]	94 °C — 30 s; 60 °C — 30 s 72 °C — 30 s; 35 cycles
<i>Td29b</i>	F: CGCACCCAGCTAGTAAGTTCG R: CCCAGCCCAGTAATAACCCAT [23]	94 °C — 30 s; 53 °C — 30 s 72 °C — 30 s; 35 cycles

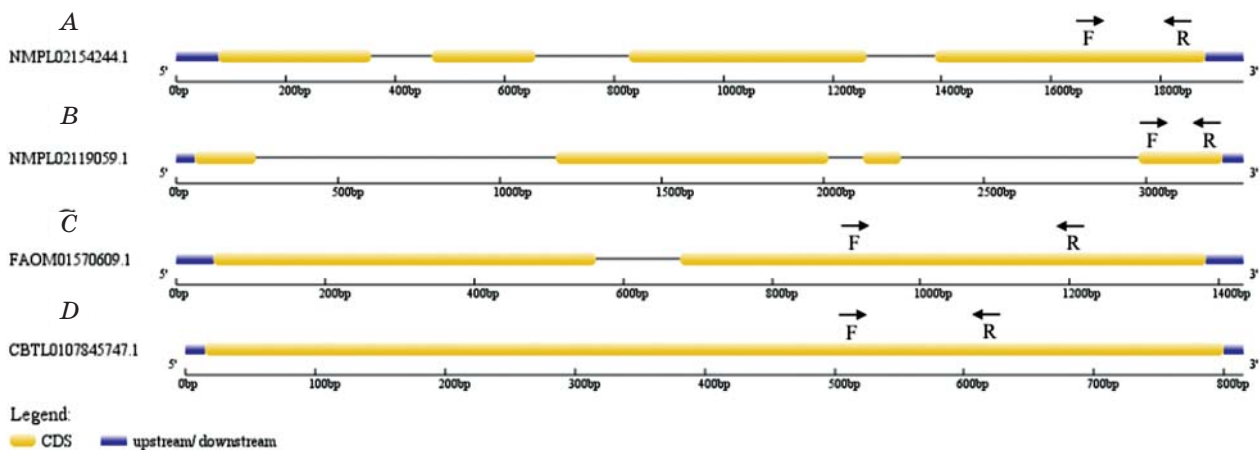
structure and location of their genes by means of alignment via BLAST tools.

Having carried out every CDS alignments in the database of wheat whole genome shotgun contigs, the gene structures and chromosomal location were defined for three studied transcription factors (*TaNAC2a*, *TaWRKY2*, *TaWRKY19*) and the dehydrin (*Td29b*) (Fig. 1) in accordance with [28]. Hence, *TaWRKY2* and *TaWRKY19* have similar structure of 4 exons and 3 introns (Fig. 1, A, B), though, they are situated in different chromosomes (*TaWRKY2* — short arm of 1D chromosome; *TaWRKY19* — short arm of 1B). Both primer pairs applied in the following DNA polymorphism study hybridized at the end of the fourth exon. The gene of TF *TaNAC2a* comprises of 2 exons and 1 intron (Fig. 1, C) and allocates at the short arm of 7A chromosome. The primer pair for this gene locus annealed at the central part of exon 2. It was established, that *Td29b* gene might have referred to single exon gene (Fig. 1, D). Its location is the short arm of 3A chromosome.

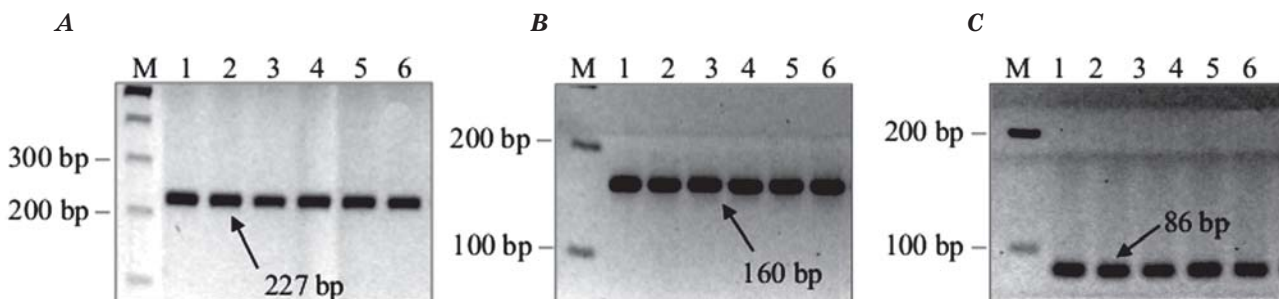
*Molecular genetic study*

To study DNA polymorphism of the selected loci of 4 genes (three TF — *TaNAC2a*, *TaWRKY2*, *TaWRKY19*; and the dehydrin gene *Td29b*) a set of 25 Ukrainian and 37 international wheat accessions from Global Wheat Program of the International Maize and Wheat Improvement Center (CIMMYT) and the Wheat Germplasm Bank was collected. By means of applying primer pairs and PCR conditions indicated in the Table 1, we observed no polymorphism for gene loci *TaNAC2a*, *TaWRKY19* and *Td29b*. There was one fragment amplified only for each sample — fragment of approximately 227 base pairs (bp) for *TaNAC2a* gene locus, 160 bp for *TaWRKY19*, 86 bp for *Td29b* (Fig. 2).

Following the amplification of total genomic DNA of all common wheat varieties of Ukrainian and foreign origin, there were two fragments detected for each sample. We observed three different genotypes in the studied *TaWRKY2* locus. The first one

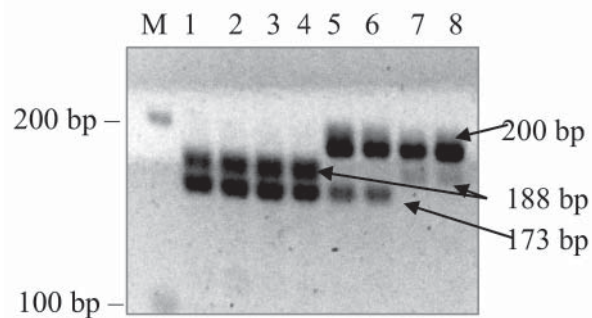


**Fig. 1. Prediction of gene exon-intron structure:**  
 A — *TaWRKY2*; B — *TaWRKY19*; C — *TaNAC2a*; D — *Td29b*



**Fig. 2. The electrophoregrams denoting PCR-products segregation of DNA marker systems:**  
 A — *TaNAC2a*; B — *TaWRKY19*; C — *Td29b*

Lanes 1–6 — common wheat varieties (Glenlea, Comanche, Wilbur, Granero Inta, Tobarito M 97, V-17);  
 M — marker of molecular weight GeneRuller™ DNA Ladder Mix



**Fig. 3. The electrophoregram depicting DNA polymorphism of *TaWRKY2* locus:** Lanes 1 — Odeska 267; 2 — Poliska 90; 3 — Darunok Podillia; 4 — Podolianka; 5 — Astarta; 6 — Kryzhynka; 7 — Sotnytsia; 8 — Zolotokolosa; M — molecular weight marker GeneRuller™ DNA Ladder Mix

represented amplified fragments of 173 and 188 bp, second — 188 and 200 bp, the third — of 173 and 200 bp (Fig. 3). Moreover, Ukrainian varieties showed greater diversity, than those foreign ones. The frequencies for each allele of amplified fragments among Ukrainian varieties are 0.52 (173+188 bp), 0.28 (173+188 bp) and 0.2 (173 + 200 bp). On the contrast, a set of amplified fragments 188+200 bp was not observed among 36 foreign varieties obtained from the CIMMYT. In addition, only two samples (Millaleau Inia and Tobarito M 97) possessed 173+200 bp pattern. Thereafter, frequencies for these two allele of amplified fragments among the CIMMYT varieties were 0.944 (173+188 bp) and 0.056 (173+200 bp). The results on the DNA polymorphism study of *TaWRKY2* locus are indicated in the Tables 2 and 3.

**Table 2. Detected DNA polymorphism of *TaWRKY2* locus in Ukrainian varieties**

Variety	Originator	Amplified fragment, bp	Variety	Originator	Amplified fragment, bp
Astarta	IPPG NASU	173, 200	Poliska 90	NSC "IA NAAS"	173, 188
Bohdana	IPPG NASU	173, 188	Shchedrivka Kyivska	IPPG NASU NSC "IA NAAS"	173, 188
Bunchak	PBGI NCSCI NAASU	173, 188	Slavna	IPPG NASU	188, 200
Darunok Podillia	IPPG NASU	173, 188	Smuhlianka	IPPG NASU	188, 200
Drevlianka	n.a.	173, 188	Solomiia	IPPG NASU	173, 188
Favorytka	IPPG NASU	173, 200	Sonata	Institute of Field and Vegetable Crops, Novi Sad, Serbia	173, 200
Hileia	IPPG NASU	188, 200	Sotnytsia	IPPG NASU	188, 200
Kryzhynka	RMIW NAASU IPPG NASU	173, 200	Spasivka	IPPG NASU	188, 200
Natalka	IPPG NASU	173, 188	Vesnianka	IPPG NASU	188, 200
Novokyivska	IPPG NASU	173, 200	Yatran 60	IPPG NASU	173, 188
Odeska 267	PBGI NCSCI NAASU	173, 188	Yednist	PBGI NCSCI NAASU	173, 188
Pereiaslavka	IPPG NASU	173, 188	Zolotokolosa	IPPG NASU	188, 200
Podolianka	IPPG NASU	173, 188			

*Note:* IPPG NASU — Institute of Plant Physiology and Genetics, National Academy of Sciences of Ukraine; PBGI NCSCI NAASU — Plant Breeding and Genetics Institute — National Center of Seed and Cultivar Investigation, the National Academy of Agrarian Sciences of Ukraine; RMIW NAASU — The V.M. Remeslo Myronivka Institute of Wheat, the National Academy of Agrarian Sciences of Ukraine; NSC "IA NAAS" — National Scientific Centre "Institute of Agriculture of the National Academy of Agrarian Sciences of Ukraine"; n.a. — not available. <http://www.wheatpedigree.net>, State register of plant varieties suitable for distribution to Ukraine of the Ministry of Agrarian Policy and Food of Ukraine <http://www.sops.gov.ua/reestr-sortiv-roslin>.



Table 3. Detected DNA polymorphism of TaWRKY2 locus in varieties from germplasm collections of the CIMMYT

Variety	Locality	Originator*	Year of registration	Amplified fragment, bp
1	2	3	4	5
AC Vista	Canada (Saskatchewan)	Agriculture and Agri-Food Canada Semi-arid Prairie Agricultural Research Centre, Swift Current	1996	173, 188
Albis	Switzerland (Zurich)	Federal Research Station for Agronomy	1983	173, 188
Anza	Mexico, USA (California)	California Agricultural Experiment Station	1971	173, 188
Batavia	Australia (Queensland)	Queensland Wheat Research Institute	1991	173, 188
Caribo	Germany	Heidenreih, Bad-Schwartau	1968	173, 188
Cenad-512	Romania	n.a.	1958	173, 188
Comanche	USA (Kansas)	Kansas Agricultural Experiment Station	1942	173, 188
D-12	Peru	n.a.	1972	173, 188
Excalibur	Australia (South-Australia)	RAGT	1990	173, 188
Gabo	Australia (New-South-Wales)	University of Sydney Plant Breeding Institute, Cobbitty	1942	173, 188
Glenlea	Canada (Manitoba)	University of Manitoba	1972	173, 188
Grande-Del-Monte	Venezuela	n.a.	n.a.	173, 188
Granero Inta	Argentina	Inta	1987	173, 188
Inia-F-66	Mexico	INIA, CIMMYT	1966	173, 188
Iskamish-K-2-Light	Afghanistan	n.a.	1975	173, 188
Janz	Australia (Queensland)	Queensland Wheat Research Institute	1989	173, 188
Katunga	Australia (Victoria)	n.a.	1992	173, 188
Ke Feng 2	China (Heilongjiang)	Keshan WRI	1979	173, 188
Kimmo	Finland	n.a.	1941	173, 188
Klein Favorito	Argentina	E. Klein	1920	173, 188
Kulin	Australia (Western-Australia)	Department of Agriculture, W.A.	1986	173, 188
Manital	Italy	Samoggia Luigi, Bologna	1981	173, 188
Millaleau Inia	Chile	INIA, CIMMYT	1982	173, 200
Recital	France	Benoist	1986	173, 188
Rokycanska sametka	Czechoslovakia	n.a.	1899	173, 188
Safed Lerma	India	Indian Agricultural Research Institute	1967	173, 188
Sakha 69	Egypt	Agricultural Research Center, Giza	1980	173, 188
Svenno	Sweden	W. Weibull	1953	173, 188
Talimka	Kyrgyzstan	Kirgizskaya GSS	1940	173, 188
Tobarito M 97	Mexico	CIMMYT	1997	173, 200

Table 3. End

1	2	3	4	5
Tobarito M 97	Mexico	CIMMYT	1997	173, 200
Tselin-naya-Yu-bileinaya	Kazakhstan	Kazakhskiy NII zernovogo khozyaystva	1988	173, 188
V-17	Mexico	CIMMYT	1968	173, 188
Wilbur	USA (Oregon)	W.J. Mariner	1897	173, 188
Zambesi	Zimbabwe	Salisbury AES	1963	173, 188
Zerdakia	Iraq	n.a.	n.a.	173, 188
Zirka	Ukraine	Plant Breeding and Genetics Institute, National Academy of Agrarian Sciences of Ukraine	1984	173, 188

Note: n.a. — not available; \* — from the Genetic Resources Information System for Wheat and Triticale (<http://www.wheatpedigree.net/>) provided by Vavilov Research Institute of Plant Industry (VIR) and International Maize and Wheat Improvement Center (CIMMYT).

According to CDS sequence of *TaWRKY2* (GenBank ID EU665425.1), the primer pair for this TF locus is likely to amplify the fragment of 188 bp long. Such a fragment was observed through the study; however, not all the wheat samples possessed it. Consequently, there must be an indel mutation, which is likely to form another allele.

The old wheat landraces is the source of potential genes of interest which can be of great value for common wheat improvement

in modern breeding programs. Thus, the following screening of a number of wheat landraces and interspecific hybrids was carried out. The data were indicated in the Table 4. As it can be seen from the table, most of them carried fragments of 173+188 bp (46 among 52 samples, frequency — 0.88). On the other hand, all the 3 fragments (173, 188 and 200 bp) were amplified from 2 wheat accessions (*T. spelta* var. *duhamelianu* Baulaender and

Table 4. Detected DNA polymorphism of *TaWRKY2* locus in old wheat species, distant and interspecific hybrids

Species/Hybrid/Cross	Subspecies	Country of originator	Amplified fragment, bp
1	2	3	4
AD	<i>T. persicum/Ae. tauschii</i>	Japan	173, 188
AD	<i>T. dicoccum/Ae. speltooides</i>	Azerbaijan	173, 188
AD	<i>Ae. ventricosa/T. dicoccum</i>	Russia	173, 188
AD	<i>T. aestivum/Ae. comosa</i>	Russia	173, 188
AD 217	<i>T. timopheevii/Ae. umbellulata</i>	Japan	173, 188
AD 7	<i>T. ispahanicum/Ae. cylindrical</i>	Azerbaijan	173
AD 8	<i>T. dicoccum/Ae. triuncialis</i>	Azerbaijan	173, 188
<i>Aegilotricum cylindroaestivum</i>	<i>Aegilops cylindrical/T. aestivum</i>	Armenia	173, 188
<i>Haynaticum</i>	<i>T. dicoccum/Dasypyrum villosum</i>	Russia	173, 188
PAH-31	<i>T. dicoccum/T. monococcum</i>	Russia	173, 188
PEAH	<i>T. dicoccum/Ae. tauschii</i>	Russia	173, 188
<i>T. dicoccum</i> Schuebl.	var. <i>rufum</i>	Sweden	173, 188

Table 4. Продолження

1	2	3	4
<i>T. dicoccum</i> Schuebl.	var. <i>aeruginosum</i>	Azerbaijan	173, 188
<i>T. dicoccum</i>	var. <i>aeruginosum</i> Runo	Russia	173, 188
<i>T. dicoccum</i>	var. <i>serbicum</i> Polba 3	Russia, Udmurtia	173, 188
<i>T. dicoccum</i>	var. <i>dicoccum</i>	Ukraine	173, 188
<i>T. dicoccum</i>	var. <i>nigroajar</i>	Ethiopia	173, 188
<i>T. dicoccum</i>	var. <i>rufum</i>	Ukraine	173, 188
<i>T. dicoccum</i>	var. <i>aeruginosum</i>	Russia, Dagestan	173, 188
<i>T. dicoccum</i>	var. <i>semicanum</i>	Germany	173, 188
<i>T. dicoccum</i>	Polba Kokchetavska	Kazakhstan	173, 188
<i>T. dicoccum</i>	var. <i>vasconicum</i> Crjunella	Spain	173, 188
<i>T. dicoccum</i>	var. <i>rufum</i>	Spain	173, 188
<i>T. dicoccum</i>	var. <i>atratum</i>	Poland	173, 188
<i>T. dicoccum</i>	n.a.	Kazakhstan	173, 188
<i>T. dicoccum</i> Schuebl.	var. <i>serbicum</i>	Belarus	173, 188
<i>T. dicoccum</i> Schuebl.	var. <i>dicoccum</i>	n.a.	173, 188
<i>T. dicoccum</i> Schuebl.	var. <i>serbicum</i> Chervona krasa	Belarus	173, 188
<i>T. dicoccum</i> Schuebl.	var. <i>haussknachtianum</i> Bolshaia holova	India	173, 188
<i>T. dicoccum</i> Schuebl.	var. <i>loganse</i> Polba Kokchetavska	n.a.	173, 188
<i>T. dicoccum</i> Schuebl.	var. <i>volgense</i> Vernal	USA	173, 188
<i>T. dicoccum</i> Schuebl.	var. <i>aeruginosum</i>	Armenia	173, 188
<i>T. dicoccum</i> Schuebl.	var. <i>serbicum</i>	Russia	173, 188
<i>T. dicoccum</i> Schuebl.	var. <i>volgense</i>	Russia	173, 188
<i>T. dicoccum</i> Schuebl.	var. <i>haussknachtianum</i>	Kazakhstan	173, 188
<i>T. dicoccum</i> Schuebl.	var. <i>haussknachtianum</i>	Azerbaijan	173, 188
<i>T. dicoccum</i> Schuebl.	var. <i>aeruginosum</i> Runo	Russia	173, 188
<i>T. ispahanicum</i>	var. <i>ispahanicum</i>	Iran	173, 188
<i>T. kiharae</i>	<i>T. timopheevii</i> × <i>Ae. tauschii</i>	Japan	173, 188
<i>T. macha</i>	var. <i>palaeoimereticum</i>	Georgia	173, 188
<i>T. sinkajae</i>	var. <i>sinskajae</i>	Russia	173
<i>T. spelta</i>	var. <i>album</i>	Canada	173, 188
<i>T. spelta</i>	var. <i>caeruleum</i> CDC Zobra	Canada	173, 188
<i>T. spelta</i>	var. <i>griseoturanorecens</i>	Tajikistan	173, 188
<i>T. spelta</i>	var. <i>duhamelianum</i>	Poland	173, 188
<i>T. spelta</i>	var. <i>duhamelianum</i> Baulaender	Germany	173, 188, 200
<i>T. spelta</i>	var. <i>duhamelianum</i> Frankenkorn	Germany	173, 188

Table 4. End

1	2	3	4
<i>T. spelta</i>	var. <i>caeruleum</i>	Azerbaijan	173, 188
<i>T. spelta</i>	var. <i>album</i>	Canada	173, 188
<i>T. vavilovii</i>	var. <i>vavilovii</i>	Armenia	173, 188, 200
<i>T. hexapolicum</i>	n.a.	Armenia	173, 200
Tritordeum 1199/09	<i>T. durum</i> / <i>Hordeum chilense</i>	Spain	188

*T. vavilovii* var. *vavilovii*) representing frequency 0.04 only. Three wheats (frequency — 0.06) had only one type of fragment (173 or 188 bp). The only sample (frequency — 0.02) (*T. hexapolicum*) had fragments of 173+200 bp.

Additionally, four rye varieties (Avgust, Khmarka, Remington and Stoir) were tested for polymorphism in *TaWRKY2* locus. As a result, the only fragment of 200 bp was detected in each sample. This fact shows that other cereal crops might have the *TaWRKY2* gene too.

The study of the genes, that impact greatly on drought response, is of great value for wheat improvement in present-day plant breeding programs. The current research reveals knowledge on DNA polymorphism of three transcriptional factors (*TaNAC2a*, *TaWRKY2*, *TaWRKY19*) and the dehydrin (*Td29b*) genes which can be applied for MAS. During the analysis the gene structure and chromosomal location were established. Thus, *TaWRKY2* and *TaWRKY19* genes comprised of 4 exons and 3 introns (2BS and

1DS, respectively); *TaNAC2a* — 2 exons and 1 intron (7AS); *Td29b* — single exon gene (3AS).

In the result of this study, no polymorphism was observed for gene loci *TaNAC2a*, *TaWRKY19* and *Td29b* by means of preselected primer pairs. In contrast, polymorphic bands were detected for *TaWRKY2* locus that did not correspond to CDS from GenBank. This fact indicated the presence of some other possible alleles of the gene.

This research was financially supported by the projects “Development of Genotyping and Marking Systems for Valuable Biological Characteristics in Crops” (state registration number 0116U000173) and “Study of Genetic Determinants and New Allele Effects of Genes to Improve Cereals under the Negative Impact of Global Climate Change” (state registration number 0117U000385) provided by the National Academy of Sciences of Ukraine. The authors express their sincere gratitude to Professor Nikolai Borisjuk for critical remarks and help with experiments.

## REFERENCES

1. *Food Outlook*: Biannual report on global food markets. November 2017. Available at: <http://www.fao.org/3/a-I8080e.pdf>.
2. Kaur V., Singh S., Behl R. K. Heat and drought tolerance in wheat: Integration of physiological and genetic platforms for better performance under stress. *Ekin J.* 2016, 2 (1), 1–14.
3. Pokhylko S. Yu., Troyanovska A. V., Stepanenko A. I., Urbanovich O. Yu., Dugan O. M., Rybalka O. I., Morhun B. V. Studies of bread wheat genotypes with transferred gene GPC-B1 of *Triticum turgidum* ssp. *dicoccoides*. *Fact. Exp. Evol. Org.* 2016, 18, 132–136. (In Ukrainian).
4. Stepanenko O. V., Stepanenko A. I., Kuzminskiy Ye. V., Morgun B. V. Identification of *Psy1* gene alleles responsible for carotenoid accumulation in wheat grains. *Biotechnol. acta.* 2017, 10 (2), 57–66. <https://doi.org/10.15407/biotech.10.02.057>.
5. Lakhneko O. R., Stepanenko A. I., Morgun B. V. Genotyping *Triticum aestivum* L. cultivars of Ukraine with microsatellite markers. *Fact. Exp. Evol. Org.* 2016, 19, 61–63.
6. Kosová K., Vitámvás P., Urban M. O., Kholová J., Prášil I. T. Breeding for enhanced drought resistance in barley and wheat — drought-associated traits, genetic resources and their potential utilization in breeding programmes. *Czech J. Genet. Plant Breed.* 2014, 50 (4), 247–261.
7. ElSayed A. I., Rafudeen M. S. Molecular marker assisted for recognition drought tolerant in some of bread wheat genotypes.



- J. Crop Sci. Biotech.* 2012, 15 (1), 17–23. doi: 10.1007/s12892-011-0051-1.
8. El-Sayed O. E., Ibrahim H. F. RAPD and ISSR markers related to drought tolerance of regenerated plants in wheat double haploids and varieties. *Middle East. Russian J. Plant Sci. Biotechnol.* 2008, 2 (2), 44–51.
  9. Kim S. H., Kim D. Y., Yacoubi I., Seo Y. W. Phenotypic and genotypic analyses of drought tolerance in Korean and Tunisian wheat cultivars. *Plant Breed. Biotech.* 2014, 2 (2), 139–150. doi: 10.9787/PBB.2014.2.2.139.
  10. Sadat S., Saeid K. A., Bihanta M. R., Torabi S., Salekdeh S. G. H., Ayeneh G. A. L. Marker assisted selection for heat tolerance in bread wheat. *World Appl. Sci. J.* 2013, 21 (8), 1181–1189. doi: 10.5829/idosi.wasj.2013.21.8.2866.
  11. Rana R. M., Rehman S. U., Ahmed J., Bilal M. A comprehensive overview of recent advances in drought stress tolerance research in wheat (*Triticum aestivum* L.). *Asian J. Agr. Biol.* 2013, 1 (1), 29–37.
  12. Niu C.-F., Wei W., Zhou Q.-Y., Tian A.-G., Hao Y.-J., Zhang W.-K., Ma B., Lin Q., Zhang Z.-B., Zhang J.-S., Chen S.-Y. Wheat WRKY genes TaWRKY2 and TaWRKY19 regulate abiotic stress tolerance in transgenic Arabidopsis plants. *Plant Cell Environ.* 2012, 35, 1156–1170. doi: 10.1111/j.1365-3040.2012.02480.x.
  13. Okay S., Derelli E., Unver T. Transcriptome-wide identification of bread wheat WRKY transcription factors in response to drought stress. *Mol. Genet. Genomics.* 2014, 289 (5), 765–781. doi: 10.1007/s00438-014-0849-x.
  14. Zhu X., Liu S., Meng C., Qin L., Kong L., Xia G. WRKY transcription factors in wheat and their induction by biotic and abiotic stress. *Plant Mol. Biol. Rep.* 2013, 31 (5), 1053–1067. doi:10.1007/s11105-013-0565-4.
  15. Pandey S. P., Somssich I. E. The role of WRKY transcription factors in plant immunity. *Plant Physiol.* 2009, 150 (4), 1648–1655. doi: 10.1104/pp.109.138990.
  16. Tang Y. M., Liu M. Y., Gao S. Q., Zhang Z., Zhao X., Zhao C. P., Zhang F. T., Chen X. P. Molecular characterization of novel TaNAC genes in wheat and overexpression of TaNAC2a confers drought tolerance in tobacco. *Physiol. Plant.* 2012, 144, 210–224. doi:10.1111/j.1399-3054.2011.01539.x.
  17. Nakashima K., Yamaguchi-Shinozaki K., Shinozaki K. The transcriptional regulatory network in the drought response and its crosstalk in abiotic stress responses including drought, cold, and heat. *Front. Plant Sci.* 2014, 5, 1–7. doi: 10.3389/fpls.2014.00170.
  18. Xue G. P., Way H. M., Richardson T., Drenth J., Joyce P. A., McIntyre C. L. Overexpression of TaNAC69 leads to enhanced transcript levels of stress up-regulated genes and dehydration tolerance in bread wheat. *Mol. Plant.* 2011, 4 (4), 697–712. doi: 10.1093/mp/ssp013.
  19. Xia N., Zhang G., Liu X. Y., Cai G. L., Zhang Y., Wang X. J., Zhao J., Huang L. L., Kang Z. S. Characterization of a novel wheat NAC transcription factor gene involved in defense response against stripe rust pathogen infection and abiotic stresses. *Mol. Biol. Rep.* 2010, 37 (8), 3703–3712. doi: 10.1007/s11033-010-0023-4.
  20. Hassan N. M., El-Bastawisy Z. M., El-Sayed A. K., Ebeed H. T., Alla M. M. N. Roles of dehydrin genes in wheat tolerance to drought stress. *J. Advanced Res.* 2015, 6, 179–188. doi: 10.1016/j.jare.2013.11.004.
  21. Min D.-H., Zhang X.-H., Xu Z.-S., Zhao Y., Chen Y., Li L.-C., Chen M., Ma Y.-Z. Induction kinetics of a novel stress-related LEA gene in wheat. *Plant Mol. Biol. Rep.* 2012, 30 (6), 1313–1321. doi: 10.1007/s11105-012-0446-2.
  22. Amara I., Zaidi I., Masmoudi K., Ludevid M., Pagès M., Goday A., Brini F. Insights into late embryogenesis abundant (LEA) proteins in plants: from structure to the functions. *Amer. J. Plant Sci.* 2014, 5 (22), 3440–3455. doi: 10.4236/ajps.2014.522360.
  23. Ali-Benali M. A., Alary R., Joudrier P., Gautier M.-F. Comparative expression of five Lea Genes during wheat seed development and in response to abiotic stresses by real-time quantitative RT-PCR. *Biochim. Biophys. Acta.* 2005, 1730, 56–65. doi: 10.1016/j.bbaexp.2005.05.011.
  24. Hu B., Jin J., Guo A.-Y., Zhang H., Luo J., Gao G. GSDS 2.0: an upgraded gene feature visualization server. *Bioinformatics.* 2015, 31 (8), 1296–1297. doi: 10.1093/bioinformatics/btu817.
  25. Murray M., Thompson W. Rapid isolation of high molecular weight plant DNA. *Nucl. Acids Res.* 1980, 8 (19), 4321–4325.
  26. Singhal H., Ren Y. R., Kern S. E. Improved DNA electrophoresis in conditions favoring polyborates and Lewis acid complexation. *PLoS One.* 2010, 5 (6), 1–6. doi: 10.1371/journal.pone.0011318.
  27. Akinina G. E., Dugar V. N., Popov V. N. Statistical analysis of genetic data using software ARLEQUIN, PHILYP, CLANN, STRUCTURE. *Kharkiv: The Plant Production Institute named by V. Ya. Yuryev, NAAS.* 2014, 100 p. (In Russian).
  28. *International Wheat Genome Sequencing Consortium (IWGSC).* A chromosome-based draft sequence of the hexaploid bread wheat (*Triticum aestivum*) genome. *Science.* 2014, 345 (6194), 1251788. doi: 10.1126/science.1251788.

**ПОЛІМОРФІЗМ ГЕНІВ ДЕЯКИХ  
ТРАНСКРИПЦІЙНИХ ФАКТОРІВ,  
ЩО ПОВ'ЯЗАНІ З ПОСУХОСТІЙКІСТЮ  
ПШЕНИЦІ**

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Метою дослідження було вивчити поліморфізм попередньо відібраних локусів генів трьох транскрипційних факторів (*TaNAC2a*, *TaWRKY2*, *TaWRKY19*) та протеїн пізнього ембріогенеза (*LEA*) дегідрину (*Td29b*), пов'язаних зі стійкістю пшениці до посухи. Структуру генів та хромосомну локалізацію було встановлено за допомогою біоінформаційних підходів. З'ясовано, що гени *TaWRKY2* та *TaWRKY19* складаються з 4 екзонів і 3 інтронів, локалізованих на плечах 2BS та 1DS хромосоми, відповідно; *TaNAC2a* містить 2 екзони та 1 інтрон 7AS. Ген *Td29b* містить один екзон 3AS. У результаті використання полімеразної ланцюгової реакції не було виявлено поліморфізму для локусів генів *TaNAC2a*, *TaWRKY19* та *Td29b* за допомогою попередньо відібраних пар праймерів. Проте для локусу *TaWRKY2* виявлено поліморфні фрагменти. Скринінг поширення поліморфних локусів проводили для набору сортів пшениці та жита, давніх пшениць та міжвидових гібридів. Поліморфізм локусу *TaWRKY2* свідчить про наявність деяких інших алелів цього гена. Ці дані є важливими для подальших досліджень посухостійкості пшениці.

**Ключові слова:** *Triticum* spp., полімеразна ланцюгова реакція, фактори транскрипції, *TaNAC2a*, *TaWRKY2*, *TaWRKY19*, *LEA*, *Td29b*, посухостійкість.

**ПОЛИМОРФИЗМ ГЕНОВ НЕКОТОРЫХ  
ТРАНСКРИПЦИОННЫХ ФАКТОРОВ,  
СВЯЗАННЫХ  
С ЗАСУХОУСТОЙЧИВОСТЬЮ ПШЕНИЦЫ**

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Целью исследования было изучение полиморфизма предварительно отобранных локусов генов трех транскрипционных факторов (*TaNAC2a*, *TaWRKY2*, *TaWRKY19*) и протеин позднего эмбриогенеза (*LEA*) дегидрина (*Td29b*), связанных с устойчивостью пшеницы к засухе. Структура генов и хромосомная локализация определены с помощью биоинформационных подходов. Установлено, что гены *TaWRKY2* и *TaWRKY19* состоят из 4 экзонов и 3 интронов, 2BS и 1DS хромосомы, соответственно; *TaNAC2a* содержит 2 экзона и 1 интрон 7AS. Ген *Td29b* состоит из одного экзона 3AS. В результате использования полимеразной цепной реакции не было выявлено полиморфизма для локусов генов *TaNAC2a*, *TaWRKY19* и *Td29b* с помощью предварительно отобранных пар праймеров. Однако для локуса *TaWRKY2* обнаружены полиморфные фрагменты. Скрининг распространения полиморфных локусов проводили для набора сортов пшеницы и ржи, древних пшениц и межвидовых гибридов. Поліморфізм локусу *TaWRKY2* свідетельствует о наличии других аллелей этого гена. Эти данные важны для дальнейших исследований устойчивости пшеницы к засухе.

**Ключевые слова:** *Triticum* spp., полимеразная цепная реакция, факторы транскрипции, *TaNAC2a*, *TaWRKY2*, *TaWRKY19*, *LEA*, *Td29b*, засухоустойчивость.