

**POLYMORPHISM OF THE *TDF_076_2D* GENE CONFERRING
MODERATE *FUSARIUM* HEAD BLIGHT RESISTANCE AMONG
COMMON WHEAT (*TRITICUM AESTIVUM* L.) CULTIVARS OF THE
STEPPE ZONE OF UKRAINE**

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*Fifty six common wheat (*Triticum aestivum* L.) cultivars developed in the Steppe zone of Ukraine were studied using the marker INDEL1 on the basis of the sequence of NPR1-like *TDF_076_2D* gene associated with moderate type II resistance to *Fusarium* head blight. We detected the resistance-associated allele in 40% of the cultivars studied. The cultivars with the resistance-associated allele of the marker may be used as the source of moderate resistance against *Fusarium* head blight.*

Key words: *common wheat, Fusarium head blight, molecular markers*

Fusarium head blight is a harmful disease of crops caused by necrotrophic fungi [2, 8, 9, 25]. Species *F. graminearum*, *F. sambucinum*, *F.culmorum*, *F. avenaceum*, *F. sporotrichiella*, *F. moniliforme* are mostly represented in Ukrainian territory and one of the predominant species is *F. graminearum* [2, 4]. The possibility and value of infestation and wheat yield losses from *Fusarium* head blight depend on weather conditions, physical parameters and the flowering time of plants of each cultivar [27]. The factors of resistance against the fungi of this genus are considered to be quantitative (QTL) meaning their additive effect providing the measured level of resistance in comparison with the plants lacking such factors [18, 21, 27]. Classification of *Fusarium* head blight resistance is following: type I (resistance against the primary infection), type II (resistance against symptom spread in head),

type III (resistance against seed infestation), type IV (tolerance) and type V (toxin resistance by their decomposition) [10, 19]. Well studied sources of type II resistance are the Chinese cultivars Sumai-3, Wuhan-1 and Ning-7840. There are five regions associated with such resistance type identified among which the QTLs on chromosomes 3B and 5A are the most important expressing dominantly and additively [15, 18, 23]. Association mapping of the resistance factors among European winter wheat cultivars was performed lately with use of significant amount of microsatellite markers. New loci affecting resistance to *Fusarium* fungi positively as well as negatively were discovered [27].

The research of model plants has been performed for evaluation of role of genes coding compounds with regulatory functions in pathogenesis of necrotrophic and, in particular, *Fusarium* fungi [12, 13, 20]. An important role in the regulation of plant interaction with pathogens belongs to the “nonexpressor of pathogenesis-related proteins 1” (*NPR1*) gene [11, 14, 24]. It was shown that for *Arabidopsis thaliana* the functional product of the gene plays the key role in the PR1 gene expression and switching between jasmonate-dependent and salicylate-dependent defence response [22, 26]. The role of *NPR1* in the pathogenesis of the *Fusarium* fungi was also carried out through transferring the gene from *A. thaliana* into wheat and studying of the transgenic plants interaction with the fungi. It was discovered that on juvenile stage the gene confers increased susceptibility to *Fusarium asiaticum* while for adult plants on the contemporary it confers resistance which testifies to bifunctional role of the gene [17].

For moderately resistant to *Fusarium* head blight European wheat genotypes Capo and ‘SVP72017’ research of expression patterns of plants interaction with *F. graminearum* та *F. culmorum* was carried out. It was discovered that the allelic states of the homoeologous *NPR1*-like genes on wheat chromosomes 2D and 2A (*TDF_076_2D* та *TDF_076_2A*) conferring with type II resistance at 14,2% and 3% correspondingly [9]. The allelic states of the genes differ by three insertions-deletions in introns. In particular with the primers flanking the first insertion-deletion (from the 5'-end, further *INDELI*) the fragments of 212 bp in size are amplified in case of the both allelic states of the *TDF_076_2A* gene but in case of susceptibility associated

allelic state of the *TDF_076_2D* gene (further allele 1) the bands of 221 bp in length are synthesized and in case of resistance-associated allelic state (further allele 2) the length of bands is also 212 bp [9].

The aim of the research was to characterize the common spring wheat cultivars of Ukrainian breeding by the marker of the *TDF_076_2D* gene conferring moderate resistance to *Fusarium* head blight.

Materials and methods. The samples of DNA extracted from seeds of 56 wheat cultivars developed in the Plant Breeding and Genetics Institute of the National Academy of Agrarian sciences of Ukraine in Odessa (further PBGI). The cultivars are listed on the table. DNA was extracted of crashed 25-35 mg batches obtained from five seeds of each cultivar. The extraction was carried out using Diatom™ DNA Prep100 Kits according to standard technique. PCR was performed in the amplifier 2720 GeneAMP System using GenPak® PCR Core Kits according the producer's technique. To determine the allelic state of the marker *INDEL1* the primers pair INDEL1-F (5'- TCATGCAGTGTTGCTTGATCT-3') and INDEL1-R (5'- CCATTCAGTTGAGCAACTTCC-3') was used. The bands of 212 bp in length were obtained in case of allele 2 and the bands of 212 bp and 221 bp in length – in case of allele 1 of the marker [23].

The bands obtained as a result of PCR were separated in 10% polyacrylamide gel (PAAGE) and stained in 0.01% AgNO₃ solution according to standard technique [5].

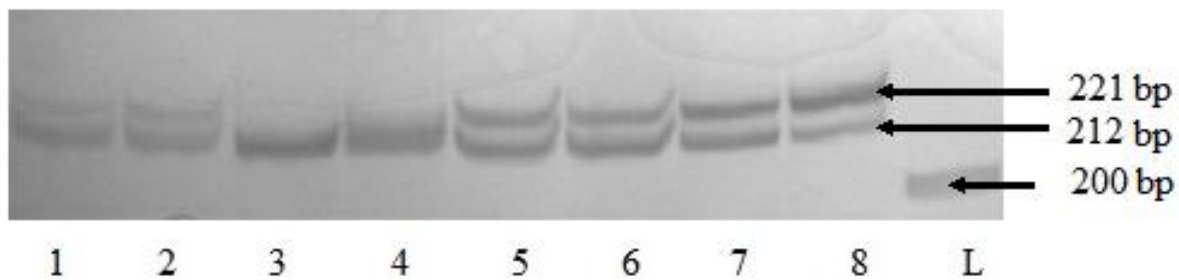
The results of the research are listed on the Table.

The example of the electrophoregrams is on the Figure

In 22 of the cultivars studied the allele associated with moderate type II resistance to *Fusarium* head blight at 14.2% which is about 40% of the cultivars (the table)

Allelic state of the marker *INDEL1* of the *TDF_076_2D* gene in the cultivars studied

Cultivar	Allele of the marker	Cultivar	Allele of the marker
Albatros-odesskii	1	Odesskaya-krasnokolosaya	2
Antonovka	1	Oksamitna	1
Bezmezhna	2	Otaman	2
Blagodarka-odeska	1	Pisanka	1
Borviy	2	Podyaka	2
Bunchuk	2	Poliovik	1
Dalnitskaya	2	Poshana	1
Dobropolka	1	Povaga	1
Dyuk	1	Selyanka	2
Epokha-odeska	1	Sirena-odesskaya	2
Goduvalnytsya-odeska	2	Skarbnitsa	1
Golubka-odeska	1	Sluzhnitsya-odeska	1
Gospodynya	1	Suputnitsya	2
Gurt	2	Turunchuk	1
Istyna	1	Ukrainka-odesskaya	1
Kiriya	2	Uzhinok	2
Knyaginya-Olga	1	Vatazhok	1
Kosovytsya	1	Vdala	1
Kuyalnik	2	Viktoriya	1
Lastivka-odeska	1	Zamozhnist	1
Lelya	2	Zaporuka	2
Liana	2	Zemlyachka-odesskaya	1
Liona	1	Zhayvir	2
Litanivka	1	Zhuravka	2
Misiya-odeska	1	Zmina	1
Nebokrai	1	Znahidka-odeska	1
Nikoniya	1	Zorepad	2
Odesskaya-267	2	Zustrich	1



The electrophoregram of bands obtained in PCR with primers flanking the marker *INDEL1* in 10% PAAGE. 1 – Albatros; 2 – Antonivka; 3 – Bezmejna; 4 – Znakhidka Oadeska; 5 – Misiya Odeska; 6 – Kosovytsia; 7 – Gospodynia; 8 – Zmina; L – 100 bp Ladder

According to comparison of the marker allelic states with cultivars resistance/sensitivity allocated by different researchers in phytopathological way (by direct inoculation of wheat plants with *Fusarium* fungi) [22, 24, 27] no strait correlation with the allelic state of the marker *INDEL1* was observed. Probably it might be explained by other resistance/susceptibility factors expressing stronger under the field tests conditions. According to association mapping the possible QTL caused by the *TDF_076_2D* gene was detected under different conditions [27]. So, although the allele of interest of the marker studied and the gen is present among wheat cultivars of Ukrainian breeding, its selection for development of new cultivars without testing by molecular-genetic markers is problematic. At the same time the allele confers 14.2% lower infestation of wheat heads by *Fusarium* head blight fungi compared to carriers of the susceptible allele and does not cause the other plant characteristics as it is peculiar for significantly resistant but non-productive cultivar Sumai-3 [Dr. George Fedak, personal communication].

Thirty cultivars carrying allele 2 of the marker also have resistance-associated allele of the molecular marker of the *Lr34/Yr18/Pm38/Sr57* gene conferring moderate resistance to rust fungi, powdery mildew and barley yellow dwarf [3, 6]. In the 16 cultivars with allele 2 of the marker *INDEL1* the allele of the marker *fcp623* of the *Tsn1* gene associated with insensitivity to toxin A of *Pyrenophora tritici-repentis* had been discovered earlier [1].

Thus the cultivars found out to carry the resistance-associated allele of the marker may be used as a source of moderate resistance to *Fusarium* head blight and other phytopathogens.

Conclusions

1. The allele of moderate type II resistance to *Fusarium* head blight was detected for 40% cultivars studied
2. The cultivars of common wheat of Ukrainian breeding may be sources of complex moderate resistance against biotrophic and necrotrophic phytopathogens.
3. Some cultivars deserve to be studied with markers associated with the QTLs and the other candidate-genes associated with that and other types of resistance to discover the genetic background for their interaction with *Fusarium* fungi.

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**ПОЛІМОРФІЗМ МАРКЕРА ГЕНА *TDF_076_2D* ПОМІРНОЇ СТІЙКОСТІ
ПРОТИ ФУЗАРІОЗУ КОЛОСУ СЕРЕД СОРТІВ ПШЕНИЦІ М'ЯКОЇ
(*TRITICUM AESTIVUM* L.) СТЕПОВОЇ ЗОНИ УКРАЇНИ**

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*Досліджували 56 сортів озимої пшениці (*Triticum aestivum* L.), створених у Степовій зоні України, за допомогою маркера INDEL1 на основі послідовності подібного до NPR1 гена TDF_076_2D, асоційованого з помірною стійкістю проти фузаріозу колоса за типом II. Визначили асоційований зі стійкістю алель у 40% досліджених сортів. Сорти, у яких був виявлений пов'язаний зі стійкістю алель маркера, можуть використовуватись як джерело помірної стійкості проти фузаріозу.*

Ключові слова: м'яка пшениця, фузаріоз колоса, молекулярні маркери.

**ПОЛІМОРФИЗМ МАРКЕРА ГЕНА *TDF_076_2D* УМЕРЕННОЙ
УСТОЙЧИВОСТИ К ФУЗАРИОЗУ КОЛОСА СРЕДИ СОРТОВ
ПШЕНИЦЫ МЯГКОЙ (*TRITICUM AESTIVUM* L.) СТЕПНОЙ ЗОНЫ
УКРАИНЫ**

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Исследовали 56 сортов озимой пшеницы (Triticum aestivum L.), созданных в Степной зоне Украины, при помощи маркера INDEL1 на основе последовательности подобного NPR1 гена TDF_076_2D, ассоциированного с умеренной устойчивостью к фузариозу колоса по типу II. Определена ассоциированная с устойчивостью аллель у 40% исследованных сортов. Сорта, у которых была выявлена связанная с устойчивостью аллель маркера, могут быть использованы как источники умеренной устойчивости к фузариозу.

Ключевые слова: *мягкая пшеница, фузариоз колоса, молекулярные маркеры*