ISSN 2518-1467 (Online),
ISSN 1991-3494 (Print)

ҚАЗАҚСТАН РЕСПУБЛИКАСЫ
ҰЛТТЫҚ ҒЫЛЫМ АКАДЕМИЯСЫНЫҢ

Х А Б А Р Ш Ы С Ы

ВЕСТНИК

НАЦИОНАЛЬНОЙ АКАДЕМИИ НАУК
РЕСПУБЛИКИ КАЗАХСТАН

THE BULLETIN

THE NATIONAL ACADEMY OF SCIENCES
OF THE REPUBLIC OF KAZAKHSTAN

PUBLISHED SINCE 1944

2

MARCH – APRIL 2020

ALMATY, NAS RK

NAS RK is pleased to announce that Bulletin of NAS RK scientific journal has been accepted for indexing in the Emerging Sources Citation Index, a new edition of Web of Science. Content in this index is under consideration by Clarivate Analytics to be accepted in the Science Citation Index Expanded, the Social Sciences Citation Index, and the Arts & Humanities Citation Index. The quality and depth of content Web of Science offers to researchers, authors, publishers, and institutions sets it apart from other research databases. The inclusion of Bulletin of NAS RK in the Emerging Sources Citation Index demonstrates our dedication to providing the most relevant and influential multidiscipline content to our community.

Қазақстан Республикасы Ұлттық ғылым академиясы "ҚР ҰҒА Хабаршысы" ғылыми журналының Web of Science-тің жаңаланған нұсқасы Emerging Sources Citation Index-те индекстелуге қабылданғанын хабарлайды. Бұл индекстелу барысында Clarivate Analytics компаниясы журналды одан әрі the Science Citation Index Expanded, the Social Sciences Citation Index және the Arts & Humanities Citation Index-ке қабылдау мәселесін қарастыруда. Web of Science зерттеушілер, авторлар, баспашылар мен мекемелерге контент тереңдігі мен сапасын ұсынады. ҚР ҰҒА Хабаршысының Emerging Sources Citation Index-ке енуі біздің қоғамдастық үшін ең өзекті және беделді мультидисциплинарлы контентке адалдығымызды білдіреді.

НАН РК сообщает, что научный журнал «Вестник НАН РК» был принят для индексирования в Emerging Sources CitationIndex, обновленной версии Web of Science. Содержание в этом индексировании находится в стадии рассмотрения компанией Clarivate Analytics для дальнейшего принятия журнала в the Science Citation Index Expanded, the Social Sciences Citation Index и the Arts & Humanities Citation Index. Web of Science предлагает качество и глубину контента для исследователей, авторов, издателей и учреждений. Включение Вестника НАН РК в Emerging Sources Citation Index демонстрирует нашу приверженность к наиболее актуальному и влиятельному мультидисциплинарному контенту для нашего сообщества.

«Қазақстан Республикасы Ұлттық ғылым академиясының Хабаршысы».
ISSN 2518-1467 (Online),
ISSN 1991-3494 (Print)
Меншіктенуші: «Қазақстан Республикасының Ұлттық ғылым академиясы»РҚБ (Алматы қ.).
Қазақстан республикасының Мәдениет пен ақпарат министрлігінің Ақпарат және мұрағат комитетінде 01.06.2006 ж. берілген **№5551-Ж** мерзімдік басылым тіркеуіне қойылу туралы куәлік.

Мерзімділігі: жылына 6 рет.
Тиражы: 2000 дана.

Редакцияның мекенжайы: 050010, Алматы қ., Шевченко көш., 28, 219 бөл., 220, тел.: 272-13-19, 272-13-18,
http://www.bulletin-science.kz/index.php/en/

Типографияның мекенжайы: «NurNaz GRACE», Алматы қ., Рысқұлов көш., 103.

«Вестник Национальной академии наук Республики Казахстан».
ISSN 2518-1467 (Online),
ISSN 1991-3494 (Print)
Собственник: РОО «Национальная академия наук Республики Казахстан» (г. Алматы).
Свидетельство о постановке на учет периодического печатного издания в Комитете информации и архивов Министерства культуры и информации Республики Казахстан **№5551-Ж**, выданное 01.06.2006 г.

Периодичность: 6 раз в год.
Тираж: 2000 экземпляров.

Адрес редакции: 050010, г. Алматы, ул. Шевченко, 28, ком. 219, 220, тел. 272-13-19, 272-13-18.
http://www.bulletin-science.kz/index.php/en/

Адрес типографии: «NurNazGRACE», г. Алматы, ул. Рыскулова, 103.

Bulletin of the National Academy of Sciences of the Republic of Kazakhstan.
ISSN 2518-1467 (Online),
ISSN 1991-3494 (Print)
Owner: RPA "National Academy of Sciences of the Republic of Kazakhstan" (Almaty).
The certificate of registration of a periodic printed publication in the Committee of Information and Archives of the Ministry of Culture and Information of the Republic of Kazakhstan N 5551-Ж, issued 01.06.2006.

Periodicity: 6 times a year.
Circulation: 2000 copies.

Editorial address: 28, Shevchenko str., of. 219, 220, Almaty, 050010, tel. 272-13-19, 272-13-18,
http://www.bulletin-science.kz/index.php/en/

Address of printing house: «NurNaz GRACE», 103, Ryskulov str, Almaty.

BULLETIN OF NATIONAL ACADEMY OF SCIENCES
OF THE REPUBLIC OF KAZAKHSTAN
ISSN 1991-3494
Volume 2, Number 384 (2020), 29 – 35 **https://doi.org/10.32014/2020.2518-1467.38**

UDC: 632.42: 633:576.3/7.086.83:581.4
IRSTI 68.37.31; 34.23.37; 34.15.23

A. Kokhmetova[1,2], M. Atishova[1]

[1]Institute of Plant Biology and Biotechnology, Almaty, Kazakhstan;
[2]Kazakh National Agrarian University, Almaty, Kazakhstan.
E-mail: gen_kalma@mail.ru, maki_87@mail.ru

IDENTIFICATION WHEAT GENOTYPES RESISTANT TO TAN SPOT *PYRENOPHORA TRITICI-REPENTIS*

Abstract. Intensified wheat production, changes in cultural practices including shifts from conventional tillage to reduced tillage practices, and wheat monoculture involving cultivation of susceptible cultivars has resulted in development of tan spot to epidemic proportions in Kazakhstan. *Pyrenophora tritici-repentis,* causal agent of tan spot on wheat. In recent years, there has been increasing distribution and harmfulness of *P. tritici-repentis* on wheat. The aim of the study was to identify and select wheat germplasm resistant to tan spot *P. tritici-repentis* using molecular markers. The results of field evaluation showed resistant reaction to tan spot in 76 wheat varieties (68%). Molecular screening of wheat germplasm was carried out based on the reaction to the fungal inoculum and to host-specific toxins (HST) produced by the *P. tritici-repentis*. The wheat germplasm insensitive to the toxins HST Ptr ToxA and Ptr ToxB was selected. As a result of molecular screening of 111 wheat genotypes using SSR marker *Xfcp623* linked to insensitivity gene to the selective toxin Ptr ToxA of tan spot, 31 carriers of effective *tsn1* gene were identified, which accounted for 27,9% of the genotypes studied. Ten samples of wheat (Jubileynaya 60, TOO11/TOOOO7, F3.71/TRM/VORONA/3/OC14, NANJTNG 82149 KAUZ, ECHA/LI115, Akmola 2, Kazakh-stanskaya rannespelaya, Kazakhstanskaya 25, 428g/MK-122A and 190-Naz/GF55) are characterized with complex resistance to the races Ptr 1 and 5, as well as to 2 toxins (ToxA and ToxB) and to the *Septoria nodorum blotch* isolate SNB7k. 20 promising wheat lines resistant to tan spot were selected. These genotypes also showed a moderate and high level of field resistance and recommended to use in the breeding programs for resistance to tan spot.

Key words: wheat, tan spot, resistance genes, molecular markers.

Introduction. The Kazakhstan is one of the major wheat producers in the world. However, diseases and pests play an important role in yield reduction in Kazakhstan. Tan spot is one of the most harmful diseases of soft and hard wheat in many agricultural regions of the world including Kazakhstan. The causative agent of this disease is the homotallic ascomycete *P. tritici-repentis* (Died.) Drechsler; imperfect stage *Drechslera tritici-repentis* (Died) Shoem.In Central Asia the disease was discovered in 1980s in Tajikistan and in recent years it has spread throughout Central Asia and Kazakhstan [1,2,3].

Five toxins (Ptr ToxA, ToxB, ToxC, and 2 toxins, united in a group Ptr ToxD) have been identified; three of them are well characterized while two are tentative. The *Tsn1* – Ptr ToxA, *Tsc2* – Ptr ToxB, and *Tsc1* – Ptr ToxC interactions have all been shown to play significant roles in the development of tan spot in common (hexaploid) wheat [4,5].

P. tritici-repentis isolates has been grouped in 8 races based on their ability to produce two distinct symptoms, necrosis and/or chlorosis on appropriate wheat differential [6]. Races 1 and 2 of *P. tritici-repentis* produce a host-selective toxin (HST), Ptr ToxA that induces necrosis symptoms [7]. Ptr ToxA is a well-characterized HST of *P. tritici-repentis* and the gene that encodes for Ptr ToxA production including the endogenous promoter has been cloned [8]. *Tsn1,* a single dominant gene that conditions sensitivity to Ptr ToxA on the long arm of chromosome 5B, has recently been cloned [9, 10]. Eight major genes for resistance to tan spot (*Tsr1-Tsr6, Tsr Har, TsrAri)* have been characterized and designated [11]. They located on chromosomes 2BS, 3A, 3BL, 3DS and 5BL.

In the CIS countries, the pathogen in Moldova, Ukraine, Belarus, Central Asia and Kazakhstan was found [12]. There are a number of studies on the racial composition of *P. tritici-repentis* in Asia and Kazakhstan. The greatest diversity was observed in Azerbaijan, where races 1, 2, 3, 5, 7, and 8 were identified, and in Syria, where races 1, 3, 5, 7, and 8 were detected; the little variation was found in the virulence of isolates from Kazakhstan (race 1 and race 2) [1]. It has been revealed that race 1 is the most widespread race in Central Asia and Kazakhstan (87%), and races 2, 3 and 4 were minor [13]. It was found that races 1 and 2 dominate in the North Caucasus in Russia, and races 1 and 8 *P. tritici-repentis* dominate in Kazakhstan [14]. The results of previous studies indicate the possibility of identifying recessive alleles of genes resistant to *P. tritici-repentis* toxins using molecular markers [15,16]. Since Ptr ToxA toxin can be synthesized in races 1, 2, 7, and 8, and race 1 is widespread in Kazakhstan, the present study is dedicated to the search for gene carriers of resistance to Ptr ToxA toxin.

Currently, there is an increasing prevalence and increased damage of wheat by tan spot in Kazakhstan. The majority of commercial wheat cultivars currently grown in Kazakhstan are still susceptible to tan spot. The presence and activation of *P. tritici-repentis* requires the rapid development of new varieties resistant to disease. The relevance of such studies is due to the need to develop genetically diverse sources of resistance, donors and promising wheat lines that can be used in breeding of resistant varieties. The main objective of this study was the identification of wheat genotypes resistant to tan spot *P. tritici*-repentis.

Materials and methods. A collection of 111 common wheat germplasms, including elite lines and cultivars of wheat from Kazakhstan, Russia and CIMMYT evaluated for *P. tritici-repentis* resistance and were characterized using the molecular markers. The field experiments conducted at the Southeast Kazakhstan, Kazakh Research Institute of Agriculture and Plant Growing (KRIAPG), Almalybak (43°13′N, 76°36′E, and 789 masl), Almaty Reg., in the 2017-2018 crop seasons. The experiment conducted in a completely randomized design with three replications. The field evaluation of adult plants assessed three times according to the scale for appraising the foliar intensity of diseases [17] in the modification for tan spot [18]. The standard international wheat differentials included Glenlea and Salamouni cultivars, as well as 6B662 and 6B365 lines and used in the field and seedling tests.

Selection of resistant wheat genotypes based on germplasm screening against fungal inoculum and HST selective toxin of *P. tritici-repentis* was carried out on the basis of HST tests. The purified HST toxins provided by foreign collaborator, Dr. Ali S., University of South Dakota, USA. Toxins injected into wheat leaves. The presence or absence of necrosis / chlorosis symptoms on the infiltrated side of the leaf indicates sensitivity or insensitivity to HST.

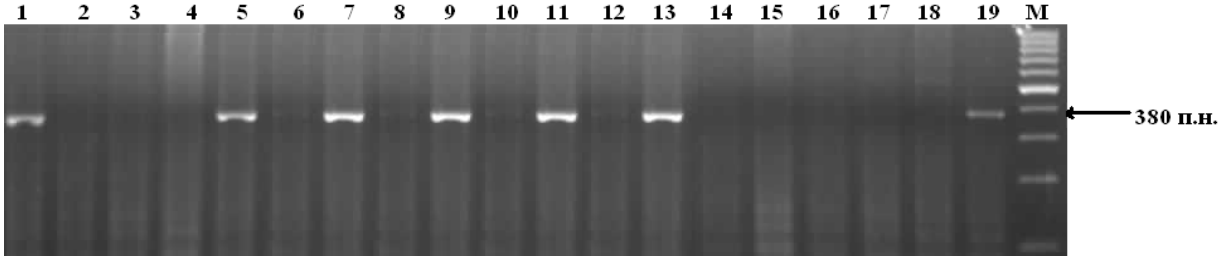
Genomic DNA extracted at two-leaf seedling stage for each individual plant using the CTAB method [19]. DNA concentration measured using a spectrophotometer SmartSpecTMPlus (Bio RAD). The DNA concentration for each sample was adjusted to 30 ng/µl. Samples were genotyped using the SSR marker *Xfcp623* designed to detect alleles of the *Tsn1* gene. The sequence of primers and PCR reaction conditions are given by [10]. The carriers of the *Tsn1* gene was also were detected using PCR protocol for SSR marker *Xfcp1* published at the WheatCAP website http://maswheat.ucdavis.edu/protocols]. The amplification products were separated on 2%-agarose gels, to determine the length of the amplification fragment 100 bp DNA Ladder (Ferments, Lithuania) was used. Gels were visualized on Gel Documentation System (Gel Doc XR+, BIO-RAD, Hercules, USA) for documentation of allele types in cultivars. Wheat entries 6B662 and Glenlea served as positive and negative controls, respectively.

Results. The results of field evaluation the resistance of 111 genotypes of wheat to tan spot showed, that immune and resistant (0 - 10%) were 76 varieties (68%) of the total number of wheat entries. The immune type of reaction to tan spot demonstrated 16 varieties of wheat, including Princes, Koksu, Opaks 18, Odesskaya 120, Pyrotriks-50, Polovchanka, Raminal, Rasad, Sanzar 8, Taza, Tyniysh, Umanka, Sharora and Erythrospermum 78. The most susceptible varieties were Azharly, Basar, Batyr, Bulava, Bezostaya 1, Kyzylbidai, Lutescens 9 and others. Thus, the results of the field evaluation of tan spot allowed to rank the material studied on the level of resistanc. Immune and resistant reaction type to tan spot showed 76 wheat cultivars.

In previous studies, we used markers *Xfcp1, Xfcp393, Xfcp394* and *Xfcp620*. Our results using these markers enabled the screening of a large number of wheat accessions for resistance to the Ptr ToxA [15, 16]. However, the repeatability and matching molecular data and phytopathological evaluations was not

always complete. With further development of PCR, we found, that the most adequate for the identification of carriers of resistance to tan spot is a molecular marker *Xfcp623* [20,21,22]. This functional marker was developed by Faris et al., 2013 as a result of mapping, sequencing and cloning of the *Tsn1* locus (intron 5). From a practical point of view this marker is the most effective and reliable [4].

In this regard, to identify genes *Tsn1* and *tsn1* in the studied wheat material the SSR marker *Xfcp623* was used. As an example, the results of the PCR amplification products, when the *Xfcp623* primers were tested on 17 wheat genotypes, are shown in the figure. Marker *Xfcp623* formed fragment size 380 bp, which is associated with a dominant allele Tsn1 sensitive to ToxA *P. tritici-repentis.*



DNA amplification profile for of wheat cultivars and lines obtained with diagnostic marker *Xfcp623* linked to the *Tsn1* gene sensitive to Ptr ToxA. 1– Kazakhstanskaya 4, 2 – Kazakhstanskaya 19, 3 – Omskaya 28, 4 – Omskaya 35, 5 – Zhalyn, 6 – Kargaly 9, 7 – Karaspan, 8 – Akmola 3, 9 – Severyanka, 10 – Albidum 31, 11 – BR35/BR14, 12 – CEP80111/VEE, 13 – ALTAR 84/AE, 14 – TPAP#1/OPATA, 15 – CROC 1AE, 16 – TALHUENJNJA, 17 – Kenzhegaly, 18 – Salamouni (the insensitive control for toxin Ptr ToxA, carrier of recessive gene t*sn1*), 19 – Glenlea (negative control), M – marker for molecular weight (Gene Ruler 100 bp DNA Ladder)

As shown in figure, 6 out of 17 samples (Kazakhstanskaya 4, Zhalyn, Karaspan, Severyanka, BR35/BR14, ALTAR 84/A) had polymorphic band identical to molecular marker *Xfcp623*, linked the dominant *Tsn1* allele conferring toxin Ptr ToxA sensitivity. Ten entries including Kazakhstanskaya 19, Omskaya 28, Omskaya 35, Kargaly 9, Akmola 3, Albidum 31, CEP80111/VEE, TPAP#1/OPATA, CROC 1AE, TALHUENJNJA, and Kenzhegaly showed null-allele, linked the recessive *tsn1* allele conferring toxin Ptr ToxA insensitivity. Thus, of the 17 analyzed promising wheat lines 10 wheat genotypes insensitive to the ToxA toxin of *P. tritici-repentis* were identified.

As a result of molecular screening of total 111 wheat genotypes using SSR marker *Xfcp623*, 31 carriers of *tsn1* gene accounted for 27,93% of the samples studied were identified. It should be noted that the phytopathological evaluation of wheat samples in which the genes for resistance to tan spot toxins were found, showed a high and moderate degree of field resistance (5-15%) to diseases. The association between resistance to tan spot and the presence of a toxin toxicity gene ToxA, tsn1 was noted. The correspondence between phenotypic and molecular analyzes was 95-98%.

Molecular screening of wheat germplasm and screening based on the reaction to the fungal inoculum and to HST toxins allowed selecting of wheat genotypes resistant to leaf spot diseases based. As an example, the table shows the 10 most resistant and the 10 most sensitive genotypes to the the 2 races and toxins of P. tritici-repentis (Ptr), and to the race of S. nodorum blotch (SNB7k). In the table the results of PCR to identify genes for resistance to toxin Ptr Tox A, as well as the response to fungal inoculum: race 1 of Ptr, toxin HST ToxA, race 5 of Ptr, toxin HST ToxV, race of Septoria SNB7k has shown.

In PCR using SSR marker *Xfcp1* first 10 genotypes formed DNA fragment of 374 bp which is associated with the presence of the recessive allele *tsn1*, insensitive to Ptr ToxA, and the next 10 wheat samples formed fragment of 402 bp, typical for dominant allele *Tsn1*, susceptible to Ptr ToxA. Thus, the complex resistance to two races of Ptr (1 and 5), two toxins (HST ToxA and HST ToxB) and the race of Septoria SNB7k were found in Kazakhstani commercial varieties (Yubileynaya 60, Jubileynaya 60, Akmola 2, Kazakhstanskaya rannespelaya, Kazakhstanskaya 25), as well as in developed in our laboratory advanced lines (428g/MK-122A and 190-Naz/GF55), and aslso lines from CIMMYT (TOO11/TOOOO7, F3.71/TRM/ VORONA/3/OC14, NANJTNG 82149 KAUZ and ECHA/LI115). Using the technology of selection for HST-toxins and marker assisted selection, a new wheat material adapted for Kazakhstan, resistant to the aggressive toxins of LSD, was selected.

Selection of disease resistant wheat genotypes based on germplasm screening using inoculum of fungi and HST toxins

Name of entry	Marker *Xfcp1*		Reaction to inoculum of Ptr				
	I	S	Race 1	HST ToxA	Race 5	HST ToxB	SNB7k
Jubileynaya 60	374 п.н.	–	1	I	1	I	1
TOO11/TOOOO7	374 п.н.	–	1	I	2	I	1
F3.71/TRM/VORONA/3/OC14	374 п.н.	–	1	I	2	I	1
NANJTNG 82149 KAUZ	374 п.н.	–	1	I	2	I	2
ECHA/LI115	374 п.н.	–	1	I	2	I	2
Akmola 2	374 п.н.	–	3	I	2	I	2
Kazakhstanskaya rannespelaya	374 п.н.	–	1	I	2	I	2
Kazakhstanskaya 25	374 п.н.	–	2	I	1	I	3
428g/MK-122A	374 п.н.	–	1	I	1	I	1
190-Naz/GF55	374 п.н.	–	1	I	1	I	1
Bogarnaya 56	–	402 п.н.	4	S	2	I	4
Bulava	–	402 п.н.	4	S	2	I	5
Mereke75	–	402 п.н.	4	S	1	I	5
Nureke	–	402 п.н.	4	S	2	I	4
Steklovinaya 24	–	402 п.н.	3	S	2	I	4
Saratovskaya 42	–	402 п.н.	5	S	2	I	4
Farabi	–	402 п.н.	4	S	2	I	4
Karlygash	–	402 п.н.	4	S	3	I	2
Gozgon	–	402 п.н.	4	S	1	I	5
Kysylbiday	–	402 п.н.	4	S	2	I	2

Note: Ptr – tan spot, SNB – Septoria, HST ToxA and ToxB – tan spot toxins, SNB7k – race od Septoria; I – insensitivity, S – sensitivity to toxins.

Discussion. ToxA toxin is synthesized by races 1, 2, 7, and 8, and the ToxB toxin is synthesized by races 2, 7, and 8 [23]. Previous studies have shown that races 1 and 8 are widespread in Kazakhstan [14]. The comparative analysis of phenotypic and molecular data on the presence or absence of resistance genes and expression of resistance to disease in the field was carried out. Sensitivity to the toxin Ptr ToxA produced by isolates of race 1 (nec+chl-), controlled by one dominant gene *Tsn1*, located on the long arm of chromosome 5B [10]. It is assumed that the sensitivity to the toxin Ptr ToxA and susceptibility to necrosis of the fungus is controlled by the same gene [24]. The *Tsn1* gene responsible for sensitivity to the protein components of plant toxins, Ptr ToxA, produced by the fungus *P. tritici-repentis*; this toxin is one of the main factors associated with the development of *P. tritici-repentis* in susceptible wheat genotypes [25].

In this regard, the present study was aimed at assessing varieties and promising lines of wheat from Kazakhstan and abroad for resistance to race 1 and race 5 and sensitivity to toxins Ptr ToxA and Ptr ToxB P. tritici-repentis. The results of field evaluation showed resistant reaction to tan spot in 76 wheat varieties (68%); almost the same results were obtained in previous study [21]. Results molecular screening for resistance to tan spot obtained in this study contradict the previous data related to field evaluation to tan spot in Kazakhstan and in South Russia that showed a low level of resistance among most commercial wheat cultivars [26]. Based on our results, it is important to carry out breeding for resistance to most common races of pathogen.

For successful selection of immunity, international cooperation with CIMMYT is of great importance. Our studies have shown that varieties resistant to tan spot must be developed using a variety of germplasm. Broad based tan spot resistance sources from the CIMMYT may contribute to the resistance observed in our study and can be utilized to develop cultivars with broad-genetic base durable resistance to wheat tan spot.

Conclusion. Results of field phytopathological screening of 111 commercial wheat varieties tan spot allowed ranking the studied material according to the level of resistance. The immune and resistant type of reaction to tan spot showed 76 varieties of wheat. As a result of molecular screening of 111 wheat genotypes using SSR marker linked to insensitivity genes to the HST Ptr ToxA, 31 carriers of effective tsn1 gene were identified, which accounted for 27,9% of the 111 samples studied. Based on the results of molecular screening and the study of the reaction of wheat samples to the inoculum and to the toxins of diseases, 10 disease-resistant lines have been identified. These genotypes also showed a moderate and high level of field resistance and recommended to use in the breeding programs for resistance to tan spot. The results of study are used in wheat breeding programs for tan spot resistance with the Marker Assisted Selection.

Acknowledgements. This study was supperted by the Kazakhstan Ministry of Education and Sciences, project AP05132540. The authors are sincerely grateful to Dr. Ali S. (Wheat Pathologist, Department of Plant Pathology, North Dakota State University, Fargo, USA) for advice on research.

А. Кохметова[1,2], М. Атишова[1]

[1]Өсімдіктер биологиясы және биотехнологиясы институты, Алматы, Қазахстан;
[2]Қазақ ұлттық аграрлық университеті, Алматы, Қазахстан

ПИРЕНОФОРОЗҒА *PYRENOPHORA TRITICI-REPENTIS* ТӨЗІМДІ БИДАЙ ГЕНОТИПТЕРІН ИДЕНТИФИКАЦИЯЛАУ

Аннотация. Бидай өндірісінің қарқынды дамуы, тәжірибие алаңында бидай өндірісінің өзгерістері, дәстүрлі топырақты өңдеуден минималды тәжірибиеге өту және төзімсіз бидай сорттарының монокультура ретінде өсіру пиренофороздың дамуының эпидемия көлеміне акеліп соқтырады. Пиренофороз дүниежүзінің көптеген ауылшаруашылық аймақтары мен Қазақстанның жұмсақ және қатты бидайының аса қауіпті ауруларының бірі болып табылады. Пиренофороздың қоздырушысы *Pyrenophora tritici-repentis*. Соңғы жылдары бидайда *P. tritici-repentis*-тің дамуы мен қауіптілігі бақылануда. Қазіргі таңда бес токсин (Ptr ToxA, ToxB, ToxC және 2 токсин, Ptr ToxD тобына біріктірілген) идентификацияланған.

Зерттеудің мақсаты – молекулалық әдістерді қолданып, *P. tritici-repentis* пиренофорозға төзімді бидай гермоплазмаларын анықтау және іріктеу. Дала жағдайында бағалау нәтижесінде бидайдың 76 сорты (68 %) пиренофорозға төзімді реакция танытты. Пиренофорозға 16 бидай сорты иммунды реакция типін көрсетті, олар Princes, Коксу, Опакс 18, Одесская 120, Пиротрикс-50, Половчанка, Раминал, Расад, Санзар 8, Таза, Тунгыш, Уманка, Шарора және Эритроспермум 78. Ажарлы, Басар, Батыр, Булава, Безостая 1, Кызылбидай, Лютеценс 9 және басқада сорттар ауруға төзімсіз болды. *P. tritici-repentis*. продуцирлейтін, (HST) ие қожайынның спецификалық токсины мен саңырауқұлақ инокулятының реакция негізінде бидайдың ұрықтық плазмасының молекулалық скринингі жүргізілді. HST *Ptr ToxA* мен *Ptr ToxB* токсиндеріне төзімді бидай гермоплазмасы іріктелінді. Пиренофороздың *Ptr ToxA* селективті токсиніне төзімді генмен байланысқан SSR типті *Xfcp623* маркерлері қолданылып, бидайдың 111 генотипіне молекулалық скрининг жүргізу нәтижесінде, эффективті *tsn1* генінің 31 тасымалдаушысы идентификацияланды, ол зерттелген генотип-тердің 27,9% құрады. Токсиндерге төзімділік гендері бар бидай үлгілерін фитопотологиялық бағалау нәтиже-сінде ауруға дала жағдайында орташа және жоғары (5-15 %) төзімділік танытты. Ауруға төзімділік пен *ToxA* токсиніне төзімді *tsn1* генінің арасындағы байланыс анықталынды. Молекулалық және фенотиптік анализ-дердің арасындағы сәйкестілік 95-98 % құрады.

SSR-типті *Xfcp1* маркерін қолданып ПЦР анализ жүргізу нәтижесінде *Ptr ToxA*-ға төзімді *tsn1* рецессивті генімен байланысқан көлемі 374 ж.н. құрайтын ДНК фрагменттері бар 10 генотип анықталынды, сонымен қатар *Ptr ToxA*-ға төзімсіз көлемі 402 ж.н. құрайтын T*sn1* доминантты гені бар бидайдың 10 үлгісі идентифи-кацияланды.

Бидайдың 10 үлгісі (Юбилейная 60, TOO11/TOOOO7, F3.71/TRM/ VORONA/3/ OC14, NANJTNG 82149 KAUZ, ECHA/LI115, Акмола 2, Казахстанская раннеспелая, Казахстанская 25, 428g/MK-122A и 190-Naz/GF55) раса *1* мен 5-ке, сонымен қатар 2 токсинге (*ToxA* мен *ToxB*) және *Septoria nodorum blotch* SNB7k изолятына кешенді төзімділігімен сипатталды. Зерттеу нәтижесінде, пиренофорозға төзімді, бидайдың перспективті линиялары таңдалынды. Бұл генотиптер дала жағдайында да орташа және жоғары төзімділік танытты және пиренофорозға төзімділік селекция бағдарламаларында қолдануға ұсынылды.

Түйін сөздер: бидай, пиренофороз, төзімділік гендері, молекулалық маркерлер.

А. Кохметова[1,2], М. Атишова[1]

[1]Институт биологии и биотехнологии растений, Алматы, Казахстан;
[2]Казахский национальный аграрный университет, Алматы, Казахстан

ИДЕНТИФИКАЦИЯ ГЕНОТИПОВ ПШЕНИЦЫ, УСТОЙЧИВЫХ К ПИРЕНОФОРОЗУ *PYRENOPHORA TRITICI-REPENTIS*

Аннотация. Интенсивное производство пшеницы, изменения в практике возделывания пшеницы, включающие переход от традиционной обработки к минимальной обработке почвы, и монокультура пшеницы, включающая возделывание восприимчивых сортов, способствуют развитию пиренофороза до масштабов эпидемии. Пиренофороз является одним из самых вредных заболеваний мягкой и твердой пшеницы во многих сельскохозяйственных регионах мира, включая Казахстан. *Pyrenophora tritici-repentis* – возбудитель пиренофороза пшеницы. В последние годы наблюдается рост распространения и вредоносности *P. tritici-repentis* на пшенице. К настоящему времени идентифицировано пять токсинов (Ptr ToxA, ToxB, ToxC и 2 токсина, объединенных в группу Ptr ToxD).

Целью исследования было выявление и отбор гермоплазмы пшеницы, устойчивой к пиренофорозу P. tritici-repentis, с использованием молекулярных маркеров. Результаты полевой оценки показали устойчивую реакцию к пиренофорозу у 76 сортов пшеницы (68%). Иммунный тип реакции к пиренофорозу показали 16 сортов пшеницы, в том числе Princes, Коксу, Опакс 18, Одесская 120, Пиротрикс-50, Половчанка, Раминал, Расад, Санзар 8, Таза, Тунгыш, Уманка, Шарора и Эритроспермум 78. Наиболее восприимчивыми оказались сорта Ажарлы, Басар, Батыр, Булава, Безостая 1, Кызылбидай, Лютеценс 9 и другие. Молекулярный скрининг гермоплазма пшеницы проводился на основе реакции к инокулюму гриба и специфические для хозяина токсины (HST), производимые P. tritici-repentis. Отобрана гермоплазма пшеницы, нечувствительная к токсинам HST Ptr ToxA и Ptr ToxB. В результате молекулярного скрининга 111 генотипов пшеницы с использованием SSR маркера Xfcp623, сцепленного с геном нечувствительности к селективному токсину Ptr ToxA пиренофороза, был идентифицирован 31 носитель эффективного гена *tsn1*, что составило 27,9% от числа изученных генотипов. Фитопатологическая оценка образцов пшеницы, в которых были обнаружены гены устойчивости к токсинам, показала высокую и умеренную степень полевой устойчивости (5-15%) к болезням. Была отмечена связь между устойчивостью к болезни и наличием нечувствительного к токсину ToxA гена tsn1. Соответствие между фенотипическим и молекулярным анализами составило 95-98%.

В результате ПЦР анализа с использованием SSR-маркера *Xfcp1* 10 генотипов формировали ДНК фрагмент размером 374 п.н., что указывает на наличие рецессивного гена *tsn1* нечувствительного к Ptr ToxA. Десять образцов пшеницы формировали фрагмент размером 402 п.н., характерный для доминантного гена *Tsn1*, чувствительного к Ptr ToxA. Выявлено десять образцов пшеницы (Юбилейная 60, TOO11/TOOOO7, F3.71/TRM/ VORONA/3/ OC14, NANJTNG 82149 KAUZ, ECHA/LI115, Акмола 2, Казахстанская раннеспелая, Казахстанская 25, 428g/MK-122A и 190-Naz/GF55), которые характеризовались комплексной устойчивостью к расам 1 и 5, к 2 токсинам (ToxA и ToxB), а также к изоляту *Septoria nodorum blotch* SNB7k. Таким образом, в результате исследований отобраны перспективные линии пшеницы, устойчивые к пиренофорозу. Эти генотипы также показали умеренный и высокий уровень полевой устойчивости и рекомендованы для использования в программах селекции на устойчивость к пиренофорозу.

Ключевые слова: пшеница, пиренофороз, гены устойчивости, молекулярные маркеры.

Information about authors:

Alma Kokhmetova, Institute of Plant Biology and Biotechnology, Kazakh National Agrarian University, Almaty, Kazakhstan; gen_kalma@mail.ru; https://orcid.org/0000-0002-0186-7832

Makpal Atishova, Institute of Plant Biology and Biotechnology, Almaty, Kazakhstan; maki_87@mail.ru; https://orcid.org/0000-0002-2270-571X

REFERENCES

[1] Lamari L., Strelkov S.E., Yahyaoui A., Amedov M., Saidov M., Djunusova M., Koichibayev M. (2005) Virulence of *Pyrenophora tritici-repentis* in the countries of the Silk Road, Can. J. Plant Pathol, 27(3):383-388. DOI:10.1080/07060660509507236.

[2] Koyshibaev M.K. (2018) Diseases of wheat [Bolezni rastenii]. Ankara: FAO. 365 p. ISBN: 978-92-5-130142-5 (in Russ.).

[3] Tagayev K.Zh., Morgounov A.I., Yessimbekova M.A., Abugalieya A.I., Bayadilova G.O. (2018) Effect of common bunt infection on agronomic traits and resistance in winter wheat lines. News NAS RK. Series of Agricultural Sciences,

5(47): 12-19. ISSN 2224-526X https://doi.org/10.32014/2018. 2224-526X.2

[4] Faris J.D., Liu Z., Xu S.S. (2013) Genetics of tan spot resistance in wheat. Theor Appl Genet, 126:2197-2217. DOI:10.1007/s00122-013-2157-y.

[5] Kariyawasam G.K., Carter A.H., Rasmussen J.B., Faris J., Xu S.S., Mergoum M., Liu Z. (2016) Genetic relationships between race-nonspecific and race-specific interactions in the wheat-Pyrenophora tritici-repentis pathosystem. Theor Appl Genet, 129(5):897-908. DOI:10.1007/s00122-016-2670-x.

[6] Lamari L., Sayoud R., Boulif M., Bernier C.C. (1995) Identification of a new race of *Pyrenophora tritici-repentis* implications for the current pathotype classification system, Can. J. Plant Pathol, 17(4):312-318. DOI:10.1080/07060669509500668.

[7] Tuori R.P., Wolpert T.J., Ciuffetti L.M. (1995) Purification and immunological characterization of toxic components from cultures of *Pyrenophora tritici-repentis*, Mol Plant Microbe Interact, 8(1):41-8. DOI:10.1094/mpmi-8-0041.

[8] Manning V.A., Pandelova I., Ciuffetti L.M. (2002) A race for anovel host-selective toxin, Phytopathology, 92: S51. ISSN: 0031-949X.

[9] Anderson J.A., Effertz R.J., Faris J.D., Francl L.J., Meinhardt S.W., Gill B.S. Genetic analysis of sensitivity to a *Pyrenophora tritici-repentis* necrosis inducing toxin in durum and common wheat, Phytopathology, 89:293-297. DOI:10.1094/PHYTO.1999.89.4.293.

[10] Faris JD, Zhang Z, Lu H, Lu S, Reddy L, Cloutier S, Fellers JP, Meinhardt SW, Rasmussen JB, Xu SS, Oliver RP, Simons KJ, Friesen TL. (2010) A unique wheat disease resistance-like gene governs effector-triggered susceptibility to necrotrophic pathogens, Proc Natl Acad Sci U S A., 107(30):13544-13549. DOI: 10.1073/pnas.1004090107.

[11] McIntosh R.A., Yamazaki Y., Dubcovsky J., Rogers J., Morris C., Appel R., Xia X.C. (2013) Catalogue of Gene Symbols for Wheat, supplement 204-2017. Available at: www.shigen.nig.ac.jp/wheat/komugi/genes.

[12] Kokhmetova A., Sapakhova Z., Urazaliev R., Yessimbekova M.,Yeleshev R., Morgounov, A. (2014) Effect of spring biomass removal on expression of agronomic traits of winter wheat, World Applied Sciences Journal, 30 (3): P. 322-329. DOI: 10.5829/idosi.wasj.2014.30.03.14026.

[13] Maraite H., Mercado-Vergnes D., Renard M.E., Zhanarbekova A., Duveiller E. (2006) Relevance of pathogen diversity in management of leaf spot and leaf blight diseases on wheat in Central Asia, Agromeridian, 2(3):105-114.

[14] Kokhmetova A.M., Kremneva O., Keyshilov Zh.S., Sultanova N.Zh. (2016) Race structure and virulence of isolates *Pyrenophora tritici-repentis* in the Republic of Kazakhstan and north Caucasus region of Russia [Rasovaja struktura i virulentnost' izoljatov Pyrenophora tritici-repentis v Respublike Kazahstan i Severo-Kavkazskom regione Rossii], Eur. J. Appl. Biotech, (3):57-66. (in Russ.).

[15] Kokhmetova A.M., Atishova M.N., Sapahova Z.B., Urazaliev R.A. (2015) Molecular screening of wheat samples for rsistance to tan spot *Pyrenophora tritici-repentis* [Molekularnyi skrining obrazcov pshenicy na ustojchivost pirenoforozu *Pyrenophora tritici-repentis*], Izvestija NAN RK, Ser. Biol. i med, 3(309):48-56. ISSN 2224-5308 (in Russ.).

[16] Kokhmetova A., Kremneva O., Volkova G., Atishova M., Sapakhova Z. (2017) Evaluation of wheat cultivars growing in Kazakhstan and Russia for resistance to tan spot, Plant Pathol, 99(1):161-167. DOI:10.4454/jpp.v99i1.3812.

[17] Saari E.E., Prescott L.M. (1975) A scale for appraising the foliar intensity of wheat diseases, Plant Dis. Reporter, 59: 377-380.

[18] Kremneva O.Yu., Volkova G.V. (2007) Diagnostics and methods for assessing the wheat resistance to the causative agent of tan spot, Guidelines, Moscow [Diagnostika i metody ocenki ustojchivosti pshenicy k vozbuditelju zheltoj pjatnistosti, Metodika, Moskva]. P. 19 (in Russ.).

[19] Riede C.R., Anderson J.A. (1996) Linkage of RFLP markers to an aluminum tolerance gene in wheat, Crop Sci, 36(4):905-909. DOI:10.2135/cropsci1996.0011183X0036000400015x.

[20] Kokhmetova A.M., Ali Sh., Sapakhova Z., Atishova M.N. (2018) Identification of genotypes-carriers of resistance to tan spot Ptr ToxA and Ptr ToxB of Pyrenophora tritici-repentis in common wheat collection [Identifikacija genotipov-nositelej ustojchivosti k toksinam pirenoforoza Ptr ToxA i Ptr ToxB Pyrenophora tritici-repentis v kollekcii mjagkoj pshenicy], Vavilov Journal of Genetics and Breeding, 22(8):978-986. DOI 10.18699/VJ18.440. (in Russian).

[21] Kokhmetova A.M, Atishova M.N, Kumarbayeva M.T., Leonova I.N., (2019) Phytopathological screening and molecular marker analysis of wheat germplasm from Kazakhstan and CIMMYT for resistance to tan spot, Vavilov Journal of Genetics and Breeding, 23(7):879-886. DOI10.18699/VJ19.562 (in Russ.).

[22] Kokhmetova A., Atishova M., Madenova A., Kumarbayeva M. (2019) Genotyping of wheat germplasm for resistance to toxins of tan spot Pyrenophora tritici-repentis, Journal of Biotechnology, 305:S53- S53. DOI: 10.1016/j.jbiotec.2019.05.188.

[23] Mikhailova LA, Terniuk IG, Mironenko NV (2010) Characteristics of populations of *Pyrenophora tritici-repentis* on the basis of virulence [Harakteristika populjacij Pyrenophora tritici-repentis na osnove virulentnosti], Mycology and phytopathology, 44(3):263-272 (in Russ.).

[24] Lamari L., Bernier C.C. (1989) Toxin of *Pyrenophora tritici-repentis*: Host-specificity, significance in disease, and inheritance of host reaction, Phytopathology, 79(7):740-744. DOI: 10.1094/Phyto-79-740.

[25] Friesen T.L., Stukenbrock E.H., Liu Z.H., Meinhardt S., Ling H., Faris J.D., Rasmussen J.B., Solomon P.S., McDonald B.A., Oliver R.P. (2006) Emergence of a new disease as a result of interspecific virulence gene transfer, Nature Genetics, 38: 953-956. DOI:10.1038/ng1839.

[26] Kremneva O.Y., Volkova G.V., (2013) ES Szeged resistant varieties of winter wheat to the tan spot pathogen, Multidisciplinary network electronic scientific journal of the Kuban State Agrarian University [Ustojchivye sorta ozimoj pshenicy k vozbuditelju zheltoj pjatnistosti E.S. Seged, Mnogoprofil'naja set', jelektronnyj nauchnyj zhurnal Kubanskogo gosudarst-vennogo agrarnogo universiteta], 7:1741-1751 (in Russ.).

Publication Ethics and Publication Malpractice in the journals of the National Academy of Sciences of the Republic of Kazakhstan

For information on Ethics in publishing and Ethical guidelines for journal publication see http://www.elsevier.com/publishingethics and http://www.elsevier.com/journal-authors/ethics.

Submission of an article to the National Academy of Sciences of the Republic of Kazakhstan implies that the described work has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis or as an electronic preprint, see http://www.elsevier.com/postingpolicy), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder. In particular, translations into English of papers already published in another language are not accepted.

No other forms of scientific misconduct are allowed, such as plagiarism, falsification, fraudulent data, incorrect interpretation of other works, incorrect citations, etc. The National Academy of Sciences of the Republic of Kazakhstan follows the Code of Conduct of the Committee on Publication Ethics (COPE), and follows the COPE Flowcharts for Resolving Cases of Suspected Misconduct (http://publicationethics.org/files/u2/New_Code.pdf). To verify originality, your article may be checked by the Cross Check originality detection service http://www.elsevier.com/editors/plagdetect.

The authors are obliged to participate in peer review process and be ready to provide corrections, clarifications, retractions and apologies when needed. All authors of a paper should have significantly contributed to the research.

The reviewers should provide objective judgments and should point out relevant published works which are not yet cited. Reviewed articles should be treated confidentially. The reviewers will be chosen in such a way that there is no conflict of interests with respect to the research, the authors and/or the research funders.

The editors have complete responsibility and authority to reject or accept a paper, and they will only accept a paper when reasonably certain. They will preserve anonymity of reviewers and promote publication of corrections, clarifications, retractions and apologies when needed. The acceptance of a paper automatically implies the copyright transfer to the National Academy of Sciences of the Republic of Kazakhstan.

The Editorial Board of the National Academy of Sciences of the Republic of Kazakhstan will monitor and safeguard publishing ethics.

Правила оформления статьи для публикации в журнале смотреть на сайте:

www:nauka-nanrk.kz

ISSN 2518-1467 (Online), ISSN 1991-3494 (Print)

http://www.bulletin-science.kz/index.php/en/

Редакторы *М. С. Ахметова, Т. А. Апендиев, Д. С. Аленов*
Верстка на компьютере *Д. А. Абдрахимовой*

Подписано в печать 10.02.2020.
Формат 60x881/8. Бумага офсетная. Печать – ризограф.
19,25 п.л. Тираж 500. Заказ 1.