АУЫЛ ШАРУАШЫЛЫҒЫ, АГРОХИМИЯ, АЗЫҚ ӨНДІРУ, АГРОЭКОЛОГИЯ
ЗЕМЛЕДЕЛИЕ, АГРОХИМИЯ, КОРМОПРОИЗВОДСТВО, АГРОЭКОЛОГИЯ
AGRICULTURE, AGROCHEMICAL, FEED PRODUCTION, AGROECOLOGY

IRSTI 68.37.31; 34.15.23 **DOI https://doi.org/10.37884/4-2023/07**

M.T. Kumarbayeva, A.M. Kokhmetova, A.A. Malysheva , A.A. Bolatbekova, A.M. Kokhmetova*

Institute of Plant Biology and Biotechnology, Almaty, Kazakhstan
madina_kumar90@mail.ru, gen_kalma@mail.ru, malysheva_angelina@list.ru, ardashka1984@mail.ru, asia.k68@mail.ru*

IDENTIFICATION OF SOURCES OF RESISTANCE TO SEPTORIA TRITICI IN WINTER WHEAT GERMOPLASM

Abstract

Septoria tritici (STB) blotch of wheat, caused by the ascomycete *Zymoseptoria tritici* (formerly *Mycosphaerella graminicola*), is one of the most serious foliar diseases of wheat. In many temperate wheat growers, STB is a devastating disease and yield losses can exceed 50% under favorable conditions. In particular the augmented use of soil management practices that leave large amounts of wheat stubble on the soil sur- face and global warming increases the chance of *Septoria tritici blotch* epidemics to emerge more frequently including in developing countries. The purpose of the study is to identify samples of wheat resistant to septoria blotch. The identification of STB-resistant spring breadwheat germplasm, in combination with and without the APR stage, will serve as an important resource in *Septoria tritici* (STB) resistance breeding efforts. According to the results of molecular screening for resistance to septoria and evaluation of the plant biomass index, 11 promising wheat lines were identified as carriers of the *Stb 7* gene and high NDVI (Normalized difference vegetation index). Identified promising lines of wheat are recommended to be used as donors in breeding programs for resistance to septoria in the Almaty region.

Key words: *wheat, septoria, resistance, promising lines, cultivar, phytopathological screening, molecular screening, NDVI biomass index, STB*

Introduction

Septoria tritici blight, caused by the ascomycete *Zymoseptoria tritici* (Desm.) Quaedvlieg & Crous (anamorph: *Septoria tritici*, teleomorph: *Mycosphaerella graminicola*), is one of the most economically important biotic factors limiting wheat production in some wheat-growing regions. *Septoria tritici* spot can be severe under certain wheat growing conditions in developing countries, particularly in northern and western Asia, northern Africa and parts of South America, including Kazakhstan [1, 2]. Crop losses due to *Septoria tritici* blotch in disease-promoting climates can be as high as 35-50% due to reduced photosynthetic area. In recent years, the increasing use of tillage practices that leave large amounts of wheat stubble and debris on the soil surface has increased the likelihood of Septoria blotch epidemics occurring under favorable climatic conditions and is therefore expected to occur more frequently in the future in developing countries. In the Almaty region in recent years, rust diseases have predominated, as well as leaf spot diseases (tan spot, septoria blight) [3-6].

In Kazakhstan, the period is 2000-2015. epiphytotic development of leaf rust separately or together with septoria occurred 8 times; with their early manifestation and strong development, the wheat yield is reduced to 20-30%. To prevent large grain losses, the crops are treated with fungicides, which requires additional costs. As a result, not only the yield of spring wheat decreases, but also the technological quality of the grain. In the southern and southeastern regions of Kazakhstan and the Kyrgyz Republic, septoria blight is the most common disease of winter wheat. In years with wet (the amount of precipitation in April and May is at least 75-100 mm) and cool spring (15-20°C) it develops

to the level of epiphytotics. Septoria blight and yellow spot occur simultaneously on the same leaf [7].

Due to global warming, rising costs of fungicide application and environmental degradation, and the emergence and prevalence of fungicide-resistant/non-susceptible strains of the pathogen, breeding for resistance provides one approach to control this disease. Inheritance of resistance to *Septoria tritici* spot in wheat can be either qualitative, isolate-specific, depending on the underlying genes, or quantitative, non-isolate-specific with polygenic inheritance. To date, 18 major resistance genes (*Stb 1-Stb 18*) have been identified, along with many quantitative genes with minor effects [8]. For effective breeding work, constant monitoring of wheat blight, including septoria blight, is necessary.

Materials and methods

The objects of research are commercial varieties and promising lines of winter wheat, cultivated or being candidates for new varieties..

The experimental material was phenotyped during the growing seasons of 2022/2023 at the Kazakh Research Institute of Agriculture and Plant Growing (KazNIIZiR), Almalybak village (N 43°14'333" E 076°41'657" B783) Almaty region. The experiments were performed in three replicates. Individual plot size was 1 m2. Treatment and fertilizer management methods were consistent with those often recommended for the area. Fertilizers were 60 and 30 kg/ha nitrogen and phosphorus oxide, respectively. Test plants were planted in mid-September in all years, and the harvest was harvested in mid-August. The irrigated foothill zone where KazNIIZiR is located is relatively well-watered; the experimental materials were watered 3 times during their development at the rate of 600 m3/ha and cleared of weeds.

Weather conditions in 2022 were more favorable for the development of leaf rust than in 2023 (http://weatherarchive.ru). In May, the amount of precipitation exceeded the norm, which led to an increase in environmental humidity and contributed to the effective infection of plants by *Septoria tritici* spores.

Phytopathological screening in the field, the degree of damage by septoria is assessed as a percentage of the leaf area occupied by yellow spot, according to the Saari and Prescott scale (1975) [9], developed for septoria, modified from Kremneva O.Yu. (2007) [10]. This scale of intensity of damage to wheat leaves uses the following gradations: 0% - very high resistance; 1-5% - high stability; 6-20% - stability; 21-30% - susceptibility; 31-50% - moderate susceptibility; 51-80% – high susceptibility; 81-100% - very high susceptibility. Methods for molecular screening of wheat STB genes for leaf rust resistance. Genomic DNA extraction was carried out according to the method proposed by Riede et al., 1996. DNA was isolated from 5-day-old wheat seedlings for each individual sample based on the CTAB method [11]. The DNA concentration was determined using the spectrophotometric method at a wavelength of 260 nm. The DNA concentration in the working solution for PCR was adjusted to 20 ng/µl. The PCR reaction mixture (25 µl) contained 2.5 µl of genomic DNA, 1 µl of each primer (1 pM/µl) (SigmaAldrich, USA), 2.5 µl of dNTP mixture (2.5 mM, dCTP, dGTP, dTTP and dATP) (ZAO Silex, Russia), 2.5 µl MgCl2 (25 mM), 0.2 µl Taq polymerase (5 units per µl) (ZAO Silex, Russia), 2.5 µl 10X PCR buffer and 12.8 µl ddH20. PCR amplification was carried out using a Mastercycler amplifier (Eppendorf, Germany). Amplification products were separated in a 2% agarose gel in TBE buffer (45 mM Tris-borate, 1 mM EDTA, pH 8) [12] with the addition of ethidium bromide. To determine the length of the amplification fragment, a 100-bp DNA marker (Fermentas, Lithuania) was used. The results were visualized using a gel documentation system (Gel Doc XR+, BIO-RAD, Hercules, USA).

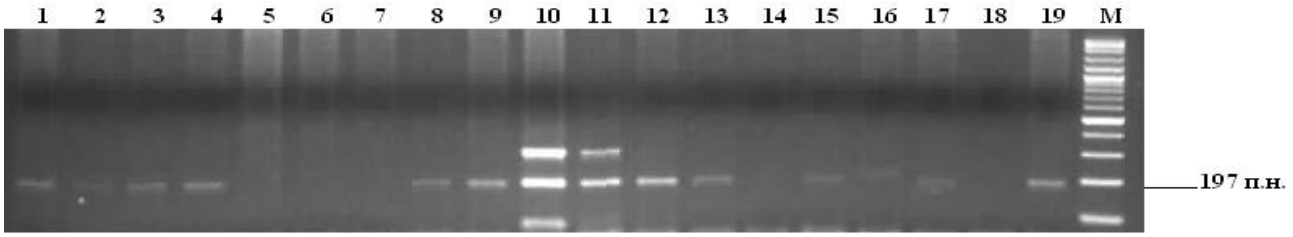
Results and their discussions

Phytopathological screening of wheat samples against a natural infectious background was carried out. According to the results of studies during the growing season 2022/2023, no signs of septoria were found in the studied samples. Typical phenotypic appearance of septoria is presented in Figure 1.

Figure 2 – *Septoria tritici* (STB) spot of wheat (Almaty region, Zhambyl district, 2022)

Based on the results of molecular screening, molecular screening was carried out to identify carriers of septoria resistance genes. Based on the analysis of the international databases GrainGenes, MASWheat, KOMUGI, a selection of molecular markers linked to septoria resistance genes was made. When molecular screening of experimental wheat material for the presence of the *Stb 7* gene complex, the molecular marker *WMC 313* was used. The Estanzuela Federal variety - *Stb 7* was used as a positive control.

During the molecular screening of experimental wheat material for the presence of the Stb 7 gene complex, the molecular marker *WMC 313* was used. The Estanzuela Federal variety - *Stb 7* was used as a positive control. The SSR marker WMC 313 amplified a product of 197 bp from the R-alleles of the *Stb 7* resistance genes. ., while the S-allele did not form a PCR product. As a result of PCR with the *WMC 313* marker, in addition to the positive control, a DNA fragment of 197 bp in size. was formed in 11 promising wheat lines. In the remaining six genotypes, such a fragment was not found (Figure 2).



1-207, 2-208, 3-209, 4-210, 5-211, 6-212, 7-213, 8-214, 9-215, 10-216, 11-217, 12-218, 13-219, 14-220, 15-221, 16-222, 17-223, 18-ddH_2O, 19-Stb 7, M- Gene –Ruler 100 bp DNA Ladder.

Figure 2 – Identification of wheat *Stb 7* carriers using the *WMC 313* SSR marker.

Table 2 presents the name of the samples, the results of molecular screening, phytopathological screening against a natural infectious background in the conditions of the Almaty region (KazNIIZiR) and an assessment of the NDVI biomass index. Genotypes 207, 209, 210, 214, 215, 216, 217, 218, 219, 221 and 223 are carriers of the *Stb 7* gene.

Table 2 – Results of studies on resistance to *Septoria tritici* (STB) in wheat.

№	Name of samples	Molecular screening		Phytopathological screening, %	NDVI biomass index assessment
		b.p.	R/S		
1	**207**- F1d.1049 d. 767 F5 (Naz x GF55) x Arap x Arap, No. 43/No. 1107 23-ICARDA-IPBB-2013(Yr5)	197 b.p.	R	0	77
2	**208**- F4(F1Д1302Д95.SILVERSTAR/4/338-K1-1//ANB/ BUC/3/GS50A/5/TAM200/KAUZ x Name) x Name, No. 40/No. 35 Moro(Yr10)	null	S	0	72
3	**209**- F4(F1д.1347Д141.NGDA146/4/YMH/ TOB//MCD/3/LIRA/5/ F130L1.12/6/GALLYA-ARAL1/7/TAM200/KAUZ) x Mereke, No. 70/No. 1103 19-ICARDA -IPBB-2013 (Yr17)	197 b.p.	R	0	81
4	**210**-282/SP-2-2012 F7 (Naz x GF66) x Ulugbek/No. 276 T.spelta (Japan-2013) (Yr5)	197 b.p.	R	0	80
5	**211**-1677 d.1051 d.783. F5(Naz x Immun78) x Arap x Arap/No. 290 Clement (W; Yr9+Yr2+?)	null	S	0	70
6	**212**-F6 (d.1030D620. F4 UlugbekhUr 4) x Mereke/No. 41 Bezostaya 1 (Yr18)	null	S	0	71
7	**213**-F4 (d.1008 (д.90 F3 (Алмалы (225) x 5353Super kraws)) x Naz/№ 1103 ICARDA-IPBB-2013 (Yr17)	null	S	0	72
8	**214**-F4 (d.1051Д783. F5 Naz x Immun78) x Arap) x Arap/№ 53 Mereke (Yr10)	197 b.p.	R	0	83
9	**215**-F4 (d.1010 (д.93 F3(N 23 x Kupava)) x Mereke /№293 Moro(W; Yr10)	197 b.p.	R	0	78
10	**216**-д.1777 Daria x №1724 F11581 x (д.807 F4 (Naz x Umanka) x Almaly) x Zymorodok, №78 x д.42 Almaly/№29 Almaly	197 b.p.	R	0	77
11	**217**-д.Сабина x д.74 Паллада/№57 Паллада	197 b.p.	R	0	79
12	**218**-д.1777 Daria x №72 Tungysh x д.133 Daria/№68 Daria	197 b.p.	R	0	80
13	**219**- d.1286 d. 79 (ARDEAL/BOEMA//F135U2-1/5/ TX69A509-2//BBY2/FOX/3/PKL70 /LIRA/4/YMH/TOB//MCD/3/LIRA)x Naz) x д.367 Lapochkina 113/DO-4 DS (Sr46)/ №379 Pavon 76 (Sr2 Ug99 complex)	197 b.p.	R	0	81
14	**220**-d.1300Д 93. AUS 4930.7/2*PASTOR/4/338-K1-1// ANB/BUC/3/GS50A/5/TAM200/KAUZ x Наз x д.367 д. Lapochkina 113/DO-4 DS (Sr46)/ №366 RL 6099 (1995) Dyck(Sr35 Ug99)	null	S	0	69
15	**221**-d. №23x Kupava x 1774 d.23-ICARDA-IPBB-2013	197 b.p.	R	0	77
16	**222**-d. №23x Kupava x1774 d. 23-ICARDA-IPBB-2013	null	S	0	71
17	**223**-d. F_5 №20 x Уманка x 1773 д.22-ICARDA-IPBB-2013	197 b.p.	R	0	82
18	ddH_2O	-	-	-	-
19	Estanzuela Federal - Stb 7	197 b.p.	R	0	77

The biomass index NDVI, Normalized Difference Vegetative Index was assessed. Based on the results of assessing the NDVI biomass index, Table 2 presents the samples that were distributed according to the NDVI value. 11 samples (57.89%) were identified with a high biomass index of 0.77-0.81, while 6 samples (31.57%) and promising lines were identified with an average NDVI value of 0.69-0.72. It is worth noting that those promising lines in which the Stb 7 gene is identified are represented by high biomass index values.

Conclusions

Based on the results of complex studies, including phytopathological screening of an adult plant, molecular screening for resistance to septoria and assessment of the plant biomass index, 11

promising wheat lines were identified as carriers of the *Stb 7* gene and high NDVI values. The results of this study are of interest for the wheat breeding program for resistance to septoria in the Almaty region.

Gratitude. The work was supported by the program of the Ministry of Science and Higher Education of the Republic of Kazakhstan, AP14869967 " Mapping of genetic factors determining resistance to *Septoria nodorum* based on genome-wide association study in the collection of hexaploid wheat" (2022-2024).

References

1. Duveiller, E. The challenges of maintain- ing wheat productivity: pests, diseases and potential epidemics [Text] / E. Duveiller, R.P. Singh, J.M. Nicol // Euphytica. – 2007. – №. 157. – P. 417–430

2. Nazari, K. Wheat rust diseases in Central and West Asia and North Africa (CWANA) and breeding for the multiple disease resistance [Text] / K. Nazari, A. Yahyaoui, O. Abdalla, M. Nachit, F. Ogbonnaya, R. Brettell, S. Rajaram // In: Presented at the international workshop on marker assisted breeding for disease resistance in wheat. – Iran: Karaj, 2009.

3. Kumarbayeva, M.T. Fitopatologicheskij skrining na ustojchivost' k pirenoforozu i ocenka hozyajstvenno-cennyh priznakov obrazcov ozimoj myagkoj pshenicy [Tekst] / M.T. Kumarbaeva, A.M. Kohmetova, A.S. Rsaliev // Izdenister, natizheler - Issledovaniya, rezul'taty. – 2020. – № 2. – S. 247-252.

4. Kejshilov, ZH.S. Kuzdik bidaj kollekciyasynyn pirenoforozga (Pyrenophora tritici-repentis) tozimdiligin bagalau [Tekst] / Zh.S.Kejshilov, A.M.Kohmetova, A.K. Madenova, M.T. Kumarbaeva, A.D. Zhigitbekova // Izdenister, natizheler - Issledovaniya, rezul'taty. – 2020. – №2. – B. 128-135.

5. Kohmetova, A.M. Kazaкstanda osiriletin bidaj sorttarynyn pirenoforoz Pyrenophora tritici-repentis auruyna tozimdiligine fitopatologiyalyk skrining zhurgizu [Tekst] / A.M. Kohmetova, Zh.S. Keishilov, K. Galymbek, M.T. Kumarbaeva // «Izdenister, natizheler-Issledovaniya, rezul'taty». –2019. – №3. – B. 213-218.

6. Kumarbayeva, M. Characterization of *Pyrenophora tritici-repentis* (tan spot of wheat) races in Kazakhstan [Text] / M. Kumarbayeva, A. Kokhmetova, N. Kovalenko, M. Atishova, Z. Keishilov, K. Aitymbetova // Phytopathologia Mediterranea. – 2022. – Vol. 61(2). – P. 243-257.

7. Kojshybaev M. Bolezni pshenicy [Tekst] / M. Kojshybaev. – Ankara: FAO, 2018. – 365 s.

8. Dreisigacker, S. Adult-plant resistance to Septoria tritici blotch in hexaploid spring wheat [Text] / S. Dreisigacker, X. Wang, B. A. Martinez Cisneros, R. Jing, P. K. Singh // Theoretical and Applied Genetics. – 2015. – Vol. 128(11). – P. 2317–2329.

9. Saari, E. E. A scale for appraising the foliar intensity of wheat diseases [Text] / E. E. Saari, L. M. Prescott // Plant Dis. Rep. – 1975. – Vol. 59. – P. 377-380.

10. Kremneva, O.YU. Diagnostika i metody ocenki ustojchivosti pshenicy k vozbuditelyu zheltoj pyatnistosti list'ev: metodicheskie rekomendacii [Tekst] / O.YU. Kremneva, G.V. Volkova. – M, 2007. – S. 12

11. Riede, C.R. Linkage of RFLP markers to an aluminum tolerance gene in wheat [Text] / C.R. Riede, J.A. Anderson // Crop Science. – 1996. – Vol. 36. – P. 905-909.

12. Chen, X. Genome scanning for resistance-gene analogs in rice, barley, and wheat by high-resolution electrophoresis [Text] / X. Chen, R. Line, H. Leung // Theor. Appl. Genet. – 1998. – 97. – P. 345–355.

М.Т. Кумарбаева, А.М. Кохметова, А.А. Малышева, А.А. Болатбекова, А.М. Кохметова
Өсімдіктер биологиясы және биотехънологиясы институты, Алматы, Қазақстан
madina_kumar90@mail.ru, gen_kalma@mail.ru, malysheva_angelina@list.ru, ardashka1984@mail.ru, asia.k68@mail.ru*

КҮЗДІК БИДАЙ ГЕРМОПЛАЗМАСЫНДАҒЫ SEPTORIA TRITICI АУРУЫНА ТӨЗІМДІЛІК КӨЗДЕРІН ИДЕНТИФИКАЦИЯЛАУ

Аңдатпа

Аскомицет *Zymoseptoria tritici* (алдынғы *Mycosphaerella graminicola*) тудыратын бидайдың *Septoria tritici* (STB) дақтары бидайдың ең қауіпті жапырақты ауруларының бірі болып табылады. Көптеген аймақтарда қоңыржай климатымен бидай өсіру STB жойқын ауру болып табылады және қолайлы жағдайларда егін шығыны 50% - дан асуы мүмкін. Атап айтқанда, топырақ бетінде үлкен көлемдегі бидай сабандарын қалдыратын топырақты басқару әдістерін кеңейту және жаһандық жылыну *Septoria tritici* індеттерінің, соның ішінде дамушы елдерде жиі пайда болу мүмкіндігін арттырады. Жаһандық жылынуға байланысты фунгицидтерді қолдану құнының өсуі мен қоршаған ортаның нашарлауына және қоздырғыштың фунгицидке төзімді/сезімсіз штаммдарының пайда болуы мен таралуына байланысты, төзімділікті өсіру осы аурумен күресудің бір тәсілін ұсынады. Зерттеудің мақсаты – септориозға төзімді бидайдың перспективті үлгілерін идентификациялау. STB төзімді күздік бидайының гермоплазмасын анықтау ересек сатсында төзімділігімен бірлескен сорттар STB төзімділігін арттыруға бағытталған бағдарламаларда маңызды ресурс ретінде пайдаланылады. Септориозға төзімділікке арналған молекулалық скрининг нәтижесінде және өсімдік биомиассасының индексін бағалау *Stb 7* генінің тасымалдаушысы ретінде және NDVI (Normalized difference vegetation index) көрсеткіштерін жоғары 11 перспективті бидай линияларын анықтады. Анықталған перспективті бидай линияларын Алматы облысы жағдайында септориозға төзімділікке бағытталған селекциялық бағдарламаларда донор ретінде пайдалану ұсынылады.

Кілт сөздер: бидай, септориоз, төзімділік, перспективалық линиялар, сорт, фитопатологиялық скрининг, молекулалық скрининг, NDVI биомасса индексі, STB

М.Т. Кумарбаева, А.М. Кохметова, А.А. Малышева, А.А. Болатбекова, А.М. Кохметова
Институт Биологии и Биотехнологии Растений, Алматы, Казахстан
madina_kumar90@mail.ru, gen_kalma@mail.ru, malysheva_angelina@list.ru, ardashka1984@mail.ru, asia.k68@mail.ru)*

ИДЕНТИФИКАЦИЯ ИСТОЧНИКОВ УСТОЙЧИВОСТИ К ПЯТНИСТОСТИ *SEPTORIA TRITICI* В ГЕРМОПЛАЗМЕ ОЗИМОЙ ПШЕНИЦЫ

Аннотация

Пятнистость *Septoria tritici* (STB) пшеницы, вызываемая аскомицетом *Zymoseptoria tritici* (ранее *Mycosphaerella graminicola*), является одной из наиболее серьезных лиственных болезней пшеницы. Во многих регионах возделывающие пшеницу с умеренным климатом STB является разрушительным заболеванием, и потери урожая могут превышать 50% при благоприятных условиях. В частности, более широкое использование методов управления почвой, при которых на поверхности почвы остается большое количество стерни пшеницы, и глобальное потепление увеличивают вероятность более частого возникновения эпидемий пятнистости *Septoria tritici*, в том числе в развивающихся странах. Цель исследования является идентификация образцов пшеницы устойчивых к септориозу. Идентификация зародышевой плазмы яровой хлебной пшеницы, устойчивой к STB, в сочетании со стадией APR и без нее послужит важным ресурсом в усилиях по селекции устойчивости к *Septoria tritici* STB. По результатам молекулярного скрининга на устойчивость к септориозу и оценка индекса биомиассы растений идентифицированы 11 перспективных линий пшеницы как носители гена *Stb 7* и высоким показателями NDVI (Normalized difference vegetation index, Нормализованный вегетационный индекс). Идентифицированные перспективные линии пшеницы рекомендуется использовать в качестве доноров в селекционных программах на устойчивость к септориозу в условиях Алматинской области.

Ключевые слова: пшеница, септориоз, устойчивость, перспективные линии, сорт, фитопатологический скрининг, молекулярный скрининг, индекса биомассы NDVI, STB