AGRICULTURAL BIOLOGY,
2023, V. 58, Iss. 5, pp. 852-863
[SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA]

ISSN 2412-0324 (English ed. Online)

ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 633.11:632.4:577.2

doi: 10.15389/agrobiology.2023.5.852eng
doi: 10.15389/agrobiology.2023.5.852rus

CHARACTERISTICS OF PERSPECTIVE WHEAT CULTIVARS, ADMITTED TO CULTIVATION IN THE LOWER VOLGA REGION OF THE RUSSIAN FEDERATION, BASED ON RESISTANCE TO PERYNOPHOROSIS AND TAN SPOT PATHOGENS

E.A. KONKOVA[1] ✉, S.V. LYASCHEVA[1], Yu.V. ZELENEVA[2], N.M. KOVALENKO[2]

[1]*Federal Center of Agriculture Research of the South-East Region,* 7, ul. Tulaikova, Saratov, 410010 Russia, e-mail baukenowaea@mail.ru (✉ corresponding author), lyaschevasveta@yandex.ru;
[2]*All-Russian Research Institute of Plant Protection*, 3, sh. Podbel'skogo, St. Petersburg, 196608 Russia, e-mail nadyakov@mail.ru, zelenewa@mail.ru
ORCID:
Konkova E.A. orcid.org/0000-0001-8607-2301
Zeleneva Yu.V. orcid.org/0000-0001-9716-288X
Lyashcheva S.V. orcid.org/0000-0002-6790-0770
Kovalenko N.M. orcid.org/0000-0001-9577-8816

The authors declare no conflict of interests
Acknowledgements:
The authors thank the reviewers for their contribution to the peer review of this work.
Supported financially by the Russian Science Foundation, Project No. 19-76-30005
Final revision received August 07, 2023
Accepted October 03, 2023

Abstract

Annual monitoring of the phytosanitary condition of wheat crops in the Saratov region evidences the active development of leaf spots and the accumulation of their infectious potential. *Pyrenophora tritici-repentis* is the causative agent of pyrenophora, or tan spot, and one of the dangerous wheat diseases. *Bipolaris sorokiniana* is the causative agent of spot blotch, a potentially dangerous wheat disease. In this work, as a result of field and laboratory assessment of 20 varieties of bread wheat (*Triticum aestivum* L.) cultivated in the Lower Volga region, samples resistant to the pathogen of tan spot, as well as moderately resistant to the pathogen of spot blotch, were identified for the first time. The results of identification of the *Tsn1*/*tsn1* gene allele using the molecular marker Xfcp623 showed that among the studied wheat samples, the *Tsn1* gene was predominant in winter wheat varieties. For the first time, statistically significant differences were revealed between the average values of field phytopathological estimates of resistance to *P. tritici-repentis* among varieties from different resistance groups (RR, R, MS and S).The purpose of the study is to assess the resistance of winter and spring bread wheat varieties (*Triticum aestivum* L.) cultivated in the Lower Volga region to tan spot and spot blotch pathogens and to identify the dominant/recessive allele of the *Tsn1* gene in plants. The work examined 13 varieties of winter bread wheat (Gostianum 237, Lutescens 230, Saratovskaya 8, Guberniya, Mironovskaya 808, Donskaya Bezostaya, Saratovskaya 90, Zhemchuzhina Povolzh'ya, Saratovskaya 17, Kalach 60, Podruga, Anastasiya, Sosedka) and 7 varieties of spring bread wheat (Favorit, Prokhorovka, Yugo-Vostochnaya 2, Saratovskaya 70, Saratovskaya 73, Belyanka, Lebedushka). Of these, 16 varieties were approved for use in 2022. Sosedka is a promising variety of winter bread wheat; Gostianum 237, Lutescens 230, Saratovskaya 8 are the Saratov selection varieties of winter bread wheat produced in the first half of the 20th century. Field trials were carried out in 2020-2022 in the breeding nursery of the Federal Center of Agriculture Research of the South- East Region (Saratov). The response of wheat varieties to natural infection by a local population of the yellow spot pathogen was assessed under field conditions using a modified and extended Saari-Prescott scale. In lab tests, plant leaves were infected with *P. tritici-repentis* and *B. sorokiniana*. The inoculum consisted of a mixture of several fungal isolates from plant infectious material 2022 from the Saratov (*P. tritici-repentis*, *ToxA*), and Leningrad (*B. sorokiniana*) regions and the Republic of Kazakhstan (*P. tritici-repentis*, *ToxB*) obtained from the collection of the All-Russian Research Institute of Plant Protection. For phytopathological assessment of *P. tritici-repentis*, a scoring scale was used that characterizes the development degree of necrosis and chlorosis. When assessing the resistance of wheat to spot blotch caused by *B. sorokiniana*, we used a scale developed at the All-Russian Research Institute of Plant Protection. Genomic DNA was isolated from leaves of 5-day-old wheat seedlings using the standard CTAB/chloroform method. After quantification, the DNA concentration was normalized to 30 ng/μl for subsequent PCR. Screening for the presence of a dominant or recessive gene allele (*Tsn1* or *tsn1*) in the varieties was carried out using PCR with primer pairs Xfcp623F/Xfcp623R (ZAO Evrogen, Russia). The proportion of varieties resistant to *P. tritici-repentis* isolates capable of producing toxins encoded by the *ToxA* and *Tox B* genes was 40 %, to the isolate with *ToxA* — 55 %, with *Tox B* — 60 %. The proportion of varieties with moderate resistance to *Bipolaris sorokiniana* was only 15 %. The genotypes of three winter bread wheat varieties (Gostianum 237, Mironovskaya 808, Podruga) and 5 genotypes of spring bread wheat (Favorit, Prokhorovka, Saratovskaya 70, Saratovskaya 73, Belyanka) which turned out to be resistant to tan spot in laboratory and three-year field trials are of the greatest interest. Molecular screening of these varieties also confirmed resistance to the PtrToxA producer, since they had *tsn1*, which indicates their resistance to the fungal toxin protein PtrToxA. These varieties should be involved into breeding programs in the region to improve the resistance of wheat to brown spot and blight pathogens.

Keywords: wheat, *Pyrenophora tritici-repentis*, *Bipolaris sorokiniana*, *ToxA*, *Tsn1*, PCR, yellow leaf spot, tan spot, spot blotch

Harmful diseases are of special factors limiting the yield of grain crops in the Saratov region. Annual monitoring of the regional phytosanitary condition of wheat crops shows the development of leaf spots and the accumulation of their infectious potential. One of the widespread wheat diseases in the region is yellow leaf spot, or pyrenophorosis.

Pyrenophorosis is an economically important disease of wheat worldwide [1, 2]. Its causative agent, the ascomycete fungus *Pyrenophora tritici-repentis* (Died.) Drechsler is known for the ability to synthesize necrotrophic effectors (NEs), including host selective toxins (HSTs) functioning as pathogenicity factors. Three necrotrophic effectors, the PtrToxA, PtrToxB and PtrToxC have been described for *P. tritici-repentis*. PtrToxA and PtrToxB are proteins, and PtrToxC is a low-molecular non-protein compound [3-5].

Relationships in the pathosystem of bread wheat—*P. tritici-repentis* are carried out in a gene-for-gene mirror pattern, that is, in a susceptible host, each individual toxin is recognized by a separate locus product, resulting in plant susceptibility and disease expression [6-8]. The uniqueness of this pathosystem is that the *ToxA* gene which determines the main pathogenicity factor (PtrToxA toxin) was introduced into the *P. tritici-repentis* genome by horizontal transfer from another wheat pathogen, the fungus *Parastagonospora nodorum* [9, 10].

Another potentially dangerous disease in the region is dark brown spot of wheat, although until now the Saratov Province was not considered the epiphytotically dangerous zone for dark brown spot. In years favorable for the pathogen, slight damage (no more than 10%) to some varieties occurred. However, due to various factors (climate change, improper crop rotation, monocultures, etc.), it can be assumed that the massive spread of this pathogen in the region is only a matter of time. If during heading it rains continuously for 5-6 days, followed by an increase in daytime temperature to 20-30 °C, the dark brown spot epiphytoty on wheat can develop very quickly [11].

Dark brown leaf spot of cereals is caused by the anamorphic fungus *Bipolaris sorokiniana* (Sacc. in Sorokin) Shoem., at the sexual stage *Cochliobolus sativus* (S. Ito & Kurib.) Drechsler ex Dastur. The disease affects barley, wheat, rye, triticale, and several wild grasses [12]. The importance of the disease is increasing in North and South America, as well as in Europe [13]. It is widespread in many regions of the Russian Federation, e.g., in the North-Western and North Caucasus regions, Tatarstan, and the Amur region [14-16]. The pathogen is a hemibiotroph, that is, in addition to its parasitic state, it is capable of saprophytic life. In seeds, soil and plant debris, the fungus remains viable for 5-7 years. Yield losses can be 20-40%. The species *B. sorokiniana* is polymorphic, it also causes helminthosporium root rot and, in combination with other pathogens, black germ of seeds [17].

Wheat resistance to *B. sorokiniana* is genetically determined. It is known that the *Sb1* gene increases quantitative (incomplete) resistance to dark brown spot. The chromosome segment in which the *Sb1* gene is located also contains the leaf rust resistance gene *Lr34* which is widely used in wheat breeding [11].

M. Lillemo et al. [18] indicate a gene for resistance to *B. sorokiniana* which is not allelic to *Sb1* and is linked to the leaf rust resistance gene *Lr46*. There is information about the presence of two or more genes for resistance to *B. sorokiniana* and the effectiveness of their pyramiding. It was found that wheat plants with erectoid and semi-drooping leaves are less affected by dark brown spot than those with drooping leaves [19].

The most rational protecting wheat from leaf spot is the cultivation and use of resistant varieties. Due to the wide distribution and harmfulness of these diseases, many scholarworks are devoted to the search for samples of grain crops that are resistant to these pathogens [20-22]. However, in most cases, the identified resistant wheat accessions are insufficient.

In this work, as a result of field and laboratory assessment of 20 varieties of bread wheat (*Triticum aestivum* L.) cultivated in the Lower Volga region, samples resistant to pyrenophorosis and moderately resistant to dark brown spot were identified for the first time. The *Tsn1/tsn1* identification with the molecular marker Xfcp623 showed that among the studied wheat samples, the *Tsn1* gene is predominantly found in winter varieties. For the first time, statistically significant differences were revealed between the average values of field phytopathological indicators in varieties from groups RR, R, MS and S that differ in resistance to *P. tritici-repentis.*

The purpose of the work is to assess the resistance of winter and spring soft wheat (*Triticum aestivum* L.) varieties cultivated in the Lower Volga region to yellow and dark brown spot pathogens and to identify the dominant/recessive allele of the *Tsn1* gene in plants.

Materials and methods. Winter soft wheat varieties Gostianum 237, Lutescens 230, Saratovskaya 8, Guberniya, Mironovskaya 808, Donskaya Bezostaya, Saratovskaya 90, Zhemchuzhina Povolzh'ya, Saratovskaya 17, Kalach 60, Podruga, Anastasiya, Sosedka (13 in total) and spring soft wheat varieties Favorit, Prokhorovka, Yugo-Vostochnaya 2, Saratovskaya 70, Saratovskaya 73, Belyanka, Lebedushka (7 in total) we studied. Of these, 16 have been approved for use in 2022 [23]. Sosedka is a promising variety of winter soft wheat; Gostianum 237, Lutescens 230, Saratovskaya 8 were originated in the Saratov region in the first half of the 20th century.

Field trials were carried out in 2020-2022 in the breeding nursery of the Federal Agrarian Research Center of the South-East (Saratov). To evaluate field resistance to the local population of the causative agent of pyrenophorosis under natural infectious load, Saari-Prescott scale (Saari & Prescott) was used with modifications and addenda [24]. All varieties tested were divided into 5 groups, the RR means highly resistant (susceptibility < 11%), R resistant (11-20%), MS moderately susceptible (21-40%), S susceptible (41-70%) and HS highly susceptible (71-100%).

In lab tests, to infect plant leaves with *P. tritici-repentis* and *B. sorokiniana*, a mixture of several fungal isolates from the collection of the All-Russian Research Institute of Plant Protection was used as the inoculum. In lab tests, a mixture of several fungal isolates (ARRIPP collection, the All-Russian Research Institute of Plant Protection) was used as an inoculum to infect plant leaves with *P. tritici-repentis* and *B. sorokiniana*. The pathogens were isolated in 2022 in Saratov (*P. tritici-repentis*, *ToxA*), Leningrad (*B. sorokiniana*) provinces and the Republic of Kazakhstan (*P. tritici-repentis*, *ToxB*).

For the phytopathological assessment of *P. tritici-repentis*, a point scale was used characterizing the degree of necrosis and chlorosis severity [25], where 1/0 means chlorosis/necrosis, 1/1 resistance (R), 1/2, 2/1, 2/2 moderate resistance

(MR), 2/3, 2/4 moderate susceptibility (MS), 3/2, 3/3, 3/4 susceptibility (S), 4/3, 4/4, 4 /5, 5/4, 5/5 high susceptibility (HS). The resistance to dark brown spot (*B. sorokiniana*) was assessed by the ARRIPP scale where 1 stands for green leaves, with dark brown dotted spots (resistant samples, R), 2 stands for green leaves, spots up to 1 mm in size (medium resistant, MR), 3 for dark brown spots up to 2 mm, merging (moderately susceptible, MS), 4 for chlorotic leaves, dark brown spots reach 3 mm (susceptible, S), and 5 for chlorotic leaves, spots larger than 3 mm, tissue maceration (highly susceptible, HS) [26].

Genomic DNA was isolated from leaves of 5-day-old seedlings by the standard CTAB/chloroform method [27]. The quality of DNA preparations was checked in a 1% agarose gel. Secondary control for purity and quality was also performed (a SmartSpecTMPlus spectrophotometer, Bio-Rad, USA). After quantification, the DNA concentration was normalized to 30 ng/µl for running PCR. The amount of DNA was consistent with the PCR protocol for the *Tsn1* gene identification [28-30].

A 25 µl reaction mixture for genomic DNA amplification contained 2.5 µl 10× PCR buffer (Biolabmix, Russia), 0.5 µl dNTP mixture (10 mM) (TransGen Biotech Co., Ltd., China), 0.5 µl each primer (25 µM) (ZAO Evrogen, Russia), 0.15 µl Taq DNA polymerase (5 units/µl, Dialat LTD, Russia), 1 µl genomic DNA and 19.85 µl diH$_2$O. PCR was carried out in a C1000 Touch Thermal Cycler (Bio-Rad, USA).

The amplified fragments were separated electrophoretically in a 1.5% agarose gel with 1× TBE buffer (pH 8.2); the gel was stained with ethidium bromide (Biolambix, Russia). To prepare an agarose gel, 200 ml of 1× TBE buffer was added to 3 g of agarose (Helicon, USA) and heated until the agarose was completely dissolved, then cooled and added with 1.8 µl ethidium bromide. To prepare 1 liter of 10× TBE buffer, 108 g of Tris-HCl (Helicon, USA), 55 g of boric acid (Helicon, USA), 9.3 g of EDTA (Helicon, USA) and diH^{2}O were used. For 1× TBE buffer, 100 ml of 10× TBE buffer was adjusted to 1 l with diH$_2$O. The size of the fragments was assessed using the Step100 plus DNA marker (Biolabmix, Russia).

Screening of isolates for dominant or recessive alleles *Tsn1*/*tsn1* was carried out by PCR with primer pairs Xfcp623F/Xfcp623R (ZAO Evrogen, Russia). PCR protocol: 3 min at 94 °C; 30 s at 94 °C, 30 s at 60 °C, 1 min at 72 °C (45 cycles); 5 min at 72 °C. The nucleotide sequence of the Xfcp623F primer is 5′-CTATTCGTAATCGTGCCTTCCG-3′, of the Xfcp623R primer 5′-CCTTCTCTCTCACCGCTATCTCATC-3′. The amplicon size is 380 bp [28-30].

Amplification of the marker is indicative of a dominant *Tsn1* allele and susceptibility to the fungal toxin protein PtrToxA, the absence of the amplification means corresponds to a recessive allele *tsn1* and resistance to PtrToxA.

Statistical processing was performed using the Statistica 12 computer program (StatSoft, Inc., USA). The average damage to the leaf blade by pyrenophorosis during field trials (*M*) and standard deviation (±SD) were calculated. To separate wheat varieties by resistance/susceptibility to pyrenophorosis, Student's *t*-test with Bonferroni correction was applied to assess the significance of differences between phytopathological scores of wheat varieties [31].

Results. As is known, climatic conditions significantly influence the development of biological systems. Increased temperatures and drought due to climate change lead to rapid senescence of leaves and an increase in the incidence of leaf spots [32]. The weather conditions of the growing seasons 2020-2022 in the region had a beneficial effect on the development of yellow leaf spot. The highest degree of damage to wheat plants by pyrenophorosis was observed in 2022 (more than 40% on susceptible varieties).

1. Resistance of wheat (*Triticum aestivum* L.) varieties cultivated in the Lower Volga region to yellow and dark brown spot pathogens

Variety (allele)	Field test estimate (*Pyrenophora tritici-repentis*), %		Lab test score, points (phenotype)		
			P. tritici-repentis		*Bipolaris sorokiniana*
	M±SD (*n* = 90)	resistance phenotype	*ToxA*	*ToxB*	
Soft winter wheat					
Gostianum 237 (*tsn1*)	4.3±1.15	RR	2/1 (MR)	2/2 (MR)	4 (S)
Lutescens 230 (*Tsn1*)	23.3±5.77	MS	1/2 (MR)	1/1 (R)	2 (MR)
Saratovskaya 8 (*Tsn1*)	18.3±2.89	R	1/1 (R)	1/1 (R)	3 (MS)
Guberniya* (*Tsn1*)	6.7±2.89	RR	3/2 (S)	3/2 (S)	2 (MR)
Mironovskaya 808* (*tsn1*)	8.3±5.77	RR	3/2 (S)	1/1 (R)	2+,3 (MS)
Donskaya Bezostaya* (*Tsn1*)	8.3±2.89	RR	3/2 (S)	1/1 (R)	3 (MS)
Saratovskaya 90* (*Tsn1*)	48.3±2.89	S	1/1 (R)	3/3 (S)	3 (MS)
Zhemchuzhina Povolzh'ya* (*Tsn1*)	41.7±5.77	S	3/3 (S)	3/3 (S)	4 (S)
Saratovskaya 17* (*Tsn1*)	41.7±5.77	S	1/1 (R)	3/3 (S)	4 (S)
Kalach 60* (*Tsn1*)	21.7±7.64	MS	1/1 (R)	1/1 (R)	2+,3 (MS)
Podruga* (*tsn1*)	3.3±2.89	RR	3/3 (S)	3/3 (S)	3 (MS)
Anastasiya* (*Tsn1*)	28.3±2.89	MS	1/1 (R)	3/3 (S)	3 (MS)
Sosedka (*Tsn1*)	8.3±5.77	RR	1/0 (R)	1/1 (R)	3 (MS)
Soft spring wheat					
Favorit* (*tsn1*)	6.7±2.89	RR	1/1(R)	1/1 (R)	4 (S)
Prokhorovka* (*tsn1*)	10.0±5.00	RR	3/3 (S)	1/1 (R)	4 (S)
Yugo-Vostochnaya 2* (*tsn1*)	23.33±5.77	MS	3/3 (S)	3/3 (S)	4 (S)
Saratovskaya 70* (*tsn1*)	11.7±2.89	R	3/3 (S)	2/2 (MR)	4 (S)
Saratovskaya 73* (*tsn1*)	8.3±2.89	RR	1/1 (R)	2/2 (MR)	3 (MS)
Belyanka* (*tsn1*)	16.7±5.77	R	1/1 (R)	1/1 (R)	2 (MR)
Lebedushka* (*Tsn1*)	26.7±2.89	MS	3/3 (S)	3/3 (S)	3 (MS)

Note. RR — high resistance, R — resistance, MR — moderate resistance, MS — moderate susceptibility, S — susceptibility. Field trials were carried out in 2020-2022 at the breeding nursery of the Federal Agrarian Research Center of the South-East (Saratov). The varieties approved for cultivation in the Lower Volga region of the Russian Federation (region 8) are marked with an asterisk [13].

According to data of 2020-2022, at the breeding nursery of the Federal Agrarian Research Center of the South-East (Saratov), the studied varieties of soft winter and spring wheat developed different resistance to the local population of *P. tritici-repentis* (Table 1). The degree of damage to the varieties Gostianum 237, Guberniya, Mironovskaya 808, Donskaya Bezostaya, Podruga, Sosedka, Favorit, Prokhorovka and Saratovskaya 73 did not exceed 8%, so they were identified as highly resistant (RR). The varieties Saratovskaya 8, Saratovskaya 70 and Belyanka turned out to be resistant (R) with an average degree of damage of no more than 18%.

Lutescens 230, Kalach 60, Anastasiya, Yugo-Vostochnaya 2 and Lebedushka showed moderate susceptibility (MS). The remaining varieties turned out to be susceptible to field damage from pyrenophorosis (see Table 1).

To establish the significance of differences between varieties ranked by resistance to pyrenophorosis (groups RR, R, MS, and S), we used Student's *t*-test with Bonferroni correction (Table 2). Note, no differences were found between varieties of the same resistance group. The exceptions were Lutescens 230 (MS) and Saratovskaya 8 (R) with the average degree of pyrenophorosis damage of 23 and 18%, respectively, and Mironovskaya 808 (RR) and Saratovskaya 70 (R), with 8 and 12%, respectively. No statistically significant differences could be detected between these pairs of varieties ($p > 0.05$).

L.A. Mikhailova et al. [25], as a result of an analysis of over 1000 samples from the VIR collection, reported that the frequency of resistant forms among winter wheat accecssions is higher than among spring wheat. Screening for resistance to yellow spot of 209 varieties of winter and 136 varieties of spring wheat from the State Register of Breeding Achievements of the Russian Federation until 2010 showed that approximately 40% of winter wheat varieties and 16% of spring wheat were characterized by varying degrees of resistance to *P. tritici-repentis*, the remaining varieties were susceptible [25].

2. Variance analysis of a posteriori comparisons of wheat (*Triticum aestivum* L.) varieties depending on the field damage by *Pyrenophora tritici-repentis*
(Student's *t*-test with Bonferroni correction; breeding nursery of the Federal Agrarian Research Center of the South-East, Saratov, **2020-2022**

Variety	Lutescens 230	Saratovskaya 8	Guberniya	Mironovskaya 808	Donskaya Bezostaya	Saratovskaya 90	Zhemchuzhina Povolzh'ya	Saratovskaya 17	Kalach 60	Podruga	Anastasiya	Sosedka	Favorit	Prokhorovka	Yugo-Vostochnaya 2	Saratovskaya 70	Saratovskaya 73	Belyanka	Lebedushka
Gostianum 237	0*	0*	0.52	0.28	0.28	0*	0*	0*	0*	0.79	0*	0.28	0.53	0.13	0*	0.04*	0.28	0*	0*
Lutescens 230		0.18	0*	0*	0*	0*	0*	0*	0.65	0*	0.18	0*	0*	0*	1	0*	0*	0.04*	0.37
Saratovskaya 8			0*	0*	0.01*	0*	0*	0*	0.04*	0*	0.01*	0.01*	0*	0.03*	0.04*	0.08	0.01*	0.65	0.03*
Guberniya				0.65	0.65	0*	0*	0*	0*	0.37	0*	0.65	1	0.37	0*	0.04*	0.65	0.01*	0*
Mironovskaya 808					1	0*	0*	0*	0*	0.18	0*	1	0.65	0.65	0*	0.37	1	0.03*	0*
Donskaya Bezostaya						0*	0*	0*	0*	0.18	0*	1	0.65	0.65	0*	0.04*	1	0.03*	0*
Saratovskaya 90							0.08	0.08	0*	0*	0*	0*	0*	0*	0*	0*	0*	0*	0*
Zhemchuzhina Povolzh'ya								1	0*	0*	0*	0*	0*	0*	0*	0*	0*	0*	0*
Saratovskaya 17									0*	0*	0*	0*	0*	0*	0*	0*	0*	0*	0*
Kalach 60										0*	0.08	0*	0*	0*	0.65	0.01*	0*	0.04*	0.18
Podruga											0*	0.18	0.37	0.08	0*	0.03*	0.18	0*	0*
Anastasiya												0*	0*	0*	0.18	0*	0*	0*	0.65
Sosedka													0.65	0.65	0*	0.04*	1	0.03*	0*
Favorit														0.37	0*	0.04*	0.65	0.01*	0*
Prokhorovka															0*	0.04*	0.65	0.04*	0*
Yugo-Vostochnaya 2																0*	0*	0.04*	0.37
Saratovskaya 70																	0.04*	0.18	0*
Saratovskaya 73																		0.03*	0*
Belyanka																			0.01*

* Differences are significant at $p \leq 0.05$.

Due to the insignificant manifestation of dark brown spot in the natural conditions of the Lower Volga region, a correct field resistance assessment of the wheat varieties was impossible. However, in the context of climate change, the consequences of which are observed in the region every year, the issue of creating promising sustainable breeding material, including resistant to dark brown spot, is undoubtedly relevant. This problem is of particular importance now, when the pathogen influenced by various factors (biological characteristics, anthropogenic impact, etc.) is expanding its area.

Despite the impressive number of wheat varieties approved for use in various regions of Russia, there are probably not enough varieties highly resistant to dark brown spot. For example, in the Republic of Tatarstan, with the strong development of dark brown spot, most varieties of spring wheat were susceptible to damage [11]. In the Amur region, under epiphytoty of dark brown spot, wheat samples varying in degrees of resistance were identified, but no barley varieties resistant to *B. sorokiniana* were found [16]. A.V. Sidorova et al. [14] did not identify samples highly resistant to dark brown leaf spot among the studied spring soft wheat accessions of the VIR world collection.

In the lab tests, we assessed the resistance of varieties to pathogens of pyrenophorous and dark brown spot. When inoculating samples, high resistance to both isolates of *P. tritici-repentis* (*ToxA* and *Tox B*) was noted in the varieties Saratovskaya 8, Kalach 60, Sosedka, Favorit, Belyanka. The varieties Saratovskaya 90, Saratovskaya 17, Anastasiya, Saratovskaya 73 showed high resistance to the isolate carrying the *ToxA* gene, and Lutescens 230, Mironovskaya 808, Donskaya Bezostaya, Prokhorovka were highly resistant to the isolate with the *Tox B* gene. Moderate resistance to both isolates of *P. tritici-repentis* was shown by Gostianum 237, Lutescens 230, to the isolate with the *ToxB* gene by Saratovskaya 70, Saratovskaya 73. The total proportion of varieties resistant to *P. tritici-repentis* producing ToxA and ToxB toxins was 40 %, to the isolate with the *ToxA* gene 55%, with *Tox B* 60%. The samples Guberniya, Zhemchuzhina Povolzh'ye, Podruga, Yugo-Vostochnaya 2 and Lebedushka were susceptible to both isolates.

N.M. Kovalenko et al. [1], when screening 70 varieties of bread wheat, first approved by the State Register of Breeding Achievements of the Russian Federation in 2018-2020, found that the proportion of varieties resistant at the seedling syaage to isolates producing ToxA and ToxB toxins was 13%, to the isolate forming ToxA 21%, ToxB 43%.

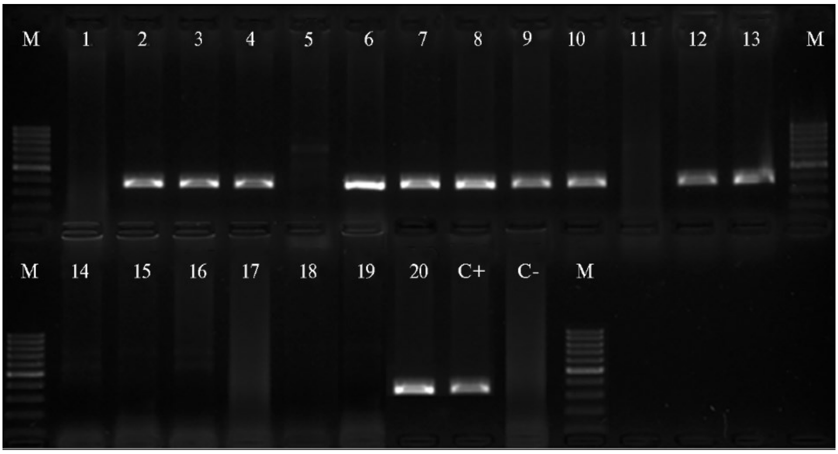
The presence of resistance donors among Russian wheat varieties creates the opportunity for successful selection for immunity to yellow spot. This is especially true currently, when the spread of diseases in grain-producing regions of Russia has become almost universal, and grain harvest losses can amount to more than 50%.

In our studies, during artificial infection of wheat varieties with the pathogen *B. sorokiniana*, no highly resistant samples were identified. However, three varieties demonstrated moderate resistance (MR), the Lutescens 230, Guberniya and Belyanka. The remaining samples were susceptible. The total proportion of varieties resistant to *B. sorokiniana* was 15%.

In addition to field and laboratory assessment of the resistance of varieties to *P. tritici-repentis*, we screened the samples for the presence of a dominant (*Tsn1*) or recessive (*tsn1*) allele in the genotype (Fig.).

Among the 20 varieties studied, only 3 varieties of winter wheat (Gostianum 237, Mironovskaya 808, Podruga) and 6 varieties of spring soft wheat (Favorit, Prokhorovka, Yugo-Vostochnaya 2, Saratovskaya 70, Saratovskaya 73, Belyanka) carried the *tsn1* allele, which indicates their resistance to PtrToxA-producing isolates at the genetic level.

The results of identification of the *Tsn1* and *tsn1* alleles using the molecular marker Xfcp623 showed the predominant presence of *Tsn1* in winter wheat varieties (see Fig.).



Electropherogram of the Xfcp623 marker amplification products in varieties of winter soft wheat (*Triticum aestivum* L.): 1 — Gostianum 237, 2 — Lutescens 230, 3 — Saratovskaya 8, 4 — Guberniya, 5 — Mironovskaya 808, 6 — Donskaya Bezostaya, 7 — Saratovskaya 90, 8 — Zhemchuzhina Povolzh'ya, 9 — Saratovskaya 17, 10 — Kalach 60, 11 — Podruga, 12 — Anastasiya, 13 — Sosedka, 14 — Favorit, 15 — Prokhorovka, 16 — Yugo-Vostochnaya 2, 17 — Saratovskaya 70, 18 — Saratovskaya 73, 19 — Belyanka, 20 — Lebedushka. M — DNA marker Step100 plus (Biolabmix, Russia). Positive control (C+) is Glenlea variety, negative control (C-) is line 6B365. The diagnostic fragment is 380 bp in size.

According to the literature, the preservation and spread of isolates with the *ToxA* gene in the population may occur due to their selection on varieties with the *Tsn1* susceptibility gene [33]. It is suggested that the sexual stage of *P. tritici-repentis* is predominantly observed on winter varieties and results in the retention of the *ToxA* gene [34].

Of the 20 cultivar—isolate *Tsn1—ToxA* pathosystems analyzed, gene interaction in almost half of the cases (45.5%) was gene-by-gene, that is, it led to damage to cultivars with the dominant *Tsn1* allele by *P. tritici-repentis* isolates with the *ToxA* gene. In the remaining 54.5% of cases, a resistance response occurred. Varieties with recessive *tsn1* alleles were affected by *ToxA* isolates in 54.5% of combinations, the rest remained resistant. Apparently, this happened because we did not use a pure toxin, but spores of a fungus that carries the *ToxA* gene and is capable of producing, in addition to PtrToxA, toxins that have not yet been identified [3, 5, 8].

N.V. Mironenko et al. [34] explain the resistance of varieties with the dominant *Tsn1* allele to *P. tritici-repentis* isolates with the *ToxA* gene by a possible decrease in the expression of the *ToxA* gene under the influence of the genetic background of a particular variety. The defeat of varieties with recessive *tsn1* alleles may indicate either the absence of gene-by-gene intraction, or the presence of other effector genes and susceptibility genes in the pathosystem.

In general, of the 20 varieties of winter and spring soft wheat that we studied, only the Belyanka variety is resistant to both pathogens at once. It showed resistance to *P. tritici-repentis* in field tests, to *P. tritici-repentis* isolates with *ToxA* and *ToxB* in lab test, and moderate resistance to *B. sorokiniana*. In addition, the Belyanka variety carries the *tsn1* gene, which indicates resistance to *P. tritici-repentis* with *ToxA*.

Thus, by screening 20 varieties of soft wheat, promising and approved for cultivation in the Lower Volga region of the Russian Federation, we identified samples resistant to the causative agent of pyrenophorous spot, and moderately resistant to dark brown spot. The varieties resistant to *Pyrenophora tritici-repentis* isolates producing toxins PtrToxA and PtrToxB account for 40%, to PtrToxA producers 55%, PtrToxB 60%. The proportion of varieties that showed average resistance to the pathogen Bipolaris sorokiniana was only 15%. The genotypes of greatest interest are three winter soft wheat varieties, the Gostianum 237, Mironovskaya 808, Podruga and five spring soft wheat varieties, the Favorit, Prokhorovka, Saratovskaya 70, Saratovskaya 73, and Belyanka which turned out to be resistant to pyrenophorosis in lab and three-year field tests. Molecular screening also confirmed immunity to PtrToxA producers in these varieties due to the *tsn1* allele which indicates their resistance to the fungal toxin protein PtrToxA. These varieties are recommended for regional programs of wheat breeding for resistance to yellow and dark brown leaf spots.

REFERENCES

1. Kovalenko N.M., Shaydayuk E.L., Gul'tyaeva E.I. *Biotekhnologiya i selektsiya rasteniy*, 2022, 5(2): 15-24 (doi: 10.30901/2658-6266-2022-2-o3) (in Russ.).
2. Bankina B., Bimšteine G., Arhipova I., Kaneps J., Darguža M. Impact of crop rotation and soil tillage on the severity of winter wheat leaf blotches. *Rural Sustainability Research*, 2021, 45(340): 21-27 (doi: 10.2478/plua-2021-0004).
3. Effertz R.J., Meinhardt S.W., Anderson J., Jordahl J.G., Francl L.J. Identification of a chlorosis-inducing toxin from *Pyrenophora tritici-repentis* and the chromosomal location of an insensitivity locus in wheat. *Phytopathology*, 2002, 92(5): 527-533 (doi: 10.1094/PHYTO.2002.92.5.527).
4. Ramos E.R., Kutcher R., Dallagnol J. *Pyrenophora tritici-repentis*: a worldwide threat to wheat. In: *Wheat* [Working Title]. IntechOpen, 2023 (doi: 10.5772/intechopen.110306).
5. Corsi B., Percival-Alwyn L., Downie R.C., Venturini L., Iagallo E.M., Mantello C.C., McCormick-Barnes Ch., Theen See P., Oliver R.P., Moffat C.S., Cockram J. Genetic analysis of wheat sensitivity to the ToxB fungal effector from *Pyrenophora tritici-repentis*, the causal agent of tan spot. *Theoretical and Applied Genetics*, 2020, 133: 935-950 (doi: 10.1007/s00122-019-03517-8).
6. Strelkov S., Lamari L. Host parasite interactions in tan spot [*Pyrenophora tritici-repentis*] of wheat. *Canadian Journal of Plant Pathology*, 2003, 25(4): 339-349 (doi: 10.1080/07060660309507089).
7. Faris J.D., Overlander M.E., Kariyawasam G.K., Carter A., Xu S.S., Liu Z. Identification of a major dominant gene for race-nonspecific tan spot resistance in wild emmer wheat. *Theoretical and Applied Genetics*, 2020, 133(3): 829-841 (doi: 10.1007/s00122-019-03509-8).
8. Gourlie R., McDonald M., Hafez M., Ortega-Polo R., Low K.E., Abbott D.W., Strelkov S. E., Daayf F., Aboukhaddou R. The pangenome of the wheat pathogen *Pyrenophora tritici-repentis* reveals novel transposons associated with necrotrophic effectors ToxA and ToxB. *BMC Biology*, 2022, 20(1): 239 (doi: 10.1186/s12915-022-01433-w).
9. Friesen T.L., Holmes D.J., Bowden R.L., Faris J.D. *ToxA* is present in the u.s. *Bipolaris sorokiniana* population and is a significant virulence factor on wheat harboring *Tsn1*. *Plant Disease*. 2018, 102(12): 2446-2452 (doi: 10.1094/PDIS-03-18-0521-RE).
10. Bertagnolli V.V., Ferreira J.R., Liu Z., Rosa A.C., Deuner C.C. Phenotypical and genotypical characterization of *Pyrenophora tritici-repentis* races in Brazil. *European Journal of Plant Pathology*, 2019, 154(4): 995-1007 (doi: 10.1007/s10658-019-01720-3).
11. Askhadullin Dan.F., Askhadullin Dam.F., Vasilova N.Z., Zharekhina T.V., Gayfullina G.R., Khusainova I.I., Bagavieva E.Z., Tazutdinova M.R. *Zashchita i karantin rasteniy*, 2018, 9: 17-19 (in Russ.).
12. Novakazi F., Afanasenko O., Lashina N., Platz G. J., Snowdon R., Loskutov I., Ordon F. Genome-wide association studies in a barley (*Hordeum vulgare*) diversity set reveal a limited number of loci for resistance to spot blotch (*Bipolaris sorokiniana*). *Plant Breeding*, 2020, 139(3): 521-535 (doi: 10.1111/pbr.12792).
13. Gupta P.K., Chand R., Vasistha N.K., Pandey S.P., Kumar U., Mishra V.K., Joshi A.K. Spot blotch disease of wheat: the current status of research on genetics and breeding. *Plant Pathology*, 2018, 67(3): 508-531 (doi: 10.1111/ppa.12781).
14. Sidorov A.V., Zakharov V.G., Tyryshkin L.G. *Izvestiya Sankt-Peterburgskogo gosudarstvennogo agrarnogo universiteta*, 2018, 53: 76-79 (doi: 10.24411/2078-1318-2018-14076) (in Russ.).
15. Kremneva O.Yu., Kostenko I.A., Pachkin A.A., Danilov R.Yu., Ponomarev A.V., Kim Yu.S. *Zernovoe khozyaystvo Rossii,* 2020, 3(69): 61-66 (doi: 10.31367/2079-8725-2020-69-3-61-66) (in Russ.).
16. Makarova M.A., Karacheva G.S., Lomakina I.V. *Zashchita i karantin rasteniy*, 2018, 8: 41-43 (in Russ.).

17. Akulov A.Yu. *Biologicheskie osobennosti Bipolaris sorokiniana (Sacc. in Sorokin) Shoem i differentsirovannaya diagnostika vozbuditeley kornevoy gnili i chernogo zarodysha yachmenya. Kandidatskaya dissertatsiya* [Biological properties of *Bipolaris sorokiniana* (Sacc. in Sorokin) Shoem and differentiated diagnosis of pathogens of root rot and black germ of barley. PhD Thesis]. Khar'kov, 2006: 232 (in Russ.).
18. Lillemo M., Joshi A.K., Prasad R., Chand R., Singh R. QTL for spot blotch resistance in bread wheat line Saar co'locate to the biotrophic disease resistance loci Lr34 and Lr46. *Theoretical and Applied Genetics*, 2013, 126(3): 711-719 (doi: 10.1007/s00122-012-2012-6).
19. Joshil A.K., Chand R. Variation and inheritance of leaf angle, and its association with spot blotch (*Bipolaris sorokiniana*) severity in wheat (*Triticum aestivum*). *Euphytica*, 2002, 124: 283-291 (doi: 10.1023/A:1015773404694).
20. Mironenko N.V., Kovalenko N.M. *Vestnik zashchity rasteniy*, 2018, 2(96): 12-16 (in Russ.).
21. Kon'kova E.A., Lyashcheva S.V. *Zernovoe khozyaystvo Rossii*, 2020, 3(69): 67-71 (doi: 10.31367/2079-8725-2020-69-3-67-71) (in Russ.).
22. Kumarbayeva M., Kokhmetova A., Kovalenko N.M., Atishova M., Keishilov Z., Aitymbetova K. Characterization of *Pyrenophora tritici-repentis* (tan spot of wheat) races in Kazakhstan. *Phytopathologia Mediterranea*, 2022, 61(2): 243-257 (doi: 10.36253/phyto-13178).
23. *Gosudarstvennyy reestr selektsionnykh dostizheniy, dopushchennykh k ispol'zovaniyu. Tom 1. «Sorta rasteniy» (ofitsial'noe izdanie)* [State register of selection achievements approved for use. Volume 1. Plant varieties (official publication)]. Moscow, 2022: 646 (in Russ.).
24. Kolomiets T.M., Pakholkova E.V., Dubovaya L.P. *Otbor iskhodnogo materiala dlya sozdaniya sortov pshenitsy s dlitel'noy ustoychivost'yu k septoriozu* [Selection of parental material for breeding wheat varieties with long-term resistance to septoria]. Moscow, 2017: 56 (in Russ.).
25. Mikhaylova L.A., Mironenko N.V., Kovalenko N.M. *Zheltaya pyatnistost' pshenitsy. Metodicheskie ukazaniya po izucheniyu vozbuditelya zheltoy pyatnistosti Pyrenophora tritici-repentis i ustoychivosti sortov* [Yellow spot of wheat. Guidelines for studying the causative agent of yellow spot *Pyrenophora tritici-repentis* and resistance of varietie]. St. Petersburg, 2012: 64 (in Russ.).
26. Smurova S.G. *Novye istochniki i donory ustoychivosti pshenitsy k Cochliobolus sativus Drechs. ex Dastur. Kandidatskaya dissertatsiya* [New donors of wheat resistance to *Cochliobolus sativus* Drechs. ex Dastu. PhD Thesis]. St. Petersburg, 2008: 236 (in Russ.).
27. Doyle J.J., Doyle J.L. Isolation of plant DNA from fresh tissue. *Focus*, 1990, 12(1): 13-15.
28. Röder M.S., Korzun V., Wendehake K., Plaschke J., Tixier M.H., Leroy Ph., Ganal M.W. A microsatellite map of wheat. *Genetics*, 1998, 149(4): 2007-2023 (doi: 10.1093/genetics/149.4.2007).
29. Zhang Z., Friesen T.L., Simons K.J., Xu S.S., Faris J.D. Development, identification, and validation of markers for marker-assisted selection against the *Stagonospora nodorum* toxin sensitivity genes *Tsn1* and *Snn2* in wheat. *Molecular Breeding*, 2009, 23: 35-49 (doi: 10.1007/s11032-008-9211-5).
30. Faris J.D., Zhang Z., Lu H.J., Lu S.W., Reddy L., Cloutier S., Fellers J.P., Meinhardt S.W., Rasmussen J.B., Xu S.S., Oliver R.P., Simons K.J., Friesen T.L. A unique wheat disease resistance-like gene governs effector-triggered susceptibility to necrotrophic pathogens. *Proceedings of the National Academy of Sciences USA*, 2010, 107(30): 13544-13549 (doi: 10.1073/pnas.1004090107).
31. Trukhacheva N.V. *Meditsinskaya statistika: uchebnoe posobie* [Medical statistics: textbook] (in Russ.). Rostov-na-Donu, 2017 (in Russ.).
32. Kokhmetova A.M., Ali S., Sapakhova Z., Atishova M.N. *Vavilovskiy zhurnal genetiki i selektsii*, 2018, 22(8): 978-986 (doi: 10.18699/VJ18.440) (in Russ.).
33. Mironenko N.V., Baranova O.A., Kovalenko N.M., Afanasenko O.S., Mikhaylova L.A. *Vestnik zashchity rasteniy*, 2017, 3 (93): 23-27 (in Russ.).
34. Mironenko N.V., Baranova O.A., Kovalenko N.M. *Mikologiya i fitopatologiya*, 2019, 53(2): 115-123 (doi: 10.1134/S0026364819020077) (in Russ.).