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RESISTANCE OF SOFT WINTER WHEAT (*Triticum aestivum* L.) VARIETIES CULTIVATED IN THE TAMBOV REGION TO TAN SPOT (*Pyrenophora tritici-repentis*)

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Abstract

The Tambov region is part of the Central Black Earth region with highly developed grain production. In the structure of cultivated area, grains make up to 50-55 % of arable land. Productivity, gross yield and quality of grain in the region vary significantly and are determined by many factors, including damage to plants by fungal pathogens. The fungus *Pyrenophora tritici-repentis* is the causative agent of tan spot, or yellow spot, a dangerous disease of wheat that progresses rapidly in grain-producing countries. The introduction of disease-resistant varieties into grain production is an economically beneficial and environmentally friendly method of plant protection that increases the efficiency of chemical and agrotechnical measures. In this work, when studying the *P. tritici-repentis* population racial composition in the Tambov region, five races of the pathogen were identified for the first time. Races producing PtrToxC predominated, and races with the *ToxA* gene encoding exotoxin were less common. For the first time, eight wheat varieties were revealed that were highly resistant to the phytopathogen in field and laboratory conditions, molecular screening also confirmed resistance to PtrToxA. The purpose of the work was to investigate the race composition of *Pyrenophora tritici-repentis* isolates from the Tambov population of 2022, to assess the resistance of winter common wheat varieties cultivated in the Tambov region to the yellow spot pathogen, and to identify their dominant/recessive allele of the *Tsn1* gene. A set of 28 varieties of winter common wheat (*Triticum aestivum* L.) comprised 20 varieties approved for use since 2022 (Antonivka, Bezenchukskaya 380, Biryuza, Dominanta, Don 93, Donera, Donskoy Surpriz, Zvonnitsa, Izyuminka, Inna, Lipetskaya Zvezda, Lgovskaya 4, Mironovskaya 100, Mironovskaya 808, Moskovskaya 39, Moskovskaya 40, Moskovskaya 56, Odesskaya 200, Sintetik, Skipetr), the remaining 8 varieties (Kosovitsa, Kruiz, Laguna, Latynevka, L'govskaya 167, Prestizh, Proza, Spartak) are not approved for zonal use. The resistance of wheat varieties to *P. tritici-repentis* was assessed in lab tests in 2022 using common methods. The infectious material was collected in 2022 in the Tambov region. From 19 affected samples of winter bread wheat, 68 monoconidial isolates of *P. tritici-repentis* were purified on the V4 nutrient medium. The response of wheat seedlings to inoculation with a suspension of *P. tritici-repentis* was assessed on days 5-6. With a set of differentiators, the Glenlea variety and the lines 6B365, 6B662 as identifiers of the toxins PtrToxA, PtrToxC and PtrToxB, the racial composition of the *P. tritici-repentis* population was identified based on the response of the leaves (necrosis/chlorosis) to the pathogen invasion. In 2020-2022, field assessments of wheat variety resistance were performed at the stationary site (the Central Russian branch of Michurin Federal Scientific Center, Tambov District, Tambov Province) under natural infection. Genomic DNA was extracted from leaves of 5-day-old wheat seedlings using the standard CTAB/chloroform procedure. DNA concentration was normalized to 30 ng/μl for PCR. Isolates were screened for the dominant *Tsn1* or recessive *tsn1* alleles using primers Xfcp623F/Xfcp623R. Among the *Pyrenophora tritici-repentis* isolates from winter bread wheat of the Tambov Province, three races were common, of which race 4 did not produce the toxins PtrToxA, PtrToxB and PtrToxC, race 3 produced

the toxin PtrToxC, and race 1 did not produce PtrToxA and PtrToxC. Races 8 (PtrToxA, PtrToxB and PtrToxC) and 2 (PtrToxA) were classified as rare. We did not find races 5 (PtrToxB), 6 (PtrToxB and PtrToxC) and 7 (PtrToxA and PtrToxB) in the population. Race 3, producing the *ToxC* gene encoded exotoxin, was the most abundant in the *P. tritici-repentis* population of the Tambov Province; races producing PtrToxA were less frequent. Practically, seven varieties, the Lipetskaya Zvezda, Moskovskaya 56, Moskovskaya 40, Bezenchukskaya 380, Biryuza, Inna, Odesskaya 200 approved for the Tambov Province by the State Register of Breeding Achievements, and the Dominanta variety approved for the North Caucasus and Ural regions are of the greatest interest. These varieties demonstrated the highest resistance to *P. tritici-repentis* in field and laboratory tests. Molecular screening also confirmed their resistance to PtrToxA. Carriers of the identified recessive allele of the *tsn1* gene which determines resistance to the PtrToxA toxin of *P. tritici-repentis* are recommended for breeding programs to increase resistance to tan spot of wheat.

Keywords: *Pyrenophoratritici-repentis*, *ToxA*, *ToxB*, *ToxC*, *Tsn1*, tan spot, yellow spot disease, PCR, wheat

The Tambov region is part of the Central Black Earth of the Russian Federation with developed agro-industry and special attention to the agricultural sector in the economy. In the sown lands of the Tambov Province, the grain crop areas are the largest. These are winter and spring wheat (on average 31% of the total area in the region), winter and spring barley (20%), corn for grain (7%) [1, 2]. According to the Federal State Rosselkhoztsentr for the Tambov Province [2], in 2020 and 2021 the most popular varieties of winter soft wheat were Moskovskaya 56, Moskovskaya 40, Skipetr, in 2022 Moskovskaya 56 and two varieties of Krasnodar selection (Alekseich and Grom).

Currently, among the harmful fungal diseases of wheat widespread in the region, yellow spot, or pyrenophorosis, is of particular economic importance. The causative agent of the disease is the fungus *Pyrenophora tritici-repentis* (Died.) Drechsler. This is one of the most harmful diseases of wheat, which occurs in all areas of crop cultivation [3-5]. *P. tritici-repentis* is capable of attacking the vegetative aerial parts of plants and grains, but is usually most noticeable on leaves. Necrosis and chlorosis on plant tissues lead to disruption of the host's metabolism and a decrease in qualitative and quantitative yield parameters. During epiphytotic years, crop losses can exceed 50% [6].

The fungus *P. tritici-repenti*s is known for its ability to synthesize necrotrophic effectors (NEs), including host selective toxins (HSTs), which function as pathogenicity factors. In *P. tritici-repentis*, three necrotrophic effectors have been described, the PtrToxA, PtrToxB and PtrToxC. There are a larger number of NEs [7-9]. PtrToxA and PtrToxB are proteins, PtrToxC is a non-protein low-molecular compound [10-12].

Based on the NEs production, *P. tritici-repentis* strains are assigned to eight races. PtrToxA is secreted by races 1, 2, 7, and 8, PtrToxB by races 5, 6, 7, and 8, and PtrToxC by races 1, 3, 6, and 8 [13]. Race 4 does not secrete any of the three known host-specific toxins and is considered avirulent according to the current model [14, 15].

In this work, five *P. tritici-repentis* races were identified in the Tambov Province for the first time. In the studied population, races producing PtrToxC predominated, while races with the exotoxin encoded by the *ToxA* gene were less common. In addition, for the first time, eight wheat varieties were detected that were highly resistant to the phytopathogen in field and in lab tests. Their resistance to PtrToxA was also confirmed by molecular screening.

The purpose of the work is to analyze the collection of *Pyrenophora tritici-repentis* isolates from the Tambov population of 2022 by race composition, to evaluate the resistance of winter soft wheat varieties to the yellow spot pathogen, and to identify the dominant/recessive allele of the *Tsn1* gene in the varieties.

Materials and methods. Samples of affected wheat leaves were collected in

2022 from the experimental and comercial fields of the Michurina Federal Scientific Center (Tambov Province, Tambov District). The weather conditions of the year were favorable for incidence of yellow spot disease. All samples were collected during grain ripening, at the stage of milky-waxy ripeness (75-85 on the Zadoks scale). Leaves with typical visual signs of the disease were collected and herbarized. A total of 19 infectious samples were collected from wheat (*Triticum aestivum* L.): varieties Bezenchukskaya 380, Biryuza, Zvonnitsa, Izyuminka, Inna, Kosovitsa, Laguna, Latynevka, Lipetskaya Zvezda, Lgovskaya 167, Lgovskaya 4, Mironovskaya 100, Mironovskaya 808, Moskavskaya 39 , Moskovskaya 40, Prestizh, Proza, Synthetik, Spartak. An infectious sample was defined as leaves with well-expressed symptoms of pyrenophorosis, collected in one field along its diagonal at equal distances at the same time [16].

The samples were investigated in lab tests. Pure culture of the fungus was isolated on the V4 nutrient medium, which consisted of 150 ml of a mixture of juices of four vegetables, 850 ml of water and 1.5 g of $CaCO_3$ [17]. Conidal isolates were used to evaluate wheat varieties for lab resistance to pyrenophorosis and to study the race composition of the fungal population.

In lab tests (2022), 28 varieties of winter soft wheat (*Triticum aestivum* L.) were assessed for resistance to pyrenophorosis. Of these, 20 varieties Antonivka, Bezenchukskaya 380, Biryuza, Dominanta, Don 93, Donera, Donskoy Syurpriz, Zvonnitsa, Izyuminka, Inna, Lipetskaya Zvezda, Lgovskaya 4, Mironovskaya 100, Mironovskaya 808, Moskovskaya 39, Moskovskaya 40, Moskovskaya 56, Odesskaya 200, Synthetic, and Skipetr were approved in 2022 for use. The remaining 8 varieties (Kosovitsa, Kruiz, Laguna, Latynevka, Lgovskaya 167, Prestizh, Proza, Spartak) are not zoned [16].

Segments of leaves of 10-day-old seedlings, 3-4 cm long, were laid out in cuvettes on glass wrapped in filter paper moistened with a 0.004% aqueous benzimidazole solution. The ends of the segments were covered with cotton rolls soaked in the same solution. Leaves, 10 for each variety, were arranged in rows and sprayed with conidial suspension ($2\text{-}3\times10^3$ units).

The cuvette was covered with glass, kept for 1 day in the dark at room temperature and placed in a light installation with LB-40 fluorescent lamps (model MIR-154, Sanyo Incubator, Japan) at 22 °C. The response to inoculation with *P. tritici-repentis* was assessed on days 5-6 based on a scale developed at the All-Russian Institute of Plant Protection [17] where 1/0 stands for chlorosis/necrosis, 1/1 for resistance (R); 1/2, 2/1, 2/2 for moderate resistance (MR); 2/3, 2/4 for moderate susceptibility (MS); 3/2, 3/3, 3/4 for susceptibility (S); 4/3, 4/4, 4/5, 5/4, 5/5 for high susceptibility (HS).

The race composition of the *P. tritici-repentis* population was identified with a set of differentiators, including the Glenlea variety and lines 6B365, 6B662 as identifiers of the toxins PtrToxA, PtrToxC and PtrToxB, by response (necrosis/chlorosis) to inoculation [14, 18]. A total of 68 fungal isolates were tested.

Field tests were performed in 2020-2022 at the North of the Central Chernozem region (the Central Russian branch of Michurin Federal Scientific Center, a stationary site, Tambov Province, Tambov District). The varieties were naturally infected. The plot area was 10 m^2 with 4-fold repetition. The seeding rate was 5 million viable seeds per 1 ha (an SFK seeder, Lemken, Germany), with the fallow as predecessor. Crop growing technology was common for the Tambov region [19].

Field resistance to pyrenophorosis was evaluated using the modified Saari and Prescott scale [20]. The varieties were identified as the highly resistant (RR, damage of < 11%), resistant (R, 11-20%), moderately susceptible (MS, 21-40%), susceptible (S, 41-70%), and highly susceptible (HS, 71-100%). On each plot, the

leaves of the main stem were examined in 30 replicates (10 stems in three places). From 1 to 3 leaves per stem were examined. Counts were carried out every 7-10 days and ended at the stage of milky-wax ripeness. The average degree of damage to the variety by the disease (%) was calculated, and the stages of development were determined according to the Zadoks scale, recording the following stages: end of heading—beginning of flowering (Zadoks scale 59-61), end of flowering—grain formation (Zadoks scale 69-71), milky-waxy ripeness (Zadoks scale 75-85).

Genomic DNA was isolated from leaves of 5-day-old wheat seedlings using the standard CTAB/chloroform method [21]. The quality of DNA samples was assessed in a 1% agarose gel. Secondary control for DNA purity and quality was performed spectrophotometrically (a Smart Spec TMPlus, Bio-Rad, USA).

After quantification, DNA concentration was normalized to 30 ng/μl for PCR. The amount of DNA was as in the PCR protocol for identifying the *Tsn1* gene [22-24].

Genomic DNA amplification (a C1000 Touch Thermal Cycler, Bio-Rad, USA) was run in a 25 μl reaction mixture. The mixture contained 2 μl genomic DNA (25 ng, acceptable from 2 to 50 ng), 1 μl of each primer (10 pM/μl) (Evrogen, Russia), 0.5 μl of the dNTPs mix (10 mM, aqueous solution of dCTP, dGTP, dTTP and dATP) (TransGen, China), 0.55 μl $MgCl_2$ (100 mM), 0.5 μl Bio Taq DNA polymerase (5U, 5 units/μl) (Dialat Ltd., Russia), 2.5 μl 10× PCR buffer (Biolabmix, Russia), 17 μl ddH_2O.

Primers Xfcp623F/Xfcp623R were used in screening for *Tsn1*/*tsn1* alleles. PCR was run as follows: 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 Xfcp623F and Xfcp623R primer sequences are 5′-CTATTCGTAATCGTGCCTTCCG-3′ and 5′-CCTTCTCTCTC-ACCGCTATCTCATC-3′, respectively, the amplicon size is 380 bp [22-24].

Data processing was carried out using the STATISTICA 12 program (StatSoft, Inc., USA). The average leaf damage by pyrenophorosis in 2020-2022 (*M*) and standard deviations (±SD) were calculated. Using the Newman-Keuls test (at $p < 0.05$), a pairwise (multiple) comparison was performed of averages for wheat variety field resistance to yellow spot over a three-year period.

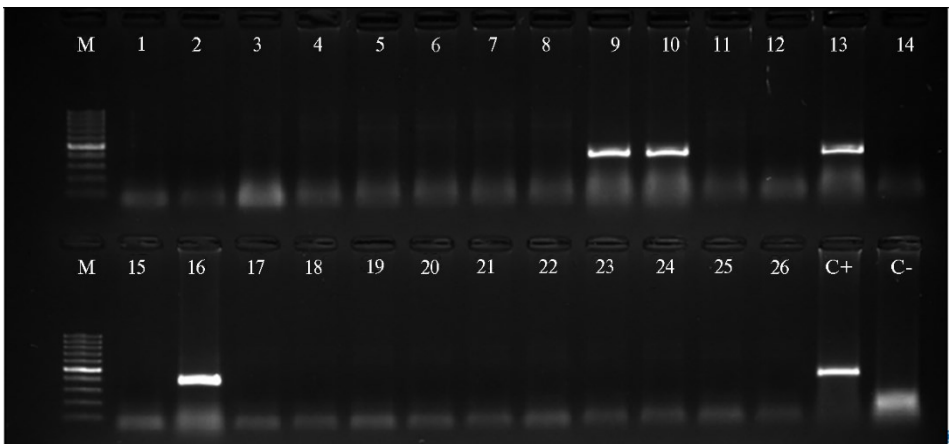
Results. Genotyping of wheat samples with the molecular marker was aimed at identifying carriers of genes that control susceptibility and resistance to the PtrToxA toxin. In Latynevka, Don 93, Synthetik and Laguna (14.3% of the set studied) and in the control variety Glenlea, a *Tsn1* carrier for the Xfcp623 marker, a 380 bp fragment associated with the *Tsn1* gene for susceptibility to the PtrToxA toxin, was amplified (Table 1, Fig.). Genotypes of the remaining 24 varieties (85.7% of the studied) contained the recessive allele *tsn1*. The *ToxA* gene is widely represented in the genotypes of Russian populations of the fungus *P. tritici-repentis* [25]. We can note the fact that 22 varieties of winter soft wheat (Table 1), including those approved for cultivation in zone 5 (the Tambov Province) are protected from PtrToxA at the genetic level due to the recessive allele *tsn1*. The toxin PtrToxA is characteristic of *P. tritici-repentis*, *Parastagonospora nodorum* and *Parastagonospora avenae* f. sp. *triticea*, causing leaf septoria and ear blight of wheat in the Tambov region [26, 27]. Our results suggest the genetic protection in released varieties from the PtrToxA toxin, synthesized by three dangerous pathogens.

The Xfcp623 marker is effective due to its location within the *Tsn1* gene, in intron 5 of this locus at positions 4901-5280 [24]. In the Komugi database (Wheat Genetic Resources DataBase, https://shigen.nig.ac.jp/wheat/komugi/), *Tsn1* registered as the HST ToxA sensitivity gene, has 8 exons and the structure S/TPK-NBS-LRR. All three domains are required for the normal functioning of the *Tsn1* gene, and the gene product does not directly interact with the *ToxA* toxin [24].

1. Resistance to the yellow spot (*Pyrenophora tritici-repentis* (Died.) Drechsler) in winter soft wheat (*Triticum aestivum* L.) varieties cultivated in the Tambov Province

Variety	*Tsn1/tsn1*	Field estimate (n = 90, 2020-2022)		Lab test estimate (N = 10, 2022)	
		leaf injury, % (M±SD)	resistance phenotype	type of response	resistance phenotype
Lipetskaya zvexda	*tsn1*	13.3±5.77	R	1/1	R
Moskovskaya 56	*tsn1*	13.3±5.77	R	2/2	MR
Dominanta	*tsn1*	16.7±5.77	R	2/2	MR
Moskovskaya 40	*tsn1*	16.7±5.77	R	2/2	MR
Bezenchukskaya 380	*tsn1*	20.0±0.00	R	1/2	MR
Biryuza	*tsn1*	20.0±0.00	R	1/2	MR
Inna	*tsn1*	20.0±0.00	R	2/2	MR
Odesskaya 200	*tsn1*	20.0±0.00	R	2/2	MR
Latynevka	*Tsn1*	23.3±5.77	MS	2/2	MR
Proza	-	23.3±5.77	MS	2/2	MR
Spartak	-	26.7±11.55	MS	2/2	MR
Don 93	*Tsn1*	26.7±11.55	MS	2/2	MR
Lgovskaya 167	*tsn1*	30.0±0.00	MS	3/3	S
Prestizh	*tsn1*	30.0±0.00	MS	3/3	S
Synthetic	*Tsn1*	30.0±10.00	MS	3/3	S
Antonivka	*tsn1*	33.3±5.77	MS	3/3	S
Donskoy Syurpriz	*tsn1*	33.3±5.77	MS	3/3	S
Laguna	*Tsn1*	33.3±5.77	MS	3/2	S
Lgovskaya 4	*tsn1*	33.3±5.77	MS	3/4	S
Scepetr	*tsn1*	33.3±20.82	MS	3/3	S
Donera	*tsn1*	36.7±5.77	MS	3/3	S
Kosovitsa	*tsn1*	36.7±11.55	MS	3/3	S
Mironovskaya 100	*tsn1*	36.7±5.77	MS	3/3	S
Izyuminka	*tsn1*	40.0±0.00	MS	3/3	S
Kruiz	*tsn1*	40.0±0.00	MS	3/3	S
Moskovskaya 39	*tsn1*	40.0±10.00	MS	3/3	S
Zvonnitsa	*tsn1*	43.3±5.77	S	3/3	S
Mironovskaya 808	*tsn1*	50.0±0.00	S	3/3	S

N o t e. R — resistance, MR — moderate resistance, MS — moderate susceptibility, S — susceptibility. Field tests were carried out at the site of the Central Russian branch of the Michurina Federal Scientific Center (Tambov Province, Tambov District). The *Tsn1/tsn1* alleles were detected by amplification of the Xfcp623 marker diagnostic fragment. Dashes mean that molecular analysis was not carried out.



Electropherogram of PCR amplification products of the Xfcp623 marker in winter soft wheat (*Triticum aestivum* L.) varieties cultivated in the Tambov Province: 1 — Lipetskaya Zvezda, 2 — Moskovskaya 56, 3 — Dominanta, 4 — Moskovskaya 40, 5 — Bezenchukskaya 380, 6 — Biryuza, 7 — Inna, 8 — Odesskaya 200, 9 — Latynevka, 10 — Don 93, 11 — Lgovskaya 167, 12 — Prestizh, 13 — Synthetik, 14 — Antonivka, 15 — Donskoy Syurpriz, 16 — Laguna, 17 — Lgovskaya 4, 18 — Skipetr, 19 — Donera, 20 — Kosovitsa, 21 — Mironovskaya 100, 22 — Izyuminka, 23 — Kruiz, 24 — Moskovskaya 39, 25 — Zvonnitsa, 26 — Mironovskaya 808. M — DNA marker Step100 plus (Biolabmix, Russia). Positive control (C+) is Glenlea variety, negative control (C–) is line 6B365. Diagnostic fragment size 380 bp.

Monoconidial pure cultures of *P. tritici-repentis* were isolated from 19 affected winter soft wheat samples on the V-4 nutrient medium. In 68 isolates, race composition was investigated using a set of wheat varieties and differentiating lines. Based on the recording leaf necrosis and chlorosis, 5 races were identified

in the population of the fungus of 2022 (Table 2).

2. Origin of monoconidial isolates of *Pyrenophora tritici-repentis* and frequency of occurrence of different races in the population (lab test, 2022)

Host variety	Number of isolates	Frequency of *Pyrenophora tritici-repentis* race occurrence, %				
		1 AC	2 A	3 C	4	8 ABC
Bezenchukskaya 380	2	0	0	50.0	50.0	0
Birtyza	4	0	0	100	0	0
Zvonnitsa	6	16.7	0	83.3	0	0
Izyuminka	4	0	0	100	0	0
Inna	4	25.0	0	75.0	0	0
Kosovitsa	4	0	0	100	0	0
Laguna	4	33.3	0	66.7	0	0
Latynevka	2	0	0	0	100	0
Lipetskaya zvezda	4	0	0	33.3	66.7	0
Lgovskaya 167	2	0	0	0	100	0
Lgovskaya 4	4	100	0	0	0	0
Mironovskaya 100	4	50.0	0	0	50.0	0
Mironovskaya 808	6	0	0	0	100	0
Moskavskaya 39	2	50.0	0	0	0	50.0
Moskovskaya 40	2	0	0	50.0	50.0	0
Prestizh	4	0	25.0	0	0	75.0
Proza	2	50	0	0	50.0	0
Synthetik	4	0	0	50.0	0	50.0
Spartak	4	50.0	0	0	50.0	0
Occurance in the populatiob, % (M±SD)	68	19.7±28.5	1.3±5.7	37.3±40.3	32.5±38.7	9.2±22.4

Примечание. A, B, C —production of the toxins PtrToxA, PtrToxB and PtrToxC, respectively, by isolates of the pathogen. Race 4 does not produce these toxins.

In the *P. tritici-repentis* population, race 4 (does not produce the toxins PtrToxA, PtrToxB and PtrToxC), race 3 (produces PtrToxC) and race 1 (PtrToxA and PtrToxC) were common. Race 8 (PtrToxA, PtrToxB and PtrToxC) and race 2 (PtrToxA) turned out to be rare. Race 5 (PtrToxB), race 6 (PtrToxB and PtrToxC) and race 7 (PtrToxA and PtrToxB) were not identified. That is, in the *P. tritici-repentis* population formed in the Tambov region in 2022, the races producing PtrToxC were the most common, and the exotoxin encoded by the *ToxA* gene was less common. We did not find any races capable of producing PtrToxB.

Our results are consistent with reports on the absence of the *ToxB* gene and the presence of the *ToxA* gene in *P. tritici-repentis* isolates from fungal populations in the Russian Federation [25, 28, 29].

N.V. Mironenko et al. [25] analyzed *P. tritici-repentis* isolates collected in 2017-2018 in the southern, northern and western Siberian regions of Russia, Finland and Kazakhstan. The race composition and the presence of the *ToxA* and *ToxB* genes were studied. The *ToxB* gene was not detected. The absence or rare occurrence of isolates producing this toxin has also been noted in other countries [30, 31]. The *ToxA* gene occuranc varied among *P. tritici-repentis* populations. Thus, in the North Caucasian population in Russia and the southeastern population in Kazakhstan, the *ToxA* gene was found in 100% isolates, while in other populations the *ToxA* occurrence varied from 5.5% (West Siberian Omsk population) to 66% (Finnish population) [25].

Previous comparison of the *P. tritici-repentis* race composition showed that in Russia, races 1, 2, 4 and 8 were identified, and in Kazakhstan races 1, 3, 4, 6 and 8. In the North Caucasus of the Russian Federation, races 1 and 2 predominated, while in Kazakhstan races 1 and 8 [29]. Races 6 and 8 are capable of producing the toxin PtrToxB which is absent in the fungal populations in Russia. E.I. Gultyaeva et al. [28] revealrd high genetic similarity between the Omsk, North Kazakhstan and Chelyabinsk populations of *P. tritici-repentis*, which indicates the

existence of a single epidemiological zone and the possibility of gene flow between the studied populations.

N.M. Kovalenko et al. [32] present the results of identifying the *Tsn1* and *tsn1* alleles using the molecular marker Xfcp623 in 35 winter wheat varieties and 31 spring wheat varieties first included in the State Register of Breeding Achievements in 2018-2020. Of these, only 9 varieties of winter wheat and 4 varieties of spring wheat carried *Tsn1*, which indicates susceptibility to PtrToxA, while the remaining varieties are protected from the toxin at the genetic level.

In this work, a comparison of the results obtained in laboratory (type of reaction) and field tests (damage intensity) revealed similarities in the nature of resistance of winter bread wheat varieties (see Table 1). Eight varieties were of greatest interest (Lipetskaya Zvezda, Moskovskaya 56, Dominanta, Moskovskaya 40, Bezenchukskaya 380, Biryuza, Inna, Odesskaya 200), which were characterized by high resistance to the pathogen both in laboratory and field conditions during three years of testing.

The varieties Latynevka (*Tsn1*), Proza, Spartak, Don 93 (*Tsn1*) showed a moderately resistant reaction in laboratory tests, and in field conditions, according to the degree of plant damage, they entered the group of moderately susceptible

Some differences in the type of variety resistance noted in field conditions during the testing years can be explained by the prevailing weather conditions which were more favorable for the pathogen in 2020 than in 2021 and 2022.

In field tests, 16 out of 28 wheat varieties, or 57.1%, showed susceptibility to pyrenophorosis. In this regard, when cultivating wheat varieties susceptible to the disease in the Tambov region, it is proposed to monitor the threshold of harmfulness of the fungus in order to take preventive protective measures, as well as to expand breeding activities to select and create donors and sources of resistance to P. tritici-repentis.

Using the Newman-Keuls test, we conducted a multiple comparison between the degree of pyrenophorosis damage e of winter bread wheat varieties under field conditions (Table 3). The results of the analysis of variance using the Newman-Keuls test revealed statistically significant differences between varieties in the degree of pyrenophorosis damage. Thus, between the susceptible variety Mironovskaya 808 (S) and the group of varieties Lipetskaya Zvezda, Moskovskaya 56, Dominanta, Moskovskaya 40, Bezenchukskaya 380, Biryuza, Inna, Odesskaya 200, Latynevka, Proza, Spartak and Don 93, which are resistant to the phytopathogen (R and MR), significant differences were established in the reaction to infection with pyrenophorosis (see Tables 1, 3). Differences were noted between the varieties Lipetskaya Zvezda, Moskovskaya 56, Dominanta, Moskovskaya 40, Bezenchukskaya 380, Biryuza, Inna, Odesskaya 200, which have field resistance (R), and the susceptible variety Zvonitsa (S). In addition, statistically significant differences were recorded between susceptible varieties (MS) Izyuminka, Cruise, Moskovskaya 39 and resistant varieties (R) Lipetskaya Zvezda, Moskovskaya 56, Dominanta, Moskovskaya 40; between susceptible varieties (MS) Donera, Kosovitsa, Mironovskaya 100 and resistant varieties (R) Lipetskaya Zvezda, Moskovskaya 56.

Sensitivity to NEs does not always determine sensitivity to *P. tritici-repentis*, and the influence of the *Tsn1*-PtrToxA interaction on disease development depends on the genetic background of the host, that is, on the specific wheat genotype [33]. The effects of *Tsn1*–ToxA interactions on pyrenophorosis in bread wheat can range from weak to very strong [34]. Some wheat genotypes possess factors that lead to changes in *ToxA* gene expression through epistasis or somehow inhibit recognition of the *ToxA* gene product by the *Tsn1* gene in plants infected with fungal spores [34].

3. Analysis of variance to compare the average phytopathological estimates of winter bread wheat (*Triticum aestivum* L.) varieties cultivated in the Tambov Province for resistance to *Pyrenophora tritici-repentis* using the Newman-Keuls test (Central Russian branch of the Michurin Federal Scientific Center, Tambov Province, Tambov District, 2020-2022)

Variety	Lipetskaya zvezda	Moskovskaya 56	Dominanta	Moskovskaya 40	Bezenchukskaya 380	Biryuza	Inna	Odesskaya 200	Latynevka	Proza	Spartak	Don 93
Donera	0.03*	0.03*	0.13	0.12	0.31	0.35	0.33	0.29	0.59	0.62	0.86	0.89
Kosovitsa	0.03*	0.03*	0.12	0.11	0.29	0.33	0.31	0.27	0.56	0.59	0.83	0.86
Mironovskaya 100	0.03*	0.03*	0.11	0.10	0.27	0.31	0.29	0.24	0.52	0.56	0.80	0.83
Izyuminka	0.01*	0.01*	0.04*	0.03*	0.12	0.13	0.13	0.11	0.31	0.33	0.63	0.66
Kruiz	0.01*	0.01*	0.03*	0.03*	0.11	0.13	0.12	0.10	0.29	0.31	0.59	0.63
Moskovskaya 39	0.01*	0.01*	0.03*	0.029*	0.10	0.12	0.11	0.09	0.27	0.29	0.56	0.59
Zvonnitsa	0.001*	0.001*	0.01*	0.007*	0.03*	0.03*	0.03*	0.03*	0.10	0.11	0.29	0.31
Mironovskaya 808	0.0002*	0.0002*	0.0003*	0.0003*	0.001*	0.001*	0.001*	0.001*	0.004*	0.01*	0.02*	0.02*

* Differences are statistically significant at $p < 0.05$.

Thus, among the *Pyrenophora tritici-repentis* isolates from samples of winter bread wheat in the Tambov region, three races were common, of which race 4 did not produce the toxins PtrToxA, PtrToxB and PtrToxC, race 3 produced the toxin PtrToxC, race 1 PtrToxA and PtrToxC. Races 8 (PtrToxA, PtrToxB and PtrToxC) and 2 (PtrToxA) were classified as rare. Races 5 (PtrToxB), 6 (PtrToxB and PtrToxC) and 7 (PtrToxA and PtrToxB) were absent in the population. Race 3 with gene ToxC for me exotoxin was the most common in the Tambov region, races producing PtrToxA were less frequently noted. From a practical point of view, the most interesting are the seven varieties of winter soft wheat approved according to the Register of Breeding Achievements for cultivation in the Tambov region, the Lipetskaya Zvezda, Moskovskaya 56, Moskovskaya 40, Bezenchukskaya 380, Biryuza, Inna, Odesskaya 200, and the Dominant variety approved for cultivation in the North Caucasus and Ural regions. These varieties demonstrated the highest resistance to *P. tritici-repentis* in field and lab tests, molecular screening also confirmed their resistance to PtrToxA. The data obtained may be used to indicate differentiator varieties for assessment of the *P. tritici-repentis* pathogenicity.

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