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Resistance of winter wheat varieties to tan spot in the North Caucasus region of Russia

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ABSTRACT

Tan spot caused by *Pyrenophora tritici-repentis* (Died.) Drechsler, in recent years, occupies an increasingly large area on the territory of Russia. Due to the wide distribution and economic significance of this disease, the search for resistant plants to the pathogen is relevant. This paper presents the results of a field assessment for 2017–2019 of 34 regionally distributed winter wheat varieties of Russian selection for resistance to *P. tritici-repentis* in the North Caucasus region of Russia. Field resistance - the development of the disease up to 30% against the background of artificial infection for three years was shown by 20.5% of the studied varieties. Wheat varieties were assessed for resistance to isolates of tan spot identified as races 1, 3, and 4 in the greenhouse at the seedling stage. The number of resistant accessions for each race was different and ranged from 12 to 20. The 12 varieties showed resistance to race 1, 14 varieties to race 3, 20 varieties to race 4. This research showed that the resistance to tan spot of studied varieties was race-specific. A functional allele of the susceptibility gene *Tsn1* to *P. tritici-repentis* isolates, producing the toxin Ptr ToxA, was diagnosed by PCR method. Of the analyzed 34 varieties, 13 had a dominant allele of the *Tsn1* (*Tsn1*⁺), and 21 had a recessive allele in the *tsn1tsn1* homozygous state. All *Tsn1*⁺ varieties, and most varieties with recessive alleles *tsn1tsn1*, were susceptible to tan spot in the field. Varieties Dolya, Gurt, Lebed and Sila, which showed field resistance, had the *tsn1tsn1* genotype. The expected reaction of varieties with different allelic composition of the *Tsn1* gene to inoculation with the isolate of race 1, according to the generally accepted model of “gene-to-gene” interaction, did not coincide with that observed in reality, which confirms the results obtained by other authors. Research results demonstrate the effect of weather conditions on the susceptibility of wheat varieties to tan spot. In years with higher humidity and higher average air temperatures, the susceptibility response to the disease was observed in more varieties than in drier years. The studies show that the main part (79.5%) of winter wheat varieties of Russian selection widely zoned in the North Caucasus region of Russia are susceptible to *P. tritici-repentis*. Varieties that have been resistant to the pathogen in the adult phase in the field for three years and to the pathogen races in which the recessive allele of the *tsn1* gene has been identified may be of interest as sources of resistance for developing new disease-resistant varieties.

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1. Introduction

Tan spot is an economically significant disease worldwide. Causal agent of tan spot is the homotallic fungus *Pyrenophora tritici-repentis* (*Ptr*) (Died.) Drechs (anamorphic: *Drechslera tritici-repentis* (Died.) Shoem.). Monoculture and cultivation of varieties with an insufficient level of resistance to the disease contribute to the accumulation of inoculum and the development of the pathogen at the epiphytotic scale in many countries. There are reports of a wide spread of the disease in Europe, Southwest Asia, Central Asia, North and South America, Africa, Australia (Oliver

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et al., 2008; Singh et al., 2010; Gamba et al., 2012; Faris et al., 2013; Momeni et al., 2014; Kokhmetova et al., 2017).

In Russia, this pathogen was first noticed in 1985. Since 2000 the disease has spread throughout Russia: it was found in Dagestan, Western Siberia, and Altai (Mikhailova et al., 2014). In the last 10 years, the spread of the disease in the North Caucasus region in some production fields of the Krasnodar, Stavropol Territories, Rostov Region reached 80–100%, and development on susceptible varieties – 60–80% (Kremneva et al., 2015; 2019).

The sources of the infection are infected seeds, plant residues from the previous season, wild cereals susceptible to the disease. Currently, this disease is economically important because of the serious impact on the yield, quantity and quality of the produced grain. The average yield loss caused by the pathogen is 5–10%, however, under conditions favorable for the fungus, losses up to 50% were observed (Wegulo et al., 2009).

The tan spot pathogen is characterized by a large genetic diversity (Lamari et al., 2005; Singh et al., 2010; Mikhailova et al., 2014). Its reproductive cycle is a very important factor in the epidemiology of the disease and the genetic variability of virulence. Based on the ability of *P. tritici-repentis* isolates to cause necrosis and chlorosis on the Glenlea variety and two lines 6B365 and 6B662, 8 races are currently described (Lamari et al., 2003). The host specific toxins: Ptr ToxA, Ptr ToxB and Ptr ToxC, are differentially produced by *P. tritici-repentis* races and serve as pathogenic factors of the fungus. Isolates of races 2, 3, and 5 produce one toxin: Ptr ToxA, Ptr ToxC, or Ptr ToxB, respectively. Isolates of races 1, 6, and 7 produce two toxins each: Ptr ToxA + Ptr ToxC, Ptr ToxB + Ptr ToxC and Ptr ToxA + Ptr ToxB, respectively. Isolates of race 8 produce all three toxins, while isolates of race 4 do not produce any known toxins and are considered non-pathogenic (Lamari et al., 2003; Singh et al., 2010). However, there are reports that *Ptr* may produce additional selective toxins that cause necrosis or chlorosis in wheat plants. (Ali and Francl, 2003; Ali and Francl, 2010; Faris et al., 2013; Guo et al., 2018).

The sensitivity of wheat to necrosis-inducing Ptr ToxA toxin is determined by the *Tsn1* gene. It is believed that a susceptibility reaction is observed during the interaction of the products of the *Tsn1* gene and the *ToxA* effector gene, which controls the synthesis of the *Ptr ToxA* toxin in the pathogen. Molecular SSR markers Xfcp1, Xfcp620, Xfcp394 have been developed that flank the *Tsn1* gene (Zhang et al., 2009) and Xfcp 623 onto the inner region of the gene (Faris et al., 2010).

The North Caucasus region is the main producer of grain in Russia - up to 20% of the total Russian volume. In Krasnodar Krai alone, more than 100 genetically heterogeneous varieties of wheat are cultivated (Romanenko et al., 2017). Analysis of the available studies indicates a widespread pathogen in the North - Caucasian region of Russia (Mikhailova et al., 2014; Kremneva and Volkova, 2007; Kremneva et al., 2019).

The aim of the research was to evaluate winter wheat varieties of the Russian breeding, occupying significant sown areas in the south of Russia, for resistance to *P. tritici-repentis* in the field on an artificial infectious background, to individual pathogen races by phytopathological testing in laboratory and greenhouse conditions, and to characterize varieties using the PCR method for the presence or absence of the dominant allele of the susceptibility gene *Tsn1*.

2. Materials and methods

The research material was 34 varieties of winter wheat of the Russian breeding of P.P. Lukyanenko National Grain Center (Krasnodar, Russia) and the “Donskoy” Scientific Center (Zernograd, Russia). The area occupied by these varieties in Krasnodar Krai in

2017, according to the Russian Agricultural Center, amounted to 92% of the total sown area of winter wheat.

Evaluation of the resistance to *P. tritici-repentis* on adult plant stage was carried out under field conditions of the North Caucasus region of Russia, All-Russian Research Institute of Biological Plant Protection (ARRIBPP), Krasnodar (45° 2.413' 0" N, 38° 58.5598' 0" E, and 29 m asl) on an artificial infectious background in the 2017–2019 crop seasons. The experiment was conducted in a completely randomized design with three replications. Varieties were sown on plots of 1 m². The rate was 100 g. of seeds per linear meter. The susceptible variety Tanya, which is a good accumulator of infection was planted after every 10 plots. Inoculation was performed with a water-conidial suspension with a concentration of 3–5 × 10³ spores/ml (load 70–100 ml/m²) in the flag leaf stage in GS 37–39, Zadoks scale, in warm, calm weather in the evening before dew.

The inoculum included conidia of *P. tritici-repentis* isolates isolated from wheat production fields in the North Caucasian region of Russia in 2017–2019. These isolates were preliminarily identified on differentiating varieties as races 1, 3 and 4 (Lamari and Bernier, 1989; Lamari et al., 2003). The percentage of Ptr-infected leaf area was determined on each leaf and the average value for all evaluated leaves was calculated for each wheat entry in order to determine the tan spot score. A rating system based on % leaf area infected developed for appraising the foliar intensity of diseases (Eyal et al., 1987) was used to categorize host reaction to *P. tritici-repentis*: 0–15% – resistant (R); 15–30% – moderately resistant (MR); 30–40% – moderately susceptible (MS); 41–100% – susceptible (S). The standard wheat differentials included Glenlea (susceptible by ToxA), 6B662 (susceptible by ToxB), 6B365 (susceptible by ToxC), Salamouni (insensitive control by ToxA) were included in the field trials. The assessment was carried out at the time of the maximum disease development - in GS 75–80 (Zadoks scale).

In a greenhouse, on seedlings 34 varieties of winter wheat plants, we studied the resistance to 1, 3 и 4 races of *P. tritici-repentis*, which we used for artificial infection of wheat plants in the field, which we used to artificially infect wheat plants in the field. Wheat plants were grown on hydroponic culture in flowerpots with a volume of 25 ml filled with sand, using a nutritious Knop solution. Plants were grown to a two-leaf phase in a greenhouse at an average temperature of 21 °C with a 16-h photoperiod. Before inoculation, the wax coating was removed from the plants and they were infected with a suspension of conidia at a concentration of 5 × 10³ per ml. Accounting for the degree of the disease development was carried out on day 7. The plants were rated for disease, using rating system based on lesion type; 1–2 represent resistance, and 3–5 represent susceptibility (Lamari and Bernier, 1989; Lamari et al., 2003).

The racial identity of *P. tritici-repentis* isolates isolated from wheat samples collected in Krasnodar Krai Russia in 2017–2019 were determined on the Canadian set of differentiators: Glenlea, Salamouni, 6B662, 6B365 (Lamari and Bernier, 1989; Lamari et al., 2003).

DNA from plant leaves was isolated by a known method using cetyl trimethyl ammonium bromide CTAB (cetyl trimethyl ammonium bromide) (Zhang et al., 2009). To identify the dominant allele of *Tsn1* gene, PCR was performed with primers for the Xfcp 623 marker diagnosing a functional dominant allele: F - CTATCG-TAATCGTGCCTTCCG; R - CTTTCTCTCACCGCTATCTCATC (Faris et al., 2010). The size of the diagnostic amplification product is 380 bp. Amplification products were separated on a 1.7% agarose gel. Amplification was carried out with the following parameters: denaturation –94 °C for 4 min; 45 cycles: 94 °C –1 min, 60 °C –1 min; 72 °C –2 min; final elongation –72 °C –10 min. As

a positive control, the Glenlea variety was used to identify the *Tsn1* gene.

Data on the degree of *P. tritici-repentis* development on different winter wheat varieties and in different years was subjected analysis of variance (ANOVA) and means were separated by Duncan test. Differences were considered statistically significant at $p < 0.05$ level. All tests were performed using Statistix 8.1v (Analytical software, 2005). The ordinary disease data was transformed into parametric data using PROC RANK followed by ANOVA and t tests of the least significant difference (LSD) in SAS 9.4 (SAS Institute, 2016) to detect the significance of the difference among different wheat genotypes.

3. Results and discussion

Analysis of data on the study of wheat varieties resistance to *P. tritici-repentis* in the field against an artificial infectious background in 2017–2019 was carried out using two-way analysis of variance, where the variety a plant and the year of the study were taken as the analysis factors. The analysis results are presented in Table 1.

The variability of the development degree of *P. tritici-repentis* on the studied varieties of winter wheat is significantly influenced by all factors taken into account in the experiment. The maximum influence on the variability of the studied trait had the belonging of the plants to a certain variety; the contribution of this factor was 78.5% of the total variance of resistance. The effect of the factor “year of study” was 9.2% of the total variability of the studied trait. The influence of the interaction of the two factors was small –0.3% of the total variance, but statistically significant. It testifies to the specific reaction of varieties to changing conditions of the year.

It was interesting to consider the variability of the development degree of yellow spot pathogen on the same variety, but in different years of the research. Thus, a statistical analysis was carried out using the Duncan test, which characterizes the statistically significant differences of each variety between the years of the study. Table 2 shows the average values for the development degree of the disease for each variety in 2017–2019.

Most varieties showed statistically significant differences in sensitivity to *Ptr* over the years of the study. The exceptions were the varieties Vassa, Dolya, Ethnos and Salamony, where significant differences in sensitivity during 2017–2019 were not identified. In varieties Antonina, Bagrat, Bezostaya 100, Grom, Gratsia, Zhiva, Sila, Urup, Stanichnaya and 6B 365, statistically significant differences in resistance were found between all three years of the study. In the remaining varieties, one year of study significantly differed in resistance from the other two. To explain the data obtained, the weather conditions in the years of the research were analyzed (Fig. 1). The figure shows the average values of temperature (°C), humidity (%) and precipitation (mm) in the months of active growing season of winter wheat (April–June) in 2017–2019. It can be seen from the figure that the average values of the presented traits differ from year to year, so in 2019, precipita-

tion during the development of the pathogen (May – June) on wheat was 2.5 times more compared to 2017 and 2018, as well as the average temperature was higher by 2–3 °C. The results of these studies confirm the previously known fact that humidity and temperature are important factors influencing the development of *P. tritici-repentis* (Duveiller et al., 2005; Fernandez et al., 2016).

Fig. 2 shows the number of varieties with a certain level of resistance to *P. tritici-repentis* according to the classification of Eyal, Z et al. (1987) over the years of the research. The largest number of susceptible varieties is observed in 2019, which was characterized as the most favorable for the development of the pathogen. There is also a difference in the number of varieties with a certain level susceptibility to the disease over the years.

The average values of the susceptibility to disease of 34 varieties of winter wheat and differentiators under field conditions for three years (2017–2019) and the standard error are shown in Fig. 3. It can be seen from the figure that the level of development of *Ptr* varied widely from 10.0% up to 72%. As a result, in the field, resistance to *Ptr* during three years was exhibited by 3 samples of wheat (8.8%) and control for resistance - Salamony, moderate resistance – by 4 samples (11.8%), moderate susceptibility – by 7 samples (13.8%), as well as lines 6B 662 and 6B 365, susceptibility - by 20 samples (58.8%).

Under greenhouse conditions at the germination phase, the studied winter wheat cultivars were evaluated on three isolates of *Ptr* identified on differentiating lines (Lamari and Bernier, 1989; Lamari et al., 2003) as races 1, 3, and 4. Table 3 shows the mean disease values from three repeats for each variety and the least significant difference (LSD) between these lines is calculated. A significant difference in value was observed in the mean disease scores between types, according to LSD. The number of resistant accessions to each race was different and ranged from 12 to 20 varieties.

In all wheat varieties, the allelic state of the susceptibility gene *Tsn1* was determined by PCR: the dominant allele of the gene was detected in 13 varieties, and the recessive one in 21 varieties (Table 3, Fig. 4).

The resistance to the *Ptr* isolate of race 1, producing *Ptr* ToxA and *Ptr* ToxC according to the rating system (Lamari and Bernier, 1989), was shown by 12 varieties (35.2%), including 4 varieties with a dominant allele of the *Tsn1* gene (Table 3). The 22 cultivars susceptible to race 1 included cultivars with the recessive allele of the *tsn1* gene (12 cultivars) and the dominant allele of the *Tsn1* (9 cultivars). For the *Ptr* isolate of race 3 forming *Ptr* Tox C (Lamari and Bernier, 1989), 14 resistant accessions (41.1%) were identified, of which 9 cultivars had the recessive allele of the *tsn1* gene, the rest were characterized by the presence of the *Tsn1* gene. To the *Ptr* isolate of race 4, that does not produce toxins (Lamari and Bernier, 1989), 20 cultivars (58.8%) showed resistance. In this group of cultivars, only 12 varieties contained the recessive allele *tsn1*, the rest contained the dominant allele of the *Tsn1* gene. The remaining 12 varieties showed a susceptibility reaction - the for-

Table 1

Two-way analysis of the variance of resistance variability of wheat varieties to *P. tritici-repentis* over three years of research (2017–2019).

Variability	df	mS	F	Variance	Share of total variance, %
Total	1709			325,1	100,0
Between the varieties	37	11530,3	296,2*	255,4	78,5
Between the years	2	17014,8	437,1*	29,8	9,2
Interaction	74	158,4	4,1*	1,0	0,3
Residual	1596	38,9		38,9	12,0

Note * - the influence of the factor is statistically significant, $p \leq 0.05$.

Df - degree of freedom, mS - mean square, F - Fisher's test.

Table 2
The reaction of wheat varieties to *P. tritici-repentis* artificial infection in the field (2017–2019).

Wheat varieties	Disease Mean, % ±SE, 2017	Disease Mean, % ±SE, 2018	Disease Mean, % ±SE, 2019	p-level
Adel	48,5 ± 2,03b	45,8 ± 2,59b	59,3 ± 2,20a	0,000*
Alexeich	45,1 ± 1,98b	42,7 ± 2,61b	51,8 ± 2,47a	0,000*
Antonina	49,3 ± 2,06b	40,5 ± 2,13c	56,5 ± 3,31 a	0,000*
Afina	40,5 ± 2,34b	37,3 ± 1,70b	46,3 ± 2,60 a	0,001*
Bagrat	61,5 ± 2,13b	55,8 ± 2,84c	68,4 ± 1,05 a	0,000*
Bezostaya 100	55,8 ± 1,91b	44,3 ± 1,77c	61,3 ± 1,42 a	0,000*
Brigada	65,5 ± 1,58b	62,2 ± 3,35b	70,3 ± 2,21 a	0,002*
Vassa	51,5 ± 2,22 a	48,3 ± 2,31 a	53,5 ± 2,29 a	0,098
Grom	20,0 ± 2,52b	15,3 ± 1,11c	28,1 ± 1,25 a	0,000*
Gracia	65,0 ± 2,98b	57,3 ± 2,79c	75,0 ± 3,49 a	0,000*
Gurt	16,3 ± 1,40 a	12,6 ± 1,13b	17,0 ± 0,66 a	0,015*
Dolya	10,5 ± 0,87 a	10,0 ± 1,09 a	12,0 ± 1,45 a	0,457
Dmitry	55,3 ± 2,10 a	45,9 ± 2,79b	58,5 ± 2,45 a	0,000*
Zhiva	39,8 ± 2,48b	35,5 ± 2,34c	45,4 ± 2,72 a	0,000*
Yesaul	32,6 ± 2,84b	30,5 ± 1,98b	39,3 ± 2,95 a	0,000*
Yeremeevna	30,5 ± 2,32b	30,0 ± 2,29b	39,5 ± 1,19 a	0,002*
Kalym	21,3 ± 1,31b	19,5 ± 0,98b	28,3 ± 0,93 a	0,000*
Kuren	48,3 ± 2,16 a	29,5 ± 1,97b	47,7 ± 2,23 a	0,000*
Laureat	28,5 ± 2,46 a	18,3 ± 2,35b	30,0 ± 1,47 a	0,000*
Lebed	20,5 ± 1,38b	23,3 ± 2,69 ab	26,5 ± 1,62 a	0,014*
Sila	15,3 ± 1,65b	12,1 ± 2,64c	20,0 ± 0,49 a	0,000*
Stan	30,3 ± 2,81b	29,0 ± 2,40b	45,5 ± 2,69 a	0,000*
Tanya	65,0 ± 3,31b	62,1 ± 3,77b	72,2 ± 3,39 a	0,050*
Tabor	41,5 ± 2,77 a	33,5 ± 2,48b	45,0 ± 1,19 a	0,000*
Trio	69,8 ± 2,65 a	50,3 ± 3,42b	72,1 ± 2,04 a	0,000*
Urup	43,1 ± 2,69b	34,7 ± 2,51c	49,5 ± 2,94 a	0,000*
Utrish	60,2 ± 3,48 a	48,5 ± 2,67b	65,1 ± 2,80 a	0,000*
Etnos	30,0 ± 2,38 a	30,1 ± 2,42 a	33,3 ± 1,63 a	0,209
Yubileinaya 100	50,1 ± 2,32b	48,8 ± 2,39b	56,7 ± 2,87 a	0,002*
Yukka	43,2 ± 2,23b	40,3 ± 2,98b	49,0 ± 2,30 a	0,007*
Asket	39,1 ± 2,87 a	30,0 ± 2,29b	44,7 ± 2,78 a	0,000*
Gubernator Dona	46,0 ± 3,30 a	40,8 ± 2,64b	49,1 ± 1,81 a	0,014*
Ermak	35,3 ± 2,33 a	29,1 ± 1,19b	38,3 ± 1,22 a	0,000*
Stanichnaya	30,3 ± 2,33b	23,5 ± 1,89c	45,5 ± 1,49 a	0,000*
Salamony	0,0 ± 0,00 a	0,0 ± 0,00 a	0,6 ± 0,13 a	0,401
Glenlea	45,3 ± 2,21 a	37,3 ± 2,87b	48,5 ± 1,43 a	0,001*
6B 662	32,3 ± 1,35 a	26,7 ± 1,31b	35,0 ± 1,20 a	0,000*
6B 365	38,3 ± 2,94b	30,3 ± 1,03c	44,3 ± 1,18 a	0,000*

Note: *Differences were considered statistically significant at $p \leq 0.05$; data represent the mean of disease progression on 25 plants and standard error. In a column, the mean values with the same letter do not differ significantly.

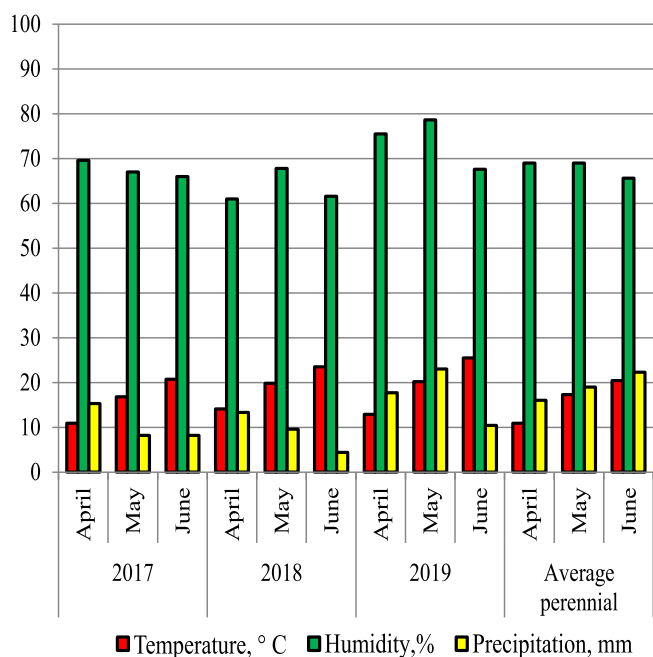


Fig. 1. Climatogram of weather conditions for the research period 2017–2019 (according to the FSBSI FRCBPP meteorological station).

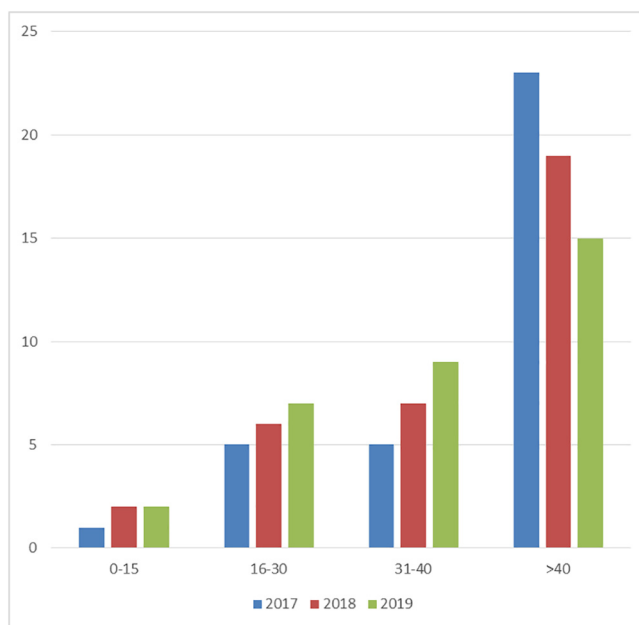


Fig. 2. Distribution of winter wheat varieties by the level of resistance to *P. tritici-repentis* (0–15 - R, 16–30 - MR, 31–40 - MS, >40 - S) in 2017–2019, according to the classification of Eyal, Z. et al. (1987).

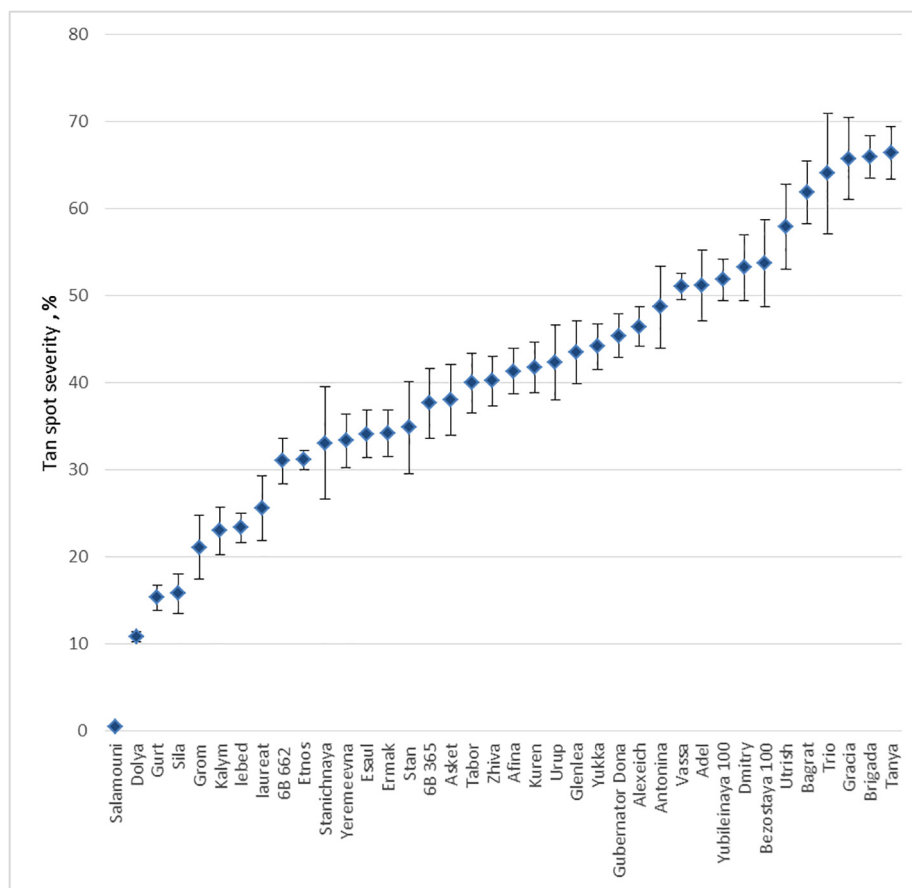


Fig. 3. The results of assessing of wheat varieties to *P. tritici-repentis* artificial infection in the field (average for 2017–2019 and standard errors).

mation of chlorosis and necrosis with a reaction type of 3–5 points. This may indicate the presence of toxins in this isolate other than those already known and previously described.

It is interesting to compare the expected response of varieties of different genotypes by the *Tsn1* gene to inoculation with an isolate of race 1 producing the Ptr ToxA toxin, according to the generally accepted “gene-to-gene” model of interaction (Ciuffetti et al., 2010) with the observed reality. The results of this comparison are presented in Table 4.

As a result of the field assessment, only seven varieties with resistance (R) and moderate resistance (MR) to artificial infection were identified: Grom, Gurt, Dolya, Kalym, Laureate, Lebed and Sila. In this group, the *Tsn1* allele was identified in three varieties, and *tsn1* in four other varieties (Table 3). The remaining varieties were susceptible to the tan spot pathogen. The development of the disease on them ranged from 30 to 72%. The control susceptible variety Tanya was affected average by 66.4%. In the group of susceptible varieties, 10 had *Tsn1*, and 15 - *tsn1*.

Wheat varieties that in adult phase under field conditions showed themselves as resistant and moderately resistant to the disease showed different responses to the *P. tritici-repentis* races in the germination phase. Resistance in the germination phase to 1, 3 and 4 races is found in 6 varieties Grom, Zhiva, Tabor, Trio, Utrish and Ermak. Variety Grom showed resistance in the adult phase, but at the same time the dominant allele *Tsn1* was found in it. The rest of the varieties showed moderately susceptible (MS) and susceptible (S) responses in the field. Differences in the susceptibility to the disease of the tested wheat varieties in the field and in the greenhouse can be associated as with different genetically controlled resistance of the host plant in different phases of the

growing season, as different climatic conditions. Despite the fact that the same fungal isolates were used to infect plants in adult phase as for inoculating wheat plants in the germination phase, field conditions are uncontrollable. We assume that natural infection of wheat crops with fungal races present in a given area may occur. As a result of studying the racial composition of the population of the pathogen of tan spot common in the North Caucasian region of Russia, we found that races 1, 2, and 8 were found in all studied areas with a frequency of 15% to 60.6% (Kremneva et al., 2019). The rest of the races were also found, but not in all areas and with a lower frequency from 2.7% to 18.5%.

4. Discussion

The results of the varieties response to infection with different races of the pathogen indicate the race-specific nature of the resistance of these varieties. It should be noted that the expected response of varieties of different genotypes by the *Tsn1* gene to inoculation with an isolate of race 1 producing the Ptr ToxA toxin, according to the generally accepted “gene-to-gene” model of interaction (Ciuffetti et al., 2010), did not match the observed reality.

The isolate defined with a phytopathological test as a producer of the toxin Ptr ToxA, i.e. assigned to race 1, equally infected varieties with both a dominant and recessive allele of the *Tsn1* gene. This result contradicts the data presented in the article by A. M. Kokhmetova et al. (Kokhmetova et al., 2018), which shows a 100% match between the molecular identification of the *Tsn1* gene alleles and the phenotypic reaction of the variety in response to inoculation with one isolate of race 1 and pure Ptr ToxA toxin. Per-

Table 3
The reaction of wheat accessions to *P. tritici-repentis* races in the greenhouse and the allelic state of the *Tsn1* gene.

Wheat accession	Race 1		Race 3		Race 4		Allelic state of the <i>Tsn1</i> gene
	Disease Mean	Rank Mean	Disease Mean	Rank Mean	Disease Mean	Rank Mean	
Salamouni	0,0	0,0a	0,0	0,0a	0,25	5,0ab	<i>tsn1</i>
Afina	0,73	14,6cde	0,67	13,3 cd	0,6	12,0bc	<i>tsn1</i>
Gracia	3,87	77,33stu	0,73	14,6cde	3,80	76,00st	<i>Tsn1</i>
Dolya	0,73	14,6cde	1,8	36,0 jk	3,47	69,33 s	<i>tsn1</i>
Tabor	0,93	18,67cdef	0,87	17,33cdef	0,8	16,0cdef	<i>tsn1</i>
Bezostaya 100	0,8	16,0cdef	3,73	74,67st	3,80	76,00st	<i>tsn1</i>
Gurt	3,80	76,00st	1,8	36,0 jk	0,8	16,0cdef	<i>tsn1</i>
Yeremeevna	1,87	37,33jk	54,67	2,73 nopqr	0,8	16,0cdef	<i>tsn1</i>
Zhiva	0,8	16,0cdef	1,07	21,33cdef	1,0	20,0cdef	<i>tsn1</i>
Sila	0,87	17,33cdef	2,80	56,00opqr	1,07	21,33cdef	<i>tsn1</i>
Grom	1,60	32,0hjk	1,8	36,0 jk	0,87	17,33cdef	<i>Tsn1</i>
Yubileinaya 100	2,53	50,67nopq	2,93	58,67qr	0,87	17,33cdef	<i>Tsn1</i>
Utrish	0,93	18,67cdef	0,87	17,33cdef	1,8	36,0 jk	<i>tsn1</i>
Trio	1,8	36,0 jk	1,07	21,33cdef	0,87	17,33cdef	<i>Tsn1</i>
Kalym	2,87	57,33pqr	3,73	74,67st	0,87	17,33cdef	<i>Tsn1</i>
Urup	4,73	94,67wx	0,87	17,33cdef	1,93	38,67jkm	<i>tsn1</i>
6B 662	0,93	18,67cdef	1,07	21,33cdef	1,19	23,757ef	<i>tsn1</i>
Etnos	3,80	76,00st	2,87	57,33pqr	0,93	18,67cdef	<i>tsn1</i>
Gubernator Dona	3,93	78,67tu	1,0	20,0cdef	2,33	46,67lmn	<i>tsn1</i>
Laureat	4,67	93,33vwxx	1,0	20,0cdef	2,53	50,67nopq	<i>Tsn1</i>
Vassa	3,80	76,00st	3,87	77,33stu	1,07	21,33cdef	<i>Tsn1</i>
Adel	3,93	78,67stu	1,03	22,67defj	1,03/	22,67defj	<i>tsn1</i>
Stanichnaya	2,33	46,67ln	3,93	78,67stu	1,03	22,67defj	<i>tsn1</i>
6B 365	2,47	49,33nop	3,87	77,33stu	1,03	/22,67defj	<i>tsn1</i>
Glenlea	2,40	48,00lno	1,20	24 efjhi	1,62	/32,31 hijk	<i>Tsn1</i>
Ermak	1,87	37,33jk	1,8	36,0 jk	1,27	25,33 fji	<i>Tsn1</i>
Alexeich	1,53	30,67ghij	2,73	54,67nopqr	1,6	32,0 hijk	<i>Tsn1</i>
Brigada	3,73	74,67st	4,87	97,33x	1,6	32hijk	<i>tsn1</i>
Asket	3,80	76,00st	3,67	73,33st	1,87	37,33jk	<i>tsn1</i>
Yukka	3,80	76,00st	3,80	76,00st	2,00	40,00 klm	<i>Tsn1</i>
Yesaul	4,40	88,00vw	2,47	49,33nop	3,93	78,67stu	<i>Tsn1</i>
Stan	3,00	60,00r	2,87	57,33pqr	2,67	53,33nopqr	<i>tsn1</i>
Kuren	3,87	77,33stu	3,80	76,00st	2,67	53,33nopqr	<i>tsn1</i>
Dmitry	2,73	54,67nopqr	4,53	90,67vwxx	4,27	85,33uv	<i>Tsn1</i>
Antonina	2,73	54,67nopqr	3,80	76,00st	4,73	94,67wx	<i>tsn1</i>
Lebed	2,80	56,00opqr	2,80	56,00opqr	3,73	74,67st	<i>tsn1</i>
Bagrat	4,53	90,67vwxx	2,87	57,33pqr	3,80	76,00st	<i>tsn1</i>
Tanya	4,67	93,33vwxx	3,87	77,33stu	3,67	73,33st	<i>Tsn1</i>
Least Significant Difference	-	7,75	-	6,99	-	7,32	-

Note: Salamouni, the insensitive control for race 1 and toxin Ptr ToxA, carrier of the recessive gene *tsn1*; Glenlea, the susceptible control for race 1 and Ptr ToxA, carrier of the dominant *Tsn1*; 6B 662, the susceptible control for Ptr ToxB; 6B 365, the susceptible control for Ptr ToxC; response to Ptr races based on Lamari and Bernier (1989) scale. Data represented mean values of 15 individuals. Because disease scores are ordinary data, disease means were transformed into rank means using PROC RANK in the SAS program for mean separation. In the rank mean column, means with the same letter are not significantly different.

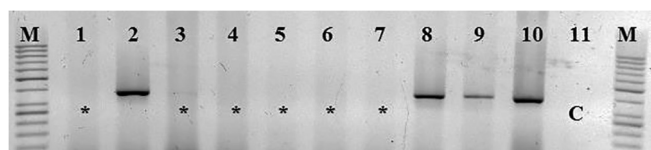


Fig. 4. Example of identification of the dominant *Tsn1* allele by PCR. Size of amplification diagnostic product is 380 bp. *refers to the zero (recessive) allele *tsn1*; the numbers correspond to the variety numbers in Table 1; C - control, without DNA; M - molecular weight marker (Gene-Ruler™; 50 bp DNA Ladder (Fermentas).

Table 4
Comparison of the results of infection of wheat varieties with different allelic states of the *Tsn1* gene with *P. tritici-repentis* race 1 isolate producing PtrToxA.

Combination of genotypes variety/isolate * (quantity)	The expected response of the variety, according to the "gene-to-gene" theory	Observed variety response, %	
		S	R
Tsn1Tsn1/PtrToxA (13)	S	26	12
tsn1tsn1/PtrToxA (21)	R	38	24

Note: S - susceptibility, R - resistance.

haps this result can be explained by the nature of the isolate of race 1 itself, used in the research (Kokhmetova et al., 2018), as a selected producer of the PtrToxA toxin. Natural isolates of *P. tritici-repentis*, in which the ToxA gene was identified by PCR, which determines the synthesis of necrosis of the Ptr ToxA inducing toxin in the mycelium and conidia of the fungus, usually do not show virulence to wheat samples with the *Tsn1* gene with a 100% probability (Chu et al., 2008). A number of studies have shown that sensitivity to toxins may not coincide with the susceptibility of the variety in response to infection with the fungus, but depends on the genotype of the variety (Chu et al., 2008; Faris et al., 2012; Kariyawasam et al., 2016).

The significance of the *ToxA - Tsn1* interaction in determining variety resistance has not been sufficiently studied. For durum wheat, preliminary results were obtained that *Tsn1-ToxA* interactions are not an essential factor for the development of the disease, and for durum wheat the significance of the interaction of these genes is determined by the genotype of the variety (Viridi et al., 2016). Another study provides evidence that *ToxA* is not a major determinant in the development of tan spot in some varieties (See et al., 2018). The authors assessed 40 Australian common wheat varieties for susceptibility to infection by the wild-type race 1 and its mutant with the *ToxA* gene deletion. Most varieties with

Tsn1 showed a lower sensitivity to isolate *Ptr* with *ToxA* mutant infection, but in some of them the symptoms caused by the wild-type isolate and the mutant did not differ. This fact indicates that additional effectors are present in the pathogen isolates. Guo J et al. (2018) used two fungal isolates that produce no known NEs and showed that they still caused disease on some durum and common wheat lines. They also identified host genetic factors that interact with the unknown NEs in these two isolates using host QTL mapping. Their work further demonstrates that the fungal pathogen produces additional NEs or other types of virulence factors besides the three known ones. Many QTL mapping studies have identified genomic regions other than three known sensitivity loci (*Tsn1*, *Tsc1*, and *Tsc2*) (Faris et al., 2012; Viridi et al., 2016; Liu et al., 2017).

The results of a field assessment of 34 varieties of winter wheat identified groups of varieties that did not change their susceptibility to the disease, despite the different weather conditions during the years of research. It was also shown that in some varieties (Grom, Eremeevna, Stan, Stanichnaya, etc.), the response of sensitivity to *Ptr* changed in different years of the study. There are studies that demonstrate the effect of agro-climatic conditions on infection and disease manifestations in the field (Duveiller et al., 2005; Fernandez et al., 2016). The works also show a different reaction of wheat varieties to *Ptr* infection in different phases of wheat plant development and in different agro-climatic regions of wheat cultivation.

To accelerate the breeding process when developing new varieties, molecular markers are increasingly being used to identify resistance genes. Researchers disagree about the possibility of using MAS (marker assisted selection) on the *Tsn1* susceptibility gene for wheat tan spot for negative selection against the dominant allele of the gene: Faris et al. (2010, 2012), Kokhmetova et al. (2017, 2018), Viridi et al. (2016) support the approach, Mironenko et al. (2015), See et al. (2018) are against it. Obviously, the feasibility of selection against the *Tsn1* gene in wheat exists, since this single gene determines the relationship with the *ToxA* effector gene not only in *P. tritici-repentis*, but also in the wheat leaf blotch pathogen *Parastagonospora nodorum* (*syn. Stagonospora nodorum*), as well as the dark brown spot pathogen *Bipolaris sorokiniana* (Friesen et al., 2018; McDonald & Solomon, 2018). This unique property of the *Tsn1* gene is explained by the fact that it interacts with almost the same *ToxA* effector gene, which, through horizontal transfer from *P. nodorum*, fell into the genomes of two other species, *P. tritici-repentis* and *Bipolaris sorokiniana* (Friesen et al., 2018; McDonald and Solomon, 2018), in which it also began to play the role of the main factor.

5. Conclusions

According to the results of the field assessment, resistant varieties Grom, Gurt, Dolya, Kalym, Laureate, Lebed and Sila (disease development up to 30% against the background of artificial infection) were identified. Using PCR analysis, we identified the genotype *tsn1tsn1* in four of these resistant varieties. Our research showed that the studied varieties have race-specific resistance to tan spot. Varieties identified as resistant to the isolate of race 1 were susceptible to different races of the fungal isolate producing other toxins (*ToxB* and *ToxC*). We did not reveal a correlation between the presence in the variety of the dominant allele of the *Tsn1* gene and its susceptibility to the isolate of race 1 producing the *Ptr ToxA* toxin, which confirms the results obtained by other authors. Research results demonstrate the effect of weather conditions on the susceptibility of wheat varieties to tan spot. In years with higher humidity and higher average air temperatures, the susceptibility response to the disease was observed in more vari-

eties than in drier years. The studies show that the main part (79.5%) of winter wheat varieties of Russian selection widely zoned in the North Caucasus region of Russia are susceptible to *Ptr*. Varieties that have been resistant to the pathogen in the adult phase in the field for three years and to the pathogen races in which the recessive allele of the *tsn1* gene has been identified may be of interest as sources of resistance for developing new disease-resistant varieties. The results obtained indicate the need for an integrated approach to studying the resistance of wheat accessions to *P. tritici-repentis*.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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