Population structure of leaf pathogens of common spring wheat in the West Asian regions of Russia and North Kazakhstan in 2017

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Wheat diseases affecting leaves like leaf rust (Puccinia triticina), tan spot (Pyrenophora triticichampas) and spot blotch (Cochliobolus sativus = Bipolaris sorokiniana) are widely spread and potentially dangerous in the West-Asian region of Russia and North Kazakhstan. The study of these pathogens’ populations is very important for genetic protection of wheat. The objective of this study was to explore the population structure of the causative agents of leaf rust and tan spot on spring wheat based on virulence traits and assessing the distribution of the causative agent of spot blotch in the West-Asian region of Russia and North Kazakhstan. The source of inoculum were wheat leaves affected by leaf rust and spot diseases collected in the West-Asian region of Russia and North Kazakhstan. Virulence analysis of P. triticina using 20 lines with known Lr genes demonstrated that all 109 monopustule isolates were avirulent on Tc Lr24. The isolates virulent on TcLr19 were identified only in the Chelyabinsk population. The prevalence of isolates virulent on TcLr2a, TcLr2b, TcLr2c, TcLr11, TcLr15, TcLr16, TcLr20 and TcLr26 was higher in the Omsk and the North Kazakhstan population, while virulence to TcLr19 was higher in Chelyabinsk. Using 20 TcLr-lines, we identified 27 virulent phenotypes of P. triticina: 12 in the Omsk, 19 in the Chelyabinsk and 8 in the North Kazakhstan population. The phenotypes TLTTR (avirulent to TcLr16, TcLr19, TcLr24, TcLr26), TCTTR (avirulent to TcLr9, TcLr16, TcLr19, TcLr24, TcLr26) and TBTR (avirulent to TcLr9, TcLr16, TcLr19, TcLr24, TcLr26) were observed in all the populations. The phenotypes TGTTR (avirulent to TcLr19, TcLr24, TcLr26) and TGTTR (avirulent to TcLr19, TcLr24, TcLr26, TcLr9, TcLr19, TcLr24, TcLr26) were common in the Omsk and the North Kazakhstan population, while THPTR (avirulent to avTcLr9, TcLr11, TcLr19, TcLr24) and TCTTQ (avirulent to TcLr9, TcLr16, TcLr19, TcLr20, TcLr24) were common in the Omsk and the Chelyabinsk population. There was a high genetic similarity in virulence and phenotypic composition between the Omsk and the North Kazakhstan population as well as between the Omsk and the Chelyabinsk population and a moderate similarity between the Chelyabinsk and the North Kazakhstan population. The prevalence of the spot blotch pathogen was higher in the material collected from the Omsk region, while none of this pathogen was identified in the North Kazakhstan.

Листовые болезни яровой пшеницы – бурная ржавчина (возбудитель – Puccinia triticina), желтая пятнистость (Pyrenophora triticichampas) и темно-бурая пятнистость (Cochliobolus sativus = Bipolaris sorokiniana) – относятся к группе распространенных и потенциально опасных болезней в западноазиатских регионах России и Северном Казахстане. Для обоснования стратегий генетической защиты пшеницы необходимы популяционные исследования фитопатогенов. Цель работы – характеристика структуры популяций возбудителей буровой ржавчины и желтой пятнистости яровой пшеницы по признакам вирулентности и оценка распространенности возбудителей темно-буровой пятнистости в западноазиатских регионах Россией и Северном Казахстане. Для обоснования стратегий генетической защиты пшеницы необходимы популяционные исследования фитопатогенов. Цель работы – характеристика структуры популяций возбудителей бурой ржавчины и желтой пятнистости яровой пшеницы по признакам вирулентности и оценка распространенности возбудителей темно-буровой пятнистости в западноазиатских регионах Россией и Северном Казахстане. Анализ вирулентности 109 изолятов P. triticina на 20 линиях-дифференциаторах показал, что все изученные монопустульные изоляты были авриентными к TcLr24. Изоляты, вирулентные к TcLr19, выявлены только в челябинской популяции. Частоты вирулентных изолятив к TcLr2a, TcLr2b, TcLr2c, TcLr11, TcLr16, TcLr20 и TcLr26 были выше в омской и североказахстанской популяциях, а к TcLr9 – в челябинской. При использовании 20 TcLr-линий определено 27 фенотипов вирулентности P. triticina: 12 в омской, 19 в челябинской, 8 в казахстанской. Фенотипы TLTTR (авиерентные (av) к TcLr16, TcLr19, TcLr24, TcLr26), TCTTQ (av: TcLr9, TcLr16, TcLr19, TcLr24, TcLr26) встречались во всех регионах. Фенотипы TQTTR (av: TcLr19, TcLr24, TcLr26) и TGTTR (av: TcLr9, TcLr19, TcLr24, TcLr26) были общими для омской и североказахстанской популяций, а THPTR (av: TcLr9, TcLr11, TcLr19, TcLr24) и TCTTQ (av: TcLr9, TcLr16, TcLr19, TcLr20, TcLr24) – для омской и челябинской. Определено высокое генетическое сходство омской популяции с североказахстанской и челябин-
Kazakhstani material. The isolates of tan spot were identified in all the regions. Five races of *P. tritici-repentis* were identified among Chelyabinsk isolates based on the toxins produced by the following pathogens: race 1 (*PtrToxA* *PtrToxC*); race 2 (*PtrToxA* *PtrToxB*), race 8 (*PtrToxA* *PtrToxB* *PtrToxC*), and race 4 (does not produce toxins). Three races were identified in the Omsk region (1 – 3) and four, in North Kazakhstan (1 – 4). A total of 26 *P. tritici-repentis* phenotypes were identified by virulence analysis using 11 differential lines: two were present in all the populations; two, in Chelyabinsk and North Kazakhstan; one, in Omsk and Chelyabinsk; and all the others were original. A high degree of similarity between the obligate pathogen *P. triticina* and the saprophytic pathogen *P. tritici-repentis* in the West-Asian region of Russia and in North Kazakhstan demonstrates that this is one epidemiological region across this wheat production area. The presence of common phenotypes suggests there is a possibility of gene exchange between the populations and this shall be considered while releasing genetically protected wheat varieties.

Key words: leaf rust; tan spot; spot blotch; spring wheat; populations; virulence; *Lr*-genes.

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Spring wheat is the main cereal crop grown in the Urals, West Siberia and North Kazakhstan. Wheat diseases affecting leaves like leaf rust, stem rust, spot blotches and tan spot significantly reduce wheat yields in these regions (Koishybaev, 2010; Shamin et al., 2016; Belan et al., 2017). Leaf rust (caused by *Puccinia triticina* Eriks.) occurs annually with severity fluctuating from moderate to epiphytic. The disease appears from flag leaf to ear-flowering stages. Stem rust (caused by *Puccinia graminis* Pers. 1. sp. *tritici* Eriks. et Hann.) develops later and prevails at grain ripening stages (Koishybaev, 2015). Until recently, glum blotch caused by *Parastagonospora nodorum* (Berk.) Quaedvl., Verkley & Crous (= *Septoria nodorum* Berk.) was the most important disease of wheat (Sanina, Pakholkova, 2002; Koishybaev, 2015). In the last ten years, however, the harmfulness of tan spot (caused by *Pyrenophora tritici-repentis* (Died.) Drechsler) has increased (Mikhailova et al., 2010, 2015; Koishybaev, 2015). Under field conditions, tan spot and septoriosis are difficult to distinguish – even for experts. The spread of tan spot is promoted by the modern gentle soil treatment, after which a large number of plants remain on the surface and serve as a habitat for the wintering of *P. tritici-repentis* pseudotocia (Mikhailova et al., 2010). *PtrToxA*, *PtrToxB*, *PtrToxC* exotoxins are the main pathogenicity factors of *P. tritici-repentis*. *Ptr ToxA* induces necrosis on susceptible plants, and both *PtrToxB* and *PtrToxC* induce chlorosis (Lamari et al., 1998). Spot blotch of wheat (caused by *Cochliobolus sativus* (S. Ito & Kurib.) Drechsler ex Dastur (= *Bipolaris sorokiniana* (Sacc.) Shoemaker)) is a more important disease in wet and warm years (Kuznetsova, 1987). These spots normally appear together at the wheat stalking stage (Koishybaev, 2010).

Fungus population studies are important for improving genetic strategies of wheat protection. With these studies, the researcher can characterize the race composition dynamics, effectiveness of resistance genes at the host plants and evaluate the influence of commercial wheat varieties on fungus population changes. The population biology of leaf rust pathogens is the most studied. By virulence and microsatellite analyses, the existence of a common *P. triticina* population in the Urals West Siberia, and Kazakhstan (Mikhailova, 2006; Kolmer, Ordoñez, 2007; Kolmer et al., 2015; Gultyaeva et al., 2017) was shown, which should be taken into account when disposing varieties with *Lr*-genes. Annual virulence surveillance of *P. triticina* populations conducted by the Chelyabinsk Scientific Research Institute of Agriculture and the Omsk State Agrarian University allows the dynamics of pathogen variability to be monitored and breeding programs to be improved.

First studies of *P. tritici-repentis* in Russia were carried out by Mikhailova et al. (2010, 2015). The existence of several *P. tritici-repentis* populations in Russia (North Caucasian, Northwestern and West Siberian) was determined according to virulence frequencies in a special wheat differential set. An independent status of the Omsk *P. tritici-repentis* population was also confirmed by microsatellite markers (Mironenko et al., 2016).

In the world literature, data about differential interactions between the plant host and *C. sativus* are controversial. The absence of differential sets significantly limits the population studies of the spot blotch pathogen based on virulence (Mikhailova et al., 2002). Mycological analysis is usually used to assess the spread of this pathogen and to estimate the prevalence of *C. sativus* isolates.
Most population studies of leaf rust and tan spot pathogens have been carried out in independent experiments. It was relevant to conduct a comprehensive analysis of the structure of pathogens that differed in parasitic type (obligate vs. hemibiotrophic), using a similar infectious material collected in geographically remote regions. The objective of this study was to explore the population structure studies of the causative agents of leaf rust and tan spot on spring wheat based on virulence and to assess the distribution of the causative agent of spot blotch in the West-Asian region of Russia and North Kazakhstan in 2017.

Materials and Method
Wheat samples with leaf rust and leaf spot symptoms were collected from the Ural (Chelyabinsk) and the East Siberian (Omsk) region of Russia and North Kazakhstan in 2017. Leaf rust severity at the sampling locations ranged from moderate to strong spots, from low to moderate.

In the Chelyabinsk region, leaves were collected from 30 spring wheat samples in the breeding nursery of the Chelyabinsk Scientific Research Institute of Agriculture. In the Omsk region, leaves were collected from 40 wheat samples growing in the experimental fields of the Omsk State Agrarian University and Cherlak and Pavlodar state variety test plots. In Kazakhstan, infectious material was collected from commercial fields at seven points of the North Kazakhstan region and at two in the Akmola region.

Leaf rust uredinia from dry leaves were renewed on a susceptible wheat variety and single pustule isolates were obtained. Isolates' multiplication for virulence analysis was carried out using a laboratory method of pathogen cultivation. Single uredinial isolates were tested for virulence to 20 near isogenic lines of Thatcher wheat that differed in single leaf rust resistance genes. Three seeds of each of these Thatcher lines were sowed to a pot filled with soil. Each set of 10–14 day-old differentials (the first leaf stage) was spray inoculated by urediniospores of each isolate (10⁶/ml) and kept in a Versatile Environmental Test Chamber (Sanyo) at optimal temperature (22 °C) and moisture (75 %) (Gultyaeva, Soloduhina, 2008). Virulent phenotypes were determined 10 days after inoculation using E.B. Mains and H.S. Jackson scale (1926), where 0 means no visible uredia; 0, hypersensitive flecks; 1, small uredia with necrosis; 2, small- to medium-sized uredia with green islands and surrounded by necrosis or chlorosis; 3, medium-sized uredia with or without chlorosis; 4, large uredia without chlorosis; X, heterogeneous, similarly distributed over the leaves. The plants with infection types 0 to 2 were classified as resistant and infection types 3 to 4 and X as susceptible.

A differential set of 20 near isogenic TcLr-lines was used for studying the leaf rust pathogen’s population structure. Each isolate was given a five-letter code based on virulence or avirulence to each of the five subsets of four differentials as adapted from the North American nomenclature for virulence in *P. triticina* (Long, Kolmer, 1989). The following order of sets was used: 1, Lr1, Lr2a, Lr2c, and Lr3a; 2, Lr9, Lr16, Lr24, and Lr26; 3, Lr3a, Lr11, Lr17, and Lr30; 4, Lr2b, Lr3bg, Lr14a, and Lr14b; 5, Lr15, Lr18, Lr19, and Lr20. The first three groups were similar to the original differential set (Long, Kolmer, 1989) widely used for *P. triticina* population studies (Kolmer, Ordoñez, 2007; Kolmer et al., 2015). Thatcher lines highly informative for differentiation of Russian populations were included in the other two groups (Gultyaeva et al., 2017). Five-letter phenotype codes, virulence frequencies, Nei (Hs) and Shennon (Sh) indexes of population diversity were determined using Virulence Analysis Tool (VAT) software package (Kosman et al., 2008).

Leaf segments with one infection spot surrounded by an area of green tissue were cut out for tan spot and spot blotch studies and put on agar medium V4 (Mikhailova et al., 2012). Dishes with leaf segments were incubated in a thermostat with UV lamps (LE-30) and at a temperature of 20 to 22 °C for three days and were then placed in a refrigerator (5–8 °C) for one day for stimulation of *P. tritici-repentis* conidia development. The frequency of *P. tritici-repentis* and *C. sativus* colonies obtained from different geographic populations was used as a criterion of the distribution of these pathogens.

Reproduction of *P. tritici-repentis* fungus culture was carried out according to L.A. Mikhailova et al. (2012). Virulence analysis was carried out using methods of cutting leaves placed on the benzimidazole solution (0.004 %).

The racial identity revealed by the ability of *P. tritici-repentis* isolates to form the toxins Ptr ToxA, Ptr ToxB and Ptr ToxC was determined by inoculation of the cultivar Glenlea, lines 6B662 and 6B365, by the presence of necrotic and chlorotic spots on wheat leaves (Lamari, Bernier, 1989; Lamari et al., 1998).

The virulence of *P. tritici-repentis* isolates was studied using the following set of cultivars: Allies (France); Norin 58, Satsukei 86, Hokkai 252, Komadi 3 (Japan); Riley 67, Clark (USA); Asiago (Italy); Salamouni (Egypt); and M3 (Canada), which differentiate the fungus isolates for their ability to produce necrosis and chlorosis. The type of infection caused by isolates was assessed using a five-point scale corresponding to the size of necrotic and chlorotic spots, according to Mikhailova et al. (2012). A comparison of the population samples on the basis of virulence was carried out according to the index of the average score of infection per isolate (the ratio of the sums of the points exhibited by isolates on tan spot wheat differential sets to the number of isolates). For the determination of phenotypes, only the indicator of the necrotic reaction evaluation was used, since it characterizes the result of the action of one (Ptr ToxA), and not two toxins, as in the case of a chlorotic reaction, when two independent traits appear that are identical in phenotype (Mikhailova et al., 2010). The results of the virulence evaluation of *P. tritici-repentis* isolates were presented as a binary matrix: 1, virulence (scores 3–5); 0, avirulence (scores 0–2).

The degree of genetic similarity between the Omsk, the Chelyabinsk and the North Kazakhstani populations of *P. triticina* and *P. tritici-repentis* for virulence was evaluated using Nei (Nei genetic distance, Nei D) and Fst indexes calculated by GenAlEx (Genetic analysis in Excel, 6.5 http://biology.anu.edu.au/GenAlEx) software package.

Results and Discussion
One hundred and nine single-pustule isolates – 30 from Chelyabinsk, 45 from Omsk and 34 from North Kazakhstan – were characterized during the leaf rust population studies. All single-pustule isolates studied were avirulent to TcLr24.
Isolates virulent to \( \text{TcLr}19 \) were detected in the Chelyabinsk population. The prevalence of isolates virulent to \( \text{TcLr}2a, \text{TcLr}2b, \text{TcLr}2c, \text{TcLr}11, \text{TcLr}15, \text{TcLr}16, \text{TcLr}20 \) and \( \text{TcLr}26 \) was higher in the Omsk and the North Kazakhstani population and of those virulent to \( \text{TcLr}9 \), in the Chelyabinsk population (Table 1). A high virulence to \( \text{Lr}9 \) in the Chelyabinsk population in comparison to the other populations studied was due to a high prevalence (10 %) of varieties with this gene in the infectious material (3 % in the Omsk population).

A high efficiency of the \( \text{Lr}24 \) gene in the regions of Russian Federation is due to the absence of commercial varieties with this gene. Nevertheless, at present, \( \text{Lr}24 \) donors are used in breeding in Russia (Tyunin et al., 2017). The world practice of cultivating varieties with the \( \text{Lr}24 \) gene shows that mass cultivation is rapidly followed the emergence of virulent races and the gene loses its effectiveness. Virulence to \( \text{Lr}24 \) occurs in \( \text{P. triticina} \) populations across North America and Australia, where wheat varieties protected by this gene are widely known (McIntosh et al., 1995).

Isolates virulent to the \( \text{Lr}19 \) gene were observed only in the Chelyabinsk population and were isolated from the line cv. Omskaya 37 or cv. Omskaya 38. Virulence to \( \text{Lr}19 \) gene is more often noted in the Volga region, where varieties with this gene are grown, but it can also occur in other regions (Kovalenko et al., 2012; Tyunin et al., 2017). All \( \text{P. triticina} \) isolates studied virulent to \( \text{TcLr}19 \) were avirulent to \( \text{TcLr}26 \). Similar observations were made for isolates virulent to \( \text{TcLr}9 \). Expanding areas with varieties carrying \( \text{Lr}9 \) provides for increase in the frequency of isolates with virulence to \( \text{Lr}9 \) in the West Asian regions of Russia, which are as powerful accumulators of infestants (Meshkova et al., 2012, Tyunin et al., 2017). To stabilize the phytosanitary situation in the Urals and West Siberia, a strategy of pyramiding the \( \text{Lr}9 \) and \( \text{Lr}19 \) genes with \( \text{Lr}26 \) and other \( \text{Lr} \)-genes may be useful, because their effective combination will help prolong the “useful life” of new varieties.

Twenty-seven virulent phenotypes (12 from Omsk, 19 from Chelyabinsk and 8 from Kazakhstan) were determined using 20 \( \text{TcLr} \) lines (Table 2). The phenotypes TLTTR, TCCTTR and TBTR were found in all the populations studied. The phenotypes TQTTR and TGTTR were common in the Omsk and the North Kazakhstani population, while THPTR and TCTTQ were common in the Omsk and the Chelyabinsk population. A high degree of similarity by the virulence phenotypes indicates gene flow between pathogen populations in the study area in 2017. In general, no significant changes in the phenotypic composition of the Omsk and the Chelyabinsk population were observed in 2017 as compared to 2014–2016 (Tyunin et al., 2017).

Analysis of the Omsk and the Chelyabinsk \( \text{P. triticina} \) population on similar sets of spring wheat showed their identical virulence on the susceptible varieties Pamyati Aziyeva, Omskaya 35, Saratovskaya 29 and Lutescens 857. Significant differences in virulence between the populations studied were observed for the following wheat samples: Lutescens 1103 (on the Tc-lines with genes \( \text{Lr}2a, \text{Lr}2b, \text{Lr}2c, \text{Lr}15, \text{Lr}16 \)), Lutescens KS14/09-2 (\( \text{Lr}2a, \text{Lr}2b, \text{Lr}2c, \text{Lr}11, \text{Lr}15, \text{Lr}16, \text{Lr}20 \)), Duet (\( \text{Lr}2a, \text{Lr}2b, \text{Lr}2c, \text{Lr}11, \text{Lr}15, \text{Lr}16, \text{Lr}20 \)), and moderate differences, for Novosibirskaya 16, Lutescens 37-17, Erythrospernum 1119 (\( \text{Lr}16 \)), Stolypinskaya 2, GVK 2127 (\( \text{Lr}16, \text{Lr}20 \)), OmGAU 100 (\( \text{Lr}11 \)), Tyumenochka (\( \text{Lr}11, \text{Lr}20 \)) and Element 22 (\( \text{Lr}11, \text{Lr}16 \)).

The Nei (Ns) and Shannon (Sh) indices, which characterize the in-population genetic diversity, showed that the Chelyabinsk population was more heterogeneous for virulence (\( Ns = 0.21 \)) and phenotypic composition (\( Sh = 0.82 \)) compared to the Omsk and the North Kazakhstani population (\( Ns = 0.09 \) and 0.06; \( Sh = 0.51 \) and 0.49, respectively).

Nei’s genetic distance (N) indicated a high similarity between the Omsk, the North Kazakhstani (\( N = 0.03 \)) and the Chelyabinsk population (\( N = 0.05 \)) and a moderate similarity between Chelyabinsk and North Kazakhstan (\( N = 0.13 \)). The results obtained in 2017 suggest there had been no changes in the structure of the populations studied compared to the previous time (Kovalenko et al., 2012; Gultyaeva et al., 2017; Tyunin et al., 2017).

For population studies in \( \text{P. tritici-repentis} \) and \( \text{C. sativus} \), we used wheat leaves with typical visual symptoms of the diseases being discussed. In the Chelyabinsk region, nine wheat cultivars were used as infectious material: Ural’skaya kukushka, Chelyaba ramnyaya, Eritrospernum 59, Iskra, Rossiyanka, Izumrudnaya, Astana 2, Tyumenochka, Tertsia; in the Omsk Region, eight: Pamyati Aziyeva, Sibakovskaya yubileynaya, OmGAU 90, Chernyava 13, Uralosibirskaya, Duet, Grani and Katyusha. In leaf samples from the North Kazakhstani region, spots was noted in six samples.

A total of 466 infected samples (segments of leaves with separate spots) were studied: 125 from Omsk, 215 from Chelyabinsk, and 126 from North Kazakhstan. The prevalence of \( \text{C. sativus} \) and \( \text{P. tritici-repentis} \) isolates was 12 % and 14 %, respectively, in Omsk samples; 3 % and 25 %, in Chelyabinsk; and 0 % and 43 % in North Kazakhstan. Thus, the presence of the causative agent of spot blotch disease of wheat was stronger in the Omsk than in Chelyabinsk region. In North Kazakhstani leave samples, \( \text{C. sativus} \) was not observed. \( \text{P. tritici-repentis} \) was noted in all regions. The prevalence of \( \text{P. tritici-repentis} \) isolates was higher in North Kazakhstani and Chelyabinsk samples and lower in Omsk.

Nineteen Chelyabinsk, 8 Omsk and 27 North Kazakhstani isolates of \( \text{P. tritici-repentis} \) were used to analyze the population structure on the basis of virulence and toxicity. \( \text{P. tritici-repentis} \) races identified in the three populations by toxicity are presented in Table 3. Five races were found in the samples of the Chelyabinsk population; three, in Omsk; and four, in North Kazakhstan.

The racial structure of Omsk \( \text{P. tritici-repentis} \) isolates was characterized by a higher diversity in 2017 than 2007, when two races were found: race 2 and race 7 (Mikhailova et al., 2010). Races 1 to 4 of \( \text{P. tritici-repentis} \), which predominate in the study populations, are also widely distributed in other Russian regions (Central European and North Caucasian) (Mikhailova et al., 2010, 2012).

In general, a high incidence of isolates producing \( \text{PtrToxA} \) (87–95 %) was noted (see Table 3), which indicates a potential harmfulness of yellow spot in the West Siberian and the Ural region of Russia and North Kazakhstan.

Twenty-six phenotypes of \( \text{P. tritici-repentis} \) were identified by virulence analysis using 11 differential cultivars (on the
### Table 1. Prevalence of isolates virulent to Thatcher lines in the Chelyabinsk, the Omsk and the North Kazakhstani P. triticina population in 2017 (%)

| Tester line | P. triticina populations | Omsk | Chelyabinsk | North Kazakhstan |
|-------------|--------------------------|------|-------------|-----------------|
| RL6064 TcLr24 |                          | 0    | 0           | 0               |
| RL6016 TcLr2a | 95 ± 0.03                | 60 ± 0.09 | 100       |
| RL6019 TcLr2b | 95 ± 0.03                | 70 ± 0.08 | 100       |
| RL6047 TcLr2c | 95 ± 0.03                | 70 ± 0.08 | 100       |
| RL6010 TcLr9  | 10 ± 0.05                | 43.3 ± 0.09 | 14.7 ± 0.06 |
| RL6053 TcLr17 | 80 ± 0.06                | 70 ± 0.08 | 100       |
| RL6052 TcLr15 | 95 ± 0.03                | 60 ± 0.09 | 100       |
| RL6005 TcLr16 | 72.5 ± 0.07              | 43.3 ± 0.09 | 67.6 ± 0.08 |
| RL6040 TcLr19 | 3.3 ± 0.03               | 3.3 ± 0.03 | 3.3 ± 0.03 |
| RL6092 TcLr20 | 92.5 ± 0.04              | 53.3 ± 0.09 | 100       |
| RL6078 TcLr26 | 80 ± 0.06                | 50 ± 0.09 | 55.9 ± 0.08 |
| RL6003 TcLr1, RL6002 TcLr3a, RL6042 TcLr3bg | 100 | 100 | 100 |
| RL6007 TcLr3a, RL6013 TcLr14a, RL6006 TcLr14b, RL6008 TcLr17, RL6009 TcLr18, RL6049 TcLr30 |

### Table 2. Phenotypic composition of P. triticina in the West Asian regions of Russia and North Kazakhstan in 2017 (%)

| Phenotype | Avirulence to TcLr lines | P. triticina populations |
|-----------|--------------------------|--------------------------|
|           |                          | Omsk | Chelyabinsk | North Kazakhstan |
| TRTR      | 19, 24                   | 0    | 0           | 2.9               |
| TRPR      | 11, 19, 24               | 2.5  | 0           | 0                 |
| TQTR      | 19, 24, 26               | 2.5  | 0           | 5.9               |
| TQTQ      | 19, 24, 26               | 2.5  | 0           | 0                 |
| TMTTR     | 16, 19, 24               | 0    | 0           | 2.9               |
| TLTR      | 16, 19, 24, 26           | 2.5  | 10          | 2.9               |
| TLPTQ     | 11, 16, 19, 20, 24, 26   | 2.5  | 0           | 0                 |
| THTTR     | 9, 19, 24                | 0    | 10          | 35.3              |
| THTQ      | 9, 19, 20, 24            | 0    | 10          | 0                 |
| THPTR     | 9, 11, 19, 24            | 12.5 | 3.3         | 0                 |
| THPTQ     | 9, 11, 19, 20, 24        | 0    | 3.3         | 0                 |
| TGTR      | 9, 19, 24, 26            | 0    | 0           | 23.5              |
| TCTTR     | 9, 16, 19, 24            | 12.5 | 3.3         | 14.7              |
| TCTTQ     | 9, 16, 19, 20, 24        | 5    | 3.3         | 0                 |
| TBTR      | 9, 16, 19, 24, 26        | 5    | 3.3         | 11.8              |
| PLPTG     | 2a, 11, 15, 16, 19, 20, 24, 26 | 0    | 3.3         | 0                 |
| PHPPG     | 2a, 9, 11, 15, 19, 20, 24 | 0    | 3.3         | 0                 |
| PCPTG     | 2a, 9, 11, 15, 16, 19, 20, 24 | 0    | 3.3         | 0                 |
| MQTKG     | 2a, 2b, 2c, 15, 19, 20, 24, 26 | 0    | 3.3         | 0                 |
| MQPKH     | 2a, 2b, 2c, 11, 15, 19, 24, 26 | 0    | 3.3         | 0                 |
| MQPKG     | 2a, 2b, 2c, 11, 15, 19, 20, 24, 26 | 0    | 3.3         | 0                 |
| MLTKH     | 2a, 2b, 2c, 15, 16, 19, 24, 26 | 0    | 6.7         | 0                 |
| MLTKG     | 2a, 2b, 2c, 15, 16, 19, 20, 24, 26 | 0    | 3.3         | 0                 |
| MLPKG     | 2a, 2b, 2c, 11, 15, 16, 19, 20, 24, 26 | 0    | 6.7         | 0                 |
| MHPKH     | 2a, 2b, 2c, 9, 11, 15, 19, 24 | 2.5  | 0           | 0                 |
| MGKHK     | 2a, 2b, 2c, 9, 15, 19, 24, 26 | 2.5  | 0           | 0                 |
| MBKHK     | 2a, 2b, 2c, 9, 15, 16, 24, 26 | 0    | 3.3         | 0                 |
basis of necrosis). Two of them were found in all regions, two were shared by the Chelyabinsk and the North Kazakhstan population, and one, by Omsk and Chelyabinsk. Forty-seven percent of Chelyabinsk isolates and 33% of North Kazakhstan isolates are represented by unique phenotypes. The presence of common phenotypes in the populations studied indicates a possible gene flow. It is believed that microevolutionary changes in the populations of the causative agent of tan spot of wheat during the occupation of new territories occur so as to expand genetic diversity and to increase virulence in comparison with the populations inhabiting developed territories (Mikhailova et al., 2010). When studying the virulence of isolates on 11 tan spot wheat differential sets, it was determined that the values of the average infection type were similar in all collections studied: 1.69 (necrosis) – 1.71 (chlorosis) in Chelyabinsk populations and 1.47–1.84; 1.71–1.55 in Omsk populations).

The indices of genetic distances of Nei and Fst indicated a high similarity between the Chelyabinsk, Omsk and North Kazakhstan isolates of *P. tritici-repentis* (N = 0.02–0.05; Fst = 0.03–0.12). This indicates the presence of a shared epiphytotic zone of *P. tritici-repentis* in the study area.

**Table 3.** *P. tritici-repentis* races identified by toxins produced in the West Asian regions of Russia and North Kazakhstan in 2017 (%)

| Races | Toxin produced | *P. tritici-repentis* populations |
|-------|----------------|----------------------------------|
|       |                | Omsk    | Chelyabinsk | North Kazakhstan |
| 1     | PtrToxA, PtrToxC | 50      | 26          | 48              |
| 2     | PtrToxA        | 37      | 53          | 26              |
| 3     | PtrToxC        | 13      | 0           | 11              |
| 4     | None           | 0       | 5           | 15              |
| 5     |_PTRToxB        | 0       | 5           | 0               |
| 6     |_PTRToxA,_PTRToxB_PTRToxC | 0       | 11          | 0               |

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**Conflict of interest**

The authors state that there is no conflict of interest.

**References**

Belan I.A., Rosseyeva L.P., Mershkova L.V., Blokhina N.P., Pershina L.A., Trubacheyev L.E., Mironenko N.V., Kazartsev I.A., Akhmetova A., Kosman E. Genetic differentiation of *Puccinia triticina* Eriks. in Russia. J. Genet. 2017;53(9):998-1005. DOI 10.1134/S1022795417070031.

Gultyaeva E.I., Aristova M.K., Shaidayuk E.L., Mironenko N.V., Kazartsev I.A., Akhmetova A., Kosman E. Genetic differentiation of *Puccinia triticina* Eriks. in Russia. J. Genet. 2017;53(9):998-1005. DOI 10.1134/S1022795417070031.

Koishybaev M. Distribution and development of yellow wheat spot in Kazakhstan. Mikologiya i fitopatologiya = Mycology and Phytopathology. 2010;45(2):177-186. (in Russian)

Koishybaev M. Development of rust and Septoria blight forms and wheat protection therefrom. Zashchita i Karantin Rasteniy = Plant Protection and Quarantine. 2015;97:21-25. (in Russian)

Kolmer J.A., Kabdulova M.G., Mustafina M.A., Zhemchuzhina N.S., Dubovoy V. Russian populations of *Puccinia triticina* in distant regions are not differentiated for virulence and molecular genotype. Pathol. Plant. 2015;64(2):329-336. DOI 0.1111/ppa.12248.

Kolmer J.A., Ordoñez M.E. Genetic differentiation of *Puccinia triticina* populations in Central Asia and the Caucasus. Phytopathology. 2007;97:1141-1149. DOI 10.1094/PHYTO-97-9-1141.

Kosman E., Dinoor A., Herrmann A., Schachtel G.A. Virulence Analysis Tool (VAT). User Manual. 2008. http://www.tau.ac.il/lifesci/departments/plants/members/kosman/VAT.html

Kovalenko E.D., Zhemchuzhina A.I., Kiseleva M.I., Kolomiets T.M., Lapochkina I.F., Khudokormova Zh.N., Bokkel’man H. Current state of leaf rust populations and creation of germplasm bank of wheat donors and sources resistant to the disease. Immunogenecheskaya zashchita sel’skokhozyaistvennykh kul’tur ot boleznei: teoriya i praktika. Materialy Mezhduunarodnoy nauchno-prakticheskoy konferentsii, posvyashchennoy 125-letiyu so dnya rozhdeniya N.I. Vavilova [Immunogenic control of plant diseases in agriculture: the theory and practice. Proceedings of the scientific and practical
conference marking the 125th anniversary of N.I. Vavilov]. Bolshie Vyazemy, Moskow region. 2012;69-80. (in Russian)

Kuznetcova T.T. Species range of diseases of cereals in Western Siberia. Nauchno-tekhnicheskii byulleten VASKhNIL, Sibirskoe odotelechie [Scientific and Technical Bulletin of the All-Russia Academy of Agricultural Sciences, Siberian Branch]. Novosibirsk: SibNIZKhim Publ., 1987;2:50-52. (in Russian)

Lamari L., Bernier C.C. Toxin of Pyrenophora tritici-repentis: host-specificity, significance in disease, and inheritance of host-reaction. Phytopathology. 1989;79:740-744. DOI 10.1094/Phyto-79-740.

Lamari L., Gilbert J., Tekauz A. Race differentiation in Pyrenophora tritici-repentis and survey of physiologic variation in western Canada. Can. J. Plant Pathol. 1998;20:396-400. DOI 10.1080/07060669809500410.

Long D.L., Kolmer J.A. North American system of nomenclature for Puccinia recondita f. sp. tritici. Phytopathology. 1989;79:525-529.

Mains E.B., Jackson H.S. Physiologic specialization in the leaf rust of wheat; Puccinia triticina Erikss. Phytopathology. 1926;16:89-120.

McIntosh R.A., Wellings C., Park R.F. Wheat Rusts: an Atlas of Resistance Genes. London: Kluwer Academic Publishers, 1995.

Meshkova L.V., Rosseeva L.P., Korenyuk E.A., Belan I.A. Dynamics of the distribution of pathotypes of the leaf rust agent virulent to cultivars with the Lr9 gene in the Omsk region. Mikologiya i fitopatologiya = Mycology and Phytopathology. 2012;46(6):397-400. (in Russian)

Mikhailova L.A., Gogoleva S.G., Gultyaeva E.I. Interactions between Bipolaris sorokiniana strains and wheat accessions. Mikologiya i fitopatologiya = Mycology and Phytopathology. 2002;36(2):63-66. (in Russian)

Mikhailova L.A., Kovalenko N.M., Mironenko N.V., Rossea L.P. Populations of Pyrenophora tritici-repentis in Russia. Mikologiya i fitopatologiya = Mycology and Phytopathology. 2015;49(4):257-261. (in Russian)

Mikhailova L.A., Mironenko N.V., Kovalenko N.M. Zheltaya pyatnost’ pshenitsy [Yellow spot of wheat]. Metodicheskie ukazaniya po izucheniyu populyatsiy vozbuditeley zheltoy pyatnosti [Methodical guidelines for the study of the populations of the causative agent of yellow spot of Pyrenophora tritici-repentis and the resistance of wheat varieties]. St-Peterburg: VIZR Publ., 2012. (in Russian)

Mikhailova L.A., Temniuk I.G., Mironenko N.V. Characterization of Pyrenophora tritici-repentis populations with regard to virulence. Mikologiya i fitopatologiya = Mycology and Phytopathology. 2010;44(3):263-272. (in Russian)

Mironenko N.V., Baranova O.A., Kovalenko N.M., Mikhailova L.A., Rossea L.P. Genetic structure of the Russian populations of Pyrenophora tritici-repentis, determined by using microsatellite markers. Genetika = Genetics (Moscow). 2016;52(8):885-895. (in Russian)

Sanina A.A., Papolkova E.V. Species structure of Septoria blight agents on wheat in different regions of Russia. Sovremennaya mikologiya v Rossii. Sbornik trudov 1-go s‘ezda mikologov Rossii [Modern mycology in Russia. Proceedings of the 1st Congress of Russian Mycologists]. Moscow, 2002. (in Russian)

Shamanin V., Salina E., Wanyera R., Zelenskiy Y., Olivera P., Morgounov A. Genetic diversity of spring wheat from Kazakhstan and Russia for resistance to stem rust Ug99. Euphytica. 2016;212(2):287-296. DOI 10.1007/s10681-016-1769-0.

Tyunin V.A., Shreyder E.R., Gultyaeva E.I., Shaydayuk E.L. Characteristics of virulence of Puccinia triticina populations and the potential of the Lr24, Lr25, LrSp genes for spring common wheat breeding in the Southern Ural. Vavilovskii Zhurnal Genetiki i Selektsi = Vavilov Journal of Genetics and Breeding. 2017;21(5):523-529. DOI 10.18699/VJ17.269. (in Russian)