Вавиловский журнал генетики и селекции. 2018;22(3):363-369
DOI 10.18699/VJ18.372



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

E.I. Gultyaeva[1] ✉, N.M. Kovalenko[1], V.P. Shamanin[2], V.A. Tyunin[3], E.R. Shreyder[3], E.L. Shaydayuk[1], A.I. Morgunov[4]

[1] All-Russian Institute of Plant Protection, St. Petersburg, Pushkin, Russia
[2] Omsk State Agrarian University named after P.A. Stolypin, Omsk, Russia
[3] Chelyabinsk Scientific Research Institute of Agriculture, Chelyabinsk region, Chebarkulsky district, Timiryazevsky, Russia
[4] International Maize and Wheat Improvement Center (CIMMYT), Ankara, Turkey

Wheat diseases affecting leaves like leaf rust (*Puccinia triticina*), tan spot (*Pyrenophora tritici-repentis*) 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 Chelyabinsk and the Omsk region of Russia and in 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 Tc*Lr19* were identified only in the Chelyabinsk population. The prevalence of isolates virulent on Tc*Lr2a*, Tc*Lr2b*, Tc*Lr2c*, Tc*Lr11*, Tc*Lr15*, Tc*Lr16*, Tc*Lr20* and Tc*Lr26* was higher in the Omsk and the North Kazakhstani population, while virulence to Tc*Lr9* was higher in Chelyabinsk. Using 20 Tc*Lr*-lines, we identified 27 virulent phenotypes of *P. triticina*: 12 in the Omsk, 19 in the Chelyabinsk and 8 in the Kazakhstani population. The phenotypes TLTTR (avirulent to Tc*Lr16*, Tc*Lr19*, Tc*Lr24*, Tc*Lr26*), TCTTR (avirulent to Tc*Lr9*, Tc*Lr16*, Tc*Lr19*, Tc*Lr24*), and TBTTR (avirulent to Tc*Lr9*, Tc*Lr16*, Tc*Lr19*, Tc*Lr24*, Tc*Lr26*) were observed in all the populations. The phenotypes TQTTR (avirulent to Tc*Lr19*, Tc*Lr24*, Tc*Lr26*) and TGTTR (avirulent to Tc*Lr9*, Tc*Lr19*, Tc*Lr24*, Tc*Lr26,* Tc*Lr*9, Tc*Lr*19, Tc*Lr*24, Tc*Lr*26) were common in the Omsk and the North Kazakhstani population, while THPTR (avirulent to avTc*Lr9*, Tc*Lr11*, Tc*Lr19*, Tc*Lr24*) and TCTTQ (avirulent to Tc*Lr9*, Tc*Lr16*, Tc*Lr19*, Tc*Lr20*, Tc*Lr24*) 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 Kazakhstani population as well as between the Omsk and the Chelyabinsk population and a moderate similarity between the Chelyabinsk and the North Kazakhstani 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

Received 04.01.2018
Accepted for publication 21.02.2018


✉ e-mail: eigultyaeva@gmail.com

## Структура популяций листовых патогенов яровой пшеницы в западноазиатских регионах России и Северном Казахстане в 2017 г.

Е.И. Гультяева[1] ✉, Н.М. Коваленко[1], В.П. Шаманин[2], В.А. Тюнин[3], Е.Р. Шрейдер[3], Е.Л. Шайдаюк[1], А.И. Моргунов[4]

[1] Всероссийский научно-исследовательский институт защиты растений, Санкт-Петербург, Пушкин, Россия
[2] Омский государственный аграрный университет им. П.А. Столыпина, Омск, Россия
[3] Челябинский научно-исследовательский институт сельского хозяйства, Челябинская область, Чебаркульский район, пос. Тимирязевский, Россия
[4] Международный центр улучшения кукурузы и пшеницы (CIMMYT), Анкара, Турция

Листовые болезни яровой пшеницы – бурая ржавчина (возбудитель – *Puccinia triticina*), желтая пятнистость (пиренофороз) (*Pyrenophora tritici-repentis*) 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 бурой ржавчины и желтой пятнистости яровой пшеницы по признакам вирулентности и оценка распространенности возбудителя темно-бурой пятнистости в западноазиатских регионах Российской Федерации и Северном Казахстане в 2017 г. Источником инфекционного материала служили пораженные бурой ржавчиной и пятнистостями листья образцов яровой пшеницы, собранные в Челябинской и Омской областях и Северном Казахстане. Анализ вирулентности 109 изолятов *P. triticina* на 20 линиях-дифференциаторах показал, что все изученные монопустульные изоляты были авирулентны к Tc*Lr24*. Изоляты, вирулентные к Tc*Lr19*, выявлены только в челябинской популяции. Частоты вирулентных изолятов к Tc*Lr2a*, Tc*Lr2b*, Tc*Lr2c*, Tc*Lr11*, Tc*Lr15*, Tc*Lr16*, Tc*Lr20* и Tc*Lr26* были выше в омской и североказахстанской популяциях, а к Tc*Lr9* – в челябинской. При использовании 20 Tc*Lr*-линий определено 27 фенотипов вирулентности *P. triticina*: 12 в омской, 19 в челябинской, 8 в казахстанской. Фенотипы TLTTR (авирулентность (av) к Tc*Lr16*, Tc*Lr19*, Tc*Lr24*, Tc*Lr26*), TCTTR (av: Tc*Lr9*, Tc*Lr16*, Tc*Lr19*, Tc*Lr24*), TBTTR (av: Tc*Lr9*, Tc*Lr16*, Tc*Lr19*, Tc*Lr24*, Tc*Lr26*) встречались во всех регионах. Фенотипы TQTTR (av: Tc*Lr19*, Tc*Lr24*, Tc*Lr26*) и TGTTR (av: Tc*Lr9*, Tc*Lr19*, Tc*Lr24*, Tc*Lr26*) были общими для омской и североказахстанской популяций, а THPTR (av: Tc*Lr9*, Tc*Lr11*, Tc*Lr19*, Tc*Lr24*) и TCTTQ (av: Tc*Lr9*, Tc*Lr16*, Tc*Lr19*, Tc*Lr20*, Tc*Lr24*) – 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); race 7 (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 the 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.

ской и умеренное между челябинской и североказахстанской популяциями. Распространенность возбудителя темно-бурой листовой пятнистости в Омской области была выше, чем в Челябинской. Из североказахстанских инфекционных образцов листьев *C. sativus* не выделен. Возбудитель желтой пятнистости обнаружен во всех изученных регионах. По признаку токсинообразования среди челябинских изолятов *P. tritici-repentis* выявлено пять рас (p1 (PtrToxA, PtrToxC); p2 (PtrToxA); p7 (PtrToxA, PtrToxB), p8 (PtrToxA, PtrToxB, PtrToxC); p4 (не образует токсины)); среди омских – три (p1, p2, p3); среди североказахстанских – четыре (p1, p2, p3, p4). 26 фенотипов *P. tritici-repentis* выявлено при анализе вирулентности на 11 сортах-дифференциаторах. Два из них были представлены во всех популяциях; два – в челябинской и североказахстанской; один – в омской и челябинской; остальные были оригинальными. Высокая степень сходства структуры популяций облигатного патогена *P. triticina* и сапротрофного *P. tritici-repentis* в западноазиатских областях Российской Федерации и Северном Казахстане указывает на единую эпидемиологическую зону. Наличие общих фенотипов патогенов свидетельствует о возможном генном потоке между изученными популяциями, что следует учитывать при размещении в этих регионах генетически защищенных сортов яровой пшеницы.

Ключевые слова: бурая ржавчина; желтая пятнистость; темно-бурая пятнистость; яровая пшеница; популяции; вирулентность; *Lr*-гены.

**HOW TO CITE THIS ARTICLE:**
Gultyaeva E.I., Kovalenko N.M., Shamanin V.P., Tyunin V.A., Shreyder E.R., Shaydayuk E.L., Morgunov A.I. Population structure of leaf pathogens of common spring wheat in the West Asian regions of Russia and North Kazakhstan in 2017. Vavilovskii Zhurnal Genetiki i Selektsii = Vavilov Journal of Genetics and Breeding. 2018;22(3):363-369. DOI 10.18699/VJ18.372

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; Shamanin et al., 2016; Belan et al., 2017). Leaf rust (caused by *Puccinia triticina* Erikss.) occurs annually with severity fluctuating from moderate to epiphytotic. The disease appears from flag leaf to ear-flowering stages. Stem rust (caused by *Puccinia graminis* Pers. f. sp. *tritici* Erikss. et Henn.) 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 serves as a habitat for the wintering of *P. tritici-repentis* pseudotecia (Mikhailova et al., 2010). PtrToxA, PtroxB, 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 lead 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 and 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 ($10^6$/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 Tc*Lr*-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, *Lr3ka*, *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 surround 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 benzimedazole 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 Tc*Lr24*.

Isolates virulent to Tc*Lr19* were detected in the Chelyabinsk population. The prevalence of isolates virulent to Tc*Lr2a,* Tc*Lr2b,* Tc*Lr2c,* Tc*Lr11,* Tc*Lr15,* Tc*Lr16,* Tc*Lr20* and Tc*Lr26* was higher in the Omsk and the North Kazakhstani population and of those virulent to *TcLr9*, in the Chelyabinsk population (Table 1). A high virulence to *Lr9* 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 *Lr24* gene in the regions of Russian Federation is due to the absence of commercial varieties with this gene. Nevertheless, at present, *Lr24* donors are used in breeding in Russia (Tyunin et al., 2017). The world practice of cultivating varieties with the *Lr24* gene shows that mass cultivation is rapidly followed the emergence of virulent races and the gene loses its effectiveness. Virulence to *Lr24* occurs in *P. triticina* populations across North America and Australia, where wheat varieties protected by this gene are widely grown (McIntosh et al., 1995).

Isolates virulent to the *Lr19* gene were observed only in the Chelyabinsk population and were isolated from the line protected by this gene. They were not detected on any wheat sample carrying *Lr19* and *Lr26* at once – not, for example, on cv. Omskaya 37 or cv. Omskaya 38. Virulence to *Lr19* 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 *P. triticina* isolates studied virulent to Tc*Lr19* were avirulent to *TcLr26*. Similar observations were made for isolates virulent to Tc*Lr9*. Expanding areas with varieties carrying *Lr9* provides for increase in the frequency of isolates with virulence to *Lr9* in the West Asian regions of Russia, which are as powerful accumulators of infection (Meshkova et al., 2012, Tyunin et al., 2017). To stabilize the phytosanitary situation in the Urals and West Siberia, a strategy of pyramiding the *Lr9* and *Lr19* genes with *Lr26* and other *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 Tc*Lr* lines (Table 2). The phenotypes TLTTR, TCTTR and TBTTR 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 *P. triticina* population on similar sets of spring wheat showed their identical virulence on the susceptible varieties Pamyati Azieva, 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 *Lr2a*, *Lr2b*, *Lr2c*, *Lr15*, *Lr16*), Lutescens KS14/09-2 (*Lr2a*, *Lr2b*, *Lr2c*, *Lr11*, *Lr15*, *Lr20*), Duet (*Lr2a*, *Lr2b*, *Lr2c*, *Lr11*, *Lr15*, *Lr16*, *Lr20*), and moderate differences, for Novosibirskaya 16, Lutescens 37-17, Erythrospermum 1119 (*Lr16*), Stolypinskaya 2, GVK 2127 (*Lr16*, *Lr20*), OmGAU 100 (*Lr11*), Tyumenochka (*Lr11*, *Lr20*) and Element 22 (*Lr11*, *Lr16*).

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 *P. tritici-repentis* and *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 rannyaya, Eritrospermum 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 Kazakhstan 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 *C. sativus* and *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, *C. sativus* was not observed. *P. tritici-repentis* was noted in all regions. The prevalence of *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 *P. tritici-repentis* were used to analyze the population structure on the basis of virulence and toxicity. *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 *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 *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 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 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 Tc*Lr24* | 0 | 0 | 0 |
| RL6016 Tc*Lr2a* | 95 ± 0.03 | 60 ± 0.09 | 100 |
| RL6019 Tc*Lr2b* | 95 ± 0.03 | 70 ± 0.08 | 100 |
| RL6047 Tc*Lr2c* | 95 ± 0.03 | 70 ± 0.08 | 100 |
| RL6010 Tc*Lr9* | 10 ± 0.05 | 43.3 ± 0.09 | 14.7 ± 0.06 |
| RL6053 Tc*Lr11* | 80 ± 0.06 | 70 ± 0.08 | 100 |
| RL6052 Tc*Lr15* | 95 ± 0.03 | 60 ± 0.09 | 100 |
| RL6005 Tc*Lr16* | 72.5 ± 0.07 | 43.3 ± 0.09 | 67.6 ± 0.08 |
| RL6040 Tc*Lr19* | 0 | 3.3 ± 0.03 | 0 |
| RL6092 Tc*Lr20* | 92.5 ± 0.04 | 53.3 ± 0.09 | 100 |
| RL6078 Tc*Lr26* | 80 ± 0.06 | 50 ± 0.09 | 55.9 ± 0.08 |
| RL6003 Tc*Lr1*, RL6002 Tc*Lr3a*, RL6042 Tc*Lr3bg*, RL6007 Tc*Lr3ka*, RL6013 Tc*Lr14a*, RL6006 Tc*Lr14b*, RL6008 Tc*Lr17*, RL6009 Tc*Lr18*, RL6049 Tc*Lr30* | 100 | 100 | 100 |

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

| Phenotype | Avirulence to Tc*Lr* lines | *P. triticina* populations | | |
|---|---|---|---|---|
| | | Omsk | Chelyabinsk | North Kazakhstan |
| TRTTR | 19, 24 | 0 | 0 | 2.9 |
| TRPTR | 11, 19, 24 | 2.5 | 0 | 0 |
| TQTTR | 19, 24, 26 | 2.5 | 0 | 5.9 |
| TQTTQ | 19, 20, 24, 26 | 0 | 3.3 | 0 |
| TMTTR | 16, 19, 24 | 0 | 0 | 2.9 |
| TLTTR | 16, 19, 24, 26 | 2.5 | 10 | 2.9 |
| TLPTQ | 11, 16, 19, 20, 24, 26 | 2.5 | 0 | 0 |
| THTTR | 9, 19, 24 | 45 | 10 | 35.3 |
| THTTQ | 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 |
| TGTTR | 9, 19, 24, 26 | 5 | 0 | 23.5 |
| TCTTR | 9, 16, 19, 24 | 12.5 | 13.3 | 14.7 |
| TCTTQ | 9, 16, 19, 20, 24 | 5 | 3.3 | 0 |
| TBTTR | 9, 16, 19, 24, 26 | 5 | 3.3 | 11.8 |
| PLPTG | 2a, 11, 15, 16, 19, 20, 24, 26 | 0 | 3.3 | 0 |
| PHPTG | 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 |
| MGTKH | 2a, 2b, 2c, 9, 15, 19, 24, 26 | 2.5 | 0 | 0 |
| MBTKK | 2a, 2b, 2c, 9, 15, 16, 24, 26 | 0 | 3.3 | 0 |

**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 |
| 7 | PtrToxA, PtrToxB | 0 | 5 | 0 |
| 8 | PtrToxA, PtrToxB, PtrToxC | 0 | 11 | 0 |

basis of necrosis). Two of them were found in all regions, two were shared by the Chelyabinsk and the North Kazakhstani population, and one, by Omsk and Chelyabinsk. Forty-seven percent of Chelyabinsk isolates and 33 % of North Kazakhstani 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 Kazakhstani 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.

Population analysis of *P. triticina* and *P. tritici-repentis*, important wheat pathogens differing in parasitism type (obligate vs. hemibiotrophic), revealed a similarity of their structure in the West Asian regions of the Russian Federation and North Kazakhstan. Data obtained should be considered for territorial zoning of genetically protected varieties in these regions. The study of new varieties should be relevant to their resistance not only to local populations of the most prevalent pathogen in a region, but also to those races that can appear in the population due to a possible air drift from neighboring regions. Such a system of studying varieties is used in cooperation programs of the Kazakhstan-Siberian Wheat Improvement Network (KASIB). The best breeding material is tested annually at various environmental settings (the Volga Region, the Urals, West Siberia and Kazakhstan). Monitoring pathogen virulence allows coordinating strategies for placing new varieties in the study regions, prolonging their "useful life" and improving the ecological situation around wheat crops.

## Acknowledgments

All population studies of the leaf rust pathogen were carried out within the state assignment of the Federal Agency for Scientific Organizations of Russia (project 0665-2018-0002). Studies of tan spot and spot blotch pathogens were supported by the Russian Foundation for Basic Research, 18-04-00128. Collection of infectious material and phytopathological assessments in the Omsk region were carried out with financial support from the Russian Science Foundation (project 16-16-10005).

## Conflict of interest

The authors state that there is no conflict of interest.

## References

Belan I.A., Rosseyeva L.P., Meshkova L.V., Blokhina N.P., Pershina L.A., Trubacheyeva N.V. Development of soft wheat varieties resistant to fungal diseases for West Siberia and the Urals. Vestnik Altayskogo Gosudarstvennogo Agrarnogo Universiteta = Bulletin of the Altai State Agricultural University. 2017;1(147):5-14. (in Russian)

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

Gultyaeva E.I., Solodukhina O.V. Rzhavchinnye bolezni zernovyh kultur organizmam [Rust diseases of cereals]. Izuchenie geneticheskih resursov zernovyh kultur po ustojchivosti k vrednym organizmam [Study of the genetic resources of crops with regard to pest resistance]. Moscow, 2008;5-31. (in Russian)

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;9:21-25. (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. Plant Pathol. 2015;64(2):328-336. 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. Immunogeneticheskaya zashchita sel'skokhozyaistvennykh kultur ot boleznei: teoriya i praktika. Materialy Mezhdunarodnoy 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-tekhnicheskiy byulleten VASKhNIL, Sibirskoe otdelenie [Scientific and Technical Bulletin of the All-Russia Academy of Agricultural Sciences, Siberian Branch]. Novosibirsk: SibNIIZKhim 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. Genetika vzaimootnosheniy vozbuditelya buroy rzhavchiny i pshenitsy. Pod red. akad. RASKhN M.M. Levitina [Levitin M.M. (Ed.) Genetics of the relationship between the brown rust agent and wheat]. St.-Peterburg: VIZR Publ., 2006. (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., Rosseva 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 pyatnistost' pshenitsy [Yellow spot of wheat]. Metodicheskie ukazaniya po izucheniyu populyatsiy vozbuditelya zheltoy pyatnistosti *Pyrenophora tritici-repentis* i ustoychivosti sortov [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., Terniuk 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., Rosseva 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., Paholkova 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 micology 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 Selektsii = Vavilov Journal of Genetics and Breeding. 2017;21(5):523-529. DOI 10.18699/VJ17.269. (in Russian)