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SHORT COMMUNICATION

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Virulence of *Puccinia triticina* in the North Caucasus region of Russia

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Abstract

Aim of study: To analyze the structure of *P. triticina* populations by a virulence survey in the North Caucasus region of Russia from 2011 to 2015.

Area of study: The North Caucasus region is a leading grain production region in Russia where wheat leaf rust causes losses in yield.

Material and methods: Uredinial samples of leaf rust were collected in all agro-climatic zones of the North Caucasus on the production sites of winter wheat and on the plots of official state trials. Single uredinial isolates (a total of 564) were tested for virulence with 41 'Thatcher' near isogenic lines with Lr resistance genes.

Main results: Clones virulent to Lr9, Lr42, Lr47 and Lr50 were not found. Isolates virulent to the Lr19, Lr24, Lr29, Lr41, Lr43 + 24, Lr45, Lr52 genes were characterized by low frequencies. The 564 fungal isolates studied were represented by 564 virulence phenotypes, the majority of them with a virulence complexity from 9 to 19. A high level of intrapopulation fungus diversity in virulence was noticed during the whole period of research (Shannon diversity index from 2.994 to 3.314). The differences in the frequencies of virulences in the years of research were small (Rogers distance from 0.001 to 0.160).

Research highlights: Due to the fact that the North Caucasus region is a zone of epiphytotic danger and high variability of the *P. triticina* population, the analysis of the genetics of the fungus population is important for the strategy of varietal distribution in the region and development of rust-resistant varieties.

Additional key words: wheat leaf rust; *Triticum aestivum;* fungal populations; *Lr*-genes; fungal isolates

Authors' contributions: GVV: critical revision of the manuscript for important intellectual content, supervising the work, coordinating the research project. OFV: performed the experiments, analyzed the data. OAK: statistical analysis, analysis and interpretation of data, drafting of the manuscript. All authors read and approved the final manuscript.

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Introduction

Leaf rust, caused by the fungus *Puccinia triticina* Erikss. is the most common disease of wheat (Kolmer *et al*., 2010). The pathogen adapts well to different environmental conditions and is widespread in all wheat cultivation regions in Russia (Gultiaeva *et al*., 2009; Volkova & Vaganova, 2016) and in the world (Kolmer, 2001; Manninger, 2006; Arslan *et al*., 2007). Despite the sufficient knowledge of the fungus, population studies are relevant due to the constant emergence and rapid accumulation of new pathotypes that overcome the resistance of varieties with effective genes (Kovalenko *et al*., 2012).

The largest research centers for the study of the virulence of *P. triticina* are in North America (Kolmer & Liu, 2000; Kolmer *et al*., 2010), Europe (Manninger, 1994), Turkey (Arslan *et al*., 2007), China (Liu

& Chen, 2012) and Africa (Terefe *et al*., 2009). The population of brown rust pathogen in grain-producing regions has also been monitored in Russia (Lebedev *et al*., 2003; Sibikeev & Druzhin, 2015). Population studies of *P. triticina* have been conducted for a long time by the staff of the All-Russian Research Institute of Plant Protection (Gultiaeva *et al*., 2007, 2009) and the All-Russian Research Institute of Phytopathology (Zhemchuzhina *et al*., 2016).

The All-Russian Research Institute of Biological Plant Protection (Krasnodar) has accumulated long-term results of the intrapopulation structure of the North Caucasian *P. triticina* population on virulence. Thus, according to Alekseeva & Smirnova (1986), in the population of the fungus in 1980-1984 absolute efficiency was shown by the Lr resistance genes 9, 19, 23, 24, 25. Anpilogova & Volkova (2000), could not find in populations of *P. triticina* in 1998-2000 isolates virulent to the Lr9 and Lr19 genes; in 1999, a single isolate virulent to Lr24 was found. In pathogen populations of 2006-2008, Volkova *et al*. (2011) detected isolates virulent to the Lr9 gene.

In general, in different years of research, a high genetic diversity of the North Caucasian populations of the pathogen was noted (Anpilogova *et al*., 1993; Volkova *et al*., 2013, 2014), what is associated with a number of different factors ―spore migration, genotypes of cultivated varieties and wide geography of the collection of infectious material of the fungus, weather conditions, the use of chemical protection products―, under the influence of which mutations in the genotype of the fungus occur. According to the theory of Vavilov (Berly-and-Kozhevnikov, 1974), the proximity of the North Caucasus to the Transcaucasian genetic center of wheat origin also plays an important role.

The purpose of this work was to analyze the structure of *P. triticina* populations by a virulence survey in the North Caucasus region of Russia.

Material and methods

Sample collection

Uredinial samples of leaf rust were collected during the period 2011-2015 annually in June in all agro-climatic zones of the North Caucasus on the production sites of winter wheat and on the plots of official state trials. The wheat development phase at the time of the research was Z82-90 according to Zadoks scale (Zadoks *et al*., 1974). In the laboratory, the initial material was multiplied on seedlings of a highly susceptible 'Michigan Amber' variety. Plants infected with the suspension of urediniospore fungus were placed overnight in a moist chamber at a temperature of 18-20 °C. Then they were moved to the greenhouse, where during the daylight hours the temperature varied between 18 and 25 °C. Eight to ten days after inoculation, single-pustule isolates were obtained, leaving one plant with one pustule in a flowerpot, which were further propagated on the 'Michigan Amber' variety in order to accumulate enough infectious material for experiments.

Virulence analysis

The analysis of the virulence of *P. triticina* isolates was carried out using 41 'Thatcher' near isogenic lines, carrying leaf rust (Lr) resistance genes. Plants of each line were grown in 50 mL pots on hydroponic culture using Knop's nutrient solution (Smirnova & Alekseeva, 1988). At the seedling stage (one leaf), these plants were inoculated with a spore suspension of a single-pustule isolate. The conditions of infection are described above. After a humid chamber, depending on the conditions, the plants were kept in a greenhouse box or climatic chamber at 18–20 °C, illumination intensity up to 15,000 lux and air humidity 60-70%. In 10-14 days, according to the Mains & Jackson (1926) scale, infection types (IT) were recorded as high (IT from 3 to 4) or low (IT from 0 to 2).

Data analysis

The diversity level of *P. triticina* phenotypes by virulence was assessed using the Shannon index (H_w) according to the formula (Kolmer *et al*., 2003):

$$H_w = -\sum p_i \ln(p_i),$$

where *pi* is the frequency of the *i*-th phenotype in a given population.

Differences between populations by virulence genes and virulence phenotypes were assessed using the Rogers index (Rogers, 1972):

$$H_r = ½ \sum(p_{i1} - p_{i2}),$$

where p_{i1} is the frequency of the *i*-th phenotype in the first population; p_{i2} is the frequency of the *i*-th phenotype in the second population.

Results and discussion

To analyze the virulence of the North Caucasian population of *P. triticina* during the period of 2011-

2015, 564 single-pustule isolates were tested (Table 1). Among 41 near-isogenic wheat lines of the 'Thatcher' variety with the Lr resistance genes used for the virulence survey, 37 showed polymorphism in response to pathogen infection. In the five years of research, isolates virulent to Lr9, Lr42, Lr47, Lr50 were not observed in the population of the fungus.

The appearance of single isolates, virulent to lines with resistance genes Lr19, Lr24, Lr29, Lr41, Lr43 + Lr45, or Lr52, was recorded. An increase in the frequency of isolates virulent to Lr1, Lr10, Lr25, Lr44 was noted. The isolates that were virulent to Lr2c, Lr3, Lr3ka, Lr11, Lr14b, Lr16, Lr26, Lr30, Lr33, Lr40, LrExch, LrB, LrKanred genes in the population of *P. triticina* remained stably high (40–90%) throughout the study period. On the lines with genes Lr2a, Lr3bg, Lr10, Lr14a, Lr17, Lr18, Lr20, Lr23, Lr28, Lr38, the variability in the response of the pathogen isolates

Table 1. Frequency of isolates with virulence genes in the North Caucasian population of *P. triticina* during 2011-2015.

Virulence on Lr genes	Frequency (%) of virulent phenotypes					
	2011	2012	2013	2014	2015	Average
1	23.7	15.7	86.0	66.0	58.0	49.9
2a	48.4	18.5	50.0	51.0	30.2	39.6
2c	68.9	43.5	86.0	74.0	56.1	65.7
3	71.6	32.4	99.0	79.0	67.7	70.0
3bg	30.0	21.3	67.0	88.0	27.7	46.8
3ka	87.4	35.2	95.0	84.0	59.4	72.2
9	0.0	0.0	0.0	0.0	0.0	0.0
10	61.6	23.1	29.0	41.0	44.3	39.8
11	90.8	27.8	66.0	68.0	59.1	62.3
14a	43.7	22.2	60.0	64.0	32.1	44.4
14b	77.4	35.2	83.0	67.0	45.2	61.6
15	7.4	0.9	21.0	34.0	4.1	13.5
16	73.2	41.7	52.0	54.0	38.1	51.8
17	48.9	38.9	64.0	70.0	11.4	46.6
18	6.8	14.8	66.0	70.0	1.5	31.8
19	0.0	0.0	0.0	3.0	4.9	1.6
20	6.3	6.5	52.0	36.0	0.0	20.2
21	2.1	16.7	22.0	13.0	27.0	16.2
23	63.2	35.2	43.0	50.0	26.0	43.5
24	0.5	0.0	1.0	5.0	0.0	1.3
25	5.8	7.4	9.0	13.0	22.8	11.6
26	58.9	42.9	60.0	44.0	36.7	48.5
28	2.1	35.2	55.0	56.0	21.4	33.9
29	0.0	0.9	2.0	6.0	1.5	2.4
30	80.5	54.6	71.0	76.0	44.9	65.4
32	1.6	1.8	5.0	2.0	13.5	4.8
33	77.9	45.4	65.0	61.0	64.2	62.7
36	33.7	28.7	17.0	36.0	34.9	30.1
38	1.1	9.3	7.0	10.0	2.0	9.6
40	71.1	49.1	79.0	80.0	48.1	65.5
41**	0.0	0.0	0.0	0.0	4.7	1.0
42	0.0	0.0	0.0	0.0	0.0	0.0
43+24	0.0	0.9	0.0	0.0	0.0	0.2
44	31.2	0.0	12.0	22.0	33.7	19.8
45	1.6	0.0	0.0	8.0	5.7	3.1
47	0.0	0.0	0.0	0.0	0.0	0.0
50	-*	-	-	-	0.0	0.0
W(52)	0.5	1.8	1.0	2.0	8.4	2.7
Exch	87.9	49.1	68.0	70.0	53.2	65.6
B	73.7	66.7	84.0	59.0	53.1	67.3
Kanred	96.0	87.9	93.0	85.0	62.4	84.9
Number of isolates, pcs	190	108	100	100	66	113
Average virulence, %	37.8	24.6	45.6	43.3	25.4	35.3

* The study was not conducted due to the absence of seeds. **Lr41 = Lr39

remained high. There was a tendency in reducing the frequency of isolates that are virulent to Lr11, Lr16, Lr23, LrExch.

For analyzing the phenotypic composition of *P. triticina*, the virulence formulas of 190, 108, 100, 100, and 66 single-pustule isolates (in 2011, 2012, 2013, 2014, and 2015 respectively) were described on 41 differentials. As a result, the same number of phenotypes with different virulence complexity was revealed, confirming once again the high heterogeneity of the North Caucasian pathogen population. Table 2 shows the percentage of *P. triticina* phenotypes with different virulence complexity.

The obtained experimental data indicate that during the growing seasons favorable for the development of the pathogen (2011, 2013, 2014), the vast majority of phenotypes in the population of the fungus were able to infect an average number (9–19) of differentials.

High rates of the Shannon index indicate a significant diversity of *P. triticina* phenotypes by virulence in the years of the research (the Shannon index ranged from 2.994 to 3.314) with the maximum value for the 2014 fungus population. According to the Rogers index, which shows differences within the populations of *P. triticina* in terms of the frequency of isolates pathogenic on differential hosts, no significant differences were found between the years of the research (Table 3).

Despite minor differences in the frequency of virulences in the years of the research, the North Caucasian population of *P. triticina* is distinguished by a high diversity in virulent phenotypes (each isolate represents a unique phenotype). The diversity of the phenotypic composition is a distinctive feature of the North Caucasian population, noted by its researchers in different years (*e.g.* Alekseeva & Smirnova, 1986; Kudinova, 2012).

We found that resistance genes Lr9, Lr42, Lr47 and Lr50 were absolute effective against North Caucasian *P. triticina* populations (Table 1). In other regions of Russia (Ural, western Siberia and central Russia), *P. triticina* isolates virulent to Lr9 were found; this is due to the large area of varieties containing Lr9 in these regions (Gultiaeva *et al*., 2015).

According to previous studies, Lr-genes 19, Lr24, Lr29, Lr41, Lr43+24, Lr45 and Lr52 lost their effectiveness, due to the appearance of virulent isolates of *P. triticina*. Isolates virulent to Lr24 were previously absent in the leaf rust pathogen population in the North Caucasus (Anpilogova *et al*., 2000), but started to appear in 2006 (Volkova & Vaganova, 2016). This may be due to an increase in the acreage of varieties protected by this resistance gene (Tyryshkin *et al*., 2014). A similar situation was also observed in the case of Lr19 resistance gene, obtained by crossing an isogenic line with *Aegilops elongatum*. Despite the fact that it is still effective in the North Caucasus region, the percentage of fungal clones that are virulent to the line with this resistance gene increases every year.

There has also been a tendency to an increase in the number of virulent isolates affecting lines carrying genes Lr1, Lr10, Lr25, or Lr44. According to Tyryshkin *et al*. (2014), the frequency of isolates virulent to Lr1 tends to increase in fungus populations from other

Table 2. Percentage of *P. triticina* phenotypes with different virulence complexity in the North Caucasus (2011-2015)

Virulence complexity in phenotypes of *P. triticina*, n	2011	2012	2013	2014	2015	Average
0-8	12.3	48.7	3.0	6.0	46.7	21.2
9-19	86.7	43.1	68.0	68.2	39.4	62.8
20-24	1.0	8.3	29.0	25.9	13.9	16.0

Table 3. Differences in virulence complexity in the North Caucasian population of *P. triticina* in 2011-2015

North Caucasian populations of *P. triticina*	2011	2012	2013	2014
	Rogers index			
2012	0.007			
2013	0.013	0.160		
2014	0.010	0.070	0.001	
2015	0.006	0.001	0.001	0.003

regions of Russia as well. As for the Lr25 gene, back in 2004-2006 it was highly effective for the North Caucasian population of brown rust pathogen (Volkova *et al*., 2014).

The high variability of types of response to pathogen isolates on lines with genes Lr2a, Lr3bg, Lr10, Lr14a, Lr17, Lr18, Lr20, Lr23, Lr28, Lr38 described above, may be due to the fact that infectious material was collected not only from the varieties in crop production, but also from breeding plots. Several hundred of different varieties and lines of wheat with a diverse spectrum of Lr genes were sown on them. Earlier (in 2004–2006), the Lr38 gene was one of the most highly efficient juvenile genes for the North Caucasian pathogen population (Volkova *et al*., 2014). The breakdown of this resistance gene, was also observed in Western Siberian population of *P. triticina* in (Meshkova *et al*., 2011).

Data on the number of phenotypes of the North Caucasian population of *P. triticina* allows us to judge its high diversity (Alekseeva & Smirnova, 1986; Kudinova, 2012). This is confirmed by the high values of the Shannon index (ranged from 2.994 to 3.314). The high diversity of the phenotypic composition is a distinctive feature of the North Caucasian population. The maximum value of the Shannon diversity index obtained for the population in 2014 can be explained by weather conditions favorable for the development of the pathogen.

Regarding the virulence complexity (Table 2), phenotypes less complex in virulence dominated in unfavorable years for the pathogen (2012 and 2015), which indicates that isolates that do not have "extra" genes survive under extreme conditions (Dyakov, 1998). In more favorable conditions, phenotypes dominate with an average number of virulence genes. This confirms the conclusion drawn earlier by Berlyand-Kozhevnikov *et al.* (1978), that isolates with an average number of virulence factors have a greater viability under natural selection conditions.

The absence of significant differences between populations in 2011-2015 for the Rogers index, despite the differences in vegetation seasons, may indicate that the population was clonal and there were no significant changes in the host population over the studied period. The largest difference was recorded between 2012 and 2013. The main reason of this phenomenon might be the unfavorable weather conditions for the development of the pathogen in 2012, which resulted in reduction in frequencies of individual virulence factors.

In summary, no isolates virulent to Lr9, Lr42, Lr47, Lr50 were observed in the North Caucasian population of the wheat brown rust pathogen. Isolates virulent to the Lr19, Lr24, Lr29, Lr41, Lr43 + 24, Lr45, LrW(52) genes were present in low frequencies. The 564 studied isolates of the fungus were represented by 564 virulence phenotypes. This is the highest level of *P. triticina* intrapopulation diversity in terms of phenotypic composition, while the differences in the frequencies of virulence in the studied years were insignificant.

Due to the fact that the North Caucasus region is a zone of epiphytotic danger and high variability of the *P. triticina* population, it is necessary to conduct an annual analysis of the genetics of the fungus population. This is important for the strategy of varietal distribution in the region and development of rust-resistant varieties.

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