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SHORT COMMUNICATION

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Virulence of barley leaf rust in the South of Russia in 2017-2019

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Abstract

Aim of study: To analyze the structure of *Puccinia hordei* populations by virulence in southern Russia during 2017-2019.

Area of study: South of Russia, the leading Russian region for barley production where barley leaf rust is an important foliar disease.

Material and methods: Uredinial samples of *P. hordei* were collected at the production sites of winter barley in the south of Russia. Single uredinial isolates (total 95) were tested for virulence with 17 differentials with *Rph* resistance genes.

Main results: No isolates were found virulent to the host line with the *Rph13* gene. There was a decrease in the number of fungal isolates virulent to the host lines with *Rph5* and *Rph7* genes. In 2017 and 2019, isolates containing a large number of virulence alleles (from 11 to 15) prevailed. In 2018, isolates with low (1-5) and medium (6-10) frequency of virulent alleles prevailed, as well as avirulent isolates. The values of the Nei index via diversity showed high similarity of the pathogen populations in 2017-2018 (N = 0.05) and minor differences in 2017-2019 and 2018-2019 (N = 0.13 and 0.16, respectively). The greatest frequency of virulence alleles in accordance with the Nei (Hs) index was noted for the 2018 population (Hs = 0.36). For the 2017 and 2019 populations, this indicator was on average Hs = 0.29 and 0.20, respectively.

Research highlights: Analysis of genetics of the *P. hordei* population is important for the strategy of varietal distribution in the region and development of rust-resistant cultivars.

Additional key words: winter barley; *Puccinia hordei*; *Rph*-genes; population; isolates.

Abbreviations used: Hs (Nei's index of population diversity); N (Nei's index of differences between populations).

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Introduction

Barley (*Hordeum vulgare* L.) is the fourth main agriculture grain in the world after wheat, maize and rice (Park *et al.*, 2015). Barley leaf rust caused by the fungus *Puccinia hordei* Otth is one of the most harmful leaf diseases in barley and causes significant crop losses in many regions of the world where it is grown (Cotterill *et al.*, 1995; Woldeab *et al.*, 2006; Murray & Brennan, 2009). The fungus spreads mainly asexually, but in the presence of an alternative host (*Ornithogalum* spp.) it can undergo sexual recombination, which leads to the emergence of a variety of new fungus pathotypes (Jost *et al.*, 2020). High damage from the disease has been reported at different times in many countries: New Zealand (Arnst *et al.*, 1979), Australia (Cotterill *et al.*, 1992), North America (Mathre, 1982; Griffey *et al.*, 1994), Czech Republic (Dreiseitl & Steffenson, 2000), Great Britain (Jenkins *et al.*, 1972; Melville *et al.*, 1976), Ethiopia (Semeane *et al.*, 1996) or South Africa (van Niekerk *et al.*, 2001). On average, yield losses from the pathogen reach 30% (Park *et al.*, 2015). With a strong development of the disease (on a susceptible variety), the yield losses may reach 62% (Cotterill *et al.*, 1992; Fazlikhan *et al.*, 2019). In Russia, barley leaf rust is most harmful in the Volga region, the North Caucasus, the Central Black Earth Region, Western and Eastern Siberia, and the Far East, where it develops almost every year. Epiphytotics occur with a frequency of 1-2 times every 10 years (Novozhilov & Zakharenko, 2000; Danilova & Volkova, 2015).

Use of fungicides, although effective, is not always economically justified and may harm the environment. Therefore, the leading role in the development of methods for the protection of barley belongs to breeding of highly productive varieties resistant to the disease. Their development and use require a comprehensive understanding of the gene pool of resistance of the host and the virulence of the pathogen.

In 2011, research of virulence of *P. horde*i population in the south of Russia in the All-Russian Research Institute of Biological Plant Protection was renewed (Danilova *et al.*, 2014). New pathotypes of fungus was identified (Danilova & Volkova, 2015). Further analysis of virulence of pathogen populations, carried out in 2014-2017, showed its diversity. For the first time it was found that the majority of *P. hordei* isolates were virulent to the host lines with *Rph* genes: *Rph1, Rph2, Rph3, Rph4, Rph5, Rph5+2, Rph6+2, Rph7, Rph8, Rph12, Rph19, Rph19+2, Rph9* (*RphC*). Isolates virulent to *Rph13* gene were not found (Volkova *et al.*, 2019). The relevance of population studies is due to the constant appearance and rapid accumulation of new pathotypes that overcome the resistance of varieties with effective genes. Since the Federal Scientific Center for Biological Plant Protection is the only scientific center where population studies of *P. hordei* are carried out, annual monitoring is an important and urgent issue for the successful breeding of barley. The aim of this work was to study the virulence of South-Russian populations of *P. hordei* in the period 2017-2019.

Material and methods

Samples of barley leaves infected with *P. hordei* were collected during the annual routine surveys of barley crops in 2017-2019. The growth phase of barley plants at the time of the study was Z82-90 on the Zadoks scale (Zadoks *et al.*, 1974). In the greenhouse, the inoculum was multiplied on the seedlings of the susceptible local variety 'Mikhailo' (bred by the P.P. Lukyanenko GZTs, Krasnodar). Plants were inoculated by suspension of urediniospores and placed in a humid chamber at 18-20 °C, 16-24 h. After inoculation, seedlings were placed in a separate box and kept under optimal conditions for pathogen: temperature +18-22 °C, intensity lighting 12-15 thousand lux for 16 h. The incubation period was 5-7 days. Eight or ten days after inoculation, isolates with single pustules were obtained. Plants with single pustule were isolated separately. Mikhailo's cultivar seedlings were infected of material from single pustule for accumulation of infectious material (Volkova *et al.*, 2018).

Fifteen varieties and lines of differentiators from international and Australian sets containing known genes for resistance to *P. hordei* were used: Sudan (*Rph1*), Peruvian (*Rph2*), Estate (*Rph3*), Gold (*Rph4*), Magnif 104 (*Rph5*), Quinn (*Rph5+2*), Bolivia (*Rph6+2*), Cebada Capa (*Rph7*), Egypt 4 (*Rph8*), Triumph (*Rph12*), PI 531849 (*Rph13*), Prior (*Rph19*), Reka 1 (*Rph19+2*), Ricardo (*Rph21+2*), and Cantala (*Rph9*). Seeds of differential lines were germinated in petri dishes. Sprouted seeds were sown with tweezers in 25 mL plastic vases with wet sand, 5 pieces each. On the 5-7th day, differentiator plants were inoculated with a water suspension of *P. hordei* spores of each isolate. On 10-14th day, infection types (in points) were assessed according to the Levin & Cherevik scale (Fig. 1). Plants with infection types 0, ;, 1 and 2 points were designated as resistant, 3 and above points - as susceptible. If infection type was slightly higher or lower than the indicated points, additional designations "+" or "-" were assigned (Levine & Cherewick, 1952; Babayants, 1988).

The diversity of *P. hordei* population of virulence alleles using the Nei index was described (Hs). To identify differences between populations, we used the Nei index for diversity (N) (Nei, 1978; Kosman & Leonard, 2007). Statistical analyses were performed using the Statistica 10.0 software (StatSoft, Inc., USA).

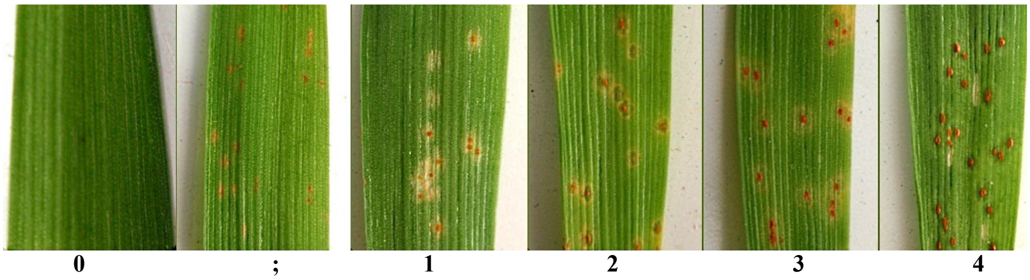

Figure 1. Range of seedling infection types for the *Puccinia hordei – Hordeum vulgare* interaction. The infection types were: 0 = no visible symptoms, ; = hypersensitive flecks, 1 = minute uredinia surrounded by mainly necrotic tissue, 2 = small to medium sized uredinia surrounded by chlorotic and/or necrotic tissue, 3 = medium to large uredinia with or without surrounding chlorosis, 4 = large uredinia without chlorosis. Infection types of 3+ or higher were considered to be compatible (*i.e.*, virulent pathogen/susceptible host).

Results and discussion

Ninety-five *P. hordei* isolates were obtained and differentiated during 2017-2019 and 75 phenotypes with different virulence were found. Phenotypes of *P. hordei* virulent to the *Rph13* genome were not identified over the years of research in the south of Russia (Table 1). Resistance gene *Rph13* has retained its effectiveness since 2012 (Danilova *et al.*, 2014; Volkova *et al.*, 2018). The source of this gene is wild barley *H. vulgare* ssp. Despite the effectiveness of *Rph13* in the south of Russia, *P. hordei* phenotypes virulent to *Rph13* have been noted in some regions of the world (Park *et al.*, 2015).

Most isolates (from 50% to 100%) virulence to isogenic lines with *Rph* genes (*Rph1, Rph2, Rph3, Rph4, Rph5, Rph5+2, Rph6+2, Rph7, Rph8, Rph19, Rph19+2, Rph21+2, Rph9*) were detected during all years of study. A decrease in *P. hordei* isolates virulent to lines with *Rph5* and *Rph7* genes was noted. A significant increase in isolates virulent to host lines with genes *Rph5+2, Rph6+2, Rph12, Rph19, Rph21+2* was observed. The response of *P. hordei* isolates with virulence to lines with genes *Rph1, Rph2, Rph3, Rph4, Rph8, Rph19+2* was variable during the years of research. Most of the known resistance genes were ineffective against *P. hordei* in southern Russia during the entire study period.

In the *P. hordei* populations in 2017 and 2019, phenotypes containing a large number (11-15) and a medium number (6-10) of virulence alleles prevailed (Table 2). Their share in the fungus population was 68.0% and 36.1% of those studied, respectively. In the 2018 population, phenotypes with a low (1-5) and medium (6-10) number of virulence alleles (42.3% and 38.5%, respectively) prevailed. The largest number (15.4%) of avirulent phenotypes were detected in the 2018 population. In the 2017 population, their number was minimal (2.8%), and no avirulent phenotypes were found in the 2019 population.

A decrease in number of virulence alleles and their virulence frequencies for most host lines could be the result of unfavorable weather conditions for development of pathogen in the growing season of 2018. On average, severity of *P. hordei* in the region did not exceed 1%, and maximum spread of disease was 5% (Ministry of Agriculture of the Russian Federation, 2020). The results obtained are consistent with the existing theory in which pathotypes with minimum amount of virulence alleles survive under adverse conditions. This is due to the fact that the abundance of "extra" alleles affects the viability of rust fungus. In conditions favorable for the pathogen, on the contrary, phenotypes with medium and high number of virulence alleles prevail (Dyakov, 1998).

According to the Nei index (Hs), which characterizes *P. hordei* population diversity, 2018 population had the

Table 1. Frequency of isolates virulent for barley (*Hordeum vulgare* L.) lines and varieties with *Rph* genes in the population of *Puccinia hordei* Otth in the south of Russia in 2017-2019

Lines and cultivars	Virulence on *Rph*-genes	Yearly frequency (%) of virulent phenotypes		
		2017	2018	2019
Sudan	*Rph1*	83.3	50.0	93.9
Peruvian	*Rph2*	72.2	38.5	90.9
Estate	*Rph3*	80.6	34.6	69.7
Gold	*Rph4*	83.3	30.8	100
Magnif 104	*Rph5*	75.0	46.2	51.5
Quinn	*Rph5+2*	36.1	38.5	90.9
Bolivia	*Rph6+2*	47.2	26.9	78.8
Cebada Capa	*Rph7*	83.3	34.6	–[a]
Egypt 4	*Rph8*	88.9	42.3	100
Cantala	*Rph9*	75.0	15.4	75.8
Triumph	*Rph12*	8.3	11.5	100
PI 531849	*Rph13*	0.0	0.0	0.0
Prior	*Rph19*	8.3	0.0	51.5
Reka 1	*Rph19+2*	86.1	34.6	90.9
Ricardo	*Rph21+2*	25.0	23.1	78.8
	No. of isolates	36	26	33

[a] Differentiation was not performed due to the absence of seeds of this line

Table 2. Frequency of isolates with different numbers of virulence alleles in the *P. hordei* population in southern Russia in 2017-2019

Number of virulence alleles	Frequency of isolates with different number of virulence alleles, %		
	2017	**2018**	**2019**
0	2.8	15.4	0.0
1-5	11.1	42.3	0.0
6-10	36.1	38.5	24.0
11-15	50.0	3.8	68.0
No. of isolates	36	26	33

highest level of diversity (Hs = 0.36), followed by 2017 population (Hs = 0.29), and 2019 population (Hs = 0.20).

The values of Nei index (N), characterizing differences between populations, indicate a high similarity of structures of fungus populations in 2017 and 2018 (N = 0.05) and its insignificant differences between populations of 2017 and 2019 (N = 0.16), and 2018 and 2019 (N = 0.13). The identified differentiation may be due to with a change in sown cultivars of barley as well as a difference in climatic conditions in the years under study.

A comparison of virulence frequency of *P. hordei* isolates with data obtained by other researchers indicates difference in effectiveness of resistance genes. For example, in Australia, no pathotypes virulent to lines with *Rph7* and *Rph14* resistance genes were detected. Genes derived from *H. vulgare* ssp. spontaneum (*Rph11, Rph13* and *Rph15*) and H. bulbosum (*Rph17, Rph18, Rph22* and *Rph26*) are also highly effective in Australia (Singh *et al.*, 2020). Barley leaf rust resistance gene *Rph7* remains effective in Europe after being used for more than 20 years, but virulent pathotypes have been found elsewhere (Dinh *et al.*, 2020). In Ethiopia, the *Rph3* and *Rph7* genes are still effective (Woldeab *et al.*, 2006). However, *Rph7* virulence has been identified in Israel (Golan *et al.*, 1978), Morocco (Parlevliet *et al.*, 1981) and North America (Steffenson *et al.*, 1993). The frequency of *P. hordei* isolates in the South Russian populations that are virulent to *Rph3* and *Rph7* genes ranged from 34.6% to 83.3% (Table 1). The *Rph13* gene, effective against the South Russian population of pathogen, was found ineffective in Europe and Australia (Czembor & Czember, 2007; Park *et al.*, 2015).

In summary, the differences in virulence between populations of *P. hordei* were insignificant (N = 0.05) during 2017-2018. This indicates the stability of the virulence of *P. hordei* population. Minor changes between the populations of 2018 and 2019 (N = 0.13) were noted. More significant differences were observed between the 2017-2019 populations (N = 0.16). For three years, no isolates virulent for lines with the *Rph13* gene have been detected. Isolates with a large and medium number of virulent alleles are prevalent in *P. hordei* population. Diversity of the South Russian populations of *P. hordei* in terms of frequencies of virulence alleles (Hs) remained in range from 0.20 to 0.36 throughout the entire study period. Due to the fact that the south of Russia is a zone of *P. hordei* epiphytotic danger, it is necessary to conduct an annual analysis of the genetics of the fungus population. Monitoring of fungal populations on crops is imperative to contain plant disease outbreaks. This is important for the strategy of developing rust resistant cultivars.

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