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THE VIRULENCE OF THE BARLEY LEAF RUST PATHOGEN IN THE NORTH CAUCASUS IN 2014-2017

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Abstract

Barley leaf rust caused by *Puccinia hordei* Otth. is a harmful disease of barley. If the crop is severely damaged, the yield loss may be of 20 to 80 %. Population studies of the pathogen abroad are actively conducted in countries for which protection against the disease is of particular importance (Australia, New Zealand, the United States, Europe, the countries of Northern Africa). This paper describes the North Caucasian *P. hordei* population virulence in 2014-2017 to 17 differentiator varieties and barley lines of international and Australian kits containing currently known pathogen resistance genes, shows. Winter barley leaves (*Hordeum vulgare* L.) of different varieties affected by *P. hordei* which were collected during route surveys in 2014-2017 in the territory of the North Caucasus served as a biomaterial. The selection and reproduction of mono-pustule isolates was carried out according to the common procedure. Barley plants were grown hydroponically with the use of Knop's nutrient solution. To assess the virulence of the fungus, 17 varieties-differentiators and lines from the international and Australian sets containing the currently known genes of resistance to the pathogen were used. A total of 208 mono-pustule isolates of the fungus were analyzed most of which were virulent to the testers with *Rph* genes, the *Rph1, Rph2, Rph3, Rph3 + Rph7, Rph4, Rph5, Rph7, Rph8, Rph 9 + Rph2.* During the four years of study, no isolates virulent to the line with *Rph13* gene were detected. In 2016-2017 as compared to 2014-2015, there was a decrease in the number of isolates virulent to the lines with genes *Rph9, Rph19,* and an increase in clones virulent to testers with genes *Rph19 + Rph2, Rph21 + Rph2.* The frequency of *P. hordei* isolates that are virulent to varieties and lines of barley with genes *Rph5 + Rph2, Rph6 + Rph2* remained moderate throughout all the years of the research. In 2016, due to unfavorable conditions for the pathogen and the collection of spore material from a limited set of winter barley varieties affected by the pathogen, the frequency of isolates virulent to varieties and lines of barley significantly reduced. In 2014, 2015 and 2017, isolates with a large number of virulence alleles, from 11 to 15, prevailed in the population and reached 52.2 %, 39.5 % and 50.0 %, respectively. The portion of isolates, avirulent to all used plants with genes *Rph*, was 1.1 %, 2.1 % and 2.8 %, respectively. In 2016, the fungal isolates with moderate and low virulence alleles prevailed. The Nei index indicates a high similarity of the structure of North Caucasian pathogen population by virulence in 2014, 2015 and 2017 (N = 0.02-0.05) and its minor changes in 2016 (N = 0.14-0.19). The obtained statistical results indicate the stability of the North Caucasian *P. hordei* population by virulence. The level of its diversity in the frequency of virulence alleles remained medium (H_s = 0.26-0.40) throughout the entire study period.

Keywords: winter barley, leaf rust, *Puccinia hordei*, virulence

Barley leaf rust (the causative agent *Puccinia hordei* Otth.) is a widespread and harmful disease of barley, which leads to grain shrinkage and yield decrease. If the plants are damaged severely the yield loss may be more than 20% [1]. The economic importance of *P. hordei* in the world agriculture depends

on the region of the crop cultivation and varies by years [2]. In recent years it has increased [3). The effect of this pathogen is especially severe in the regions of the East and Midwest of the USA, North Africa, New Zealand, Europe, Australia, and some Asian countries [4]. In a number of countries, especially in the regions where crops ripen late, significant yield losses have been observed in the susceptible varieties [5, 6]. In Russia, the barley leaf rust is most harmful in the Volga, North Caucasus and Central Black Earth regions, Western and Eastern Siberia and in the Far East where it emerges almost annually. The epiphytoties in the North Caucasus, Central Black Earth and Volga regions occur 1-2 times per 10 years [7, 8].

Breeding and cultivation of highly productive varieties resistant to diseases including the barley leaf rust possess the leading role among barley protection methods. Their creation and application require a comprehensive study of the gene pool of the host plant resistance and of the pathogen virulence. To date, 21 genes conferring juvenile resistance (*Rph1-Rph19*, *Rph21* and *Rph22*) and 3 genes for age-related resistance (*Rph20*, *Rph23* and *Rph24*) which have been found in *Hordeum vulgare*, *H. vulgare subsp. spontaneum* and *H. bulbosum* are known [9, 10].

Abroad, the population studies of the pathogen are actively conducted in the countries for which the protection against the disease is especially urgent. Cotterill et al. [11] have proved that most of the known genes are ineffective against the isolates identified in Australia from 1966 to 1995, and only *Rph3* and *Rph7* genes remained effective. Subsequently, the *P. hordei* isolates which are virulent for the lines with these genes emerged in different countries [12, 13]. The virulence of the fungal isolates to the line having the *Rph15* gene has been reported by Sun [14]. Nevertheless, the gene of a wild barley species *H. vulgare* subsp. *spontaneum*, like the *Rph16* gene [15] is still considered effective and is actively used in breeding programs [16). The juvenile genes *Rph11* and *Rph14* are ineffective, and the isolates which are virulent for their carriers are found in many parts of the world [17]. Currently, *Rph20*, *Rph23*, and *Rph24* are recognized effective genes of adult plant resistance to *P. hordei* [18].

In the territory of the former USSR, until 1964, the race and phenotypic composition of *P. hordei* has not been studied. The first investigations of its race composition with a set of differentiator varieties selected by Levin and Cherevik were published in 1968 by Rogozhina et al. [19]. A total of 18 races, 16 of which were listed in the international registry and two ones (X and Y) were described for the first time were discovered. Subsequently, the study of the race diversity of different geographical populations of the pathogen was continued [20, 21], and another two new races (1L, 2L) and two races listed in the international register [22, 23] were found.

In this study, we for the first time established that most of the mono-pustule isolates of the barley leaf rust causative agent from the North Caucasus population are virulent to the testers with *Rph1*, *Rph2*, *Rph3*, *Rph3* + *Rph7*, *Rph4*, *Rph5, Rph7*, *Rph8*, and *Rph9* + *Rph2* genes. No virulent isolates for the line with *Rph13* gene have been found.

The objective of this work was to study the structure of the North Caucasian population of barley leaf rust causative agent (*Puccinia hordei*) for virulence.

Techniques. The leaves of different varieties of winter barley (*Hordeum vulgare* L.) affected by *P. hordei* were collected in the North Caucasus in the course of routine surveys in 2014-2017. A total of 208 mono-pustule isolates of the fungus have been isolated and analyzed.

The isolation, reproduction, and differentiation of mono-pustule isolates were performed according to the described technique [24]. The barley plants

were grown hydroponically using the Knop's nutrient solution [22].

To assess virulence, 17 differentiator varieties and lines from the international and Australian kits [18] which contain the known to date genes of resistance to the pathogen were used. Seeds of the differentiators were germinated in Petri dishes. The germinated seeds were sown, 5 seeds per 25 ml plastic flowerpots with wet sand. On Days 5 to 7, the differentiator plants were inoculated with aqueous suspension of *P. hordei* spores of each mono-pustule isolate. On Days 10 to 14, the types of plant response (in points) were evaluated according to the Levin and Cherevik scales. The plants with response types of 0, 0; 1 and 2 points were classified as resistant, with 3 and 4 points — as susceptible. In case if the type of reaction was slightly higher or lower than the above points, the additional designations "+" or "–" were introduced [25].

The diversity of the *P. hordei* population was described for virulence genes using Nei's H_s index [26]:

$$H_s(P) = \Sigma[1 - q_i^2 - (1 - q_i)^2]/k,\ 1 \le i \le k,$$

where q_i is the frequency of the i allele in the population, k is the number of alleles.

The inter-population differences (Nei's N index) were evaluated as the frequency of virulence alleles using the genetic distance (D) according to Nei [26, 27]:

$$D = -\ln IN,$$

$$IN = \Sigma\Sigma\, x_{ij}\, y_{ij}/\sqrt{\Sigma\Sigma\, x_{ij}^2\, \Sigma\Sigma\, y_{ij}^2},$$

where x_{ij} and y_{ij} are the frequencies of the i allele of the j year in the compared populations x and y.

The statistical processing was performed using Statistica 10.0 software (StatSoft, Inc., USA).

Results. The barley differentiator varieties and lines used in the work are shown in Table 1.

1. The differentiator varieties and lines of winter barley (*Hordeum vulgare* L.) with known genes of resistance to barley leaf rust used to investigate virulence of *Puccinia hordei* Otth. North Caucasian population isolates

Set of differentiators	Variety/line	Origin	Resistance gene(s)
International	Sudan	C.I. 6489	*Rph1*
International	Peruvian	*Hordeum vulgare*	*Rph2*
International	Estate	*Hordeum vulgare*	*Rph3*
International	Midas	*Hordeum vulgare*	*Rph3 + Rph7*
International	Gold	*Hordeum vulgare*	*Rph4*
International	Magnif 104	*Hordeum vulgare*	*Rph5*
Additional Australian	Quinn	*Mains' Quinn,* C.I. 1024	*Rph5 + Rph2*
International	Bolivia	*Hordeum vulgare*	*Rph6 + Rph2*
International	Cebada Capa	*Hordeum vulgare*	*Rph7*
International	Egypt 4	*Hordeum vulgare*	*Rph8*
International	Triumph	*H. vulgare* ssp. *spontaneum*	*Rph12*
International	Abyssinian	*Hordeum vulgare*	*Rph9*
Additional Australian	PI 531849	*H. vulgare* ssp. *spontaneum*	*Rph13*
Additional Australian	Prior	*Hordeum vulgare*	*Rph19*
Additional Australian	Reka 1	C.I. 5051	*Rph19 + Rph2*
Additional Australian	Ricardo	*Hordeum vulgare*	*Rph21 + Rph2*
Additional Australian	Cantala	*Hordeum vulgare*	*RphC*

In 2014, 2015 and 2017, the conditions for the pathogen development were favorable, while 2016 was unfavorable due to weather conditions.

Over the four years of study, the *Rph13* gene had been demonstrating the absolute effectiveness (Table 2). The source of this gene is the wild species of barley *H. vulgare* ssp. *spontaneum*. However, despite its effectiveness in the North Caucasus, the isolates which are virulent for carriers of this gene have been found in several regions of the world [18]. In all years of study, most testers with *Rph1*, *Rph2*, *Rph3*, *Rph3 + Rph7*, *Rph4, Rph5*, *Rph7*, *Rph8*, and *Rph9 + Rph2*

resistance genes were affected by isolates of the North Caucasian *P. hordei* population with the high frequency. In 2016-2017, compared to 2014-2015, there was a decrease in the number of isolates virulent to the lines with the *Rph9* and *Rph19* genes, and an increase in the number of clones virulent for the testers with *Rph19* + *Rph2* and *Rph21* + *Rph2* genes. The number of isolates virulent for the barley varieties and lines with *Rph5* + *Rph2* and *Rph6* + *Rph2* genes remained medium in all years of research. In 2016, due to the conditions unfavorable for the pathogen and due to the collection of spore material from the limited set of varieties affected by the pathogen, the frequency of isolates virulent to barley varieties and lines was significantly lower.

2. Frequency of *Puccinia hordei* Otth. isolates of North Caucasian population virulent to winter barley (*Hordeum vulgare* L.) lines and varieties with *Rph* genes

Testers with *Rph* genes	Frequency, %			
	2014	2015	2016	2017
Rph1	76.1	60.5	51.3	83.3
Rph2	75.0	73.7	33.3	72.2
Rph3	81.5	63.2	15.4	80.6
Rph3 + *Rph7*	79.3	60.5	20.5	86.1
Rph4	68.5	63.2	28.2	83.3
Rph5	73.9	55.3	30.8	75.0
Rph5 + *Rph2*	30.4	50.0	35.9	36.1
Rph6 + *Rph2*	34.8	36.8	12.8	47.2
Rph7	80.4	55.3	15.4	83.3
Rph8	78.3	57.9	25.6	88.9
Rph9	56.5	47.4	2.6	8.3
Rph12	50.0	52.6	5.1	38.9
Rph13	0.0	0.0	0.0	0.0
Rph19	26.1	10.5	2.6	8.3
Rph19 + *Rph2*	42.4	50.0	46.2	86.1
Rph21+ *Rph2*	0.0	0.0	0.0	25.0
Rph C	41.3	34.2	2.6	75.0
Number of isolates	92	38	39	39
Intrapopulation diversity index H_s	0.36	0.40	0.26	0.30

Therefore, most of the currently known resistance genes were ineffective against *P. hordei* in all years of researches. The *Rph13* gene, no virulent isolates to which have been detected since 2012, retained its high efficiency [8, 21].

The statistical analysis of the pathogen population diversity for the frequencies of virulence alleles showed that in 2016 the population was the least diverse (Hs = 0.26) that was determined by the conditions unfavorable for the pathogen (Table 2). In general, the diversity of the North Caucasian population of *P. hordei* remained medium (Hs = 0.26-0.40 (Hs = 0.26-0.40) as per the frequencies of virulence alleles throughout the entire survey period.

3. Frequency of isolates with different number of virulence alleles attacking lines and varieties of winter barley (*Hordeum vulgare* L.)

Number of virulence alleles	Isolates with different number of virulence alleles, %			
	2014	2015	2016	2017
0	1.1	2.1	30.8	2.8
1-5	22.8	28.9	35.9	11.1
6-10	23.9	26.3	33.3	36.1
11-15	52.2	39.5	0.0	50.0
Number of isolates	92	38	39	39

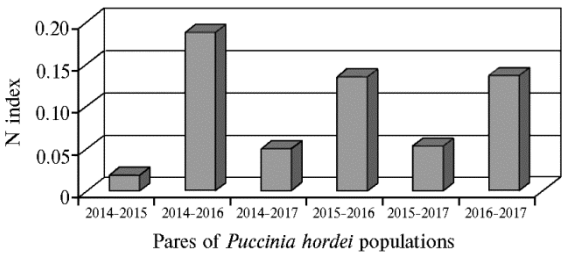
The dynamics of frequency of *P. hordei* isolates with different number of virulence alleles showed that in 2014, 2015 and 2017, the isolates with a large number of virulence alleles (11 to 15) predominated in the pathogen population (Table 3), with 1.1 to 2.8% of isolates avirulent to all the lines with *Rph* genes

used in our researches. In 2016, 33.3-35.9% isolates had medium and low number of virulence alleles, and no isolates with high number of virulence alleles (11-15 genes) were found. Moreover, almost a third of the isolates were avirulent to all studied testers with *Rph* genes.

The decrease in the number of virulence alleles and their frequencies toward most testers could be the consequence of the weather conditions unfavorable for the pathogen during the growing season of 2016. In average, the emergence of *P. hordei* throughout the region had not exceed 5%; the spores were collected from the limited set of winter barley varieties affected by the pathogen that could also affect the reduction of the pathogen population diversity. The obtained results are consistent with the existing theory that the isolates with minimum number of virulence alleles survive under adverse conditions. This is due to the fact that the abundance of "excessive" alleles affects the viability of the rust causative agents. On the contrary, if the conditions are favorable for the pathogen, the isolates with medium and high number of virulence alleles predominate (28].

The values of the Nei's N index which characterizes the differences between populations points out on the high similarity of the structure of the pathogen North Caucasian population by virulence in 2014, 2015 and 2017 (N = 0.02-0.05) and its insignificant changes in 2016 (0.14-0.19) (Fig.).

The revealed differentiation may be due to the reasons described above.



The values of the Nei's N index characterizing the differences between the North Caucasian populations of *Puccinia hordei* Otth. in the frequency of virulence alleles.

The comparison of the frequency of *P. hordei* isolates of the North Caucasian population which are virulent to the carriers of *Rph* genes with the data obtained by other researchers testifies about the discordance in the effectiveness of most resistance genes. Thus, in Australia, *Rph7, Rph11*, *Rph14*, *Rph15*, and *Rph18* [29] genes and later mapped *Rph21* gene [23) remain effective. The *Rph3* gene has retained its effectiveness in Australia since 1992 [9], but in 2009 the isolates virulent to lines with this gene were discovered [30]. *Rph7* and *Rph16* genes in Europe [2, 3] and *Rph3* and *Rph7* genes in Ethiopia [31] are deemed effective. The frequency of *P. hordei* isolates of the North Caucasian population virulent to lines with *Rph3* and *Rph7* genes ranged from 15.4% to 83.3% (see Table 2). The *Rph13* gene effective against the pathogen of North Caucasian population is ineffective in Europe and Australia [3, 9, 29].

So, the differences in virulence of the North Caucasian population of *Puccinia hordei* Otth. in 2014, 2015 and 2017 were insignificant (N = 0.02-0.05) that testifies about stability of the pathogen in virulence. In 2016 which was unfavorable for *P. hordei*, insignificant intrapopulation changes occurred (N = 0.14-0.19). During four years, no isolates virulent to the line with the *Rph13* gene were found. Most isolates turned out to be saturated with virulent alleles. The diversity of the North Caucasian population of *P. hordei* in the virulence allele frequency (H_s) ranged from 0.26 to 0.40 throughout the entire survey period.

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