PLANT PROTECTION

Diversity of Virulence Phenotypes of *Puccinia Triticina* in Different Agroclimatic Zones of the North Caucasus

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Abstract—The phenotypic composition of the North Caucasian population of wheat leaf rust pathogen (*Puccinia triticina* Erikks.) in various agroclimatic zones of the region in 2016–2018 is analyzed. Under study were 233 single pustule isolates, of which 212 virulence phenotypes were identified. In all the years of research, a high level of population diversity was established (the Shannon index (Sh) was 0.92–0.99). In 2016, the PHRS phenotype, which was identified in the southern foothill, western Azov, and eastern steppe agroclimatic zones, dominated. In the population of 2016, phenotypes with a high and medium number of virulence genes prevailed. In 2017, the phenotypes of DCRL, LBLL (Western Azov zone), and PCQB (Northern zone) were best represented. Avirulent phenotype BBBB was common for populations of 2016–2018. In 2016, a phenotype with virulence to *Lr9* (TLGS) was first detected. In 2017 and 2018, phenotypes virulent to the Lr24 gene (PKTT, SFGQ, CFPQ, TKTS, MKTT, LKSR) were detected in the populations of the fungus. A high level of population differences in phenotypic composition between the years of research was established (Rogers index (R) was 0.96–0.99).

INTRODUCTION

The North Caucasus is the main region for the cultivation of winter wheat, the sown area of which is 55% of all winter crops in the Russian Federation. This crop is susceptible to a number of dangerous phytopathogens, including brown rust (*Puccinia triticina* Erikks.), one of the malicious and common phytopathogens [1]. Due to the favorable climate for the development of the pathogen and large areas of cultivation of the host plant, brown rust remains a significant wheat disease in the North Caucasus [2]. Therefore, the development of effective methods of protection against this harmful pathogen is especially relevant for this region. Traditionally, over large areas of cultivation, the disease is controlled through the use of effective fungicides. But the growing greening of agricultural production requires safe methods of protecting wheat from brown rust, such as cultivating varieties protected by disease resistance genes [3]. It is known that a scientifically based strategy of varietal displacement by aerial landscape niches and proper varietal change is necessary for the correct and effective use of the potential of various varieties [4]. Knowledge of the dynamics of the phenotypic composition of the pathogen's population in various agroclimatic zones, differing in heat and moisture supply, is also important. Therefore, annual studies of virulence and phenotypic composition of the brown rust population are carried out in various regions of the world [5–7]. In Russia, similar work is carried out in the main grain-producing regions. Fungal virulence is studied in the Lower Volga region, in the Urals, in the West Siberian, Northwest, and Central regions [8–12]. In the North Caucasus, the virulence of the brown rust population in various agroclimatic zones is described for 2006–2015 [2, 13, 14].

The aims of this work were to analyze the phenotypic composition of the North Caucasian population of *P. triticina* in 2016–2018 in five agroclimatic zones, to identify common and dominant phenotypes, and to assess the phenotypic diversity and the level of differences in the fungal population by years of research.

MATERIALS AND METHODS

To collect the infectious material, routine examinations were performed annually (in the phase of milk-wax ripeness of grain) for breeding and production crops of winter wheat in five agroclimatic zones of the region, differing in heat and moisture supply (Fig. 1). The leaves affected by the disease were collected on wheat crops in each agroclimatic zone and used to isolate monopodule isolates of the fungus. The preparation of monopustular isolates and their reproduction was carried out according to existing techniques [14].
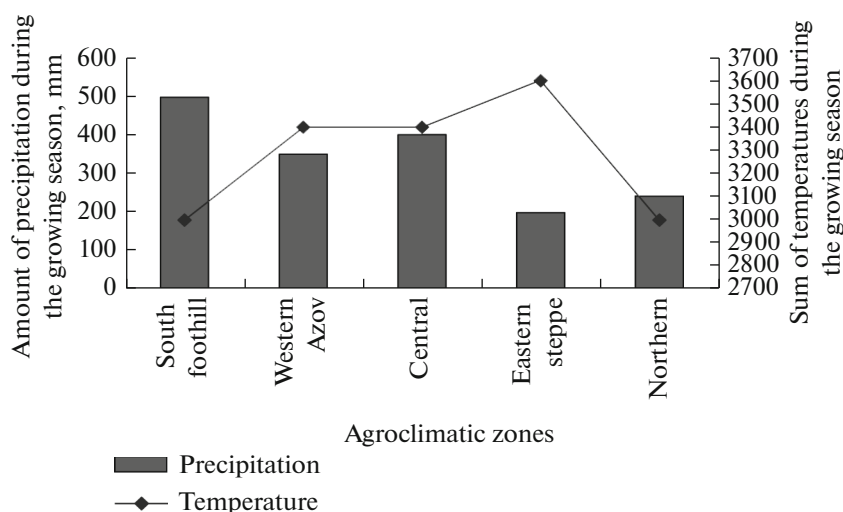
Fig. 1. Description of agroclimatic zones of the North Caucasus by heat and moisture supply [20].

To analyze the virulence of *P. triticina* isolates, 16 closely isogenic Thatcher lines with *Lr* genes—*1*, *2a*, *2c*, *3*, *9*, *16*, *24*, *26*, *3ka*, *11*, *17*, *30*, *B*, *10*, *14a*, and *18*—were used. the lines were grown in flowerpots (50 mL) hydroponically using Knop nutrient solution [15]. At the age of one leaf, they were inoculated with a spore suspension of a monopuscular isolate. After the wet chamber, the plants were kept at 18−20°C, illumination intensities of up to 15000 lux and air humidity of 60−70%. On the 10th−14th day, infectious types (IT) were recorded according to the Mains and Jackson [16] scales as high (IT from 3 to 4) or low (IT from 0 to 2).

The phenotypes were labeled according to the North American nomenclature [17]. The diversity of the population by phenotypic composition was evaluated by the Shannon index [18]. Differences between populations by virulence phenotypes were evaluated using the Rogers index [19]:

$$H_r = 1/2 \sum (pi\,1 - pi\,2),$$

where *pi* 1 is the frequency of the *i*th phenotype in the first population; *pi* 2 is the frequency of the *i*th phenotype in the second population.

RESULTS AND DISCUSSION

In 2016, the prevailing weather conditions favored the development of phytopathogens on winter cereal crops. Brown rust of wheat was noted in all five agroclimatic zones of the region. In the 2017 growing season, the conditions of early spring were favorable for the growth and development of winter crops. However, frequent rains in June and a low temperature regime delayed the maturation of wheat and created conditions for the development of diseases. Brown rust was detected in four agroclimatic zones (except for the eastern steppe) with maximum development in the central zone (6.8%). The following year, 2018, was unfavorable for the development of phytopathogens. Against the background of the abnormally hot weather of April−June, a significant deficit of precipitation was observed (20−30% of the norm), which contributed to the development of soil and atmospheric droughts, which had reached the criteria for a dangerous phenomenon by the end of June. Relative humidity decreased to 45−55%, which was 15−20% below the norm. In this regard, brown rust was noted in three agroclimatic zones: the southern foothill, western Azov, and central zone (with a development of up to 1−2%).

In 2016−2018, 233 monopodular isolates of *P. triticina* were studied, of which 212 virulence phenotypes were identified. As in previous years [13, 14], the brown rust pathogen population in the region continued to be highly diverse (Table 1). At the same time, the maximum diversity in phenotypic composition was revealed in the population of 2017. Despite the

Table 1. Characteristics of the diversity of the *P. triticina* population in the North Caucasus by phenotypic composition

Characteristics of a population	2016	2017	2018
Number of isolates, pcs.	90	82	61
Number of phenotypes, pcs.	76	79	57
Number of unique phenotypes, pcs.	70	76	54
Diversity index, Sh	0.92	0.99	0.97

Table 2. Phenotypic composition of the North Caucasian population of *P. triticina* in various agroclimatic zones, 2016–2018

Year	Agroclimatic zone	Number of isolates	Phenotypes
2016	South foothill	17	**MHTT***, TLGS, THJJ, THTT, **PHRS, PGRS**, PBQG, PGCT, PRGS, **PBRQ**, RHHS, RCTT, RGRS, NBCP, FGNQ
	Western Azov	11	**PHRS**, **PGRS**, PCQJ, SBMN, CBMQ, FHMG, DBCL, **PBMJ**, LBRL
	Central	28	MCPB, MCKJ, TGPD, **THRS**, THTS, THRR, THTQ, TCTS, PDLG, PBSS, PQBB, PCTT, PCTS, PHTS, PHSJ, **PHRS**, **BBBB**, BCCB, LHLH, CGPD, NHKL, NCCJ, NHTL, FGTD, FGTG, QHPH, QGRQ, GBRS
	Eastern steppe	18	MHRS, MCRQ, **THRS**, THRQ, TBQS, **PHRS**, PHMQ, **PHRQ**, PCQQ, PHRN, **PGRS**, LBBL, FHRG, FCMJ, FHHS, FCMJ,
	Northern	16	MBGG, MBGL, PHRT, PGTN, PGKL, **PGRL**, **BBBB**, BBBL, LBCL, NHSJ, FHTQ, KBML, HHTG, HHRN, DBNB, TCCS
2017	South foothill	24	MBLS, MBBG, TBBD, TBBL, TBBB, BBBG, BBCB, RBHB, RBHB, CBCL, CBLG, NCRQ, NGBD, NBGM, NBCB, FGBG, FBHB, FBBB, KBBD, QBLL, QBBB, HBBG, HBCN, GBBL
	Western Azov	26	BCBM, **LBLL**, LDBD, CGRP, NBHN, NHGR, FGPL, FBQB, FBTB, FBRG, HCMQ, DBRG, **DCRL**, DGBL, DGBG, DCBG, DGNL
	Central	17	MBSG, TGRJ, TBHS, PBCB, PGTQ, **PHRQ**, **PGRL**, RGRN, RHTG , LCLN , NHRH, FCTS, FGTK, FCTG, FBMG, GGRS, DCTQ
	Northern	14	THQT, TCQQ, **PCQB**, **BBBB**, SHBL, LCLB, CCGB, CBLG, NGBB, FHGB, FGLB, FBLB, JBLB
2018	South foothill	18	MJTM, MHJP, MGLS, TKTS, PBJS, PKPQ, BHLQ, **BBBB**, BBDT, BBBL, BHDT, LHSC, LHRQ, LGTR, LGRT, CHMT, NHPT
	Western Azov	20	**MHTT**, MHMH, MDTT, MBTL, MGTP, MBTR, MJPT, MKTT, MHPT, MCLK, PHTP, **PKTT**, RHHT, LHTT, LKSR, CGTS,
	Central	21	MHTR, MMRG, MHTN, MHST, TGPT, PCTD, PHQL, PHTD, PHTQ, PHTG, PHTK, PCTQ, PHTT, PCPR, PCTL, KGHS, BCDR, SHTT, SFGQ, CFPQ, NCSN

* Repeating phenotypes within or between zones.

unfavorable conditions of the 2018 growing season, the diversity of *P. triticina* remained at a high level.

Virulence phenotypes in five agroclimatic zones are given in Table 2, and virulence formulas (ineffective genes) of dominant and repeating phenotypes are given in Table 3. In 2016, out of 90 monoplastic isolates, 76 phenotypes were identified. The PHRS, THRS, PGRS, PBMJ, PBRQ, BBBB, and MHTT phenotypes were repeating, while the remaining were

unique. In 2016, the PHRS phenotype dominated, which was identified in the southern foothill, western Azov, and eastern steppe agroclimatic zones. The THRS phenotype was found in the central and eastern steppe zones, PGRS in the western Azov and eastern steppe zones, and PBRQ in the southern foothill zone. These phenotypes contain an average number of virulence alleles. In addition to virulent ones, avirulent (BBBB) and weakly virulent (PBMJ) phenotypes were

Table 3. Virulence and frequency (%) of repeating phenotypes of the North Caucasian population of *P. Triticina*, 2016–2018

Virulence phenotype	Virulence (ineffective genes)	Pcs./frequency, %		
		2016	2017	2018
PHRS	1, 2c, 3, 16, 26, 3ka, 11, B, 10, 14a	9/9.9	0	0
THRS	1, 2a, 2c, 3, 16, 26, 3ka, 11, B, 10, 14a	2/2.2	0	0
PGRS	1, 2c, 3, 16, 3ka, 11, B, 10, 14a	3/3.3	0	0
PHRQ	1, 2c, 3, 16, 26, 3ka, 11, B, 10	1/1.1	1/1.2	0
PGRL	1, 2c, 3, 16, 3ka, 11, B	1/1,1	1/1.2	0
PBMJ	1, 2c, 3, 11, 10, 14a	2/2.2	0	0
PBRQ	1, 2c, 3, 16, 26, 3ka, 11, B, 10	2/2.2	0	0
BBBB	0	2/2.2	1/1.2	2/3.3
DCRL	2c, 26, 3ka, 11, B	0	2/2.4	0
LBLL	1, 3ka, B	0	2/2.4	0
PCQB	1, 2c, 3, 26, 3ka, 11	0	2/2.4	0
MHTT	1, 3, 16, 26, 3ka, 11, 17, 30, B, 10, 14a, 18	2/2.2	0	1/1.6
PKTT	1, 2c, 3, 16, 24, 26, 3ka, 11, 17, 30, B, 10, 14a, 18	0	0	3/4.9

also repeated in 2016. Generally, phenotypes with a high and average number of virulence alleles prevailed in the 2016 population.

In 2017, the phenotypes DCRL, LBLL (western Azov zone) and PCQB (northern zone) dominated. The avirulent phenotype of BBBB was found, as in 2016, in the northern zone with insufficient heat and moisture supply (Fig. 1). In 2017 and 2018, phenotypes of group B (BBBB, BBBL, BBDT, BHDT, BHLQ, BBBG, BBCB, FBBB, KBBD, QBLL, QBBB) containing the minimum number of virulence alleles prevailed in the southern piedmont zone. This may be due to both biotic (varietal composition) and abiotic (reduced heat supply of the zone) factors.

Phenotypes with a high content of virulence alleles (MHTT, MDTT, SHTT, PHTT, PHTQ, etc.) prevailed in the western Azov and central zones. Table 3 shows that the phenotypes of *P. triticina*, which are avirulent (BBBB) or contain a large number of virulence alleles (MHTT, PKTT), are repeated in 2018. In 2017 and 2018, phenotypes virulent to the line with the *Lr24* gene (for example, PKTT, SFGQ, CFPQ, TKTS, MKTT, LKSR) were noted in fungal populations. Single isolates virulent to this line were also noted in the North Caucasian population in previous years [13, 14]. In 2016, a phenotype with virulence to the line containing *Lr9* (TLGS) was first discovered. In previous years, this phenotype was not observed [13], although this gene has already lost its effectiveness in other regions [9, 11]. In general, differences in the phenotypic composition of the fungus between years, estimated using the Rogers index (R), are significant for each pair of compared populations (for 2016 and 2017, R = 0.96, 2017 and 2018–0.97, 2016 and 2018–0.99).

CONCLUSIONS

Thus, the phenotypic composition of the *P. triticina* population was monitored in 2016−2018 in various agroclimatic zones of the North Caucasus. Of the 233 monopodular isolates of the fungus analyzed, 212 phenotypes were identified. Recurring phenotypes were identified in each agroclimatic zone. The phenotype with virulence to the line containing *Lr9* (TLGS) was detected for the first time. A high level of phenotypic diversity of the North Caucasian fungal population (Sh = 0.92−0.99) and a significant level of differences in the *P. triticina* population by phenotypic composition between years of research (R = 0.96−0.99) were established.

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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