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DIVERSITY AND VIRULENCE OF LEAF AND STEM RUST PATHOGENS OF WHEAT IN KAZAKHSTAN

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Abstract. Phytosanitary security is crucial for ensuring the food and national security of Kazakhstan, especially in combating high-risk cereal diseases like leaf rust and stem rust. This study assessed the virulence and race composition of *Puccinia triticina* and *Puccinia graminis* populations collected from different regions of Kazakhstan. A total of 30 isolates of leaf and 31 isolates of stem rust were analyzed against Lr- and Sr- near-isogenic lines to determine the effectiveness of resistance genes. Results revealed significant variability in race composition and virulence between northern and southern populations, with some races identified as common to both regions.

Keywords: leaf rust, stem rust, wheat, race, resistance.

INTRODUCTION

Phytosanitary security is a vital part of the country's food security, helping to prevent the spread of dangerous crop diseases. Tackling these challenges involves identifying threats, understanding current conditions, and implementing measures to control high-risk diseases in cereal crops. A modern approach includes developing high-yield, disease-resistant cereal varieties using advanced breeding, plant pathology, molecular genetics, and genomics techniques [1-4].

However, wheat production faces significant threats from various fungal diseases, with leaf rust and stem rust being among the most destructive. Leaf rust, caused by the biotrophic fungus *Puccinia triticina*, is a widespread and common disease of bread wheat. In Kazakhstan, leaf rust occurs annually, especially in the northern, eastern, and western regions where adequate moisture levels favor the disease's proliferation on spring wheat crops. Between 2001 and 2016, leaf rust developed epidemically eight times, either alone or alongside septoria, significantly impacting wheat yield [6]. The majority of commercial wheat varieties grown in Kazakhstan lack resistance, resulting in severe outbreaks that can affect up to 1.5-2.0 million hectares, reducing yields by 15-20% during years of early onset and aggressive spread [7].

Similarly, stem rust, caused by *Puccinia graminis* has historically been the most damaging wheat disease worldwide. In 2015 and 2016, a major stem rust epidemic struck the northern regions of Kazakhstan and the adjacent Omsk region of Russia, affecting approximately one million hectares in Kazakhstan and two million hectares in Russia [8]. The disease resurfaced in 2017-2018, affecting northern and eastern Kazakhstan as well as the Omsk, Novosibirsk, and Altai regions of Russia, leading to marked reductions in yield and grain quality [9]. During this period, disease severity and incidence in Kazakhstan's main wheat-growing areas reached unprecedented levels of up to 90% and 70%, respectively, highlighting the urgent need for improved management strategies and resistant wheat varieties [10].

Addressing the threats posed by these diseases is critical for ensuring sustainable wheat production and food security in Kazakhstan. Developing high-yielding, disease-resistant wheat varieties through advanced breeding techniques, plant pathology, molecular genetics, and genomics is a modern approach that offers a promising solution to these challenges. Population studies of wheat pathogens are a crucial step in developing scientifically coordinated breeding strategies and deploying resistant varieties. The use of genetically uniform donors in breeding often leads to a rapid loss of resistance due to the emergence of new races and changes in the phenotypic composition of pathogen populations. Additionally, the effectiveness of the same resistance gene can vary between regions, depending on the composition of the pathogen population [7]. The relevance of studying wheat rust populations is further heightened by ongoing changes in wheat production. This study aims to assess the status of wheat rusts in Kazakhstan and evaluate potential strategies for enhancing wheat resilience against these pervasive threats.

MATERIALS AND METHODS

Sampling of pathogen populations, including biotrophic and hemibiotrophic pathogens of cereal crops, was conducted in the southern and northern regions of Kazakhstan on commercial fields and experimental plots. The collection and propagation of single-pustule isolates of P. triticina and P. graminis were carried out under greenhouse conditions according to established protocols [11]. To determine the race composition and virulence of leaf rust populations, 20 near-isogenic lines of Thatcher with Lr genes (TcLr) were used [12]. Stem rust races were identified using the North American nomenclature, which is based on five sets of near-isogenic lines [13].

The letter codes for races, virulence frequencies of populations, and fungal race indices, as well as genetic distance indices, were determined using the Virulence Analysis Tools (VAT) software package [14]. Multidimensional diagrams were generated using Principal Coordinates Analysis (PCoA) in the GeneAIEx software package [15] to assess genetic distances among pathogen populations based on virulence profiles. PCoA allowed for the visualization of relationships and clustering patterns, highlighting the degree of similarity or divergence in virulence structure between regional populations. To analyze virulence, the Virulence Analysis Tools (VAT) software package was used. It calculates the frequencies of virulent isolates, diversity indices (Shannon, Nei, and Kosman), and genetic distances between populations, which served as the basis for clustering and population structure analysis. Intra-population diversity was assessed using Nei's (Hs), Shannon's (Sh), and Kosman's (KW) indices. To evaluate inter-population differences in virulence, Nei's (N) and Rogers' (R) distance indices were applied. Additionally, the Gst index was used to estimate genetic differentiation among populations [15].

RESULTS

A total of 30 isolates of the pathogen P. triticina were propagated from herbarium samples of wheat, and 31 isolates of P. graminis were similarly obtained from herbarium samples of wheat and barley, collected in the Kostanay, Turkestan, and Zhambyl regions of Kazakhstan (Table 1).

Table 1 – Origin and number of single-pustule isolates of *Puccinia triticina* and *Puccinia graminis*

Region	District (village, organization)	Culture (variety)	Number of isolates, pcs	
			P. triticiana	*P. graminis*
Zhambyl	Baizaksky (Akzhar, commercial fields)	Spring wheat (unknown)	10	8
Turkestan	Tulkibassky (Tausagyz, production field)	Spring wheat (unknown)	10	8
Kostanay	Karabalykskу (Scientific, Karabalyk AES)	Spring wheat (unknown)	10	6
		Spring barley (Virage)	0	5
		Spring barley (Giant)	0	4
Total			30	31

Using the isolated P. triticiana and P. graminis isolates, the virulence of wheat leaf and stem rust populations was evaluated using Lr and Sr near-isogenic lines. Lr near-isogenic lines were inoculated with single-pustule leaf rust isolates in greenhouse compartments enabled the assessment of the virulence potential of these fungal populations (Table 2).

Table 2 – Virulence frequency of leaf rust populations to isogenic L r -lines

Lines with genes	Frequency of isolates (%) virulent to *Lr* near-isogenic lines (regions):		
	Kostanay	Turkestan	Zhambyl
Ts *Lr1*	100	100	100
Ts *Lr2a*	100	100	100

Ts *Lr2c*	100	100	100
Ts *Lr3 a*	100	100	100
Ts *Lr9*	0	0	0
Ts *Lr16*	100	100	100
Ts *Lr24*	0	0	0
Ts *Lr26*	50	50	40
Ts *Lr3ka*	70	70	60
Ts *Lr11*	100	100	100
Ts *Lr17*	100	100	100
Ts *Lr30*	80	70	70
Ts *Lr19*	10	0	20
Ts *Lr20*	100	100	100
Ts *Lr25*	0	20	10
Ts *Lr29*	0	0	0
Ts *Lr2b*	100	100	100
Ts *Lr3bg*	100	100	100
Ts *Lr14a*	100	100	100
Ts *Lr15*	100	100	100

Among the twenty Lr lines used for virulence analysis, five showed variability in infection type when inoculated with different leaf rust populations. All tested isolates were avirulent on the lines TcLr9, TcLr24 and TcLr29; virulent to TcLr1, TcLr2a, TcLr2c, TcLr3a, TcLr16, TcLr11, TcLr17, TcLr20, TcLr2b, TcLr3bg, TcLr14a and TcLr15. The frequency of isolates virulent to lines with the genes TcLr3ka and TcLr30 was high across all populations, reaching 60-80%. The coefficients of variation in the frequency of isolates virulent to TcLr26 ranged from 40-50%. Lines TcLr19 and TcLr25 showed strong resistance, with 80–100% of isolates failing to cause infection. Based on the experimental results, the genes TcLr9, TcLr19, TcLr24, TcLr25, and TcLr29 were identified as effective against various pathogen populations (Table 2).

The characteristics of intra-population diversity of leaf rust based on statistical indices are presented in Table 3.

Table 3 – Table 3 – Characteristics of intra-population diversity of *Puccinia triticina* based on virulence

Indexes	Populations		
	Kostanay	Turkestan	Zhambyl
Hs	0.071	0.083	0.094
Sh	0.676	0.593	0.556
KW_m	0.110	0.130	0.140

According to the three diversity indices—Shannon (Sh), Nei (Hs), and Kosman (KWm)—populations from Zhambyl (Hs = 0.094; KWm = 0.140) and Kostanay (Sh = 0.676) regions showed relatively high heterogeneity.

Stem rust populations from three regions of Kazakhstan showed high virulence against lines with the genes Sr5, Sr21, Sr7b, Sr9g, Sr36, Sr17, Sr9a, Sr9d, Sr10, SrTmp, Sr38 and SrMcN across all populations (Table 4). Virulence to Sr9e and Sr6 was lower in the southern regions (12.5-62.5%) compared to the Kostanay region (40.0-86.7%). Virulent isolates of the pathogen to Sr8a were found in all populations with a frequency of 13.3-50.0%.

Table 4 – Virulence frequency of stem rust populations to isogenic S r -lines

Lines with genes	Frequency of isolates (%) virulent to *Sr* -lineages in populations (areas):		
	Kostanay	Turkestan	Zhambyl
Sr5	100.0	100.0	100.0
Sr21	93.3	100.0	100.0
Sr9e	40.0	12.5	25.0
Sr7b	73.3	75.0	87.5

Sr11	0.0	0.0	0.0
Sr6	86.7	62.5	50.0
Sr8a	13.3	37.5	50.0
Sr9g	100.0	100.0	100.0
Sr36	66.7	75.0	87.5
Sr9b	40.0	62.5	62.5
Sr30	0.0	0.0	0.0
Sr17	100.0	100.0	100.0
Sr9a	100.0	100.0	100.0
Sr9d	100.0	100.0	100.0
Sr10	86.7	75.0	87.5
SrTmp	73.3	75.0	87.5
Sr24	0.0	0.0	0.0
Sr31	0.0	0.0	0.0
Sr38	80.0	100.0	100.0
SrMcn	100.0	100.0	100.0

Intrapopulation diversity of populations and statistical indices of differences between populations of P. graminis are presented in Table 5.

Table 5 – Characteristics of intra-population diversity of Puccinia graminis based on virulence

Indexes	Populations		
	Kostanay	Turkestan	Zhambyl
Hs	0.166	0.156	0.136
Sh	0.625	0.635	0.583
KW_m	0.233	0.225	0.213

The results indicate that intra-population diversity in phenotypic composition is higher in the Kostanay region (Hs=0.166; Sh=0.625; KWm=0.233) and comparatively lower in the southern regions (Hs=0.136-0.156; Sh=0.583-0.635; KWm=0.213-0.225).

Analysis of 30 single-pustule isolates of P. triticina in Kazakhstan's regions identified seven races of the fungus, which were unevenly distributed among the populations (Table 6). Out of the total races found, only two were present in all studied populations, although with varying frequencies. Among them, race TGKGT, which is avirulent to Lr9, Lr24, Lr26, Lr3ka, Lr19, Lr25, and Lr29, was frequently observed in all regions (30-40%), and race THSGT, avirulent to Lr9, Lr24, Lr30, Lr19, Lr25, and Lr29, appeared at a frequency of 20-30%.

Additionally, two other races were found in two of the studied regions: TGTGT, which is avirulent to Lr9, Lr24, Lr26, Lr19, Lr25, and Lr29, was detected in the Kostanay and Turkestan regions, while race THTJT, avirulent to Lr9, Lr24, Lr19, and Lr29, was found in the southern regions of the country.

Unique races, absent from other populations, were identified in two of the studied regions, often represented by single isolates, though there were exceptions. Race TGTQT, found in the Zhambyl region population at a frequency of 20%, was absent in other populations. Races THTQT and THTGT were found only in the Kostanay population (10–20%) and not detected elsewhere.

Table 6 – Frequency of leaf rust races in populations from different regions of Kazakhstan

Races	Avirulence to Thatcher lines with *Lr* genes	Frequency of occurrence of races (%) in populations:		
		Kostanay	Turkestan	Zhambyl
TGKGT	*9, 24, 26, 3ka, 19, 25, 29*	30	30	40
THSGT	*9, 24, 30, 19, 25, 29*	20	30	30
TGTGT	*9, 24, 26, 19, 25, 29*	20	20	0
THTJT	*9, 24, 19, 29*	0	20	10
TGTQT	*9, 24, 26, 25, 29*	0	0	20
THTGT	*9, 24 19, 25, 29*	20	0	0

THTQT	*9, 24, 25, 29*	10	0	0

Most of the identified races were registered in the population from the Kostanay region, with samples collected near wheat fields at the Karabalyk Agricultural Research Station.

The summary results of the study on the race composition of Kazakhstan's P. triticina populations are presented in Figure 1.

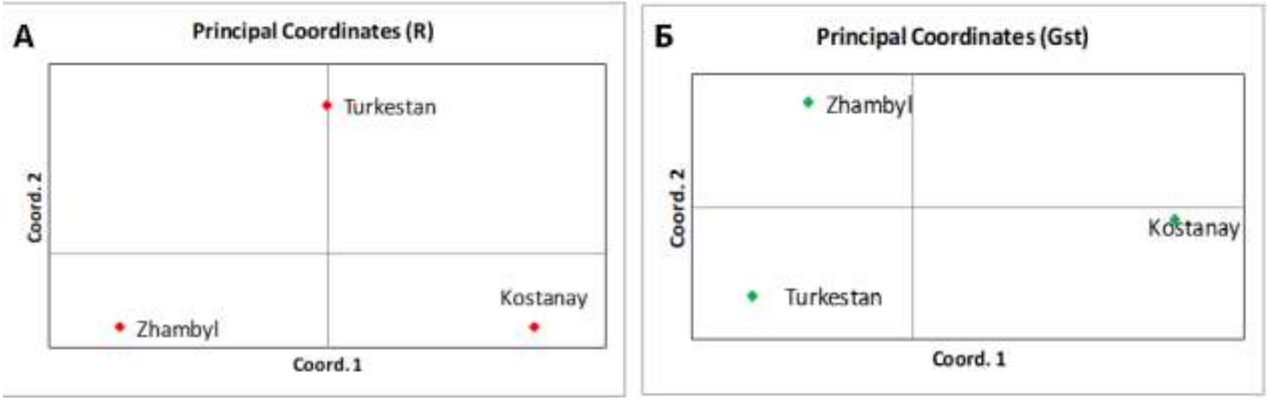

Figure 1 – Multivariate similarity diagram of regional populations of *Puccinia triticina* in Kazakhstan by by Rogers (A) and Gst (B) indices

According to Rogers' index (R), the regional populations of P. triticina were differentiated into groups on a multidimensional diagram: northern (Kostanay) and southern Kazakhstan (Turkestan and Zhambyl) (Figure 1A). Similar results were obtained using the genetic distance index Gst (Figure 1B).

Analysis of 31 single-pustule isolates of P. graminis identified six races of stem rust, each having 10 to 14 virulence genes (Table 7). The frequency of these races varied significantly among the regional populations studied. Out of all the races detected in Kazakhstan, only two races, QHHSF and THMTF, were found in all the populations studied. In the northern regions, races RHMRF, RFRTF, THMTC, and MHCTC were also observed, comprising 6.7-13.3% of the population.

In the southern regions of Kazakhstan, where winter wheat predominates, stem rust occurs less frequently than in other regions. Urediniospore samples of P. graminis were collected in the Turkestan and Zhambyl regions. Analysis of the isolates from this region identified four races, with RFRTF being the predominant race (37.5-50.0%). The races QHHSF, THMTF, and RHMRF (12.5-25.0%) were also found and were present in the Kostanay region as well.

Table 7 – Frequency of occurrence of stem rust races in populations in the regions of Kazakhstan

Races	Avirulence to *Sr* genes	Frequency of occurrence of races (%) in populations:		
		Kostanay	Turkestan	Zhambyl
QHHSF	*9e, 7b, 11, 8a, 36, 30, Tmp , 24, 31*	26.7	25.0	12.5
THMT	*11, 8a, 9b, 30, 24, 31*	26.7	12.5	25.0
RHMRF	*9e, 11, 8a, 9b, 30, 10, 24, 31*	13.3	25.0	12.5
RFRTF	*9th, 11th, 6th, 30th, 24th, 31st*	13.3	37.5	50.0
THMTC	*11, 8a, 9b, 30, 24, 31, 38*	13.3	0,0	0,0
MHCTC	*21, 9e, 11, 8a, 36, 9b, 30, 24, 31, 38*	6.7	0,0	0,0

The races of P. graminis identified in the regions of Kazakhstan were mostly avirulent to lines with genes Sr11, Sr24, Sr30, and Sr31.

The multidimensional similarity diagram between regional populations, based on Rogers' index values, which characterize the differences in population structure by race composition, is presented in Figure 2.

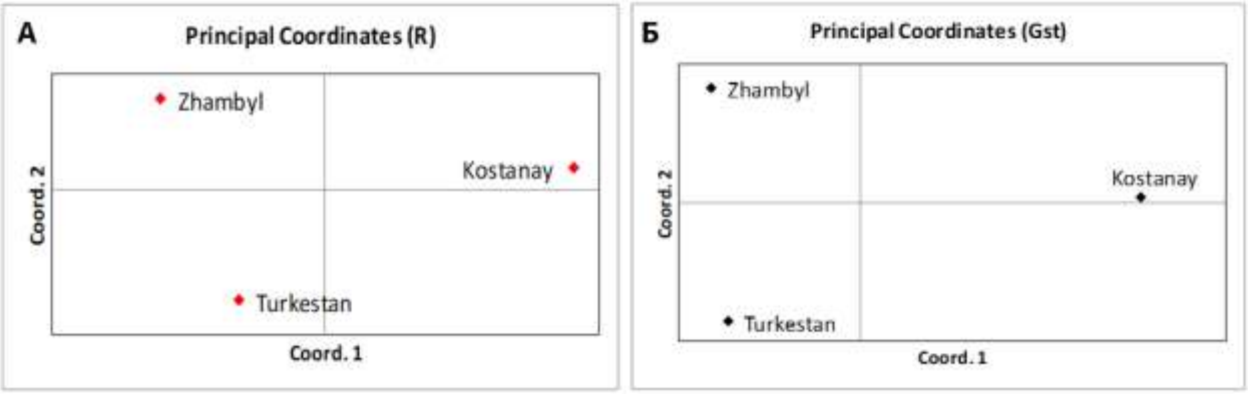

Figure 2 – Multivariate similarity diagram of regional populations of *Puccinia graminis* f. sp. tritici in Kazakhstan based on Rogers (A) and Gst (B) indices

The studied regional population samples were grouped into two clusters. One cluster included the Kostanay population, while the second cluster consisted of the Turkestan and Zhambyl populations (Figure 2A). A similar differentiation of the Kazakhstani regional populations of P. graminis was observed using the Gst index (Figure 2B).

DISCUSSION

It has been previously noted that P. triticina populations from the southern and northern regions of Kazakhstan show high similarity in virulence and race composition [16]. In our previous studies, we identified the effective genes TcLr19, TcLr24, TcLr25, and TcLr29, for which the number of isolates capable of overcoming resistance conferred by these genes was low. Notably, virulent races of the pathogen against lines with Lr9 and Lr19 genes were first detected in Kazakhstan in 2005-2006 [17]. The low genetic differentiation observed may result from spore dispersal by air currents due to the absence of geographical barriers between northern and southern Kazakhstan [18]. Additionally, in 2017, high genetic similarity was observed between the Omsk and North Kazakhstan populations, while the Chelyabinsk population showed moderate similarity [7]. The full life cycle development of the pathogen may also be facilitated by the presence of the alternate hosts, Isopyrum fumarioides or Thalictrum spp., in the regions of Kazakhstan. Consequently, the high infectious potential of the fungus, sustained in nature on the alternate host, does not rule out the mass occurrence of leaf rust under favorable conditions [18].

To assess the relationship between races identified in Kazakhstan and known global races, the findings were compared with published data. The race QHHSF, found in all regions of Kazakhstan, has also been reported in the Omsk and Novosibirsk regions of Russia. As a result, some Kazakh races were found to be identical to those in Western Siberia and the Altai Krai of Russia; for example, the RFRTF race has been registered in the Omsk region of Russia [9, 19]. However, in most cases, Kazakh races differ from those found in other regions and countries worldwide. Various variants of the Ug99 race, rapidly spreading in African and Asian countries, have not been detected in Kazakhstan. The races identified in Kazakhstan also do not correspond to the globally widespread TKTTF, TTTTF, TRTTF, and TTRTF races, which have caused epidemics in many countries.

No virulent isolates against the Sr11, Sr30, Sr24, and Sr31 lines were detected in the regions of Kazakhstan. Therefore, in breeding wheat for race-specific immunity, sources of effective resistance genes (Sr11, Sr24, Sr30, and Sr31) against stem rust should be selected. The virulence data of stem rust populations align with studies on virulence from previous years [20].

Most of the predominant races in Kazakhstan are closely related and polymorphic for virulence to specific Sr resistance genes. For example, race THMTF is similar to RHMRF with additional virulence to Sr9e and Sr10, while race RFRTF is virulent to Sr8a, Sr9b, and Sr10 but, unlike RHMRF, is avirulent to Sr6. Race THMTC was identical to MHCTC with additional virulence to Sr21, Sr9e, and Sr36. Such single-step changes in virulence are reported to be a key evolutionary process in pathogen populations. The obtained results provide breeders, phytopathologists, and geneticists with new effective tools to enhance breeding programs for developing new resistant wheat varieties.

CONCLUSION

The study evaluated the virulence of wheat leaf rust and stem rust populations against near-isogenic Lr and Sr lines. The most effective lines were those carrying the genes Lr9, Lr19, Lr24, Lr25, and Lr29 for leaf rust resistance, and Sr11, Sr24, Sr30, and Sr31 for stem rust. A total of 7 leaf rust and 6 stem rust races were identified, each with varying frequencies and differing in their virulence levels on differentiator varieties. Races TGKGT and THSGT were found in all studied populations, while races TGTGT and THTJT were present in two regions of Kazakhstan. In addition, stem rust races QHHSF, THMTF, RHMRF, RFRTF, and RKRTF were observed across all studied regions, with additional races THMTC and MHCTC identified in the Kostanay region.

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ҚАЗАҚСТАНДАҒЫ ЖАПЫРАҚ ЖӘНЕ САБАҚ ТАТЫ ҚОЗДЫРҒЫШТАРЫНЫҢ АЛУАНТҮРЛІЛІГІ МЕН ВИРУЛЕНТТІЛІГІ

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Аннотация. Фитосанитариялық қауіпсіздік — Қазақстанның азық-түлік пен ұлттық қауіпсіздігін қамтамасыз етудің маңызды бөлігі. Әсіресе жапырақ және сабақ таты сияқты қауіпті дәнді дақылдар ауруларымен күресте оның маңызы ерекше. Бұл зерттеуде Қазақстанның әртүрлі өңірлерінен жиналған *Puccinia triticina* және *Puccinia graminis* популяцияларының вируленттілігі мен расалық құрамы зерттелді. Барлығы 30 P. triticina және 31 P. graminis изоляты Lr- және Sr- гендері бар изогендік линияларға қарсы талданып, осы гендердің тиімділігі анықталды. Нәтижелер солтүстік және оңтүстік популяциялар арасындағы расалық құрамы мен вируленттілігінде елеулі айырмашылықтар бар екенін көрсетті, сондай-ақ кейбір расалар екі өңірге де ортақ болып шықты.

Түйін сөздер: жапырақ таты, сабақ таты, бидай, раса, төзімділік.

РАЗНООБРАЗИЕ И ВИРУЛЕНТНОСТЬ ПОПУЛЯЦИЙ ЛИСТОВОЙ И СТЕБЛЕВОЙ РЖАВЧИНЫ В КАЗАХСТАНЕ

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Аннотация. Фитосанитарная безопасность, как неотъемлемая часть продовольственной безопасности страны, имеет ключевое значение для обеспечения биологической и национальной безопасности республики. В настоящем исследовании оценивались вирулентность и расовый состав популяций *Puccinia triticina* и *Puccinia graminis*, собранных в различных регионах Казахстана. Всего было проанализировано 30 изолятов P. triticina и 31 изолят P. graminis на изогенных линиях с генами устойчивости Lr и Sr с целью определения эффективности указанных генов. Полученные результаты показали значительные различия в расовом составе и вирулентности между северными и южными популяциями; при этом некоторые расы были общими для обоих регионов.

Ключевые слова: листовая ржавчина, стеблевая ржавчина, пшеница, раса, устойчивость.