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Virulence and race structure of *Puccinia graminis* f. sp. *tritici* in Kazakhstan

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Abstract: Severe epidemics of wheat stem rust caused by *Puccinia graminis* f. sp. *tritici* (Pgt) have been observed in recent years in major spring wheat producing regions in Kazakhstan. However, information on the virulence structure and race composition of Pgt is currently not available. Stem rust samples were collected in 2015–2018 in three regions of Kazakhstan to determine the virulence diversity and race distribution in the Pgt populations. A total of 203 single-pustule isolates were derived and evaluated on the stem rust differential and supplemental lines and 38 races were identified. Among them, the races QHHSF and THMTF were found in all the regions and in all the years. The races RFRTF, RHMRF, TKRPF and MHCTC were the most common races in the Akmola and Kostanay regions, and the races LHCSF, QKCSF and LKCSF were only widely distributed in East Kazakhstan. The virulence complexity (*i.e.,* number of *Sr* genes on which the races were virulent) ranged from 5 to 16, with about 40% of the races having 14 or more virulence. The stem rust resistance genes *Sr11*, *Sr13*, *Sr22*, *Sr26*, *Sr31*, *Sr33* and *Sr35* were found to confer resistance to all the races identified during the study period. Hence, these genes can be used as sources of resistance in wheat breeding programmes in Kazakhstan.

Keywords: *Triticum* spp.; wheat stem rust; population; isolate; resistance gene

Stem rust of wheat caused by *Puccinia graminis* f. sp. *tritici* (Pgt) is historically the most damaging wheat disease worldwide (Roelfs et al. 1992; Singh et al. 2015). Over the last 20 years, there has been significant concern about the spread of the Pgt aggressive race Ug99, which was first detected in Uganda in 1998 (Pretorius et al. 2000). To date, 13 races within the Ug99 race group have been identified in Eastern Africa and the Middle East, and it is projected to spread further, threatening critical wheat growing regions in the world (Singh et al. 2015; Patpour et al. 2016). In recent years, new races, that are not members of the Ug99 race group, have caused disease outbreaks on wheat crops in Europe (Bhattacharya 2017; Lewis et al. 2018), Africa (Olivera et al. 2015) and the Caucasus region of Eurasia (Olivera et al. 2019).

Recently, in the northern regions of Kazakhstan and in Western Siberia where spring wheat is mainly cultivated, stem rust has become a major disease. In 2015 and 2016 in the northern regions of Kazakhstan, as well as in the adjacent Omsk region of Russia, a major stem rust epidemic occurred, affecting, respectively, approximately one and two million ha of wheat (Shamanin et al. 2016, 2020; Koyshybaev 2018; Rsaliyev & Rsaliyev 2018). Stem rust occurred again in 2017–2018 in the northern and eastern regions

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of Kazakhstan and Omsk, Novosibirsk and the Altai Krai regions of Russia, and not only resulted in a marked yield reduction, but also in lower grain quality (Koishybaev 2018; Gultyaeva et al. 2020; Shamanin et al. 2020; Skolotneva et al. 2020). During 2015–2018 disease severity and incidence in the main wheat growing regions of Kazakhstan were up to 90 and 70%, respectively, and was substantially higher than found in previous years (Koyshybaev 2018; Rsaliyev et al. 2019; Yskakova & Rsaliyev 2019).

In a recent report (Hodson et al. 2017), researchers from the International Maize and Wheat Improvement Center (CIMMYT) and the Global Rust Reference Center (GRRC) called for urgent further research, both to understand the pathogen dynamics and to increase the proportion of resistant cultivars in these regions. With favourable weather conditions and an apparent high race diversity, the scale of the reported epidemics has serious implications for neighbouring regions and beyond (Hodson et al. 2017). The Kazakhstan-Siberian Network for Wheat Improvement responded to the threat of stem rust by systematically screening their bread and durum wheat varieties and locally-developed germplasm in Kazakhstan, Western Siberia and Kenya. An overwhelming majority of the locally-cultivated and new varieties were shown to be highly susceptible (Shamanin et al. 2016; Yskakova & Rsaliyev 2019; Gultyaeva et al. 2020). However, several years of screening resulted in a set of stem rust-resistant new spring bread and durum wheat varieties and breeding lines from Kazakhstan and Russia, which were analysed for stem rust resistance across several locations (including Kenya) (Shamanin et al. 2016; Gultyaeva et al. 2020).

Recently, the pathogenic variability of the Pgt population has been investigated across Western Siberia. The virulence structure of the stem rust population spread in the Omsk, Novosibirsk and Altai Krai regions were identified at the GRRC (Denmark), and in the laboratory of molecular phytopathology at the Institute of Cytology and Genetics (Russia) (Hovmøller 2017; Shamanin et al. 2020; Skolotneva et al. 2020). Despite the increasing importance of stem rust in Kazakhstan, the population structure of Pgt in this region has not been characterised and, so far, there were no attempts to determine the effectiveness of the *Sr* genes for the future application in local breeding programmes. The present study was, therefore, undertaken to describe the characteristics of the race structure of the Pgt populations and the effectiveness of the *Sr* genes in the major spring wheat regions of Kazakhstan in 2015–2018.

MATERIAL AND METHODS

Pathogen sample collection. The surveys on stem rust were conducted from 2015 to 2018 in a total of 11 districts across three regions in the Republic of Kazakhstan, namely Akmola, Kostanay and East Kazakhstan. The commercial wheat fields and experimental plots were observed during the period of high disease pressure from July to August. The stem rust severity on the wheat cultivars was evaluated using the modified Cobb scale (Peterson et al. 1948) and host response to infection as described in Roelfs et al. (1992). About five to six pieces of stem tissue of about 10 cm in length bearing moderately susceptible to susceptible pustules were collected at each point. The samples were air-dried at room temperature for at least 24 h and then stored in paper bags. The dried samples were transported to the Research Institute for Biological Safety Problems and were kept in a refrigerator at 4 °C until further use in the isolation process. A total of 85 stem rust samples (15, 22, 25 and 23 in 2015, 2016, 2017 and 2018, respectively) were collected. The passport data of the samples (the locations, disease severity on the wheat cultivars and number of Pgt isolates) are given in Supplemental Table S1 (ESM).

Production of single-pustule isolates and their multiplication. The development of single-pustule isolates and multiplication of these isolates was undertaken on the universally rust susceptible variety Morocco. Five to six seeds of the susceptible wheat cultivar were planted into 11 cm diameter plastic pots containing soil, sand, and compost mixtures in a 2 : 1 : 1 (v:v:v) ratio. Bulked urediniospores from each stem rust sample was suspended in a lightweight mineral oil, Soltrol 170 and sprayed onto the seven-day-old Morocco seedlings. The seedlings were incubated in a dew chamber for 14 h at 18 °C in the dark, and then for an additional period of 3 to 4 h under fluorescent light. The inoculated plants were placed on a greenhouse bench at 20 ± 4°C with a photoperiod of 16 hours (Jin et al. 2007; Admassu et al. 2009). After seven to ten days of inoculation, leaves containing a single fleck about to develop into a single pustule were selected from the base of the leaves and the remaining seedlings within the pots were removed using scissors. Only the leaves containing single pustules were separately covered with cellophane bags and tied up at the base with a rubber band to avoid cross-contamination (Fetch & Dunsmore 2004).

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Two weeks later (when the pustule was well developed), the spores from each pustule were collected. One to seven single-pustule isolates were derived from each original sample. The seven-day-old seedlings of the susceptible variety Morocco were inoculated with a suspension, prepared by mixing the urediniospores with Soltrol 170, for multiplication for each of the single-pustules in separate pots following the procedures mentioned earlier. The spore multiplication procedure was repeated 2–3 times until a sufficient number of spores were produced for experimentation. The spores of each single-pustule isolate were collected in separate test tubes and stored at 4 °C as described by Rsaliyev and Rsaliyev (2018) for future use.

Race identification and virulence characterisation. The North American stem rust differential set was used for the race identification (Roelfs & Martens 1988; Jin et al. 2008). The differential wheat lines possessed the resistance genes *Sr5, Sr21, Sr9e, Sr7b, Sr11, Sr6, Sr8a, Sr9g, Sr36, Sr9b, Sr30, Sr17+Sr13, Sr9a, Sr9d, Sr10, SrTmp, Sr24, Sr31, Sr38* and *SrMcN.*, Also, additional lines including *Sr13* (ST464), *Sr22* (SwSr22T.B), *Sr26* (Eagle), *Sr33* (Tetra Canthatch/*Ae. tauschii*) and *Sr35* (Mq(2)5XG2919) resistance genes were used to determine the virulence with respect to these resistance genes. The urediniospores of each Pgt isolate from storage at 4 °C were heat shocked at 40 °C for 10 min and placed in a rehydration chamber for 2 to 4 h, where approximately 80% relative humidity was maintained by a KOH solution (Jin et al. 2007). Each isolate was inoculated onto the differential and additional lines set following the procedure described above.

The infection type (IT) on the seedlings of each differential line was assessed 14 days after inoculation using a 0–4 scale (Stakman et al. 1962), where ITs of 0, 1, 2, or combinations thereof are considered as a low IT and ITs of 3 or higher are considered as a high IT. The IT 2– or higher on the line Combination VII indicates virulent to *Sr17,* but avirulent to *Sr13* (Olivera et al. 2015, 2019). Each single-pustule isolate was evaluated on the differential lines twice before a race was designated. The race designation was performed based on the letter code proposed by Roelfs and Martens (1988).

Data analysis. The descriptive analysis of the populations included calculation of the following parameters: the race frequencies, virulence frequencies for each differential line, and the average virulence complexity for the races. The structure and relationships of the populations were analysed using methods of diversity analyses based on the assignment of isolates according to the dissimilarity between their virulence patterns. The corresponding assignment-based Kosman diversity (*KW*) within (Kosman 1996), Gleason index of richness (*G*) (Gleason 1922), evenness (*E*) (Sheldon 1969) between the populations (regions) with regard to a simple mismatch coefficient. All the above-written parameters were analysed using the Virulence Analysis Tool program (version SW 2018) (Kosman et al. 2008).

RESULTS

Virulence frequencies. The virulence frequencies to the *Sr* genes differed among the regional populations of Pgt in Kazakhstan (Table 1). Most isolates were virulent to *Sr5, Sr6, Sr9g, Sr9a, Sr9d, Sr10, Sr17, Sr38* and *SrMcN* (69.6–100%) in all regions of Kazakhstan in 2015–2018. Virulence to *Sr7b, Sr9b, Sr36* and *SrTmp* was considerably lower in the East Kazakhstan region (0–30.8%), compared to the Akmola and Kostanay regions (45.0–95.5%). Virulence to *Sr21* was also lower in the East Kazakhstan region (37.5–53.3%) compared with the other two regions (75–90%). Virulence to *SrTmp* increased from 61.5% in 2015 to 95.5% in 2018 in the Akmola region. Virulence to *Sr9b, Sr10, SrTmp* and *Sr38* varied significantly between the years in the Kostanay region. Virulence to *Sr8a* was between 23.1 and 50.0% in all the regions and all the years. The isolates virulent to *Sr9e* occurred at low frequencies during 2015–2017 (0–30%) in all the regions, but only in 2018, it increased (35–45.5%) in the Akmola and Kostanay regions. All the tested isolates were avirulent to *Sr30* in 2015 and 2016, but since 2017, virulence to *Sr30* occurred only in the Akmola region (up to 9.1%). The frequencies of virulence to *Sr24* occurred at lower frequencies (4.3–10%) in the Akmola and Kostanay regions and were not present in the East Kazakhstan region. None of the isolates across Kazakhstan had any virulence to the stem rust resistance genes *Sr11, Sr13, Sr22, Sr26, Sr31, Sr33* and *Sr35* (Table 1).

Frequency and virulence patterns of the Pgt races. A total of 38 races were isolated among the 203 isolates collected during 2015–2018 (Tables 2 and 3). Among them, 29 races were unique, present in only one of the surveyed regions, seven were detected in only two regions and two races were present in all three regions. The races RFRTF, RHMRF, THMTF, TKRPF, MHCTC, QHHSF, QKCSF LHCSF and LKCSF, were the most frequent, with

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Table 1. Frequency of isolates (%) of *Puccinia graminis* f. sp. *tritici* with virulence to stem rust resistance (*Sr*) genes collected in three regions of Kazakhstan from 2015 to 2018

Line	Resistance gene(s)	Akmola region				Kostanay region				East Kazakhstan region			
		2015	2016	2017	2018	2015	2016	2017	2018	2015	2016	2017	2018
ISr5-Ra	Sr5	84.6	93.3	95.7	95.5	93.8	95.0	100	100	100	92.3	100	100
CnS_T_mono_deriv	*Sr21*	76.9	80.0	82.6	81.8	81.3	75.0	90.0	85.0	37.5	46.2	53.3	50.0
Vernstein	*Sr9e*	15.4	26.7	21.7	45.5	25.0	20.0	30.0	35.0	0.0	23.1	13.3	16.7
ISr7b-Ra	*Sr7b*	84.6	86.7	87.0	95.5	81.3	90.0	90.0	90.0	0.0	30.8	26.7	16.7
ISr11-Ra	*Sr11*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
ISr6-Ra	*Sr6*	76.9	73.3	69.6	86.4	75.0	85.0	85.0	90.0	100	100	100	94.4
ISr8a-Ra	*Sr8a*	38.5	40.0	43.5	50.0	37.5	30.0	40.0	45.0	37.5	23.1	26.7	44.4
CnSr9g	*Sr9g*	100	100	100	95.5	100	100	100	100	100	100	100	94.4
W2691SrTt-1	*Sr36*	53.8	66.7	52.2	72.7	68.8	70.0	80.0	70.0	0.0	15.4	13.3	11.1
W2691Sr9b	*Sr9b*	46.2	60.0	47.8	50.0	50.0	45.0	60.0	60.0	25.0	30.8	13.3	16.7
BtSr30Wst	*Sr30*	0.0	0.0	4.3	9.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Combination VII	*Sr17* (+*Sr13*)	76.9	93.3	95.7	95.5	81.3	80.0	90.0	95.0	100	92.3	86.7	94.4
ISr9a-Ra	*Sr9a*	92.3	93.3	100	100	93.8	95.0	100	95.0	100	100	100	100
ISr9d-Ra	*Sr9d*	92.3	86.7	95.7	81.8	81.3	90.0	90.0	85.0	100	100	100	100
W2691Sr10	Sr10	84.6	73.3	78.3	81.8	68.8	80.0	90.0	90.0	100	92.3	86.7	94.4
CnsSrTmp	*SrTmp*	61.5	80.0	87.0	95.5	68.8	80.0	90.0	85.0	0.0	30.8	13.3	16.7
LcSr24Ag	*Sr24*	0.0	6.7	4.3	4.5	6.3	10.0	5.0	10.0	0.0	0.0	0.0	0.0
Sr31/6*LMPG	*Sr31*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
VPM-1	*Sr38*	84.6	80.0	60.9	81.8	68.8	70.0	85.0	85.0	100	92.3	86.7	94.4
McNair 701	*McN*	100	100	100	100	100	100	100	100	100	100	100	100
ST464*	*Sr13*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
SwSr22T.B*	*Sr22*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Eagle*	*Sr26*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tetra Canthatch/ *Ae. tauschii**	*Sr33*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mq(2)5XG2919*	*Sr35*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

*additional lines carrying resistance genes *Sr13, Sr22, Sr26, Sr33* and *Sr35*

QHHSF and THMTF being present in all three regions. The race QHHSF with virulence to the genes *Sr5, Sr21, Sr6, Sr9g, Sr9b, Sr17, Sr9a, Sr9d, Sr10, Sr38* and *SrMcn* comprised 4.5–15.4% of the isolates from Akmola, 10–12.5% from Kostanay and 11.1–25% from East Kazakhstan in 2015-2018. The race THMTF with virulence to *Sr5, Sr21, Sr9e, Sr7b, Sr6, Sr36, Sr9g, Sr9b, Sr17, Sr9a, Sr9d, Sr10, Sr38* and *SrMcn*, comprised about 5–15.4% of the isolates in all the regions.

In the Akmola and Kostanay regions, there were 20 races each among the 73 and 76 isolates tested, respectively (Tables 2 and 3). The Akmola and Kostanay regions only had seven races, CHBGC, MHCTC, RFRTF, RHMRF, RHRTP, THRTC and TKRPF, in common. Among them, the most common races in the northern regions of Kazakhstan were RFRTF (10–23.1%), RHMRF (5–17.4%), TKRPF (4.3–15%) and MHCTC (6.3–13%). It is also interesting to note that two of the races (TKRTF and RKRTF) that were unique to the Akmola and Kostanay regions were the most dominant ones in 2017 and 2018, respectively (Table 2).

The race pattern in Eastern Kazakhstan was different from that of the other two regions. In this region, nine races were found among the 54 isolates tested (Tables 2 and 3). Of these, the most abundant races were LHCSF (23.1–37.5%), QKCSF (12.5–27.8%)

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Table 2. Number, frequency (%) and virulence spectrum of the races of *Puccinia graminis* f. sp. *tritici* identified in three Kazakhstan regions from 2015 to 2018

Races*	Virulence spectrum (ineffective *Sr* resistance genes)	No. of isolates**	Akmola region				Kostanay region				East Kazakhstan region			
			2015	2016	2017	2018	2015	2016	2017	2018	2015	2016	2017	2018
QHHSF	*5, 21, 6, 9g, 9b,17, 9a, 9d, 10, 38, Mcn*	25	15.4	13.3	13.0	4.5	12.5	10.0	10.0	10.0	25.0	23.1	13.3	11.1
RFRTF	*5, 21, 7b, 8a, 9g, 36, 9b, 17, 9a, 9d, 10, Tmp, 38, Mcn*	24	23.1	26.7	13.0	13.6	18.8	15.0	15.0	10.0	–	–	–	–
THMTF	*5, 21, 9e, 7b, 6, 9g, 36,17, 9a, 9d, 10, Tmp, 38, Mcn*	18	7.7	6.7	–***	13.6	6.3	5.0	10.0	15.0	–	15.4	13.3	11.1
RHMRF	*5, 21, 7b, 6, 9g, 36, 17, 9a, 9d, Tmp, 38, Mcn*	17	7.7	13.3	17.4	13.6	12.5	10.0	10.0	5.0	–	–	–	–
TKRPF	*5, 21, 9e, 7b, 6, 8a, 9g, 36, 9b, 17, 9a, 10, Tmp, 38, Mcn*	16	7.7	13.3	4.3	13.6	12.5	10.0	10.0	15.0	–	–	–	–
LHCSF	*5, 6, 9g, 17, 9a, 9d, 10, 38, Mcn*	16	–	–	–	–	–	–	–	–	37.5	23.1	33.3	27.8
MHCTC	*5, 7b, 6, 9g, 17, 9a, 9d, 10, Tmp, Mcn*	14	7.7	6.7	13.0	9.1	6.3	10.0	10.0	10.0	–	–	–	–
RHRTP	*5, 21, 7b, 6, 9g, 36, 9b, 17, 9a, 9d, 10, Tmp, 24, 38, Mcn*	9	–	6.7	4.3	4.5	6.3	10.0	5.0	10.0	–	–	–	–
QKCSF	*5, 21, 6, 8a, 9g, 17, 9a, 9d, 10, 38, Mcn*	8	–	–	–	–	–	–	–	–	12.5	–	13.3	27.8
LKCSF	*5, 6, 8a, 9g, 17, 9a, 9d, 10, 38, Mcn*	8	–	–	–	–	–	–	–	–	25.0	15.4	13.3	11.1
RKRTF	5, 21, 7b, 6, 8a, 9g, 36, 9b, 17, 9a, 9d, 10, Tmp, 38, Mcn	6	–	–	–	–	–	–	15.0	15.0	–	–	–	–
TKRTF	*5, 21, 9e, 7b, 6, 8a, 9g, 36, 9b, 17, 9a, 9d, 10, Tmp, 38, Mcn*	4	–	–	4.3	13.6	–	–	–	–	–	–	–	–
CHBGC	*7b, 6, 9g, 9d, Mcn*	4	7.7	6.7	–	–	6.3	5.0	–	–	–	–	–	–
RFCTC	*5, 21, 7b, 8a, 9g, 17, 9a, 9d, 10, Tmp, Mcn*	3	–	–	13.0	–	–	–	–	–	–	–	–	–
RHBQC	*5, 21, 7b, 6, 9g, 9a, 9d, Mcn*	3	–	–	–	–	–	–	–	–		7.7	13.3	
THRTC	*5, 21, 9e, 7b, 6, 9g, 36, 9b, 17, 9a, 9d, 10, Tmp, Mcn*	2	–	–	4.3	–	–	–	5.0	–	–	–	–	–
RHLTF	*5, 21, 7b, 6, 9g, 36, 9a, 9d, 10, Tmp, 38, Mcn*	2	–	–	–	–	–	5.0	5.0	–	–	–	–	–
PHCRC	*5, 9e, 7b, 6, 9g, 17, 9a, 9d, Tmp, Mcn*	2	–	6.7		4.5	–	–	–	–	–	–	–	–
NKHTF	*5, 9e, 6, 8a, 9g, 9b, 17, 9a, 9d, 10, Tmp, 38, Mcn*	2	–	–	–	–	–	–	–	–	–	7.7		5.6
MHMTC	*5, 7b, 6, 9g, 36, 17, 9a, 9d, 10, Tmp, Mcn*	2	–	–	–	–	–	10.0	–	–	–	–	–	–
CHCTF	*7b, 6, 9g, 17, 9a, 9d, 10, Tmp, 38, Mcn*	1	–	–	–	–	–	–	–	–	–	7.7	–	–
FKBTC	*9e, 7b, 6, 8a, 9g, 9a, 9d, 10, Tmp, Mcn*	1			4.3	–	–	–	–	–	–	–	–	–
HHMSF	*21, 7b, 6, 9g, 36, 17, 9a, 9d, 10, 38, Mcn*	1	7.7	–	–	–	–	–	–	–	–	–	–	–
HJFPF	*21, 7b, 6, 8a, 30, 17, 9a, 10, Tmp, 38, Mcn*	1	–	–	–	4.5	–	–	–	–	–	–	–	–
LCLLC	*5, 9g, 36, 9a, Mcn*	1	–	–	–	–	6.3	–	–	–	–	–	–	–
MBBQC	*5, 7b, 9a, 9d, Mcn*	1	–	–	–	–	–	–	–	–	–	–	–	5.6
MHBGC	*5, 7b, 6, 9g, 9d, Mcn*	1	–	–	–	–	–	–	–	5.0	–	–	–	–
MKBTF	*5, 7b, 6, 8a, 9g, 9a, 9d, 10, Tmp, 38, Mcn*	1	7.7	–	–	–	–	–	–	–	–			
MKDTC	*5, 7b, 6, 8a, 9g, 30, 9a, 9d, 10, Tmp, Mcn*	1	–	–	–	4.5	–	–	–	–	–	–	–	–

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Table 2. continue

Races*	Virulence spectrum (ineffective *Sr* resistance genes)	No. of isolates**	Akmola region				Kostanay region				East Kazakhstan region			
			2015	2016	2017	2018	2015	2016	2017	2018	2015	2016	2017	2018
RHBSF	*5, 21, 7b, 6, 9g, 9a, 9d, 10, 38, Mcn*	1	7.7	–	–	–	–	–	–	–	–	–	–	–
RHMTF	*5, 21, 7b, 6, 9g, 36, 17, 9a, 9d, 10, Tmp, 38, Mcn*	1	–	–	4.3	–	–	–	–	–	–	–	–	–
RKBQC	*5, 21, 7b, 6, 8a, 9g, 9a, 9d, Mcn*	1	–	–	–	–	6.3	–	–	–	–	–	–	–
RKLTF	*5, 21, 7b, 6, 8a, 9g, 36, 9a, 9d, 10, Tmp, 38, Mcn*	1	–	–	–	–	–	5.0	–	–	–	–	–	–
TFKRC	*5, 21, 9e, 7b, 8a, 9g, 9b, 30, 17, 9a, 9d, Tmp, Mcn*	1	–	–	4.3	–	–	–	–	–	–	–	–	–
THBQC	*5, 21, 9e, 7b, 6, 9g, 9a, 9d, Mcn*	1	–	–	–	–	–	5.0	–	–	–	–	–	–
THLTF	*5, 21, 9e, 7b, 6, 9g, 36, 9a, 9d, 10, Tmp, 38, Mcn*	1	–	–	–	–	–		5.0	–	–	–	–	–
THMTC	*5, 21, 9e, 7b, 6, 9g, 36, 17, 9a, 9d, 10, Tmp, Mcn*	1	–	–	–	–	6.3	–	–	–	–	–	–	–
TKCTF	*5, 21, 9e, 7b, 6, 8a, 9g, 17, 9a, 9d, 10, Tmp, 38, Mcn*	1	–	–	–	–	0.0	–	–	5.0	–	–	–	–

*races ranked by frequency in the regions; ** the number of isolates within each race in all the regions and years; ***race not detected in the region in a given sampling year

and LKCSF (11.1–25%), each of which were only detected in the East Kazakhstan region.

The virulence complexity of the races varied from 5 to 16. A proportion of races detected in the study (39.4%) had a virulence complexity of 14–16, which is indicative of a highly complex virulence structure in Kazakhstan. The race TKRTF has the widest virulence spectrum (a virulence complexity equal to 16) of all the races identified in Kazakhstan. The races typed as RHRTP were the only ones showing virulence to *Sr24* and were found in the regions of Akmola (4.1–6.7%) and Kostanay (5–10%). The other races are restricted to certain years or certain regions (Table 2).

Descriptive parameters. The parameters of intrapopulation diversity and statistical indices of the difference of Pgt between the Kazakhstan regions are shown in detail in Table 3. Of the 29 races present in only one region, 11 were present in Akmola only, 11 in Kostanay and seven in the East Kazakhstan region. The average virulence complexity of the races in the regions under study varied from 11.85 (Akmola region) to 10.11 (East Kazakhstan region), and, in three regions, this value was equal to 11.13.

According to the Kosman and Gleason indices, the intrapopulation diversity of the race structure was higher in the Akmola region ($KW = 0.26$, $G = 4.42$) and significantly lower ($KW = 0.16$, $G = 2.00$) in the East Kazakhstan region. The evenness was quite uniform in all the Kazakhstan regions (Ev= 0.87, 0.88, 0.86 in the Akmola, Kostanay, East Kazakhstan regions, respectively). The total KW, G and E of all the regions was just 0.27, 6.96 and 0.82, respectively (Table 3).

DISCUSSION

Historical studies on the race composition of the pathogen in Kazakhstan have recently been summarised (Rsaliyev & Rsaliyev 2018). In the same document, it is stated that the use of the Sermon old standard differential set (Stakman et al. 1962) and an incomplete North American system of race nomenclature (Roelfs & Martens 1988) in the experiments prevents measuring the similarity between the Kazakhstani races and the known worldwide races of the pathogen (Rsaliyev & Rsaliyev 2018). According to Shamanin et al. (2016) there was an obvious change of pathogen virulence (and possibly aggressiveness) in Kazakhstan and Western Siberia from 2015, compared to previous years. Taking the deteriorating phytopathological situation associated with stem rust epidemics occurring in 2015–2018

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Table 3. Population parameters of the *Puccinia graminis* f. sp. *tritici* in three regions of Kazakhstan in 2015–2018

Regions	Number of			Complexity*	Diversity parameters**		
	isolates	races	original races		*KW*	*G*	*E*
Akmola	73	20	11	11.85	0.26	4.42	0.87
Kostanay	76	20	11	11.50	0.24	4.39	0.88
East Kazakhstan	54	9	7	10.11	0.16	2.00	0.86
Total	203	38	29	11.13	0.27	6.96	0.82

*average virulence complexity of races; **KW – the Kosman index of diversity; G = gleason – the index of richness; E – a measure of genetic evenness

into account, the virulence and racial composition of Pgt in the regions of Kazakhstan were studied.

A total of 38 races were identified in three major spring wheat regions of Kazakhstan. Among them, the races QHHSF and THMTF were found in all the regions and in all the years. The two adjacent regions, Akmola and Kostanay, had seven similar races out of the twenty races detected. Of these, the races RFRTF, RHMRF, TKRPF and MHCTC were widespread in the northern Kazakhstan regions in all the years. The races TKRTF and RKRTF were only observed in both 2017 and 2018 and were the most common races in the Akmola and Kostanay regions, respectively (Table 2). Their geographic proximity, an absence of barriers and cultivation of similar spring bread wheat cultivars between the two regions likely significantly contributed to the high race similarity.

In the East Kazakhstan region, nine races were found. Among these races, LHCSF, QKCSF and LKCSF were the three most common races in this area. The difference in the race composition in Eastern Kazakhstan compared to the Akmola and Kostanay regions could possibly also be explained by the cultivar differences and the severity on the collected wheat samples in the Kazakhstan regions. Most isolates were obtained from the commercial wheat cultivars Akmola 2, Omskaya 18, Omskaya 36, Karabalykskaya 92, Karabalykskaya 90 and Asyl Sapa in the Akmola and Kostanay regions and the bulk of East Kazakhstan isolates were isolated from other spring wheat cultivars including Omskaya 18, Altay, Daria and the breeding lines (GVK 2227/2, GVK 2104/3) (Supplementary Table S1). Besides, the stem rust severity and host response to infection varies between the years, locations, and cultivars in the survey regions of Kazakhstan. During 2015–2018, in the Akmola and Kostanay regions, a majority of the commercial cultivars (Astana, Akmola 2, Omskaya 36, Omskaya 18, Karabalykskaya 92, Karabalykskaya 90, Augustina, Asyl Sapa and other varieties) were highly susceptible (60–90S) and moderately susceptible (30–40MS) to stem rust in all the years. In 2015 and 2016, the disease severity in the East Kazakhstan region was up to 40%, whereas, in 2017 and 2018, the disease severity was up to 60% (ESM).

The different classes of wheat cultivars may be heterogeneous for stem rust resistance genes; thus, the selection pressures may differ among the regional populations. Since it is not known which resistance genes are contained in the commercial wheat cultivars grown in Kazakhstan, it is not possible to determine their effect on the race composition of the Pgt population. Recently, it was found that the genetic basis of resistance in the new spring wheat genotypes from Kazakhstan and Russia is generally limited to *Sr25, Sr31, Sr36, Sr6Ai, Sr6Ai#2*, and some unknown major genes (Shamanin et al. 2016). However, these new varieties and lines of wheat with *Sr* genes are not yet commercially used in Kazakhstan. Virulence diversities within the Pgt populations were also reported from countries, such as Russia (Skolotneva et al. 2020), Ethiopia (Admassu et al. 2009), Mexico (Singh 1991) and Canada (Fetch 2004). The results of the population composition comparison in Western Siberia of Russia suggest that the Omsk, Novosibirsk and Altai subpopulations have relatively independent sources of genetic diversity (Shamanin et al. 2020; Skolotneva et al. 2020).

Most of the predominant races in Kazakhstan varied from one another by single-gene changes. The race TKRTF was similar to RKRTF with an additional virulence to *Sr9e*, and the race RKRTF was similar to RFRTF with an additional virulence to *Sr6*. The race TKRPF is also virulent for *Sr9e*, but unlike TKRTF, it is avirulent for *Sr9d*. The race LKCSF was similar to LHCSF, and LHCSF to QKCSF with an additional virulence to *Sr8a* and *Sr21*, respectively

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(Table 2). Such single-step changes in the virulence were reported to be the main process of the evolutionary change in the Pgt populations (Green 1975). The race TKRTF has the widest virulence spectrum of all the races identified in Kazakhstan, combining virulence to a number of important resistance genes including *Sr21, Sr36, Sr38* and *SrTmp*.

In this study, the pathogen population structure was variable during the study period with some races being absent over time, with new ones arising during the same period and only a few races occurring consistently during the study period (Table 2). A similar phenomenon is also very well-known from previous research in Georgia (Olivera et al. 2019) where the Pgt population virulence structure was highly variable from 2013 to 2015. Such variation over time is not uncommon, as the races prevalent in a specific season depend on the type of wheat cultivars grown in the season (Singh 1991), and, to some extent, on the predominant environmental conditions, especially the temperature (Roelfs et al. 1992).

A comparison of the races identified in the present study with other countries revealed some differences. In the regions of Kazakhstan, variants of the Ug99 race were not found, which are rapidly spreading throughout the countries of Africa and Asia (Singh et al. 2015; Patpour et al. 2016). The races in Kazakhstan also do not resemble the TKTTF, TTTTF, TRTTF and TTRTF races responsible for epidemics in many countries of the world (Olivera et al. 2015, 2019; Bhattacharya 2017; Lewis et al. 2018). Many of these races were not identified in other neighbouring countries, such as Russia (Hovmøller 2017; Shamanin et al. 2020; Skolotneva et al. 2020) and China (Li et al. 2018). An isolate assigned as race TTTTF in the Omsk region proved to be significantly different from the isolates of this race, which appeared widespread in stem rust epidemics in Sicily (Hovmøller 2017). The race TTRTF with a wide range of virulence was one of the dominant races in Georgia in 2014 and 2015 (Olivera et al. 2019).

However, the individual races from Kazakhstan were identical to the races of Western Siberia. The dominant races QHHSF and TKRPF in the regions of Kazakhstan are also found in the Omsk and Novosibirsk regions (Hovmøller 2017; Shamanin et al. 2020; Skolotneva et al. 2020). The most virulent races RFRTF and TKRTF in the Kostanay and Akmola regions were registered at the same period in the Omsk region (Hovmøller 2017; Shamanin et al. 2020). The races LKCSF, LHCSF and QKCSF identified from the population of the East-Kazakhstan region only were identified in the samples of the population of the Altai Krai (Shamanin et al. 2020; Skolotneva et al. 2020). In the northern regions of Kazakhstan, only one race, RHRTP, with virulence to *Sr24*, was identified, which was also found in the Omsk region (Hovmøller 2017; Shamanin et al. 2020).

Based on the research presented here, we assume that the Pgt races in Kazakhstan and Western Siberia have a common origin and differ from the races of other countries. This may be due to the similar climate and close distances between the regions, as well as the uniformity of the cultivated varieties of spring wheat on the resistance to stem rust in the regions. The Russian West Siberia region is directly adjacent to the North Kazakhstan regions and similar types of spring wheat are grown in these regions (Kolmer et al. 2014). In particular, most wheat cultivars in Kazakhstan have been developed in collaboration with Russian breeders using Russian wheat genetic resources based on bilateral projects (Martynov et al. 2005; Morgounov et al. 2007). Similar results were recently obtained when studying the population of wheat leaf rust (*P. triticina*) in various regions of Kazakhstan and Russia. These studies did not reveal any significant differences in the virulence between the isolates of *P. triticina* from Northern Kazakhstan and Western Siberia (Kolmer et al. 2014).

The majority of the resistance genes were ineffective against most of the races identified in this study. For instance, most isolates or races were virulent to *Sr5, Sr6, Sr9g, Sr17, Sr9a, Sr9d* and *Sr10* in all the regions. The races virulent to *Sr7b, Sr9e, Sr9b, Sr21, Sr36* and *SrTmp*, were predominately found in the Akmola and Kostanay regions, and less so in the East Kazakhstan region (Tables 1 and 2). Some stem rust resistance genes, namely *Sr24* and *Sr30*, were found to confer resistance to most of the races prevalent in Kazakhstan. Wheat plants carrying *Sr11, Sr13, Sr22, Sr26, Sr31, Sr33* and *Sr35* were resistant to all the *Pgt* races detected during the study. These genes could be used in breeding for resistance to stem rust in Kazakhstan and the neighbouring regions of Western Siberia and Central Asia.

The continual cultivation of susceptible wheat varieties to stem rust on large areas and the general trend of climate warming in the wheat production areas of Kazakhstan could be the main reasons for the evolution and high virulence diversity of the pathogen in the country. In particular, the years 2015–2018

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were the four warmest in the history of the observations on the background of preserving the long-term warming trend in Kazakhstan (Ilyakova et al. 2019). The fifth Intergovernmental Panel on Climate Change report largely confirms that temperatures are rising in Central Asia, including in Kazakhstan. The report also indicates that precipitation has increased at higher altitudes, including in Siberia and northern Kazakhstan (Broka et al. 2016). The deterioration of the phytosanitary situation in the West Siberian regions is also due to the climate warming, cultivation of susceptible wheat varieties and appearance of new Pgt races (Shamanin et al. 2020).

The present study provides a foundation for future studies on the pathogenic variability within the Pgt populations in Kazakhstan and addresses the knowledge gap on the virulence structure and race composition of Pgt in Central Asia.

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