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Characterization of *Pyrenophora tritici-repentis* (tan spot of wheat) races in Kazakhstan

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Summary. Tan spot, caused by *Pyrenophora tritici-repentis*, is an economically important foliar disease of wheat in Kazakhstan. Population structure of the pathogen changes every year due to climate change. This study aimed to characterize the race structure of *P. tritici-repentis* isolates recovered from wheat in south and north Kazakhstan, and identify tan spot resistance in host genotypes based on disease phenotypes and molecular screening. Virulence profiles were determined within 40 isolates of the pathogen from wheat crops during the 2020 growing season. Seven races, (1, 3, 4, 5, 6, 7 and 8) were identified. A collection of 80 wheat accessions, including promising lines and cultivars from Kazakhstan and Russia, were evaluated for their reactions to races 1 and 5 of the pathogen, and to Ptr ToxA and Ptr ToxB, using greenhouse assessments and molecular markers diagnostic for the *Tsn1* and *Tsc2* genes. From a practical viewpoint, 18 wheat genotypes were insensitive to the two races and the two Ptr toxins. This resistant germplasm can be used in breeding programmes aiming to develop wheat varieties resistant to *P. tritici-repentis*.

Keywords. Tan spot, Ptr ToxA, Ptr ToxB, Race 1, Race 5.

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INTRODUCTION

Wheat is grown in many countries as the main source of nutrition for almost 40% of the global population, and provides 20% of dietary protein and calories (Giraldo *et al*., 2019). Global wheat use was projected to increase by 1.5 million tons in 2019-2020 compared to 2018-2019, mainly due to a 3.5% increase in feed demand (FAO, 2019). However, climate change and the onset of severe plant disease epidemics will probably reduce wheat yields and grain quality (Gurung *et al*., 2014). Between 5 and 14% of wheat yields are lost each year due to diseases Tan spot is a major wheat disease, which

occurs in temperate and warm wheat growing areas, including Kazakhstan (Duveiller *et al*., 1998; Phuke *et al*., 2020). This country suffers from crop losses due to common bunt, and yellow, leaf and stem rusts, but in recent years tan spot has been causing increased damage (Kokhmetova *et al*., 2016a; Kokhmetova *et al*., 2017; Kokhmetova *et al*., 2018a; Kokhmetova *et al*., 2019a; Kokhmetova *et al*., 2020a; Kokhmetova and Atishova, 2020; Gultyaeva *et al*., 2020; Madenova *et al*., 2021).

Tan spot is caused by the necrotrophic fungus *Pyrenophora tritici-repentis* (d.) Dreches (anamorph *Dreschslera tritici-repentis* (d.) Shoemaker). The tan spot pathogen was first described in 1823 (Hosford, 1982), and subsequently outbreaks of this disease were reported in Europe, USA, and Japan in early 1900, where the pathogen was considered to be a saprophyte causing minor to severe spotting in wheat (Wegulo, 2011). Tan spot epidemics were first reported in 1970s in Canada, the United States, Australia, and South Africa (Hosford, 1971; Tekauz, 1976; Rees and Platz, 1992; Lamari *et al*., 2005a), and then spread throughout Central Asia. The tan spot pathogen infects entire plants, but is usually most noticeable on leaves, as well as stems and head tissues. These infections lead to reductions in photosynthesis and ultimately to decreased crop yields and deterioration of grain quality. In severe cases, crop losses can exceed 50% (Wegulo, 2011). In recent years, this necrotrophic pathogen has caused increased wheat crop losses, which have been associated with reduction in tillage practices, as *P. tritici-repentis* overwinters in wheat stubble (Cotuna *et al*., 2015).

Pyrenophora tritici-repentis infects susceptible host germplasm due to host-selective toxins produced by different races, which induce necrotic or chlorotic symptoms (Lamari and Bernier, 1991) (Lamari and Barnier, 1989; Strelkov *et al*., 1999; Lamari *et al*., 2003). Three host-specific toxins, Ptr ToxA, Ptr ToxB and Ptr ToxC, have been identified and characterized in the eighth known pathogen races, while race 4 does not produce any known toxins and are non-pathogenic (Lamari and Strelkov, 2010). Ptr ToxA induces necrosis on sensitive wheat cultivars (Balance *et al*., 1989; Toma's *et al*., 1990; Zhang *et al*., 1997), and is produced by races 1, 2, 7 and 8 (Lamari *et al*., 2003). Ptr ToxB causes chlorosis in sensitive wheat genotypes, and was identified in isolates of races 5 (Oralaza *et al*., 1995), 6, 7 and 8 (Strelkov and Lamari, 2003). Ptr ToxC, causes extensive host chlorosis and was found to be produced by races 1, 3, 6 and 8 (Strelkov and Lamari, 2003).

There are three known effector-dominant susceptibility gene interactions: ToxA-*Tsn1*, which induces necrotic symptoms, ToxB-*Tsc2* and ToxC-*Tsc1*, both causing chloroses (Faris *et al*., 2013). The *Tsn1*-ToxA interaction in development of tan spot is dependent on the host genetic background, and the wheat *Tsn1* gene is a major determinant for susceptibility to the disease (Mofat *et al*., 2014). Lamari *et al*. (2003) noted that this interaction follows the inverse gene-for-gene model. Genotypes without the *Tsn1* gene are insensitive to the toxin (Lamari and Barnier, 1991; Faris *et al*., 1996; Gamba *et al*., 1998; Anderson *et al*., 1999; Friesen *et al*., 2003). However, Adhikari *et al*. (2009) proposed that recognition of ToxA through *Tsn1* may activate important genes involved in host defense response and signaling pathways. The Ptr ToxB-*Tsc2* interaction has accounts for up to 69% of the phenotypic variation in disease caused by race 5 (Friesen and Faris, 2004), so a compatible Ptr ToxB-*Tsc2* interaction plays a major role in tan spot development (Abeysekara *et al*., 2010).

Surveys of wheat fields in Central Asia and Kazakhstan in 2003 showed that tan spot was most common on winter wheat, with the severity that could reach 50% to 100% (Koyshybayev, 2002; Lamari *et al*., 2005b). Analysis of the available studies indicates a widespread pathogen in Kazakhstan (Kokhmetova *et al*., 2016b; Kokhmetova *et al*., 2017). Investigation of *P. tritici-repentis* Ptr population structure in Kazakhstan have drawn attention since the beginning of 2000s, and continued in recent years (Zhanarbekova *et al*., 2005; Maraite *et al*., 2006; Kokhmetova *et al*., 2016b; Kokhmetova *et al*., 2017). As previous varies in different years in Kazakhstan by geographical and climatic zones, and in recent years it has become more widespread globally. The races 1, 3, 4, 6 and 8 were identified in 2013–2015 (Kokhmetova *et al*., 2016b; Kokhmetova *et al*., 2017), and races 1, 2, 3, 7 and 8 in 2018 (Kokhmetova *et al*., 2020b). In both years, races 1 and 8 were dominant. In these years, races 1 and 8 were dominant (Table 1).

Previous study of germplasm resistance (Kokhmetova *et al.,* 2019b) allowed identification of high-yielding wheat genotypes resistant to *P. tritici-repentis*. In 2018, 27 genotypes (42% of those assessed) were insensitive to ToxA, and showed field resistance to the pathogen. In 2020, 20 advanced wheat lines (18% of those assessed) showed moderate to high levels of field resistance to tan spot, and these were selected and recommended for use in the resistance breeding (Kokhmetova and Atishova, 2020c). In 2021, 48 entries (27% of those assessed) with the lowest field assessed tan spot severities were confirmed to be insensitive o Ptr ToxA in the molecular screening. Entries which were resistant under field conditions had similar levels of seedling resistance. Of the 103 host entries evaluated, 28 can be directly used in breeding programmes to improve

Table 1. The frequency of occurrence of *P. tritici-repentis* races in Kazakhstan.

Years	Race								References
	1	2	3	4	5	6	7	8	
2001	+	+	-	-	-	-	-	-	Lamari *et al*., 2005b
2003–2004	+	+	+	+	-	-	-	-	Maraite *et al*., 2006
2013–2015	+	-	+	+	-	+	-	+	Kokhmetova *et al*., 2016b
2018	+	+	+	-	-	-	+	+	Kokhmetova *et al*., 2020b

tan spot resistance and productivity of winter wheat (Kokhmetova *et al*., 2021b).

Integrated plant disease management requires a combination of several strategies to effectively combat disease. For tan spot, the use of resistant wheat varieties is the best option to sustainably manage the disease. In addition, utilizing host resistance it is the most cost-effective and environmentally friendly method for disease control. To this end, the breeding of resistant wheat varieties should be a major objective for tan spot control, which should include assessment of germplasm disease susceptibility (Engle *et al*., 2006).

The objectives of the present study were; 1) to characterize race structure of *P. tritici-repentis* isolates recovered from wheat in south and north Kazakhstan, and 2) to identify the tan spot resistance in wheat cultivars based on disease phenotypes and molecular screening. The results of this study will provide knowledge for regional wheat breeders and plant pathologists involved in development of tan spot management strategies.

MATERIALS AND METHODS

Plant material and field disease phenotyping

This study assessed 80 winter wheat genotypes. These included: 13 cultivars (Almaly, Daulet, Egemen 20, Dana, Diana, Dinara, Krasnovodopadskaya 25, Krasnovodopadskaya 210, 2 Matay, President, Zhadyra, Zhetisu Pirotrix 50), 47 elite lines (10204_1KSI, 10204_2KSI, 10204_3KSI, 10205_2KSI, 10205_3KSI, 601_SP2, 605_SP2, 612_SP2, 620_SP2, 621_SP2, 624_SP2, 630_SP2, 631_SP2, 632_SP2, 634_SP2, 636_SP2, 637_SP2, 638_SP2, 640_SP2, GF_1_CP, GF_2_CP, GF_3_CP, GF_4_CP, GF_5_CP, GF_6_CP, GF_7_CP, GF_8_CP, GF_9_CP, GF_10_CP, 4_PSI, 9_PSI, 1_PSI, 2_PSI, 3_PSI, 5_PSI, 6_PSI, 7_PSI, 8_PSI, 602_SP2, 607_SP2, 609_SP2, 613_SP2, 618_SP2, 635_SP2, 633_SP2, 639_SP2, 10205_1KSI) from Kazakhstan, and 20 cultivars (Aragella, Priirtyshskaya, Danaya, Obskaya ozimaya, Veselka, Povolzhskaya-Niva, Darina, Bazis, Leonida, Turanus, Clavdiya 2, Italmas, Voronezhskaya 18, Kalixo, Streletskaya 12, Universiya, Likamero, Sonett Rima, Obskaya ozimaya) from Russia (Table 3).

Evaluation of adult plant resistance to *P. tritici-repentis* was carried out under field conditions at the Kazakh Research Institute of Agriculture and Plant Growing (KRIAPG), Almalybak (43°13'N, 76°36'E, 789 masl), Almaty Region in southeast Kazakhstan, during the growing seasons of 2019 and 2020. The experiments were completely randomized with three replicate plots. Individual plot size was 3 m^2 (3 m by 7 rows at 15 cm spacings). The source of infection within the field experiments was from naturally colonized wheat straw. In October, before sowing, the infected straw (1 kg m^{-2}) was incorporated into the soil. The growing seasons were favourable for pathogen infection and disease development. Mean daily temperature and relative humidity measurements showed similar trends in both years, although average temperatures were lower in the 2020 than in 2019 growing season. The average maximum air temperature for mid-May in 2019 was 18.6°C and in 2020 was 14.5°C. From April, May and June 2019, mean daily temperatures were, respectively, 11.4°C, 16.9°C and 22.3°C, and in 2020 were 14.0°C, 16.7°C and 21.6°C. For April, May and June 2019 the monthly rainfalls were, respectively, 168, 39 and 72 mm, and mean relative humidity (RH) was 84.13%. In April, May and June 2020, monthly rainfalls were, respectively, 140, 74 and 30 mm, and mean RH was 81.52%, (www. pogodaiklimat.ru/monitor.php, accessed 15 June, 2021). These climatic conditions were highly conducive for tan spot infection and development. Disease was assessed three times at ZGS 75–80 (Zadoks *et al.,* 1974), until maximum disease development was reached. The amounts of plant damage were evaluated as a percentage of leaf area occupied by tan spot. The foliar disease intensity scale of Saari and Prescott (1975), as modified for tan spot (Kremneva and Volkova, 2007) was used for these assessments. Wheat germplasm lines were classified into five groups according to tan spot severity as follows: resistant (R), 5–10%; moderately resistant (MR), 11–20%; moderately susceptible (MS), 21–30%; susceptible (S), 31%+, or immune (I),

0%. The cultivars Salamouni and Glenlea were included as, respectively, susceptible and resistant controls.

Wheat differential lines

Four hexaploid wheat genotypes (Glenlea, 6B662, 6B365, and Salamouni) were included as a differential set, which is effective for the differentiation of eight currently known races of *P. tritici-repentis* (Lamari *et al*., 2003). Seeds of each genotype were sown in 10 cm diam. plastic pots filled with the potting mix at six seeds per pot. The resulting seedlings were maintained in a growth cabinet at 20°C/18°C (day/night) with a 16 h daily photoperiod at 180 mmol m^{-2} s^{-1}, until they were inoculated at the two- to three-leaf stage. Seedlings were assessed 6 d after inoculation and were evaluated based on the development of necrosis or chlorosis or absence of symptoms.

Survey and fungal isolations

Surveys were carried out in the main wheat-growing regions of Kazakhstan during 2019 and 2020 cropping seasons. Each survey sample consisted of 40 leaves exhibiting typical tan spot symptoms, and these were collected randomly from wheat fields in south and north Kazakhstan. Several different wheat fields were surveyed in each region. In south Kazakhstan, 16 fields were surveyed (including disease monitoring in the Karasai, Talgar and Zhambyl regions), while in north Kazakhstan, six fields were surveyed (Karabalyk region). Wheat growth stages at the time of the survey ranged from the beginning of stem elongation (ZGS 30) to the milk stage (ZGS77) (Zadoks *et al*., 1974). Leaves showing symptoms of tan spot were carefully cut and placed in paper envelopes, which were left to air dry at room temperature. Fungal isolations and inoculum production were carried out as described by Lamari and Bernier (1989). Leaves were cut into 1 to 2 cm pieces, surface-sterilized with 30% alcohol for 20 sec then 1% sodium hypochlorite solution for 2 min, and then washed three times (1 min each), with sterile distilled water (Gilchrist-Saavedra *et al*., 2006). The tissue pieces were then placed in Petri dishes, each containing two layers of sterile filter paper moistened with sterile distilled water to maintain high humidity. The dishes were then kept in the dark and incubated for 24 h at 15°C to induce the formation of conidia on the tips of the conidiophores (Lamari and Bernier, 1989). After incubation, the leaf tissue pieces were examined using ×40 binocular magnifiers, and individual conidia identified as *P. tritici-repentis* were placed onto V8-PDA medium (150 mL of V8 juice, 10 g of Potato Dextrose Agar, 3 g of CaCO3, 10 g of water agar, and 850 mL of distilled water) and incubated at 20°C until colonies reached approx. 4 cm diam. A total of 186 single-conidium isolates of *P. tritici-repentis* were obtained, with 122 isolates recovered from south Kazakhstan and 64 from north Kazakhstan. These isolates were subsequently phenotypically characterized on the wheat differential set. A subset of 40 isolates was selected for further characterization (Table 2).

Inoculum production, inoculation, disease assessments and toxin infiltration

The *P. tritici-repentis* cultures were incubated on V8-PDA medium in the dark for 7 to 8 d at 20°C, until colonies reached approx. 4 cm diam. The cultures were then incubated for 24 h under light at room temperature (20–22°C), followed by 24 h at 15°C in the dark. Mycelium plugs (0.5 cm diam.) were then excised from the colonies and transferred singly to 9 cm diam. Petri dishes each containing 25 mL of V8-PDA. Conidia were then harvested by flooding the Petri dishes with sterile distilled water and dislodging the conidia with a wire loop. The inoculum concentration was adjusted to 3,000 conidia mL^{-1} (assessed with a hemocytometer), and a drop of Tween 20 was added per 100 mL to reduce surface tension in the conidium suspensions (Lamari and Barnier, 1989).

Wheat seedlings at the two-leaf stage were sprayed with conidium suspensions to run off, using a hand sprayer. Precautions were taken to avoid cross-infection of isolates. The inoculated seedlings were incubated in a dew chamber for 24 h at 20°C (day) and 18°C (night) with a 16 h daily photoperiod, and 90% relative humidity (Lamari *et al*., 2005b). The seedlings were evaluated for symptom development 7 d after inoculation. Tan spot severity was assessed using the 1 to 5 scale developed by Lamari and Bernier (1989), where: 1 = small, dark-brown to black spots, without any surrounding chlorosis or tan necrosis; 2 = small dark-brown to black spots, with very little chloroses or tan necroses; 3 = small, dark-brown to black spots, completely surrounded by distinct chlorotic or tan necrotic rings, not coalescing; 4 = small, dark-brown to black spots, completely surrounded by tanned chlorotic or necrotic zones, sometimes coalesced; and 5 = most lesions consisting of coalescing chlorotic or tan necrotic tissue. Seedlings with lesion types 1 to 2 were considered to be resistant (–), whereas those with scores of 3 to 5 were classified as susceptible to a given trait (+). For analyses, the seedlings were assigned the following binomials (+,–), (+,+), (–),

and (–,+) to indicate, respectively, the presence (+) or absence (–) of necrosis and chlorosis (Lamari and Bernier, 1991).

Infiltration with toxins was carried out on wheat seedlings at the two-leaf stage (Oralaza *et al*., 1995; Faris *et al*., 1996), which were grown in the conditions described above. The second leaf of each plant (three plants from each genotype) was infiltrated with 25 µL of the purified toxins Ptr ToxA or Ptr ToxB, using a 1 mL capacity syringe. Four leaves of each genotype were treated twice with the culture filtrate of each of the two toxins. The infiltrated plants were then placed in a growth chamber set at 21°C and 16 h daily photoperiod. Plants were evaluated 4 d after infiltration. The leaves of experimental control plants were each infiltrated with 25 µL of sterile distilled water. The leaves were evaluated as sensitive or insensitive to ToxA as presence/absence of necroses, or as sensitive or insensitive to ToxB as presence/absence of chlorosis, on the infiltrated side of each leaf (Faris *et al*., 1996).

Virulence was determined for 186 single conidium *P. tritici-repentis* isolates, which were obtained from infected plants collected from Kazakhstan wheat fields during the 2020 growing season. A total of 40 single conidium isolates were recovered and characterized (Table 2).

Identication of Tsn1 *and* Tsc2 *genes in wheat genotypes*

Genomic DNA was extracted from 5-d-old wheat seedlings using the CTAB method (Riede and Anderson, 1996). To identify the carriers of resistance genes, PCR protocols were used, with primers flanking diagnostic gene markers and DNA samples from the 80 wheat genotypes. Leaf samples from all entries, including the two reference cultivars, were genotyped with the SSR marker *Xfcp623* designed to detect alleles of the *Tsn1* gene. The primers and PCR conditions corresponded to those of Faris *et al*. (2010). The marker had two alleles: 380 bp (the dominant allele of the *Tsn1* gene linked to sensitivity) and the null allele (the recessive allele of the *tsn1* gene linked to insensitivity to Ptr ToxA) (Zhang *et al*., 2009). The sequence of primers for the *Xfcp623* marker (5'–3') were F – CTATTCGTAATCGTGCCTTCCG; R - CCTTCTCTCTCACCGCTATCTCATC (Faris *et al*., 2010), and the *XBE444541* – STS marker for the *Tsc2* locus sensitive to Ptr ToxB. The marker has two alleles: 340 bp (the dominant allele of the *Tsc2* gene linked to sensitivity to the Ptr ToxA) and 505 bp (recessive allele of the *tsc2* gene linked to resistance to the Ptr ToxB). Sequence of primers for marker *XBE444541* (5'–3') were F – TGGACCAGTATGAGA; R – TTCTGGAGGATGTTGAGCAC (Abeysekara *et al*., 2010). PCR reactions were carried out in a T100TM Thermal Cycler (Bio-Rad). Each PCR mixture (25 µL) contained 2.5 µL of genomic DNA (30 ng), 1 µL of each primer (1 pM μL^{-1}) (Sigma Aldrich), 2.5 µL of dNTP mixture (2.5 mM, dCTP, dGTP, dTTP and dATP aqueous solution) (ZAO), 2.5 µL $MgCl_2$ (25 mM), 0.2 µL Taq polymerase (5 units μL^{-1}) (ZAO), 2.5 µL 10× PCR buffer and 12.8 µL ddH_20. PCR amplification was performed with a Mastercycler (Eppendorf), with initial denaturation at 94°C for 3 min, 45 cycles: 94°C for 1 min, annealing at 60°C for 1 min, 72°C for 2 min, and final elongation at 72°C for 10 min. The amplification products were separated on 2% agarose gel in TBE buffer (45 mM Tris-borate, 1 mM EDTA, pH 8) (Chen *et al*., 1998) with the addition of ethidium bromide. A 100-bp DNA ladder (Fermentas) was included to determine amplification lengths. Results were visualized using the Gel Documentation System (Gel Doc XR, BioRad).

RESULTS

Race characterization

The majority of the tested isolates from south Kazakhstan (21.2%) induced chlorosis on the wheat differential line 6B365, but lines 6B662 and "Glenlea" each exhibited a resistant reaction, symptoms typical for race 3 of *P. tritici-repentis*. In these isolates, race 1 (15.1%), race 4 (12.1%), race 5 (6.1%), race 6 (10.0%), race 7 (18.2%) and race 8 (18.2%) were also identified in 2020. Analysis of the virulence of isolates from north Kazakhstan in 2020 showed that they belonged to two races, race 4 (71.4%) and race 7 (28.6%) (Table 2).

Fifty-six wheat genotypes, representing 70% of those assessed, showed insensitivity to both race 1 and race 5 of *P. tritici-repentis*. The most interesting were the 15 entries GF_1_CP, GF_2_CP, GF_5_SP2, GF_6_SP2, GF_7_SP2, GF_10_CP, 10204_3_KSI, 10205_1_KSI, 10205_2_KSI, 601_SP2, 620_SP2, 624_SP2 and 640_SP2 from the Kazakhstan collection and the three entries Danaya, Povolozhskaya Niva, and Darina) from the Russian collection, which were insensitive to the pathogen (severity scores 1 to 1.6), to two races, and to the two toxins (Ptr ToxA and Ptr ToxB).

The purpose of genotyping wheat genotypes using a molecular marker was to identify carriers of genes that control sensitivity to the toxins. The *Xfcp623* marker amplified a fragment of 380 bp associated with the *Tsn1* gene, which demonstrates host sensitivity to the toxin. The results of genotyping with marker *Xfcp623* are presented in Table 3.

Table 2. Reactions of differential *Triticum aestivum* genotypes to inoculation with 40 *Pyrenophora tritici-repentis* isolates collected from Kazakhstan in 2020.

Isolate	Geographic origin	Reaction of differential genotypes to the PTR inoculation			Race number
		Glenlea	6B365	6B662	
KZ-29-S-2020	Almalybak, Almaty oblast.	N	C	R	1
KZ-30-S-2020	Almalybak, Almaty oblast.	N	C	R	1
KZ-7-S-2020	Almalybak, Almaty oblast.	N	C	R	1
KZ-28-S-2020	Almalybak, Almaty oblast.	N	C	R	1
KZ-40-S-2020	Almalybak, Almaty oblast.	N	C	R	1
KZ-23-S-2020	Almalybak, Almaty oblast.	R	C	R	3
KZ-24-S-2020	Almalybak, Almaty oblast.	R	C	R	3
KZ-26-S-2020	Almalybak, Almaty oblast.	R	C	R	3
KZ-31-S-2020	Almalybak, Almaty oblast.	R	C	R	3
KZ-32-S-2020	Almalybak, Almaty oblast.	R	C	R	3
KZ-33-S-2020	Almalybak, Almaty oblast.	R	C	R	3
KZ-34-S-2020	Almalybak, Almaty oblast.	R	C	R	3
KZ-5-S-2020	Almalybak, Almaty oblast.	R	R	R	4
KZ-1-N-2020	Karabalyk agricultural experimental station, Kostanay oblast	R	R	R	4
KZ-2-N-2020	Karabalyk agricultural experimental station, Kostanay oblast	R	R	R	4
KZ-5-N-2020	Karabalyk agricultural experimental station, Kostanay oblast	R	R	R	4
KZ-6-N-2020	Karabalyk agricultural experimental station, Kostanay oblast	R	R	R	4
KZ-8-N-2020	Karabalyk agricultural experimental station, Kostanay oblast	R	R	R	4
KZ-21-S-2020	Almalybak, Almaty oblast.	R	R	R	4
KZ-22-S-2020	Almalybak, Almaty oblast.	R	R	R	4
KZ-27-S-2020	Almalybak, Almaty oblast.	R	R	R	4
KZ-3-S-2020	Almalybak, Almaty oblast.	R	R	C	5
KZ-41-S-2020	Almalybak, Almaty oblast.	R	R	C	5
KZ-4-S-2020	Almalybak, Almaty oblast.	R	C	C	6
KZ-25-S-2020	Almalybak, Almaty oblast.	R	C	C	6
KZ-39-S-2020	Almalybak, Almaty oblast.	R	C	C	6
KZ-1-S-2020	Almalybak, Almaty oblast.	N	R	C	7
KZ-2-S-2020	Almalybak, Almaty oblast.	N	R	C	7
KZ-6-S-2020	Almalybak, Almaty oblast.	N	R	C	7
KZ-47-S-2020	Almalybak, Almaty oblast.	N	R	C	7
KZ-8-S-2020	Almalybak, Almaty oblast.	N	R	C	7
KZ-9-S-2020	Almalybak, Almaty oblast.	N	R	C	7
KZ-3-N-2020	Karabalyk agricultural experimental station, Kostanay oblast	N	R	C	7
KZ-4-N-2020	Karabalyk agricultural experimental station, Kostanay oblast	N	R	C	7
KZ-11-S-2020	Almalybak, Almaty oblast.	N	C	C	8
KZ-12-S-2020	Almalybak, Almaty oblast.	N	C	C	8
KZ-35-S-2020	Almalybak, Almaty oblast.	N	C	C	8
KZ-36-S-2020	Almalybak, Almaty oblast.	N	C	C	8
KZ-37-S-2020	Almalybak, Almaty oblast.	N	C	C	8
KZ-38-S-2020	Almalybak, Almaty oblast.	N	C	C	8

The field evaluations of resistance of 80 wheat genotypes tan spot showed that five lines or cultivars were immune (severity = 0%), and 44 (55%) were resistant (severity = 5–10%). wheat genotypes. The five genotypes identified with immunity to tan spot were GF_2_CP, GF_10_CP, 637_SP2, Matay and President. Table 3 presents average field assessment data for 2018, 2019 and 2020. These field tan spot evaluation results allowed the genotype levels of resistance to be assessed.

Table 3. Reactions of wheat genotypes to *Pyrenophora tritici-repentis* races 1 and 5, and the toxins ToxA and ToxB, in molecular screening and field evaluations.

Wheat genotype	Geographic origin	*Xfcp623, Tsn1*	*XBE444541, Tsc2*	Response to isolates of races and HST toxins				Tan spot field evaluation %
				Race 1 #KZ-7-S-6	Ptr ToxA	Race 5 #KZ-41-N-2019	Ptr ToxB	
GF_2_CP	KZ	*tsn1*	*tsc2*	1.0	I	1.3	I	0
GF_10_CP	KZ	*tsn1*	*tsc2*	1.0	I	1.1	I	0
637_SP2	KZ	*tsn1*	*tsc2*	2.3	I	2.3	I	0
Matay	KZ	*tsn1*	*tsc2*	2.3	I	2.3	I	0
President	KZ	*Tsn1*	*tsc2*	3.3	S	1.0	I	0
GF_6_CP	KZ	*tsn1*	*tsc2*	1.0	I	1.2	I	5
GF_7_CP	KZ	*tsn1*	*tsc2*	1.0	I	1.1	I	5
GF_9_CP	KZ	*Tsn1*	*tsc2*	3.2	S	1.0	I	5
10204_2KSI	KZ	*tsn1*	*tsc2*	2.0	I	2.2	I	5
10204_3KSI	KZ	*tsn1*	*tsc2*	1.0	I	1.1	I	5
601_SP2	KZ	*tsn1*	*tsc2*	1.0	I	1.0	I	5
624_SP2	KZ	*tsn1*	*tsc2*	1.0	I	1.2	I	5
630_SP2	KZ	*tsn1*	*tsc2*	2.2	I	2.0	I	5
631_SP2	KZ	*Tsn1*	*tsc2*	3.5	S	2.3	I	5
Zhadyra	KZ	*Tsn1*	*tsc2*	3.2	S	1.0	I	5
Zhetisu	KZ	*tsn1*	*tsc2*	2.2	I	2.3	I	5
GF_1_CP	KZ	*tsn1*	*tsc2*	1.0	I	1.0	I	10
GF_4_CP	KZ	*tsn1*	*Tsc2*	2.0	I	3.5	S	10
GF_5_CP	KZ	*tsn1*	*tsc2*	1.0	I	1.1	I	10
GF_8_CP	KZ	*tsn1*	*tsc2*	2.1	I	2.1	I	10
4_PSI	KZ	*tsn1*	*tsc2*	2.2	I	2.1	I	10
9_PSI	KZ	*Tsn1*	*tsc2*	3.3	S	1.2	I	10
10204_1KSI	KZ	*tsn1*	*tsc2*	2.1	I	2.2	I	10
10205_2KSI	KZ	*tsn1*	*tsc2*	1.0	I	1.1	I	10
10205_3KSI	KZ	*Tsn1*	*tsc2*	3.2	S	1.1	I	10
605_SP2	KZ	*tsn1*	*Tsc2*	2.2	I	2.5	I	10
612_SP2	KZ	*tsn1*	*Tsc2*	2.2	I	3.3	S	10
620_SP2	KZ	*tsn1*	*tsc2*	1.0	I	1.2	I	10
621_SP2	KZ	*tsn1*	*tsc2*	2.1	I	2.2	I	10
632_SP2	KZ	*tsn1*	*tsc2*	2.1	I	2.3	I	10
634_SP2	KZ	*Tsn1*	*tsc2*	3.2	S	1.0	I	10
636_SP2	KZ	*tsn1*	*tsc2*	2.2	I	2.2	I	10
638_SP2	KZ	*tsn1*	*tsc2*	2.2	I	2.4	I	10
640_SP2	KZ	*tsn1*	*tsc2*	1.0	I	1.1	I	10
Dana	KZ	*tsn1*	*tsc2*	2.2	I	2.2	I	10
Diana	KZ	*tsn1*	*tsc2*	2.2	I	2.2	I	10
Krasnovodopadskaya 210	KZ	*tsn1*	*tsc2*	1.0	I	1.1	I	10
Aragella	RU	*tsn1*	*tsc2*	2.3	I	2.5	I	10
Priirtyshskaya	RU	*tsn1*	*tsc2*	2.1	I	2.1	I	10
Danaya	RU	*tsn1*	*tsc2*	1.0	I	1.6	I	10
Obskaya ozimaya	RU	*Tsn1*	*tsc2*	3.2	S	1.3	I	10
Veselka	RU	*tsn1*	*Tsc2*	2.1	I	3.2	S	10
Povolzhskaya-Niva	RU	*tsn1*	*tsc2*	1.0	I	1.2	I	10
Darina	RU	*tsn1*	*tsc2*	1.0	I	1.5	I	10
Bazis	RU	*Tsn1*	*tsc2*	3.6	S	1.6	I	10
Leonida	RU	*tsn1*	*tsc2*	2.3	I	2.3	I	10

(Continued)

Table 3. (Continued).

Wheat genotype	Geographic origin	*Xfcp623, Tsn1*	*XBE444541, Tsc2*	Response to isolates of races and HST toxins				Tan spot field evaluation %
				Race 1 #KZ-7-S-6	Ptr ToxA	Race 5 #KZ-41-N-2019	Ptr ToxB	
Turanus	RU	*tsn1*	*tsc2*	2.2	I	2.5	I	10
Clavdiya 2	RU	*tsn1*	*tsc2*	2.3	I	2.0	I	10
Italmas	RU	*tsn1*	*Tsc2*	1.0	I	3.5	S	10
Voronezhskaya 18	RU	*Tsn1*	*tsc2*	3.3	S	1.3	I	15
Kalixo	RU	*Tsn1*	*tsc2*	3.4	S	1.1	I	15
Streletskaya 12	RU	*tsn1*	*tsc2*	2.2	I	2.1	I	15
Universiya	RU	*tsn1*	*tsc2*	1.0	I	2.0	I	15
7_PSI	KZ	*tsn1*	*tsc2*	2.2	I	2.1	I	20
633_SP2	KZ	*Tsn1*	*tsc2*	3.4	S	1.3	I	20
Dinara	KZ	*tsn1*	*tsc2*	2.1	I	2.1	I	20
Krasnovodopadskaya 25	KZ	*tsn1*	*tsc2*	1.0	I	2.3	I	20
Pirotrix 50	KZ	*tsn1*	*tsc2*	2.2	I	2.3	I	20
GF_3_CP	KZ	*tsn1*	*tsc2*	1.0	I	2.2	I	25
3_PSI	KZ	*tsn1*	*tsc2*	2.4	I	2.0	I	25
5_PSI	KZ	*tsn1*	*tsc2*	2.2	I	2.1	I	25
6_PSI	KZ	*tsn1*	*tsc2*	2.1	I	2.1	I	25
8_PSI	KZ	*tsn1*	*tsc2*	2.3	I	2.0	I	25
10205_1KSI	KZ	*tsn1*	*tsc2*	1.0	I	1.1	I	25
609_SP2	KZ	*tsn1*	*tsc2*	1.0	I	2.2	I	25
613_SP2	KZ	*tsn1*	*tsc2*	2.3	I	2.2	I	25
635_SP2	KZ	*tsn1*	*tsc2*	2.2	I	2.0	I	25
Likamero	RU	*Tsn1*	*tsc2*	3.4	S	2.2	I	25
Sonett	RU	*Tsn1*	*tsc2*	3.3	S	2.1	I	25
1_PSI	KZ	*tsn1*	*tsc2*	2.3	I	2.2	I	30
2_PSI	KZ	*tsn1*	*tsc2*	2.5	I	2.0	I	30
602_SP2	KZ	*Tsn1*	*tsc2*	2.8	S	2.3	I	30
607_SP2	KZ	*tsn1*	*Tsc2*	2.1	I	2.2	I	30
618_SP2	KZ	*Tsn1*	*tsc2*	3.0	S	1.1	I	30
639_SP2	KZ	*Tsn1*	*Tsc2*	3.3	S	3.4	S	30
Daulet	KZ	*tsn1*	*tsc2*	1.0	I	1.2	I	30
Almaly	KZ	*Tsn1*	*tsc2*	2.3	I	2.2	I	30
Rima	RU	*Tsn1*	*Tsc2*	3.5	S	2.0	I	30
Obskaya ozimaya 2	RU	*Tsn1*	*tsc2*	3.6	S	1.4	I	30
Egemen 20	KZ	*Tsn1*	*tsc2*	3.3	S	2.2	I	40
Salamouni	Lebanon	*tsn1*	*tsc2*	1.0	I	1.0	I	5
Glenlea	Canada	*Tsn1*	-	3.8	S	1.0	I	40
6B662	Unknow	-	*Tsc2*	-	-	3.8	S	35

Notes: KZ: Kazakhstan; RU: Russia; *Xfcp623* is the SSR marker to the *Tsn1* locus sensitive to Ptr ToxA, amplifies a 380 bp DNA fragment; *XBE444541,* the STS marker to the *Tsc2* locus, amplifies a 340 bp DNA fragment in wheat entries sensitive to ToxB and 505 bp in insensitive; Salamouni, the insensitive control for races 1 and 5, toxins Ptr ToxA, and Ptr ToxB, carrier of the recessive genes *tsn1* and *tsc2*; Glenlea, the susceptible control for race 1 and Ptr ToxA, carrier of the dominant *Tsn1* gene; 6B662, susceptible control for race 5 and Ptr ToxB, carrier of the dominant *Tsc2* gene. Lesion types 1–5 based on the Lamari and Bernier's scale (1989); 1–2 indicates resistance, and 3–5, susceptibility. The reaction to toxin infiltration: I, insensitivity; S, susceptibility. Tan spot field evaluation Ptr, % based on the intensity scale of Kremneva and Volkova, 2007. Wheat germplasm was classified into five groups according to tan spot severity as follows: resistant (R): 5–10%, moderately resistant (MR): 11–20%, moderately susceptible (MS): 21–30%, susceptible (S): 31%+ and Immune (I):0%.

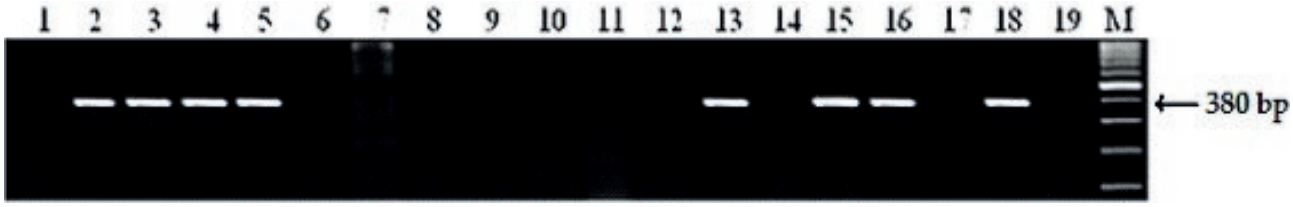

Figure 1. DNA amplification products for wheat cultivars and elite lines obtained with diagnostic marker *Xfcp623* linked to the *Tsn1* gene sensitive to Ptr ToxA. Lane: 1, GF_3_CP; 2, GF_9_CP; 3, 10205_3KSI; 4, 631_SP2; 5, 634_SP2; 6, 1_PSI; 7, Daulet; 8, Dinara; 9, Streletskaya 12; 10, Pirotrix 50; 11, Zhetisu; 12, Aragella; 13, President; 14, Matay; 15, Voronezhskaya 18; 16, Sonett; 17, Salamouni (resistant reference cultivar for race 1, insensitive to Ptr ToxA, with recessive gene *tsnl*); 18, Glenlea (susceptible reference cultivar for race 1, sensitive to Ptr ToxA, with dominant gene *Tsnl*); 19, ddH_2O; M, molecular weight marker (Gen-RulerTM; 100 bp DNA Ladder). Fragments amplified by *Xfcp623* were separated in 2% agarose gels. The bands are 380 bp for the *Tsn1* allele (lanes 2, 3, 4, 5, 13, 15, 16 and 18, control), sensitive to Ptr ToxA; and null allele for the *tsn1* allele, insensitive to Ptr ToxA (lanes 1, 6, 7, 8, 9, 10, 11, 12, 14 and 17 control).

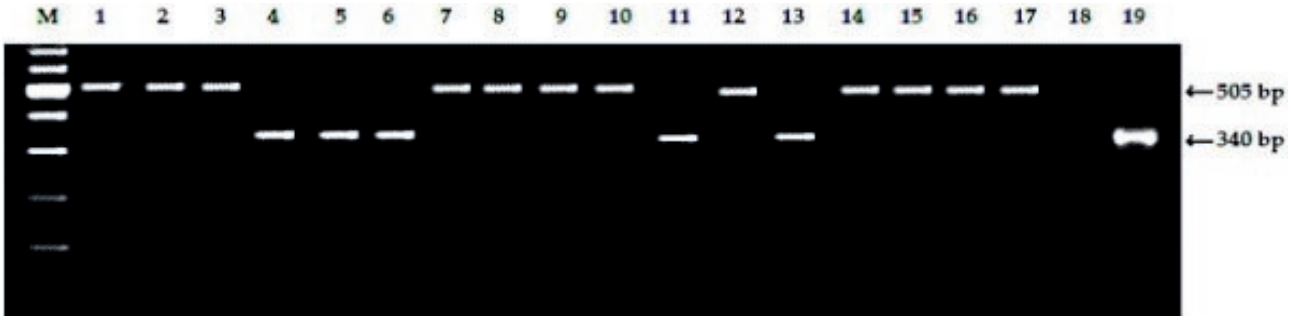

Figure 2. DNA amplification products for wheat cultivars and elite lines obtained with diagnostic marker *XEE444541* linked to the *Tsc2* gene sensitive to Ptr ToxB. Lane: M, molecular weight marker (Gen-RulerTM; 100 bp DNA Ladder), 1, GF_1_CP; 2, GF_10_CP; 3, 609_SP2; 4, GF_4_CP; 5, 612_SP2; 6, 639_SP2; 7, Egemen 20; 8, Almaly; 9, Danaya; 10, 601_SP2; 11, Veselka; 12, Dinara; 13, Italmas; 14, Zhadyra; 15, Pirotrix 50; 16, 640_SP2; 17, 10204_1KSI; 18, ddH_2O; 19, 6B662 (susceptible reference genotype for race 5, sensitive to Ptr ToxB with dominant gene *Tsc2*). Fragments amplified by *XEE444541* were separated in 2% agarose gels. The bands are 340 bp for the *Tsc2* allele (lanes 4, 5, 6, 11, 13 and 19), sensitive to Ptr ToxB and null allele for the *tsc2* allele, insensitive to Ptr ToxB (lanes 1, 2, 3, 7, 8, 9, 10, 12, 14, 15, 16 and 17).

The proportion of genotypes insensitive to Ptr ToxA (*tsn1*) was high, with 59 of the 80 tested genotypes insensitive to the toxin. The genotypes included 47 from Kazakhstan (78%) and 12 from Russia (60%) (Table 3). Examples of PCR results for 18 host genotypes are shown in Figure 1. Seven genotypes (GF_9_CP, 10205_3KSI, 631_SP2, 634_SP2, President, Voronezhskaya 18, and Sonett) had 380 bp fragments, indicative of the dominant *Tsn1* allele conferring toxin Ptr ToxA sensitivity. Nine genotypes (GF_3_CP, 1_PSI, Daulet, Dinara, Streletskaya 12, Pirotrix 50, Zhetisu, Aragella, and Matay) gave no amplification products (null allele), indicative of the recessive tsn1 allele conferring insensitivity to the toxin Ptr ToxA (Figure 1).

The *XBE444541* marker amplified a 340 bp fragment linked to the *Tsc2* allele, which controls sensitivity to the toxin in eight wheat entries (GF_4_CP, 605_SP2, 607_SP2, 612_SP2, 639_SP2, Rima, Veselka, Italmas and in control 6B662). Twelve host genotypes (GF_1_CP, GF_10_SP, 609_SP2, Egemen, Almaly, Danaya, 601_SP2, Dinara, Zhadyra, Pirotrix 50, 640_SP2, 10204_1KSI) had the amplification product (505 bp) indicative of the recessive *tsc2* allele, conferring toxin Ptr ToxB insensitivity (Figure 2). These 12 genotypes all showed insensitivity to race 5 and Ptr ToxB toxin when screened using the race 5 isolate and HST Ptr ToxB infiltrate. In general, the proportion of the examined genotypes insensitive to Ptr ToxB was high, at 72 of the 80 tested genotypes. The degree of linkage of the marker *XBE444541* with insensitivity to race 5 and Ptr ToxB was 90% (Table 3).

Identification of genotypes resistant to *P. tritici-repentis* was based on the results of molecular analyses, screening of the wheat genotypes for reaction to races 1 and 5 of the pathogen, as well as reactions to the toxins Ptr ToxA and Ptr ToxB. The reactions of wheat genotypes to isolates of races and Ptr ToxA and Ptr ToxB are presented in Table 3. In general, the frequency of genotypes resistant to race 1 and race 5 in the wheat collection was high at 70%. Eighteen wheat entries presented the were the most resistant. These were: GF_1_CP,

GF_2_CP, GF_5_CP, GF_6_CP, GF_7_CP, GF_10_CP, 10204_3KSI, 10205_1KSI, 10205_2KSI, 601_SP2, 620_SP2, 624_SP2, 640_SP2, Daulet, Krasnovodopadskaya 210, Danaya, Povolzhskaya-Niva, and Darina. These host lines showed insensitivity (scores 1–1.6 point) to both races of the pathogen and to two toxins (Ptr ToxA and Ptr ToxB), and were also insensitive to the toxins as indicated in the molecular screening (Table 3). A moderate degree of insensitivity to pathogen races and the toxins was observed in 38 wheat entries. Susceptibility to race 1 and ToxA was found in twenty host genotypes (25%), including twelve Kazakh lines and eight Russian cultivars. Susceptibility to race 5 and ToxB was detected in only five of the host genotypes.

DISCUSSION

The race population structure of *P. tritici-repentis* in Kazakhstan has had large fluctuations in recent years (Kokhmetova *et al*., 2016b., Kokhmetova *et al*., 2020b). Population structure and race composition of the pathogen has been studied in many geographic regions in the world. In North American pathogen collections, Lamari *et al*. (1995) first identified races 1 to 4, with prevailing races 1 and 2 (Lamari *et al.,*1998). Later, these races were identified in mainly wheat growing regions. Race 1 was identified in Azerbaijan, Kyrgyzstan, Kazakhstan, Uzbekistan and Syria, and race 2 was found in Azerbaijan and Kazakhstan and in South America (Lamari *et al.,* 2005a, Kokhmetova *et al.,* 2018b, Kokhmetova *et al.,* 2019a, Gamba *et al.,* 2012). Studies conducted in 2016 to determine the racial composition of the pathogen in Kazakhstan showed that races 1 and 8 were dominant (Kokhnetova *et al*., 2016b). Benslimane *et al.* (2011) showed that six PTR races were identified in Algeria (races 1, 4, 5, 6, 7 and 8). Four of these (races 1, 4, 7 and 8) are described in Algeria for the first time. Lamari *et al.* (1998) were the first to report race 5 in Algeria, and this race has since been reported in Canada (Strelkov *et al*., 2002), the United States of America (Ali *et al*.,1999), Syria and Azerbaijan (Lamari *et al*., 2005b). In contrast, race 6 was found in Algeria and Morocco (Strelkov *et al*., 2002; Benslimane, 2018; Gamba *et al*., 2017), while races 7 and 8 were found only in the Middle East, Caucasus and Algeria, and Kazakhstan in 2018 and 2020 (Kokhmetova *et al*., 2020b; Benslimane, 2018, Ouaar *et al*., 2022). In 2021, in the North Caucasus region of Russia, races 1, 3 and 4 were identified (Kremneva *et al*., 2021). Races 2, 4, 5 and 7 were found in Tunisia by Kamel *et al*. (2019).

Studies carried out in 2018 on reaction of wheat germplasm to inoculation and toxin infiltration made it possible to identify more than 78% of entries that are simultaneously resistant to *P. tritici-repentis* races 1 and 5 and to the Ptr ToxA and Ptr ToxB (Kokhmetova *et al*., 2018b). In previous studies in Kazakhstan in 2019, the present authors found positive correlations between seedling and field scores (Kokhmetova *et al.,* 2019b).

Races 1 and 8 were predominant in 2016 in isolates from southeast Kazakhstan. (Kokhmetova *et al.,* 2016b). In 2018, five races of *P. tritici-repentis* were identified in Kazakhstan, including races 1, 2, 3, 7 and 8 (Kokhmetova *et al.,* 2020b). The results from the present study indicate the presence of seven races, 1, 3, 4, 5, 6, 7 and 8 in this country. Race 2, found in the 2018 studies, was absent, but additional races 4, 5 and 6 were found. These differences in *P. tritici-repentis* population structure in Kazakhstan indicate the need for annual monitoring, and study of the distribution of tan spot. This would enhance understanding of the dynamics of variability and distribution of *P. tritici-repentis* and the disease this pathogen causes.

In 2020 most of the wheat cultivars from Kazakhstan (72.6%) showed sensitive responses to race 1 of *P. tritici-repentis*, while 67.5% of the lines were resistant to race 5. As a result of this study, 25 lines with the best combinations of SNP alleles associated with resistance to races 1 and 5 were identified, for use as candidates for future wheat variety selection and release (Kokhmetova *et al.,*2021a).

In the present study, a collection of 80 common wheat accessions, including promising lines and cultivars from Kazakhstan and Russia, were evaluated for reaction to race 1 and 5 of *P. tritici-repentis*, and to Ptr ToxA, and Ptr ToxB, and were characterized using the *Xfcp623* and *XBE444541* molecular markers diagnostic for the *Tsn1* and *Tsc2* genes. The *XBE444541* marker amplified a 340 bp fragment linked to the *Tsc2* allele, which controls sensitivity to the toxin in eight wheat entries. However, the race 5 isolate did not always cause chlorosis in wheat genotypes, for which the presence of a dominant allele of the *Tsc2* gene, sensitive to Ptr ToxB, was assumed. Thus, a resistant reaction to race 5 and the Ptr ToxB, instead of the expected susceptible reaction, was found in the wheat lines 605_SP2 and 607_SP2, and in Rima. This is consistent with the results of a number of studies on the interaction of genes *Tsn1* and *Tsc2* and toxins of *P. tritici-repentis*, where it has been shown that sensitivity to toxins does not always determine sensitivity to tan spot and depends on the genetic background of the host, i.e., on a specific wheat genotype (Chu *et al.,* 2008, Kariyawasam *et al*., 2016). Zhang *et al.* (2009) have also observed differential responses to toxins and conidium inoculations. Durum and common wheat breed-

ers alike should strive to remove both Tsc1 and Tsc2 from their materials, using marker-assisted selection to achieve disease resistance (Virdi *et al.,* 2016).

From a disease management point of view, 18 wheat entries were shown to have resistance to races 1 and 5 of *P. tritici-repentis*, and confirmed resistance to Ptr ToxA in molecular screening. These include fifteen wheat cultivars from Kazakhstan and three from Russia. Susceptibility to Ptr ToxA did not always correlate with susceptibility to race 1 of the pathogen, and depended on the genetic background of the hosts. In the previous study, 19 winter wheat entries were highly resistant to race 1 and resistant under field conditions, so it is recommended that these genotypes are used to deploy resistance genes in wheat breeding programmes (Kokhmetova *et al*., 2021b). Evolution of virulence involves the generation of genetic variation, followed by selection. Genetic variation arises by mutation, chromosomal rearrangement, recombination, and inter- and intra-species hybridization (Burnett, 2003).

The present study has shown the prevalence of a diverse population of *P. tritici-repentis* in regions of Kazakhstan. Differences in results in regions may depend on wheat varietal characteristics and climatic conditions, which differed in each region. The obtained data indicate that annual studies should continue to recognize the population dynamics of *P. tritici-repentis*, as well race distribution areas. The pathogen should also be periodically monitored for any virulence changes. The identification of six *P. tritici-repentis* races on wheat demonstrates high diversity of the pathogen population in Kazakhstan, which requires further in-depth characterization. The results of genotyping and screening of wheat entries for resistance to the most common races of *P. tritici-repentis* in Kazakhstan will increase efficiency of breeding, based on the elimination of carriers of dominant alleles of the *Tsn1* gene, which provides sensitivity to the aggressive toxin Ptr ToxA toxin from breeding material. Carriers of the identified *tsn1* gene for resistance to Ptr ToxA can be used in breeding programmes for pyramiding of genes for resistance to wheat diseases.

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AUTHOR CONTRIBUTIONS

AK and MK conceived the manuscript and designed the research. MK and NK analyzed the data and wrote the manuscript. MA, KA, NM and ZhK generated the phenotypic and genotyping data. All authors reviewed the manuscript.

LITERATURE CITED

Abeysekara N.S., Friesen T.L., Liu Z., McClean P.E., Faris J.D., 2010. Marker development and saturation mapping of the tan spot Ptr ToxB sensitivity locus *Tsc2* in hexaploid wheat. *Plant Genome* 3: 179189. https://doi.org/10.3835/plantgenome2010.07.0017.

Adhikari T.B., Bai J., Meinhardt S.W., Gurung S., Myrfeld M.…Rasmussen, J.B., 2009. *Tsn1*-mediated host responses to ToxA from *Pyrenophora tritici-repentis. Molecular Plant-Microbe Interactions* 22: 1056–1068. https://doi.org/10.1094/MPMI-22-9-1056

Ali S., Francl L.J., De Wolf E.D., 1999. First report of *Pyrenophora tritici-repentis* race 5 from North America. *Plant Disease* 83: 591–591. https://doi.org/10.1094/pdis.1999.83.6.591a

Anderson J.A., Efertz R.J., Faris J.D., Francl L.J., Meinhardt S.W., 1999. Genetic analysis of sensitivity to *Pyrenophora tritici-repentis necrosis-inducing toxin in durum and common wheat*. *Phytopathology* 89, 293–297. https://doi.org/10.1094/PHYTO.1999.89.4.293

Balance G.M., Lamari L., Bernier C.C., 1989. Purifcation and characterization of a host selective necrosis toxin from *Pyrenophora tritici-repentis*. *Physiological and Molecular Plant Pathology* 35: 203–213.https://doi.org/10.1016/0885-5765(89)90051-9

Benslimane H., Lamari L., Benbelkacem,A., Sayoud R., Bouznad Z., 2011. Distribution of races of *Pyrenophora tritici-repentis* in Algeria and identication of a new virulence type. *Phytopathologia Mediterranea* 50: 203–211. https://doi.org/10.14601/Phytopathol_Mediterr-8746

Benslimane H., 2018. Virulence phenotyping and molecular characterization of a new virulence type of *Pyrenophora tritici-repentis* the causal agent of Tan Spot. *Journal of Plant Pathology* 34: 139–142. https://doi.org/10.5423/PPJ.NT.07.2017.0150

Burnett J., 2003. Fungal Populations Species. New York: Oxford University Press.

Chen X., Line R., Leung H., 1998. Genome scanning for resistance gene analogs in rice, barley, and wheat by high resolution electrophoresis. *Theoretical and Applied Genetics* 97: 345–355. https://doi.org/10.1007/s001220050905

Chu C.G., Friesen T.L., Faris J.D., Xu S.S., 2008. Evaluation of seedling resistance to tan spot and *Stagonospora nodorum* blotch in tetraploid wheat. *Crop Science* 48: 1107–1116. https://doi.org/10.2135/cropsci2007.09.0516.

Cotuna O., Paraschivu M., Paraschivu A., Sarateanu V., 2015. The influence of tillage, crop rotation and residue management on tan spot *Drechslera tritici repentis*. Died. Shoemaker in winter wheat. *Research Journal of Agricultural Sciences* 47: 13–21.

Duveiller E., Dubin H.J., Reeves J., McNab A., 1998. *Helminthosporium Blights of Wheat: Spot Blotch and Tan Spot.* El Batan, Mexico: CIMMYT.

Engle J.S., Madden L.V. Lipps P.E., 2006. Distribution and pathogenic characterization of *Pyrenophora tritici-repentis* and *Stagonospora nodorum* in Ohio. *Phytopathology* 96: 1355–1362. https://doi.org/10.1094/PHYTO-96-1355

FAO, IFAD, UNICEF, WFP, and WHO, 2019. The State of Food Security and Nutrition in the World 2019. Safeguarding against economic slowdowns and downturns (Rome: FAO). Licence: CC BY-NC-SA 3.0 IGO. http://www.fao.org/3/ca5162en/ca5162en.pdf

Faris J.D., Anderson J.A., Francl, L.J Jordahl, J.G., 1996. Chromosomal location of a gene conditioning insensitivity in wheat to a necrosis-inducing culture fltrate from *Pyrenophora tritici-repentis*. *Phytopathology* 86: 459–463. https://doi.org/10.1094/Phyto-86-459

Faris J.D., Zhang Z., Lu H., Lu S. Reddy L., 2010. A unique wheat disease resistance-like gene, governs effector-triggered susceptibility to necrotrophic pathogens. *Proceedings of the National Academy of Sciences of the USA*, 107: 13544–13549. https://doi.org/10.1073/pnas.1004090107.

Faris J.D., Liu Z., Xu S.S., 2013. Genetics of tan spot resistance in wheat. *Theoretical and Applied Genetics* 126: 2197–2217. https://doi.org/10.1007/s00122-013-2157-y

Friesen T.L., Ali S., Kianian S., Francl L.J., Rasmussen J.B., 2003. Role of host sensitivity to Ptr ToxA in development of tan spot of wheat. *Phytopathology* 93: 397–401. https://doi.org/10.1094/PHYTO.2003.93.4.397

Friesen T.L., Faris J.D., 2004. Molecular mapping of resistance to *Pyrenophora tritici-repentis* race 5 and sensitivity to Ptr ToxB in wheat. *Theoretical and Applied Genetics* 109: 464–471. https://doi.org/10.1007/s00122-004-1678-9

Gamba F.M., Lamari L., Brülé-Babel A.L., 1998. Inheritance of race-specifc necrotic and chlorotic reactions induced by *Pyrenophora tritici-repentis* in hexaploïd wheats. *Canadian Journal of Plant Pathology* 20: 401–407. https://doi.org/10.1080/07060669809500411

Gamba F.M., Strelkov S.E., Lamari L., 2012. Virulence of *Pyrenophora tritici-repentis* in the Southern Cone Region of South America. *Canadian Journal of Plant Pathology* 34: 545–550. https://doi.org/10.1080/07060661.2012.695750

Gamba F.M., Bassi F.M., Finckh M.R., 2017. Race structure of *Pyrenophora tritici-repentis* in Morocco. *Phytopathologia Mediterranea* 56: 119–126. https://doi.org/10.14601/Phytopathol_Mediterr-18830

Gilchrist-Saavedra L., Fuentes-Dávila G., Martínez-Cano C., López-Atilano R.M., Duveiller E.,… García A.I., 2006. Practical Guide to the Identification of Selected Diseases of Wheat and Barley. Second edition. Mexico, D.F.: CIMMYT

Giraldo P., Benavente E., Manzano-Agugliaro F., Gimenez E., 2019**.** Worldwide research trends on wheat and barley: A bibliometric comparative analysis. *Agronomy* 9: 352. https://doi.org/10.3390/agronomy9070352

Gultyaeva E.I., Kokhmetova A.M., Shreyder E.R., Shaydayuk,E.L., Atishova., M.N.,…Galymbek, K., 2020. Genetic variability of perspective btreeding material of spring bread wheat for resistance to leaf rust in russia and Kazakhstan. *Bulletin of NAS RK* 3: 60-68. https://doi.org/10.32014/2020.2518-1467.70

Gurung S., Mamidi S., Bonman J.M., Xiong M., Brown-Guedira G., 2014. Genome-wide association study reveals novel quantitative trait loci associated with resistance to multiple leaf spot diseases of spring wheat. *PloS One* 9. https://doi.org/10.1371/journal.pone.0108179

Hosford R.M.J., 1971. A form of *Pyrenophora trichostoma* pathogenic to wheat and other grasses. *Phytopathology* 61: 28–32. https://doi.org/10.1094/Phyto-61-28

Hosford R.M.J., 1982. Tan spot-developing knowledge 1902–1981, virulent races and differentials, methodology, rating systems, other leaf diseases, literature. Tan spot of wheat and related diseases workshop. Ed. R.M. Hosford Jr. Fargo, NDSU, 1–24 pp.

Kamel S., Cherif M., Hafez M., Despins T., Aboukhaddour R., 2019. *Pyrenophora tritici–repentis* in Tunisia: Race Structure and Effector Genes. *Frontiers in Plant Science* 10: 1562. https://doi.org/10.3389/fpls.2019.01562

Kariyawasam G.K., Carter A.H., Rasmussen J.B., Faris J.D., Xu S.S., 2016. Genetic relationships between

race-nonspecifc and race-specifc interactions in the wheat *Pyrenophora tritici-repentis* pathosystem. *Theoretical and Applied Genetics* 129: 897908. https://doi.org/0.1007/s001220162670x.

Koishybaev M.K., 2002. *Diseases of Crops.* Almaty: Bastau Publ. (in Russian)

Kokhmetova A., Madenova A., Kampitova G., Urazaliev R., Yessimbekova M., …Purnhauser L., 2016a. Identification of leaf rust resistance genes in wheat cultivars produced in Kazakhstan. *Cereal Research Communications* 44(2): 240–250. https://doi.org/10.1556/0806.43.2015.056.

Kokhmetova A.M., Kremneva, O.Yu., Keyshilov, Zh.S., Sultanova, N.Zh., 2016b. Race range and virulence of *Pyrenophora tritici-repentis* isolates in the Republic of Kazakhstan and the North Caucasus region of Russia. *Eurasian Journal of Applied Biotechnology* 3: 57–66. (in Russian)

Kokhmetova A., Kremneva, O., Volkova, G., Atishova, M., Sapakhova, Z., 2017. Evaluation of wheat cultivars growing in Kazakhstan and Russia for resistance to tan spot. *Journal of Plant Pathology* 99: 161–167. https://doi.org/10.4454/jpp.v99i1.3812.

Kokhmetova A., Sharma, R., Rsaliyev S., Galymbek, K., Baymagambetova, K., …Morgounov A., 2018a. Evaluation of Central Asian wheat germplasm for stripe rust resistance. *Plant Genetic Resources* 16(2): 178–184. https://doi.org/10.1017/S1479262117000132.

Kokhmetova A.M., Ali S., Sapakhova Z., Atishova M.N., 2018b. Identification of genotypes-carriers of resistance to tan spot Ptr ToxA and Ptr ToxB of *Pyrenophora tritici*-repentis in common wheat collection. *Vavilov Journal of Genetics and Breeding* 22: 978–986. https://doi.org/10.18699/vj18.440

Kokhmetova A.M., Atishova M.N., Madenova A.K., Kumarbayeva M.T., 2019a. Genotyping of wheat germplasm for resistance to toxins of tan spot *Pyrenophora tritici-repentis*. *Journal of Biotechnology* 305: S5, https://doi.org/10.1016/j.jbiotec.2019.05.188

Kokhmetova A., Atishova M., Kumarbayeva M., Leonova I.N., 2019b. Phytopathological screening and molecular marker analysis of wheat germplasm from Kazakhstan and CIMMYT for resistance to tan spot. *Vavilov Journal of Genetics and Breeding* 23: 879–886. https://doi.org/10.18699/vj19.562

Kokhmetova A.M., Atishova M.N., Galymbek K., 2020a. Identification of wheat germplasm resistant to leaf, stripe and stem rust using molecular markers. *Bulletin of NAS RK* 2(384): 45–52. https://doi.org/10.32014/2020.2518-1467.40

Kokhmetova A.M., Kovalenko N.M., Kumarbaeva M.T., 2020b. *Pyrenophora tritici-repentis*population structure in the Republic of Kazakhstan and identification of wheat germplasm resistant to tan spot. *Vavilov Journal of Genetics and Breeding* 24(7): 722–729. https://doi.org/10.18699/VJ20.666

Kokhmetova A., Atishova M., 2020c. Identification wheat genotypes resistant to tan spot *Pyrenophora tritici-repentis*. *Bulletin of NAS RK* 2(384): 29–35. https://doi.org/10.32014/2020.2518-1467.38

Kokhmetova, A., Sehgal, D., Ali, S., Atishova, M., Kumarbayeva, M., …Dreisigacker, S., 2021a. Genome-Wide Association Study of Tan Spot Resistance in a Hexaploid Wheat Collection From Kazakhstan. *Frontiers in Genetics* 11: 581214. https://doi.org/10.3389/fgene.2020.581214

Kokhmetova,A., Kumarbayeva M., Atishova M., Nehe A., …Morgounov A., 2021b. Identification of high-yielding wheat genotypes resistant to *Pyrenophora tritici-repentis* (tan spot). *Euphytica* 217: 97. https://doi.org/10.1007/s10681-021-02822-y

Kremneva O.Y., Mironenko N.V., Volkova G.V., Baranova O.A., Kim Y.S., Kovalenko N.M., 2021. Resistance of winter wheat varieties to tan spot in the North Caucasus region of Russia. *Saudi Journal of Biological Sciences* 28(3): 1787–1794. https://doi.org/10.1016/j.sjbs.2020.12.021.

Kremneva O.Y., Volkova G.V., 2007. Diagnostics and methods for assessing the wheat resistance to the causative agent of tan spot. Guidelines. Russian Agricultural Academy Printing House, Moscow (in Russian).

Lamari L., Bernier C.C., 1989. Evaluation of wheat lines and cultivars to tan spot (*P. tritici-repentis*) based on lesion type. *Canadian Journal of Plant Pathology* 11: 49–56. https://doi.org/10.1080/07060668909501146

Lamari L., Bernier C.C., 1991. Genetics of tan necrosis and extensive chlorosis in tan spot of wheat caused by *Pyrenophora tritici-repentis*. *Phytopathology* 81: 1092–1095. https://doi.org/10.1094/Phyto-81-1092

Lamari L., Sayoud R., Boulif, M., Bernier C.C., 1995. Identification of a new race in *Pyrenophora tritici-repentis:* implications for the current pathotype classification system. *Canadian Journal of Plant Pathology* 17(4): 312–318. https://doi.org/10.1080/07060669509500668

Lamari L., Gilbert J., Tekauz A., 1998. Race differentiation in *Pyrenophora tritici-repentis* and survey of physiologic variation in western Canada. *Canadian Journal of Plant Pathology* 20(4): 396–400.

Lamari L., Strelkov S.E., Yahyaoui A., Orabi J., Smith R.B., 2003. The identifcation of two new races of *Pyrenophora tritici-repentis* from the host center of diversity confrms a one-to-one relationship in tan

spot of wheat. *Phytopathology* 93: 391–396. https://doi.org/10.1094/PHYTO.2003.93.4.391

Lamari L., McCallum B.D., Depauw R.M., 2005a. Forensic pathology of Canadian bread wheat: the case of tan spot. *Phytopathology* 95: 144–152. https://doi.org/10.1094/phyto-95-0144

Lamari L., Strelkov, S.E., Yahyaoui A., Amedov,M., Saidov, M., …Koichibayev, M., 2005b. Virulence of *Pyrenophora triticirepentis* in the countries of the Silk Road. *Canadian Journal of Plant Pathology* 27: 383–388. https://doi.org/10.1080/07060661.2012.695750.

Lamari L., Strelkov S.E., 2010. The wheat/*Pyrenophora tritici-repentis* interaction: progress towards an understanding of tan spot disease. *Canadian Journal of Plant Pathology* 32, 4–10.

Madenova A., Sapakhova Z., Bakirov S., Galymbek K., Yernazarova G., …Keishilov Zh., 2021. Screening of wheat genotypes for the presence of common bunt resistance genes. *Saudi Journal of Biological Sciences* 28: 2816–2823. https://doi.org/10.1016/j.sjbs.2021.02.013

Maraite H., Mercado-Vergnes D., Renard M.-E., Zhanarbekova A., Duveiller E., 2006. Relevance of pathogen diversity in management of leaf spot and leaf blight diseases on wheat in Central Asia. *Agromeridian* 2: 105–114.

Mofat C.S., See P.T., Oliver R.P., 2014. Generation of a ToxA knockout strain of the wheat tan spot pathogen *Pyrenophora tritici-repentis*. *Molecular Plant Pathology* 15: 918–926. https://doi.org/10.1111/mpp.12154

Orolaza N.P., Lamari L., Balance G.M., 1995. Evidence of a host-specifc chlorosis toxin from *Pyrenophora tritici-repentis*, the causal agent of tan spot of wheat. *Phytopathology* 85: 1282–1287. https://doi.org/10.1094/Phyto-85-1282

Ouaar N., Benbelkacem A., Singh P.K., Oumata *S.*, Benslimane H., 2022. Reaction of Algerian and international germplasm of wheat against races 1 and 5 of *Pyrenophora tritici-repentis* the causal agent of tan spot. *Cereal Research Communications* 50(1): 75–84. https://doi.org/10.1007/s42976-021-00161-1

Phuke R.M., He X., Juliana P., Bishnoi S.K., Singh G.P., …Singh, P.K., 2020. Association Mapping of Seedling Resistance to Tan Spot (*Pyrenophora tritici-repentis* Race 1) in "CIMMYT and South Asian Wheat Germplasm. *Frontiers in Plant Science* 11: 1309. https://doi.org/10.3389/fpls.2020.01309

Rees R.G., Platz G.J., 1992. Tan spot and its control - some Australian experiences, in Advances in tan spot research. (L.J. Francl, J.M. Krupinsky, M.P. McMullen, ed.). North Dakota Agricultural Experiment Station, Fargo, ND, USA. *Agriculture* 1992, pp. 1–15.

Riede C.R., Anderson J.A., 1996. Linkage of RFLP markers to an aluminum tolerance gene in wheat. *Crop Science* 36(4): 905–909.

Saari E.E., Prescott L.M., 1975. A scale for appraising the foliar intensity of wheat diseases. *Plant Disease Reporter* 59, 377–380.

Strelkov S.E., Lamari L., Balance G.M., 1999. Characterization of a host specifc protein toxin (Ptr ToxB) from *Pyrenophora tritici-repentis*. *Molecular Plant-Microbe Interactions* 12: 728–732. https://doi.org/10.1094/MPMI.1999.12.8.728

Strelkov S.E., Lamari L., Sayoud R., Smith R.B., 2002. Comparative virulence of chlorosis-inducing races of *Pyrenophora tritici-repentis*. *Canadian Journal of Plant Pathology* 24: 29–35. https://doi.org/10.1080/07060660109506967

Strelkov S.E., Lamari L., 2003. Host-parasite interaction in tan spot *Pyrenophora tritici-repentis* of wheat. *Canadian Journal of Plant Pathology* 25: 339–349. https://doi.org/10.1080/07060660309507089

Tekauz A., 1976. Distribution, severity and relative importance of leaf spot disease of wheat in western Canada in 1974. *Canadian Plant Disease Survey* 56: 36–40.

Toma's A., Feng G.H., Reeck G.R., Bockus W.W., Leach J.E., 1990. Purifcation of a cultivar specifc toxin from, *Pyrenophora tritici-repentis*, causal agent of tan spot of wheat. *Molecular Plant-Microbe Interactions* 3: 221–224. https://doi.org/10.1094/MPMI-3-221

Virdi S. K., Liu Z., Overlander M. E., Zhang Z., Xu S. S., 2016. New Insights into the Roles of Host Gene-Necrotrophic Effector Interactions in Governing Susceptibility of Durum Wheat to Tan Spot and Septoria nodorum Blotch. G3 (Bethesda, Md.), 6(12), 4139–4150. https://doi.org/10.1534/g3.116.036525

Wegulo S.N., 2011. Tan spot of cereals. *The Plant Health Instructor* https://doi.org/10.1094/PHI-I-2011-0426-01

Zadoks J.C., Chang T.T., Konzak M.M., 1974. A decimal code for the growth stages of wheat. *Weed Research* 14: 415–421. https://doi.org/10.1111/j.1365-3180.1974.tb01084.x

Zhanarbekova A.B., Koishibayev M., Maraite H., Duveiller E., Mercado D.M., 2005. The distribution of tan spot on wheat and race structure of *Drechslera tritici-repentis* in Kazakhstan and neighboring CIS countries. In: *Proceedings International Scientific Conference "Modern Problems of Plant Protection and Quarantine"*. Almaty, Kazakhstan, 2005, 371–376.

Zhang H.F., Francl LJ., Jordahl J.G., Meinhardt S.W., 1997. Structural and physical properties of necrosis inducing toxin from *Pyrenophora tritici-repentis*. *Phytopathology* 87: 154–160. https://doi.org/10.1094/phyto.1997.87.2.154

Zhang Z., Friesen T.L., Simons K.J., Xu S.S., Faris J.D., 2009. Development, identification, and validation of markers for marker assisted selection against the *Stagonospora nodorum* toxin sensitivity genes *Tsn1* and *Snn2* in wheat. *Molecular Breeding* 23: 3549. https://doi.org/10.1007/s1103200892115.