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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.) Drechs (anamorph Dreschlera 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 Bernier, 1989; Strelkov et al., 1999; Lamari et al., 2003). Three host-specific toxins, Ptr ToxA, Ptr ToxB andPtr 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 susceptible 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% (Koysybayev, 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 to 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
Pyrenophora tritici-repentis races in Kazakhstan

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 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, 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, 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, 639_SP2, 10205_1KSI) from Kazakhstan, and 20 cultivars (Aragella, Priirtyshskaya, Danaya, Obskaya ozymaya, Veselka, Povolzhskaya-Niva, Darina, Bazis, Leonida, Turanus, Clavdiya 2, Italmas, Voronezhskaya 18, Kalixo, Streletskaia 12, Universiya, Likamero, Sonett Rima, Obskaya ozymaya) 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 m² (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 (Kremnev 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),

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

| Years       | Race | References          |
|-------------|------|---------------------|
| 2001        | +    | + - - - - - - - -   |
| 2003–2004   | +    | + + + - - - - - -   |
| 2013–2015   | +    | - + + + - - - + +   |
| 2018        | +    | + + + - - - - + +   |
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 Bernier, 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 Barnier, 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).

Identification 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 – CTATTCGTAATCGTGACCTTCCCG; R – CTTCTCTCTCAGGCAACCTCTCTCATC (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 – TGGACCAGATGAGAGA; R – TTCTG-

GAGGATGTTGAGCACA (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 MgCl₂ (25 mM), 0.2 μL Taq polymerase (5 units μL⁻¹) (ZAO), 2.5 μL 10× PCR buffer and 12.8 μL ddH₂O. 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.
The field evaluations of resistance of 80 wheat genotypes to 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 | XBE4444541, Tsc2 | Race 1 #KZ-7-S-6 | Race 5 #KZ-41-N-2019 | ToxA | ToxB | Tan spot field evaluation % |
|---------------------|-------------------|---------------|------------------|-------------------|----------------------|------|------|-----------------------------|
| 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               |
| Zhetitsu            | 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      | I 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      | I 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)
| Wheat genotype       | Geographic origin | Xfcp623, Tsn1 | XBE444541, Tsc2 | Race 1 #KZ-7-S-6 | Race 5 #KZ-41-N-2019 | Tan spot field evaluation % |
|----------------------|------------------|--------------|-----------------|------------------|-----------------------|----------------------------|
| Turanus              | RU               | tsn1         | tsc2            | 2.2              | I                     | 2.5                        |
| Claudiya 2           | RU               | tsn1         | tsc2            | 2.3              | I                     | 2.0                        |
| Italmas              | RU               | tsn1         | Tsc2            | 1.0              | I                     | 3.5                        |
| Voronezhskaya 18     | RU               | Tsn1         | tsc2            | 3.3              | S                     | 1.3                        |
| Kalixo               | RU               | Tsn1         | tsc2            | 3.4              | S                     | 1.1                        |
| Streletskaia 12      | RU               | tsn1         | tsc2            | 2.2              | I                     | 2.1                        |
| Universiya           | RU               | tsn1         | tsc2            | 1.0              | I                     | 2.0                        |
| 7_PSI                | KZ               | tsn1         | tsc2            | 2.2              | I                     | 2.1                        |
| 633_SP2              | KZ               | Tsn1         | tsc2            | 3.4              | S                     | 1.3                        |
| Dinara               | KZ               | tsn1         | tsc2            | 2.1              | I                     | 2.1                        |
| Krasnovodopadskaya 25| KZ               | tsn1         | tsc2            | 1.0              | I                     | 2.3                        |
| Pirotrix 50          | KZ               | Tsn1         | tsc2            | 2.2              | I                     | 2.3                        |
| GF_3_CP              | KZ               | tsn1         | tsc2            | 1.0              | I                     | 2.2                        |
| 3_PSI                | KZ               | tsn1         | tsc2            | 2.4              | I                     | 2.0                        |
| 5_PSI                | KZ               | tsn1         | tsc2            | 2.2              | I                     | 2.1                        |
| 6_PSI                | KZ               | tsn1         | tsc2            | 2.1              | I                     | 2.1                        |
| 8_PSI                | KZ               | tsn1         | tsc2            | 2.3              | I                     | 2.0                        |
| 10205_1KSI           | KZ               | tsn1         | tsc2            | 1.0              | I                     | 1.1                        |
| 609_SP2              | KZ               | tsn1         | tsc2            | 1.0              | I                     | 2.2                        |
| 613_SP2              | KZ               | tsn1         | tsc2            | 2.3              | I                     | 2.2                        |
| 635_SP2              | KZ               | tsn1         | tsc2            | 2.2              | I                     | 2.0                        |
| Likamero             | RU               | Tsn1         | tsc2            | 3.4              | S                     | 2.2                        |
| Sonett               | RU               | Tsn1         | tsc2            | 3.3              | S                     | 2.1                        |
| 1_PSI                | KZ               | tsn1         | tsc2            | 2.3              | I                     | 2.2                        |
| 2_PSI                | KZ               | tsn1         | tsc2            | 2.5              | I                     | 2.0                        |
| 602_SP2              | KZ               | Tsn1         | tsc2            | 2.8              | S                     | 2.3                        |
| 607_SP2              | KZ               | Tsn1         | Tsc2            | 2.1              | I                     | 2.2                        |
| 618_SP2              | KZ               | Tsn1         | tsc2            | 3.0              | S                     | 1.1                        |
| 639_SP2              | KZ               | Tsn1         | Tsc2            | 3.3              | S                     | 3.4                        |
| Daulet               | KZ               | tsn1         | tsc2            | 1.0              | I                     | 1.2                        |
| Almaly               | KZ               | Tsn1         | tsc2            | 2.3              | I                     | 2.2                        |
| Rima                 | RU               | Tsn1         | Tsc2            | 3.5              | S                     | 2.0                        |
| Obskaya ozimaya 2    | RU               | Tsn1         | tsc2            | 3.6              | S                     | 1.4                        |
| Egemen 20            | KZ               | Tsn1         | tsc2            | 3.3              | S                     | 2.2                        |
| Salamouni Lebanon     | KZ               | tsn1         | tsc2            | 1.0              | I                     | 1.0                        |
| Glenlea              | Canada           | Tsn1         |                | 3.8              | S                     | 1.0                        |
| 6B662                | Unknow           |             | Tsc2            |                |                      | 3.8                        |

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%.
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, 61_PS1, Daulet, Dinara; 9, Streletskaia 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 $tsn1$); 18, Glenlea (susceptible reference cultivar for race 1, 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).

The proportion of genotypes insensitive to Ptr ToxB ($tsc2$) was high, with 72 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, 61_PS1, Daulet, Dinara; 9, Streletskaia 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 $tsn1$); 18, Glenlea (susceptible reference cultivar for race 1, 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).
Sequence: A196

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 (Kokhmetova 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.

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