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Phytopathological screening and molecular marker analysis of wheat germplasm from Kazakhstan and CIMMYT for resistance to tan spot

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Tan spot caused by the fungus *Pyrenophora tritici-repentis* is an important leaf spot disease in wheat growing areas throughout the world. The study aims to identify wheat germplasm resistant to tan spot based on phytopathological screening and molecular marker analysis. A collection of 64 common wheat germplasms, including cultivars and breeding lines from Kazakhstan and CIMMYT, was assessed for tan spot resistance in greenhouse conditions and characterized using the *Xfcp623* molecular marker, diagnostic for the *Tsn1* gene. All wheat cultivars/lines varied in their reaction to tan spot isolate race 1, ranging from susceptible to resistant. Most accessions studied (53 %) were susceptible to Ptr race 1. Spring wheat cultivars were more susceptible to race 1 than winter wheat cultivars. As a result of genotyping, an insensitive reaction to Ptr ToxA was predicted in 41 wheat cultivars (64 %). The *tsn1* gene carriers identified included 27 Kazakhstani and 14 CIMMYT cultivars/lines, demonstrating insensitivity to Ptr ToxA. The majority of the *Tsn1* genotype were sensitive to race 1 and showed susceptibility to the pathogen in the field. Disease scores from seedling stage positively correlated with field disease ratings. Of particular interest are 27 wheat accessions that demonstrated resistance to spore inoculation by Ptr race 1, were characterized by insensitivity to ToxA and showed field resistance to the pathogen. The results of this study will contribute to wheat breeding programs for tan spot resistance with Marker Assisted Selection using the closely flanking markers.
Key words: wheat; molecular markers; *Pyrenophora tritici-repentis*; tan spot; *Tsn1*; ToxA.

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Фитопатологический скрининг и молекулярный анализ гермоплазмы пшеницы из Казахстана и CIMMYT на устойчивость к пиренофорозу

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Пиренофороз, возбудитель *Pyrenophora tritici-repentis*, является важным заболеванием листовых пятнистостей в регионах выращивания пшеницы по всему миру. Цель исследования – идентифицировать и отобрать гермоплазму пшеницы, устойчивую к пиренофорозу *P. tritici-repentis*, с использованием молекулярных маркеров. Коллекция из 64 образцов мягкой пшеницы, включающая зарегистрированные сорта и элитные селекционные линии пшеницы из Казахстана и CIMMYT, была подвергнута оценке устойчивости к *P. tritici-repentis* в теплице и охарактеризована с помощью молекулярного маркера *Xfcp623*, диагностического для гена *Tsn1*. Все сорта/линии пшеницы различались по реакции на изолят расы 1 Ptr, проявляя широкий спектр реакции – от восприимчивой до устойчивой. Большинство исследованных образцов (53 %) оказались восприимчивы к изоляту расы 1 Ptr. Сорта яровой пшеницы были более восприимчивы к расе 1, чем сорта озимой пшеницы. В результате генотипирования нечувствительная реакция к токсину Ptr ToxA была предсказана у 41 сорта пшеницы (64 %). Идентифицированные носители гена *tsn1* включали 27 казахстанских и 14 сортов/линий CIMMYT, демонстрировавших нечувствительность к Ptr ToxA. Большинство образцов с генотипом *Tsn1* были чувствительны к расе 1 и показали восприимчивость к патогену в полевых условиях. Оценки заболеваемости на стадии проростков положительно коррелировали с оценками в полевых условиях. Особый интерес представляют 27 образцов пшеницы, которые проявили устойчивость к инокуляции спор расы 1

P. tritici-repentis, характеризовались нечувствительностью к токсину ToxA и демонстрировали устойчивость к патогену в полевых условиях. Полученные результаты внесут вклад в программы селекции пшеницы на устойчивость к пиренофорозу на основе Marker Assisted Selection с использованием тесно фланкирующих маркеров.
Ключевые слова: пшеница; молекулярные маркеры; *Pyrenophora tritici-repentis*; пиренофороз; *Tsn1*; ToxA.

Introduction

Ensuring food security is the most important priority of Kazakhstan's economic strategy. Wheat production is limited to a number of biotic stresses, including leaf spot diseases. Tan spot, caused by the fungus *Pyrenophora tritici-repentis* (Died.) Drechsler (anamorph), is an important foliar blight disease in temperate and warmer wheat growing areas throughout the world (Duveiller et al., 1998), including Kazakhstan (Koyshibayev, 2018). Under conditions favorable for tan spot development, yield losses can rise beyond 50 % (Rees et al., 1988). This pathogen infects leaf, stem and head tissue resulting in reduced photosynthetic area, increased transpiration and reduced accumulation of organic matter and causes considerable reduction in yield and quality of wheat. Modern industrial farming technologies contribute to the development of the disease: minimal tillage of the soil with the preservation of stubble on its surface, monoculture and cultivation of wheat cultivars with insufficient resistance to pathogen. The infected seeds, plant residues of the previous growing season, infected self-seeding plants and wild cereals susceptible to this disease could serve as a source of infection to winter wheat seedlings in autumn. Integrated disease control strategies, such as the cultivation of resistant varieties, combined with desired crop rotations and management practices, are the most effective, environmentally friendly and cost-effective tools to combat wheat tan spot (Mikhailova et al., 2012).

Inheritance to tan spot resistance has both quantitative and qualitative nature, while the toxins resistance genes and quantitative trait loci (QTL) are race specific and they control the processes that reduce the sensitivity to toxins (Mikhailova et al., 2012; Faris et al., 2013; Liu et al., 2017). To date according to Catalogue of Gene Symbols eight major resistance genes (*Tsr1*, *Tsr2*, *Tsr3*, *Tsr4*, *Tsr5*, *Tsr6*, *TsrHar*, *TsrAri*) located on chromosomes 2BS, 3A, 3BL, 3DS, and 5BL have been identified (McIntosh et al., 2013). Numerous genetic studies performing quantitative trait loci analysis, have reported resistance to tan spot as a polygenic trait however the major racespecific genes frequently underlines these QTLs. Additional QTLs have been identified and located on chromosome arms 1AL, 2AS, 3AS, 4AL, 5AL, 1BS, 2BL, 3BS, 2DS, 2DL, 6A, 7A, and 7DS (Chu et al., 2010; Singh et al., 2010, 2016; Xiao-Chun et al., 2010; Kalia et al., 2018). Recent studies using associative mapping allowed the detection of loci that determine resistance to different Ptr races (Gurung et al., 2011; Kollers et al., 2014; Juliana et al., 2018).

In order to increase effectiveness of wheat breeding subject to resistance to tan spot, it is essential to understand the genetic basis of resistance to the disease. *Pyrenophora tritici-repentis* induces on susceptible cultivars two different symptoms, necrosis and chlorosis. Genetically, both symptoms are under independent host control. Based on the ability to induce necrosis and chlorosis symptoms, 8 races of Ptr were identified (Lamari, Bernier, 1989a; Strelkov, Lamari, 2003). It has been found that the fungus produces a number of host-selective toxins (HSTs) known as Ptr ToxA, Ptr ToxB and Ptr ToxC, etc., which interact directly or indirectly with the products of the dominant plant genes *Tsn1*, *Tsc2* and *Tsc1*, respectively (Ballance et al., 1989). Recent studies on cloning and characterization of the *Tsn1* gene have shown that the pathogen utilizes HST-toxins to weaken resistance mechanisms of the host and cause the disease. However, in addition to host-HST interactions, a wide range of QTLs responsible for race-non-specific resistance and recessively inherited genes of "qualitative" resistance have been identified (Faris, Friesen, 2005; Singh et al., 2016). Molecular markers for the HST resistance genes and for race non-specific QTLs intended for use in marker assisted selection (MAS) have been developed (Faris et al., 2012; https://maswheat.ucdavis.edu/protocols). Diversity Arrays Technology (DArT) WPT-3049 (2.9 cM) and WPT-0289 (4.6 cM) markers were closely linked to Tsr1 and Tsr6, respectively (Singh et al., 2016).

There are a number of studies on the racial composition of *P. tritici-repentis* in Asia and Kazakhstan. The greatest diversity was observed in Azerbaijan, where races 1, 2, 3, 5, 7, and 8 were identified, and in Syria, where races 1, 3, 5, 7, and 8 were detected; the little variation was found in the virulence of isolates from Kazakhstan (race 1 and race 2) (Lamari et al., 2005). It has been revealed that race 1 is the most widespread race in Central Asia and Kazakhstan (87 %), and races 2, 3 and 4 were minor (Zhanarbekova et al., 2005; Maraite et al., 2006). It was found that races 1 and 2 dominate in the North Caucasus region of Russia, and races 1 and 8 *P. tritici-repentis* dominate in Kazakhstan (Kokhmetova et al., 2016). Studies on the racial composition of *P. tritici-repentis* in Kazakhstan indicate the necessity to identify the wheat germplasm resistant to the prevailing races of the disease among promising lines and wheat varieties cultivated in Kazakhstan. The results of previous studies indicate the possibility of postulating recessive alleles of genes for resistance to *P. tritici-repentis* toxins using molecular markers (Kokhmetova et al., 2017, 2018).

Conventional phytopathological methods are not always effective for identification of pathogen resistance genes. The situation is complicated by the fact that different leaf spot pathogens occur together in the field, which make more difficult disease evaluation. In this case, the use of molecular markers associated with disease resistance will be effective for identification of disease resistance factors. The presence of effective molecular markers closely linked to the genes of resistance to toxins makes it possible to conduct molecular screening of wheat breeding material. The main objectives of this study were (i) phytopathological and molecular screening elite cultivars and wheat breeding lines for resistance to tan spot, (ii) identification of resistance sources effective against tan spot.

Materials and methods

A collection of 64 common wheat germplasms, including 46 registered cultivars and elite wheat breeding lines from Ka-

Table 1. *Pyrenophora tritici-repentis* isolate (29A-11) served as representative of race 1

Isolate	Glenlea	6B-662	6B-365	Race*	Source	Location
29A-11	N (ToxA)	R	C (ToxC)	1 (nec+chl+)	Winter wheat	Almaty region of Kazakhstan

* Based on Lamari et al., 2005: N (ToxA) – disease inducing factor (Ptr ToxA), causing necrosis in Glenlea; C (ToxC) – disease inducing factor (Ptr ToxC), causing chlorosis in 6B-365; R – stand for resistance to toxins of *P. tritici-repentis*; nec+ – presence of necrosis; chl+ – presence of chlorosis.

zakhstan and 18 entries from CIMMYT was used in the work (see Table 2). Evaluation of the resistance to *P. tritici-repentis* on adult plant stage was carried out under field conditions of Southeast Kazakhstan, Kazakh Research Institute of Agriculture and Plant Growing (KRIAPG), Almalybak (43°13′ N, 76°36′ E, and 789 m asl), Almaty region, in the 2017–2018 crop seasons. The experiment was conducted in a completely randomized design with three replications. The field evaluation (from naturally ocurring infections) to tan spot resistance was assessed three times according to the scale for appraising the foliar intensity of diseases (Saari, Prescott, 1975) in the modification for tan spot (Kremneva, Volkova, 2007).

The standard wheat differentials included Glenlea and Salamouni cultivars, as well as 6B662 and 6B365 lines. Seedling resistance of the wheat cultivars were assayed in the greenhouse conditions at the two-leaf seedling stage. Three seedlings of each differential line and tested wheat cultivars were produced in plastic cones filled with soil and grown in the greenhouse at an average temperature of 21 °C with a 16-h photoperiod. The seedlings were inoculated with spore suspension with 4,000 spores per ml of each isolate individually until run off. Inoculated seedlings were moved to a mist chamber at 21 °C with a 16-h photoperiod for 24 h. Thereafter, the plants were moved to a growth chamber at 22 °C with a 16-h photoperiod for 24 h (light for 16 h and darkness for 8 h). The plants were rated for disease, using rating system based on lesion type; 1–2 represent resistance, and 3–5 represent susceptibility (Lamari, Bernier, 1989a).

The samples of *P. tritici-repentis* were randomly collected from hexaploid wheat in Almaty region of Kazakhstan. The Petri dishes with leaf pieces with lesions were incubated at 20 °C for 12–18 h under fluorescent lights (~80 $\mu mol \cdot m^{-2} \cdot s^{-1}$), followed by an additional 12–18 h in the dark at 15 °C (Lamari et al., 1995). Two to three isolates from each field were tested. All *P. tritici-repentis* isolates were typed by their respective race through inoculating them individually on the wheat differential set developed by Lamari, Bernier (1989b) and Strelkov, Lamari (2003). It was found, that *P. tritici-repentis* isolate 29A-11 was related to race 1, since it was produced necrosis on Glenlea and chlorosis on 6B-365 and possible characterized by the production of two toxins: ToxA and ToxC (Table 1).

P. tritici-repentis isolate 29A-11, representing race 1 was used for inoculation. Culture of *P. tritici-repentis* isolate 29A-18 race 1 was grown on V4 agar (150 ml V4 juice in a ratio of 4:3:2:1 parts beet juice, parsley, tomato and carrot, respectively, 20 g agar, 1.5 g $CaCO_3$ and 850 ml distilled water) in the dark at 20–22 °C for 6 days (Mikhailova et al., 2012). The plates were filled with sterile distilled water; the mycelium flattened the base of a sterile test tube and excess water poured off. To induce conidiophore production, the plates were incubated under continuous light at room temperature for two days followed by 1 day in the dark in an incubator at 16 °C to induce conidia production. The plates were filled with distilled water and the conidia were suspended in the distilled water by gentle brushing the mycelium to dislodge the conidia from the conidiophores. In order to reduce surface tension 2–3 drops of Tween 20 were added per liter of spore suspension. Spore concentration was measured and adjusted to 4,000 spores per ml. Wheat accessions were screened for toxin ToxA reaction. Plants were infiltrated at the second leaf stage as desciebed in Liu et al. (2006) and were scored as sensitive or insensitive 3 days later based on presence or absence of necrosis, respectively.

Genomic DNA was extracted at two-leaf seedling stage for each individual plant using the CTAB method (Riede, Anderson, 1996). DNA concentration was measured using a spectrophotometer SmartSpecTMPlus (BioRAD). The DNA concentration for each sample was adjusted to 30 ng/µl. Samples were genotyped using the SSR marker *Xfcp623* designed to detect alleles of the *Tsn1* gene. The primer sequence and PCR conditions are given by Faris et al. (2010). The amplification products were separated on 2%-agarose gels, to determine the length of the amplification fragment 100 bp DNA Ladder (Ferments, Lithuania) was used. Gels were visualized on GelDoc BIO-PRINT MEGA for documentation of allele types in cultivars. Wheat entries 6B662 and Glenlea served as positive and negative controls, respectively.

Results

Wheat germplasm reaction to race 1 of *Pyrenophora tritici-repentis*. Seedlings and adult plant response of wheat germplasm to *P. tritici-repentis* are presented in Table 2.

The reaction of wheat cultivars and lines representing the range of lesion types to race 1 using rating system (Lamari, Bernier, 1989a) based on two phenotypically distinct symptoms: tan necrosis (N) and chlorosis (C) was carried out. Evaluation to race 1 showed that lesion type varied greately amongst wheat cultivars. It was found that 30 entries out of 64 (46.9 %) had average disease reaction type, less than 2 and considered as resistant to this isolate (see Table 2). A type of symptom consisting of small dark spots without any surrounding chlorosis or tan necrosis (rating 1–2, R) was observed in about 19 % of the accessions tested. A type symptom consisting of minute dark spots with very little chlorosis or tan necrosis (rating 2, R-N), showed 19 (30.6 %) accessions. The chlorosis or tan necrosis symptoms (rating 3–4, S-N, S-C, S-NC), were observed in 34 (55.1 %) of the accessions tested.

Fifteen of the 64 entries were sensitive to production of chlorosis and this indicate that they may be sensitive to Ptr ToxB or Ptr ToxC, but resistant to Ptr ToxA. Relatively large proportion of resistant cultivars was presented in entries from CIMMYT (72.22 %). The number of Kazakhstani samples resistant to race 1 was significantly less (36.96 %). So, the majority of studied Kazakhstani and CIMMYT entries were

Table 2. The reaction of wheat accessions to race 1 *P. tritici-repentis* and the allelic state of the *Tsn1* gene

Accession	Origin	Growth habit	Response to race 1		Response to Ptr ToxA	Allelic state of molecular marker	Field evaluation Ptr, %
			Lesion type	Reaction			
Sapaly	KZ	Winter	3	S-C	I	–	10
Reke	KZ		2	R-N	I	–	15
Taza	KZ		2	R	I	–	10
Zhenis	KZ	Spring	2	R-N	I	–	10
Karabalykskaya 101	KZ		3	S-N	I	–	5
Akmola 40	KZ		4	S-N	I	–	5
Celinnaya Jubileynaya	KZ		3	S-C	I	–	10
Kazakstanskaya 3	KZ		4	S-N	S	–	15
Shortandinskaya 2007	KZ		3	S-N	I	–	10
Ishimskaya 92	KZ		2	R	I	–	0
Karagandinskaya 22	KZ		2	R-N	I	–	10
Karabalykskaya 90	KZ		3	S-N	I	–	15
Kazakstanskaya 19	KZ		3	S-N	I	–	10
Kazakstanskaya 20	KZ		3	S-N	I	–	10
Celinnaya 90	KZ		2	R-N	I	–	10
Kazakstanskaya 15	KZ		2	R-N	S	–	15
Pavlodarskaya 93	KZ		3	S-NC	I	–	15
Kargaly 9	KZ		2	R	I	–	0
Express	KZ		2	R-N	I	–	10
KP33	KZ	Winter	1	R-N	I	–	10
KP34	KZ		2	R	I	–	5
KP36	KZ		1	R	I	–	5
KSI6	KZ		1	R	I	–	5
KSI 9/374	KZ		2	R-N	I	–	10
KSI 16	KZ		1	R-N	I	–	10
KSI 17	KZ		2	R-N	I	–	5
Bogarnaya 56	KZ		4	S-NC	S	+	35
Mereke 70	KZ		3	S-N	I	+	50
Raminal	KZ		4	S-N	I	+	35
Nureke	KZ		4	S-C	S	+	50
Tungysh	KZ		2	R-N	S	+	50
Aray	KZ		4	S-N	S	+	35
Alem	KZ		4	S-N	S	+	35
Progress	KZ		3	S-N	S	+	50
Zhalyn	KZ		3	S-N	S	+	35
Karaspan	KZ		4	S-NC	S	+	30
Kazakstanskaya 4	KZ		4	S-NC	S	+	35
Anara	KZ		3	S-N	S	+	35
Akbidai	KZ		4	S-NC	S	+	50
Rausin	KZ		3	S-N	S	+	35
Zhadyra	KZ		3	S-N	S	+	35
KP 4	KZ		3	S-N	S	+	35
KP 18	KZ		3	S-NC	S	+	30
KP 35	KZ		3	S-N	S	+	50
Celinnaya 26	KZ	Spring	2	R-N	I	+	30
Akmola 2	KZ		4	S-NC	S	+	35
Bobwhite 04	CIMMYT		2	R-N	S	+	15

Table 2 (end)

Accession	Origin	Growth habit	Response to race 1		Response to Ptr ToxA	Allelic state of molecular marker	Field evaluation Ptr, %
			Lesion type	Reaction			
F133/SHA5//OPATA	CIMMYT	Winter	2	R-N	S	+	25
BR35/BR14	CIMMYT		1	R-N	I	–	20
F3.71/TRM/VORONA/3/OC14	CIMMYT		1	R-N	I	–	10
CEP80111/VEE	CIMMYT		1	R-N	I	–	15
TPAP#1/OPATA	CIMMYT		3	S-NC	I	–	10
P83-5112/V82274	CIMMYT		3	S-NC	S	+	0
JAC161/TEMU51.80	CIMMYT		1	R	I	–	0
CATBIRD	CIMMYT	Spring	1	R	I	–	20
GAN/AE.437SOVARROSA	CIMMYT	Winter	1	R-N	I	–	25
GAN/AE (408)	CIMMYT		1	R	I	–	15
EG, AUS/H 567.71//4* EG AVS/3/2	CIMMYT		4	S-NC	S	+	25
EFED/F5.83 7792 (BAJAS)	CIMMYT		3	S-NC	I	–	20
L.A.CJAT(SANTACARUS)	CIMMYT		3	S-NC	I	–	0
TALHUENJNJA	CIMMYT		1	R-N	I	–	15
EFED/22150	CIMMYT		1	R	I	–	15
TOO11/TOOOO7	CIMMYT		1	R	I	–	20
RECURRENT SELECTION 1	CIMMYT		1	R	I	–	25
Salomouni	Egypt	Spring	1	R	I	–	0
Glenlea	Canada		4	S-N	S	+	40

Note: *Xfcp623* is the SSR marker to the *Tsn1* locus; "+" indicates sensitive to Ptr ToxA samples, contain *Tsn1* allele, 380 bp DNA fragment; "–" indicates insensitive to Ptr ToxA samples, contain *tsn1*, null allele; Salamouni, the insensitive control for race 1 and toxin Ptr ToxA, carrier of the ressive gene *tsn1*; Glenlea, the susceptible control for race 1 and Ptr ToxA, carrier of the dominant *Tsn1*; 1–5 are the lesion type rating based on Lamari and Bernier (1989a) scale; 1, 2 – indicates resistance, and 3–5 – susceptibility; the reaction to Ptr ToxA: I, insensitivity, S, sensitivity to Ptr ToxA.

susceptible to isolate 29A-18 related to race 1. In general, a higher number of spring wheat cultivars exhibited suceptibility to race 1 as compared to winter wheat cultivars.

Field evaluation to tan spot. The results of the field evaluation (from naturally ocurring infections) to tan spot resistance showed that the severity level to *P. tritici-repentis* varied widely, from 0 to 50 % (see Table 2, Fig. 1). In the field, considerable disease (>30 %) developed on plants of 21 cultivars (32.8 %). High level of lesion development from naturally ocurring infections was observed in cultivars Mereke 70, Nureke, Progress, Akbidai and KP 35. Field resistance (<15 %) was observed in 37 (57.8 %) of the accessions tested. There was a tendency towards lower rating in the field, most cultivar had similar reactions when tested in the greenhouse and in the field.

Genotyping of wheat accessions with *Xfcp623* marker. The wheat cultivars were genotyped with *Xfcp623* marker to predict reaction to the Ptr ToxA. The marker of *Xfcp623* has two alleles: in the presence of *Tsn1* allele (Ptr ToxA sensitivity) in the genome, 380 bp fragment is amplified, the absence of a fragment of amplification (null-allele) suggests the presence of the recessive *tsn1* allele (Ptr ToxA insensitivity). The results of genotyping of wheat cultivars and lines with the *Xfcp623* marker are presented in Table 2. As an example, the results of the PCR amplification with *Xfcp623* marker on 16 wheat genotypes, are shown in the Figure 2. According to Figure 2, seven out of 16 entries (Bogarnaya 56, KP 18, Kazakstanskaya 4, Akmola 2, KP 4, P83-5112/V82274 and Anara) had

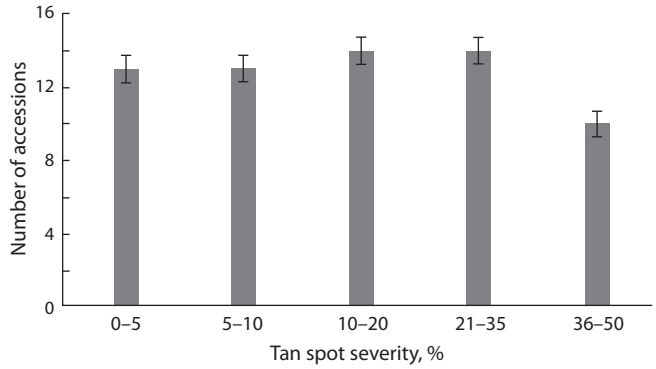

Fig. 1. Frequency distribution of wheat accession for severity to *P. tritici-repentis*, field evaluation, Almaty region.

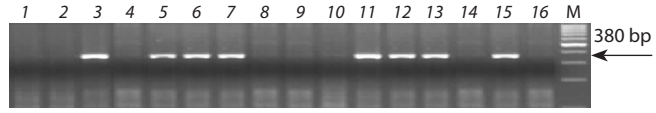

Fig. 2. DNA amplification profile for wheat cultivars and elite lines obtained with *Xfcp623* marker.

Lane: *1*, KSI 16; *2*, GAN/AE (408); *3*, Bogarnaya 56; *4*, KP 17; *5*, KP 18; *6*, Kazakstanskaya 4; *7*, Akmola 2; *8*, Taza; *9*, Kargaly 9; *10*, EFED/22150; *11*, KP 4; *12*, P83-5112/V82274; *13*, Anara; *14*, KP33; *15*, Glenlea (the sensitive control for race 1 and toxin Ptr ToxA, carrier of the dominant gene *Tsn1)*; *16*, Salamouni (the insensitive control for race 1 and toxin Ptr ToxA, carrier of the ressive gene *tsn1)*; M, DNA Ladder.

380 bp fragment, which allows us to postulate the presence of the dominant *Tsn1* allele conferring toxin Ptr ToxA sensitivity. Seven entries including KSI 16, GAN/AE (408), KP 17, Taza, Kargaly 9, EFED/22150 and KP33 showed a lack of amplification (null-allele) and suggests that these samples contain recessive *tsn1* allele conferring toxin Ptr ToxA insensitivity.

The results of genotyping showed that the frequency of *Tsn1* allele was 37.5 % (24 cultivars of 64 analyzed). Analysis of molecular and phytopathological data shows that carriers of the *tsn1* gene characterized by high field resistance with severity level ranging from 0 to 15 %. The carriers of the *Tsn1* gene, showed in the field susceptibility to the disease comprised at 30–45 %.

Twenty-seven wheat entries are of the greatest interest since they demonstrated resistance to the seedling inoculation by the race 1 of *P. tritici-repentis*, showed resistance to the pathogen in the field and were Ptr ToxA insensitive. This set of accessions includes 16 cultivars and lines from Kazakhstan (Reke, Taza, Zhenis, Ishimskaya 92, Karagandinskaya 22, Karabalykskaya 90, Celinnaya 90, Kargaly 9, Express, KP33, KP34, KP36, KSI6, KSI 9/374, KSI 16, KSI 17) and 11 CIMMYT lines (BR35/BR14, F3.71/TRM/VORONA/3/OC14, CEP80111/VEE, JAC161/TEMU51.80, CATBIRD, GAN/AE.437SOVARROSA, GAN/AE (408), TALHUENJNJA, EFED/22150, TOO11/TOOOO7, RECURRENT SELECTION 1).

Discussion

Ptr ToxA toxin is known to be one of the main factors associated with the development of *P. tritici-repentis* in susceptible wheat genotypes (Friesen et al., 2006). The new *P. tritici-repentis* races are emerging through natural selection, therefore development of new resistance sources is imperative (Ali et al., 2010). Several reports indicate the global prevalence of race 1 (Singh et al., 2010; Abdullah et al., 2017a). Since it was previously shown that the race 1 is the most prevalent race in Kazakhstan (Zhanarbekova et al., 2005; Maraite et al., 2006; Kokhmetova et al., 2016), in the present study, we searched for carriers of resistance to race 1 and Ptr ToxA toxin among wheat germplasm. The particular value of the experiment is the breeding material, which was developed and selected in Kazakhstan, as well as in CIMMYT. This material is representing different genetic background, including carriers of resistance to tan spot.

Molecular markers *Xfcp393*, *Xfcp394* and *Xfcp623* were developed as diagnostics for detection of insensitivity to Ptr ToxA and Sn ToxA (Zhang et al., 2009; Faris et al., 2010). On the basis of sequencing the marker *Xfcp623* was proposed as diagnostic for *Tsn1* gene. It was located in intron 5 of the locus in position 4901…5280 (Faris et al., 2010). The reliability of the diagnostic marker *Xfcp623* for identifying wheat genotypes with resistance to the fungus and insensitivity to Ptr ToxA was shown in some studies (Karelov et al., 2015; Kokhmetova et al., 2017, 2018; Mironenko et al., 2017). Taking into consideration the higher efficiency of the *Xfcp623* marker, wheat germplasm in our study were genotyped with this marker. In this study, the frequency of *tsn1* allele of the marker *Xfcp623* for Ptr ToxA insensitivity was 62.5 %. Sensitive reaction to the Ptr ToxA was predicted for the 20 remainder of the tested genotypes (37.5 %).

The majority of studied cultivars, 53.1 % were susceptible to isolate 29A-18 related to race 1. In general, the most part of spring wheat cultivars exhibited susceptibility to race 1 as compared to winter wheat cultivars. The same response to race 1 in a set of spring and winter wheat cultivars was observed by Abdullah et al. (2017b), who indicated that the high resistance of winter wheat to race 1 tan spot minimizes their role in establishing race 1 in the region.

Disease scores from seedling stage, assessed herein, positively correlated with field disease ratings. The most of the wheat entries with the *Tsn1* genotype (90.5 %) were sensitive to race 1 and showed susceptibility to the pathogen in the field. The exception was 2 varieties (Tungysh and Celinnaya 26), which showed an insensitive reaction to the race 1 of the fungus.

Among the studied wheat material 59 accessions (92.2 %) exhibited "sensitive" or "insensitive" alleles at marker loci *Xfcp623* in Ptr ToxA sensitive and insensitive accessions, respectively. In other words, no recombination was observed within the segment harboring the marker *Xfcp623* and *Tsn1* among these 59 accessions. Only 5 genotypes had recombination events between *Xfcp623* and *Tsn1*. In the cultivars Kazakstanskaya 3 and Kazakstanskaya 15 the sensitivity to Ptr ToxA and the presence of resistance gene *tsn1* was observed. The cultivars Mereke 70, Raminal and Celinnaya 26 showed the insensitivity to Ptr ToxA, but characterized by the presence of susceptible gene *Tsn1*. Apparently, recombinations are possible in the segment harboring the *Xfcp623* and *Tsn1* marker among these wheat cultivars.

The results of our study are in agreement with a previous research, suggesting that ToxA is not the major determinant in tan spot disease development in some host backgrounds and indicates the presence of additional effectors (Oliver et al., 2014; Rybak et al., 2017; See et al., 2018). The ToxA–*Tsn1* interaction alone is not a prerequisite for pathogenicity of race 1 Ptr isolates, and pathologists have started to recognize that race 1 Ptr isolates harbour additional uncharacterized effectors in addition to ToxA and ToxC (Manning, Ciuffetti, 2015). Ptr interacts with the host in a complex and intricate manner, leading to a variety of disease reactions that are dependent or independent of the ToxA–*Tsn1* interaction (See et al., 2018). ToxA is found ubiquitously in Australian Ptr isolates and the removal of ToxA sensitivity gene from wheat has been shown to have no effect on yield penalty (Oliver et al., 2014). Although the removal of the *ToxA* gene in Ptr does not severely impede the ability of the pathogen to infect in all varieties, the absence of the *Tsn1* gene in the wheat germplasm does generally improve resistance to tan spot disease (See et al., 2018).

Conclusion

In summary, the results presented in this study indicate that it is necessary to continue breeding for development of carriers of *tsn1* gene insensitive to Ptr ToxA toxin. The obtained results will contribute to wheat breeding by pyramiding Ptr ToxA insensitivity genes using the closely linked marker *Xfcp623* into the desired germplasm. The group of disease resistance germplasm identified in this study can be utilized to develop cultivars with broad-genetic base durable resistance to wheat tan spot.

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