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Race Diversity of *Pyrenophora tritici-repentis* in South Dakota and Response of Predominant Wheat Cultivars to Tan Spot

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Abstract

The fungus *Pyrenophora tritici-repentis* (*Ptr*) causing tan spot (TS) is an important pathogen of wheat in the US Northern-Great-Plains. Knowledge of physiological variation in the pathogen population is essential in the development of durable TS resistant cultivars. Eight *Ptr* races have been identified based on three host selective toxins (Ptr ToxA/Ptr ToxB/Ptr ToxC), which are associated with necrosis and chlorosis symptoms. The information about *Ptr* race structure and reaction of wheat cultivars grown in SD to tan spot is scarcely available. In this study, 569 isolates of *Ptr* collected from wheat were genotyped for *Ptr ToxA* and *Ptr ToxB* genes and a subset of 134 isolates were evaluated for their race identity on a wheat differential set. *Ptr ToxA* and *Ptr ToxB* genes were amplified in 89.6% and 0.4% isolates, respectively. The remaining 57 (10%) isolates lacked both toxins genes. The characterization of 134 isolates exhibited diverse race structure with 74.6%, 18.7%, 1.49% , and <1% isolates categorized as race 1, 4, 5, and 2, respectively. Another six (4.5%) isolates behaved like race 2 but lacked *Ptr ToxA* gene, hence could not fit under the currently known eight races. Our results determine the diversity of *Ptr* population that exists in SD and establish the presence of race 5 in SD for the first time. Since races 1 and 5 are most prevalent in the region, we screened 45 most predominant wheat cultivars against these races and *Ptr ToxA*. We observed eleven cultivars resistant or moderately resistant to both races, however, seven spring wheat cultivars showed susceptibility to both races 1 and 5. Continued cultivation of wheat cultivars susceptible to both races could play a role in the establishment and development of new races. Continuous germplasm enhancement and periodically monitoring *Ptr* population can help in better TS management.

Keywords: Tan spot; *Drechslera tritici-repentis*; Wheat; Host-selective toxins; Ptr ToxA; Ptr ToxB; Race 1; Race 5

Introduction

Tan spot caused by an ascomycete fungus *Pyrenophora tritici-repentis* (Died.) Drechs. (anamorph: *Drechslera tritici-repentis* Shoe.) is an important foliar disease of wheat in the US Northern Great Plains (NGP) [1]. In addition to wheat, a primary and economical host, the fungus has a wide host range that includes cereals such as barley, oat, rye and many non-cereal grasses [2-4]. The fungus produces oval-shaped tan necrotic spots with a chlorotic halo and a small black spot in the center of the leaves of susceptible cultivars. Tan spot can cause a significant yield loss up to 50%, attributed to low 1000 KWT, shriveled kernels, decrease in leaf area for photosynthesis, and number of grains/spike [5-7]. The fungus overwinters from one growing season to the next on infested wheat residue primarily in the form of sexual fruiting bodies "Pseudothecia" in the region [8]. These fruiting bodies produce ascospores under cool and wet conditions in spring, which serve as a primary source of inoculum for disease initiation. Further, these sexual fruiting bodies provide ample opportunities for the fungal sexual recombination that could lead to change in the pathogen virulence, especially when two distinct races reside on the residue of a cultivar susceptible to multiple races. Virulence variation in *P. tritici-repentis* has been observed based on an isolate's ability to produce necrosis and/or chlorosis symptoms on appropriate wheat differential genotype. So far, the isolates from wheat and alternative host plants have been grouped into eight races [2,9-11] and race 1 was the most prevalent race observed in Africa, Asia, Europe, North and South America [2,9,11-15]. Further, *P. tritici-repentis* produces three host-selective toxins, *Ptr ToxA, Ptr ToxB,* and *Ptr ToxC*, which are associated with necrosis and chlorosis symptoms in toxins sensitive wheat genotypes [11,16-18]. At present gene-specific molecular markers are available for *Ptr ToxA* (associated with necrosis symptom) and *Ptr ToxB* (chlorosis) and can be used to determine if an isolate carries these genes. However, presence or absence of *Ptr ToxA* and *Ptr ToxB* genes in the isolate allows us to potentially categories it into group 1 (races 1, 2, 5, 7): which carries one or both of these genes or group 2 (race 3 or 4): which lacks these genes or group 3: as new race(s) [11]. Lack of availability of race 3 (*Ptr ToxC*) and race 4 specific molecular markers still warrants for phenotyping the isolates on tan spot wheat differential set to confirm the race identity [11].

Wheat is the second most important crop in SD planted on 2.7 million acres with the total production of 103.16 million bushels [19]. Wheat productivity is impacted by various pests and diseases, including TS in the US NGP. TS alone can cause 5% to 29% yield reduction in South Dakota depending on the inoculum level and cultivar susceptibility under suitable environment for disease development [5]. Excessive use of fungicide could make the pathogen fungicide resistant, reduce its utility and ultimately impact crop productivity [20,21]. In this scenario, development of TS resistant cultivars seems to be the best approach for disease management, especially for a marginally profitable crop like wheat.

A comprehensive knowledge of physiological variation in the pathogen population and reaction of commonly grown cultivars in the region is essential for developing TS management strategies including durable disease resistant cultivars. Limited information is available on *P. tritici-repentis* race structure prevalent in wheat and reaction of wheat cultivars to TS in South Dakota. The objectives of this study were to 1) characterize race structure of *P. tritici-repentis* isolates recovered from wheat in South Dakota and 2) evaluate the current status of TS

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resistance in wheat cultivars widely grown in South Dakota and the region. The information obtained in this study would help the regional wheat breeders and pathologists in the development of TS management strategies.

Materials and Methods

Leaf samples collection and recovery of *P. tritici-repentis* isolates

Leaf samples with tan spot symptoms were collected from 55 locations during the 2012 (n=18), 2013 (n=19), and 2014 (n=18) growing seasons in South Dakota. About 15 to 20 leaves were randomly collected from each location. The samples were collected from both South Dakota State University Experimental Research Stations and commercial field plots when the crop was mostly at milk-stage (Feekes 10.54-11.1). The leaves were cut into about 2 cm long segments with at least one TS lesion/segment, placed in paper bags (one sample/bag), and stored in a refrigerator until used. *P. tritici-repentis* isolates were recovered from the leaf segments following the method described by Ali and Francl [22]. In short, 40-50 segments randomly selected of each sample were placed in 9 cm Petri plates with three layers of moist filter paper. Plates were incubated under fluorescent light for 24 h at room temperature (22°C ± 1°C) and 24 h in dark at 16°C for spores formation. The segments were examined under a stereoscope for *P. tritici-repentis* conidia. Thereafter 10-15 single conidia were collected with a flamed steel needle and transferred individually onto V8PDA plates [23]. The cultures were grown for 5-6 days in the dark and then stored at -20°C following the procedure of Jodhal and Francl [24] until they were characterized for their race structure. In total 569 isolates, including 8 isolates from Nebraska, were recovered in 2012 (n=138), 2013 (n=176) and 2014 (n=255) over 3-year period.

Genotypic characterization of *P. tritici-repentis* isolates for *Ptr ToxA* and *Ptr ToxB* genes

Fresh cultures of all 569 *P. tritici-repentis* isolates were obtained by growing them from their stored cultures at -20°C individually on V8PDA medium for 5 days in the dark. Each isolate's mycelia were removed from the agar surface with a sterile scalpel and placed in a 2 ml micro-centrifuge tube, and dried overnight in a water bath at 37°C. Thereafter, the mycelia were ground into a fine powder using a first prep machine Retsch MM 301 (Retsch., Clifton, NJ). DNA from all the isolates was recovered by following the method of Moreno et al. [25]. The DNA concentration was adjusted to 25 ng/µl using a Nano drop (Counterpane Inc. Tacoma, WA) and run on a 0.8% agarose gel to verify the DNA quality. The genotype of the *P. tritici-repentis* isolates for *Ptr ToxA* and *Ptr ToxB* genes was determined using the *Ptr ToxA* and *Ptr ToxB* genes specific primers developed by Andrie et al. [26]. The conformity of the isolates of *P. tritici-repentis* was further verified by using two *P. tritici-repentis* mating type genes (*MAT1-1* and/or *MAT1-2*) specific primers [27]. PCR reactions were performed in total 20 µl volume (2 µl genomic DNA @25 ng/µl, 0.8 µl of each primer (10 mM), 0.5 µl dNTP (200 µM), 2 µl 10x thermophol buffer, 0.2 µl 10 U/ml Taq DNA Polymerase and 13.7 µl of molecular biology grade water). PCR reaction was conducted in a S-1000 thermal cycler (Bio-Rad, Hercules, CA) using amplification steps of 94°C for 1 min, followed by 30 cycles of 94°C for 45 sec, 55°C for 30 sec and 72°C for 1 min with a final extension of 72°C for 7 min. We pooled PCR products from housekeeping genes (*MAT1-1 or MAT1-2*) with Ptr ToxA or Ptr ToxB specific PCR products as a positive amplification control for each isolate. The amplified products were electrophoresed on 1.5% agarose gel and scored with reference to 1 Kb ladder (New England Biolabs, Ipswich, MA). *P. tritici-repentis* isolates, Pti2 (race 1) and DW7 (race 5) were used as positive controls for *Ptr ToxA* and *Ptr ToxB* genes, respectively.

Phenotypic characterization of *P. tritici-repentis* isolates

Fresh cultures of randomly selected 134 of 569 isolates genotyped for *Ptr ToxA* and *Ptr ToxB* genes were obtained by following the procedure described by Ali and Francl [22]. In short, all 134 isolates cultures were initiated by plating their frozen dry plugs on fresh V8PDA Petri plates. The plugs of each isolate were placed in 3 plates (one plug/plate) and were incubated in dark for 5 days. The plates were flooded with distilled sterile water (DSW) and the mycelial growth was flattened with a sterile test tube. The excess water was decanted from the plates and then placed under fluorescent light for 24 h at room temperature (22°C ± 1°C) and for 24 h in dark at 16°C for spore production. The conidia were harvested with sterile disposable plastic inoculating loops (Fisher Scientific, Asheville, NC) by adding about 30 ml of DSW into each plate. The final spore concentration was adjusted to 2500 spores/ml prior to inoculations. *P. tritici-repentis* isolates, Pti2 (race 1) and DW7 (race 5) were included as positive checks for validation of inoculation procedure and race identification. Two-week-old seedlings of all 4 differentials genotypes Glenlea, 6B365, 6B662, and Salamouni [11] were raised in 3 × 9 cm plastic containers (Stuewe & Sons, Inc. Tangent, OR) filled with Sunshine Mix 1 (Agawam, MA) with three replications inoculated individually with all 134 isolates by spraying their spore suspension with a handheld CO_2 pressurized sprayer (Preval, Coal City, IL). Three seedlings/container were maintained throughout the experimentation. The seedlings were placed in a humidity chamber at 100% humidity for 24 h for enhancing the chances of fungal infection. Thereafter, the seedlings were moved to a greenhouse bench at South Dakota State University (SDSU) for seven days for symptom development. The isolates were grouped under appropriate race based on their ability to produce necrosis and chlorosis symptoms on the differentials genotype [11].

Evaluation of wheat cultivars for their reaction to tan spot using race 1 and 5 and *Ptr ToxA*

Two-week-old seedlings of 41 (hard red spring=29 and hard red winter=12) wheat cultivars which have been planted as popular varieties in South Dakota (Tables 1a and 1b) were raised in plastic containers as described under section A. In addition, four spring wheat advanced lines were included in the experiment. All cultivars seed was kindly provided by SDSU spring and winter wheat breeding programs. The purpose of the cultivars evaluation was to know if any of the cultivars are susceptible to both race 1 and 5 or race 5 that may play any role in the establishment of *P. tritici-repentis* race 5. Tan spot wheat differential genotypes Glenlea, 6B365, 6B662, and Salamouni were included in the experiment as checks for inoculation procedure validation and disease rating. Seedlings of all 45 wheat genotypes were inoculated individually using race 1 and race 5 spore's suspension. Nine seedlings (3 seedlings/container) of each cultivar were inoculated and the experiment was repeated once. The inoculated seedlings were rated for their reaction based on the 1-5 rating scale [23].

All 45 wheat genotypes were also evaluated for Ptr ToxA reaction by following the procedure of Faris et al. [28]. Briefly, three fully expanded second leaves of three two-week-old seedlings of all 45 genotypes was infiltrated with Ptr ToxA (10 µg/ml), using a needleless syringe and infiltrated leaf area was marked by a non-toxic permanent marker. The seedlings were rated as sensitive/insensitive based on presence/absence of necrosis, respectively. Wheat differential genotypes Glenlea (Ptr ToxA sensitive) and Salamouni (insensitive) were included in the experiment as positive controls. The experiment was repeated once. Ptr ToxA was kindly provided by Dr. S. Mienhardt, Department of Plant Pathology, North Dakota State University, Fargo, North Dakota.

Number	Cultivar/Line	*P. tritici-repentis* races				Ptr ToxA
		Race 1		Race 5		
		Lesion type	Reaction	Lesion type	Reaction	
1	Advance	1.8	MR	2.3	MR	+
2	Barlow	1.5	R	1.9	MR	-
3	Breaker	1.0	R	2.0	MR	+
4	Brick	1.0	R	2.2	MR	+
5	Briggs	2.0	MR	2.6	MR	+
6	Chris	1.0	R	4.3	S	-
7	Elgin-ND	1.1	R	3.8	MS	+
8	Faller	1.0	R	3.6	MS	-
9	Forefront	3.7	MS	4.4	S	+
10	LCS Albany	1.1	R	1.4	R	+
11	LCS Breakaway	4.0	S	4.0	S	-
12	LCS Powerplay	1.2	R	1.8	MR	+
13	Linkert	2.0	MR	4.1	S	-
14	Mott	3.5	MS	1.8	MR	-
15	Norden	3.7	MS	4.0	S	+
16	Oxen	3.1	MS	1.1	R	+
17	Prosper	2.0	MR	3.6	MS	-
18	RB07	1.7	MR	1.3	R	+
19	Rollag	1.1	R	3.9	S	-
20	Russ	3.9	S	4.0	S	+
21	Sabin	1.3	R	1.0	R	+
22	Samson	1.1	R	1.2	R	-
23	SD4189	3.8	MS	4.0	S	+
24	SD4215	1.9	MR	1.0	R	+
25	Select	4.0	S	3.8	MS	+
26	SY Soren	1.0	R	1.0	R	-
27	SY Rowyn	3.0	MS	1.2	R	+
28	Transverse	3.3	MS	2.1	MR	+
29	Vantage	1.7	MR	1.3	R	-
30	Velva	1.2	R	3.8	MS	-
31	WB Mayville	4.0	S	1.1	R	+
32	SD4148	1.6	MR	3	MS	+
33	SD4011	1.1	R	2.1	MR	+

Table 1a: Reaction of hard red spring wheat cultivars/lines to *P. tritici-repentis* race 1, race 5, and Ptr ToxA (Tan spot).

Number	Cultivar/Line	*P. tritici-repentis* races				Ptr ToxA
		Race 1		Race 5		
		Lesion type	Reaction	Lesion type	Reaction	
1	Alice	3.0	MS	1.1	R	+
2	Expedition	3.3	MS	1.7	MR	+
3	Freeman	1.6	MR	1.0	R	-
4	Grainfield	3.0	MS	1.0	R	+
5	Ideal	4.0	S	1.0	R	+
6	Lyman	1.9	MR	1.0	R	+
7	Millennium	2.8	MR	1.2	R	+
8	Overland	3.2	MS	1.2	R	+
9	Redfield	2.1	MR	1.0	R	-
10	SY Wolf	1.0	R	1.0	R	-
11	WB Matlock	3.8	MS	1.3	R	+
12	Wesley	2.2	MR	1.0	R	-

Table 1b: Reaction of hard red winter wheat cultivars to *P. tritici-repentis* race 1, race 5, and Ptr ToxA (Tan spot).

Results and Discussion

Molecular evaluation of *P. tritici-repentis* isolates for *Ptr ToxA* and *Ptr ToxB* genes

We confirmed all 569 isolates were *P. tritici-repentis* by mating type (*MAT1-1 or MAT1-2*) primers. Further, amplification of *Ptr ToxA* (585 bp) and *Ptr ToxB* (295 bp) genes specific PCR products from isolates Pti2 (race 1) and DW7 (race 5) validated the success of PCR assay (Figure 1). All isolates were then scored for presence or absence of the *Ptr ToxA* and *Ptr ToxB* genes and the isolates were divided into three groups and that include: 1) isolates carrying *Ptr ToxA* gene, 2) isolates carrying *Ptr ToxB* gene, and 3) the isolates without *Ptr ToxA* and *Ptr ToxB* genes (Figure

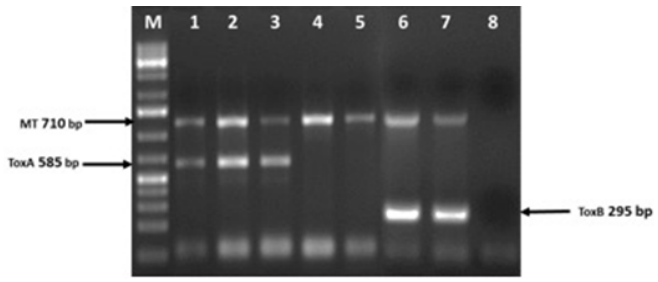

Figure 1: Characterization *P. tritici-repentis* isolates based on *ToxA*, *ToxB* primer amplification and Mating type (*MT*) genes. Lane 1-3: *Ptr ToxA* gene from unknown isolates SD13-101, SD12-S26-SN27-P2, and Pti2 (race1 positive control); Lane 4-5: isolates 12-S11-SN11-P7.2 and 12-S11-SN11-P7.3 without *ToxA/ToxB*; Lane 6-7: isolates SD13-103 (unknown) and DW-7 (race 5 positive control) amplified *Ptr ToxB* and Lane 8: water (as negative control).

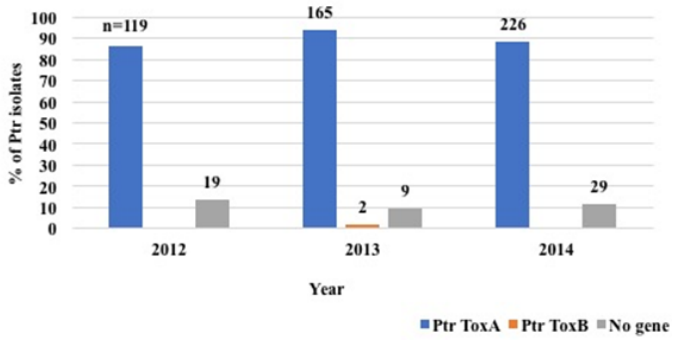

Figure 2: Genotypic characterization of *P. tritici repentis* isolates for *Ptr ToxA* and *Ptr ToxB* genes prevalent in wheat in South Dakota in 2012-2014.

2). The isolates (n=502, 89.5%) belonging to group 1 can potentially be race 1, 2, and 8 as these races carry *Ptr ToxA* gene, however the lack of *Ptr ToxB* gene in all these isolates eliminates the possibility of race 8 [11]. In addition, all eight isolates from Nebraska also amplified *Ptr ToxA* gene and were placed in group 1. Further, the frequency of isolates with *Ptr ToxA* gene was higher in all three years, 2012 (n=119, 86.2%), 2013 (n=165, 93.8%), and 2014 (n=226, 88.3%) compared to isolates with *Ptr ToxB* gene (Figure 2). The presence of *Ptr ToxA* gene at high frequency in the evaluated isolates shows predominance of race 1 in South Dakota which is similar to *P. tritici-repentis* race structure reported from different countries [2,13,14,29]. Only two (0.4%) isolates that amplified *Ptr ToxB* gene (group 2) could possibly be of either race 5 (*Ptr ToxB*) or race 6 (*Ptr ToxB* and *Ptr ToxC*) depending on the phenotypic reaction of these isolates (chlorosis on differential genotype 6B662- race 5 or chlorosis on both genotypes 6B662 and 6B365-race 6). Ours results suggest that though only two isolates with *Ptr ToxB* gene were observed from one location in 2013, there is prevalence of either race 5 or race 6 in South Dakota. Though earlier we have reported race 5 in North Dakota [30], this is the first time we observed race 5 in South Dakota suggesting that the race is expanding and this could aggravate the challenges in tan spot management in the region.

Lack of molecular markers for *Ptr ToxC* gene that distinguishes race 1 (*Ptr ToxA* and *Ptr ToxC*) from 2 (*Ptr ToxA*); race 3 (*Ptr ToxC*) from race 4 (carries neither of the three toxins genes); and race 5 (*Ptr ToxB*) from 6 (*Ptr ToxB* and *Ptr ToxC*) is a bottleneck in determining the isolate's absolute race.

We categorized 57 (10%) isolates into group 3 that were devoid of both *Ptr ToxA* and *Ptr ToxB* genes and potentially be of race 3 and 4 [11], or a new unknown virulent race similar to those reported in previous studies [9,26,31,32]. The isolates without *Ptr ToxA* and *PtrToxB* genes were observed in all three years 2012, (19 isolates, 13.8%), 2013 (9 isolates, 5.1%) and in 2014 (29 isolates, 11.7%) but they varied in their proportion (Figure 2). Our results indicate that majority (n=502) of the isolates collected over the three-year period from South Dakota carry *Ptr ToxA* gene that validate reports indicating prevalence of *Ptr ToxA* carrying isolates in abundance in South Dakota [12,31,32]. Availability of *P. tritici-repentis* whole genome information [33] may help in accelerating the development of molecular markers for race 3 and 4 and minimize the dependency on phenotyping which is a time-consuming procedure and requires a well-trained person for observing the isolates phenotypic reaction. However, presently the exact race identification of the isolates is only possible through their phenotyping on the differential set [11,34].

Phenotypic evaluation of *P. tritici-repentis* isolates for race identification

A subset of randomly chosen 134 of 569 *P. tritici-repentis* isolates studied for *Ptr ToxA* and *Ptr ToxB* genes were further characterized for their race structure on tan spot wheat differential set. The 134 isolates were grouped into races 1, 2, 4, and 5 (Figure 3) based on phenotypic reaction on the differential set indicating the prevalence of diverse population of *P. tritici-repentis* in wheat in South Dakota. As expected Pti2 (race 1) produced necrosis and chlorosis and DW7 (race 5) produced chlorosis on appropriate differential genotypes [11] validating the inoculation procedure. Among these four races, the majority of the isolates from 2012 (40%), 2013 (84%), and 2014 (80%) were identified as race 1. However, during 2012 in addition to race 1, a significant proportion of isolates were identified as an unknown race (Figure 3). Prevalence of race 1 at high frequency in the state can be expected as most of the cultivars grown in the South Dakota exhibit some level of susceptibility to tan spot race 1 (Tables 1a and 1b). Also, our phenotyping results conform with the previous studies that reported race 1 as the most prevalent race in many countries including

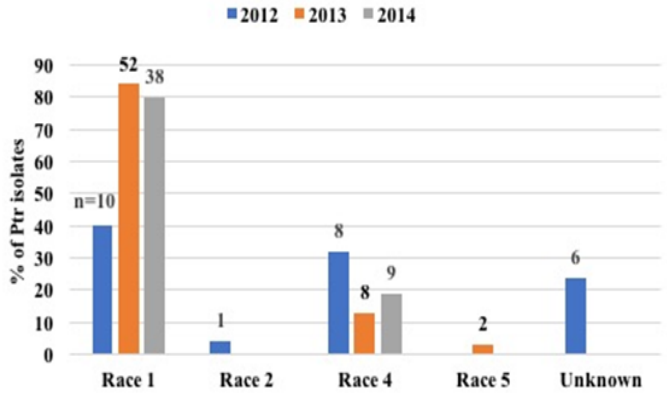

Figure 3: Race characterization of *P. tritici-repentis* isolates prevalent on wheat in South Dakota in 2012-2014.

Figure 4: Reaction of Ptr in wheat. (A) Isolate SD12-S11-P7.2 (Lacks in *Ptr ToxA* gene) produced necrosis on Glenlea (B) no chlorosis on 6B365.

the US [2,9,11,14,29,35]. One reason for dominance of race 1 over other races observed in various countries may be deployment of tan spot susceptible cultivars to race 1 on a larger acreage, as documented from two independent studies where screening with multiple *P. tritici-repentis* races identified majority of genotypes were susceptible to race 1 [36,37]. However, predominance of race 1 does not completely eliminate the possibility of other races and their prevalence at low frequency has been reported [2,10,11,13,29].

Further, 25 (18.65%) isolates were identified as race 4 and were the second most prevalent race in all three years, 2012 (32%), 2013 (13%), and 2014 (19%) (Figure 3). We observed a higher proportion of race 4 (13% to 32%) in our study as compared to around 5% reported in some earlier studies [2,10,13,29]. A higher prevalence of *P. tritici-repentis* race 4 has also been reported from the fungal alternative hosts like non-cereal grasses, smooth bromegrass, intermediate wheatgrass, western wheatgrass etc. [2] and rye [38], in the US NGP. In rye, 78% of 103 isolates characterized for their race structure were identified as race 4 [38]. Recovery of race 4 in a slight higher frequency in wheat in this study could be the race 4 isolates transferring from rye and establishing as a weak pathogen on wheat. With rye being mostly planted adjacent to wheat field plots in the NGP, it is highly likely that race 4 isolates from rye may survive on wheat crop.

Only 1.4% of the 134 isolates we phenotypically evaluated on differential set were race 5 (Figure 3). Although race 5 was detected at very low frequency and only in 2013, however, this is the first report of prevalence of race 5 in SD. Our leaf sampling was random in all three years, however, no detection of race 5 in 2012 and 2014 could be due to the majority of leaf samples likely collected from race 5 resistant cultivars. Only one isolate of the 134 isolates characterized was identified as race 2. We categorized 31 isolates that lacked *Ptr ToxA* and *Ptr ToxB* genes to be either of race 3 or race 4, however, phenotypic characterization showed six isolates did not fit under any of the 8 races. These isolates produced necrosis (Figure 4) like race 2, however, lacked Ptr ToxA so were categorized as unknown races. Recovery of these types of *P. tritici-repentis* isolates is not surprising as they have been noted in other independent studies as well [9,15,26,31]. Further, the occurrence of necrosis producing isolates without *Ptr ToxA* gene on the *Ptr ToxA* sensitive wheat genotype indicates presence of additional toxin(s) responsible for necrosis symptoms. These isolates further need to be investigated for their mechanism of host-pathogen interaction.

Reaction of wheat cultivars to tan spot (*P. tritici-repentis* race 1, race 5, and *Ptr ToxA*)

In this study, we evaluated 45 wheat cultivars (hard red spring=33 and hard red winter=12) including four advanced lines to tan spot using race 1 and race 5 to determine the potentially prevalent races in the region based on their susceptibility. Wheat cultivars belonging to both spring and winter wheat classes exhibited diverse reaction, ranging from susceptible to resistant, to both races (Tables 1a and 1b). In general, a higher number of spring wheat cultivars exhibited susceptibility to race 1 and race 5 as compared to winter wheat cultivars. Seven of the 33 spring wheat cultivars/lines, Forefront, LCS Breakaway, Norden, Russ, Select, and SD4189 exhibited susceptibility to both races 1 and 5 (Table 1a). Fourteen cultivars (42%) showed susceptible to moderately susceptible reaction to race 5, whereas 10 cultivars (30%) were susceptible to race 1. Wheat cultivars susceptible to both race 1 and 5 could play an important role in the development of new virulent race(s) such as race 6 (a combination of *Ptr ToxB* and *Ptr ToxC*), race 7 (*Ptr ToxA* and *Ptr ToxB*), and race 8 (*Ptr ToxA, Ptr ToxB, and Ptr ToxC*) as they are the combination of race 1 (*Ptr ToxA* and *Ptr ToxC*) and race 5 (*Ptr ToxB*). This is highly possible to occur, as the fungus *P. tritici-repentis* is residue-borne in nature and present year-round on wheat residue due to minimum tillage practices in South Dakota and the region. Presence of two different races (1 and 5) in the same leaf and/or in different plants within the same field provides ample opportunities to the fungus for sexual reproduction and lead to evolution of a new race.

In contrast to spring wheat, about 50% (n=6) winter wheat cultivars

exhibited susceptibility to tan spot race 1 and developed lesion type 3-3.8 (moderately susceptible) except Ideal (lesion type 4; susceptible). All winter wheat cultivars were moderately resistant to resistant to race 5 as they developed lesion type 1-2 (Table 1b). The majority of the winter wheat cultivars exhibited resistance to tan spot (race 5) which minimizes their role in the race 5 establishment in the state.

These results indicate that spring wheat cultivars that are susceptible to both races may be a potential source for survival of both races 1 and 5 in the region. Though race 5 was detected from only one location in 2013, monitoring the fungal isolates on wheat cultivars susceptible to both race 1 and 5 or only race 5 may provide better information on the race 5 prevalence in South Dakota.

All 45 wheat cultivars/lines varied in their reaction to Ptr ToxA as they developed either necrosis (sensitive) or no necrosis (insensitive). We found a very weak correlation (r=0.24) between the susceptibility-sensitivity and resistance-insensitivity to the toxin in spring wheat, however a good correlation (r=0.72) was observed in winter wheat (Tables 1a and 1b). Nine spring wheat cultivars were susceptible; whereas two cultivars were susceptible to race 1 but insensitive to the toxin. Also, 10 cultivars were resistant to race 1 and insensitive to the toxin; whereas 12 cultivars showed resistance to race 1 but sensitivity to Ptr ToxA (Table 1a). For example, spring wheat cultivar 'LCS Breakway' showed susceptibility (lesion type 4) to race 1 (Ptr ToxA producers) but exhibited insensitivity to the toxin; whereas cultivar 'Select' developed susceptible reaction (lesion type 4) to race 1 and exhibited sensitivity to the toxin. Six winter wheat cultivars that developed a susceptible reaction to race 1 were also developed necrosis (sensitive) to Ptr ToxA. Four cultivars showed resistance to race 1 were also insensitive to the toxin; whereas two winter wheat cultivars exhibited a resistant reaction to race 1 but were sensitive to Ptr ToxA. Our results are in accordance with previous studies where all 4 possible combinations of tan spot susceptibility and Ptr ToxA sensitivity (susceptibility-sensitivity, susceptibility-insensitivity; resistant-sensitivity, and resistant-insensitivity) were observed in wheat genotypes [9,16,39,40].

The results of this study indicate that a diverse population of *P. tritici-repentis* exists on wheat in South Dakota with race 1 being the most prevalent. In addition, we observed race 5 for the first time in South Dakota and identified wheat cultivars susceptible to race 1 and 5. Planting of such cultivars on a large acreage coupled with favorable climatic conditions might play an important role in the development of more virulent races. Further identification of isolates lacking *Ptr ToxA* gene can help in understanding the mechanism of pathogenicity/virulence and help in development of better TS management strategies. Our results suggest that screening of germplasm against both races 1 and 5 should be performed prior to using them in the development of tan spot resistant cultivars and regular monitoring of the pathogen population in the region can lead to better management of leaf spot diseases like tan spot.

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