

Insights into Tan Spot and Stem Rust Resistance and Susceptibility by Studying the Pre-Green Revolution Global Collection of Wheat

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Tan spot (TS), caused by the fungus *Pyrenophora tritici-repentis* (Died) Drechs, is an important foliar disease of wheat and has become a threat to world wheat production since the 1970s. In this study a globally diverse pre-1940s collection of 247 wheat genotypes was evaluated against Ptr ToxA, *P. tritici-repentis* race 1, and stem rust to determine if; (i) acquisition of Ptr ToxA by the *P. tritici-repentis* from *Stagonospora nodorum* led to increased pathogen virulence or (ii) incorporation of TS susceptibility during development stem rust resistant cultivars led to an increase in TS epidemics globally. Most genotypes were susceptible to stem rust; however, a range of reactions to TS and Ptr ToxA were observed. Four combinations of disease-toxin reactions were observed among the genotypes; TS susceptible-Ptr ToxA sensitive, TS susceptible-Ptr ToxA insensitive, TS resistant-Ptr ToxA insensitive, and TS resistant-Ptr ToxA toxin sensitive. A weak correlation ($r = 0.14$ for bread wheat and -0.082 for durum) was observed between stem rust susceptibility and TS resistance. Even though there were no reported epidemics in the pre-1940s, TS sensitive genotypes were widely grown in that period, suggesting that Ptr

ToxA may not be an important factor responsible for enhanced prevalence of TS.

Keywords : foliar disease, host-selective toxin, *Puccinia graminis* f. sp. *tritici*, wheat, yellow spot

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Introduction of a new pathogen/race or change in virulence in an already existing pathogen population, deployment of a high yielding but susceptible cultivar on a large area, and changes in cultural practices that aids pathogen survival could change the status of a minor disease to a major one and create a disease epidemic in a region. This scenario is probably responsible for an increase in tan spot in major wheat growing regions since the 1970s. Tan spot (yellow spot), caused by the fungus *Pyrenophora tritici-repentis* (Died) Drechs. (anamorph = *Drechslera tritici-repentis* (Died.) Shoem.) is an important foliar disease of wheat in major wheat growing countries, especially in the US northern Great Plains (Hosford, 1982). In addition to wheat, the pathogen can survive and infect many non-cereal grasses (Ali and Francl, 2003; Hosford, 1982; Krupinsky, 1992; Sprague, 1950). *Pyrenophora tritici-repentis* (Ptr) can survive on infested crop residue from one growing season to the next, through infected seed, and can thus be dispersed over a long distance from one country to another (Hosford, 1982; Schilder and Bergstrom, 1995). The fungus produces lens shaped necrotic lesions encircled by a chlorotic halo with a pinhead size black spot in the center of leaves of tan spot susceptible wheat cultivars. Losses due to reduction of leaf photosynthetic area caused by tan spot have been reported to be up to 50%, depending upon the cultivar, pathogen virulence, growth stage, and favorable environment for

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the disease development (Rees and Platz, 1983; Shabeer and Bockus, 1988). The fungus was initially reported in the 1850s from Germany on *Agropyron repens* (Diedicke, 1902); however, it was first reported causing leaf spot in wheat in Japan (Nisikado, 1928), and soon after in Canada (Conners, 1932) and India (Mitra, 1934). Thereafter, the disease was observed in Australia, the USA, and in many other wheat-growing countries (Barrus, 1942; De Wolf et al., 1998; Rees and Platz, 1983). Though the disease was observed on wheat in the 1920s, it was not considered a major disease or a potential threat to wheat production until the 1970s (De Wolf et al., 1998; Hosford, 1982; Moreno et al., 2012; Rees and Platz, 1983). Today, tan spot has become a major threat to sustainable wheat production with several epidemics in the post green revolution.

Eight races of *P. tritici-repentis* have been reported to date (Lamari et al., 2003), of which races 1 through 5 exist in the USA (Ali and Francl, 2003; Engle et al., 2006) with race 1 being the most prevalent in the USA and worldwide (Ali and Francl, 2003). Three host-selective effectors (toxins: Ptr ToxA, Ptr ToxB, and Ptr ToxC) produced by the fungus and considered to be associated with two distinctly identified symptoms (necrosis and chlorosis) were reported (Balance et al., 1989; Orolaza et al., 1995; Strelkov and Lamari, 2003; Tomas et al., 1990; Tuori et al., 1995). Ptr ToxA has been reported to serve as the pathogenicity or virulence factor (Friesen et al., 2006; Tuori et al., 1995).

The change in tan spot status from a minor to major disease after the 1970s is believed to be the result of several factors including (i) adoption of zero tillage from conventional tillage practices in the 1970s, to avoid soil erosion, manage soil moisture, and save fuel costs in major wheat-growing countries including the USA. This change in tillage practice leaves a large amount of crop residue that may help *P. tritici-repentis* survive from one season to the next growing season (Conners, 1940); (ii) an increase in the pathogen virulence due to a horizontal transfer of *Ptr ToxA* gene from *Stagnospora nodorum* to *P. tritici-repentis* (Friesen et al., 2006; Oliver et al., 2008); (iii) an accidental insertion of genes for tan spot susceptibility during the deployment of stem rust resistant cultivars after the 1930s and 1950s stem rust epidemics in the USA and other major wheat growing countries (De Wolf et al., 1998). Two studies have been conducted to find the link if any, between the genotype sensitivity to Ptr ToxA and the increase in tan spot in Australia (Oliver et al., 2008); and potential sources of susceptibility and sensitivity to Ptr ToxA in Canadian wheat and the increase in tan spot in the 1970s (Lamari et al., 2005), but mixed results were reported. To our knowledge, a global collection of wheat

cultivars/genotypes developed prior to major stem rust epidemics in the USA in the 1930s and 1950s, including countries representing wheat origin/center of diversity, have not been investigated for their reaction to tan spot and Ptr ToxA toxin. The objectives of this study were (i) to understand if stem rust resistance/susceptibility is associated with increased susceptibility to tan spot; (ii) to study if acquisition of Ptr ToxA from *S. nodorum* to *P. tritici-repentis* is major factor leading to increased susceptibility of bread and durum wheat to tan spot.

Materials and Methods

Plant materials. Two hundred and forty-seven wheat genotypes (bread wheat = 156; durum = 91) belonging to 6 continents Africa, Asia, Australia, Europe, North America, and South America (Supplementary Fig. 1), which were developed and/or utilized commercially in thirty-nine countries prior to the 1930s, and 1950s stem rust epidemics in the US Great Plains were obtained from United States Department of Agriculture (USDA) National Small Grains Collection, Aberdeen, Idaho (Supplementary Fig. 1). A few durum cultivars post 1970 from the USA were also characterized. Wheat genotypes Glenlea and Salamouni were included in the experiment as tan spot susceptible, Ptr ToxA sensitive and tan spot resistant, and Ptr ToxA insensitive checks, respectively. Two-week-old seedlings of all 247 wheat genotypes including checks were raised in 5 × 23 cm containers (Stuewe & Sons Inc., Tangent, OR, USA) filled with Sunshine Mix 1 (Sun Gro Horticulture, Agawam, MA, USA) and tested for their reaction to tan spot with *P. tritici-repentis* race 1, and sensitivity to Ptr ToxA. Nine seedlings (three seedlings/cone) of each genotype were evaluated for reaction to tan spot, and Ptr ToxA. The seedlings were watered and fertilized as needed. The seedlings were kept in a greenhouse at 21–22°C with 16 hours' photoperiod until the experiment was terminated.

Inoculum preparation, plants inoculation and disease rating. A single spore of *P. tritici-repentis* race 1 isolate "SD13-101-1" recovered from wheat in South Dakota, was used for inoculum production throughout the experimentation. A fresh culture of the isolate was initiated by plating the isolate dry plugs stored at –20°C on V8PDA (V8 Juice = 150 ml; CaCO₃ = 3 g; potato dextrose agar = 10 g; agar = 10 g; distilled water = 850 ml) to prepare spore suspension as described in Ali and Francl (2001). Briefly, ten dry plugs (one plug/plate) were placed in the center on fresh V8PDA plates, wrapped with aluminum foil paper, and incubated for 5–6 days at room temperature. After 6 days when the cultures had grown approxi-

mately 3 cm from the center, about 30 ml of distilled sterilized water was added into each plate and the mycelial growth was knocked down with the help of a flamed sterile test tube bottom. The plates were incubated under fluorescent grow lights for 24 h and then in the dark at 16°C for 24 h to induce conidiophores and conidia development, respectively. Thereafter, about 30 ml of distilled sterile water was added to each plate, and conidia were collected with a loop wired needle and spore suspension was adjusted to 3,000 spores/ml prior to inoculations.

Two-week-old seedlings of each of genotypes were inoculated with the race 1 spore suspension with a CO₂ pressurized hand sprayer (Power Sprayer, Preval; Chicago Aerosol, Coal City, IL, USA) and placed in humidity chambers at 100% humidity for 24 h for infection initiation. The seedlings were then moved from the humidity chambers to the greenhouse bench. Seven days post-inoculations, the seedlings were rated for disease reactions using a 1–5 rating scale where lesion type 1–2 is resistant to moderately resistant, and 3–5 is moderately susceptible to susceptible (Lamari and Bernier, 1989).

Screening for stem rust. Seedlings of 247 wheat genotypes were raised in jiffy pots containing potting soil. The screening was done at Cereal Disease Laboratory, USDA-Agricultural Research Service (USDA-ARS), St. Paul, MN, USA. Seven to 9-day-old seedlings were inoculated with urediniospores when the first leaf was fully expanded. Seedlings were evaluated against a mixture of *P. graminis tritici* races (QFCSC, QTHJC, MCCFC, RCRSC, RKQOC, TPMKC, TTTTF, TTKST, TRTTF, and TKTTC) commonly prevalent in the US Great Plains and race TTKSK by following the method described by Jin et al. (2007). The seedlings were incubated in the humidity chamber at 18°C for 14 h in dark and 4 h in light. The inoculated plants were placed on a greenhouse bench at 18 ± 2°C. The plants were rated for infection types (IT) described by Stakman et al. (1962).

Toxin bioassays. All bread and durum genotypes tested for tan spot in section “A” above were evaluated for their reaction to Ptr ToxA (Supplementary Table 1, Fig. 1). To avoid any discrepancy in screening results due to using different plants for the fungal and toxin reaction, nine fully expanded first leaves of each genotype, prior to inoculation with race 1 at two-leaf-stage, were infiltrated with purified Ptr ToxA at 10 µg/ml using a needle-less syringe as described by Faris et al. (1996). The leaves were examined 72 h post-toxin infiltration for necrosis development and rated as “+” (toxin sensitive) and “-” (insensitive). The purified Ptr ToxA was kindly provided by Dr. Steven Meinhardt, Department of Plant Pathology, North Dakota

State University, Fargo, ND, USA. Tan spot wheat differentials Glenlea (Ptr ToxA sensitive) and Salamouni (insensitive) were included as controls in the experiment. Three leaves of randomly chosen 50 genotypes were also infiltrated with toxin without race 1 spore inoculation and inoculated with race 1 spore suspension without the toxin infiltration to verify if the toxin infiltration into the first leaf would not impact the inoculation results.

Results

Reaction of bread wheat genotypes to *P. tritici-repentis* race 1 and Ptr ToxA. We evaluated 156 bread wheat genotypes with tan spot (race 1) and reactions ranging from resistant to susceptible disease were observed. Of the 156 genotypes, 104 genotypes (66.7%) developed lesion type ranging from 3–5 and were rated moderately susceptible to susceptible to tan spot; whereas, 52 genotypes (33.3%) were rated from resistant to moderately resistant showing lesion type of 1–2 (Supplementary Table 1, Fig. 1). The same genotypes were also screened for reaction to Ptr ToxA; 50.6% (n = 79) of the genotypes exhibited necrosis in the toxin infiltrated leaf area and were rated sensitive (Fig. 1). The other 49.4% (n = 77) were rated as toxin insensitive as they did not develop necrosis symptoms (Fig. 1). The wheat genotypes that were rated as tan spot susceptible (n = 104) were evaluated for ToxA reaction, 62.5% (n = 65) were sensitive and 37.5% (n = 39) insensitive to Ptr ToxA. Whereas, 52 genotypes that exhibited resistance reaction to spore inoculations, 26.9% (n = 14) were sensitive and 73.1% (n = 38) were insensitive to Ptr ToxA (Fig. 1, 2).

When looking at the genotypes from different continents, 35.9% (n = 14) of the wheat genotypes from Asia

		Bread wheat (156)		Durum wheat (91)	
		Ptr ToxA reaction			
		I (77)	Sen (79)	I (40)	Sen (51)
Tan spot reaction	S (104)	39 (37.5%)	65 (62.5%)	30 (42.3%)	41 (57.7%)
	R (52)	38 (73.1%)	14 (26.9%)	10 (50.0%)	10 (50.0%)
A				B	

Fig. 1. Reaction of bread wheat (A) and durum wheat (B) genotypes to tan spot (*Pyrenophora tritici-repentis* race 1) and Ptr ToxA. R, resistant; S, susceptible; I, insensitive to Ptr ToxA; Sen, sensitive to Ptr ToxA.

and Europe exhibited susceptibility to tan spot but were insensitive to Ptr ToxA (Table 1). However, an opposite trend was observed in susceptible genotypes belonging to Australia and North America where 56.4% (n = 53) were sensitive to the toxin and susceptible to tan spot (Table 1). Seventeen percent (n = 16) of wheat genotypes exhibited

resistance to race 1 and were also insensitive to the toxin.

Reaction of durum wheat genotypes to *P. tritici-repentis* race 1 and Ptr ToxA. In total, 91 durum genotypes were screened for their reaction to Ptr ToxA and tan spot using race 1 (produces Ptr ToxA). Nearly, 78.0% (n = 71) of the evaluated genotypes exhibited susceptibility to tan spot; whereas, the other 20 were resistant to race 1 (Fig. 1). All 91 genotypes were also evaluated for their reaction to Ptr ToxA, of which 56.0% (n = 51) turned out to be sensitive and the other 44.0% (n = 40) exhibited insensitivity to the toxin (Fig. 1). Of 71 genotypes susceptible to tan spot, 57.7% (n = 41) and 42.3% (n = 30) were sensitive and insensitive to Ptr ToxA, respectively (Fig. 1, 2). Half (n = 10) of the genotypes that exhibited resistance to tan spot race 1 were also insensitive to the toxin, whereas other half were resistant to tan spot (race 1) but were sensitive to Ptr ToxA (Fig. 1). Durum genotypes from North America were largely susceptible to tan spot and were also sensitive to the toxin; however, an opposite trend was observed in the genotypes from Europe where 75.0% of genotypes were susceptible to the fungus but were insensitive to Ptr ToxA (Table 2). Of the durum genotypes from Africa that were rated as susceptible, 57.9% were sensitive and 42.1% insensitive to the toxin reaction (Table 2).

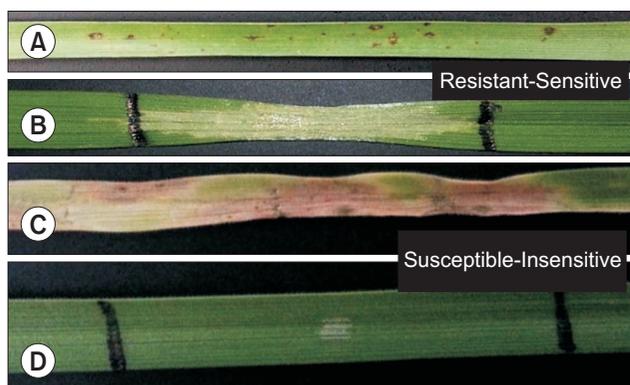


Fig. 2. (A) Bread wheat cultivar ('Molein Mis' released in 1923) showing resistance to fungus while inoculated with *Pyrenophora tritici-repentis* race 1, (B) exhibiting sensitivity to Ptr ToxA toxin, and (C) susceptible reaction with fungus to durum wheat line ('Capelli 38' released in 1946). (D) But insensitive to Ptr ToxA toxin.

Table 1. Reaction of 156 bread wheat genotypes from six continents to *Pyrenophora tritici-repentis* race 1 and Ptr ToxA

Continent	Genotypes	R/I (%)	R/Sen (%)	S/I (%)	S/Sen (%)
Africa	16	7 (43.8)	3 (18.8)	4 (25.0)	2 (12.5)
Asia	16	2 (12.5)	0	9 (56.3)	5 (31.3)
Australia	8	0	0	1 (12.5)	7 (87.5)
Europe	23	11 (47.8)	4 (17.4)	5 (21.7)	3 (13.0)
North America	86	16 (18.6)	6 (7.0)	18 (20.9)	46 (53.5)
South America	7	2 (28.6)	1 (14.3)	2 (28.6)	2 (28.6)
Total	156	38 (24.4)	14 (9.0)	39 (25.0)	65 (41.7)

R, resistant; I, insensitive to Ptr ToxA; Sen, sensitive to Ptr ToxA; S, susceptible.

Table 2. Reaction of 91 durum wheat genotypes from six continents to *Pyrenophora tritici-repentis* race 1 and Ptr ToxA

Continent	Genotypes	R/I (%)	R/Sen (%)	S/I (%)	S/Sen (%)
Africa	48	5 (10.4)	5 (10.4)	16 (33.3)	22 (45.8)
Asia	7	2 (28.6)	0	3 (42.9)	2 (28.6)
Australia	1	0	0	0	1 (100)
Europe	12	2 (16.7)	0	7 (58.3)	3 (25.0)
North America	21	1 (4.8)	5 (23.8)	3 (14.3)	12 (57.1)
South America	2	0	0	1 (50.0)	1 (50.0)
Total	91	10 (11.0)	10 (11.0)	30 (33.0)	41 (45.1)

R, resistant; I, insensitive to Ptr ToxA; Sen, sensitive to Ptr ToxA; S, susceptible.

Reaction of 247 wheat genotypes to stem rust. All 156 bread wheat and 91 durum genotypes were evaluated against stem rust using the bulk of local races. Nearly 98.7% ($n = 154$) were susceptible with IT ranging from (3–4) on the Stakman rating scale (Stakman et al., 1962). Only two genotypes developed IT fleck to 1 and were rated as resistant (Fig. 3). Of these 154 stem rust susceptible genotypes, 64.3% ($n = 99$) were rated as susceptible and 35.7% ($n = 55$) resistant to tan spot (Fig. 3). Additionally, comparing the reaction of stem rust susceptible genotypes ($n = 154$) to Ptr ToxA demonstrated 52.6% ($n = 81$) genotypes were sensitive and 47.4% ($n = 73$) were insensitive to Ptr ToxA (Fig. 3).

In durum wheat, 79.1% ($n = 72$) of 91 genotypes were rated as stem rust susceptible and the other 20.9% ($n = 19$) were resistant (Fig. 3). Of those 72 stem rust susceptible genotypes, 75.0% ($n = 54$) and 25.0% ($n = 18$) were susceptible and resistant to tan spot, respectively. Also, 73.6% ($n = 53$) of the stem rust susceptible genotypes were sensitive and the other 26.4% ($n = 19$) were insensitive to the toxin Ptr ToxA (Fig. 3). Of the 19 stem rust resistant durum genotypes, 78.9% ($n = 15$) were susceptible, whereas, 21.1% ($n = 4$) were resistant to tan spot. Of the stem rust resistant durums, 94.7% ($n = 18$) durums showed sensitivity and 5.3% ($n = 1$) exhibited insensitivity to the toxin when infiltrated with Ptr ToxA (Fig. 3). In this study we observed a large number of varieties/genotypes that exhibit susceptibility to stem rust, exhibit

both resistant and susceptible reaction to tan spot and also display both sensitive and insensitive reaction to toxin Ptr ToxA; thus, a weak ($r = 0.145$ for bread wheat and $r = -0.082$ for durum wheat) to no correlation was observed between the stem rust susceptibility and tan spot resistance.

Discussion

A change in tan spot status from a minor disease prior 1960s to a major disease in the 1970s presents a serious threat to wheat production in the USA and the world. Plant pathologists and breeders are continuously developing superior cultivars with better resistance, and developing management strategies to minimize losses due to tan spot. An increase in tan spot occurrence could be a result of change in the pathogen virulence, and/or a gene insertion of susceptibility in the wheat varieties during deployment of stem rust resistance after rust epidemic in the 1930s and 1950s in the US and elsewhere (De Wolf et al., 1998). There is limited information on the reaction of bread wheat and durum wheat genotypes/cultivars developed prior to rust epidemics in the USA and elsewhere. Evaluating older genotypes for reaction to stem rust, tan spot, Ptr ToxA (toxin sensitivity), and the role of host selective toxins (HSTs) in the disease development can address two major questions; (i) is stem rust resistance/susceptibility associated with increased susceptibility to tan spot; (ii) or, was acquisition of Ptr ToxA from *S. nodorum* to *P. tritici-repentis* a major factor in increased susceptibility of wheat to tan spot? In this study we evaluated pre-epidemic cultivars of bread wheat ($n = 156$) and durum wheat ($n = 91$), against stem rust, tan spot (*P. tritici-repentis* race 1), the Ptr ToxA producer and most prevalent race worldwide (Ali and Francl, 2003; Ali et al., 2010; Sarova et al., 2005). Our results did not show any correlation between stem rust susceptibility and tan spot resistance as well as the role of Ptr ToxA in the disease development. We observed multiple interactions where wheat cultivars were (i) stem rust susceptible and tan spot susceptible (Fig. 3); (ii) stem rust resistant and tan spot resistant; (iii) susceptible to race 1 and sensitive to the toxin; (iv) resistant to race 1 and insensitive to the toxin; (v) susceptible to the race 1 and insensitive to the toxin; and (vi) resistant to the race 1 and sensitive to the toxin (Fig. 3). A weak ($r = 0.145$ for bread wheat and $r = -0.082$ for durum wheat) to no correlation between stem rust susceptibility and tan spot resistance and susceptibility was observed. Of the 154 wheat genotypes that were susceptible to stem rust, 55 (35.7%) of them were resistant to tan spot and 99 (64.3%) were susceptible, and similar results were observed in durum genotypes (Fig. 3). Thus,

		Tan spot reaction		Ptr ToxA reaction		
Bread wheat (156)	Stem rust reaction	R (56) S (100)		I (73) Sen (83)		
		R (154) S (154)	55 (35.7%) 99 (64.3%)	R (154) S (154)	73 (47.4%) 81 (52.6%)	
	A	R (2) S (154)	1 (50.0%) 1 (50.0%)	B	R (2) S (154)	0 (0%) 2 (100%)
Durum wheat (91)	Stem rust reaction	R (22) S (69)		I (20) Sen (71)		
		R (72) S (72)	18 (25.0%) 54 (75.0%)	R (72) S (72)	19 (26.4%) 53 (73.6%)	
	C	R (19) S (72)	4 (21.1%) 15 (78.9%)	D	R (19) S (72)	1 (5.3%) 18 (94.7%)

Fig. 3. Comparative analysis of bread wheat genotypes for their reaction to, (A) tan spot and stem rust; (B) Ptr ToxA and stem rust. Comparative analysis of durum wheat genotypes for their reaction to, (C) tan spot and stem rust; (D) Ptr ToxA and stem rust. R, resistant; S, susceptible; I, insensitive to Ptr ToxA; Sen, sensitive to Ptr ToxA.

the data suggests that resistance/susceptibility to stem rust is not likely associated with an increased susceptibility to tan spot.

However, in a previous study (Lamari et al., 2005), 86 wheat genotypes/cultivars developed and deployed from pre and post-stem rust epidemics in North America were screened against *P. tritici-repentis* races 2 and 5 and host-selective toxins Ptr ToxA and Ptr ToxB in Canada to investigate the sources of tan spot susceptibility and the toxins sensitivity (Lamari et al., 2005). They reported that sensitivity and susceptibility to tan spot may come from three bread wheat cultivars Red Fife, Hard Red Calcutta, Marquis, and one tetraploid wheat Yaroslav Emmer, utilized as a source of stem resistance in the development of first bread wheat cultivar “Hope” to combat stem rust in the US and beyond. All four cultivars were tan spot susceptible and Ptr ToxA sensitive. The majority of the cultivars were sensitive to race 5 and Ptr ToxB and a good correlation was observed between the genotype tan spot susceptibility and the toxin sensitivity, but why tan spot was not a problem before the 1970s while most of the commercial cultivars were susceptible to tan spot was not explained or investigated. In contrast, our results using a large collection of 247 genotypes did not follow this pattern as more than 50% of wheat genotypes exhibited susceptibility to race 1 (Ptr ToxA producers) but insensitivity to Ptr ToxA. In addition, some genotypes exhibited resistance to the fungal inoculation but were sensitive to the toxin.

In addition, we tested 247 wheat genotypes against stem rust, tan spot race 1, and Ptr ToxA, collected from various countries and continents with none of the genotype collection following any specific pattern; thus, indicating that stem rust resistance and tan spot susceptibility (race 1) and sensitivity to Ptr ToxA may not necessarily be always true in the wheat-tan spot host-pathogen system. Additionally, we identified wheat genotypes that exhibited resistance to spore inoculation but were sensitive to Ptr ToxA; these genotypes may have an alternative mechanism for resistance to tan spot. Our results suggest that tan spot susceptibility-toxin(s) sensitivity and/or resistance-toxin insensitivity may not be ubiquitous (Lamari et al., 2005), and this discrepancy may be due to wheat genotypes collected and tested in the two studies may have been from different countries. The primary objective of Lamari et al. (2005) was to trace the sources of tan spot susceptibility that was incorporated into the Canadian wheat cultivars, and while ours were to understand if tan spot susceptible/sensitivity was incorporated in wheat while breeding for stem rust resistance and if acquiring *Ptr ToxA* gene from *S. nodorum* has any major role in the tan spot increase.

Further, if acquisition of Ptr ToxA from *S. nodorum* to *P. tritici-repentis* was a major factor leading to increased susceptibility of wheat to tan spot, most of the genotypes should be expected to be sensitive to Ptr ToxA; however, we observed all four combinations (tan spot susceptible-Ptr ToxA insensitive, tan spot susceptible-Ptr ToxA sensitive, tan spot resistant-Ptr ToxA insensitive, tan spot resistant-Ptr ToxA sensitive) suggesting that Ptr ToxA was not the major factor responsible in increased incidence of tan spot (Fig. 1). Our results further suggest that the role of toxin in the disease development is a genotype dependent host-pathogen interaction. Similar kinds of interactions have been observed among post 1970s modern bread wheat and durum wheat genotypes when they were challenged with race 1 or race 2 (both races produce Ptr ToxA) spore inoculation and Ptr ToxA (Ali et al., 2007, 2010; Friesen et al., 2003; Noriel et al., 2011).

The toxin Ptr ToxA might play a role in aggressiveness, not in pathogenicity was also suggested by Friesen et al. (2003) when wheat mutants (tan susceptible but ToxA insensitive) developed from Ptr ToxA sensitive bread wheat genotype ND495 showed slower development of disease initially in the mutants as compared to the wild type inoculated with race 2; however, similar disease level was observed at the final disease rating time point. Another study (Andrie et al., 2007) reported two isolates SO3 and PT82 which did not harbour *ToxA* and *ToxB* genes, respectively, but produced necrosis and chlorosis on wheat differential lines Glenlea (Ptr ToxA sensitive) and 6B662 (Ptr ToxB sensitive). These studies suggest there might be some other pathogenicity factors involved in addition to current HST which make the fungal population virulent or aggressive towards the 1970s. Our results, using genotypes older than 1970 shows that the all four combinations (tan spot susceptible-Ptr ToxA insensitive, tan spot susceptible-Ptr ToxA sensitive, tan spot resistant-Ptr ToxA insensitive, and tan spot resistant-Ptr ToxA insensitive) existed pre-1970, validating our earlier observation (Ali et al., 2007, 2010) and other studies (Friesen et al., 2003; Noriel et al., 2011; Oliver et al., 2008), and suggests that Ptr ToxA is likely not a major factor in tan spot increase in USA and elsewhere.

In our study, 64.0% (n = 14) of the bread wheat genotypes from Asia and Europe exhibited susceptibility to tan spot but were insensitive to Ptr ToxA. However, an opposite trend was observed in susceptible genotypes belonging to Australia and North America where 78.0% of susceptible genotypes were also sensitive to the toxin (Table 1). Similarly, in durum wheat, 81.0% (n = 13) of the durum genotypes from North America and Australia that showed a susceptible reaction to the fungus were also sensitive to the toxin (Table 2). In Africa, tan spot suscep-

tible and toxin insensitive cultivars were more prevalent. This suggests that primarily two types of germplasm, tan spot susceptible-toxin sensitive and tan spot susceptible-toxin insensitive, were utilized in the development of pre- and post-stem rust epidemics durum and bread wheat cultivars globally; however, the ratio of the two germplasms utilized varied from continent to continent. Similar observations were reported by Noriel et al. (2011) while studying post 1970s genotypes. Additionally, the pathogen population with or without Ptr ToxA may have varied in different continents and thus decreased the toxin significance in tan spot development. Although tan spot has been reported from 6 continents, losses due to tan spot are greater in North America and Australia where the majority of the bread wheat genotypes were both susceptible to tan spot and sensitive to Ptr ToxA.

In another study (Ali et al., 2010), several isolates were identified which lacked *Ptr ToxA* gene but still induce necrosis, again suggesting Ptr ToxA was not the only factor responsible for tan spot incidence. Further, if insertion of Ptr ToxA from *S. nodorum* into *P. tritici-repentis* happened in the 1940s (Friesen et al., 2006), which is very recent event, it probably occurred on a very low scale. It may be very likely that the fungus can be transported over long distances through an infected seed, but it seems difficult if not impossible for the fungal population harboring Ptr ToxA to establish globally in such a short period. Several reports indicate the global prevalence of race 1, which characteristically shows both necrosis and chlorosis symptoms are due to Ptr ToxA and Ptr ToxC (Ali and Francl, 2003; Sarova et al., 2005; Singh et al., 2010). However, the pathogen population prevalent prior to the 1940s needs to be further characterized to understand of virulence mechanism in tan spot and if it carried Ptr ToxA. Our study showed tan spot susceptible and sensitive varieties were widely grown prior to the green revolution. Existence of wheat genotypes and their diverse reaction to the fungal isolates for tan spot symptoms (necrosis) with or without ToxA in our current study indicate a low association in tan spot outbreaks in the 1970s and later.

A tan spot epidemic was observed in 1937 and 1939 in Manitoba, and in 1941 in Saskatchewan (Conners, 1940, 1941). The upsurge in disease in those years were thought to be due to the use of a one-way disk for soil preparation which may have left a lot of residue on the soil which favored the pathogen overwintering. Similar observations on an increase in tan spot and other stubble borne wheat diseases were reported when farmers adopted zero tillage, moving away from conventional tillage practices to avoid soil erosion, improve moisture retention, and save on fuel expenses (De Wolf et al., 1998; Hosford, 1982; Odvody

et al., 1982).

Suggesting retention of infested crop residue due to a change in tillage practices may have a significant role in upsurge of tan spot. Our study shows neither incorporation of stem rust resistance during breeding nor horizontal transfer Ptr ToxA led to increased susceptibility of wheat cultivars in northern Great Plains.

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