Genetic Analysis of Resistance to *Pyrenophora tritici-repentis* Races 1 and 5 in Tetraploid and Hexaploid Wheat

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ABSTRACT

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Tan spot of wheat, caused by the fungus *Pyrenophora tritici-repentis*, is a destructive disease worldwide that can lead to serious losses in quality and quantity of wheat grain production. Resistance to multiple races of *P. tritici-repentis* was identified in a wide range of genetically diverse genotypes, including three different species *Triticum aestivum* (AABBDD), *T. spelta* (AABBDD), and *T. turgidum* (AABB). The major objectives of this study were to determine the genetic control of resistance to *P. tritici-repentis* races 1 and 5 in 12 newly identified sources of resistance. The parents, F₁, F₂, and F_{2:3} or F_{2:5} families of each cross were

analyzed for the allelism tests and/or inheritance studies. Plants were inoculated at the two-leaf stage under controlled environmental conditions and disease reaction was assessed based on lesion-type rating scale. A single recessive gene controlled resistance to necrosis caused by *P. tritici-repentis* race 1 in both tetraploid and hexaploid resistant genotypes. The lack of segregation in the inter- and intra-specific crosses between the resistant tetraploid and hexaploid genotypes indicated that they possess the same genes for resistance to tan necrosis and chlorosis induced by *P. tritici-repentis* race 1. A single dominant gene for chlorosis in hexaploid wheat and a single recessive gene for necrosis in tetraploid wheat, controlled resistance to *P. tritici-repentis* race 5.

Additional keywords: host resistance, *Triticum* species.

Tan spot, a foliar disease of durum and common wheat, is caused by the fungus *Pyrenophora tritici-repentis* (Died.) Drechs. This fungus on susceptible durum and common wheat induces two distinct symptoms; tan necrosis and chlorosis. On an average, tan spot causes 5 to 15% yield losses; however, it can cause up to 50% yield loss under conditions favorable for disease development (2,20). Tan spot also adversely affects the quality of grain by causing grain shrivelling, dark smudge, and black point (2,9). Recent increases in the severity and incidence of tan spot have been attributed to changes in cultural practices such as shifts from conventional tillage and stubble burning to conservation tillage systems, shorter crop rotations, continuous wheat cultivation, and use of susceptible wheat cultivars (2,9). Adoption of a number of cultural practices in association with application of fungicides is effective in controlling tan spot but they have their limitations. Growing resistant cultivars is the most practical, environmentally safe, effective, and economical means of controlling this disease (2,20).

Currently, eight races of *P. tritici-repentis* have been identified based on their ability to induce necrosis and/or chlorosis on a set of differential cultivars (17). To date, three host-specific toxins (e.g., Ptr ToxA, Ptr ToxB, and Ptr ToxC) produced by the tan spot fungus have been identified and characterized. Genetic studies have established that the same gene controls sensitivity to the toxins and susceptibility to their producer races (3,12).

Evaluation of germplasm could lead to identification of potential donors for a given trait. Information coming out of genetic studies reveals which germplasm and breeding strategy is to be adopted to develop cultivars containing the desired traits. Various

host-pathogen interaction studies on tan spot of wheat have reported resistance to be inherited qualitatively (1,15,22) or quantitatively (4,7,18). The same single recessive gene conditioning resistance to necrosis induced by *P. tritici-repentis* races 1 and 2, in both tetraploid and hexaploid wheat, is mapped on the long arm of chromosome 5B and designated as *tsn1* (1,6). The relationship between other resistance genes for tan spot in tetraploid and hexaploid wheat is still unknown.

Singh et al. (21) mapped the gene, *tsn2*, controlling resistance to necrosis induced by *P. tritici-repentis* race 3 in tetraploid wheat to the long arm of chromosome 3B. Tadesse et al. (27) identified single recessive genes, *tsn3* and *tsn-syn1*, effective against tan spot caused by *P. tritici-repentis* race 1 on chromosome 3D in the hexaploid synthetic wheat lines XX41 and XX110, respectively. Additionally, they also observed a dominant gene, designated *Tsn-syn2*, on chromosome 3D in the synthetic line XX45. In a recent report, Tadesse et al. (28) reported resistance to tan spot induced by *P. tritici-repentis* race 1 in the hexaploid resistant genotype Salamouni to be located on chromosome 3A and designated the gene as *tsn4*. Singh et al. (25) reported that a single gene located on chromosome 3B, designated *tsn5*, controls resistance to necrosis induced by *P. tritici-repentis* race 5 in tetraploid wheat.

Working with hexaploid wheat, Effertz et al. (3) mapped the locus, *tsc1*, conditioning insensitivity to chlorosis caused by toxin Ptr ToxC (*P. tritici-repentis* race 1) to the short arm of chromosome 1A. Faris et al. (7) reported that resistance to chlorosis caused by *P. tritici-repentis* race 3 in hexaploid wheat was quantitative with a major quantitative trait loci (QTL) located on chromosome 1A. Further, Singh and Hughes (23) confirmed that resistance to chlorosis caused by *P. tritici-repentis* races 1 and 3 in hexaploid wheat was controlled by the same gene. Friesen and Faris (10), using a hexaploid wheat population, identified the Ptr ToxB insensitivity gene, *tsc2*, at the distal tip of the short arm of

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chromosome 2B. This gene accounted for 69% of the phenotypic variation caused by *P. tritici-repentis* race 5. Faris and Friesen (8), studying hexaploid wheat, for the first time observed race non-specific QTLs on chromosome arms 1BS and 3BL effective against *P. tritici-repentis* races 1, 2, 3, and 5.

Singh et al. (24) screened 975 accessions of wheat and its related species and identified new sources of resistance effective against *P. tritici-repentis* races 1, 2, 3, and 5. The objectives of this study were to (i) determine the genetic basis of resistance to *P. tritici-repentis* races 1 and 5 in the newly identified sources of resistance coming from different *Triticum* species including synthetic wheat, (ii) clarify through allelic studies if these uncharacterized resistant sources possess resistance genes not identified previously, and (iii) determine the relationship between resistance genes in tetraploid and hexaploid wheat.

MATERIALS AND METHODS

Population development. A total of 17 genotypes were selected for this study based on their genetic make-up and reaction to *P. tritici-repentis* races 1 and 5 (Table 1). These genotypes included seven synthetic hexaploid wheat genotypes namely, Synthetic Hexaploid Elite (SHE) #9, SHE #25, SHE #67, SHE #85, SHE #89, Altar Synthetic, and Septoria Synthetic #57; seven hexaploid wheat genotypes, Kenyon, Katepwa, 98W1147, Erik, CIMMYT Line #18, Intros Line #7, and 92MREHTR 28B; one hexaploid spelt wheat, 2000 Spelt #20; one tetraploid accession, *T. turgidum* #283 (PI352519); and the durum cultivar Coulter. These genotypes were selected based on the findings of Singh et al. (24). Twenty cross populations including 13 resistant/susceptible (R/S), 5 resistant/resistant (R/R), and 2 susceptible/susceptible (S/S), were made using the 17 selected genotypes. Each F₁ plant grown to produce F₂ seed was harvested separately. The individual F₂ populations of each cross were tested for disease reaction to eliminate nonhybrid populations. The F₂ plants were grown to produce F_{2,3} families. Advance generations (F_{2,5} families) were produced by single seed descent in each of the crosses studied.

To study the inheritance and allelic relationship of the resistance genes for tan spot, the parents, F₁, F₂, and F_{2,3} or F_{2,5}

families were screened individually with *P. tritici-repentis* race 1 or 5. Depending upon the cross, 7 to 9 F₁ plants per cross and F₂ populations ranging from 76 to 269 plants per cross were screened. At least 70 families per cross were tested for the genetic studies. A family size of 16 to 18 plants was used for the genetic studies. This gave a 99% probability of being able to distinguish between a segregating and a nonsegregating family assuming a single gene controlled resistance (13).

Disease assessment. The conidial inoculum was produced using a modification of the method of Lamari and Bernier (14). The isolates Ptr D-2000-HT VI-5 (*P. tritici-repentis* race 1) and Ptr DW-13 (*P. tritici-repentis* race 5) were used for inoculum production. Additionally, the cross *T. turgidum* #283/Coulter was screened with *P. tritici-repentis* race 2 isolate Ptr 86-124. The inoculum of *P. tritici-repentis* races 1, 2, and 5 was obtained by placing 0.5-cm-diameter dried mycelial plugs of the isolate, previously stored at -20°C, on 10-cm petri plates containing V8-potato dextrose agar (PDA) (150 ml of V8-juice, 10 g of PDA, 10 g of agar, 3 g of CaCO₃, and 850 ml of distilled water). These cultures were incubated in the dark at 20 to 22°C for 6 days. The plates were then flooded with sterile distilled water and the mycelium was flattened with the base of a sterile test tube. Excess water was decanted from the dishes and the plates were incubated under continuous light at 22 to 24°C for 2 days followed by 1 day in the dark in an incubator at 16°C to induce conidiophore and conidia production, respectively. The plates were flooded with sterile distilled water and the conidia were dislodged with a camel-hair brush. Spore concentration was measured with a haemocytometer and adjusted to 3,000 conidia per ml before inoculation. In order to reduce surface tension, 2 drops of Tween 20 (polyxyethylene sorbitan monolaurate) were added per liter of the spore suspension.

To optimize uniform emergence, seeds were pregerminated and seeds at a similar stage of germination were planted in 6-in-diameter pots containing soilless mix (Redi-Earth, W.R. Grace and Co. of Canada Ltd., Ajax, ON, Canada). Appropriate checks including the resistant and susceptible parents used in the development of the cross were included in each test to verify the validity of the race and inoculation process. For the genetic studies, nine seeds per pot were planted. All disease tests were

TABLE 1. Genotype, species/genome, pedigree/accession, and reaction to *Pyrenophora tritici-repentis* races 1 and 5 of 17 *Triticum aestivum* and one each of *T. spelta*, *T. turgidum*, and *T. durum* genotypes evaluated in the greenhouse at University of Saskatchewan, Saskatoon (2002 to 2004) and North Dakota State University, Fargo (2004 to 2005)

Genotype	Species/genome	Pedigree/accessions	Race 1		Race 5	
			Necrosis	Chlorosis	Necrosis	Chlorosis
Synthetic Hexaploid Elite #9	<i>T. aestivum</i> /ABD	Altar 84/A. <i>tauschii</i> (211)	Resistant ^a	Resistant	Resistant	Resistant
Synthetic Hexaploid Elite #25	<i>T. aestivum</i> /ABD	Altar 84/A. <i>tauschii</i> (224)	Resistant	Resistant	Resistant	Resistant
Synthetic Hexaploid Elite #67	<i>T. aestivum</i> /ABD	Snipe/YAV79//Dack/Teal/3/A. <i>tauschii</i> (629)	Resistant	Resistant	Resistant	Resistant
Synthetic Hexaploid Elite #85	<i>T. aestivum</i> /ABD	Ceta/A. <i>tauschii</i> (174)	Resistant	Resistant	Resistant	Resistant
Synthetic Hexaploid Elite #89	<i>T. aestivum</i> /ABD	STY-US/Celta//Pals/3/SRN5/4/A. <i>tauschii</i> (502)	Resistant	Resistant	Resistant	Resistant
Septoria Synthetic #57	<i>T. aestivum</i> /ABD	BCN//GAN/A. <i>squarrosa</i> (408)	Resistant	Resistant	Resistant	Resistant
ALTAR Synthetic	<i>T. aestivum</i> /ABD	Altar 84*5/A. <i>squarrosa</i> (219)//YACO	Resistant	Resistant	Resistant	Resistant
92MREHTR 28B	<i>T. aestivum</i> /ABD	Car853/Coc/Vee'S'/3/E7408/Pam'S'//Hor	Resistant	Resistant	Resistant	Resistant
CIMMYT Line #18	<i>T. aestivum</i> /ABD	Unknown (origin Mexico)	Resistant	Resistant	Resistant	Resistant
INTROS Line #7	<i>T. aestivum</i> /ABD	Unknown (origin Mexico)	Resistant	Resistant	Resistant	Resistant
Katepwa	<i>T. aestivum</i> /ABD	Neepawa*6/RL-2938/3/Neepawa*6//CI-8154/2*Frocor	Susceptible ^b	Resistant	Resistant	Susceptible
Kenyon	<i>T. aestivum</i> /ABD	Neepawa*5/Buck Manantial	Susceptible	Resistant	Resistant	Susceptible
98W1147	<i>T. aestivum</i> /ABD	HY413/93FHB#21//ID3775	Susceptible	Resistant	Resistant	Resistant
2000 Spelt #20	<i>T. spelta</i> /ABD	PI348771/Oberkulm	Resistant	Resistant	Resistant	Resistant
<i>Triticum turgidum</i> #283	<i>T. turgidum</i> /AB	PI352519	Resistant	Resistant	Resistant	Resistant
Coulter	<i>T. durum</i> /AB	DT-188/Dt-224//DT-182	Susceptible	Resistant	Susceptible	Resistant
Erik (Check)	<i>T. aestivum</i> /ABD	Kitt/2/Waldron/Era	Resistant	Resistant	Resistant	Resistant
6B-365 (Check)	<i>T. aestivum</i> /ABD	Unknown	Resistant	Susceptible	Resistant	Resistant
6B-662 (Check)	<i>T. aestivum</i> /ABD	Unknown	Resistant	Resistant	Resistant	Susceptible
Glenlea (Check)	<i>T. aestivum</i> /ABD	Pemina*2/Bage/2/CB100	Susceptible	Resistant	Resistant	Resistant

^a Genotype showing resistant reaction and a rating of 1 to 2 on a 1-to-5 lesion-type scale.

^b Genotype showing susceptible reaction and a rating of 3 to 5 on a 1-to-5 lesion-type scale.

conducted using a completely randomized design with two replicates. Using a hand sprayer, plants at the two-leaf stage were sprayed until runoff with the conidial suspension of the race tested. Following inoculation, the seedlings were incubated for 24 h maintaining continuous leaf wetness in a mist chamber located in a growth room at 22/17°C (day/night) with a 16-h photoperiod and then returned to benches in the same growth room. Eight days after inoculation, the seedlings were rated for disease reaction based on the 1-to-5 lesion-type rating scale developed by Lamari and Bernier (14), with plants having ratings of 1 and 2 considered resistant and those with ratings of 3 to 5 classified as susceptible.

Statistical analysis. Segregation data observed for the F₂ plants and F_{2:3} families were analyzed using the chi-square test for goodness-of-fit to various genetic models to determine the number of genes controlling resistance to tan spot of wheat (26).

RESULTS

Reaction of the 17 selected genotypes to *P. tritici-repentis* races 1 and 5 were consistent in all tests. Kenyon, 98W1147, Katepwa, and Coulter were susceptible showing necrotic symptoms but were resistant to chlorosis induced by *P. tritici-repentis* race 1 (Table 1). All the remaining 12 genotypes were resistant to both the necrosis and chlorosis components of tan spot induced by *P. tritici-repentis* race 1. Kenyon and Katepwa showed chlorosis while Coulter exhibited necrotic symptoms upon inoculation with *P. tritici-repentis* race 5. All the other genotypes were resistant to *P. tritici-repentis* race 5 (Table 1). The checks genotypes; Erik, Glenlea, 3B-365, and 6B-662, reacted as expected upon inoculation with either *P. tritici-repentis* race 1 or race 5 (16).

Genetics of resistance to *P. tritici-repentis* race 1: Inheritance studies. All 13 R/S crosses studied were observed to segregate for the necrosis component of tan spot caused by *P. tritici-repentis* race 1 and segregation did not occur for the chlorosis component. All the F₁ plants of the R/S cross among tetraploid

wheat were susceptible, indicating that resistance to necrosis was recessive in tetraploid wheat (Table 2). In the inter-specific R/S cross between *T. turgidum* #283 (tetraploid) and 98W1147 (hexaploid), all F₁ plants were susceptible. The two susceptible hexaploid genotypes, Kenyon and 98W1147, were crossed with a diverse set of resistant hexaploid parents including synthetic and spelt wheat; however, all F₁ plants of these R/S crosses were susceptible indicating resistance to necrosis induced by *P. tritici-repentis* race 1 in the hexaploid wheat was controlled by recessive gene(s).

The F₂ generation of the 13 R/S inter-and intra-specific crosses segregated in a 1 resistant:3 susceptible ratio, indicating that a single gene controlled resistance and confirming that resistance to necrosis was recessive in all crosses studied (Table 2). The F_{2:3} families of all R/S crosses segregated in the ratio 1 homozygous resistant:2 segregating:1 homozygous susceptible, thus confirming the hypothesis of monogenic control of resistance to necrosis in tetraploid wheat, hexaploid wheat, and an inter-specific cross between tetraploid and hexaploid wheat (Table 3).

Allelism studies. All the 16 parents involved in development of the 19 crosses studied with *P. tritici-repentis* race 1, were resistant to chlorosis (Table 1). In each of the 19 crosses, which were R/R crosses for segregation to chlorosis by *P. tritici-repentis* race 1, all F₁ and F₂ plants tested were resistant (Table 2). Confirmation of the lack of segregation of single-plant data was done by screening F_{2:3} families of all the crosses except the cross Coulter/Kenyon. The lack of segregation for chlorosis confirms that the resistant sources from both tetraploid and hexaploid wheat shared the resistance gene(s) controlling chlorosis induced by *P. tritici-repentis* race 1 (Table 3).

All F₁ plants of the R/R crosses of five resistant hexaploid genotypes with the resistant hexaploid check cultivar Erik were resistant to necrosis induced by *P. tritici-repentis* race 1 (Table 2). However, while most F₂ plants of these crosses were resistant, a few plants in four of the five crosses gave a moderately susceptible necrotic reaction (rating 3). None of the segregations of the

TABLE 2. Reaction of F₁ and F₂ plants of 19 wheat crosses to necrosis caused by *Pyrenophora tritici-repentis* race 1 evaluated in the greenhouse at University of Saskatchewan, Saskatoon in 2002 to 2004

Cross	F ₁		F ₂		Ratio tested	χ^2 value	P value ^b
	R ^a	S	R	S			
Resistant (hexaploid)/susceptible (hexaploid)							
Syn. Hex. Elite #9/98W1147	0	9	55	173	1:3	0.94	0.76
Syn. Hex. Elite #25/98W1147	0	8	79	190	1:3	2.74	0.09
Syn. Hex. Elite #67/98W1147	0	9	72	166	1:3	3.50	0.06
Syn. Hex. Elite #85/98W1147	0	7	76	180	1:3	3.00	0.08
Syn. Hex. Elite #89/98W1147	0	7	52	124	1:3	1.94	0.16
Septoria Syn. #57/Kenyon	0	8	75	175	1:3	3.33	0.07
2000 Spelt #20/Kenyon	0	7	74	177	1:3	2.69	0.10
Intros Line #7/Kenyon	0	8	60	196	1:3	0.33	0.56
CIMMYT Line #18/Kenyon	0	9	61	198	1:3	0.29	0.59
Altar Synthetic/Kenyon	0	8	54	202	1:3	2.08	0.14
92MREHTR28B/Kenyon	0	7	60	197	1:3	0.37	0.54
Resistant (tetraploid)/susceptible (tetraploid)							
<i>Triticum turgidum</i> #283/Coulter	0	7	74	181	1:3	2.19	0.13
Resistant (tetraploid)/susceptible (hexaploid)							
<i>T. turgidum</i> #283/98W1147	0	7	20	54	1:3	0.16	0.68
Resistant (hexaploid)/resistant (hexaploid)							
2000 Spelt #20/Erik	8	0	178	2	1:0	—	—
Intros Line #7/Erik	9	0	256	0	1:0	—	—
CIMMYT Line #18/Erik	8	0	248	8	1:0	—	—
Altar Synthetic/Erik	9	0	255	1	1:0	—	—
Resistant (tetraploid)/resistant (hexaploid)							
<i>T. turgidum</i> #283/Erik	7	0	192	0	1:0	—	—
Susceptible (tetraploid)/susceptible (hexaploid)							
Coulter/Kenyon	0	8	0	168	0:1	—	—

^a Plants were rated on a 1-to-5 lesion-type rating scale. Plants with ratings of 1 and 2 were classified as resistant (R) and those with ratings of 3 to 5 were classified as susceptible (S).

^b Probability of obtaining deviations from the expected ratio by chance alone. A probability value greater than 0.05 indicates that the observed population does not differ significantly from expected hypothesis.

R/R crosses fit a ratio indicative that the resistant sources possessed different resistance genes. The F₁ and F₂ single-plant segregation data were confirmed by screening the F_{2:3} families of each of these crosses. All the F_{2:3} families of each of the crosses tested were homozygous resistant, thus confirming that all the six resistant parents shared the same resistance gene (Table 3). All F₁ and F₂ generations and F_{2:3} families of the cross between resistant tetraploid genotype *T. turgidum* #283 and resistant hexaploid cultivar Erik did not segregate for resistance (Tables 2 and 3). This confirms that the tetraploid and hexaploid resistant geno-

types studied shared the same single gene for resistance to necrosis induced by *P. tritici-repentis* race 1.

Genetics of resistance to *P. tritici-repentis* race 5: Inheritance studies. All F₁ plants of the four R/S crosses among the hexaploid wheat genotypes were resistant, indicating that resistance to chlorosis caused by *P. tritici-repentis* race 5 was dominant (Table 4). The F₂ generations of the R/S intra-specific crosses segregated in a 3 resistant:1 susceptible ratio, indicating that a single gene controls resistance and confirming that resistance to chlorosis was dominant in all the crosses studied. The F_{2:5}

TABLE 3. Reaction of F_{2:3} families of 18 wheat crosses to necrosis caused by *Pyrenophora tritici-repentis* race 1 evaluated in the greenhouse at University of Saskatchewan, Saskatoon in 2002 to 2004

Cross	F _{2:3} families ^a			Ratio tested	χ^2 value	P value ^b
	R	Seg	S			
Resistant (hexaploid)/susceptible (hexaploid)						
Syn. Hex. Elite #9/98W1147	17	45	18	1:2:1	1.18	0.56
Syn. Hex. Elite #25/98W1147	22	45	13	1:2:1	2.05	0.36
Syn. Hex. Elite #67/98W1147	22	44	14	1:2:1	1.50	0.47
Syn. Hex. Elite #85/98W1147	14	47	19	1:2:1	3.05	0.22
Syn. Hex. Elite #89/98W1147	17	45	18	1:2:1	1.18	0.56
Sep. Syn. #57/Kenyon	22	40	18	1:2:1	0.30	0.86
2000 Spelt #20/Kenyon	15	45	20	1:2:1	1.87	0.39
Intros Line #7/Kenyon	14	44	22	1:2:1	1.50	0.47
CIMMYT Line #18/Kenyon	14	44	22	1:2:1	1.50	0.47
Altar Synthetic/Kenyon	13	42	25	1:2:1	3.13	0.21
92MREHTR28B/Kenyon	14	45	21	1:2:1	2.45	0.29
Resistant (tetraploid)/susceptible (tetraploid)						
<i>Triticum turgidum</i> #283/Coulter	17	33	20	1:2:1	0.36	0.86
Resistant (tetraploid)/susceptible (hexaploid)						
<i>T. turgidum</i> #283/98W1147	20	36	14	1:2:1	0.74	0.69
Resistant (hexaploid)/resistant (hexaploid)						
2000 Spelt #20/Erik	80	0	0	1:0:0	—	—
Intros Line #7/Erik	80	0	0	1:0:0	—	—
CIMMYT Line #18/Erik	80	0	0	1:0:0	—	—
Altar Synthetic/Erik	80	0	0	1:0:0	—	—
Resistant (tetraploid)/resistant (hexaploid)						
<i>T. turgidum</i> #283/Erik	70	0	0	1:0:0	—	—

^a Plants were rated on a 1-to-5 lesion-type rating scale with families having plants with ratings of 1 and 2 classified as resistant (R), ratings of 3 to 5 as susceptible (S), and those with both resistant and susceptible plants as segregating (Seg).

^b Probability of obtaining deviations from the expected ratio by chance alone. A probability value greater than 0.05 indicates that the observed population does not differ significantly from expected hypothesis.

TABLE 4. Reaction of F₁ and F₂ plants of 11 wheat crosses to necrosis and chlorosis caused by *Pyrenophora tritici-repentis* race 5 evaluated in the greenhouse at University of Saskatchewan, Saskatoon (2002 to 2004) and North Dakota State University, Fargo (2004 to 2005)^a

Cross	F ₁		F ₂		Ratio tested	χ^2 value	P value ^c
	R ^b	S	R	S			
Resistant (hexaploid)/susceptible (hexaploid)							
Sep. Syn. #57/Kenyon	9	0	160	49	3:1	0.27	0.60
2000 Spelt #20/Kenyon	7	0	149	59	3:1	1.26	0.26
CIMMYT Line #18/Kenyon	8	0	150	58	3:1	0.92	0.34
Altar Synthetic/Kenyon	7	0	157	63	3:1	1.55	0.21
Resistant (hexaploid)/resistant (hexaploid)							
Sep. Syn. #57/Erik	9	0	207	0	1:0	—	—
2000 Spelt #20/Erik	7	0	220	0	1:0	—	—
CIMMYT Line #18/Erik	9	0	201	0	1:0	—	—
Altar Synthetic/Erik	8	0	212	0	1:0	—	—
Resistant (tetraploid)/resistant (hexaploid)							
<i>Triticum turgidum</i> #283/Erik	9	0	221	0	1:0	—	—
Susceptible (tetraploid)/susceptible (hexaploid)							
Coulter/Katepwa	0	9	13	63	3:13	0.13	0.72
Resistant (tetraploid)/susceptible (tetraploid) inoculated by race 5							
<i>T. turgidum</i> #283/Coulter	0	8	45	172	1:3	2.10	0.15
Resistant (tetraploid)/susceptible (tetraploid) inoculated by race 3							
<i>T. turgidum</i> #283/Coulter	0	7	63	167	1:3	0.70	0.40
Resistant (tetraploid)/susceptible (tetraploid) inoculated by race 2							
<i>T. turgidum</i> #283/Coulter	0	8	66	172	1:3	0.95	0.33

^a Necrotic reaction of F₁ and F₂ plants of cross *Triticum turgidum* #283/Coulter to races 2 and 3 is presented.

^b Plants were rated on a 1-to-5 lesion-type rating scale. Plants with ratings of 1 and 2 were classified as resistant (R) and those with ratings of 3 to 5 were classified as susceptible (S).

^c Probability of obtaining deviations from the expected ratio by chance alone. A probability value greater than 0.05 indicates that the observed population does not differ significantly from expected hypothesis.

families of all four R/S crosses segregated in the ratio 7 homozygous resistant:2 segregating:7 homozygous susceptible, thus confirming the hypothesis of monogenic control of resistance to chlorosis induced by *P. tritici-repentis* race 5 in hexaploid wheat (Table 5). In previous studies, resistance to necrosis induced by *P. tritici-repentis* races 3 and 5 in the tetraploid cross *T. turgidum* #283/Coulter was reported to be controlled by single recessive genes (21,25). In this study, the same cross was additionally screened with *P. tritici-repentis* race 2 and the segregation of the F₁ and F₂ generations and F_{2.5} families indicated that a single recessive gene control (Tables 4 and 5).

Allelism studies. All F₁ and F₂ generations of the four R/R crosses involving resistant hexaploid wheat genotypes when tested with *P. tritici-repentis* race 5 showed a resistant reaction (Table 4). Individual plant data were confirmed by screening F_{2.3} families of each of the R/R crosses and all the families were resistant confirming that all four resistant sources possessed the same gene for resistance to chlorosis induced by *P. tritici-repentis* race 5 (Table 5). All the F₁ and F₂ plants and F_{2.3} families of the inter-specific cross between the resistant tetraploid (*T. turgidum* #283) and the resistant hexaploid (Erik) sources showed a resistant reaction confirming that resistance to *P. tritici-repentis* race 5 in both tetraploid and hexaploid wheat were controlled by the same gene (Tables 4 and 5).

The genetic studies with *P. tritici-repentis* race 5 between susceptible genotypes Coulter (tetraploid) and Katepwa (hexaploid) gave interesting results. Coulter is susceptible to necrosis while Katepwa is susceptible to chlorosis caused by *P. tritici-repentis* race 5 (Table 1). All F₁ plants of the cross Coulter/Katepwa were susceptible showing only necrotic symptoms. Plants of the F₂ generation of this cross segregated for both necrosis and chlorosis. Rating plants for both necrosis and chlorosis components separately are error prone and hence disease scoring for resistant and susceptible (necrosis and/or chlorosis) reaction was performed. The F₂ generation of this inter-specific S/S cross segregated in a 3 resistant:13 susceptible ratio indicating different genes control susceptibility to *P. tritici-repentis* race 5 in this cross (Table 4). The F_{2.3} families of this cross segregated for 1 homozygous resistant:15 susceptible (homozygous and hetero-

zygous families) confirming different genes control susceptibility to necrosis (tetraploid) and chlorosis (hexaploid) caused by *P. tritici-repentis* race 5 in this cross (Table 5).

DISCUSSION

The identification and differentiation between the two distinct symptoms, tan necrosis and chlorosis, induced by *P. tritici-repentis* on susceptible wheat cultivars has led to reliable and accurate assessment of resistance and susceptibility (14,22). Determination of the genetics of resistance to tan spot was further enhanced by the identification of *P. tritici-repentis* races inducing either necrosis or chlorosis symptoms and the development of the necrosis-chlorosis model of tan spot (14,15). In the last two decades, various genetic studies have reported resistance to tan spot to be quantitatively (4,7,18) or qualitatively (11,12,19) inherited. However, accurate comparison of these conflicting studies cannot be made due to the lack of standardization of techniques such as method of inoculation, rating scales, symptoms studied, races/isolates used, and the environmental conditions for disease development.

The resistant parents studied were genetically diverse and included synthetic wheats and representative of three different species; *T. aestivum*, *T. spelta*, and *T. turgidum*; however, they all possessed the same resistance gene(s) for necrosis and chlorosis induced by *P. tritici-repentis* race 1. Genetic and mapping studies reveal that the gene, *tsn1*, controlling resistance to necrosis caused by *P. tritici-repentis* races 1 and 2 is located on chromosome 5B (1,6) and a major QTL controlling resistance to chlorosis caused by *P. tritici-repentis* race 1 is located on chromosome 1A (3). Tetraploid and hexaploid wheat possess both A and B genomes, which may explain the occurrence of the same resistance genes. The only prior interspecific genetic study (1) also demonstrated that the same single recessive gene controlled resistance to necrosis caused by the toxin Ptr ToxA, isolated from race 2, in both tetraploid and hexaploid wheat.

Although recent studies by Tadesse et al. (27,28) reveal that resistance to *P. tritici-repentis* race 1 is located on chromosomes 3A and D, similar novel results were not observed in this study.

TABLE 5. Reaction of F_{2.3} or F_{2.5} families of 11 wheat crosses to necrosis and chlorosis caused by *Pyrenophora tritici-repentis* race 5 evaluated in the greenhouse at University of Saskatchewan, Saskatoon (2002 to 2004) and North Dakota State University, Fargo (2004 to 2005)^a

Cross	F _{2.3} or F _{2.5} families ^b			Ratio tested	χ^2 value	P value ^c
	R	Seg	S			
Resistant (hexaploid)/susceptible (hexaploid) (F _{2.5} families)						
Sep. Syn. #57/Kenyon	38	11	31	7:2:7	0.81	0.67
2000 Spelt #20/Kenyon	33	14	33	7:2:7	1.83	0.40
CIMMYT Line #18/Kenyon	38	14	28	7:2:7	3.26	0.20
Altair Synthetic/Kenyon	34	16	30	7:2:7	4.34	0.11
Resistant (hexaploid)/resistant (hexaploid) (F _{2.3} families)						
Sep. Syn. #57/Erik	80	0	0	1:0:0	—	—
2000 Spelt #20/Erik	80	0	0	1:0:0	—	—
CIMMYT Line #18/Erik	80	0	0	1:0:0	—	—
Altair Synthetic/Erik	80	0	0	1:0:0	—	—
Resistant (tetraploid)/resistant (hexaploid) (F _{2.3} families)						
<i>Triticum turgidum</i> #283/Erik	70	0	0	1:0:0	—	—
Susceptible (tetraploid)/susceptible (hexaploid) (F _{2.3} families)						
Coulter/Katepwa	4		66	1:15	0.03	0.85
Resistant (tetraploid)/susceptible (tetraploid) F _{2.5} families inoculated by race 5						
<i>T. turgidum</i> #283/Coulter	32	11	37	7:2:7	0.47	0.79
Resistant (tetraploid)/susceptible (tetraploid) F _{2.5} families inoculated by race 3						
<i>T. turgidum</i> #283/Coulter	31	9	40	7:2:7	1.27	0.53
Resistant (tetraploid)/susceptible (tetraploid) F _{2.5} families inoculated by race 2						
<i>T. turgidum</i> #283/Coulter	33	13	34	7:2:7	1.04	0.59

^a Necrotic reaction of F_{2.5} families of cross *Triticum turgidum* #283/Coulter to races 2 and 3 is presented.

^b Plants were rated on a 1-to-5 lesion-type rating scale with families having plants with ratings of 1 and 2 classified as resistant (R), ratings of 3 to 5 as susceptible (S) and those with both resistant and susceptible plants as segregating (Seg).

^c Probability of obtaining deviations from the expected ratio by chance alone. A probability value greater than 0.05 indicates that the observed population does not differ significantly from expected hypothesis.

Differences in the findings of this study to that of Tadesse et al. (27,28) could be due to differences in the resistant genotypes and isolates used and the environmental conditions of disease development and its assessment. Additionally, the virulence data provided by Tadesse et al. (27,28) clearly indicates the isolates they had used were not race 1 even though they interpreted the isolates as *P. tritici-repentis* race 1. Additional research needs to be conducted to resolve the difference in the findings of Tadesse et al. (27,28) and other researchers (1,6,12).

The finding of a single recessive gene controlling resistance in tetraploid wheat to tan necrosis induced by *P. tritici-repentis* race 5 was also observed by Gamba and Lamari (11). In this study a single dominant gene was found to control resistance to chlorosis induced by *P. tritici-repentis* race 5 in hexaploid wheat; however, previous findings reveal resistance to be controlled by a single recessive gene (12,19) or to be quantitative in nature (10). The mode of inheritance of resistance to chlorosis varies from recessive, partially dominant to completely dominant depending upon the parental genotypes (23). The expression of the chlorotic symptom of tan spot is continuous (14) and highly influenced by environmental conditions (23). Additionally, it is possible that Kenyon, the susceptible parents in all the crosses studied with *P. tritici-repentis* race 5, may possess a number of modifier genes which may contribute to the differing results.

The resistant parents analyzed in this study were genetically very diverse but the allelic studies for seedling resistance to *P. tritici-repentis* races 1 and 5 indicate that all the resistant sources possessed the same gene(s) for resistance to tan spot. These findings agree with earlier studies (1,11,12) wherein different sources of resistance had no allelic differences for tan spot resistance. Although not all of these studies (12,22,23) contained the resistant cultivar Erik, the allelic findings strongly suggest that the wheat gene pool possesses very narrow genetic diversity for resistance to tan spot and hence rigorous efforts should continue to seek new and different genes for resistance.

Lamari et al. (17) suggested that the wheat-*P. tritici-repentis* pathosystem conforms to the toxin model of gene-for-gene hypothesis originally proposed by Ellingboe (5). In this model, a compatible interaction between host plant and pathogen leads to susceptibility. The susceptibility interaction occurs due to the production of a toxin by the pathogen and the presence of a 'toxin-receptor' site in the host. If the presence of such a site is considered, the product of a susceptibility gene, then the resistance in the wheat-*P. tritici-repentis* pathosystem is due to lack of a susceptibility gene(s) rather than the presence of a resistance gene(s) (17,22). This could be the reason why most studies have identified the same resistance gene(s) using the known races of *P. tritici-repentis*. Future allelic studies should be conducted among susceptible genotypes rather than resistant genotypes as in the wheat-*P. tritici-repentis* host pathosystem the susceptibility genes play major roles in the disease development.

Assuming hexaploid wheat-*P. tritici-repentis* pathosystem followed the toxin model of gene-for-gene hypothesis (17), prior genetic (22,23), molecular (3,6,7), and virulence (16,17) studies indicate that the virulence of *P. tritici-repentis* race 1 is the addition of virulence of race 2 and race 3. However, the single gene inheritance of resistance in the cross *T. turgidum* #283/Coulter when challenged with *P. tritici-repentis* races 1, 2, and 3 indicates that in the case of tetraploid wheat-*P. tritici-repentis* host pathosystem the virulence of *P. tritici-repentis* race 1 is not the addition of virulence of race 2 and race 3. In tetraploid wheat, race 1 causes necrosis similar to race 2 and the same gene controls resistance (11). Similar results were observed in this study. Although the segregation for chlorosis induced by *P. tritici-repentis* race 1 in the cross *T. turgidum* #283/Coulter was not observed, there was single gene segregation for resistance to necrosis induced by race 3 (21). Coulter is susceptible and *T. turgidum* #283 is resistant to *P. tritici-repentis* races 1, 2, and 3

and hence if virulence of race 1 is addition of races 2 and 3 and tetraploid wheat-*P. tritici-repentis* follows the gene-for-gene hypothesis, then the cross *T. turgidum* #283/Coulter should show two gene segregation when challenged with race 1. However, a single gene segregation was observed in the cross *T. turgidum* #283/Coulter indicating race 1 lacks the virulence factor present in race 3. This is supported by the differential symptoms observed in tetraploid line 4B-160 to races 1 and 3 (11).

Friesen and Faris (10) mapped the resistance to chlorosis induced by *P. tritici-repentis* race 5 to the distal tip of chromosome 2B in hexaploid wheat while Singh et al. (25) mapped the resistance to necrosis in tetraploid wheat to the long arm of chromosome 3B. Studying the interspecific cross between two susceptible genotypes Katepwa (chlorotic and hexaploid) and Coulter (necrotic and tetraploid), the genetic analysis indicates that there are two independent susceptibility genes for *P. tritici-repentis* race 5, one for necrosis in tetraploid and the other for chlorosis in hexaploid wheat. Assuming wheat-*P. tritici-repentis* follows the gene-for-gene hypothesis, this genetic finding for the first time confirms that *P. tritici-repentis* race 5 possesses two virulence genes. The identification of multiple major genes for resistance to tan spot and no interaction among them indicates that in order to develop complete resistance to all races of *P. tritici-repentis*, wheat breeders need to incorporate all the resistance genes.

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