A Novel Source of Resistance in Wheat to *Pyrenophora tritici-repentis* Race 1

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ABSTRACT

Singh, S., Bockus, W. W., Sharma, I., and Bowden, R. L. 2008. A novel source of resistance in wheat to *Pyrenophora tritici-repentis* race 1. Plant Dis. 92:91-95.

Tan spot, caused by the fungus *Pyrenophora tritici-repentis*, causes serious yield losses in wheat (*Triticum aestivum*) and many other grasses. Race 1 of the fungus, which produces the necrosis toxin Ptr ToxA and the chlorosis toxin Ptr ToxC, is the most prevalent race in the Great Plains of the United States. Wheat genotypes with useful levels of resistance to race 1 have been deployed, but this resistance reduces damage by only 50 to 75%. Therefore, new sources of resistance to *P. tritici-repentis* are needed. Recombinant inbred lines developed from a cross between the Indian spring wheat cvs. WH542 (resistant) and HD29 (moderately susceptible) were evaluated for reaction to race 1 of the fungus. Composite interval mapping revealed quantitative trait loci (QTL) on the short arm of chromosome 3A explaining 23% of the phenotypic variation, and the long arm of chromosome 5B explaining 27% of the variation. Both resistance alleles were contributed by the WH542 parent. The QTL on 5B is probably *tsn1*, which was described previously. The 3AS QTL (*QTs.ksu-3AS*) on 3AS is a novel QTL for resistance to *P. tritici-repentis* race 1. The QTL region is located in the most distal bin of chromosome 3AS in a 2.2-centimorgan marker interval. Flanking markers *Xbarc45* and *Xbarc86* are suitable for marker-assisted selection for tan spot resistance.

Additional keywords: *Drechslera tritici-repentis*

Tan spot is an important disease of wheat (*Triticum aestivum* L.) that occurs throughout the world. It also occurs on many other species of native and cultivated grasses (13,17) and is caused by the ascomycete fungus *Pyrenophora tritici-repentis* (Died.) Drechsler (anamorph *Drechslera tritici-repentis* (Died.) Shoemaker). It is especially severe under reduced tillage (8) because the pathogen survives between cropping seasons on the previous year's colonized host residue on or above the soil surface. From these residues, the fungus produces ascospores or conidia that initiate the epidemic on lower wheat leaves early in the season. Secondary inoculum, in the form of conidia, then is produced from these infected leaves and advances the epidemic.

Tan spot has been one of the most important diseases on wheat in the Great

Plains of the United States (1,7). It can be managed by several means, including rotations to nonhost species (8), destroying or burying the infested residue (9), and applying foliar fungicides (6). However, wheat producers in Kansas often wish to cultivate wheat in the same field year after year and maintain residue on the soil surface to conserve soil and soil moisture, and do not want the expense of applying a foliar fungicide. In these situations, host plant resistance is the best management method.

Good resistance to tan spot occurs in wheat and has been deployed in popular commercial cultivars. In Kansas, adoption of resistant cultivars resulted in a 63% decline in tan spot between 1976 and 2000 (7) and the downward trend has continued to the 75% reduction level through 2006 (W. W. Bockus, *unpublished*). Resistance is still effective despite the planting of over 70% of the wheat hectareage to resistant cultivars (5 or lower on KSU Extension 1-to-9 scale) for several years. However, current sources of resistance typically reduce damage by only 50 to 75% (7). Therefore, new sources of resistance to *P. tritici-repentis* are needed.

Resistance to tan spot has been reported to be inherited qualitatively (22,24,25) or quantitatively (17,19,20) and usually is associated with a lack of sensitivity to specific toxins produced by *P. tritici-repentis*. Ptr ToxA is a 13.2-kDa protein

that produces a necrotic symptom in susceptible wheat cultivars (4,44). Insensitivity to this toxin (*tsn1*) is inherited recessively (2,18) and is located on the long arm of chromosome 5B in hexaploid wheat (18,42). Ptr ToxB is a 6.6-kDa protein that produces extensive leaf chlorosis, and an insensitivity gene (*tsc2*) to this toxin was mapped on the short arm of chromosome 2B (21,43). Ptr ToxC is a nonionic, polar, low molecular weight molecule that produces chlorosis, and a recessive insensitivity gene (*tsc1*) to this toxin was mapped to the short arm of chromosome 1A (16,19). Interestingly, race 3 (which produces Ptr ToxC) caused necrosis rather than chlorosis in tetraploid wheat and a resistance gene (*tsn2*) was mapped to chromosome arm 3BL (37). Races of *P. tritici-repentis* have been defined based upon whether they produce the necrosis or chlorosis phenotype on certain cultivars because of the production of one or more of the three toxins (3,12,26).

In several cases, resistance to tan spot was not associated with insensitivity to fungal toxins. Faris and Friesen (20) reported race-nonspecific quantitative resistance to tan spot on chromosomes 1B and 3B of hexaploid wheat. These two quantitative trait loci (QTL) provided resistance against races 1, 2, 3, and 5, which produce Ptr ToxA and Ptr ToxC, Ptr ToxA, Ptr ToxC, and Ptr ToxB, respectively. Effertz et al. (16) reported possible resistance on chromosome 3AS to chlorosis induced by race 1; however, resistance was not associated with insensitivity to the chlorosis toxin Ptr ToxC. It is possible that additional toxic compounds are produced by the fungus that could account for these results (3,16).

In preliminary tests, the Indian spring wheat line WH542 was shown to possess a high level of resistance to *P. tritici-repentis* race 1, which is the most common race in Kansas (W. W. Bockus, *unpublished*). This research sought to determine whether the resistance in WH542 was different from other published sources of resistance to race 1, with the long-term goal of augmenting that which has already been deployed in commercial wheat cultivars.

MATERIALS AND METHODS

Plant material and experimental design. A segregating population of F_{6:11} recombinant inbred lines (RILs) was de-

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Accepted for publication 22 August 2007.

Table 1. Wheat parents of recombinant inbred lines (RILs) and check cultivars used to locate a novel source of resistance to race 1 of *Pyrenophora tritici-repentis* and their reactions to tan spot

Entry	Description	Known reaction to tan spot ^a	Leaf area affected (%) ^b
WH542	Resistant parent	...	10.0
HD29	Moderately susceptible parent	...	45.4
Karl 92	Resistant check	3.25	12.2
TAM 105	Highly susceptible check	8.85	68.3
LSD ^c	...	...	11.6

^a Based upon numerous observations over many years and reported in Kansas State University Cooperative Extension publications on a 1-to-9 scale where 1 is highly resistant and 9 is highly susceptible (14).

^b Mean of 20 replications with one plant per replication and the bottom three leaves of each plant evaluated for damage due to tan spot and averaged.

^c Least significant difference ($P = 0.05$).

Fig. 1. Frequency distribution of tan spot disease severities (percentage leaf area affected) for 96 recombinant inbred lines (RILs) developed from the cross of wheat parents WH542 (resistant) and HD29 (moderately susceptible). The range containing the parents is shown on the figure.

veloped from a cross between the Indian spring wheat cvs. WH542 and HD29 (36). WH542 is resistant and HD29 moderately susceptible to race 1 of *P. tritici-repentis*. The reactions of 96 RILs, the two parent cultivars, and two winter wheat check cultivars to tan spot were determined in the greenhouse using standard methods (10). The 100 entries were arranged in a randomized complete block design with 20 blocks. Four blocks were planted on each of five consecutive days. Each experimental unit within a block consisted of a single plant grown in a 2.5-by-13-cm plastic cone (Stuewe and Sons, Corvallis, OR) in a soil/vermiculite mix (50:50) in the greenhouse (30 and 18°C, high and low temperatures, respectively) with supplemental light provided by metal-halide growth lights. The check cultivars were Karl 92 (resistant check) and TAM 105 (susceptible check). After many years of observation in the field and greenhouse, Karl 92 has a known reaction to tan spot of 3.25 on a 1-to-9 scale and TAM 105 has a known reaction of 8.85 (Table 1). This scale, where 1 is highly resistant and 9 is highly susceptible, is used by the Kansas State University Cooperative Extension Service to report the relative reaction of cultivars to diseases (14).

Fungal inoculation. A spore suspension (10,000 spores/ml) of a single isolate of *P.*

tritici-repentis was used to inoculate plants at the four-leaf stage (about 4 weeks after sowing). Of the 20 blocks, 4 were inoculated on each of five consecutive days. The isolate was obtained from a commercial field in Kansas and had a virulence pattern consistent with race 1 based upon inoculation trials on differential cultivars (3). A DeVilbiss atomizer (Micromedics Inc., St. Paul, MN) powered with air pressure of 172 kPa was used to apply 35 ml of the suspension to each block. The suspension was prepared by transferring a plug of mycelium from a plate of one-fourth-strength potato-dextrose agar to the center of a plate containing V8 agar (150 ml of V8 juice, 850 ml of distilled water, 3.0 g of CaCO_3 , and 15 g of agar), incubating at room temperature (22 to 25°C) in the dark for 5 days, knocking down the aerial mycelium with a sterile bent-glass rod, and incubating for 12 h at 22°C 30 cm below fluorescent lights followed by 12 h in the dark at 17°C (34). Spores were harvested from plates by flooding with distilled water, gently scraping the colony with a steel microbial transfer instrument, passing the resulting suspension through one layer of cheesecloth, and diluting to the desired concentration with distilled water. Inoculated plants were placed into a plastic-covered chamber in the greenhouse (30

and 18°C, high and low temperatures, respectively) where continual leaf wetness was maintained for 48 h and then were returned to the greenhouse bench. Seven days after inoculation, the bottom three leaves of each plant were assessed visually for percent leaf area affected by disease (chlorosis or necrosis) and averaged. Therefore, the disease severity value for the 100 entries was based upon the average rating of three leaves on each of 20 replicate plants.

Molecular markers. DNA was isolated from the parents and each RIL as described by Singh et al. (38). Polymerase chain reaction (PCR)-based marker sets *Xbarc* (20 sets) (40), *Xgwm* (54 sets) (35), *Xfcp* (2 sets) (20), and *Xgdm* (9 sets) (33) were amplified following the procedures of Röder et al. (35) and separated on 2.3% GenePure HiRes Agarose (ISC Bioexpress, Kaysville, UT) gels or an ABI 3100 Prism Genetic Analyzer (Applied Biosystems, Foster City, CA) using 36-cm capillaries. Genescan and Genotyper analysis software (Applied Biosystems) were used to score the individuals (32). PCR products from 25 EST-STS primers (*unpublished*) were separated using the mutation detection enhancement (MDE) matrix (Cambrex, Rockland, ME; 5,29). The computer program MAPMAKER, version 2.0 for Macintosh (27), was used to assemble marker linkage groups.

QTL analysis. The computer program QGENE v.4.0 (31; J. C. Nelson, *unpublished*; <http://www.qgene.org/>) was used for simple interval mapping (SIM) and composite interval mapping (CIM). For CIM, cofactor markers were selected manually. Interval mapping was carried out and logarithm of the odds (LOD) thresholds were determined by 1,000 permutation tests. The effect of a marker was considered significant when $P \leq 0.0001$; this threshold corresponded to a highly conservative test.

RESULTS AND DISCUSSION

Phenotypic evaluation of RILs against race 1 of the pathogen showed that disease scores were continuously distributed (Fig. 1), suggesting multiple gene control of resistance. Average tan spot severities for the resistant check cv. Karl 92 and the highly susceptible check cv. TAM 105 were consistent with previous reports (Table 1) (14). The resistance of cv. WH542 was not significantly different from Karl 92 and the reaction of HD29 was intermediate between Karl 92 and TAM 105.

In all, 110 polymorphic markers were identified and scored in the RIL population. Marker segregation was distorted, with WH542 alleles accounting for 37% of all genotypes. This distortion was spread fairly uniformly over all linkage groups. A full description of marker genomic coverage for this mapping population was published previously (39).

Fig. 2. Quantitative trait loci interval map of wheat chromosome 3AS (*QTs.ksu-3AS*) associated with tan spot resistance in recombinant inbred lines derived from the cross WH542/HD29. The logarithm of the odds (LOD) score is indicated on the y-axis. The dotted line represents the LOD scores for composite interval mapping. LOD threshold value is 3.33 after 1,000 permutation tests.

SIM indicated that five wheat chromosomes (1B, 3A, 3B, 5B, and 6B) were associated with resistance to tan spot in the population (Table 2). However, CIM analysis confirmed only two of the five QTL, one each on chromosome 3AS and 5BL. In both cases, resistance alleles were contributed by WH542.

The QTL identified on chromosome arm 3AS is a novel QTL (*QTs.ksu-3AS*) for resistance to tan spot caused by race 1 of *P. tritici-repentis* (Table 3; Fig. 2). It accounted for 23% of the phenotypic variation for the disease (Table 3). The QTL is flanked by markers *Xbarc86* and *Xbarc45*, spanned 2.2 centimorgans (cM) (Fig. 2), and lies in the most distal chromosome bin of 3AS (41).

Effertz et al. (16) associated the restriction fragment length polymorphism marker *Xcdo395* on chromosome 3AS with a portion of the insensitivity of Opata 85 to chlorosis-inducing crude culture filtrate of *P. tritici-repentis*. However, the effect was not found with purified chlorosis toxin (Ptr ToxC) and they suggested that their result could be an experimental artifact. Our results provide conclusive evidence for a tan spot resistance QTL in this region (3AS). Furthermore, the potential toxin insensitivity locus reported by Effertz et al. (16) and *QTs.ksu-3AS* could be the same because Opata 85 and WH542 are closely related and share Jupateco and Bluejay in their pedigrees. This chromosomal region may be a source of multiple-disease resistance because it has been reported to carry a QTL for resistance to Fusarium head blight in tetraploid wheat (11).

Marker *Xfcp393* on the long arm of chromosome 5B was significantly associated with resistance to race 1 and ex-

Table 2. Simple interval mapping for tan spot resistance in the population derived from the cross of wheat parents WH542 (resistant) and HD29 (moderately susceptible)

Map interval (markers)	QTL ^a	Chromosome	R ²	LOD (P values) ^b
<i>Xgwm273</i> – <i>Xgwm153</i>	<i>QTs.ksu-1B</i>	1B	18.0	4.0 (<0.0001)
<i>Xbarc45</i> – <i>Xbarc86</i>	<i>QTs.ksu-3AS</i>	3AS	23.0	5.4 (<0.0001)
<i>Xgwm285</i> – <i>Xgwm108</i>	<i>QTs.ksu-3BL</i>	3BL	19.0	4.4 (<0.0001)
<i>Xgwm499</i> – <i>Xest-stsbe968</i>	<i>QTs.ksu-5BL</i>	5BL	18.0	4.4 (<0.0001)
<i>Xwmc105</i> – <i>Xbarc198</i>	<i>QTs.ksu-6BS</i>	6BS	14.3	3.0 (<0.0001)

^a QTL = quantitative trait loci.

^b LOD = logarithm of the odds.

Table 3. Composite interval mapping for tan spot resistance in the population derived from the cross of wheat parents WH542 (resistant) and HD29 (moderately susceptible)

Map interval (markers)	QTL ^a	Chromosome	LOD ^b	R ² (%) ^c
<i>Xbarc45</i> – <i>Xbarc86</i>	<i>QTs.ksu-3AS</i>	3AS	5.4	23
<i>Xgwm499</i> – <i>Xest-stsbe968</i>	<i>QTs.ksu-5BL</i>	5BL	6.5	27

^a Quantitative trait loci.

^b Logarithm of the odds.

^c Phenotypic variation explained.

Fig. 3. Quantitative trait loci interval map of wheat chromosome 5BL (*QTs.ksu-5BL*) associated with tan spot resistance in recombinant inbred lines derived from the cross WH542/HD29. The logarithm of the odds (LOD) score is indicated on the y-axis. The dotted line represents the LOD scores for composite interval mapping. LOD threshold value is 3.33 after 1,000 permutation tests.

plained 27% of the phenotypic variation. The region was flanked by PCR-based markers *Xgwm499* and *Xest-stsbe968* (Table 3; Fig. 3). A toxin sensitivity gene (*Tsn1*) in this interval has been reported previously in a mapping population from the cross of Chinese Spring (CS) and the CS-*Triticum dicoccoides* chromosome 5B disomic substitution line (23). Marker *Xfcp393* is only 0.2 cM from *Tsn1* (<http://maswheat.ucdavis.edu/protocols/Ts>

[n1/index.htm](http://maswheat.ucdavis.edu/protocols/Ts)); therefore, these two resistance loci are very likely the same. This chromosome region is a gene-rich region and harbors genes for several other important traits, including kernel length (15) and resistance to Karnal bunt (39), Fusarium head blight (28), and aphids (30).

Three other QTL reported here had significant negative effects on tan spot severity and were revealed by SIM but not with CIM. One of these QTL was detected

within interval markers *Xgwm273-Xgwm153* on chromosome 1B and accounted for 18% of the variation for resistance (Table 2). Another QTL detected on the long arm of chromosome 3B explained 19% of the variation for the disease. QTL from chromosomes 1BS and 3BL associated with resistance to race 1 of the pathogen have been reported previously (19,20). Another QTL was associated with marker *Xbarc146* on the short arm of chromosome 6B and explained 14.3% of the variation for the disease using SIM but was not detected with CIM (Table 2).

Marker-assisted selection to incorporate the tan spot resistance QTL from chromosome 3AS into elite lines should be possible using the closely flanking markers *Xbarc45* and *Xbarc86*. The markers also should be useful when combined with previously identified markers for *tsn1*, *tscl*, and *tscl2* (16,20,21,23) to pyramid insensitivity genes and QTL into desirable germplasm.

Commercial cultivars in Kansas with useful levels of resistance to tan spot have been deployed for about 20 years (7). Resistance in these cultivars has been to race 1 of *P. tritici-repentis* because it was the dominant race in Kansas during this time (W. W. Bockus, unpublished). Extension ratings for these cultivars have been 3 to 5 on the 1-to-9 scale; therefore, there is room to improve the resistance response of commercial cultivars in Kansas. Because the resistance QTL on 3AS appears to be a novel source of resistance, its addition to these cultivars should enhance their overall resistance to tan spot.

ACKNOWLEDGMENTS

This is contribution number 08-16-J from the Kansas Agricultural Experiment Station. We thank M. Davis for technical support and the Kansas Wheat Commission for partial financial support.

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