Quantitative Trait Loci for Resistance to *Pyrenophora tritici-repentis* Race 1 in a Chinese Wheat

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ABSTRACT

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Tan spot, caused by *Pyrenophora tritici-repentis*, is an economically important foliar disease of wheat worldwide. Eight races of the pathogen have been characterized on the basis of their ability to cause necrosis or chlorosis in a set of differential wheat lines. Race 1 produces two host-selective toxins, Ptr ToxA and Ptr ToxC, that induce necrosis and chlorosis, respectively, on leaves of sensitive wheat genotypes. A population of recombinant inbred lines was developed from a cross between Chinese landrace Wangshuibai (resistant) and Chinese breeding line Ning7840 (highly susceptible) to identify chromosome regions harboring quantitative trait loci (QTL) or genes for tan spot resistance. Plants were inoculated at the four-leaf stage in a greenhouse and percent leaf area diseased was scored 7 days after inoculation. Two QTL for resistance to race 1 were mapped to the short arms of chromosomes 1A and 2B in the population. The QTL on 1AS, designated as *QTs.ksu-1AS,* showed a major effect and accounted for 39% of the phenotypic variation; the QTL on 2BS, designated as *QTs.ksu-2BS,* explained 4% of the phenotypic variation for resistance. A toxin infiltration experiment demonstrated that both parents were insensitive to Ptr ToxA, suggesting that the population was most likely segregating for reaction to chlorosis, not necrosis. The markers closely linked to the QTL should be useful for marker-assisted selection in wheat-breeding programs.

Tan spot, caused by the ascomycete fungus *Pyrenophora tritici-repentis* (Died.) Drechsler (anamorph: *Drechslera tritici-repentis* (Died.) Shoemaker), is an important foliar disease of common wheat (*Triticum aestivum* L.) and durum wheat (*T. turgidum* L. subsp. *durum*) throughout the world. The symptoms of the disease are small, dark-brown spots that become tan elliptical or diamond-shaped lesions with a small, dark-brown spot in the center surrounded by a chlorotic halo.

Several strategies have been proposed for reducing yield losses caused by tan spot, including burying or destroying crop residues (6), implementing crop rotations involving nonhost species (5), and applying foliar fungicides. Growing resistant cultivars is a preferred approach when farmers plant wheat under reduced tillage to conserve soil and soil moisture with minimum investment in fungicide application. Even if some commercial cultivars have resistance, many of the currently used cultivars are susceptible (4). Therefore, identification of new sources containing novel resistance to *P. tritici-repentis* may facilitate genetic improvement of commercial cultivars.

Resistance to tan spot has been reported to cosegregate with insensitivity to host-selective toxins (HSTs) produced by *P. tritici-repentis* races (22). To date, eight races have been defined on the basis of their virulence patterns on five differential hexaploid wheat accessions (Glenlea, Katepwa, Salamouni, 6B365, and 6B662) and the specific HST they produce (2,28). Among three known HSTs, Ptr ToxA is the best characterized and produces

necrosis symptoms on the leaves of susceptible wheat (3,45). A single recessive gene, *tsn1*, was discovered to confer insensitivity to Ptr ToxA and is located on the long arm of chromosome 5B in hexaploid wheat (16,40). Although both Ptr ToxB and Ptr ToxC produce extensive chlorosis on susceptible wheat, they have different chemical characteristics. Ptr ToxB is a 6.6-kDa protein and a recessive wheat gene, *tsc2*, on the short arm of chromosome 2B controls insensitivity to the toxin (20,41); whereas Ptr ToxC is a nonionic, polar, low molecular weight protein and specifically interacts with *tsc1*, a recessive insensitivity gene on the short arm of chromosome 1A (13).

Several quantitative trait loci (QTL) for tan spot resistance were reported. A major QTL on the long arm of chromosome 5B was associated with resistance to necrosis caused by race 1 (8) and a major QTL on chromosome 1AS was associated with resistance to chlorosis induced by the same race (17). Friesen and Faris (20) found a major QTL on 2BS resistant to race 5 that induced chlorosis. However, sources of resistance to tan spot have been reported mainly in American and European cultivars to date (13,14,16–20). The International Triticeae Mapping Initiative (ITMI) population derived from the cross between W-7984 (a synthetic hexaploid wheat line) and Opata 85 (a CIMMYT spring wheat line; P1591776) has been extensively used for mapping tan spot resistance genes (14,17,19,20). More recently, Singh et al. (37) reported a novel resistance QTL in a line from India and Tadesse et al. (42) found a resistance gene in Ethiopian wheat cultivars. However, resistance to tan spot in Chinese sources has not been well characterized to date. In this study, we developed a recombinant inbred line (RIL) population from the cross between Chinese landrace Wangshuibai (WSB) and breeding line Ning7840 to characterize QTL for resistance to *P. tritici-repentis* race 1 from this source. Race 1 is the most prevalent race in North America and produces both Ptr ToxA and Ptr ToxC (1).

MATERIALS AND METHODS

Plant materials and disease evaluation. In total, 288 F$_{2:6}$ RILs were developed from the cross between WSB and Ning7840 by single-seed descent. WSB is resistant to *P. tritici-repentis* race 1, and Ning7840 is highly susceptible. WSB showed a similar leaf area diseased (LAD) (54%) to the resistant check, Karl 92 (55%), and Ning7840 had a similar LAD (84%) to the susceptible check, TAM 105 (88%). Karl 92 and TAM 105 have shown stable contrast in resistance to race 1 and have been used as long-term resistant and susceptible controls, respectively, for more than a decade in Kansas State University (10). Therefore, the mean and standard error of both controls were used to determine the cut-off values for resistance and susceptibility (Table 1). Initially, the first population of 96 F$_{2:6}$ RILs was developed for preliminary QTL screening. To evaluate tan spot resistance, all RILs along with the two parents and two controls were planted in a rack holding 100 66-ml plastic cone tubes (Stuewe and Sons, Corvallis, OR) filled with a mixture of steamed soil:vermiculite (50/50) with a cotton ball in the bottom of each tube. One seed per RIL or cultivar was planted in each tube, and the experiment was arranged in a randomized complete block design with 20 blocks (racks) (7). Because mist chamber space was limited, four racks were planted each day for five consecutive days. To further validate the initial QTL mapping result, a second population of 192 F$_{2:6}$ RILs was developed from the same parents and phenotyped as described for the initial population, except that 10 blocks per RIL were evaluated.

Plants were grown under light for 12 h at 25°C and darkness for 12 h at 21°C. At the four-leaf stage (≈4 weeks after planting), plants were inoculated with a spore suspension (≈5,000 spores/ml) from the isolate AZ-00 of *P. tritici-repentis*. The isolate AZ-00 belongs to *P. tritici-repentis* race 1 (2). Spores were produced by transferring a small agar disc of mycelium of the fungus from a one-quarter potato dextrose agar plate to the center of V8 agar plates (150 ml of V-8 juice, 3 g of CaCO$_3$, 15 g of agar, and 850 ml water) and flattening aerial hyphae with a sterile bent-glass rod around the perimeter when the colony reached ≈4 to 5 cm in diameter (≈5 days in the dark at 21 to 24°C). The V8 plates were placed at 21 to 24°C for 12 h under light (≈40 cm below four fluorescent tubes) followed by 12 h of darkness at 16°C. Spores were harvested by flooding the plates with distilled water, scraping the surface of colonies with a fungal transfer spatula, filtering and rinsing the suspension through one layer of cheesecloth into a container, and diluting the suspension to a desired concentration with distilled water. For inoculation, a DeVilbis atomizer (Micromedics Inc., St. Paul, MN) connected to an air compressor was used to uniformly apply 35 ml of the suspension to each rack. After inoculation, the racks with plants were immediately placed in a mist chamber at 100% relative humidity created by a cool humidifier for 48 h at 20 to 28°C with a 12-h photoperiod. After the mist period, plants were returned to the greenhouse benches. Seven days after inoculation, the bottom three leaves of each plant were scored for LAD by visually estimating the percentage of leaf area with necrosis or chlorosis, and an average percent LAD across three leaves of the same plant was used as the overall LAD score for the plant. The scores from all plants in each genotype were combined for QTL analysis.

Analysis of variance was conducted by using the PROC GLM procedure of SAS (version 9.1; SAS Institute, Inc., Cary, NC) using a mean percent LAD across 20 plants per line in the first experiment or 10 plants per line in the second experiment. The model is $Y_{ij} = \mu + \tau_i + \rho_j + e_{ij}$, where μ is population mean, τ_i is a fixed effect representing treatment (genotype), ρ_j is a fixed effect representing block (greenhouse spatial effect), and $e_{ij} \approx N(0,\sigma^2)$ is random residual effect. Fisher's least significant difference at α = 0.05 was used to separate the genotype means.

Toxin infiltration. Toxin Ptr ToxA was extracted from *P. tritici-repentis* race 1 as described by Tomas et al. (45). After wheat plants were scored for tan spot symptoms, ≈100 μl of Ptr ToxA (44) was infiltrated into the middle of the youngest fully expanded leaves of the two parents and two checks using a Hagborg device (23). The water-soaked area of the infiltrated region was immediately delimited with a permanent felt marker. At 3 to 5 days after infiltration, presence or absence of necrosis on the infiltrated leaves was recorded to reflect sensitive reaction (+) or insensitive reaction (–) to Ptr ToxA infiltration. Infiltrations were performed in at least three plants per cultivar.

Molecular marker analysis. One-week-old leaf tissue was collected in 1.1-ml eight-strip tubes, dried for 2 days in a freeze drier (Thermo Fisher, Waltham, MA), and ground to fine powder in a Mixer Mill (Retsch GmbH, Rheinische Strasse 36, Germany) by shaking strip tubes with a 3.2-mm stainless steel bead at 25 times/s for 5 min. Genomic DNA was extracted from leaves of parents and RILs by using the cetyltrimethyl ammonium bromide method (34). Polymerase cycle reaction (PCR) amplifications were performed in a Tetrad Peltier DNA Engine (Bio-Rad Laboratories, Hercules, CA). A 12-μl PCR mixture contained 1.2 μl of 10× NH$_4$ buffer (Bioline Inc. Taunton, MA), 2.5 mM MgCl$_2$, 200 μM each dNTP, 100 nM forward-tailed primer, 200 nM reverse primer, 100 nM M13 fluorescent-dye-labeled primer, 1 U of *Taq* DNA polymerase, and 50 ng of template DNA. A modified touchdown program (29) was used for amplification of PCR. In brief, the reaction was incubated at 95°C for 5 min; then continued for five cycles of 1 min of denaturing at 96°C and 5 min of annealing at 68°C, with a decrease of 2°C in each of the subsequent cycles; and 1 min of extension at 72°C. For another five cycles, the annealing temperature started at 58°C for 2 min, with a decrease of 2°C for each subsequent cycle. PCR went through an additional 25 cycles of 1 min at 96°C, 1 min at 50°C, and 1 min at 72°C, with a final extension at 72°C for 5 min. PCR products were separated in an ABI 3730 DNA analyzer (Applied Biosystems, Foster City, CA) and data were scored using GeneMarker (version 1.6; SoftGenetics LLC. State College, PA).

Bulked segregant analysis (30) was performed to screen polymorphic simple-sequence repeats (SSRs) associated with tan spot resistance. Equal amounts of DNA were pooled separately from five resistant and five susceptible RILs. In total, 1,500 microsatellite or SSR markers covering all 21 wheat chromosomes (33,38) were screened between the parents and between the two bulks. Polymorphic markers between the bulks were further analyzed for linkage mapping and QTL analysis in the populations.

Linkage and QTL analysis. A linkage map was constructed with the JoinMap program, version 3.0 (47). The logarithm of the odds (LOD) threshold value was set at 3.0 for grouping linked markers. Ripple was performed each time after adding a new marker. Kosambi (24) centimorgans (cM) were used to convert recombination frequencies into genetic distance.

Combined means were used for interval mapping (IM) and composite interval mapping (CIM) with the QTL Cartographer V2.5 (48). CIM was implemented by using the standard model 6,

TABLE 1. Range and means for the percentage of leaf area diseased (LAD) after inoculation with *Pyrenophora tritici-repentis* race 1 and reaction to Ptr ToxA toxin assay for two parents (Wangshuibai and Ning7840) and two check cultivars (Karl 92 and TAM 105)

Entry	LAD (%) (range)	Mean LAD (%) ± standard deviation	Reaction to ToxA
Wangshuibai	47.9–64.0	53.9 ± 4.1	Insensitive
Ning7840	70.3–86.3	83.8 ± 4.1	Insensitive
Karl 92	46.6–62.7	54.7 ± 4.1	Insensitive
TAM 105	79.6–95.7	87.7 ± 4.1	Sensitive
LSD (α = 0.05)^z	…	11.1	…

^{z} LSD = least significant difference.

starting with default values of five cofactors obtained by a forward regression to control genetic background. A window size of 10 cM was used to block a chromosome region between the markers flanking the test site. In both IM and CIM, the walking speed for a genome-wide QTL scan was set at 1.0 cM and the LOD thresholds to declare a significant QTL were determined based on the result of 1,000 permutations. For each QTL, the coefficient of determination (R^2), which was the proportion of total phenotypic variance explained by a QTL, was determined on the basis of the R^2 for the single marker that was the closest to the target QTL. The total R^2 that represents the phenotypic variation explained by the model was calculated through multiple linear regressions using the SAS REG procedure. All loci that had significant main effects were tested against all other markers to detect significant interactions ($P < 0.01$).

RESULTS

Reactions of the RILs to isolate AZ-00 and toxin bioassay. All parents, the RILs, and the two checks were inoculated with conidia of AZ-00 (nec+ chl+), an isolate of race 1. The reactions of both resistant and susceptible parents to isolate AZ-00 were not significantly different from their corresponding checks (Table 1). The RILs segregated for reactions to isolate AZ-00. Resistant genotypes developed small, dark-brown spots with no or little encompassing chlorosis, whereas the susceptible ones had extensive chlorosis over the entire leaf. Some RILs showed moderate

Fig. 1. Histograms of the percent leaf area diseased (LAD) caused by *Pyrenophora tritici-repentis* race 1 in the Wangshuibai (WSB)/Ning7840 (WN) population. **A,** Percent LAD means across a subset of the WN population; **B,** percent LAD means across the entire WN population.

resistance (<50% LAD) or susceptibility (>60% LAD). The frequency distribution of percent LAD in the population was continuous but had two major peaks, indicating that a QTL with a major effect may contribute to tan spot resistance in the population and that the presence of other QTL with minor effects or environmental factors may also affect tan spot resistance in the population (Fig. 1).

In the toxin infiltration assay, the susceptible check TAM 105 showed susceptibility to Ptr ToxA on the inoculated leaves but the resistant check Karl 92 and both parents (WSB and Ning7840) (Table 1) did not, suggesting that Ptr ToxA was not the virulence factor responsible for the tan spot segregation in the WSB/Ning7840 population.

Initial QTL localization in WSB. Of the 1,500 SSR markers screened, 169 were polymorphic between parents and 58 of 169 were polymorphic between bulks; therefore, these 58 markers were used for screening the initial population containing 96 lines for linkage mapping. Linkage analysis identified nine linkage groups (data not shown). A further QTL scan using the linkage maps and percent LAD means across 20 plants identified two QTL on two chromosomes by both the IM and CIM methods. IM and CIM detected one major effect QTL (R^2 = 0.56 and 0.40, respectively) on the short arm of chromosome 1A. The QTL was flanked by the markers *Xcfa2153* and *Xgwm33* (Fig. 2), designated as *QTs.ksu-1AS*. A second QTL, designated as *QTs.ksu-2BS,* was detected on 2BS with a minor effect. This QTL was flanked by *Xbarc7* and *Xbarc55* and explained 13 and 10% of the phenotypic variation in IM and CIM, respectively (Table 2; Fig. 2). A slight difference in QTL locations was observed between IM and CIM: *Xbarc7* was the closest marker to the QTL in IM and *Xbarc55* was the closest marker in CIM. In either case, alleles decreasing the percent LAD were from the resistant parent, WSB. Because CIM reduces the genetic noise and enhances the QTL detection power by incorporating cofactors in the model, the confidence intervals determined by the drop of the LOD value around the maximum LOD value tend to be smaller in CIM than IM (Table 2). For this reason, only the results from CIM were used for further data interpretation.

Validation of QTL in a large population. A new population of 192 F_6 RILs from the same cross was used to validate the results derived from the initial mapping population. All markers linked to QTL on chromosome 1AS and 2BS were screened on the population. Both *QTs.ksu-1AS* and *QTs.ksu-2BS* were significant in IM and CIM. The *QTs.ksu-1AS* explained ≈40 and 39% of the phenotypic variation in IM and CIM, respectively (Table 2; Fig. 2). The *QTs.ksu-2BS* explained 5 and 4% of the phenotypic variation in IM and CIM, respectively (Table 2; Fig. 2). As expected, all alleles decreasing the percent LAD were from the resistant parent WSB. Markers *Xcfa2153* and *Xbarc7* were assembled into multiple regression models and, together, explained 39% of the phenotypic variation for resistance to isolate AZ-00; interaction between the two loci was not significant (Table 3). Markers *Xcfa2153* and *Xbarc7* associated with the two QTL had significant effects on the percent LAD means (Table 2), indicating the significant differences in tan spot severity between RILs that have only WSB alleles of marker *Xcfa2153* or *Xbarc7* and those that have Ning7840 alleles of both markers (Table 4).

Two markers, *Xcfa2153* and *Xbarc7*, were chosen to estimate their effectiveness for marker-assisted selection (Table 4). The replacement of the Ning7840 allele of *Xcfa2153* with the corresponding WSB allele led to an 18% decrease in mean percent LAD and replacement of the Ning7840 allele of *Xbarc7* with the corresponding WSB allele led to a 5.4% decrease in mean percent LAD. When both marker alleles of Ning7840 were replaced by corresponding WSB alleles, the mean percent LAD decreased by 21.4%. The results indicated that marker-assisted selection for the two QTL using a single marker per QTL can significantly
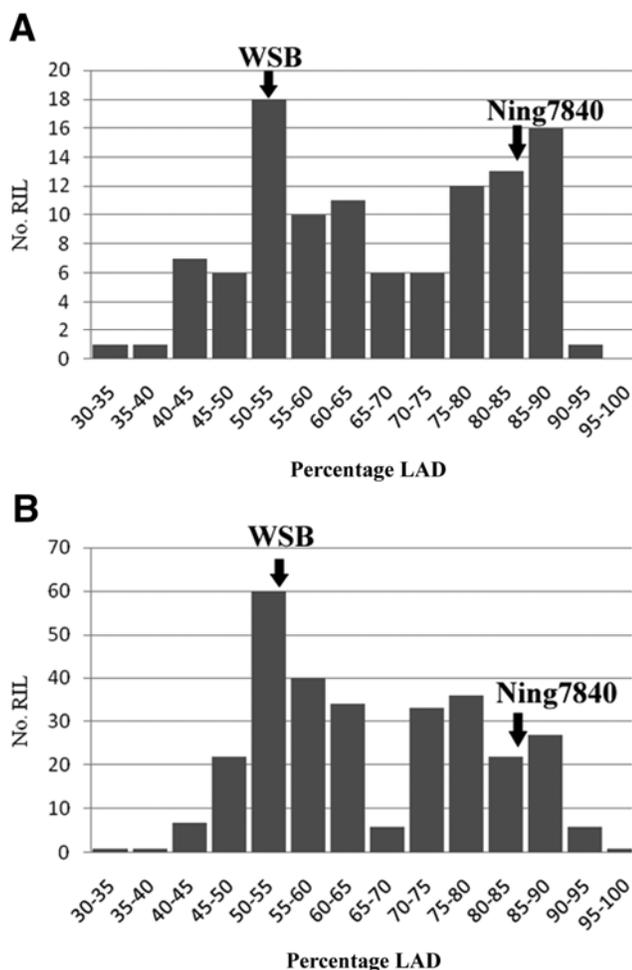
decrease percent LAD and that *QTs.ksu-1AS* is probably a major QTL responsible for tan spot resistance in WSB.

DISCUSSION

Tan spot is one of the important foliar diseases of wheat in the Great Plains of the United States, and yield losses ≤50% have been reported (32). Breeding for tan spot resistance is regarded as the most economical approach to disease control. Resistance to tan spot has been reported to be under either polygenic (8,15, 17,18,20) or oligogenic control (11,12,21,22,25,26,35,43). Previously, QTL associated with resistance to tan spot races 1, 2, 3, and 5 have been reported (13,16,17,20). This study identified two QTL, *QTs.ksu-1AS* and *QTs.ksu-2BS,* associated with tan spot

resistance to *P. tritici-repentis* race 1 (isolate AZ-00) in a recombinant inbred population derived from Chinese landrace WSB and line Ning7840.

Although many environmental factors may influence tan spot symptom development, testing seedlings in a greenhouse is highly repeatable if proper rating scale and experimental design are used. Two common rating scales have been used for tan spot evaluation, a 1-to-5 lesion-type rating scale and percent LAD (7,9,16–18,25). A high correlation was observed between the two rating scales (17) and they both provide accurate rating of tan spot damage in wheat leaves. However, estimation of LAD is easy to learn whereas a lesion-type scale needs more experience to get accurate readings (7). In this study, we used average of percent LAD across the bottom three leaves, and the disease ratings of two
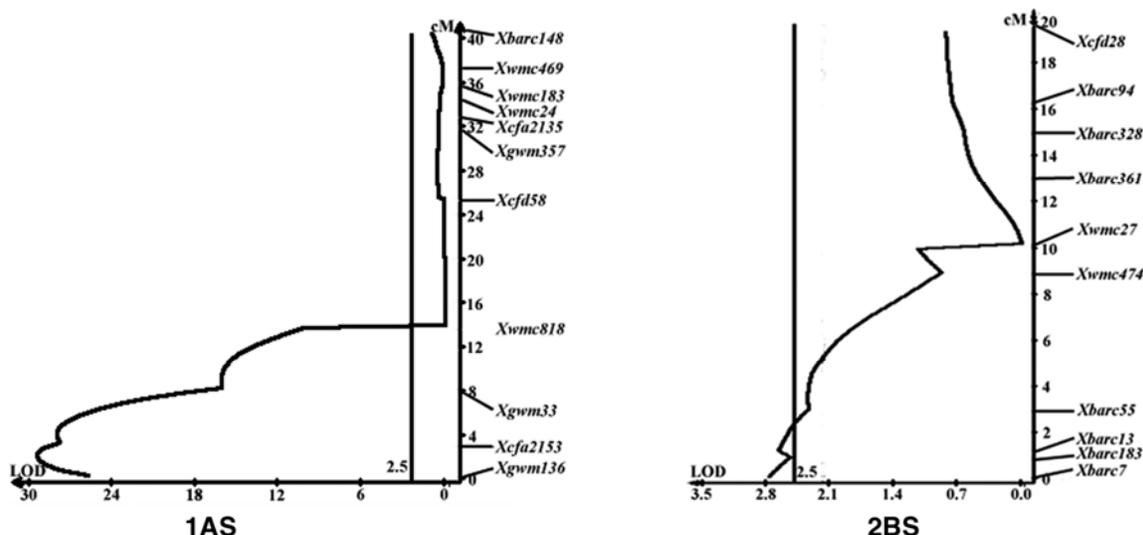

Fig. 2. Composite interval mapping of quantitative trait loci (QTL) associated with tan spot resistance contributed by Wangshuibai in the large population. Positions of markers and centimorgan (cM) distances between loci are shown to the right of the linkage maps. Vertical solid line represents the logarithm of the odds (LOD) significance threshold of 2.5. The LOD, additive effect, and R^2 values for each QTL are listed in Table 2.

TABLE 2. Interval mapping (IM) and composite interval mapping (CIM) analysis of quantitative trait loci (QTL) associated with resistance to race 1 of *Pyrenophora tritici-repentis*[w]

QTL[x]	Method	Chr	Position (cM)	Marker[y]	LOD	Additive effect	LOD Threshold (5%, 1%)	R^2	LOD interval (cM)[z]
Initial									
QTs.ksu-1AS	IM	1AS	3.4	*Xcfa2153*	15.5	−11.6	2.2, 3.6	0.56	1.8–4.4
	CIM	1AS	3.4	*Xcfa2153*	11.9	−11.5	2.2, 3.2	0.40	0–10
QTs.ksu-2BS	IM	2BS	0.0	*Xbarc7*	3.2	−5.7	2.2, 3.6	0.13	0–2.5
	CIM	2BS	2.9	*Xbarc55*	4.1	−8.2	2.2, 3.2	0.10	1.2–6.2
Entire									
QTs.ksu-1AS	IM	1AS	2.0	*Xcfa2153*	28.9	−8.9	1.6, 2.4	0.40	0.8–2.8
	CIM	1AS	2.0	*Xcfa2153*	29.5	−8.9	1.6, 2.5	0.39	0.8–2.6
QTs.ksu-2BS	IM	2BS	0.0	*Xbarc7*	2.5	−2.9	1.6, 2.4	0.05	0–2.8
	CIM	2BS	0.0	*Xbarc7*	2.8	−3.0	1.6, 2.5	0.04	0–2.7

[w] Chr and Position refer to the chromosome and map position (in centimorgans [cM]) of the QTL at the maximum logarithm of the odds (LOD) score for IM and CIM.

[x] QTL populations: Initial = 96 lines and Entire = 288 lines.

[y] Closest markers to the LOD peaks.

[z] LOD confidence intervals in cM for drop of 1 LOD from the LOD peak.

TABLE 3. Analysis of variance for the percentage of leaf area diseased estimated from the greenhouse experiments[z]

Source	DF	SS	MS	*F* value	*P* > *F*
Model	3	19,655.26279	6,551.75426	53.11	<0.0001
Xcfa2153	1	17,980.47711	17,980.47711	145.75	<0.0001
Xbarc7	1	1,197.17395	1,197.17395	9.7	0.0021
Xcfa2153 × Xbac7	1	66.66608	66.66608	0.54	0.463
Error	248	30,594.71068	123.36577	…	…
Corrected total	251	50,249.97347	…	…	…

[z] Abbreviations: DF = degree of freedom, SS = sum of squares, MS = mean square.

control cultivars were similar to previous ones; therefore, the disease data were accurate for QTL mapping. The randomized complete block design in this study successfully helped control experimental variation due to spatial and temporal effects. Analysis of variance showed that the percent LAD means of resistant and susceptible checks generated from the two experiments were not significantly different (data not shown). Also, the mean percent LAD across 10 plants/line was not significantly different from the mean percent LAD across 20 plants/line; thus, there were no significant experiment–treatment interactions, and the disease data from both experiments were combined for QTL analysis.

Variation in QTL effects were observed with population size and mapping methods (Table 2). In the initial QTL screening with 96 RILs, the effects of QTL were larger than those detected in the large population of 288 lines (Table 2). However, Vales et al. (46) suggested that small population sizes used for detecting QTL could overestimate QTL effects. When population size increased from 96 to 288 RILs, the QTL detection power increased and precision was significantly improved for both methods. Furthermore, CIM may provide better detection power than IM and appears to be more reliable in predicting QTL because it considers the background effect by taking cofactors into account. Therefore, the results obtained in this study should be useful to breeding programs.

To date, three HSTs, Ptr ToxA (44,45), Ptr ToxB (31,41), and Ptr ToxC (14), have been characterized in different races of *P. tritici-repentis*. *P. tritici-repentis* race 1 produces both Ptr ToxA and Ptr ToxC. In the present study, the toxin infiltration results excluded Ptr ToxA as the toxin responsible for tan spot resistance segregation in the current mapping population. Meanwhile, the population segregated for extensive chlorosis, which is the typical symptom caused by Ptr ToxC (27). Therefore, Ptr ToxC was most likely the toxin responsible for tan spot resistance segregation in the mapping population.

Previous studies have identified a major QTL (*QTsc.ndsu-1A*) associated with resistance to chlorosis caused by *P. tritici-repentis* race 1 and an insensitivity gene (*tsc1*) to partially purified chlorosis toxin (Ptr ToxC), and both were mapped on chromosome 1AS using the ITMI population (14,17). A restriction fragment length polymorphism (RFLP) marker, *XGli1*, was mapped close to *QTsc.ndsu-1A* and located at 5.7 cM from *tsc1*. The QTL *QTs.ksu-1AS* from this study was mapped to a position similar to that for *QTsc.ndsu-1A*. According to the maps developed in this study and others (17,33,38,39), SSR markers *Xgwm136*, *Xgwm33*, and *Xcfa2153* reside in the region flanked by RFLP markers *XksuD14.1* and *XksuD14.2* mapped by Faris et al. (17) at the distal end of chromosome 1A. Therefore, the two QTL are most likely the same QTL mapped in different populations; however, markers developed in this study are closer to the QTL *QTs.ksu-1AS* and are more breeder friendly for marker-assisted selection. Furthermore, Chu et al. (9) mapped the *Ne2* gene on chromosome 2BS with a distance of 3.2 cM away from marker *Xbarc55*. However, the *Ne2* gene is believed to interact with *Ne1*, which is a complementary gene on chromosome 5BL for controlling hybrid necrosis (9). Hybrid necrosis was not observed in the study, and *Ne2* has not yet been found to be associated with resistance to chlorosis caused by *P. tritici-repentis* race 1; therefore, further work needs to be done to establish the relationship between *QTs.ksu-2BS* and *Ne2*.

In this study, we found two QTL associated with resistance to tan spot in a Chinese landrace that has not been used for exploring novel genetic sources. The results indicate that both QTL are located in chromosome locations similar to previously discovered QTL or genes residing in U.S. sources, demonstrating that similar genes exist between U.S. and Chinese germplasm even though they are far from each other in geographic origin. This hypothesis is supported by Singh et al. (36), who suggested that limited genetic diversity for resistance to tan spot existed in the wheat gene pool. However, whether they are the same or different alleles remains to be investigated.

Molecular markers linked to QTL reported in this study could facilitate marker-assisted selection for the QTL resistant to tan spot from chromosome 1AS and 2BS. RFLP markers for tan spot resistance reported from previous studies have not been used in marker-assisted breeding due to the complicated procedure for RFLP analysis. In this study, we reported several breeder-friendly SSR markers closely linked to QTL *QTs.ksu-1AS* and *QTs.ksu-2BS*. These markers, especially *Xcfa2153* and *Xbarc7,* are highly polymorphic in a collection of germplasm (*unpublished data*) and could be readily used for marker-assisted breeding. Primer CFA2153 amplified a 212-bp fragment in 1AS of WSB and a 218-bp fragment in Ning7840, and primer BARC7 amplified 293- and 288-bp fragments in 2BS of WSB and Ning7840, respectively. To select for plants with low tan spot, breeders could select for the marker alleles of a resistant parent or select against the marker alleles of a susceptible parent in breeding populations. Furthermore, we mapped *QTs.ksu-1AS* within a 3-cM interval; the markers in the interval should be useful for further high-resolution mapping, assuming that there are enough polymorphic markers at the distal end of chromosome 1AS.

ACKNOWLEDGMENTS

LITERATURE CITED

1. Ali, S., and Francl, L. J. 2003. Population race structure of *Pyrenophora tritici-repentis* prevalent on wheat and noncereal grasses in the Great Plains. Plant Dis. 87:418-422.
2. Andrie, R. M., Pandelova, I., and Ciuffetti, L. M. 2007. A combination of phenotypic and genotypic characterization strengthens *Pyrenophora tritici-repentis r*ace identification. Phytopathology 97:694-701.
3. Ballance, G. M., Lamari, L., and C, B. C. 1989. Purification and characterization of a host selective toxin from *Pyrenophora tritici-repentis*. Physiol. Mol. Plant Pathol. 35:203-213.
4. Bockus, W. W., Appel, J. A., Bowden, R. L., Fritz, A. K., Gill, B. S., Martin, T. J., Sears, R. G., Seifers, D. L., Brown-Guedira, G. L., and Eversmeyer, M. G. 2001. Success stories: Breeding for wheat disease resistance in Kansas. Plant Dis. 85:453-461.
5. Bockus, W. W., and Claassen, M. M. 1992. Effects of crop rotation and residue management practices on severity of tan spot of winter wheat. Plant Dis. 76:633-636.
6. Bockus, W. W., and Shroyer, J. P. 1998. The impact of reduced tillage on soilborne plant pathogens. Annu. Rev. Phytopathol. 36:485-500.
7. Bockus, W. W., Su, Z., Garrett, K. A., Gill, B. S., Stack, J. P., Fritz, A. K., Roozeboom, K. L., and Martin, T. J. 2007. Number of experiments needed to determine wheat disease phenotypes for four wheat diseases.

TABLE 4. Number of observations and percent leaf area diseased (LAD) of recombinant inbred lines of the Wangshuibai (WSB)/Ning7840 (NING) population for the four allelic state combinations for markers *Xcfa2153* and *Xbarc7* after inoculation with conidia of race 1 of *Pyrenophora tritici-repentis*[x]

Xcfa2153	*Xbarc7*	No. of lines[y]	LAD (%)[z]
WSB	WSB	69	55.6 a
NING	WSB	53	71.6 b
WSB	NING	69	59.0 a
NING	NING	61	77.0 c

[x] Least significant difference ($P = 0.05$) = 3.92.

[y] Number of lines with the marker allele combination.

[z] Means with the same letter are not significantly different at $P_{0.05}$.

Plant Dis. 91:103-108.

8. Cheong, J., Wallwork, H., and Williams, K. J. 2004. Identification of a major QTL for yellow leaf spot resistance in the wheat varieties Brookton and Cranbrook. Aust. J. Agric. Res. 55:315-319.

9. Chu, C. G., Faris, J. D., Friesen, T. L., and Xu, S. S. 2006. Molecular mapping of hybrid necrosis genes *Ne1* and *Ne2* in hexaploid wheat using microsatellite markers. Theor. Appl. Genet. 112:1374-1381.

10. De Wolf, E. E., and Sloderbeck, P. E. 2009. Wheat variety disease and insect ratings 2009. Kans. State Univ. Agric. Exp. Stn. Coop. Ext. Serv. Publ. MF-991.

11. Duguid, S. D., and Brûlé-Babel, A. L. 2001. Inheritance and interaction of spring wheat (*Triticum aestivum* L.) resistance to race 2 and race 3 of *Pyrenophora tritici-repentis* (Died.) Drechs. Can. J. Plant Sci. 81:527-533.

12. Duguid, S. D., and Brûlé-Babel, A. L. 2001. Inheritance of resistance to a necrosis-and chlorosis-inducing isolate from race 1 of *Pyrenophora tritici-repentis* in spring wheat. Can. J. Plant Sci. 81:519-525.

13. Effertz, R. J., Anderson, J. A., and Francl, L. J. 2001. Restriction fragment length polymorphism mapping of resistance to two races of *Pyrenophora tritici-repentis* in adult and seedling wheat. Phytopathology 91:572-578.

14. Effertz, R. J., Meinhardt, S. W., Anderson, J. A., Jordahl, J. G., and Francl, L. J. 2002. Identification of a chlorosis-inducing toxin from *Pyrenophora tritici-repentis* and the chromosomal location of an insensitivity locus in wheat. Phytopathology 92:527-533.

15. Elias, E., Cantrell, R. G., and Hosford, R. M., Jr. 1989. Heritability of resistance to tan spot in durum wheat and its association with other agronomic traits. Crop Sci. 29:299-304.

16. Faris, J. D., Anderson, J. A., Francl, L. J., and Jordahl, J. G. 1996. Chromosomal location of a gene conditioning insensitivity in wheat to a necrosis-inducing culture filtrate from *Pyrenophora tritici-repentis.* Phytopathology 86:459-463.

17. Faris, J. D., Anderson, J. A., Francl, L. J., and Jordahl, J. G. 1997. RFLP mapping of resistance to chlorosis induction by *Pyrenophora tritici-repentis* in wheat. Theor. Appl. Genet. 94:98-103.

18. Faris, J. D., and Friesen, T. L. 2005. Identification of quantitative trait loci for race-nonspecific resistance to tan spot in wheat. Theor. Appl. Genet. 111:386-392.

19. Faris, J. D., Li, W. L., Liu, D. J., Chen, P. D., and Gill, B. S. 1999. Candidate gene analysis of quantitative disease resistance in wheat. Theor. Appl. Genet. 98:219-225.

20. Friesen, T. L., and Faris, J. D. 2004. Molecular mapping of resistance to *Pyrenophora tritici-repentis* race 5 and sensitivity to Ptr ToxB in wheat. Theor. Appl. Genet. 109:464-471.

21. Gamba, F. M., and Lamari, L. 1998. Mendelian inheritance of resistance to tan spot (*Pyrenophora tritici-repentis*) in selected genotypes of durum wheat (*Triticum turgidum*). Can. J. Plant Pathol. 20:408-414.

22. Gamba, F. M., Lamari, L., and Brule-Babel, A. L. 1998. Inheritance of race-specific necrotic and chlorotic reactions induced by *Pyrenophora tritici-repentis* in hexaploid wheats. Can. J. Plant Pathol. 20:401-407.

23. Hagborg, W. A. F. 1970. A device for injecting solutions and suspensions into thin leaves of plants. Can. J. Bot. 48:1135-1136.

24. Kosambi, D. D. 1944. The estimation of map distance from recombination values. Ann. Eugen. 12:172-175.

25. Lamari, L., and Bernier, C. C. 1989. Toxin of *Pyrenophora tritici-repentis*: Host-specificity, significance in disease, and inheritance of host reaction. Phytopathology 79:740-744.

26. Lamari, L., and Bernier, C. C. 1991. Genetics of tan necrosis and extensive chlorosis in tan spot of wheat caused by *Pyrenophora tritici-repentis*. Phytopathology 81:1092-1095.

27. Lamari, L., and Gilbert, J. 1998. Toward a coherent model of host-pathogen interactions in tan spot of wheat: An epilogue to the third international workshop on tan spot of wheat. Can. J. Plant Pathol. 20:440-443.

28. Lamari, L., Strelkov, S. E., Yahyaoui, A., Orabi, J., and Smith, R. B. 2003. The identification of two new races of *Pyrenophora tritici-repentis* from the host center of diversity confirms a one-to-one relationship in tan spot of wheat. Phytopathology 93:391-396.

29. Ma, H. X., Bai, G. H., Carver, B. F., and Zhou, L. L. 2005. Molecular mapping of a quantitative trait locus for aluminum tolerance in wheat cultivar Atlas 66. Theor. Appl. Genet. 112:51-57.

30. Michelmore, R. W., Paran, I., and Kesseli, R. V. 1991. Identification of markers linked to disease resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. Proc. Natl. Acad. Sci. USA 88:9828-9832.

31. Orolaza, N. P., Lamari, L., and Ballance, G. M. 1995. Evidence of a host-specific chlorosis toxin from *Pyrenophora tritici-repenti*s, the causal agent of tan spot of wheat. Phytopathology 85:1282-1287.

32. Riede, C. R., Francl, L. J., Anderson, J. A., Jordahl, J. G., and Meinhardt, S. W. 1996. Additional sources of resistance to tan spot of wheat. Crop Sci. 36:771-777.

33. Roder, M. S., Korzun, V., Wendehake, K., Plaschke, J., Tixier, M.-H., Leroy, P., and Ganal, M. W. 1998. A microsatellite map of wheat. genetics 149:2007-2023.

34. Saghai-Maroof, M. A., Soliman, K. M., Jorgensen, R. A., and Allard, R. W. 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc. Natl. Acad. Sci. USA 81:8014-8018.

35. Singh, P. K., and Hughes, G. R. 2005. Genetic control of resistance to tan necrosis induced by *Pyrenophora tritici-repentis*, races 1 and 2, in spring and winter wheat genotypes. Phytopathology 95:172-177.

36. Singh, P. K., Mergoum, M., Ali, S., Adhikari, T. B., and Hughes, G. R. 2008. Genetic analysis of resistance to *Pyrenophora tritici-repentis* races 1 and 5 in tetraploid and hexaploid wheat. Phytopathology 98:702-708.

37. 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.

38. Somers, D. J., Isaac, P., and Edwards, K. 2004. A high-density micro-satellite consensus map for bread wheat (*Triticum aestivum* L.). Theor. Appl. Genet. 109:1105-1114.

39. Sourdille, P., Singh, S., Cadalen, T., Brown-Guedira, G., Gay, G., Qi, L. L., Gill, B. S., Dufour, P., Murigneux, A., and Bernard, M. 2004. Microsatellite-based deletion bin system for the establishment of genetic-physical map relationships in wheat (*Triticum aestivum* L.). Funct. Integr. Genomics 4:12-25.

40. Stock, W. S., Brule-Babel, A. L., and Penner, G. A. 1996. A gene for resistance to a necrosis-inducing isolate of *Pyrenophora tritici-repentis* located on 5BL of *Triticum aestivum* cv. Chinese Spring. Genome 39:598-604.

41. Strelkov, S. E., Lamari, L., and Ballance, G. M. 1999. Characterization of a host-specific protein toxin (Ptr ToxB) from *Pyrenophora tritici-repentis*. Mol. Plant-Microbe Interact. 12:728-732.

42. Tadesse, W., Hsam, S., Wenzel, G., and Zeller, F. 2008. Chromosome location of a gene conferring resistance to *Pyrenophora tritici-repentis* in Ethiopian wheat cultivars. Euphytica 162:423-430.

43. Tadesse, W., Schmolke, M., Hsam, S. L., Mohler, V., Wenzel, G., and Zeller, F. J. 2007. Molecular mapping of resistance genes to tan spot (*Pyrenophora tritici-repentis* race 1) in synthetic wheat lines. Theor. Appl. Genet. 114:855-862.

44. Tomas, A., and Bockus , W. W. 1987. Cultivar-specific toxicity of culture filtrates of *Pyrenophora tritici-repentis*. Phytopathology 77:1337-1366.

45. Tomas, A., Feng, G. H., Reeck, G. R., Bockus, W. W., and Leach, J. E. 1990. Purification of a cultivar-specific toxin from *Pyrenophora tritici-repentis*, causal agent of tan spot of wheat. Mol. Plant-Microbe Interact. 3:221-224.

46. Vales, M., Schön, C., Capettini, F., Chen, X., Corey, A., Mather, D., Mundt, C., Richardson, K., Sandoval-Islas, J., Utz, H., and Hayes, P. 2005. Effect of population size on the estimation of QTL: A test using resistance to barley stripe rust. Theor. Appl. Genet. 111:1260-1270.

47. Van Ooijen, J. W., and Voorrips, R. E. 2001. JoinMap 3.0. PRI. SoftGenetics LLC, Wageningen, The Netherlands.

48. Wang, S., Basten, C. J., and Zeng, Z. B. 2007. Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh.