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ORIGINAL PAPER

Identification of novel tan spot resistance loci beyond the known host-selective toxin insensitivity genes in wheat

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Abstract Tan spot, caused by *Pyrenophora tritici-repentis*, is a destructive foliar disease of wheat causing significant yield reduction in major wheat growing areas throughout the world. The objective of this study was to identify quantitative trait loci (QTL) conferring resistance to tan spot in the synthetic hexaploid wheat (SHW) line TA4152-60. A doubled haploid (DH) mapping population derived from TA4152-60 × ND495 was inoculated with conidia produced by isolates of each of four virulent races of *P. tritici-repentis* found in North America. QTL analysis revealed a total of five genomic regions significantly associated with tan spot resistance, all of which were contributed by the SHW line. Among them, two novel QTLs located on chromosome arms 2AS and 5BL conferred resistance to all isolates tested. Another novel QTL on chromosome arm 5AL conferred resistance to isolates of races 1, 2 and 5, and a QTL specific to a race 3 isolate was detected on chromosome arm 4AL. None of these QTLs corresponded to known host selective toxin (HST) insensitivity loci, but a second QTL on chromosome arm 5BL conferred resistance to the Ptr ToxA producing isolates of races 1 and 2 and corresponded to the *Tsn1* (Ptr ToxA sensitivity) locus. This indicates that the wheat-*P. tritici-repentis* pathosystem is much more complex than previously thought and that selecting for toxin insensitivity alone will not necessarily lead to tan spot resistance. The markers associated with the QTLs identified in this work will be useful for deploying the SHW line as a tan spot resistance source in wheat breeding.

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Introduction

Tan spot, caused by the fungus *Pyrenophora tritici-repentis* (Died.) Drechs. [anamorph *Drechslera tritici-repentis* (Died.) Shoem.], is a destructive foliar disease of common wheat (*Triticum aestivum* L.) and durum wheat (*T. turgidum* L. var. *durum*), as well as other grass species (Ali and Francl 2003; Hosford 1971; Krupinsky 1992). Riede et al. (1996) reported that yield losses caused by tan spot were capable of approaching 50%. Disease incidence has become more common in recent years due in part to the application of reduced tillage practices in cereal growing regions of the world (Strelkov and Lamari 2003).

Isolates of *P. tritici-repentis* differ in virulence. Susceptibility of wheat to *P. tritici-repentis* is manifested by the development of necrosis and/or chlorosis depending on the race of the pathogen. Isolates of *P. tritici-repentis* have been grouped into eight races based on their virulence pattern on five differential wheat lines: Glenlea, Katepwa, Salamouni, 6B365, and 6B662 (Lamari et al. 1995, 2003; Strelkov et al. 2002; Strelkov and Lamari, 2003) and on the presence of genes coding for specific host-selective toxins (HSTs) (Andrie et al. 2007). Races 1–5 have all been found in North America (Ali et al. 1999; Lamari et al. 2003), with

races 1 and 2 being the most prevalent (Ali and Francl 2003; Lamari et al. 1998; Lamari and Bernier 1989a).

Reports of tan spot resistance in wheat range from qualitative (Gamba and Lamari 1998; Gamba et al. 1998; Lee and Gough 1984; Lamari and Bernier 1989b, 1991; Sykes and Bernier 1991; Singh et al. 2006a; Tadesse et al. 2007) to quantitative (Cheong et al. 2004; Elias et al. 1989; Faris et al. 1997, 1999; Friesen et al. 2003; Faris and Friesen 2005; Nagle et al. 1982). *P. tritici-repentis* is known to produce at least three HSTs (Effertz et al. 2002; Lamari and Bernier 1989b; Orolaza et al. 1995; Tomás and Bockus 1987; Tuori et al. 1995) that interact with specific host sensitivity genes to cause disease (Effertz et al. 2002; Friesen et al. 2003; Friesen and Faris 2004; Lamari and Bernier 1989b). Among the identified HSTs, Ptr ToxA, a well-characterized HST produced by races 1 and 2 (Tomás and Bockus 1987; Tuori et al. 1995), was shown to be responsible for the development of necrosis (Lamari and Bernier 1989b). Sensitivity to Ptr ToxA is conditioned by a single dominant gene named *Tsn1* (Lamari and Bernier 1989b) on chromosome arm 5BL (Faris et al. 1996; Anderson et al. 1999). Insensitivity to Ptr ToxA is highly associated with resistance (Cheong et al. 2004; Friesen et al. 2003; Lamari and Bernier 1989b).

Ptr ToxB (Orolaza et al. 1995) and Ptr ToxC (Effertz et al. 2002) are both chlorosis-inducing HSTs. The Ptr ToxB-sensitivity gene (*Tsc2*) was mapped to chromosome arm 2BS and was shown to confer susceptibility to the race 5 isolate DW5 (Friesen and Faris 2004). Sensitivity to Ptr ToxC is conditioned by the gene *Tsc1*, found on the short arm of chromosome 1A (Effertz et al. 2002). Sensitivity to Ptr ToxC has also been shown to be associated with tan spot susceptibility (Effertz et al. 1998, 2002; Faris et al. 1997, 1999). Therefore, several major resistance genes and quantitative trait loci (QTLs) identified have been due to insensitivity to HSTs produced by the various races of *P. tritici-repentis*.

In addition to the known toxin sensitivity genes, Faris and Friesen (2005) identified resistance QTLs on chromosome arms 1BS and 3BL in a recombinant inbred population derived from Grandin × BR34. Neither of these QTLs has been shown to be associated with toxin insensitivity genes. They also found that the *Tsn1*–Ptr ToxA interaction was not a significant factor in tan spot development in that population. This result, along with other recent studies (Andrie et al. 2007; Friesen et al. 2002, 2003; Riede et al. 1996; Singh et al. 2006a, b; Tadesse et al. 2007) has indicated the potential for additional complexity in the wheat-*P. tritici-repentis* pathosystem.

Synthetic hexaploid wheat (SHW) (×*Aegilotriticum* spp., $2n = 6x = 42$, AABBDD) is the induced amphiploid from the hybrid between tetraploid wheat (*Triticum turgidum* L., $2n = 4x = 28$, AABB) and *Aegilops tauschii* Coss. ($2n = 2x = 14$, DD), and is commonly used as bridging germplasm in the introgression of desirable genes from *Ae. tauschii* to common wheat (reviewed by Cox, 1998). Xu et al. (2004) identified 41 SHW lines resistant to a tan spot race 1 isolate, and among them, TA4152-60 showed a high level of resistance. In this study, we evaluated a doubled haploid (DH) population derived from the cross between TA4152-60 and an elite North Dakota hard red spring wheat line (ND495) for reaction to races 1, 2, 3 and 5 of *P. tritici-repentis* and used QTL analysis to identify genomic regions and molecular markers associated with resistance.

Materials and methods

Plant materials

A mapping population consisting of 120 DH lines derived from the SHW line TA4152-60 and the North Dakota breeding line ND495, which was used for developing whole genome linkage maps (Chu et al. 2008), was used for the identification of tan spot resistance QTLs. TA4152-60 was developed at the International Maize and Wheat Improvement Center (CIMMYT) from a cross between the durum wheat variety Scoop 1 and the *Ae. tauschii* accession WPI358 (TA2516). TA4152-60 is insensitive to Ptr ToxA and resistant to Pti2, a race 1 isolate of *P. tritici-repentis* (Xu et al. 2004). ND495 is a selection from ‘Justin*2/3/ND 259/Conley//ND 112’, and is sensitive to Ptr ToxA and susceptible to Pti2 (race 1). Our tests also indicated that ND495 and TA4152-60 showed different reactions to isolates of race 2 (86–124), race 3 (OH99) and race 5 (DW5) (Table 1).

Fungal isolates, inoculation, and rating

Because races 1, 2, 3 and 5 are all found in North America and isolates of race 4 are avirulent on wheat (Ali et al. 1999; Lamari et al. 2003), we selected isolates Pti2 (race 1) (Friesen et al. 2002), 86–124 (race 2) (Friesen et al. 2003), OH99 (race 3) (Engle et al. 2006; Faris and Friesen 2005), and DW5 (race 5) (Ali et al. 1999; Friesen and Faris 2004) for this study. All isolates have been race characterized based on the standard differential set as proposed by Lamari et al. (2003). In addition, the presence of *ToxA* has been evaluated in each isolate used, with *ToxA* being present in races 1 and 2 and absent in races 3 and 5 (data not shown). For evaluation of disease reaction, all plants were inoculated with conidia of each isolate separately. Disease inoculation was conducted in three experiments under controlled conditions using procedures described in Friesen et al. (2003). For each experiment, nine seeds of each line were planted in three super-cell cones (Stuewe and Sons, Inc.,

Table 1 Reaction type means of TA4152-60, ND495 and the doubled haploid population (DHP) to conidial inoculation of four isolates representing races 1, 2, 3, and 5 of *P. tritici-repentis*

Isolate	ND495	TA4152-60	DHP AVG	DHP range
Pti 2 (race 1)	4.05	1.32	2.87	1.50–4.50
86–124 (race 2)	4.25	1.11	2.37	1.17–4.17
OH99 (race 3)	3.00	1.00	1.84	1.00–3.50
DW5 (race 5)	4.19	1.17	2.88	1.17–4.67

Reaction types were rated 7-day post-inoculation using the 1–5 rating scale developed by Lamari and Bernier (1989a), where 1 = resistant, 2 = moderately resistant, 3 = moderately resistant to moderately susceptible, 4 = moderately susceptible, and 5 = susceptible

Corvallis, OR, USA) with three seeds per cone. Cones were placed in RL98 trays (Stuewe and Sons, Inc., Corvallis, OR, USA). To eliminate any edge effect, the susceptible cultivar Grandin was planted in all the cones around the borders on each RL98 tray except for six cones, which were used for planting the parents. Therefore, a total of 27 plants were used for each line with each isolate. Fungi were grown and conidia were harvested as described by Lamari and Bernier (1989a). Spore inoculum was adjusted to 3,000 spores/ml, and two drops of Tween-20 were added per 100 ml of inoculum. Plants were inoculated until runoff and placed in 100% relative humidity in the light at 21°C for 24 h, and then placed in a growth chamber under a 12-h photoperiod at 21°C. Reaction types were rated 7-day post-inoculation using the 1–5 reaction type rating scale developed by Lamari and Bernier (1989a), where 1 = resistant, 2 = moderately resistant, 3 = moderately resistant to moderately susceptible, 4 = moderately susceptible, and 5 = susceptible. Lines showing equal numbers of two reaction types were given an intermediate score (e.g. lines showing equal numbers of reaction type 1 and 2 were scored as 1.5). Ptr ToxA sensitivity of each DH line was obtained previously (Chu et al. 2008). Purified Ptr ToxB and Ptr ToxC were not available for this research.

Molecular mapping and QTL analysis

The linkage maps developed for this DH population were previously reported and consisted of 632 markers and spanned 3,811.5 cM with an average density of one marker per 6.03 cM (Chu et al. 2008). A subset of 449 markers spaced approximately 5–20 cM apart and giving the most complete genome coverage was selected and used for QTL detection.

Bartlett's χ^2 was calculated to test the homogeneity of variances among different experiments using the SAS program (SAS Institute 1999), and data from homogeneous experiments were combined for QTL analysis. Individual markers significantly ($P < 0.001$) associated with tan spot resistance were identified through single-factor regression analysis using the computer program QGENE (Nelson 1997). Simple interval mapping (SIM) and composite interval-regression mapping (CIM) were performed using the computer program Map Manager QTX (Manly et al. 2001) to evaluate marker intervals putatively associated with trait phenotypes. A permutation test with 5,000 permutations was conducted to determine that the critical LOD threshold of 3.0 in this DH population yields an experiment-wise significance level of 0.05. Markers with significant ($P < 0.001$) main effects were tested against all other markers to identify significant ($P < 0.000001$) interactions (Manly et al. 2001). Markers with the most significant effect for each QTL and the interactions were assembled into multiple regression models to determine the coefficient of determination (R^2), which is the total amount of variation explained by the model.

Results

Evaluation of tan spot in the DH population

Tan spot reaction types caused by the four isolates of *P. tritici-repentis* were recorded 7-day post-inoculation for each DH line. Homogeneity tests indicated the data from the three replicated experiments for each isolate were homogeneous. Bartlett's χ^2 was 2.67, 1.87, 3.84 and 4.61, and the associated P values with 2 degrees of freedom were 0.26, 0.39, 0.15 and 0.10 for reaction to isolates Pti2 (race 1), 86–124 (race 2), OH99 (race 3), and DW5 (race 5), respectively. Thus, the reaction type data for each isolate were combined and the means for each isolate were used for QTL analysis. The SHW line TA4152-60 was highly resistant to all isolates and had average reaction types of 1.0–1.3, whereas ND495 showed average reaction types of 3.0 for reaction to OH99 and 4.0–4.3 for Pti2, 86–124, and DW5 (Table 1, Fig. 1). Therefore, ND495 was moderately susceptible to the race 3 isolate and susceptible to the isolates of races 1, 2 and 5.

Distribution of the reaction type means in the DH population indicated that resistance to all four isolates was quantitatively controlled, and little transgressive segregation

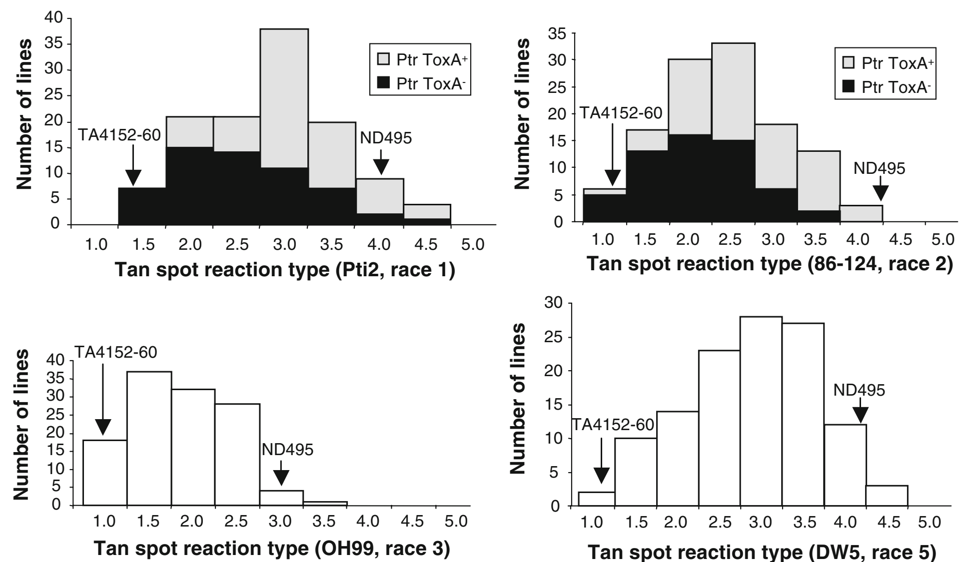

Fig. 1 Histograms of average reaction type distribution in the doubled haploid population after inoculation with the four isolates/races of *P. tritici-repentis*. For isolates representing races 1 and 2, Ptr ToxA insensitive and sensitive lines are indicated in *gray* and *black*, respectively. *White bars* for isolates representing races 3 and 5 refer to the whole population

Table 2 Single factor regression analysis of individual markers associated with resistance to isolates of four races of *P. tritici-repentis* conditioned by TA4152-60 in the doubled haploid population derived from TA4152-60 × ND495

Marker or interaction	Chromosome location	R^2 value			
		Pti2 (race 1)	86–124 (race 2)	OH99 (race 3)	DW5 (race 5)
Xbarc10	2AS	0.12***	0.20***	0.12**	0.19***
Xbarc1047	4AL	NS	NS	0.09*	NS
Xfcp412	5AL	0.14***	0.11**	NS	0.18***
Xbarc128.2	5BL	0.20***	0.20***	0.24***	0.12**
Xcfa2106	7BS	NS	NS	NS	NS
Xbarc128.2 × *Xcfa2106*	5BL and 7BS	NS	NS	NS	0.13***
Tsn1	5BL	0.17***	0.15***	NS	NS
Xfcp547	2BL	NS	NS	NS	NS
Tsn1 × *Xfcp547*	5BL and 2BL	0.10***	0.09***	NS	NS
Multiple regression[a]		0.49***	0.54**	0.45**	0.48**

Significance levels: *** $P < 0.0001$, ** $P < 0.001$, * $P < 0.005$, NS non-significant

[a] Mutiple regression models included only the significant markers and interactions for each isolate

was observed (Fig. 1). Because races 1 and 2 produce Ptr ToxA (Tomás and Bockus 1987; Tuori et al. 1995), and Ptr ToxA insensitivity is known to be associated with resistance (Friesen et al. 2003; Lamari and Bernier 1989b), we compared the previously obtained Ptr ToxA sensitivity data (Chu et al. 2008) with the distribution of Pti2 and 86–124 reaction types among Ptr ToxA sensitive and insensitive DH lines (Fig. 1). Of the 57 Ptr ToxA insensitive DH lines, 32 (56%) and 44 (77%) showed reaction type means less than 2.5 for reaction to Pti2 and 86–124, respectively, and among the 63 Ptr ToxA sensitive lines, 47 (75%) and 33 (52%) had mean reaction types greater than 3.0 for reaction to Pti2 and 86–124, respectively. This suggests that a compatible *Tsn1*–Ptr ToxA interaction plays a significant role in disease development in this population, which corroborates the results of the QTL analysis (see below).

QTL Identification

A total of five genomic regions harboring QTL were revealed through single-factor regression, SIM, and CIM, and they were all significantly associated with the resistance contributed by TA4152-60 (Tables 2, 3; Fig. 2). Two of the QTL were located on chromosome arm 5BL (designated as *QTs.fcu-5BL.1* and *QTs.fcu-5BL.2*), and the remaining three were on chromosome arms 2AS (*QTs.fcu-2AS*), 4AL (*QTs.fcu-4AL*) and 5AL (*QTs.fcu-5AL*) (Fig. 2).

Four of the five QTLs, *QTs.fcu-2AS*, *QTs.fcu-5AL*, *QTs.fcu-5BL.1* and *QTs.fcu-5BL.2*, were significantly associated with the resistance to Pti2 (race 1) of *P. tritici-repentis* (Table 3; Fig. 2). The interval between *Xgwm71.2* and *Xfcp526* defined the peak of *QTs.fcu-2AS*, which explained 14% of the phenotypic variation using CIM (Table 3).

Table 3 Composite interval mapping analysis of QTLs associated with resistance to isolates Pti2 (race 1), 86–124 (race 2), OH99 (race 3) and DW5 (race 5) of *P. tritici-repentis* in the TA4152-60 × ND495 derived doubled haploid population

QTL	Marker interval[a]	Chromosome-peak position (cM)[b]	R^2 value				Logarithm of the odds (LOD)			
			Pti2	86–124	OH99	DW5	Pti2	86–124	OH99	DW5
QTs.fcu-2AS	*Xgwm515–Xfcp526*	84.0–85.5	0.14	0.22	0.14	0.19	5.08	8.55	5.27	4.95
QTs.fcu-4AL	*Xbarc236–Xgwm644*	151.8	NS	NS	0.10	NS	NS	NS	3.56	NS
QTs.fcu-5AL	*Xbarc1061–Xcfa2185*	138.4–140.1	0.10	0.09	NS	0.14	3.47	3.47	NS	4.77
QTs.fcu-5BL.1	*Xbarc138–Xgwm260*	57.6–59.7	0.22	0.22	0.26	0.14	7.43	8.44	9.02	4.88
QTs.fcu-5BL.2	*Xfcp615–Xbarc142*	105.2–107.1	0.17	0.14	NS	NS	5.75	5.49	NS	NS

NS non-significant

[a] Marker intervals for the isolate having the largest interval for each QTL are given

[b] A range of the chromosome-peak position indicates the position was slightly different among the different isolates

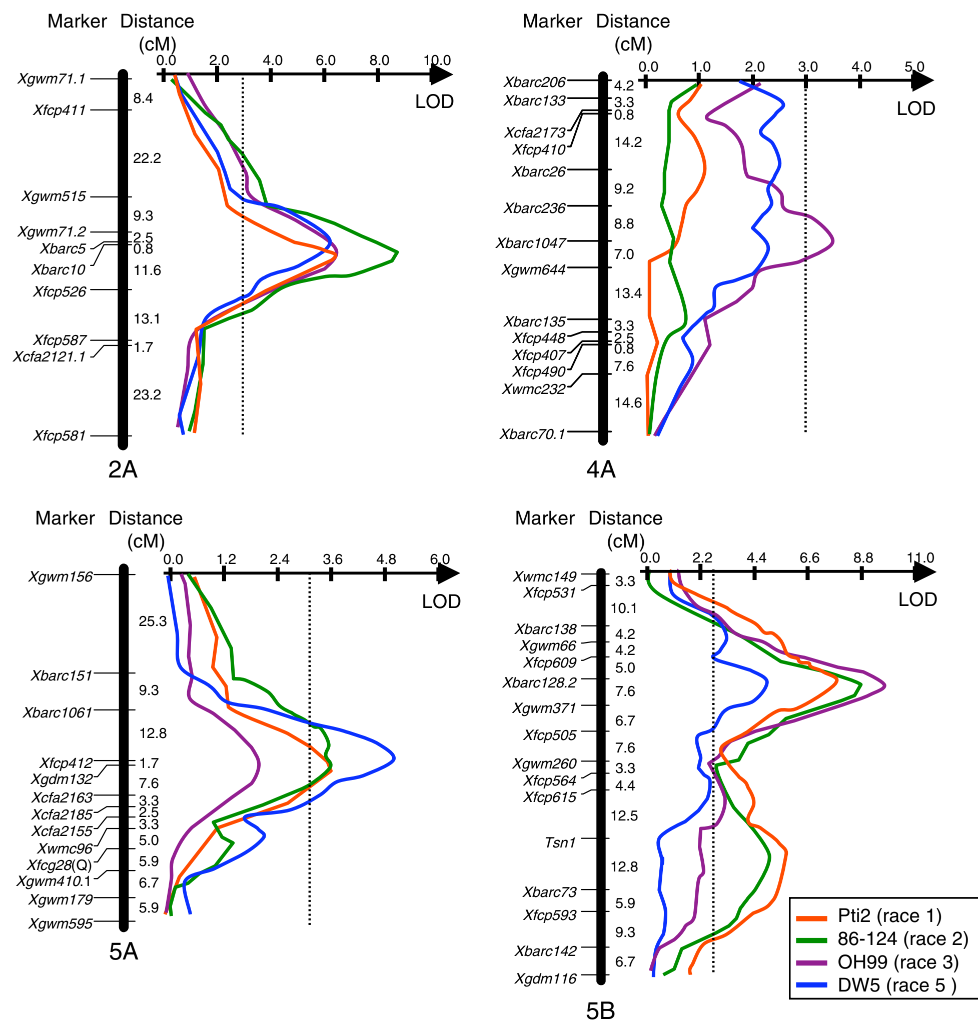

Fig. 2 Composite interval regression maps of QTLs associated with the resistance contributed by TA4152-60. The positions of marker loci are shown to the left of the linkage groups and centiMorgan (*cM*) distances between loci are shown along the *right*. *Red*, *green*, *purple*, and *blue lines* indicate QTLs for resistance to isolates Pti2 (race 1), 86–124 (race 2), OH99 (race 3), and DW5 (race 5), respectively. The *vertical dotted line* represents the logarithm of the odds (*LOD*) significance threshold of 3.0. The LOD and R^2 values for each QTL are listed in Table 3

Xbarc10 was the most significant marker within the interval explaining 12% of the variation (Table 2). *QTs.fcu-5AL* peaked within the interval defined by markers *Xbarc1061* and *Xcfa2163*, and it explained 10% of the phenotypic variation in CIM (Table 3). *Xfcp412* was the marker most significantly associated with *QTs.fcu-5AL* for resistance to Pti2, and it explained 14% of the variation in single-factor regression (Table 2). The two QTLs on chromosome arm 5BL, *QTs.fcu-5BL.1* and *QTs.fcu-5BL.2*, explained 22 and 17% of the variation in CIM, respectively (Table 3, Fig. 2). *QTs.fcu-5BL.1* was located in the proximal region of 5BL and peaked at the interval defined by *Xbarc138* and

Xfcp505 (Table 3, Fig. 2). *Xbarc128.2* was the most significant marker within the *QTs.fcu-5BL.1* interval and explained 20% of the variation in single-factor regression (Table 2). *QTs.fcu-5BL.2* was located more distal and peaked in the interval between *Tsn1* and *Xbarc142* (Fig. 2). In single-factor regression, the *Tsn1* locus explained 17% of the variation in disease caused by Pti2 (Table 2). A significant interaction between the marker *Xfcp547* located on chromosome arm 2BL and *Tsn1* on 5BL was also identified, and the interaction explained 10% of the variation (Table 2). The most significant marker associated with each of the four QTLs as well as the interaction were assembled into a multiple regression model, which explained a total of 49% of the variation for resistance to Pti2 (Table 2).

Resistance to isolate 86–124 (race 2) was governed by the same four QTLs associated with resistance to Pti2 (race 1) (Table 3, Fig. 2). *QTs.fcu-2AS* peaked between *Xgwm515* and *Xfcp526* and explained 22% of phenotypic variation in CIM (Table 3, Fig. 2). The most significant marker within the interval was *Xbarc10*, and it explained 20% of the variation in single-factor regression (Table 2). *QTs.fcu-5BL.1* also explained 22% of phenotypic variation in CIM (Table 3) and peaked between markers *Xgwm66* and *Xfcp505* (Fig. 2). The most significant marker within the interval was *Xbarc128.2*, which explained 20% of the variation in single-factor regression (Table 2). *QTs.fcu-5AL* peaked between *Xbarc1061* and *Xcfa2163* on 5AL, and it explained 9% of the phenotypic variation in CIM (Table 3). The marker *Xfcp412* was the most significant and explained 11% of the variation in single-factor regression (Table 2). *QTs.fcu-5BL.2* explained 14% of the variation in CIM (Table 3), and peaked between *Tsn1* and *Xbarc142*, the interval similar to that for resistance to Pti2. The *Tsn1* locus had the most significant effect within the *QTs.fcu-5BL.2* region and explained 15% of the variation in single-factor regression (Table 2). The interaction between *Xfcp547* and *Tsn1*, which was significantly associated with resistance to Pti2 was also significantly associated with resistance to 86–124, and it explained 9% of the trait variation (Table 2). Markers associated with the four significant QTLs and the interaction were all significant in a multiple regression model, which explained a total of 54% of the phenotypic variation for resistance to 86–124 (race 2) (Table 2).

Resistance to isolate OH99 (race 3) was conditioned by *QTs.fcu-2AS*, *QTs.fcu-4AL* and *QTs.fcu-5BL.1*, and they explained 14, 10 and 26% of the phenotypic variation in CIM, respectively (Table 3, Fig. 2). *QTs.fcu-2AS* peaked at the same marker interval as that for resistance to 86–124, and the most significant marker within the interval was *Xbarc10*, which explained 12% of the variation in single-factor regression (Table 2). *QTs.fcu-4AL* was located near the middle of chromosome arm 4AL and peaked within the interval defined by markers *Xbarc236* and *Xgwm644* (Fig. 2). The marker *Xbarc1047* was the most significant and explained 9% of the variation in single-factor regression (Table 2). *QTs.fcu-5BL.1* was the most significant QTL for resistance to OH99, and was flanked by markers *Xbarc138* and *Xfcp505*, an interval similar to that observed for resistance to Pti2 and 86–124. As with resistance to Pti2 and 86–124, *Xbarc128.2* was the most significant marker associated with *QTs.fcu-5BL.1* for resistance to OH99, and it explained 24% of the variation in single-factor regression (Table 2). No significant interactions between markers with significant main effects and other loci were found. Markers *Xbarc10*, *Xbarc1047*, and *Xbarc128.2* were assembled into a multiple regression model and explained a total of 45% of the variation for resistance to isolate OH99 (race 3) (Table 2).

For isolate DW5 (race 5), three QTLs, *QTs.fcu-2AS*, *QTs.fcu-5AL* and *QTs.fcu-5BL.1* were all significantly associated with the resistance, and they explained 19, 14, and 14% of the trait variation in CIM, respectively (Table 3, Fig. 2). *QTs.fcu-2AS* peaked in the same region as observed for resistance to isolates Pti2, 86–124, and OH99 (Fig. 2), and the most significant marker was again *Xbarc10* explaining 19% of the variation in single-factor regression (Table 2). *QTs.fcu-5AL* was within the interval defined by markers *Xbarc1061* and *Xcfa2185*, which is the same region as observed for resistance to Pti2 and 86–124 (Fig. 2). *Xfcp412* was the most significant marker within the interval and explained 18% variation in single-factor regression (Table 2). *QTs.fcu-5BL.1* peaked within the same genomic region as observed for resistance to Pti2, 86–124, and OH99, and again, *Xbarc128.2* was the most significant marker and explained 12% of the variation in single-factor regression (Table 2). A significant interaction between the marker *Xcfa2106* located on chromosome arm 7BS and *Xbarc128.2* was also identified, and the interaction explained 13% of the variation in single-factor regression (Table 2). The three QTLs and the interaction were all significant in the multiple regression model and together they explained 48% (Table 2) of the phenotypic variation.

Discussion

In this research, we identified a total of five QTLs that conferred resistance to tan spot contributed by TA4152-60. *QTs.fcu-2AS* and *QTs.fcu-5BL.1* were significantly associated with resistance to isolates representing all four races. *QTs.fcu-5AL* was significantly associated with resistance to the isolates representing races 1, 2 and 5. *QTs.fcu-5BL.2*, which harbors the *Tsn1* locus governing reaction to Ptr ToxA, was associated with resistance to the isolates representing races 1 and 2, which are the only two races that produce Ptr ToxA (Lamari and Bernier 1989b, Tomás and

Bockus 1987; Tuori et al. 1995). *QTs.fcu-4AL* was significantly associated with resistance to the race 3 isolate only (Table 3).

Friesen and Faris (2004) identified a QTL on chromosome arm 2AS conferring resistance to the same race 5 isolate used here and the marker *Xcdo447* showed the most significant effects. In our research, *QTs.fcu-2AS* was located in a more proximal position of the arm and *Xbarc10* had the most significant effects. *Xcdo447* and *Xbarc10* are located in different deletion bins (Erayman et al. 2004; Sourdille et al. 2004), which suggests that *QTs.fcu-2AS* is not the same as the one identified by Friesen and Faris (2004). The QTL *QTs.fcu-4AL* identified in this study conferred resistance only to the race 3 isolate OH99 (Fig. 2). Based on the position of the common marker *Xbarc206* among the maps of Faris et al. (1997, 1999), Sourdille et al. (2004), and ours, *QTs.fcu-4AL* is probably the same as that previously identified (Faris et al. 1997, 1999; Faris and Friesen 2005; Friesen and Faris 2004). However, this QTL was reported to confer resistance to a race 1 isolate (Faris et al. 1997, 1999) and a race 5 isolate (Friesen and Faris 2004; Faris and Friesen 2005). Although closely linked, different genes conferring tan spot resistance might exist in this region, or different alleles of the same locus might have the ability to recognize different races/isolates. From the position of common marker *Xcfa2163* on maps of Liu et al. (2005) and ours, *QTs.fcu-5AL* identified in this research was different from the 5AL QTL reported in Faris and Friesen (2005). As for the genomic region containing *QTs.fcu-5BL.1*, there were no previous reports regarding its association with either disease resistance or insensitivity to HSTs. Therefore, *QTs.fcu-2AS*, *QTs.fcu-5AL* and *QTs.fcu-5BL.1* are probably novel tan spot resistance QTLs derived from the SHW line.

By definition, races 3 and 5 produce Ptr ToxC and Ptr ToxB, respectively, and the *Tsc1* and *Tsc2* genes govern sensitivity to Ptr ToxC and Ptr ToxB, respectively (Effertz et al. 2002; Friesen and Faris 2004). We identified no QTLs within the genomic regions of 1AS and 2BS, which are known to harbor the *Tsc1* and *Tsc2* genes. Purified Ptr ToxC and Ptr ToxB were not available for this project, but the fact that no QTLs corresponding to the known genomic regions of *Tsc1* and *Tsc2* were identified indicates that either these genes are not segregating in our population, or Ptr ToxB and Ptr ToxC were not significant factors in causing disease. In either case, our results indicate that isolates OH99 and DW5 produce virulence factors in addition to Ptr ToxC and Ptr ToxB.

Four out of the five resistance QTLs detected in this study were not associated with any known toxin insensitivity locus. It is possible that underlying the four QTLs are toxin insensitivity genes which have yet to be discovered. Two independent research groups have reported the finding of a fourth and fifth toxin, which were both referred to as Ptr ToxD (Manning et al. 2002, Meinhardt et al. 2003). Lines containing host sensitivity to these two toxins have been reported but chromosomal locations of the sensitivity loci have not been identified. Therefore, other toxins probably exist and might be associated with any of these four QTLs. It is also possible that these QTLs are non-toxin associated, and that some other mechanism of resistance other than toxin insensitivity is conferred by the SHW line similar to that proposed by Faris and Friesen (2005). It is also possible that a combination of these scenarios exist. Regardless, it is apparent that each of the isolates used in this work harbor virulence factors in addition to the known toxins. This fact may have implications on the race classification system. Andrie et al. (2007) reported that the current differential set may need to be expanded due to its insufficiency in classification based on the additional complexity beyond the HSTs described. It was also noted that genotyping of the isolates for the known HST-producing genes (i.e. *ToxA* and *ToxB*) is also needed to increase confidence in race classification.

All the resistance QTLs identified in this study were located in the A and B genome chromosomes of the SHW line. It was somewhat surprising that no QTLs were detected in the D genome because the tetraploid parent (Scoop 1), which donated the A and B genomes, showed a mean reaction type of 2.8 in response to isolate Pti2 (race 1) (Xu et al. 2004) indicating Scoop 1 was moderately resistant to moderately susceptible. Tadesse et al. (2007) reported resistance to the race 1 isolate ASC1b on chromosome arm 3DS derived from three SHW lines. Therefore, we expected the high level of resistance in TA4152-60 to be due to genes in the D genome donated by the *Ae. tauschii* accession WPI358 (TA2516). However, after inoculating the *Ae. tauschii* accession with Pti2, it showed a reaction type of 3–4 indicating it was also susceptible to tan spot (data not shown). This substantiates the lack of resistance identified on the D genome in our population.

He et al. (2003) compared global gene expression of a SHW line with its diploid and tetraploid parents and found that a significant number of genes had altered expression levels in the hexaploid. Islam et al. (2003) compared the proteome patterns of diploid, tetraploid and hexaploid wheat and found the expression of proteins in hexaploid wheat is affected by interactions among the diploid genomes when they coincide within the hexaploid nucleus. The durum cultivar Scoop 1 carries the resistance genes that underlie the QTLs identified in this research, but it is possible that these genes are not active or are expressed at lower levels in the tetraploid. Once the A and B genomes of the tetraploid are combined with the D genome from *Ae. tauschii*, the resistance genes may be activated or up regulated leading to higher levels of resistance in TA4152-60.

Therefore, comparing the expression level of resistance genes in the tetraploid parent with that of the SHW line might shed light on the issue.

In conclusion, our research revealed five QTLs conferring resistance to tan spot in the SHW line, three of which are novel. Four of the QTLs do not correspond to known toxin insensitivity loci that were previously shown to play significant roles in disease development for the corresponding races. This finding indicates that the wheat-tan spot system is more complex than previously thought, and that isolates harbor numerous virulence factors (possibly unidentified HSTs) in addition to those HSTs currently used to define races. The results of this research and that of Faris and Friesen (2005) indicate that genotypes insensitive to the known tan spot toxins are not always resistant to the disease and that additional and/or alternate factors are involved. Although the currently identified toxins, which define the *P. tritici-repentis* race classification system, have been shown to be highly important in disease and should be used in selection of the most resistant wheat varieties, our previous (Faris and Friesen 2005) and current research shows that wheat breeders should not rely solely on these toxins, since other virulence factors are highly important in disease development. This research may have implications for the tan spot race classification system but additional research on the pathogen side to define these new virulence factors will be necessary. The molecular markers identified in this research will be useful for deploying the SHW-derived tan spot resistance QTLs in wheat breeding.

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