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Identification of quantitative trait loci conferring resistance to tan spot in a biparental population derived from two Nebraska hard red winter wheat cultivars

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Abstract Tan spot, caused by *Pyrenophora tritici-repentis* (*Ptr*), is a destructive foliar disease in all types of cultivated wheat worldwide. Genetics of tan spot resistance in wheat is complex, involving insensitivity to fungal-produced necrotrophic effectors (NEs), major resistance genes, and quantitative trait loci (QTL) conferring race-nonspecific and race-specific resistance. The Nebraska hard red winter wheat (HRWW) cultivar 'Wesley' is insensitive to Ptr ToxA and highly resistant to multiple *Ptr* races, but the genetics of resistance in this cultivar is unknown. In this study, we used a recombinant inbred line (RIL) population derived from a cross between Wesley and another Nebraska cultivar 'Harry' (Ptr ToxA sensitive and highly susceptible) to identify QTL associated with reaction to tan spot caused by multiple races/isolates. Sensitivity to Ptr ToxA conferred by the *Tsn1* gene was mapped to chromosome 5B as expected. The *Tsn1* locus was a major susceptibility QTL for the race 1 and race 2 isolates, but not for the race 2 isolate with the *ToxA* gene deleted. A second major susceptibility QTL was identified for all the Ptr ToxC-producing isolates and located to the distal end of the chromosome 1A, which likely corresponds to the *Tsc1* locus. Three additional QTL with minor effects were identified on chromosomes 7A, 7B, and 7D. This work indicates that both Ptr ToxA-*Tsn1* and Ptr ToxC-*Tsc1* interactions are important for tan spot development in winter wheat, and Wesley is highly resistant largely due to the absence of the two tan spot sensitivity genes.

Keywords Wheat leaf spot diseases · Host-selective toxin · Sensitivity gene · Marker-assisted selection

Abbreviations

LOD	Log of odds ratio
QTL	Quantitative trait locus
MAS	Marker-assisted selection
NE	Necrotrophic effector
RIL	Recombinant inbred line
Ptr	*Pyrenophora tritici-repentis*
S	Sensitivity gene

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SNP Single-nucleotide polymorphism
Tsn Tan spot necrosis
Tsc Tan spot chlorosis

Introduction

Tan or yellow spot, caused by *Pyrenophora tritici-repentis* (*Ptr*), can occur on all cultivated wheat crops including bread wheat (*Triticum aestivum* L.) and durum wheat (*T. turgidum* L.). The foliar symptom of the disease is a characteristic tan-colored and elliptical necrotic lesion, often with a yellow halo (Friskop and Liu 2016). The fungal pathogen overwinters on wheat residues, and thus, it is believed that a wide adoption of no or reduced tillage production systems has increased disease incidence and made tan spot one of the most important diseases in most wheat-growing regions (Hosford 1982; Murray and Brennan 2009; Faris et al. 2013). Tan spot has been one of the most common diseases in North Dakota and surrounding areas where the majority of US hard red spring and durum wheat are produced (Friskop and Liu 2016).

Yield losses due to tan spot can reach 50% on highly susceptible cultivars when environmental conditions are favorable (Rees et al. 1982; Lamari and Bernier 1989). The disease can also diminish grain quality by causing pink to red discoloration of the grain, known as red smudge (Schilder and Bergstrom 1994). Disease management strategies for wheat tan spot include crop rotation, residue removal, and timely application of protective and systemic fungicides (Friskop and Liu 2016). Developing and deploying resistant cultivars is the most desirable way to control tan spot of wheat. However, the majority of wheat cultivars in North Dakota and surrounding areas are susceptible (Singh et al. 2006a; Liu et al. 2015; Friskop and Liu 2016). Breeding for tan spot resistance has been difficult due to the diverse and complex nature of pathogen virulence, host resistance, and host-pathogen interactions.

Ptr is known to produce three necrotrophic effectors (NE), namely Ptr ToxA, Ptr ToxB, and Ptr ToxC, which interact with their wheat sensitivity genes to induce necrosis or chlorosis disease symptoms (Ciuffetti et al. 2010). The global *Ptr* isolates have been grouped into eight races according to their ability to produce combinations of the three NEs (Strelkov and Lamari 2003). However, new evidence has strongly suggested the existence of additional races (Ali et al. 2010; Mereno et al. 2015) as well as the presence of additional NEs in the current races (Friesen et al. 2002; Moffat et al. 2014). In addition, Ptr ToxA has been shown to have an epistatic effect on other unidentified NEs (Manning and Ciuffetti 2015; See et al. 2018).

Wheat sensitivity genes for the three *Ptr* NEs have been identified and mapped to wheat chromosome arms, which are *Tsn1* on 5BL for Ptr ToxA (Faris et al. 1996), *Tsc1* on 1AS for Ptr ToxC (Effertz et al. 2002), and *Tsc2* on 2BS for Ptr ToxB (Friesen and Faris 2004; Abeysekara et al. 2009). Among them, *Tsn1* has been isolated from wheat and shown to be a NBS-LRR, resistance-like gene (Faris et al. 2010). Because each NE and host sensitivity gene interaction can lead to susceptibility/disease, and their effects can be additive, resistance is often seen as the lack of sensitivity genes, and removal of these sensitivity genes from wheat cultivars could reduce the levels of susceptibility (Liu et al. 2017). However, the effect of each pair of NE and host sensitivity gene interaction on disease can be highly variable, depending on the host genetic background and the isolate used (Faris et al. 2012; Virdi et al. 2016).

In addition to the three major sensitivity (susceptibility) genes, several studies have identified qualitative and recessive resistance genes against specific races/isolates of *Ptr*, including *tsr2* on 3BL (Singh et al. 2006b), *tsr3* on 3DL (Tadesse et al. 2006a), *tsr4* on 3AL (Tadesse et al. 2006b), and *tsr5* on 3BL (Singh et al. 2008). Furthermore, many additional QTL conferring resistance/susceptibility to tan spot have also been identified using biparental and association mapping studies (Faris et al. 2013 review; Virdi et al. 2016; Kariyawasam et al. 2016; Liu et al. 2015, 2017). It is interesting that some of the identified QTL are race-nonspecific, conferring resistance to multiple or all races (Faris and Friesen 2005; Chu et al. 2008; Kariyawasam et al. 2016).

Hard red winter wheat (HRWW) accounts for 3 to 10% of total wheat production in North Dakota (North Dakota Wheat Commission, www.ndwheat.com, accessed on July 5th 2018). Although growing HRWW in North Dakota is risky because of harsh winter conditions, HRWW has gained an increased interest due to its higher yield and the ability to spread seasonal workloads. 'Jerry', developed by North Dakota State University and the USDA-ARS and released in 2001 (Peel et al. 2004), has been the leading HRWW cultivar in the state. However, Jerry is highly susceptible to tan spot (Liu et al. 2015). The HRWW cultivar

'Wesley' from Nebraska has demonstrated resistance to multiple races of *Ptr* (Liu et al. 2015). To better utilize Wesley in breeding programs, resistance in this cultivar needed to be characterized. The objectives of this study were to map Wesley's resistance to multiple *Ptr* races of tan spot using a recombinant inbred line (RIL) population derived from the cross between Harry and Wesley (Hussain et al. 2017) and to investigate the role of NE-wheat sensitivity gene interactions in the development of tan spot disease in winter wheat.

Materials and methods

Plant materials

The population derived from Harry/Wesley, hereafter referred to as HW population, consisted of 194 recombinant inbred lines (RILs). Both Harry and Wesley are HRWW cultivars developed by Nebraska Agricultural Experiment Station in collaboration with the USDA-ARS. The HW population was originally developed for the mapping of drought tolerance (Hussain et al. 2017). In a previous study, we found that Wesley is insensitive to Ptr ToxA and highly resistant to major *Ptr* races while Harry is sensitive and highly susceptible (Liu et al. 2015). The two parental lines and all the RILs were evaluated for disease resistance using multiple races/isolates and NE infiltrations. In addition, four tan spot differential lines: Salamouni (insensitive to all three NEs), Glenlea (Ptr ToxA sensitive), 6B365 (Ptr ToxC sensitive), and 6B662 (Ptr ToxB sensitive) were also included making a total of 200 entries for each evaluation. Planting and growing the seedling plants followed the same protocols described in Liu et al. (2015). Briefly, seeds were sown in super-cell containers (Stuewe & Sons, Inc., Corvallis, OR) that were filled with Sunshine SB100 soil (Sun Grow Horticulture, Bellenvue, WA) and placed on RL98 trays (Stuewe & Sons, Inc., Corvallis, OR). The cultivar Jerry, highly susceptible to tan spot, was planted along the borders of the each RL98 rack to minimize the potential edge effect. The disease evaluations and NE infiltrations were conducted on the plants at the two to three leaf seedling stage, which required approximately 2 weeks of growth under temperatures ranging from 20 to 25 °C after seeds were sown. Three biological replications were performed with a randomized complete block design (RCBD) for each isolate and NE evaluation.

Fungal inoculations and NE infiltrations

Five *Ptr* isolates were tested individually on the HW population, including Pti2, 86-124, 331-9, DW5, and AR CrossB10, which represented races 1, 2, 3, 5, and new race, respectively. These isolates were classified as different races based on the production of NEs or virulence on the differential lines (Table 1). The isolates 86-124 (race 2), 331-9 (race 3), and DW5 (race 5) each produce a single, known NE (Ptr ToxA, Ptr ToxC and Ptr ToxB, respectively). The isolate Pti2 (race 1) produces both Ptr ToxA and Ptr ToxC. AR CrossB10 was characterized as a new race because it produces no Ptr ToxA but is virulent on Glenlea (Ptr ToxA sensitive) (Ali et al. 2010). However, this isolate produces Ptr ToxC (Kariyawasam et al. 2016). The fungal strain 86-

Table 1 Reaction of the parental lines and the HW population to *Pyrenphora tritici-repentis* races/isolates

Isolate (race)[a]	NE produced[b]	Harry[c]	Wesley[c]	HW population mean	HW population range
Pti2 (race 1)	Ptr ToxA, Ptr ToxC	4.00	1.67	2.98	1.33–4.33
86-124 (race 2)	Ptr ToxA	4.00	1.33	3.00	1.33–4.17
86-124ΔToxA	–	4.00	2.00	2.76	1.17–4.17
331-9 (race 3)	Ptr ToxC	4.50	2.00	3.20	1.83–4.67
DW5 (race 5)	Ptr ToxB	4.00	1.33	2.74	1.17–4.17
AR CrossB10 (New)	Ptr ToxC	4.00	2.00	3.13	1.83–4.33

[a] Six isolates representing different *P. tritici-repentis* races were used to evaluate the HW population and parental lines. Fungal strain 86-124ΔToxA derives from 86 to 124 but lacks the *ToxA* gene (Kariyawasam et al. 2016)

[b] The Ptr races are known to produce different necrotrophic effectors (NEs): Ptr ToxA, Ptr ToxB, and Ptr ToxC. 86-124ΔToxA does not produce Ptr ToxA, but it might produce other unknown effectors

[c] Disease was scored using a 1 to 5 scale with 1 being resistant and 5 being susceptible. Means of three replicates are given

124ΔToxA was genetically modified from 86 to 124 (race 2) through deletion of the *ToxA* gene, thus producing no Ptr ToxA (Kariyawasam et al. 2016). Strain 86-124ΔToxA was used to test whether the effect of the 5B QTL was due to a Ptr ToxA-*Tsn1* interaction.

Fungal culturing and inoculum preparation followed the procedure described in Lamari and Bernier (1989). Briefly, the fungus was grown in dark for 5 days followed by the sporulation treatments. The conidiospores were harvested from the plates by adding sterilized distilled water to the plates and gently scrapping the surface of the fungal cultures. The concentration of the inoculum was defined by spore counting under microscope and adjusted to approximately 3000 spores per mL and Tween-20 was added at a rate of two drops per 100 mL of the spore suspension before spraying inoculum. Plants were inoculated with the spore suspension using a paint sprayer (Husky; Home Depot) that was connected to an air pump with a pressure set at 1.0 bar. Inocula were applied till the leaves of all the plants were uniformly covered with water drops. Inoculated plants were transferred to a mist chamber with a 100% relative humidity and incubated for 24 h at 21 °C. Then, they were moved to and grown in a growth chamber with 12-h photoperiod at 21 °C for 7 days. Disease severity was rated using a lesion type-based scale from 1 to 5 where 1 is highly resistant and 5 is highly susceptible (Lamari and Bernier 1989). An intermediate score was given if two types of reactions were observed. The disease score lower than 2.5 was considered to be resistant.

The HW population was also evaluated for reaction to Ptr ToxA and Ptr ToxB, which were produced from genetically modified *Pichia pastoris* yeast strain X33 expressing the individual NE gene (Liu et al. 2009; Abeysekara et al. 2010). The yeast *P. pastoris* strains were cultured for 48 h at 30 °C and the culture filtrates were harvested by centrifuging the yeast cells. Approximately 20 μl of the culture filtrate was infiltrated into the fully expanded secondary leaf by using a 1-ml syringe without the needle. The infiltrated region was marked with a felt pen, and infiltrated plants were kept in a growth chamber at 21 °C with 12-h photoperiod. Reactions to NE were scored on the 5th day as 1 (sensitive, necrosis, or chlorosis developed on the marked area) or 0 (insensitive, no reaction developed on the marked area). The scored data were transformed into marker data which were used for mapping the sensitivity locus.

Statistical analysis and QTL mapping

Normality of the disease data for each isolate was evaluated using the Shapiro-Wilk test in PROC UNIVARIATE in SAS 9.4 Software (SAS Institute 2016). Disease data from different replicates were tested for homogeneity using Bartlett's Chi-squared test (Snedecor and Cochran 1989) if the data fitted a normal distribution, or by Levene's test (Levene 1960) if the data did not fit a normal distribution. Analyses of variance were conducted using PROC GLM (SAS Institute 2016). The data from homogeneous replications were combined to compute disease means for each RIL, which were then used in QTL analysis.

The genetic linkage map of the HW population contained 3641 SNP markers from genotyping by sequencing (GBS) and covered all 21 wheat chromosomes with a total genetic distance of 1959 cM and a marker density of 1.8 cM per marker (Hussain et al. 2017). For the QTL analysis, the linkage maps were reconstructed to remove most co-segregating markers and some tightly linked markers without affecting the quality of the maps using MapDisto 1.7.7 (Lorieux 2012). The resulting map consisted of 2749 markers that spanned 1911.84 cM with marker density at 1.43 cM. Because the population segregated for reaction to Ptr ToxA, sensitivity to Ptr ToxA was also mapped as a qualitative trait in the previous linkage map using MapDisto (Lorieux 2012). QTL mapping was conducted using QGene 4.4.0 (Joehanes and Nelson 2008). Simple interval mapping (SIM) was used initially to identify the genomic region associated with tan spot reaction and to quantify the disease variations explained by the QTL (R^2). Composite interval mapping (CIM) was then performed to define the genomic locations. A permutation test with 1000 iterations resulted in a LOD threshold of 4.2 for an experiment-wise significance level of 0.05.

Results

Reactions of the parental lines to fungal inoculations and NE infiltrations

Wesley exhibited black to brown colored, small size lesions on the secondary leaves for all the isolates tested, and it had average disease ratings ranging from 1.33 to 2.00 (Fig. 1, Table 1), indicating high levels of resistance. In contrast, Harry developed large necrotic

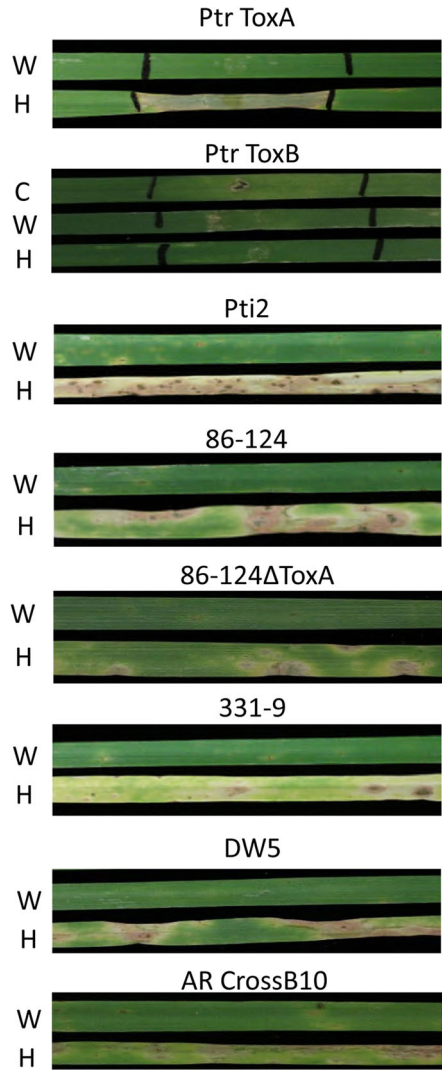


Fig. 1 Reaction of the parental lines to different *Pyrenophora tritici-repentis* race/isolate inoculations and necrotrophic effector infiltrations. The *P. tritici-repentis* races/isolates included Pti2 (race 1), 86-124 (race 2), 86-124ΔToxA, 331-9 (race 3), DW5 (race 5), and AR CrossB10 (new race) and *P. tritici-repentis*. The NEs included Ptr ToxA and Ptr ToxB. W: Wesley, H: Harry. C: control 6B662 (for the Ptr ToxB infiltration only)

lesions or extensive chlorosis on the secondary leaves and its disease rating ranged from 4.00 to 4.50, which was highly susceptible (Fig. 1, Table 1). For Ptr ToxA infiltration, Wesley was insensitive while Harry was sensitive (Fig. 1). Although the differential line 6B662 had a sensitive reaction to Ptr ToxB, neither Wesley nor Harry was sensitive to Ptr ToxB (Fig. 1). Extensive chlorosis developed on the leaves of Harry when inoculated with Pti2, 331-9, and AR CrossB10, but did not develop when inoculated with 86-124, 86-124ΔToxA, and DW5 (Fig. 1). As Pti2, 331-9, and AR CrossB10 all produce Ptr ToxC, and extensive chlorosis is characteristic of the Ptr ToxC-*Tsc1* interaction, Harry must carry *Tsc1* conferring sensitivity to Ptr ToxC.

Reactions of the HW population to Ptr ToxA infiltration and mapping of sensitivity to Ptr ToxA

The HW population segregated for reaction to Ptr ToxA as 92 sensitive to 100 insensitive, which fits a 1:1 ratio ($\chi^2 = 0.33$, $P = 0.56$). Sensitivity to Ptr ToxA was mapped to chromosome 5B as expected. The newly constructed chromosome 5B map was 135.2 cM in length and *Tsn1* was located at 47.1 cM, between markers HWGBS3693 and HWGBS3680.

Reaction of the HW population to fungal inoculations

The HW population segregated for reaction to tan spot caused by all the isolates tested. The mean disease severity for the whole population ranged from 2.74 (isolate DW5) to 3.20 (isolate 331-9) (Table 1, Fig. 2). No obvious transgressive segregation was observed. For all the isolates tested, the majority of RILs had intermediate reactions and only a few RILs had reactions similar to the resistant or susceptible parents (Fig. 2). The fungal strain 86-124ΔToxA produces no known NE, but still caused disease on Harry and susceptible RILs strongly indicating the presence of an unidentified NE(s) or another virulence factor(s) (Fig. 2). Normality tests rejected a normal distribution of the disease reaction to all the isolates except the race1 isolate Pti2 ($P = 0.07$) and race5 isolate DW5 ($P = 0.26$).

QTL identification

Homogeneity analysis with Barlett's Chi-squared test (for Pti2 and DW5) and Levene's test (for the remaining isolates) indicated that the variance among the replicates for each isolate was not significant ($P = 0.06$–0.17). Therefore, the means of the three replicates for each isolate were used in QTL identification. A total of five QTL associated with reaction to tan spot were identified in the HW population. These QTL were located on chromosomes 1A, 5B, 7A, 7B, and 7D, and were designated as *QTs.zhl-1A*, *QTs.zhl-5B*, *QTs.zhl-7A*, *QTs.zhl-7B*, and *QTs.zhl-7D*, respectively (Table 2). The resistance alleles for these QTL are all from Wesley, the resistant parent (Table 2).

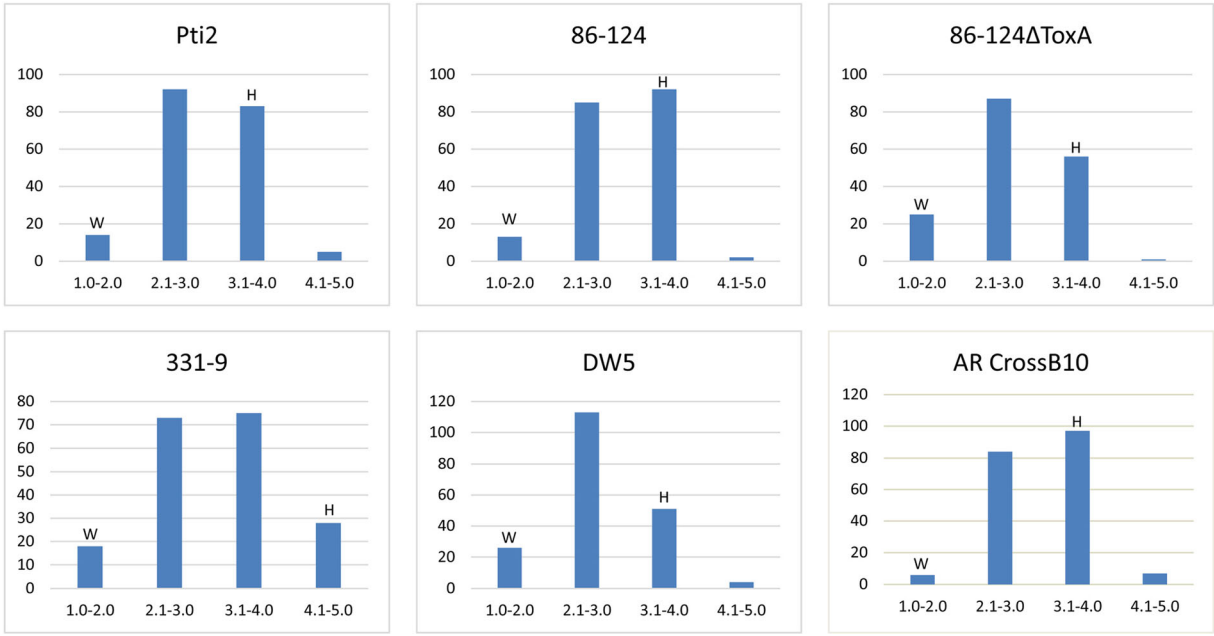


Fig. 2 Histograms showing the disease reaction of the Harry × Wesley population to individual *Pyrenophora tritici-repentis* races/isolates. The races/isolates used for the evaluations included Pti2 (race1), 86-124 (race 2), 86124ΔToxA, 331-9 (race 3), DW5 (race 5), and AR CrossB10 (new race). The disease phenotype was rated using a 1–5 scale with 1 being highly resistant and 5 being highly susceptible. The *x*-axis is the disease scale, and the *y*-axis is the number of recombinant inbred lines

QTs.zhl-1A.1 was located on the distal end of 1AS between markers *HWGBS60* and *HWGBS5150* and was significant for Pti2, 331-9, and AR CrossB10, all of which produce Ptr ToxC (Fig. 3, Table 2). The other markers *HWGBS58* and *HWGBS59* co-segregated with HWGBS60. This QTL is likely due to the Ptr ToxC-*Tsc1* interaction, had LOD values ranging from 9.9 to 46.9, and accounted for 10 to 64% of the variation in disease. *QTs.zhl-5B.1* was identified for Pti2 and 86-124 which produce Ptr ToxA and mapped at the *Tsn1* locus which confers sensitivity to Ptr ToxA (Fig. 3, Table 1). This QTL was not associated with reactions to strain 86-124ΔToxA, which does not produce Ptr ToxA (Fig. 3). These results indicate that *QTs.zhl-5B.1* is due to the Ptr ToxA-*Tsn1* interaction. The QTL had similar LOD and R^2 values for Pti2 and 86-124. *QTs.zhl-7B.1* is a minor QTL located on the short arm of chromosome 7B, and it was the only QTL associated with reaction to DW5 (Fig. 3). The QTL explained 8% of the disease variation with a LOD value of 6.6 (Table 2). This QTL was flanked by *HWGBS5696* and *HWGBS5992* and two co-segregating markers: *HWGBS5678* and *HWGBS5672* mapped very closely to *HWGBS5696* (Table 2 and Fig. 3). The QTL on 7A and 7D: *QTs.zhl-7A.1* and *QTs.zhl-7D.1* were identified for 86-124ΔToxA, the isolate producing no known NE. *QTs.zhl-7A.1* was flanked by *HWGBS5420* and *HWGBS5422* explaining 12% of the disease variation and *QTs.zhl-7D.1* was located between *HWGBS6047* and *HWGBS6066* explaining 13% of the disease variation (Fig. 3, Table 2). Three other markers *HWGBS6029*, *HWGBS6031*, and *HWGBS6046* co-segregated with *HWGBS6047* on 7D. Interestingly, the two QTL were not identified using its wild-type isolate 86-124 (Fig. 3).

The additive effect of the identified QTL

Because *QTs.zhl-1A* and *QTs.zhl-5B* are the two major QTL identified and they are due to the NE-wheat sensitivity gene interactions, we also investigated the genetic relationships between these two interactions by categorizing the RILs based on the genotype at the two loci and comparing the disease means in the reaction to Pti2 which produces both Ptr ToxA and Ptr ToxC. There are four genotypic groups based on the combination of the parental alleles at two QTL including the Harry allele at both loci (H,H), the Wesley allele at both loci (W,W), and the Harry allele at one locus and Wesley at the other locus (W,H and H,W) (Table 3). Significant differences were obtained for the disease means among all four groups

Table 2 QTL associated with reaction to tan spot caused by different *Pyrenophora tritici-repentis* races/isolates in the HW population

QTL	Interval (cM)	Flanking markers	LOD (R^2)[a]						Source[b]
			Pti2	86-124	86124ΔToxA	331-9	DW5	AR crossB10	
QTs.zhl-1A	0.0–2.0	*HWGBS60-HWGBS150*	9.9 (0.10)	–	–	46.9 (0.64)	–	18.6 (0.31)	W
QTs.zhl-5B	20.0–52.0	*HWGBS3693-HWGBS3672*	16.9 (0.28)	14.9 (0.22)	–	–	–	–	W
QTs.zhl-7A	124.0–130.0	*HWGBS5420-HWGBS5422*	–	–	8.8 (0.12)	–	–	–	W
QTs.zhl-7B	18.0–26.0	*HWGBS5696-HWGBS5992*	–	–	–	–	6.6 (0.08)	–	W
QTs.zhl-7D	176.0–180.0	*HWGBS6047-HWGBS6066*	–	–	8.2(0.13)	–	–	–	W

[a] A permutation test with 1000 iterations yielded a LOD value of 4.2 and it was used as the cut-off to identify significant QTL. R^2 values are given in parenthesis for each QTL, indicating the amount of phenotypic variation explained by the QTL

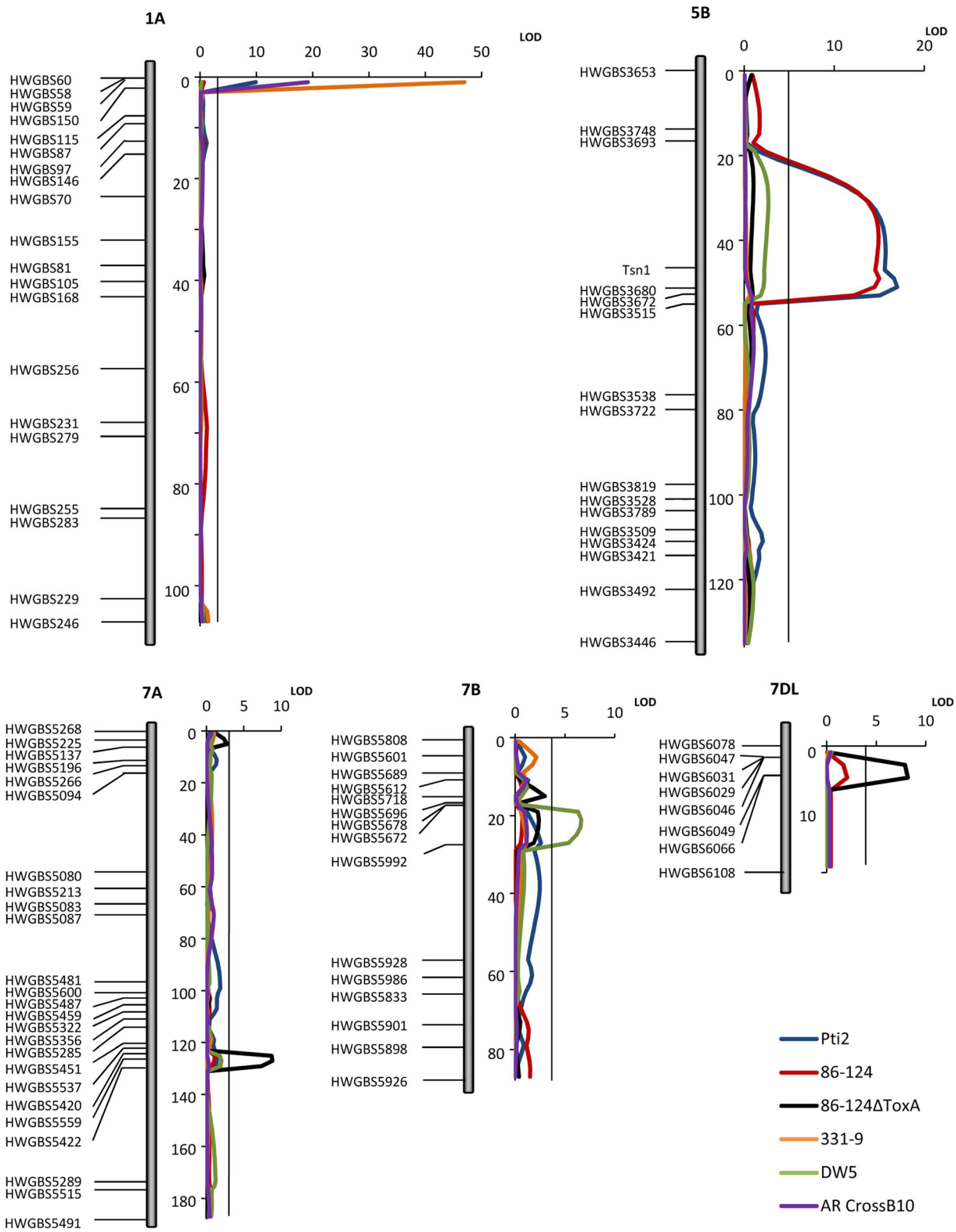
[b] The parental line that contributed the resistant allele where 'H' = Harry and 'W' = Wesley

with the genotypic group with Harry the allele at both loci having the highest disease mean (3.51) and that with the Wesley allele at both loci having the lowest disease mean (2.54) (Table 3). It is very interesting to notice that Wesley has a significant low disease mean than the group carrying Wesley's allele at both loci. This might be due to the fact that Wesley does not have the susceptibility QTL on 7A, 7B, and 7D.

Discussion

Genetic resistance to tan spot has been shown to involve multiple factors, including the lack of NE sensitivity genes, the presence of race-nonspecific resistance QTL, the presence of qualitative recessive resistance genes, and other less well-characterized QTL (Faris et al. 2013 for review; Liu et al. 2017). Wesley, which was highly resistant to multiple races of tan spot pathogen, could be a good source of tan spot resistance in breeding programs for winter wheat. Using QTL analysis in a segregating winter wheat population derived from Harry and Wesley, we characterized genetic resistance in Wesley. Reaction to tan spot in this population was primarily due to the two NE-wheat sensitivity gene interactions: Ptr ToxA-*Tsn1* and Ptr ToxC-*Tsc1*, which indicate that resistance in Wesley is largely due to the lack of NE sensitivity genes *Tsn1* and *Tsc1*, rather than the presence of any active resistance genes. Therefore, breeders should place strong emphasis on selection for the absence of the two NE sensitivity genes in segregating populations. *Tsn1* has been cloned and a perfect marker, *Xfcp623*, has been developed from the gene itself, which can be used in marker-assisted selection (Faris et al. 2010). However, *Tsc1* has not yet been cloned and the closest marker developed so far is 4.7 cM away from it (Faris et al. 2013). Three co-segregating GBS markers: *HWGBS58*, *HWGBS59*, and *HWGBS60* mapped in the HW population were found to underlie the peak of the 1AS QTL (Fig. 3), which might be very close to *Tsc1*. These GBS markers can be converted into PCR-based KASP or STARP (Semi-Thermal Asymmetric Reverse PCR, Long et al. 2017) markers for marker-aided selection against *Tsc1*.

The significance of the two major QTL, *QTs.zhl-1A* and *QTs.zhl-5B*, in the respective Ptr ToxC-*Tsc1* and Ptr ToxA-*Tsn1* interactions of this study, confirms their important role in tan spot development in winter wheat genetic backgrounds. Many studies have been


1A
LOD
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HWGBS60
HWGBS58
HWGBS59
HWGBS150
HWGBS115
HWGBS87
HWGBS97
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HWGBS70
HWGBS155
HWGBS81
HWGBS105
HWGBS168
HWGBS256
HWGBS231
HWGBS279
HWGBS255
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HWGBS3680
HWGBS3672
HWGBS3515
HWGBS3538
HWGBS3722
HWGBS3819
HWGBS3528
HWGBS3789
HWGBS3509
HWGBS3424
HWGBS3421
HWGBS3492
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HWGBS5422
HWGBS5289
HWGBS5515
HWGBS5491
7B
LOD
0 5 10
HWGBS5808
HWGBS5601
HWGBS5689
HWGBS5612
HWGBS5718
HWGBS5696
HWGBS5678
HWGBS5672
HWGBS5992
HWGBS5928
HWGBS5986
HWGBS5833
HWGBS5901
HWGBS5898
HWGBS5926
7DL
LOD
0 5 10
HWGBS6078
HWGBS6047
HWGBS6031
HWGBS6029
HWGBS6046
HWGBS6049
HWGBS6066
HWGBS6108
Pti2
86-124
86-124ΔToxA
331-9
DW5
AR CrossB10

◀ **Fig. 3** Composite interval regression maps of chromosomes 1A, 5B, 7A, 7B, and 7D containing QTLs significantly associated with reaction to tan spot in the HW population. QTL mapping was conducted on the HW population for different *Pyrenophora tritici-repentis* races/isolates, which are represented by different colors, including Pti2 (race 1), 86-124 (race 2), 86124ΔToxA, 331-9 (race 3), DW5 (race 5), and AR crossB10 (new race). The positions of marker loci are shown to the left of the linkage groups and genetic scales in centiMorgan (cM) are shown to the right of each chromosome. A solid line represents the logarithm of the odds (LOD) significance threshold of 4.2. The LOD and R_2 values for each QTL are presented in Table 2

conducted to investigate the role of the Ptr ToxA-*Tsn1* interaction in spring wheat germplasm and populations (Faris et al. 2013; Dinglasan et al. 2018). Although the Ptr ToxA-*Tsn1* interaction usually plays a significant role in hexaploid wheat backgrounds, it has never been shown to be important in tetraploid wheat backgrounds (Faris et al. 2013 for review; Virdi et al. 2016). Very interestingly, SnToxA-*Tsn1* interactions in the wheat-*Parastagonospora nodorum* system have always been shown to be important regardless of wheat polyploid levels and host genotypes (Friesen et al. 2006; Virdi et al. 2016). Sensitivity to Ptr ToxA has been found to significantly correlate with susceptibility to Ptr ToxA-producing races in winter wheat germplasm indicating the importance of the Ptr ToxA-*Tsn1* interaction in disease in winter wheat backgrounds (Noriel et al. 2011; Kollers et al. 2014; Liu et al. 2015). In this study, we used QTL mapping in a biparental population to further confirm that Ptr ToxA-*Tsn1* interaction is important for tan spot development in winter wheat genetic backgrounds.

Table 3 Disease means of four categories of RILs based on alleles at *QTs.zhl-1A.1* and *QTs.zl-5B.1* for the reaction caused by race 1 isolate Pti2

Allele at *QTs.zhl-1A*, *QTs.zhl-5B*[a]	No. of RILs	Pti2 (Race 1) [b]
H,H	49	3.51a
W,H	43	3.07b
H,W	49	2.83c
W,W	53	2.54d
Wesley	–	1.67e
Harry	–	4.00a

[a] The source of the allele at each QTL where H and W are the alleles from Harry and Wesley, respectively. The parental lines were included as controls

[b] Means with different letters were significantly different

Because Ptr ToxC cannot be easily obtained and purified, the role of the Ptr ToxC-*Tsc1* interaction in disease has not been extensively investigated except for a few QTL mapping studies, which suggested its important role (Faris et al. 1997; Effertz et al. 2001, 2002; Sun et al. 2010; Kariyawasam et al. 2016; Liu et al. 2017). Here, we demonstrated that the Ptr ToxC-*Tsc1* interaction is also important for disease in winter wheat backgrounds. However, the effect of the interaction on disease, which was measured by R^2, was variable depending on the race/isolate used, i.e., 10% for Pti2, 31% for AR CrossB10, and 64% for 331-9 (Table 2, Fig. 3). A similar result was obtained in a study performed by Kariyawasam et al. (2016) using a spring wheat population. Liu et al. (2017) demonstrated that the Ptr ToxA-*Tsn1* interaction and the Ptr ToxC-*Tsc1* interaction made additive contributions to the level of disease in a spring wheat population when both interactions were present. Here, we showed that the two interactions can also have an additive effect on disease development in winter wheat backgrounds (Table 3). This observation has been commonly found in the wheat-*P. nodorum* system where multiple NE-sensitivity gene interactions have been identified (Oliver et al. 2012 for review). Therefore, for these necrotrophic pathogens, the part of the disease system that is based on inverse gene-for-gene interactions involving multiple NE-host sensitivity gene combinations, these interactions often have an additive effect and produce quantitative differences in disease development and resistance responses (Friesen and Faris 2010). Thus, in breeding programs, the sensitivity loci should be removed systematically in order to obtain higher levels of tan spot resistance.

The wheat-*Ptr* system has also been shown to involve QTL conferring resistance to multiple or all *Ptr* races, which was referred to as race-nonspecific resistance QTL (Faris and Friesen 2005). Race-nonspecific resistance QTL has been identified in hexaploid spring wheat lines which showed resistance to multiple races (Faris and Friesen 2005; Chu et al. 2010; Faris et al. 2012; Kariyawasam et al. 2016). Some race-nonspecific resistance QTL can have complete epistasis on the effect of the Ptr ToxA-*Tsn1* interaction, but partial epistasis on the Ptr ToxC-*Tsc1* interaction (Kariyawasam et al. 2016). This type of resistance should be very useful in breeding programs to develop wheat cultivars with resistance to multiple races. Wesley is highly resistant to multiple races, but we did not identify any QTL conferring

resistance to all the races tested in the population (Table 3), indicating that Wesley does not carry race-nonspecific resistance. The high levels of resistance to multiple races in Wesley are most likely due to its insensitivity to the three known NEs: Ptr ToxA, Ptr ToxB, and Ptr ToxC, as well as other possibly unidentified NEs. It remains unknown whether or not race-nonspecific resistance is present in winter wheat germplasm.

The race 5 isolate DW5 produces Ptr ToxB, which interacts with the sensitivity gene *Tsc2* on 2BS to induce chlorosis (Strelkov et al. 1999; Martinez et al. 2004; Friesen and Faris 2004; Abeysekara et al. 2010). For this isolate, we only identified a minor QTL (*Qts.zhl-7B*) on 7B (Table 3). The fact that no QTL were identified at the *Tsc2* locus (2BS) is due to the lack of Ptr ToxB sensitivity in both Wesley and Harry (Fig. 1). There are two possible reasons that can explain why no major QTL was identified for DW5. First, it is possible that DW5 produced multiple unidentified NEs, but effects of which are too small to detect in this population. Second, the genetic linkage map developed in the HW population has a poor coverage in most D genome chromosomes (Hussain et al. 2017) and it is possible that some major or minor QTL could be missed or not identified. Liu et al. (2015) conducted an association mapping in a collection of winter wheat germplasm which included Wesley and Harry, revealing a QTL on 7B for DW5. This QTL might be the same as *Qts.zhl-7B.1* identified in the HW population. Tan spot resistance/susceptibility QTL on 7B have been reported before, but the previous studies used different races (Faris et al. 2012; Kollers et al. 2014).

For AR CrossB10, *QTs.zhl-1A*, which is involved in the Ptr ToxC-*Tsn1* interaction, is the only QTL identified in the HW population. AR CrossB10 was defined as a new race because it does not produce Ptr ToxA, but caused necrosis symptoms on the Ptr ToxA differential line Glenlea (Ali et al. 2010). This suggests that AR CrossB10 produces a different NE(s) which interacts with an unidentified wheat sensitivity gene(s). Previous studies using biparental mapping or association mapping have revealed QTL on a number of other wheat chromosomes (Patel et al. 2013; Liu et al. 2015, 2017; Kariyawasam et al. 2016). However, none of those QTL was identified in the HW population, which might be due to no segregation for these loci or the low coverage in some areas of the genetic linkage maps in the HW population.

The two fungal strains 86-124 and 86-124ΔToxA are nearly identical except that 86-124ΔToxA is deficient in the production of Ptr ToxA compared to the wild-type 86-124 (Kariyawasam et al. 2016). The *Tsn1* locus was associated with a major QTL for 86-124, but not for 86-124ΔToxA which strongly indicates that this QTL involves the Ptr ToxA-*Tsn1* interaction. On the contrary, two QTL, *QTs.zhl-7A* and *QTs.zhl-7D*, were identified for 86-124ΔToxA, but not for 86-124 in this population (Table 2). This suggests that the effect of these QTL is masked by that of the Ptr ToxA-*Tsn1* interaction. Epitasis of the Ptr ToxA-*Tsn1* interaction over other interactions has been reported in the wheat-*Ptr* system (Manning and Ciuffetti 2015; See et al. 2018). As mentioned above, the effect of Ptr ToxA-*Tsn1* interaction can be completely masked by the action of race-nonspecific resistance (Kariyawasam et al. 2016). These epistasis mechanisms remain unknown, which hinders breeding of tan spot-resistant cultivars.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical standards All experiments complied with the ethical standards of the university.

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