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ORIGINAL ARTICLE

Genetic relationships between race-nonspecific and race-specific interactions in the wheat–*Pyrenophora tritici-repentis* pathosystem

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Abstract

Key message **We identified a major QTL conferring race-nonspecific resistance and revealed its relationships with race-specific interactions in the wheat–*Pyrenophora tritici-repentis* pathosystem.**

Abstract Tan spot, caused by the fungus *Pyrenophora tritici-repentis* (*Ptr*), is a destructive disease of wheat worldwide. The disease system is known to include inverse gene-for-gene, race-specific interactions involving the recognition of fungal-produced necrotrophic effectors (NEs) by corresponding host sensitivity genes. However, quantitative trait loci (QTLs) conferring race-nonspecific resistance have also been identified. In this work, we identified a major race-nonspecific resistance QTL and characterized its genetic relationships with the NE-host gene interactions Ptr ToxA-*Tsn1* and Ptr ToxC-*Tsc1* in a recombinant inbred wheat population derived from the cross between 'Louise' and 'Penawawa.' Both parental lines were sensitive to Ptr ToxA, but Penawawa and Louise were highly resistant and susceptible, respectively, to conidial inoculations of all races. Resistance was predominantly governed by a major race-nonspecific QTL on chromosome arm 3BL for resistance to all races. Another significant QTL was detected at the distal end of chromosome arm 1AS for resistance to the Ptr ToxC-producing isolates, which corresponded to the known location of the *Tsc1* locus. The effects of the 3B and 1A QTLs were largely additive, and the 3B resistance QTL was epistatic to the Ptr ToxA-*Tsn1* interaction. Resistance to race 2 in F_1 plants was completely dominant; however, race 3-inoculated F_1 plants were only moderately resistant because they developed chlorosis presumably due to the Ptr ToxC-*Tsc1* interaction. This work provides further understanding of genetic resistance in the wheat-tan spot system as well as important guidance for tan spot resistance breeding.

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Abbreviations

CIM	Composite interval mapping
ETI	Effector-triggered immunity
NETS	Necrotrophic effector-triggered susceptibility
HST	Host-selective toxin
ITMI	International triticeae mapping initiative
LOD	Log of odds ratio
QTL	Quantitative trait locus
MAS	Marker assisted selection
NE	Necrotrophic effector
RIL	Recombinant inbred line
Ptr	*Pyrenophora tritici-repentis*
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeat
SWSW	Soft white spring wheat

Introduction

Tan spot, also known as yellow leaf spot, is caused by the fungus *Pyrenophora tritici-repentis* (*Ptr*) and can occur on

both common wheat (*Triticum aestivum* L.) and durum (*T. turgidum* L. var. *durum*). In the last century, the disease has evolved from a minor problem to a major threat to wheat production around the world (Hosford 1982; Murray and Brennan 2009; Faris et al. 2013). The wide adoption of minimum tillage practices is thought to be the main reason for the increase of tan spot because the fungus overwinters on wheat residue left from the previous year's crop providing a direct source of inoculum. If infestation is severe, tan spot can cause yield losses approaching 50 % and negatively affect grain quality (Rees et al. 1982; Schilder and Bergstrom 1994). Although crop rotation and fungicide applications can be used to reduce losses due to tan spot, the development and deployment of resistant varieties is the most economical, environmentally friendly, and sustainable way to manage the disease. To breed tan spot resistant cultivars, a good understanding of genetic resistance/susceptibility and associated mechanisms in the wheat–*Ptr* system is needed.

Ptr is a necrotrophic pathogen, meaning that it requires dead or dying tissue to acquire nutrients and proliferate. Necrotrophic specialists such as *Ptr* are known to produce necrotrophic effectors (NE), previously known as host-selective toxins (HSTs). The NEs are recognized by corresponding sensitivity/susceptibility genes in the host in an inverse gene-for-gene manner (Wolpert et al. 2002; Friesen et al. 2008; Ciuffetti et al. 2010). In this model, recognition of an NE by the corresponding host sensitivity gene leads to a compatible interaction and ultimately necrotrophic effector-triggered susceptibility (NETS) (Liu et al. 2009). If the pathogen does not produce the NE, or if the host does not possess the corresponding sensitivity gene, an incompatible interaction occurs resulting in resistance. This scenario is in contrast to the classic gene-for-gene model (Flor 1956) where resistance occurs upon the recognition of an avirulence gene product by the corresponding plant resistance gene product. The interaction leads to resistance and is known as effector-triggered immunity (ETI). Therefore, resistance in disease systems involving necrotrophic fungi is usually recessive and largely due to the absence of NE recognition by the host. However, multiple NE-host gene interactions are usually present in a given system and their effects are mostly additive (Friesen and Faris 2010). Therefore, resistance/susceptibility in these systems is often best characterized as a quantitative trait.

Three NE-host gene interactions have been identified in the wheat–*Ptr* pathosystem, including Ptr ToxA-*Tsn1*, Ptr ToxB-*Tsc2* and Ptr ToxC-*Tsc1* (Ciuffetti et al. 2010; Faris et al. 2013 for review). Among them, the Ptr ToxA-*Tsn1* interaction leads to necrosis, while the other two induce chlorosis. *Ptr* isolates have been classified into eight races based on the NEs they produce and/or their virulence toward differentials that carry individual host sensitivity genes (Lamari and Strelkov 2010; Faris et al. 2013 for review). Because the NE-host gene interaction determines race specificity in tan spot, they are considered as race-specific interactions. The host genes *Tsn1*, *Tsc1* and *Tsc2* have been mapped to wheat chromosome arms 5BL (Faris et al. 1996), 1AS (Effertz et al. 2001) and 2BS (Friesen and Faris 2004; Abeysekara et al. 2009), respectively. Among them, only the *Tsn1* gene has been cloned, and it encodes a plant resistance gene-like protein containing protein kinase, nucleotide binding, and leucine-rich repeat domains (Faris et al. 2010).

In addition to these susceptibility genes, four other qualitative genes conditioning tan spot resistance (*tsr*) were also identified, including *tsr2* (Singh et al. 2006), *tsr3* (Tadesse et al. 2006a), *tsr4* (Tadesse et al. 2006b) and *tsr5* (Singh et al. 2008). Both *tsr2* and *tsr5* were identified in tetraploid wheat and mapped to chromosome arm 3BL in a close proximity, whereas *tsr3* and *tsr4* were mapped to chromosome arms 3DL and 3AL, respectively, in hexaploid wheat. Because these resistance genes were shown to be recessive, it is possible that they also represent host susceptibility loci that interact with unidentified fungal NEs (Faris et al. 2013 for review). Manning and Ciuffetti (2015) recently demonstrated the presence of novel NE-host susceptibility gene interactions in this pathosystem, the effect of which could be masked by Ptr ToxA-*Tsn1* if they co-exist in a certain genotype.

QTL mapping using bi-parental or natural populations has also been conducted to identify genomic regions involved in tan spot resistance. All 21 wheat chromosomes except 4B and 6D have been reported to harbor QTLs conferring resistance to tan spot (Faris et al. 2013 for review; Patel et al. 2013; Kollers et al. 2014; Liu et al. 2015). Some QTLs have coincided with the locations of the three NE sensitivity loci strongly indicating these NE-host sensitivity gene interactions are important in the development of tan spot (Cheong et al. 2004; Singh et al. 2008; Faris et al. 1997, 2012; Friesen and Faris 2004; Sun et al. 2010). However, there are also many QTLs that were identified in genomic regions other than the three sensitivity loci (Faris et al. 2013 for review). One study revealed no significant role for the Ptr ToxA-*Tsn1* interaction in the development of tan spot caused by the Ptr ToxA-producing races 1 and 2 (Faris and Friesen 2005). Instead, they identified genomic regions on chromosomes 1B and 3B conferring resistance to multiple races, and these have been referred to as race-nonspecific resistance QTLs (Faris and Friesen 2005; Faris et al. 2013 for review).

Many tan spot resistant genotypes identified so far are insensitive to Ptr ToxA, and in most cases, they have been crossed with Ptr ToxA-sensitive, disease susceptible lines to develop bi-parental populations for characterizing tan spot resistance (Faris et al. 2013 for review). In our effort

to screen the US spring wheat elite lines for reaction to tan spot (Liu et al. unpublished data), we found that the soft white spring wheat (SWSW) cultivar 'Penawawa' is sensitive to Ptr ToxA, but highly resistant to all races of tan spot. To our knowledge, no study has been done to characterize the genetics of a cultivar that is sensitive to Ptr ToxA, but highly resistant to all races including races 1 and 2, which produce Ptr ToxA. This cultivar had been crossed with another SWSW cultivar 'Louise' to develop a recombinant inbred line (RIL) population and used to map high temperature adult plant stripe rust resistance QTLs and seed-expressed polyphenol oxidase genes (Carter et al. 2009; Beecher et al. 2012). Here, we used this population to identify Penawawa-derived tan spot resistance QTLs and to characterize the relationships between race-nonspecific resistance and race-specific interactions including Ptr ToxA-*Tsn1* and Ptr ToxC-*Tsc1*.

Materials and methods

Plant materials

The Louise/Penawawa population used in this study, hereafter referred to as the LP population, consisted of 188 RILs and has been described in Carter et al. (2009). Both Louise and Penawawa are SWSW cultivars that were highly adapted to the Pacific-Northwest region of the USA. The population was initially developed to map high temperature adult plant stripe rust resistance in Louise (Carter et al. 2009). Our preliminary data showed that Penawawa was highly resistant to tan spot while Louise was highly susceptible. The two parental lines and all RILs were used for fungal NE sensitivity and disease evaluations. Four wheat lines known as the tan spot differential lines including Salamouni, Glenlea, 6B365 and 6B662 were also included in disease evaluations. To determine the nature of resistance in Penawawa, F_1 plants of Louise/Penawawa were tested for reaction to tan spot along with the four differential lines.

Seeds of each RIL, the parental lines, F_1 and differential lines were planted in super-cell containers (Stuewe & Sons, Inc., Corvallis, OR) that were filled with Sunshine SB100 soil (Sun Grow Horticulture, Bellevue, WA). Three seeds per container and one container per line were used for planting. RL98 trays (Stuewe & Sons, Inc., Corvallis, OR) were used to hold the planted containers. Following planting, all planted containers were given equal amounts of Osmocote Plus 15-19-12 fertilizer (Scotts Sierra Horticultural Product Company, Maysville, OH). The highly susceptible (Liu et al. 2015) North Dakota hard red winter wheat cultivar Jerry was planted in the containers along the borders of each RL 98 tray to reduce the edge effect. The plants were grown in a greenhouse room with the temperature ranging from 20 to 25 °C. When the plants reached the two- to three-leaf stage (around 14 days after planting under our greenhouse conditions), the plants were used for NE infiltrations or fungal inoculations. At least three biological replications were conducted for NE and disease evaluations following a randomized complete block design (RCBD).

Necrotrophic effector infiltration

The parental lines and RILs were evaluated for reaction to NEs Ptr ToxA and Ptr ToxB. Both NE genes have been cloned and transformed into *Pichia pastoris* yeast strain X33 (Liu et al. 2009; Abeysekara et al. 2010). The corresponding genetically modified X33 strains were used to produce each NE. The strains were cultured in yeast potato dextrose broth for 24–48 h at 30 °C with vigorous shaking and the resulting cultures were centrifuged to collect the culture filtrates for infiltration. Approximately 20 µl of NE culture filtrates was infiltrated into the fully expanded secondary leaf of a wheat seedling using a 1 ml syringe with the needle removed. The infiltrated areas were marked using a felt pen and plants were placed in a growth chamber at 21 °C with a 12-h photoperiod. The reactions were scored 5 days after infiltration as sensitive (necrosis or chlorosis developed in the marked area) or insensitive (no reaction in the marked area).

Fungal inoculations and disease evaluation

Five natural *Ptr* isolates were used to evaluate the LP population, including Pti2, 86-124, 331-9, DW5 and AR CrossB10. Of the three known *Ptr* NEs, Pti2 produces Ptr ToxA and Ptr ToxC, 86-124 produces only Ptr ToxA, 331-9 produces only Ptr ToxC, and DW5 produces only Ptr ToxB. Therefore, these isolates have been classified as races 1, 2, 3 and 5, respectively. Isolate AR CrossB10 does not conform to the race classification system because it causes necrosis on the differential line Glenlea even though it does not produce Ptr ToxA (Ali et al. 2010). Based on our observations, AR CrossB10 causes extensive chlorosis on 6B365, thus indicating that it likely produces Ptr ToxC (ZH Liu, unpublished). All these isolates were collected from North America (Friesen et al. 2003; Ali et al. 2010).

To examine the role of the Ptr ToxA-*Tsn1* interaction in disease, a *ToxA* knockout strain of 86-124 (86-124Δ*ToxA*) was also include in the experiment to evaluate the LP population. This knockout strain was obtained from the genetic modification of 86-124 by replacing the whole *ToxA* coding region with the hygromycin resistance gene (JB Rasmussen et al. unpublished data). We have confirmed that the strain

does not contain the *ToxA* gene and does not produce Ptr ToxA in culture (data not shown).

Inoculum was prepared as described in Lamari and Bernier (1989). Briefly, fungal isolates were grown in the dark for 5 days on V8-potato dextrose agar at room temperature. After being flooded with sterilized distilled water and flattened using the bottom of a flame sterilized test tube, the cultures were moved to a light bank and kept under continuous light for 24 h at room temperature. Then, the cultures were incubated in the dark for 24 h at 16 °C to induce sporulation. Conidia were harvested by flooding the plate with sterilized distilled water followed by gentle scraping of the surface of the culture with an inoculation loop. The harvested spore solution was adjusted to a concentration of approximately 3000 spores/ml and two drops of Tween-20 per 100 ml were added before inoculations.

Plants were inoculated and then kept in a mist chamber with 100 % humidity as described in Liu et al. (2015). Plants were then placed in a growth chamber with 12-h photoperiod at 21 °C for plant growth and disease development. Disease reactions were evaluated 7 days after inoculation using a lesion type-based 1–5 rating scale with 1 being highly resistant and 5 being highly susceptible (Lamari and Bernier 1989). If a line had equal amounts of two reaction types, an intermediate score was given.

Statistical analysis and QTL mapping

These statistical analyses were conducted using SAS program with corresponding command codes (SAS Institute 2011). The disease data for each isolate were first tested for normal distribution using Shapiro–Wilk in the PROC UNIVARIATE procedure (SAS Institute 2011). Homogeneity of variance among different experiments was then performed using Bartlett's χ^2 test if the data fit a normal distribution (Snedecor and Cochran 1989), or Levene's test if it did not (Levene 1960). Data from homogeneous experiments were combined and used to calculate the disease means, which were used for QTL detection and subsequent analysis. Disease means of the LP population caused by different isolates were compared using Fisher's protected least significant difference (LSD) at $P < 0.05$.

The linkage map of the LP population was initially developed using 295 SSRs and one SNP marker (Carter et al. 2009). Later, the map was reconstructed by the addition of 1434 SNP markers, covering all 21 chromosomes with an average marker density of 2.2 cM per marker (Beecher et al. 2012). For QTL mapping, we removed redundant and closely linked markers, and reconstructed the linkage maps using MapDisto (Lorieux 2012). The resulting maps consisted of 21 linkage groups corresponding to the 21 wheat chromosomes and contained a total of 596 markers spanning 3163.7 cM in genetic distance. This new map was employed to identify markers associated with resistance to tan spot using QGene 4.0 (Joehanes and Nelson 2008).

A permutation test consisting of 1000 permutations yielded an LOD threshold of 3.2 for an experiment-wise significance level of 0.05. Composite interval mapping (Zeng 1994) was also performed as described in Faris et al. (2014) to identify genomic regions significantly associated with tan spot resistance.

To dissect the genetic relationships between race-nonspecific resistance QTL with NETS caused by the Ptr ToxA-*Tsn1* and Ptr ToxC-*Tsc1* interactions, we compared the disease means of different groups of RILs in the LP population that were classified based on the presence or absence of the 3B QTL and individual host insensitivity genes. All comparisons were done using Fisher's protected least significant difference (LSD) at $P < 0.05$ (SAS Institute 2011).

Results

Reaction of parental lines and the LP population to NEs and fungal isolates

Both Louise and Penawawa were sensitive to Ptr ToxA and insensitive to Ptr ToxB (Fig. 1). However, the two lines differed in their reactions to conidial inoculations. Penawawa developed small pinpoint dark spots on the leaves demonstrating high levels of resistance to all races and it had average disease reactions that ranged from 1.00 for DW5 (race 5) to 1.58 for Pti2 (race 1) (Fig. 1; Table 1). In contrast, Louise developed large necrotic and/or chlorotic lesions in reaction to all isolates indicating that it was highly susceptible to all of them (Fig. 1). The average disease score for Louise ranged from 3.08 for 86-124 (race 2) to 4.17 for AR CrossB10 (Table 1). AR CrossB10 caused chlorosis on Louise similar to that caused by the Ptr ToxC-producing isolates Pti2 (race 1) and 331-9 (race 3) suggesting that AR CrossB10 likely produce Ptr ToxC as well.

The whole population was also tested for reaction to Ptr ToxA and as expected, all 188 RILs were sensitive. However, the LP population segregated for disease reactions from highly resistant to highly susceptible for all races (Table 1). The average disease scores of the population were 2.83, 2.32, 2.42, 2.56 and 2.89 for races 1, 2, 3, 5, and AR CrossB10, respectively. A normality test rejected the hypothesis that the disease reaction of the LP population to all isolates fit a normal distribution. Disease histograms also suggested non-normal distribution for disease reactions to all isolates (Fig. 2). In addition, the shapes of the histograms for all isolates differed with the Pti2 histogram

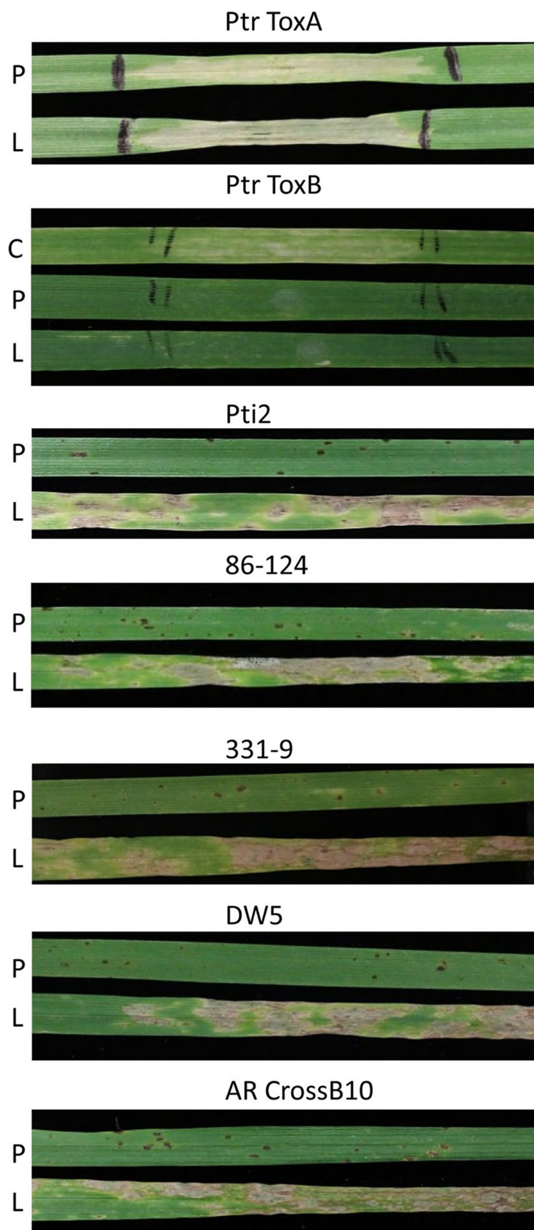

Fig. 1 Reaction of Louise and Penawawa to necrotrophic effector infiltrations and individual isolate inoculations. The *Pyrenophora tritici-repentis* NEs Ptr ToxA and Ptr ToxB, and five fungal isolates representing different races including Pti2 (race 1), 86-124 (race 2), 331-9 (race 3), DW5 (race 5) and AR crossB10 (unclassified isolate) were used. *P* Penawawa, *L* Louise, and *C* 6B662, used as a positive control for Ptr ToxB infiltration

showing relatively more susceptible RILs and the DW5 histogram showing more resistant RILs (Fig. 2).

QTL identification

Because disease reactions of the LP population significantly deviated from a normal distribution, Levene's test was used to determine the homogeneity of variances of disease ratings among the three experiments for each isolate. The results indicated the data from different experiments were homogeneous ($P = 0.07$–0.58, $df = 2$) for all isolates. Therefore, the average disease scores of each RIL from the three experiments were computed and used in subsequent QTL analyses.

In total, four QTLs associated with tan spot resistance in the LP population were identified, and the resistance alleles at all four QTLs were contributed by the resistant parent Penawawa. These QTLs were distributed on chromosome arms 1AS, 2DL, 3BL, and 5AL and designated *QTs.zhl-1A*, *QTs.zhl-2D*, *QTs.zhl-3B*, and *QTs.zhl-5A*, respectively (Table 2). *QTs.zhl-3B* and *QTs.zhl-5A* were significantly associated with resistance to all five isolates, whereas *QTs.zhl-1A* was significantly associated with disease caused by the Ptr ToxC-producing isolates Pti2, 331-9, and AR CrossB10 and *QTs.zhl-2D* associated with disease by all isolates except DW5.

QTs.zhl-3B conferred resistance to all isolates and had the largest effect among all QTLs identified. It had a LOD value ranging from 13.6 (AR CrossB10) to 44.0 (86-124) and the effect of the QTL explained from 22 (AR CrossB10) to 53 % (86-124) of the disease variation (Table 2). The genomic region harboring this QTL was flanked by the SNP markers *Xiwa1383* and *Xiwa4613*. The SSR marker *Xwmc69* was the closest to the peak position of the QTL (Fig. 3).

QTs.zhl-1A was the second most significant QTL and it was located at a position between markers *Xiwa6644* and *Xpsp2999* on the distal end of the 1AS chromosome arm (Fig. 2). This position is near the known location of the *Tsc1* gene. As mentioned above, this QTL was significantly associated with resistance to only the Ptr ToxC-producing isolates, for which it had LOD values that ranged from 14.4 to 23.1 and R^2 values that ranged from 0.09 to 0.22 (Table 2).

The other race-nonspecific QTL, *QTs.zhl-5A,* was flanked by the markers *Xiwa7025* and *Xiwa5173* and explained from 6 % of the disease variation for 331-9 to 14 % of the disease variation for DW5 (Fig. 2; Table 2). *QTs.zhl-2D* was located approximately on the end of the long arm of chromosome 2D, flanked by the markers *Xwmc41* and *Xgwm608* and accounted for 3 to 9 % of the disease variation.

Disease reactions of RILs for the different allelic states at *QTs.zhl-3B* and *QTs.zhl-1A*

QTs.zhl-1A and *QTs.zhl-3B* had major effects associated with disease caused by the isolates Pti2, 331-9 and AR CrossB10, which produce Ptr ToxC. To investigate the genetic relationships between the two QTLs, the RILs were grouped into four categories based on their allelic state at the two loci, and

Table 1 Lesion type means of Louise, Penawawa, and the Louise × Penawawa recombinant inbred line population to conidial inoculations of *Pyrenophora tritici-repentis* races 1, 2, 3, 5 and isolate AR CrossB10

Isolates[a]	Louise[b]	Penawawa[b]	LP population average	LP population range
Pti2 (race 1) (Ptr ToxA+, Ptr ToxB−, Ptr ToxC+)	4.12	1.58	2.83	1.00–4.50
86-124 (race 2) (Ptr ToxA+, Ptr ToxB− Ptr ToxC−)	3.08	1.13	2.32	1.00–4.13
331–9 (race 3) (Ptr ToxA−, Ptr ToxB−, Ptr ToxC+)	3.83	1.16	2.42	1.00–4.67
DW5 (race 5) (Ptr ToxA−, Ptr ToxB+, Ptr ToxC−)	3.75	1.00	2.56	1.00–4.88
AR CrossB10 (Ptr ToxA−, Ptr ToxB−, Ptr ToxC+)	4.17	1.33	2.89	1.00–4.14

[a] Five isolates representing different *Pyrenophora tritici-repentis* races were used to evaluate the LP population and *parental lines* for reaction to tan spot. The NEs they produce are indicated in parenthesis where '+' = production of the NE and '−' = no production of the NE

[b] Disease was scored using a 1–5 scale with 1 being highly resistant and 5 being highly susceptible

the disease means of these groups were compared (Table 3). The group of RILs that had Penawawa alleles at both loci was highly resistant with mean reaction types less than 2.0, whereas RILs with Louise alleles at both loci were highly susceptible with mean reaction types greater than 3.5.

RILs with Penawawa alleles at *QTs.zhl-3B* and Louise alleles at *QTs.zhl-1A*, or vice versa, were moderately resistant to moderately susceptible. Comparisons among the mean disease reaction types of isolates Pti2 and 331-9 for these two allelic classes indicated that RILs with Louise alleles at *QTs.zhl-1A* and Penawawa alleles at *QTs.zhl-3B* were significantly more resistant than RILs with Penawawa alleles at *QTs.zhl-1A* and Louise alleles at *QTs.zhl-3B* (Table 3). However, no significant difference between these two classes was observed for average disease reactions types obtained with isolate AR CrossB10.

Reaction of the LP population to a race 2 *ToxA* knockout strain

The LP population was also evaluated with a race 2 *ToxA* knockout strain (86-124ΔToxA), which does not produce Ptr ToxA. The average disease reaction types obtained from this modified strain were compared to those obtained from isolate 86-124 to make direct comparisons between isolates that only differ by the production of Ptr ToxA, whereby 86-124 produces Ptr ToxA and 86-124ΔToxA does not. The disease means of RILs with the *QTs.zhl-3B* resistance allele from Penawawa were 1.65 for 86-124ΔToxA and 1.71 for 86-124, and they were not significant different (Table 4). However, RILs having the Louise allele at *QTs.zhl-3B* had average disease reaction types of 2.88 for 86-124ΔToxA and 3.02 for 86-124, which were significantly different ($P = 0.03$).

Reactions of Louise × Penawawa F_1 plants to tan spot

We tested the F_1 plants derived from Louise and Penawawa along with the two parental lines for reaction to 86-124 (race 2) and 331-9 (race 3) to determine the genetic nature of resistance. For 86-124, all F_1 plants were as resistant as Penawawa and only developed pinpoint lesions, whereas Louise developed large necrotic lesions as observed before (Fig. 4). For isolate 331-9, Penawawa again had pinpoint lesions and was classified as highly resistant whereas Louise was highly susceptible with the development of large necrotic lesions with chlorosis (Figs. 1, 4). However, the F_1 plants were considered moderately resistant because although they exhibited pinpoint dark lesions they also showed chlorosis across the inoculated area (Fig. 4).

Discussion

We identified a total of four genomic regions associated with resistance to tan spot in the LP population, all of which were derived from the resistant parent Penawawa. Among them, the QTLs on the chromosome arms 3BL (*QTs.zhl-3B*) and 5AL (*QTs.zhl-5A*) confer resistance to all races tested. In agreement with the terminology previously used in wheat-tan spot system, we refer to this QTL as race-nonspecific. Race-nonspecific resistance was first reported by Faris and Friesen (2005) in the common wheat variety 'BR34', and it was largely controlled by two QTLs with one on 1BS and the other on 3BL. Chu et al. (2008) subsequently reported chromosome arms 2AS and 5BL harboring QTLs for race-nonspecific resistance in a synthetic wheat accession. Faris et al. (2012) recently identified two QTLs on chromosome arms 5DL and 7BS also as being race-nonspecific in the wheat landrace Salamouni. Together, these results provide strong evidence that the wheat-tan spot system involves race-nonspecific resistance, and further indicates that this type of resistance may commonly occur in wheat germplasm.

QTs.zhl-3B may be the same as *QTs.fcu-3BL* identified by Faris and Friesen (2005) because both QTLs appear to exist within the same region of chromosome 3B (Fig. 3; Faris and Friesen 2005). However, a lack of markers in

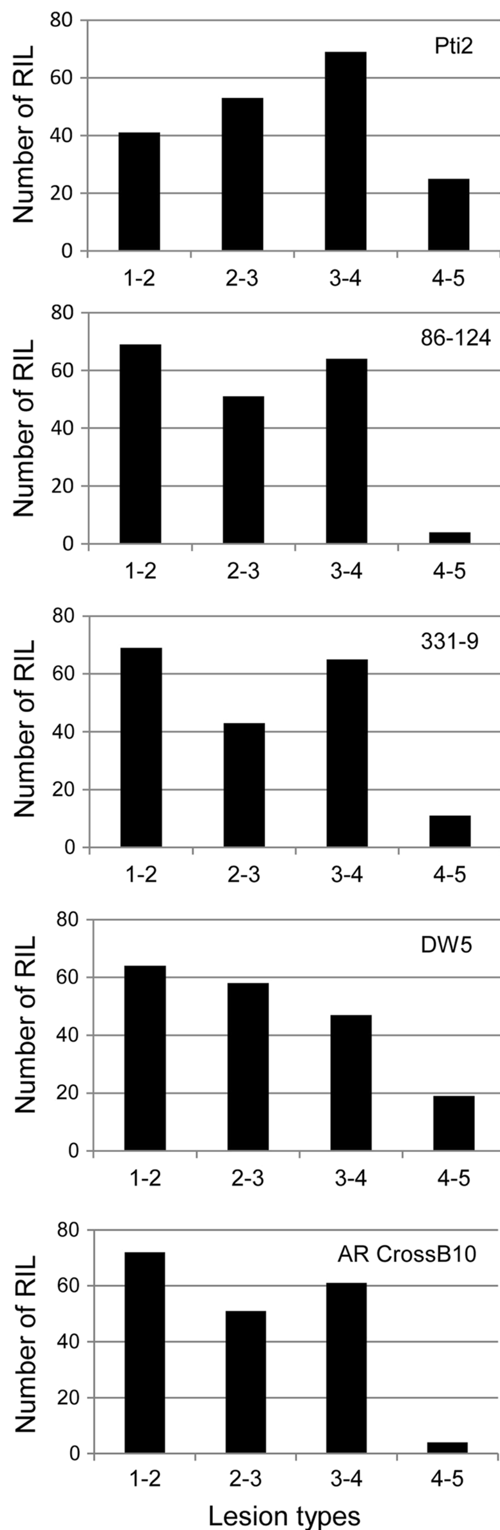

Fig. 2 Histograms of disease reaction of the Louise × Penawawa population to individual isolates. The LP population was evaluated with five isolates representing different races, including Pti2 (race 1), 86-124 (race 2), 331-9 (race 3), DW5 (race 5) and AR crossB10 (unclassified isolate). The disease was scored using a 1–5 lesion type-based scale with 1 being highly resistant and 5 being highly susceptible. The *x* axis is the disease scale and *y* axis is the number of recombinant *inbred lines*

common between the two 3B maps makes it difficult to draw conclusions. Mapping with more common markers within this genomic region in both mapping populations is needed to determine if *QTs.zhl-3B* and *QTs.fcu-3BL* are the same, or if different genes underlie them.

It is also interesting to note that *QTs.zhl-3B* appears to be close to the positions of the tan spot resistance genes *tsn*2 (Singh et al. 2006) and *tsn5* (Singh et al. 2008) (now designated as *tsr2* and *tsr5*, respectively; Faris et al. 2013). However, *tsr2* and *tsr5* were reported to be recessive resistance genes and specifically effective against races 3 and 5, respectively, which would suggest they are different from the gene underlying *QTs.zhl-3B* identified in the current research. It is possible that this genomic region of the chromosome 3B may contain multiple genes that have major effects on tan spot resistance/susceptibility.

Ptr ToxA was shown to be a major disease determinant for *Ptr* (Ciuffetti et al. 1997) and many QTL mapping studies have indicated that the Ptr ToxA-*Tsn1* interaction plays an important role in disease caused by races 1 and 2 (Cheong et al. 2004; Singh et al. 2008; Sun et al. 2010; Chu et al. 2008; Faris et al. 2012). However, QTL mapping by Faris and Friesen (2005) led to the identification of QTLs conferring race-nonspecific resistance with no detection of the *tsn1* locus as a significant QTL for races 1 and 2 even though the population they used segregated for *Tsn1*. It was speculated that race-nonspecific resistance QTLs might act upstream of the Ptr ToxA-*Tsn1* interaction precluding the development of necrosis. Wheat genotypes that are sensitive to Ptr ToxA, but highly resistant to races 1 and/or 2 have been reported previously (Noriel et al. 2011; Liu et al. 2015). However, genetic resistance in these genotypes has not been characterized. Using the LP population, we demonstrated that genotypes such as Penawawa carry race-nonspecific resistance. Although the entire LP population was sensitive to Ptr ToxA, most RILs carrying *QTs.zhl-3B* Penawawa alleles were as resistant as Penawawa to race 2. In addition, we evaluated the LP population with a race 2 *ToxA* knockout strain (86-124ΔToxA) and found that it caused significantly less disease than wild-type 86-124 only on the RILs lacking the *QTs.zhl-3B* resistance allele. These results indicate that NETS from the Ptr ToxA-*Tsn1* interaction was prohibited by the effects of the race-nonspecific resistance QTL *QTs.zhl-3B*. In other words, *QTs.zhl-3B* has an epistatic effect on the Ptr ToxA-*Tsn1* interaction in the LP population.

We identified a QTL on the distal end of chromosomal arm 1AS (*QTs.zhl-1A*) conferring resistance to races 1 and 3 as well as AR CrossB10, all of which produce Ptr ToxC. Thus, *QTs.zhl-1A* likely corresponds to the *Tsc1* locus which conditions sensitivity to Ptr ToxC (Effertz et al. 2002). Faris et al. (1997) and Effertz et al. (2001) also identified QTLs for resistance to races 1 and/or 3 on 1AS

Table 2 Composite interval mapping analysis of QTLs associated with resistance to tan spot caused by *Pyrenophora tritici-repentis* races 1, 2, 3, 5 and isolate AR CrossB10 in the Louise × Penawawa recombinant inbred line population

QTL	Interval (cM)	Flanking markers	R^{2a}					LOD[b]					Source[c]
			Pti2	86-124	331-9	DW5	AR	Pti2	86-124	331-9	DW5	AR	
QTs.zhl-1A	0.0–6.0	*Xiwa6644-Xpsp2999*	0.09	NS	0.22	NS	0.14	14.7	NS	23.1	NS	14.4	P
QTs.zhl-2D	144.0–152.0	*Xwmc41-Xgwm608*	0.09	0.07	0.03	NS	0.05	8.6	6.4	4.7	NS	5.5	P
QTs.zhl-3B	72.0–78.0	*Xiwa1383-Xiwa4613*	0.30	0.53	0.41	0.46	0.22	18.4	44.0	34.3	36.3	13.6	P
QTs.zhl-5A	154.0–160.0	*Xiwa7025-Xiwa5173*	0.13	0.13	0.06	0.14	0.08	12.7	13.9	5.2	18.2	7.9	P

[a] R^2 the coefficient of determination. The R^2 value × 100 represents the amount of phenotypic variation explained. *NS* indicates the QTL was not significant

[b] LOD was determined by the execution of 1000 permutations on marker and phenotypic datasets, which yielded a value of 3.2 as the cutoff for the detection of significant QTLs

[c] The source of each *QTL* indicates the resistance allele was contributed by one of the parental lines with L being Louise and P being Penawawa

at the *Tsc1* locus. Together, these results indicate that the Ptr ToxC-*Tsc1* interaction is important for disease caused by Ptr ToxC-producing races/isolates. Disease dissection in the LP population showed that reactions of RILs to Ptr ToxC-producing isolates were largely dependent on the allele types at both *QTs.zhl-3B* and *QTs.zhl-1A*. RILs with Penawawa alleles at both loci had the lowest disease means followed by those that carried Penawawa alleles at only one locus, and then by those that did not carry Penawawa alleles at either locus (Table 3). This indicates that the presence of *QTs.zhl-1A* (absence of *Tsc1*) is additive to *QTs.zhl-3B*.

Friesen and Faris (2004) were the first to map *Tsc2* and showed that the Ptr ToxB-*Tsc2* interaction explained as much as 69 % of the variation in disease caused by race 5 using the ITMI population. Abeysekara et al. (2010) confirmed the role of the Ptr ToxB-*Tsc2* interaction in disease caused by race 5 using a population of RILs derived from Salamouni × Kepatawa. The LP population does not segregate at the *Tsc2* locus; therefore, it was not possible to assess the relationship of the effect of the Ptr ToxB-*Tsc2* interaction with *QTs.zhl-3B*. A population derived from Penawawa and 6B662 (sensitive to Ptr ToxB, susceptible to race 5) would serve as a good resource for this purpose.

The F_1 plants of Louise and Penawawa were highly resistant to 86-124 (race 2), but moderately resistant to 331-9 (race 3). The results indicate resistance in Penawawa, mainly conferred by the 3BL QTL, is completely dominant to susceptibility caused by race 2, but partially dominant to susceptibility by race 3. Based on the reaction to race 2, Ptr ToxA-induced necrosis in the F_1 was completely prohibited further indicating that *QTs.zhl-3B* was epistatic to the Ptr ToxA-*Tsn1* interaction. For the race 3 inoculation, F_1 plants developed mainly chlorosis across the leaves indicative of a compatible Ptr ToxC-*Tsc1* interaction indicating that the Ptr ToxC-*Tsc1* interaction is independent of the effect of *QTs.zhl-3B* as shown by the analysis of the LP population data for Ptr ToxC-producing isolates (see above).

Several lines of evidence from our research suggest the presence of additional susceptibility factors in the host besides *Tsn1*, *Tsc1* and *Tsc2*. First, the race 2 *ToxA* knockout strain 86-124ΔToxA, which does not produce any of the three known *Ptr* NEs, was still able to cause disease in Louise and the LP population with an average lesion type of nearly 3.0 among RILs lacking the Penawawa allele at *QTs.zhl-3B*. Second, DW5, which produces only Ptr ToxB, caused average reaction types of 3.83 and 2.42 on Louise and the LP population, respectively, even though all were insensitive to Ptr ToxB. Third, Louise and some RILs developed strong necrosis (Figs. 1, 4) after being inoculated with 331-9 which is only known to produce Ptr ToxC, a chlorosis-inducing NE. It is possible that *QTs.zhl-2D* and *QTs.zhl-5A* may represent susceptibility factors, which might consist of novel NE sensitivity genes that recognize yet unidentified NEs produced by these isolates. The presence of additional unidentified NE-host sensitivity interactions has been suggested in a number of other studies as well (Ciuffetti et al. 2003; Meinhardt et al. 2003; Friesen et al. 2003; Manning and Ciuffetti 2015). More work is needed to identify and characterize those potentially new interactions.

QTs.zhl-5A is the second QTL identified to confer race-nonspecific resistance in the LP population. Based on the chromosome position and common markers, we believe that *QTs.zhl-5A* is the same as *QTs.fcu-5AL* and *QTs.fcu-5A.1* that were identified in the TA4152-60/ND495 and Lebsock/PI 94749 populations, respectively (Chu et al. 2008, 2010). However, *QTs.zhl-5A* had relatively smaller effects compared to the other two. No QTL has previously been reported on 2DL using a bi-parental population, and thus *QTs.zhl-2D* might be novel.

Our work highlights the complexity of the wheat–*Ptr* pathosystem, which not only involves inverse

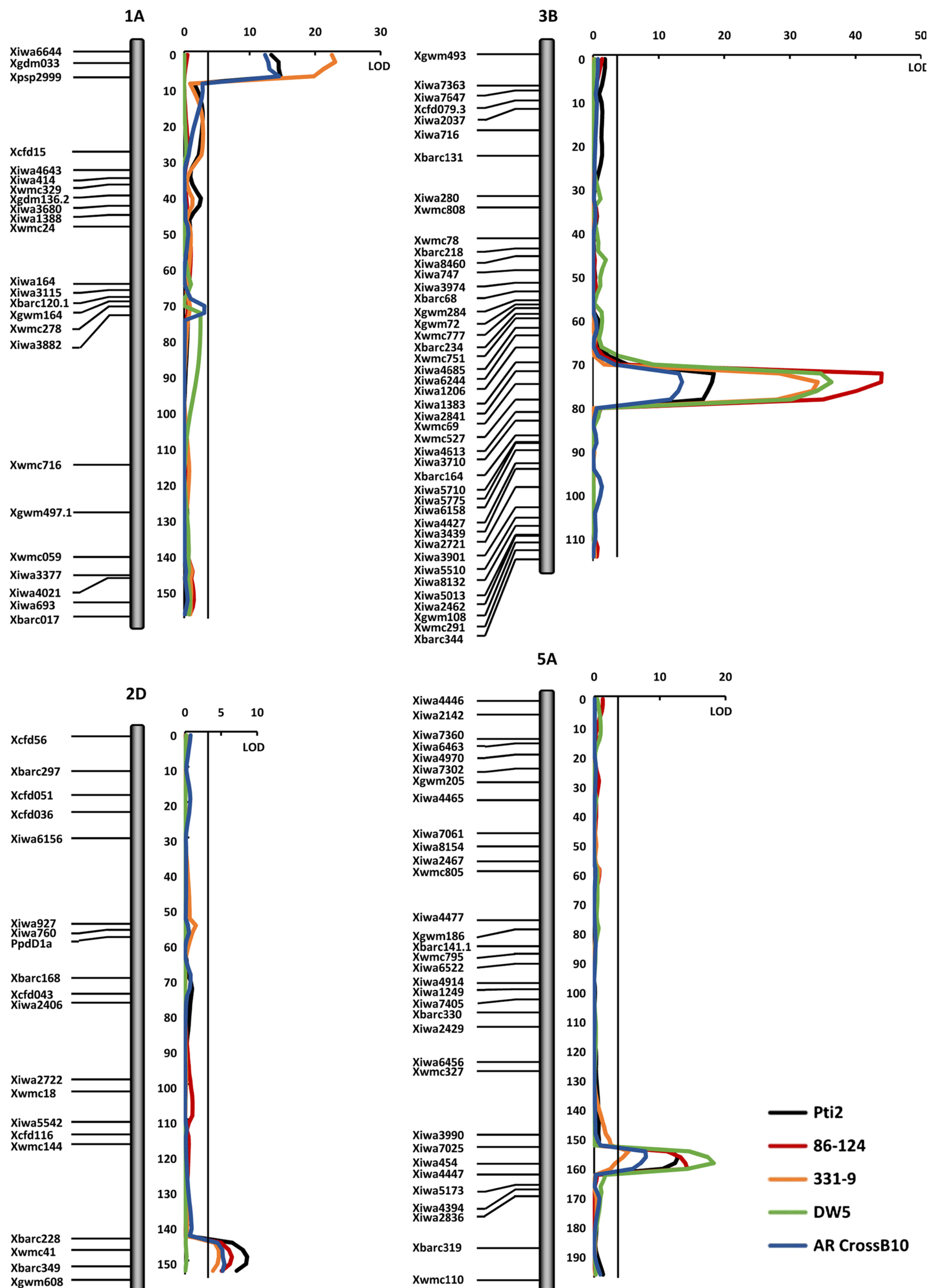

Fig. 3 Composite interval regression maps of chromosomes 1A, 2D, 3B and 5A containing QTLs significantly associated with resistance to tan spot. QTL mapping was conducted on the LP population for five *Pyrenophora tritici-repentis* isolates representing different races including Pti2 (race 1, *black*), 86-124 (race 2, *red*), 331-9 (race 3, *orange*), DW5 (race 5, *green*) and AR crossB10 (unclassified isolate, *blue*). The positions of marker loci are shown to the left of the linkage groups and genetic scales in centiMorgan (cM) are shown along the right of each chromosome. A *solid line* represents the logarithm of the odds (LOD) significance threshold of 3.2. The LOD and R^2 values for each QTL are presented in Table 2

Table 3 Comparison of the disease means of the recombinant inbred lines grouped based on their allelic state at *QTs.zhl-1A* and *QTs.zhl-3B* in the Louise × Penawawa population

Allele at *QTs.zhl-1A*, *QTs.zhl-3B*[a]	No. of RILs (n)	Pti2 (Race1)[b]	331–9 (Race 3)[b]	AR CrossB10[b]
L, L	50	3.50a	3.53a	3.58a
P, L	47	3.18b	2.65b	3.06b
L, P	43	2.74c	2.31c	2.94b
P, P	48	1.87d	1.18d	1.96c

[a] The allele type is indicated by L (Louise allele) and P (Penawawa allele) at the corresponding locus

[b] Numbers in the same column followed by the same letter are not significantly different at $P = 0.05$ as determined by LSD

Table 4 Comparison of the recombinant inbred lines grouped on the presence or absence of the 3BL QTL in the Louise × Penawawa population for reaction to the race 2 isolate and its *ToxA* knock out strain

Allele type at *QTs.zhl-3B*[a]	No. of RILs	Isolate/strain[b]	Disease mean[c]
P	97	86-124	1.71a
		86-124ΔToxA	1.65a
L	91	86-124	3.02b
		86-124ΔToxA	2.88c

[a] The presence of allele type at *QTs.zhl-3B*, *L* Louise allele and *P* Penawawa allele

[b] 86-124 was used to generate the *ToxA* knockout strain 86-124ΔToxA. The knockout strain has been proved to not produce Ptr ToxA

[c] Numbers followed by the same letter are not significantly different at the 0.05 level of probability as determined by LSD

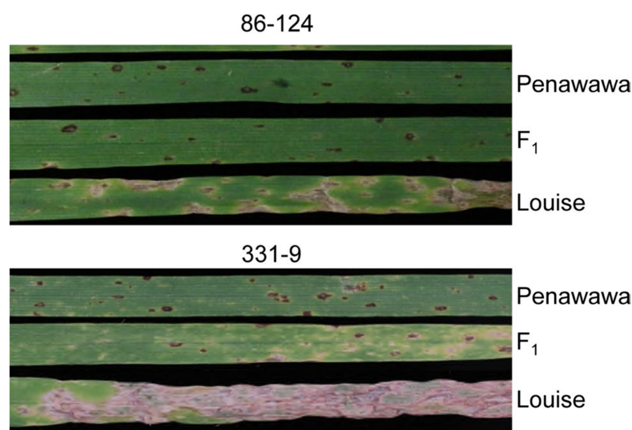

Fig. 4 Reaction of F_1 plants between Louise and Penawawa to races 2 and 3. F_1 plants between Louise and Penawawa were inoculated with isolates 86-124 and 331-9 representing races 2 and 3, respectively. The leaves were photographed 7 days after inoculation

gene-for-gene, race-specific interactions determined by the fungal-produced NEs and host sensitivity genes that leads to NETS, but also a major QTL for race-nonspecific resistance. In addition, many minor QTLs, either race-specific or race-nonspecific, might also exist to modify these two types of reactions. We provided here the first comprehensive view of how a major race-nonspecific resistance QTL is related to NETS caused by the NE and host gene interaction, which has an important application in breeding for tan spot resistance. To obtain more complete resistance, breeders should incorporate the major race-nonspecific resistance QTLs into elite lines and remove NE sensitivity genes, especially those not affected by race-nonspecific resistance, such as *Tsc1*. The molecular markers associated with the major race-nonspecific resistance QTL (*QTs.zhl-3B*) should be useful to move this QTL into breeding lines via MAS. Similarly, molecular markers linked to *QTs.zhl-1A* can be used to remove *Tsc1* from breeding lines. Nevertheless, more research is needed to investigate the genetic relationships of race-nonspecific resistance and NETS in a wide range of genetic backgrounds for a broad utilization of the race-nonspecific resistance genes/QTLs.

Author contribution statement Conceived and designed the experiments: ZHL, AHC. Performed the experiments: GK. Analyzed the data: GK, SSX, JDF. Contributed reagents/materials/analysis tools: AHC, JBR, MM. Wrote the paper: GK, ZHL, JDF.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest for this article.

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

References

Abeysekara NS, Friesen TL, Keller B, Faris JD (2009) Identification and characterization of a novel host–toxin interaction in the wheat–*Stagonospora nodorum* pathosystem. Theor Appl Genet 120:117–126

Abeysekara NS, Friesen TL, Liu ZH, McClean PE, Faris JD (2010) Marker development and saturation mapping of the tan spot Ptr ToxB sensitivity locus *Tsc2* in hexaploid wheat. Plant Genome 3:179–189

Ali S, Gurung S, Adhikari TB (2010) Identification and characterization of novel isolates of *Pyrenophora tritici-repentis* from Arkansas. Plant Dis 94:229–235

Beecher BS, Carter AH, See DR (2012) Genetic mapping of new seed-expressed polyphenol oxidase genes in wheat (*Triticum aestivum* L.). Theor Appl Genet 124:1463–1473

Carter AH, Chen XM, Garland-Campbell K, Kidwell KK (2009) Identifying QTL for high-temperature adult-plant resistance to stripe rust (*Puccinia striiformis* f. sp. *tritici*) in the spring wheat (*Triticum aestivum* L.) cultivar 'Louise'. Theor Appl Genet 119:1119–1128

Cheong J, Wallwork H, Williams KJ (2004) Identification of a major QTL for yellow leaf spot resistance in the wheat varieties Brookton and Cranbook. Aust J Agric Res 55:315–319

Chu CG, Friesen TL, Xu SS, Faris JD (2008) Identification of novel tan spot resistance loci beyond the known host-selective toxin insensitivity genes in wheat. Theor Appl Genet 117:873–881

Chu CG, Chao S, Friesen TL, Faris JD, Zhong S, Xu SS (2010) Identification of novel tan spot resistance QTLs using an SSR-based linkage map of tetraploid wheat. Mol Breed 25:327–338

Ciuffetti LM, Tuori RP, Gaventa JM (1997) A single gene encodes a selective toxin causal to the development of tan spot of wheat. Plant Cell 9:135–144

Ciuffetti LM, Manning VA, Martinez JP, Pandelova I, Andrie RM (2003) Proteinaceous toxins of *Pyrenophora tritici-repentis* and investigation of the site-of-action of Ptr ToxA. In: Rasmussen JB, Friesen TL, Ali S (eds) Proceedings of the fourth international wheat tan spot and spot blotch workshop. N D Agric Exp Stn, Fargo, pp 96–102

Ciuffetti LM, Manning VA, Pandelova I, Betts MF, Martinez JP (2010) Host-selective toxins, Ptr ToxA and Ptr ToxB, as necrotrophic effectors in the *Pyrenophora tritici-repentis*-wheat interaction. New Phytol 187:911–919

Effertz RJ, Anderson JA, Francl LJ (2001) Restriction fragment length polymorphism mapping of resistance to two races of *Pyrenophora tritici-repentis* in adult and seedling wheat. Phytopathology 91:572–578

Effertz RJ, Meinhardt SW, Anderson JA, Jordahl JG, Francl LJ (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

Faris JD, Friesen TL (2005) Identification of quantitative trait loci for race-nonspecific resistance to tan spot in wheat. Theor Appl Genet 111:386–392

Faris JD, Anderson JA, Francl LJ, Jordahl JG (1996) Chromosomal location of a gene conditioning insensitivity in wheat to a necrosis-inducing culture filtrate from *Pyrenophora tritici-repentis*. Phytopathology 86:459–463

Faris JD, Anderson JA, Francl LJ, Jordahl JG (1997) RFLP mapping of resistance to chlorosis induction by *Pyrenophora tritici-repentis* in wheat. Theor Appl Genet 94:98–103

Faris JD, Zhang Z, Lu HJ, Lu SW, Reddy L, Cloutier S, Fellers JP, Meinhardt SW, Rasmussen JB, Xu SS, Oliver RP, Simons KJ, Friesen TL (2010) A unique wheat disease resistance-like gene governs effector-triggered susceptibility to necrotrophic pathogens. Proc Natl Acad Sci USA 107:13544–13549

Faris JD, Abeysekara NS, McClean PE, Xu SS, Friesen TL (2012) Tan spot susceptibility governed by the *Tsn1* locus and race-nonspecific resistance quantitative trait loci in a population derived from the wheat lines Salamouni and Katepwa. Mol Breed 30:1669–1678

Faris JD, Liu Z, Xu SS (2013) Genetics of tan spot resistance in wheat. Theor Appl Genet 126:2197–2217

Faris JD, Zhang QJ, Chao S, Zhang Z, Xu SS (2014) Analysis of agronomic and domestication traits in a durum x cultivated emmer wheat population using a high-density single nucleotide polymorphism-based linkage map. Theor Appl Genet 127:2333–2348

Flor HH (1956) The complimentary genetics systems in flax and flax rust. Adv Genet 8:29–54

Friesen TL, Faris JD (2004) Molecular mapping of resistance to *Pyrenophora tritici-repentis* race 5 and sensitivity to Ptr ToxB in wheat. Theor Appl Genet 109:464–471

Friesen TL, Faris JD (2010) Characterization of the wheat-*Stagonospora nodorum* disease system: what is the molecular basis of this quantitative necrotrophic disease interaction. Can J Plant Pathol 32:20–28

Friesen TL, Ali S, Kianian S, Francl LJ, Rasmussen JB (2003) Role of host sensitivity to Ptr ToxA in development of tan spot of wheat. Phytopathology 93:397–401

Friesen TL, Xu SS, Harris MO (2008) Stem rust, tan spot, *Stagonospora nodorum* blotch, and hessian fly resistance in Langdon durum-synthetic hexaploid wheat lines. Crop Sci 48:1062–1070

Hosford RM Jr (1982) Tan spot-developing knowledge 1902–1981, virulent races and differentials, methodology, rating systems, other leaf diseases, literature. In: Hosford RM Jr (ed) Tan spot of wheat and related diseases workshop. N D Agric Exp Stn, Fargo, pp 1–24

Joehanes R, Nelson JC (2008) QGene 4.0, an extensible Java QTL-analysis platform. Bioinformatics 24:2788–2789

Kollers S, Rodemann B, Ling J, Korzun V, Ebmeyer E, Argillier O, Hinze M, Plieske J, Kulosa D, Ganal MW, Röder MS (2014) Genome-wide association mapping of tan spot resistance (*Pyrenophora tritici-repentis*) in European winter wheat. Mol Breed 34:363–371

Lamari L, Bernier CC (1989) Evaluation of wheat lines and cultivars to tan spot *Pyrenophora tritici-repentis* based on lesion type. Can J Plant Pathol 11:49–56

Lamari L, Strelkov SE (2010) The wheat/*Pyrenophora tritici-repentis* interaction: progress towards an understanding of tan spot disease. Can J Plant Pathol 32:4–10

Levene H (1960) Robust tests for equality of variances. In: Olkin I, Ghurye SH, Hoeffding W, Madow WG, Maan HB (eds) Contributions to probability and statistics: essays in honor of Harold Hotelling. Stanford University Press, Stanford, pp 278–292

Liu ZH, Faris JD, Oliver RP, Tan K, Solomon PS, Mcdonald MC, Mcdonald BA, Nunez A, Lu S, Rasmussen JB, Friesen TL (2009) SnTox3 acts in effector triggered susceptibility to induce disease on wheat carrying the *Snn3* gene. PLoS Pathog 5:1–15

Liu ZH, El-Basyoni I, Kariyawasam G, Zhang G, Fritz A, Hansen JM, Marais F, Friskop AJ, Chao S, Akhunov E, Baenziger PS (2015) Evaluation and association mapping of resistance to tan spot and *Stagonospora nodorum* blotch in adapted winter wheat germplasm. Plant Dis 99:1333–1341

Lorieux M (2012) MapDisto: fast and efficient computation of genetic linkage maps. Mol Breed 30:1231–1235

Manning V, Ciuffetti LM (2015) Necrotrophic effector epistasis in the *Pyrenophora tritici-repentis*-wheat interaction. PLoS One 10:e0123548. doi:10.1371/journal.pone.0123548

Meinhardt SW, Ali S, Ling H, Francl LJ (2003) A new race of *Pyrenophora tritici-repentis* that produces a putative host-selective toxin. In: Rasmussen JB, Friesen TL, Ali S (eds) Proceedings of the fourth international wheat tan spot and spot blotch workshop. N D Agric Exp Stn, Fargo, pp 117–119

Murray GM, Brennan JP (2009) Estimating disease losses to the Australian wheat industry. Aust Plant Pathol 38:558–570

Noriel AJ, Sun XC, Bockus W, Bai G (2011) Resistance to tan spot and insensitivity to Ptr ToxA in wheat. Crop Sci 51:1059–1067

Patel JS, Mamidi S, Bonman JM, Adhikari TB (2013) Identification of QTL in spring wheat associated with resistance to a novel isolate of *Pyrenophora tritici-repentis*. Crop Sci 53:842–852

Rees RG, Platz GJ, Mayer RJ (1982) Yield losses in wheat from yellow spot: comparison of estimates derived from single tillers and plots. Aust J Agric Res 33:899–908

SAS Institute (2011) SAS/IML 9.3 User's Guide. SAS Institute, Cary, NC

Schilder AMC, Bergstrom GC (1994) Infection of wheat seed by *Pyrenophora tritici-repentis*. Can J Bot 72:510–519

Singh PK, Gonzalez-Hernandez JL, Mergoum M, Ali S, Adhikari TB, Kianian SF, Elias EM, Hughes GR (2006) Identification and molecular mapping of a gene conferring resistance to *Pyrenophora tritici-repentis* race 3 in tetraploid wheat. Phytopathology 96:885–889

Singh PK, Mergoum M, Gonzalez-Hernandez JL, Ali S, Adhikari TB, Kianian SF, Elias EM, Hughes GR (2008) Genetics and molecular mapping of resistance to necrosis inducing race 5 of *Pyrenophora tritici-repentis* in tetraploid wheat. Mol Breed 21:293–304

Snedecor GW, Cochran WG (1989) Statistical methods, 8th edn. Iowa State University Press, Ames, Iowa

Sun X-C, Bockus WW, Bai GH (2010) Quantitative trait loci for resistance to *Pyrenophora tritici-repentis* race 1 in a Chinese wheat. Phytopathology 100:468–473

Tadesse W, Hsam SL, Wenzel G, Zeller FJ (2006a) Identification and monosomic analysis of tan spot resistance genes in synthetic wheat lines (*Triticum turgidum* L. × *Aegilops tauschii* Coss.). Crop Sci 46:1212–1217

Tadesse W, Hsam SLK, Zeller FJ (2006b) Evaluation of common wheat cultivars for tan spot resistance and chromosomal location of a resistance gene in the cultivar 'Salamouni'. Plant Breed 125:318–322

Wolpert TJ, Dunkle LD, Ciuffetti LM (2002) Host-selective toxins and avirulence determinants: what's in a name? Ann Rev Phytopathol 40:251–285

Zeng Z-B (1994) Precision mapping of quantitative trait loci. Genetics 136:1457–1468