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ORIGINAL PAPER

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Molecular mapping of resistance to *Pyrenophora tritici-repentis* race 5 and sensitivity to Ptr ToxB in wheat

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Abstract Tan spot, caused by *Pyrenophora tritici-repentis* (Ptr), is an economically important foliar disease in the major wheat growing areas of the world. Multiple races of the pathogen have been characterized based on their ability to cause necrosis and/or chlorosis in differential wheat lines. Isolates of race 5 cause chlorosis only, and they produce a host-selective toxin designated Ptr ToxB that induces chlorosis when infiltrated into sensitive genotypes. The international Triticeae mapping initiative (ITMI) mapping population was used to identify genomic regions harboring QTLs for resistance to fungal inoculations of Ptr race 5 and to determine the chromosomal location of the gene conditioning sensitivity to Ptr ToxB. The toxin-insensitivity gene, which we are designating *tsc2*, mapped to the distal tip of the short arm of chromosome 2B. This gene was responsible for the effects of a major QTL associated with resistance to the race 5 fungus and accounted for 69% of the phenotypic variation. Additional minor QTLs were identified on the short arm of 2A, the long arm of 4A, and on the long arm of chromosome 2B. Together, the major QTL on 2BS identified by *tsc2* and the QTL on 4AL explained 73% of the total phenotypic variation for resistance to Ptr race 5. The results of this research indicate that Ptr ToxB is a major virulence factor, and the markers closely linked to *tsc2* and the 4A QTL should be useful for introgression of resistance into adapted germplasm.

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Introduction

Pyrenophora tritici-repentis (Ptr) (Died.) Drechs. [anamorph *Drechslera tritici-repentis* (Died.) Shoem.], the causal agent of tan spot, is a major foliar disease of wheat (*Triticum aestivum* L.) in the US and major wheat growing areas throughout the world (Weise 1987). Typical symptoms include a tan colored, diamond shaped necrotic lesion with a small, dark brown infection site. Lesions are often surrounded by chlorotic halos (Weise 1987). Genetic resistance tends to reduce or eliminate the size of the necrotic and/or chlorotic area, but the small dark brown infection site remains (Lamari and Bernier 1989a).

Lamari et al. (1995) proposed a race classification system for *P. tritici-repentis* isolates. Isolates that produced necrosis on the differential cultivar Glenlea and chlorosis on the differential line 6B365 were designated as race 1. Isolates that produced necrosis on Glenlea only and chlorosis on 6B365 only were designated as races 2 and 3, respectively. Race 4 isolates are avirulent on wheat, and race 5 isolates produce chlorotic symptoms similar to race 3 but on cv. Katepwa. Race 6 combines the virulences of races 3 and 5. Race 7 combines the virulences of races 2 and 5, and race 8 combines the virulences of races 2, 3, and 5 (Lamari et al. 2003).

Four toxins of *P. tritici-repentis* (Ptr ToxA, Ptr ToxB, Ptr ToxC, and Ptr ToxD) have been reported (Tomás and Bockus 1987; Orolaza et al. 1995; Effertz et al. 2002; Manning et al. 2002), of which Ptr ToxA (Ballance et al. 1989; Tomás et al. 1990; Brown and Hunger 1993; Tuori et al. 1995; Zhang et al. 1997) and Ptr ToxB (Strelkov et al. 1999; Martinez et al. 2001) have been well characterized. According to the race classification system proposed by Lamari et al. (2003), Ptr ToxA is produced by races 1, 2, 7 and 8; Ptr ToxB is produced by races 5, 6, 7 and 8; and Ptr ToxC is produced by races 1, 3, 6 and 8.

Orolaza et al. (1995) partially purified a toxic compound from culture filtrates of a race 5 isolate, and they demonstrated that it was a hydrophilic molecule that was stable when exposed to organics and had a molecular mass

of at least 3.5 kDa. The progeny of a cross between resistant and susceptible genotypes were used to show that a single dominant gene in the host controlled the reaction to race 5 of the fungus and the partially purified toxin. Strelkov et al. (1999) later demonstrated that the toxin, Ptr ToxB, was a heat-stable, 6.61 kDa protein that was active at concentrations as low as 14 nM. Martinez et al. (2001) cloned and characterized the *ToxB* gene that encodes the 64 amino acid HST, Ptr ToxB.

Reports regarding the inheritance of resistance to tan spot have ranged from qualitative (Lamari and Bernier 1989b, 1991; Sykes and Bernier 1991; Gamba and Lamari 1998; Lee and Gough 1984) to quantitative (Elias et al. 1989; Faris et al. 1997; Friesen et al. 2003; Nagle et al. 1982). Insensitivity to Ptr ToxA produced by race 2 (nec+, chl−) isolates was found to be conditioned by a single recessive gene in the host (Lamari and Bernier 1989b). This gene, designated *Tsn1*, was mapped to the long arm of chromosome 5B in common wheat (Faris et al. 1996) and in durum wheat (Anderson et al. 1999). It was suggested that sensitivity to Ptr ToxA and susceptibility to tan necrosis caused by the fungus were controlled by a common gene (Lamari and Bernier 1989b). However, more recent experiments have indicated that Ptr ToxA is a virulence factor because *Tsn1* accounted for only 24.4% of the phenotypic variation for disease, and toxin-insensitive mutants were not resistant to the fungus but developed disease more slowly than the wild-types (Friesen et al. 2003).

Faris et al. (1997) investigated resistance to chlorosis induction produced by race 1 (nec+, chl+) in a population of recombinant inbreds derived from the synthetic hexaploid wheat W-7984 × Opata 85 [international Triticeae mapping initiative (ITMI population)]. They identified a QTL with major effects on the short arm of chromosome 1A (*QTsc.ndsu-1A*), a minor QTL on the long arm of chromosome 4A, and an epistatic interaction, which together accounted for 49% of the phenotypic variation. Later, Li et al. (1999) mapped a collection of defense response genes in the ITMI population, which included a gene encoding oxalate oxidase that mapped to the minor QTL on 4AL. Using the oxalate oxidase 4AL marker, the 1A marker, and an epistatic interaction, Faris et al. (1999) were able to explain 58% of the total variation for resistance to race 1. Effertz et al. (2001) confirmed that *QTsc.ndsu-1A* was also the predominant QTL associated with resistance to chlorosis in adult plants of the same population.

Resistance to chlorosis produced by race 3 (nec−, chl+) isolates was investigated in a different recombinant inbred population and found to be predominantly controlled by *QTsc.ndsu-1A* with minor effects observed at a locus on 4AL (Effertz et al. 2001), which may be the same as the 4AL locus identified by Faris et al. (1997, 1999) for resistance to race 1. In addition, the gene (*Tsc1*) conditioning sensitivity to Ptr ToxC was mapped to the *QTsc.ndsu-1A* locus in the ITMI population (Effertz et al. 2002).

Here, we determined the chromosomal location of the gene conditioning sensitivity to the chlorosis-inducing toxin Ptr ToxB, identified putative minor QTLs associated with resistance to the race 5 (nec−, chl+) isolate DW5 and investigated the role of Ptr ToxB in the development of disease.

Materials and methods

Plant materials

A subset of the ITMI population consisting of 104 recombinant inbred (RI) lines was used for this study. This RI population was derived from crossing the synthetic hexaploid wheat W-7984 and the CIMMYT (International Maize and Wheat Improvement Center)-bred hard red spring wheat Opata 85 (PI591776) as described in Nelson et al. (1995c). The population of RI lines was provided by M. E. Sorrells, Cornell University, Ithaca, N.Y., USA. Both parents of the population are moderately susceptible to tan spot induced by race 5, but W-7984 is sensitive to Ptr ToxB while Opata 85 is insensitive (Fig. 1). Checks used in the experiment included both parents and the tan spot susceptible line ND495.

Fungal cultures, culture filtrate production and identification of toxin properties

The *P. tritici-repentis* race 5 isolates DW5 and DW7 were collected from durum wheat fields in North Dakota in 1998 and were used for production of culture filtrates that contained Ptr ToxB. Isolate DW7 was used by Martinez et al. (2001) to purify Ptr ToxB and to clone the gene encoding it. Isolates were grown in Petri plates on V8-PDA for 5 days at which time they were flattened and put through a 24 h light (room temperature) and 24 h dark (16°C) cycle for the production of conidia. Plates were saturated with sterile distilled water, and conidia were harvested using a sterile loop. One milliliter of the spore suspension was added to 50 ml quantities of liquid Fries media (5 g ammonium tartrate, 1 g ammonium nitrate, 0.5 g magnesium sulfate, 1.3 g potassium phosphate (dibasic), 2.6 g potassium phosphate (monobasic), 30 g sucrose, 1 g yeast extract, dissolved in 1000 ml water) and placed on an orbital shaker at 80 rpm for 48 h followed by 3 weeks of stationary growth in the dark. Culture filtrates were passed through two layers of cheesecloth followed by vacuum filtration through a Whatman number one filter and a 0.45 μm Whatman cellulose nitrate filter.

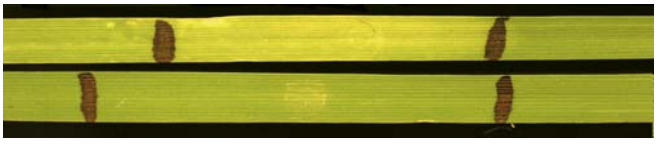

Fig. 1 Reaction of W-7984 (*top*) and Opata 85 (*bottom*) to culture filtrates of *P. tritici-repentis* race 5 isolate DW5 containing Ptr ToxB. *Marker lines* indicate the boundaries of infiltration sites

Size-based filtration was done to help confirm that the 6.61 kDa Ptr ToxB was present in the culture filtrates. Culture filtrates were initially filtered through a 10 kDa filter and checked for activity. The filtrate was then put through a 5 kDa filter and both the rediluted concentrate and the filtrate were assayed. All samples were assayed on the Ptr ToxB-sensitive wheat differential line Katepwa.

Because Ptr ToxB is a protein, an overnight proteinase K treatment was completed at 50°C on both the DW5 and DW7 culture filtrates to confirm that the chlorosis response was from a protein. Treatments and controls using both DW5 and DW7 consisted of raw culture filtrates treated with proteinase K (final concentration, 1 mg/ml), size-filtered culture filtrates treated with proteinase K (final concentration, 1 mg/ml), untreated raw culture filtrates, untreated size-purified culture filtrates, and proteinase K in Fries media alone.

Bioassay for Ptr ToxB sensitivity

For assaying the ITMI population, culture filtrates of both DW5 and DW7 were size-filtered as described earlier. A bioassay, based on chlorosis development, was used to characterize the response of lines to Ptr ToxB. Two replicates of three seeds of each RI line were planted and grown in a controlled chamber under a 12 h photoperiod at 21°C. The second leaf of each plant was infiltrated with DW5 culture filtrates using a 1 ml syringe with the needle removed. Experiments were replicated using DW7 size-purified culture filtrates to confirm the sensitivity of Ptr ToxB. Chlorosis development was evaluated on the 5th day, and each line was assigned a toxin-sensitive or toxin-insensitive reaction type. To differentiate between fungal reactions and toxin reactions, fungal reactions will be referred to as susceptible and resistant and toxin reactions will be referred to as sensitive and insensitive.

Conidial inoculation and rating

For disease analysis, RI lines were inoculated with conidia of isolate DW5. Inoculations were done at the two-to-three-leaf stage. Individual lines of the ITMI population were planted along with parents using three conetainers (Stuewe and Sons, Inc., Corvallis, Ore., USA) per line and three plants per conetainer. Plants were placed in racks of 98 consisting of 20 lines and a border of wheat plants used to eliminate any edge effect. Conidia were grown and harvested as described by Lamari and Bernier (1989a). Conidia were diluted to 3,000 spores/ml, and 2 drops of Tween 20 (polyoxyethylene sorbitan monolaurate) were added per 100 ml of inoculum. Plants were inoculated until runoff. Following inoculation plants were placed in 100% relative humidity in the dark at 21°C for 24 h, and then placed in a controlled chamber under a 12 h photoperiod at 21°C. Disease readings were taken on the 7th day post-inoculation using the 1–5 scale developed by Lamari and Bernier (1989a). Three replicates were completed for the entire population along with parental lines and resistant and susceptible checks.

Molecular mapping and QTL analysis

Extensive molecular marker-based genetic linkage maps exist for the ITMI population (Nelson et al. 1995a, b , c; Marino et al. 1996; Van Deynze et al. 1995; Li et al. 1999; Röder et al. 1998). For this study, we used a subset of 524 markers that gave the most complete genome coverage without redundancy. Reactions of RI lines to culture filtrates were scored as either parental type and treated as a marker to determine linkage to existing markers. The phenotypic marker was tested for linkage to all other markers in the data set using the computer program Mapmaker (Lander et al. 1987) v2.0 for Macintosh and the 'TRY' command. Linkage was assessed using a minimum log likelihood ratio (LOD)=3.0 and the Kosambi mapping function (Kosambi 1944).

The subset of 525 markers (original subset plus the phenotypic marker for reaction to culture filtrate) was used to identify associations with lesion-type scores. Methods for detecting QTLs were performed essentially as described in Faris et al. (1997, 1999). Briefly, the computer program QGENE (Nelson 1997) was used to conduct simple linear regression, interval regression (Haley and Knott 1992), and multiple regression. Simple linear regression was used to identify individual markers significantly ($P<0.001$) associated with resistance. For a data set of this size and using a significance threshold of $P<0.001$, there is a possibility of detecting significant marker-phenotype associations by random chance. However, we chose the given significance threshold to reduce the chances of committing a Type II error (declaring a significant QTL as nonsignificant). Interval regression mapping was used to infer the best position for QTLs between marker loci. A LOD threshold of about 3.0 in this RI population yields an experiment-wise significance level of 0.05 (Lander and Botstein 1989). Markers with significant main effects were tested against all other markers for significant ($P<0.001$) interactions. Markers with significant main effects were assembled into a multiple regression model. Markers that failed to retain significance in the model were eliminated. The coefficient of determination (R^2) is the proportion of the total phenotypic variation explained by the markers in the model.

Results

Toxin properties

Culture filtrates passed through a 10 kDa filter were shown to induce chlorosis on Katepwa indicating the toxin is less than 10 kDa. Culture filtrates passed through a 5 kDa cutoff filter did not induce chlorosis on Katepwa, but the re-diluted concentrate did induce chlorosis indi-

cating the presence of a toxin in the range of 5–10 kDa as expected. Also as expected, proteinase K treatment of culture filtrates eliminated chlorotic symptoms compared with culture filtrates alone demonstrating that the toxin is a protein. Culture filtrates from the race 5 isolates DW7, which was used by Martinez et al. (2001) to purify Ptr ToxB and clone the gene responsible for its production, showed identical reactions when infiltrated side by side with culture filtrates of DW5. Size-purified culture filtrate infiltrations of the ITMI population from isolates DW5 and DW7 indicated that a single toxic compound present in both DW5 and DW7 culture filtrates was responsible for causing chlorosis. This was evidenced by the co-segregation of sensitivity in this population to filtrates of both isolates.

Reaction to culture filtrates and molecular mapping of sensitivity

Infiltration of the culture filtrates into wheat leaves produced sensitive or insensitive reactions. Compatible reactions on sensitive genotypes could be detected after 2 days, and were clearly evident after 5 days (Fig. 1). Reactions of sensitive and insensitive RI lines resembled the reactions of W-7984 and Opata 85, respectively.

The ITMI population of RI lines co-segregated for reaction to culture filtrates of *P. tritici-repentis* race 5 isolates DW5 and DW7 at a ratio of 63:41 for insensitive: sensitive, respectively. This segregation ratio is not significantly different from the expected 1:1 ratio at the 0.05 level of probability.

The phenotypic reactions of the RI lines to the culture filtrate were used to determine linkage of the gene for sensitivity with existing markers in the dataset. The gene was tested for linkage with all 524 markers in the dataset and found to be located at the very distal tip of the short arm of chromosome 2B and linked at 1.9 cM from the marker *Xfbb274*.

Although the segregation ratio of the population was not significantly different from 1:1 at $P<0.05$, it was slightly skewed in favor of sensitivity (Opata 85 alleles). Nearly all of the markers on chromosome 2B from the distal tip of the short arm to marker *Xmwg546* on the long arm had segregation ratios that were skewed in favor of Opata 85 alleles (data not shown). These results indicate that, despite its slightly skewed segregation ratio, the gene conditioning sensitivity to Ptr ToxB is nuclear and located at the tip of 2BS. We propose the symbol *Tsc2* to designate this gene.

Reaction to conidial inoculations of race 5

The average reactions of W-7984 and Opata 85 to conidial inoculations of race 5 isolate DW5 were 3.11 and 3.25, respectively, but the reaction types were dissimilar. W-7984 developed large chlorotic lesions, whereas Opata 85 developed lesions of similar size that consisted of tan colored necrosis (Fig. 2). Lesion types in the RI lines did not necessarily mimic the reactions of the parents. Analysis of variance indicated highly significant differences among RI lines, and mean lesion types of RI lines ranged from 1.5 to 4.5 (Fig. 3) with toxin insensitive lines ranging from 1.5 to 4 and toxin sensitive lines ranging from 3 to 4.5. Many genotypes were highly resistant (lesion types 1–2) or highly susceptible (lesion types 4–5), but a significant portion of the population was moderately resistant to moderately susceptible (lesion types between 2 and 4) (Fig. 3).

QTL analysis

Lesion type means were regressed on the molecular marker data to identify loci significantly associated with resistance to tan spot caused by race 5 isolate DW5. Simple linear regression analysis and interval regression mapping revealed four genomic regions significantly associated with resistance to the fungus (Table 1, Fig. 4). By far the most significant factor was *tsc2*, which was located at the distal tip of chromosome 2BS. The effects *tsc2* alone explained 69.2% of the phenotypic variation for resistance to tan spot caused by race 5 isolate DW5.

Interval regression mapping of chromosome 2B indicated the presence of two putative minor QTLs in addition to the major QTL (Fig. 4). One minor QTL was near the major QTL on the short arm of chromosome 2B. The marker with the most significant effects underlying this QTL was *Xmwg950*, which mapped 44.3 cM proximal to the Ptr ToxB sensitivity locus and accounted for 14.5% of the phenotypic variation. A second minor QTL on chromosome 2B was on the long arm (Fig. 4). The most significant marker underlying this QTL was *Xmwg2025*, which accounted for 11.9% of the phenotypic variation (Table 1). To further investigate the plausibility of the minor QTLs on 2B, we reconstructed the linkage map of 2B using markers spaced at 10–15 cM intervals and a LOD threshold of 3.0 (data not shown). We then repeated

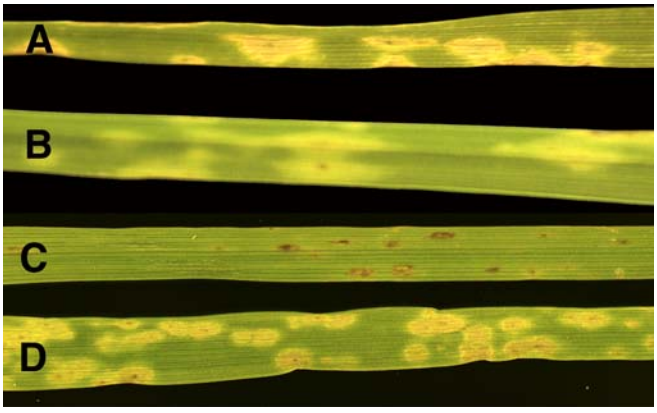

Fig. 2A–D Reaction of lines and parents of the ITMI mapping population to conidial inoculation of *Pyrenophora tritici-repentis* race 5 isolate DW5. Opata 85 (**A**) and W7984 (**B**) along with resistant (**C**) and susceptible (**D**) lines showing transgressive segregation

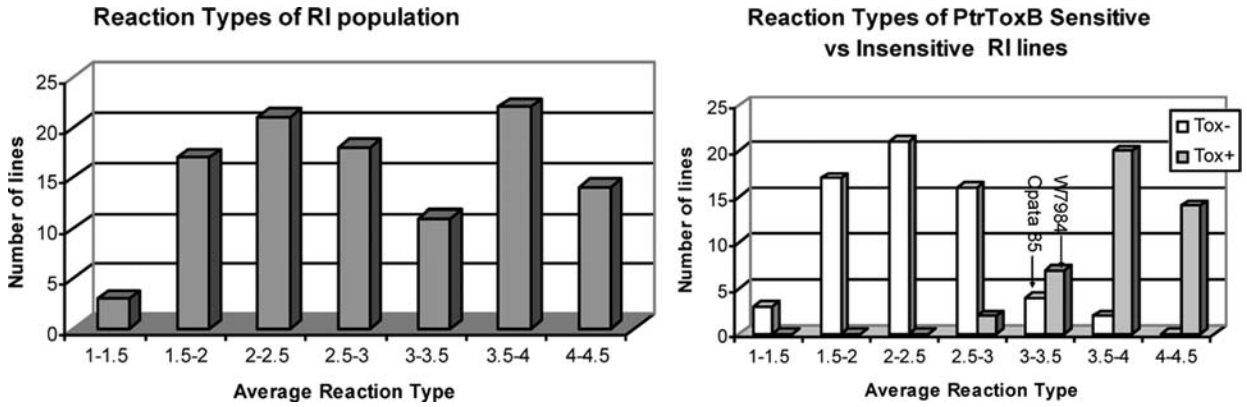

Fig. 3 Histogram of lesion type means of the ITMI recombinant inbred population after inoculation with conidia of *P. tritici-repentis* race 5 isolate DW5 (*left*). Lesion type means separated based on reaction to culture filtrates containing Ptr ToxB (*right*)

the interval regression analysis using this dataset, but found that the three QTLs, one major and two minor, remained significant. However, regression analysis using only RI lines that were insensitive to Ptr ToxB revealed that the major (*tsc2*) and minor (*Xmwg950*) QTLs on the short arm were not significantly associated with the variation among these lines while the minor QTL on 2BL remained significant. This suggests that the minor QTL on 2BS may not be independent from the major QTL detected by *tsc2*, and therefore was not considered further.

A minor QTL was also detected on the short arm of chromosome 2A (Fig. 4), and the marker with the most significant effects was *Xcdo447*. This marker alone explained 11.7% of the variation, and resistance at this locus was contributed by Opata 85 (Table 1).

A resistance QTL contributed by W-7984 was located on the long arm of chromosome 4A (Fig. 4). The most significant marker at this locus was *Xksu916(Oxo)*, which explained 20% of the phenotypic variation (Table 1). *Xksu916(Oxo)* was mapped by Li et al. (1999) as an RFLP probe, and it represents a cloned fragment of a gene that encodes oxalate oxidase from barley.

The markers with significant main effects were assembled into a multiple regression model to determine the total amount of variation explained by the model. Markers *Xmwg2025* (chromosome 2BL) and *Xcdo447* (chromosome 2AS) did not retain significance in the model and were therefore eliminated. The Ptr ToxB insensitivity locus (*tsc2*) from 2BS and the marker *Xksu916(Oxo)* on chromosome 4AL together explained 73% of the total phenotypic variation for resistance to tan spot caused by race 5 isolate DW5.

Discussion

In this study, we identified the chromosomal location of the gene conditioning sensitivity to partially purified culture filtrates containing the proteinaceous toxin Ptr ToxB produced by *P. tritici-repentis* race 5 isolates DW5 and DW7, and we identified QTLs associated with resistance to tan spot caused by DW5. Race 5 isolates were first collected from Algeria (Lamari et al. 1995) and have since been collected from wheat fields in the US (Ali et al. 1999). Isolates from both locations show the same reactions on the standard tan spot differential set (Oralaza et al. 1995; Ali et al. 1999) and the same toxin (Ptr ToxB) has been isolated and characterized from isolates from both locations (Strelkov et al. 1999; Martinez et al. 2001).

Ptr ToxB is a 6.61 kDa proteinaceous host selective toxin and although we did not use purified Ptr ToxB, evidence indicates that our culture filtrates contained Ptr ToxB. Differential lines for *P. tritici-repentis* showed a response typical of race 5 and Ptr ToxB. One of the race 5 isolates (DW7) was also used by Martinez et al. (2001) to purify Ptr ToxB, confirming that Ptr ToxB is produced by this isolate. Sensitivity to DW5 and DW7 culture filtrates co-segregated in the ITMI population indicating that a single toxic compound was responsible for causing chlorosis. Size based filtration using 10 and 5 kDa cutoff filters and proteinase K treatment confirmed that the toxic compound present in culture filtrates was a protein between 5 and 10 kDa in size. In addition, the chlorotic reaction on Katepwa, which is the differential cultivar used in differentiating race 5 isolates, provides further evidence

Table 1 Chromosomal location, parental source, R^2 values, and significance values of four markers associated with resistance in the ITMI population to *Pyrenophora tritici-repentis* race 5

Marker	Chromosome	Resistance source	R^2	*P* (single marker)	*P* (multiple regression model, R^2=0.730)
tsc2 (ToxB insensitivity)	2BS	Opata 85	0.692	<0.00001	<0.00001
Xksu916(Oxo)	4AL	W-7984	0.200	<0.00001	0.0002
Xmwg2025	2BL	Opata 85	0.119	0.0004	–
Xcdo447	2AS	Opata 85	0.117	0.0004	–

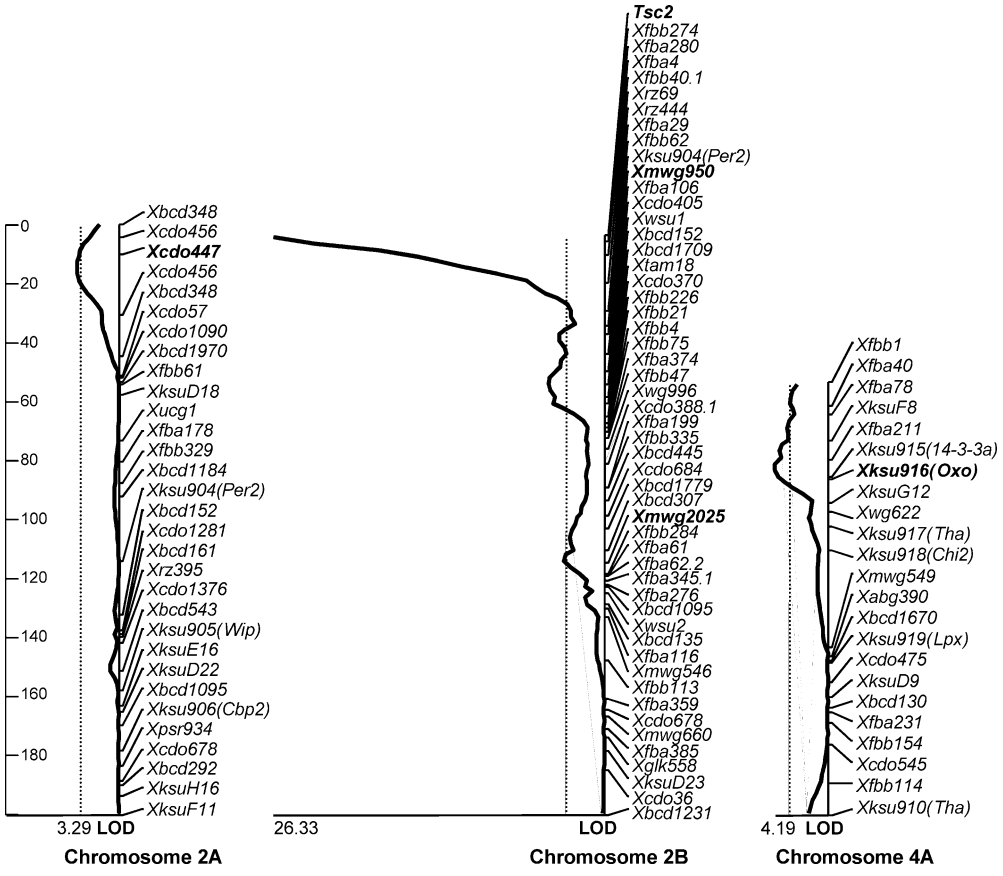

Fig. 4 Interval regression maps of chromosomes 2A, 2B, and 4A possessing QTLs significantly associated with resistance to tan spot caused by *P. tritici-repentis* race 5 isolate DW5. The *dotted line* indicates the LOD threshold of 3.0. Markers with the most significant single factor effects for individual QTLs are in *bold*. The LOD values of these markers are given below the *horizontal lines* at the *bottom* of the maps

that the partially purified culture filtrates contained Ptr ToxB as the sole toxic component.

Gamba et al. (1998) used F_1 and F_2 progeny of a cross between the tan spot differential line Katepwa (race 5 susceptible and Ptr ToxB sensitive) and the race 5 resistant line Erik to show that race 5 pathogenicity was strictly correlated with Ptr ToxB sensitivity, and susceptibility/sensitivity was governed by a single dominant gene. In our research, a chlorotic disease phenotype was evident when sensitive lines were inoculated with conidia of a race 5 isolate (Fig. 2), but the average disease reaction of the Ptr ToxB insensitive parent Opata 85 was 3.11, indicating that although Opata 85 was insensitive to Ptr ToxB, it was susceptible to race 5. Furthermore, average disease reactions for insensitive and sensitive lines were 2.4 and 3.9, respectively, with ranges of 1.5–4.0 for insensitive lines and 3.0–4.5 for sensitive lines. Therefore, no lines showing sensitivity to the toxin were resistant to the fungus but some lines showing insensitivity to the toxin were still susceptible to the fungus. This shows that the presence of *tsc2* is sufficient for susceptibility to the race 5 isolate DW5 but that both parents of the ITMI population possess additional minor factors that affect resistance/susceptibility.

In this research, our goal was to determine the chromosomal location of the toxin sensitivity gene and investigate its role in disease using a QTL mapping approach. Our results indicate that insensitivity to Ptr ToxB is a major factor for resistance to race 5, but other relatively minor QTLs are also involved. To date, QTL mapping has only been conducted to identify resistance to *P. tritici-repentis* races 1 and 3 (Faris et al. 1997; Effertz et al. 2001). In the ITMI population, the majority of resistance was contributed by W-7984, which was highly resistant to both races (Faris et al. 1997). In our research, both parents were moderately susceptible to the race 5 isolate DW5, but most of the resistance QTLs identified, including the toxin insensitivity locus, were contributed by Opata 85. Although W-7984 was sensitive to Ptr ToxB, it may harbor minor resistance factors that went undetected in our analysis due to their relatively minor effects. In addition, it is possible that interactions are involved but went undetected. Epistatic interactions are more difficult to identify than single marker effects because linkage of markers and QTLs at more than one locus is involved. Significant associations may go undetected if marker linkages are not tight enough. The D-genome chromosomes in this population are less saturated with molecular markers than the A- and B-genome chromosomes, and they contain several gaps of more than 20 cM. Therefore, it is possible that minor QTLs and interactions involving factors on the D-genome chromosomes may have gone undetected. Analysis of a much larger population with highly saturated maps is needed to confirm that the putative minor QTLs are real and to detect epistatic interactions that may be associated with resistance.

The one resistance QTL contributed by W-7984 was detected by *Xksu916(Oxo)* on the long arm of chromo-

some 4A, and it explained 20% of the phenotypic variation. There is extensive evidence that this QTL is not only associated with resistance to tan spot, but also to other pathogens. The marker *Xksu916(Oxo)* represents a gene encoding oxalate oxidase (Li et al. 1999), and the probe used to identify this locus was a fragment of an oxalate oxidase gene cloned from barley (Zhou et al. 1998). Faris et al. (1999) showed that this locus was significantly associated with resistance to tan spot caused by a race 1 isolate, and it explained 17.5% of the variation. Effertz et al. (2001) also identified a minor QTL on chromosome 4AL for resistance to a tan spot race 3 isolate. A different mapping population was used and the *Xksu916(Oxo)* marker was not included, but the 4AL marker that they identified (*Xwg622*) maps in close proximity to *Xksu916(Oxo)* in the ITMI population. Therefore, it is likely that the minor QTL identified on 4AL by Effertz et al. (2001) is the same as that identified by Faris et al. (1997; 1999) and the one identified in this research.

In addition to having associations with resistance to tan spot, the oxalate oxidase locus [*Xksu916(Oxo)*] was found to be significantly associated with resistance to various pathotypes of leaf rust at the seedling and adult stages (Faris et al. 1999) and to powdery mildew (J. Faris, unpublished). Oxalate oxidase has been shown to be induced in barley and wheat seedlings following inoculation with the powdery mildew fungus (Dumas et al. 1995; Zhang et al. 1995; Hurkman and Tanaka 1996). Oxalate oxidase is a H_2O_2-generating enzyme that is thought to play a central role in a signal transduction pathway for regulation of the hypersensitive-response to pathogen attack (Zhou et al. 1998). Therefore, it seems likely that the oxalate oxidase gene on chromosome 4AL of W-7984 may play a significant role in conferring resistance to multiple pathogens, and it may be beneficial to introgress this allele into adapted germplasm.

It should be relatively efficient to select individual tan spot resistant RI lines of this population for production of resistant germplasm through backcrossing. Multiple RI lines possess the Ptr ToxB insensitivity allele from Opata 85 and the oxalate oxidase and Ptr ToxC insensitivity alleles from W-7984. Furthermore, the entire population is insensitive to Ptr ToxA and resistant to necrosis caused by race 2 isolates (Faris et al. 1997). Therefore, there are RI lines in this population that possess major factors for resistance to *P. tritici-repentis* races 1, 2, 3, and 5. Furthermore, the population also segregates for sensitivity to a host-selective toxin produced by the *Stagonospora nodorum* isolate Sn2000 which was shown to underlie a major QTL for resistance to *S. nodorum* blotch caused by the same isolate (Z.H. Liu, T.L. Friesen, S.W. Meinhardt, S. Ali, J.B. Rasmussen and J.D. Faris, personal communications). Toxin infiltration assays are simple and can be done at the seedling stage. Therefore, the toxins themselves can be used as selectable markers allowing for direct selection of the desired alleles. Assuming the oxalate oxidase gene is responsible for the effects observed on chromosome 4A, the marker *Xksu916(Oxo)* can also be used for direct selection of the W-7984 allele in backcrossing schemes. These markers should allow for the efficient development of germplasm that is resistant to tan spot and other diseases.

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