

The *Tsn1*–ToxA interaction in the wheat–*Stagonospora nodorum* pathosystem parallels that of the wheat–tan spot system

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Abstract: The wheat tan spot fungus (*Pyrenophora tritici-repentis*) produces a well-characterized host-selective toxin (HST) known as Ptr ToxA, which induces necrosis in genotypes that harbor the *Tsn1* gene on chromosome 5B. In previous work, we showed that the *Stagonospora nodorum* isolate Sn2000 produces at least 2 HSTs (SnTox1 and SnToxA). Sensitivity to SnTox1 is governed by the *Snn1* gene on chromosome 1B in wheat. SnToxA is encoded by a gene with a high degree of similarity to the *Ptr ToxA* gene. Here, we evaluate toxin sensitivity and resistance to *S. nodorum* blotch (SNB) caused by Sn2000 in a recombinant inbred population that does not segregate for *Snn1*. Sensitivity to the Sn2000 toxin preparation cosegregated with sensitivity to Ptr ToxA at the *Tsn1* locus. *Tsn1*-disrupted mutants were insensitive to both Ptr ToxA and SnToxA, suggesting that the 2 toxins are functionally similar, because they recognize the same locus in the host to induce necrosis. The locus harboring the *tsn1* allele underlies a major quantitative trait locus (QTL) for resistance to SNB caused by Sn2000, and explains 62% of the phenotypic variation, indicating that the toxin is an important virulence factor for this fungus. The *Tsn1* locus and several minor QTLs together explained 77% of the phenotypic variation. Therefore, the *Tsn1*–ToxA interaction in the wheat–*S. nodorum* pathosystem parallels that of the wheat–tan spot system, and the wheat *Tsn1* gene serves as a major determinant for susceptibility to both SNB and tan spot.

Key words: wheat, tan spot, *Stagonospora nodorum*, *Tsn1*, disease resistance.

Résumé : Le champignon causant la tache auréolée chez le blé (*Pyrenophora tritici-repentis*) produit une toxine spécifique de l'hôte (HST), connue sous le nom de Ptr ToxA, laquelle induit une nécrose chez les génotypes portant le gène *Tsn1* sur le chromosome 5B. Dans un travail antérieur, les auteurs avaient montré que l'isolat Sn2000 du *Stagonospora nodorum* produit au moins deux HST, SnTox1 et SnToxA. La sensibilité à SnTox1 est déterminée par le gène *Snn1* situé sur le chromosome 1B du blé. SnToxA est codée par un gène présentant une grande similarité avec le gène codant pour Ptr ToxA. Ici, les auteurs évaluent la sensibilité à la toxine et la résistance à la moucheture (SNB) causée par Sn2000 chez une population de lignées recombinantes fixées qui n'est pas en ségrégation pour *Snn1*. La ségrégation pour la sensibilité à la toxine Sn2000 était identique à celle pour la sensibilité à Ptr ToxA au locus *Tsn1*. Des mutants pour *Tsn1* étaient insensibles à Ptr ToxA et à SnToxA ce qui suggère que les deux toxines sont fonctionnellement semblables puisqu'elles reconnaissent le même locus chez l'hôte lors de l'induction de la nécrose. Le locus comprenant l'allèle *tsn1* sous-tend un QTL majeur pour la résistance à la moucheture causée par le Sn2000 et expliquait 62 % de la variation phénotypique, ce qui indique que la toxine constitue un important facteur de virulence pour ce champignon. Le locus *Tsn1* et plusieurs QTL mineurs expliquent ensemble 77 % de la variation phénotypique. Ainsi, l'interaction *Tsn1*–ToxA dans le pathosystème blé–*S. nodorum* est semblable à celle existant dans le système blé-tache auréolée et le gène *Tsn1* du blé constitue un déterminant majeur de la sensibilité à la fois à la moucheture et à la tache auréolée.

Mots clés : blé, tache auréolée, *Stagonosporum nodorum*, *Tsn1*, résistance à la maladie.

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Introduction

Stagonospora nodorum blotch (SNB) is caused by the fungus *Phaeosphaeria nodorum* (E. Mull.) Hedjar (anamorph: *Stagonospora nodorum*). It is a major foliar and glume disease of common wheat (*Triticum aestivum* L., $2n = 6x = 42$, AABBDD genomes) and durum (*T. turgidum* L., $2n = 4x = 28$, AABB genomes), and has the potential to cause yield losses up to 50% (King et al. 1983; Wicki et al. 1999). Use of host resistance is the most effective and preferred method to control disease. It has been reported that the inheritance of resistance to SNB is qualitative (Kleijer et al. 1977; Ma and Hughes 1995; Murphy et al. 2000; Kim et al. 2004), but most reports have found it to be quantitative (Scott et al. 1982; Fried and Meister 1987; Bostwick et al. 1993; Du et al. 1999; Wicki et al. 1999).

Quantitative trait loci (QTLs) mapping has been conducted in many crops, including wheat, and it is used to analyze disease resistance and agronomic traits (Gupta et al. 1999). Recently, this technique has been used to identify genomic regions governing SNB resistance. Czembor et al. (2003) identified, on chromosomes 2B, 3B, 5B, and 5D, QTLs associated with partial resistance to SNB in a doubled haploid population derived from a cross between the partially resistant 'Liwilla' and the susceptible 'Begra'. Schnurbusch et al. (2003) detected, on chromosomes 3BS, 4BL, and 5BL, QTLs associated with Stagonospora glume blotch resistance in Swiss winter wheat, and Arseniuk et al. (2004) identified a QTL on chromosome 6AL and a putative QTL on chromosome 6D associated with seedling resistance in the winter wheat 'Alba'. Liu et al. (2004b) identified a major QTL associated with seedling resistance to SNB in the International Triticeae Mapping Initiative (ITMI) population on chromosome 1BS, in addition to several minor QTLs. The detection of QTLs on multiple chromosomes in different studies indicates that different genes are associated with resistance in different tissues and in developmental stages.

Host-selective toxins are important in plant–fungal interactions because of their specificity in causing disease. The wheat tan spot pathogen *Pyrenophora tritici-repentis* produces multiple proteinaceous host-selective toxins, including Ptr ToxA (Tomás and Bockus 1987; Tomás et al. 1990), Ptr ToxB (Orolaza et al. 1995; Strelkov et al. 1999), and Ptr ToxD (Manning et al. 2002). Ptr ToxA, one of the best-characterized toxins, is a small protein, with a molecular mass of 13.2 kDa, that induces necrosis when infiltrated into sensitive wheat genotypes (Tuori et al. 1995). The fungal gene responsible for the production of the toxin has been isolated and characterized (Ballance et al. 1996; Ciuffetti et al. 1997). Host sensitivity to Ptr ToxA is associated with susceptibility to the fungus (Lamari and Bernier 1989), and conditioned by a single dominant gene, designated *Tsn1*, on the long arm of chromosome 5B (Faris et al. 1996; 2000; Anderson et al. 1999; Haen et al. 2004). Ptr ToxA has been implicated as the primary determinant of pathogenicity in races producing this toxin (Lamari and Bernier 1989; Ciuffetti et al. 1997), but recent study has shown that Ptr ToxA is a virulence factor rather than a pathogenicity factor in some wheat genotypes (Friesen et al.

2003), and that it has no significance in other genotypes (Faris and Friesen 2005).

A proteinaceous host-selective toxin (SnTox1) has been identified from a partially purified toxin preparation of *S. nodorum* isolate Sn2000. A single dominant gene, *Snn1*, which conditions sensitivity to partially purified SnTox1, has been mapped to the short arm of chromosome 1B in the host, using the ITMI population and wheat aneuploid stocks (Liu et al. 2004a). The toxin was determined to be a major virulence factor because the toxin-sensitivity locus accounted for as much as 58% of the phenotypic variation in disease caused by *S. nodorum* (Liu et al. 2004b). Recently, the whole-genome sequence of 1 isolate of *S. nodorum* was released, and a gene with a high degree of similarity to the *PtrToxA* gene was identified in the *S. nodorum* sequence (Friesen et al. 2006). Therefore, the toxin preparation used to identify SnTox1 also contained another host-selective toxin, designated SnToxA. Friesen et al. (2006) showed that the *ToxA* gene was native to *S. nodorum*, and was likely acquired by *P. tritici-repentis* through interspecific gene transfer, leading to the establishment of the tan spot pathogen.

The objectives of this study were to determine the chromosomal location of the gene conferring sensitivity to the Sn2000 toxin preparation in an intervarietal hard red spring wheat population that does not segregate for *Snn1*, to determine if *Tsn1* confers sensitivity to both Ptr ToxA and SnToxA, to evaluate the role of SnToxA in causing SNB, and to identify QTLs associated with seedling resistance to Sn2000.

Materials and methods

Plant materials

A segregating population of recombinant inbred lines was developed by James A. Anderson at the University of Minnesota, in St. Paul, from a cross between the Brazilian hard red spring wheat variety 'BR34' and the North Dakota hard red spring wheat variety 'Grandin'; it consists of 118 F_{7:9} lines (and is hereafter referred to as the BG population). The entire recombinant inbred line population, parents, F₁ plants, and 18 additional wheat genotypes (including common wheat, synthetic hexaploid wheat, and durum wheat), were infiltrated with Ptr ToxA and the Sn2000 toxin preparation, and inoculated with conidia of isolate Sn2000. *Tsn1*-disrupted mutants were generated using the hexaploid wheat cultivar 'Kulm' and the tetraploid durum wheat cultivar 'Langdon' (see section Generation and validation of *Tsn1* mutants).

Generation and validation of *Tsn1* mutants

A total of 5 ethylmethane-sulfonate- and 2 fast-neutron-induced *Tsn1*-disrupted mutants were evaluated for reaction to Ptr ToxA and the Sn2000 toxin preparation. The ethylmethane-sulfonate-induced toxin-insensitive mutant M103, developed in the Ptr ToxA-sensitive hexaploid cultivar 'Kulm', is described elsewhere (Friesen et al. 2003). An additional ethylmethane-sulfonate-induced mutant was developed in the sensitive tetraploid wheat 'Langdon' (LDNm937), using the methods described by Simons et al. (2006).

The 'Kulm' ethylmethane-sulfonate mutant M103 was crossed with the toxin-sensitive synthetic hexaploid wheat

TA4152-62 (developed at CYMMIT, in Mexico, and obtained from the Wheat Genetics and Genomics Resource Center in Manhattan, Kans.) to develop a segregating F_2 population. The population, consisting of 100 F_2 plants, was evaluated for reaction to Ptr ToxA. DNA was isolated from the F_2 plants, as described by Faris et al. (2000), and the population was genotyped using simple sequence repeat (SSR) markers *Xfcp1* and *Xfcp2*, developed by Lu et al. (2006), which are tightly linked to *Tsn1*. Cosegregation of markers with the phenotypic reaction to Ptr ToxA confirmed that the *Tsn1* gene was disrupted. In a similar manner, the LDNm937 mutant was validated by crossing it with the sensitive *Triticum dicoccoides* accession 36-12 (obtained from E. Nevo, Haifa, Israel), and evaluating Ptr ToxA reactions and the flanking SSR markers in the corresponding F_2 population.

For the generation of fast-neutron mutants, approximately 10000 'Langdon' seeds were exposed to fast neutrons (5 Gy) at the International Atomic Energy Agency, in Vienna, Austria. A subset of 700 M2 plants were screened for reaction to Ptr ToxA, which led to the identification of the *Tsn1*-deletion mutants LDNfn1733 and LDNfn2411. The fast-neutron mutants were screened with markers found to be tightly linked to *Tsn1* by Haen et al. (2004) and with the SSR markers described by Lu et al. (2006) to verify that a chromosomal segment encompassing the *Tsn1* locus was deleted.

Genotyping of 'Kulm' and 'Langdon' for *Snn1*

Because the Sn2000 toxin preparation contains both SnTox1 and SnToxA, we wanted to determine the genotypes of 'Langdon' and 'Kulm' at the *Snn1* locus to verify that these genotypes were sensitive only to SnToxA and not to SnTox1. 'Langdon' harbors the *Tsn1* gene on chromosome 5B. Therefore, the 'Langdon'–*T. dicoccoides* disomic chromosome 5B substitution line (LDN-DIC 5B), which is insensitive to Ptr ToxA because it has the *tsn1* allele, was tested for reaction to the Sn2000 toxin preparation to determine if 'Langdon' carries *Snn1* on chromosome 1B. To determine the genotype of 'Kulm' at the *Snn1* locus, we developed an F_2 population, consisting of 122 plants derived from 'Kulm' \times W-7976. W-7976 is a synthetic hexaploid wheat, developed at CYMMIT, and is sensitive to the Sn2000 toxin preparation but insensitive to Ptr ToxA, and therefore has the genotype *Snn1Snn1/tsn1tsn1*. The population was screened for reaction to both Ptr ToxA and the Sn2000 toxin preparation, and segregation ratios were analyzed to determine the genotype of 'Kulm' at the *Snn1* locus.

Toxin bioassays

Ptr ToxA was produced and purified from a race 2 isolate, as described by Zhang et al. (1997). The toxin preparation, containing SnToxA and SnTox1, was partially purified from culture filtrates of the *S. nodorum* isolate Sn2000, as described by Liu et al. (2004a). When the secondary leaf was fully expanded, it was assayed by infiltrating approximately 25 μ L of toxin, using a 1 mL syringe with the needle removed. The boundaries of the infiltration site were marked with a nontoxic felt pen before water-soaking disappeared. After infiltration, all plants were moved to a growth cham-

ber, at 21 °C, with a 12 h photoperiod. Leaves were evaluated 3 days after infiltration and scored as insensitive or sensitive. Sensitive reactions were characterized by the presence of necrosis throughout the infiltrated area of the leaf; insensitive reactions showed no visible necrosis. All experiments were replicated 3 times.

Plant inoculation and disease assessment

Three replications were used for disease evaluation. For each replication, 9 plants from each line were grown in cones, with 3 individuals per cone. Cones were placed into racks of 98, bordered by the susceptible genotype ND495 or 'Grandin'. All plants were grown in the greenhouse, at an average temperature of 21 °C, with a 16 h photoperiod. Plants were inoculated at the 2- or 3-leaf stage. Inoculum preparation and inoculations were done as described by Liu et al. (2004b). After inoculation, plants were placed in a mist chamber with a relative humidity of 100% for 24 h, followed by 6 days in a controlled growth chamber at 21 °C with a 12 h photoperiod. For disease evaluations, lesion types were scored on the 2nd leaf, using the 0–5 scale described by Liu et al. (2004b), where 0 is highly resistant and 5 is highly susceptible. All disease ratings were done 7 days after inoculation.

Genetic mapping

More than 700 molecular markers, spanning the entire genome, have been mapped in the BG population; details regarding molecular markers and map construction are described by Liu et al. (2005). For this research, reactions of recombinant inbred lines to the toxins were assigned genotypic values; they were placed using the TRY command in the Mapmaker program, v. 2.0, for Macintosh (Lander et al. 1987).

Statistical analysis

Segregation ratios of the 'Kulm' \times W-7976 population for reaction to Ptr ToxA and the Sn2000 toxin preparation were tested for fit to 1 insensitive : 3 sensitive and 1 insensitive : 15 sensitive ratios, respectively, using a χ^2 analysis ($P < 0.05$). Segregation ratios of toxin reactions in the BG population were tested for fit to a 1:1 ratio. Methods for QTL detection and analysis in the BG population were the same as those described by Faris and Friesen (2005) for detecting QTLs associated with tan spot resistance.

Results

Mapping the toxin-sensitivity locus

The BG population segregated for reaction to Ptr ToxA in a ratio of 59 sensitive : 59 insensitive, and exactly fit the expected 1:1 ratio for a single gene ($\chi^2 = 0$). The reactions to Ptr ToxA were converted to genotypic scores and mapped as *Tsn1* to the long arm of chromosome 5B, between markers *Xfcp300* and *Xfcp101*, at a logarithmic odds score (LOD) > 10.0 , at a position similar to that reported by Faris et al. (1996). Reactions to the Sn2000 toxin preparations containing SnToxA cosegregated perfectly with *Tsn1*.

Table 1. Reactions of parents, checks, and other wheat cultivars and lines to Ptr ToxA, the Sn2000 toxin preparation (SnTox1 + SnToxA), and conidial inoculations with the *Stagonospora nodorum* isolate Sn2000.

Cultivar or line	Wheat type and origin	Ptr ToxA	Sn2000 toxins*	Sn2000 [†]
'BR34'	HRSW, Brazil	I	I	0.0a
'Grandin'	HRSW, N.D.	S	S	4.50gh
'Atlas 66'	Winter wheat, N.C.	I	I	0.83b
'Bobwhite'	Spring wheat, CYMMIT	S	S	4.33gh
'Cheyenne'	Winter wheat, Nebr.	S	S	3.17e
'Chinese Spring'	Spring wheat, China	I	S	4.67h
'Erik'	HRSW, Colo	I	I	1.5c
'Glenlea'	HRSW, Canada	S	S	4.83h
'Hope'	HRSW, S.D.	S	S	4.0gh
'Katepwa'	HRSW, Canada	S	S	4.5gh
'Kulm'	HRSW, N.D.	S	S	4.33gh
'Langdon'	Durum, N.D.	S	S	3.5ef
ND2709	HRSW, N.D.	S	S	4.5gh
ND459	HRSW, N.D.	S	S	4.17gh
ND688	HRSW, N.D.	I	I	2.17d
'Opata 85'	HRSW, CYMMIT	I	I	2.17d
'Sumai 3'	Winter wheat, China	S	S	3.17e
'Timstein'	Spring wheat, Australia	S	S	3.67ef
W-7976	Synthetic wheat, CYMMIT	I	S	2.0cd
W-7984	Synthetic wheat, CYMMIT	I	S	2.0cd

Note: HRSW, hard red spring wheat; I, insensitive; S, sensitive; least significant difference = 0.61.

*Toxins partially purified from Sn2000 and contain SnTox1 and SnToxA.

[†]Conidial reactions. Numbers followed by the same letter are not significantly different at $P = 0.05$.

Validation that SnToxA and Ptr ToxA both recognize *Tsn1*

The ethylmethane-sulfonate- and fast-neutron-induced *Tsn1*-disrupted mutants were screened for reaction to both Ptr ToxA and the SnToxA-containing preparation from isolate Sn2000. All mutants were insensitive to both toxins, whereas the corresponding wild types were sensitive to both toxins (data not shown). The LDN-DIC 5B substitution line was insensitive to both Ptr ToxA and the Sn2000 toxin preparation, indicating that 'Langdon' has the genotype *snn1snn1/Tsn1Tsn1*. Therefore, the *Snn1*-SnTox1 interaction is not recognized in 'Langdon' or 'Langdon' mutants.

The 'Kulm' × W-7976 F₂ population segregated in a ratio of 90 sensitive:32 insensitive for reaction to Ptr ToxA, which fit the expected 3:1 ratio for a single gene (*Tsn1*) controlling the reaction ($\chi^2 = 0.99$, $0.90 > P > 0.75$). The same population segregated in a ratio of 112:10 for reaction to the Sn2000 toxin preparation, which fit the expected 15:1 ratio ($\chi^2 = 1.23$, $0.50 > P > 0.25$) for the independent segregation of 2 genes (*Snn1* and *Tsn1*). Therefore, 'Kulm' has the same genotype as 'Langdon' (*snn1snn1/Tsn1Tsn1*), whereas W-7976 has the genotype *Snn1Snn1/tsn1tsn1*. These data confirm that the Sn2000 toxin preparation contains 2 toxins (SnToxA and SnTox1), and they demonstrate that SnToxA and Ptr ToxA both recognize the product of the *Tsn1* gene in the host to induce necrosis.

Reactions of various wheat genotypes to toxins

Most wheat lines tested showed sensitive reactions (Table 1), suggesting that they harbor either *Snn1* or *Tsn1*. 'Chinese Spring' and the synthetic hexaploids W-7984 and

W-7976 are insensitive to Ptr ToxA and do not carry *Tsn1*, but do contain *Snn1*, which makes them sensitive to the Sn2000 toxin preparation. Five wheat lines ('BR34', 'Erik', 'Atlas 66', 'Opata 85', and 'ND688') were insensitive to both toxins, indicating that they do not contain *Snn1* or *Tsn1*. The remaining 12 lines were sensitive to Ptr ToxA and, therefore, also sensitive to the Sn2000 toxin preparation, which contains SnToxA. Of these 12 sensitive lines, we determined that 'Langdon', 'Kulm', and 'Grandin' carry the *snn1* allele and are therefore insensitive to SnTox1; the allelic state of the *Snn1* locus for the remaining 9 lines that are sensitive to both Ptr ToxA and the Sn2000 toxin preparation is unknown. The F₁ plants derived from 'BR34' × 'Grandin' were sensitive to both toxins (Fig. 1), indicating that sensitivity to both Ptr ToxA and SnToxA is dominant.

Reactions to Sn2000 conidial inoculations

The reactions of wheat genotypes to Sn2000 varied, and lesion types ranged from 0.0 to 4.83 (Table 1). Most wheat genotypes that were sensitive to the Sn2000 toxin preparation were susceptible to Sn2000. However, the synthetic hexaploid wheat lines W-7984 and W-7976 were sensitive and moderately resistant to Sn2000. 'BR34' was the most resistant among all tested genotypes, displaying a near-immune reaction to Sn2000. 'Grandin' was highly susceptible, with large coalescing necrotic/chlorotic lesions; it had a mean lesion type of 4.5. The F₁ plants developed lesion types of 3.0 after inoculation and were moderately susceptible, which suggests that overall susceptibility is not completely dominant.

For the BG population, analysis of variance indicated

Fig. 1. Reactions of ‘Grandin’, ‘BR34’, and their F₁ plants to infiltration with Ptr ToxA and SnToxA. Leaves A, B, and C were infiltrated with Ptr ToxA and leaves D, E, and F were infiltrated with the Sn2000 toxin preparation containing SnToxA. Leaves A and D, F₁ of ‘Grandin’/‘BR34’; leaves B and E, ‘Grandin’; and leaves C and F, ‘BR34’.

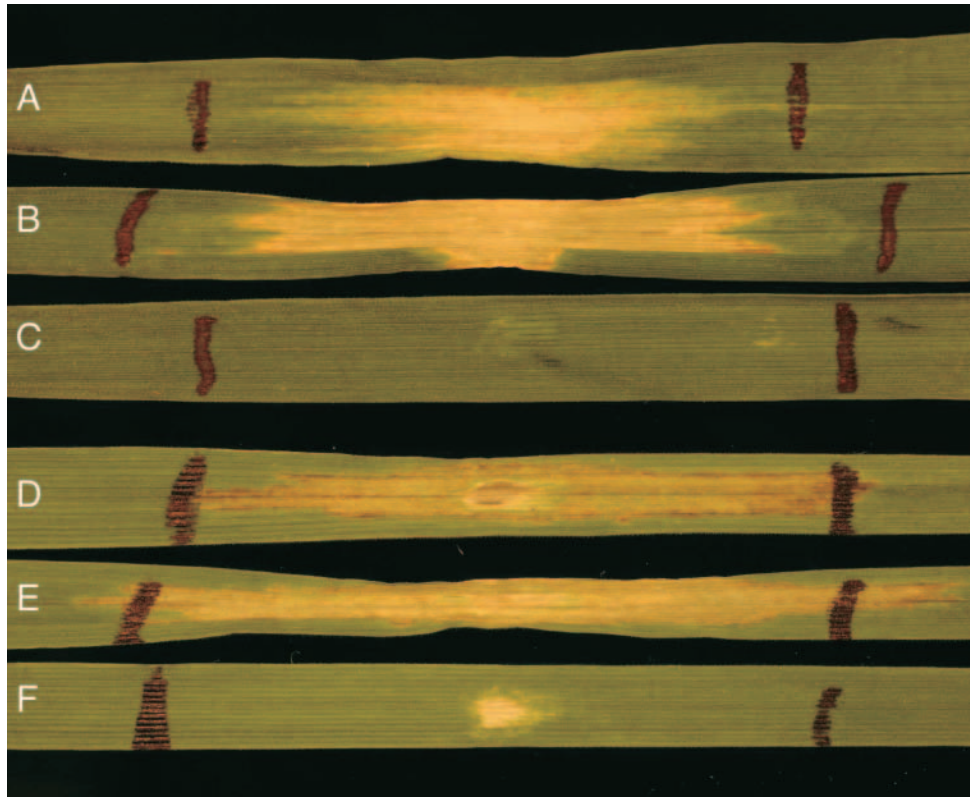
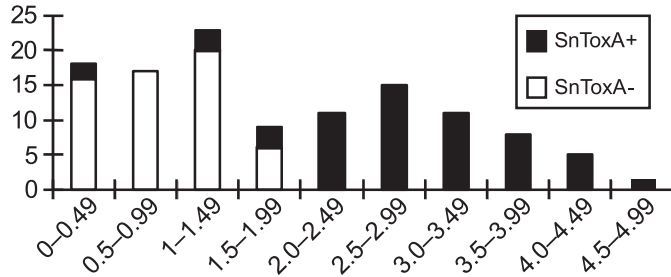


Fig. 2. Histogram of lesion-type means of the BG (cross between the Brazilian hard red spring wheat variety ‘BR34’ and the North Dakota hard red spring wheat variety ‘Grandin’) recombinant inbred population after inoculation with the *Stagonospora nodorum* isolate Sn2000.



highly significant differences among recombinant inbred lines for reaction to Sn2000. The average lesion type for the population was 1.75, but mean lesion types ranged from 0 to 4.5, indicating no transgressive segregation (Fig. 2). Toxin-insensitive lines were highly or moderately resistant, and their lesion types ranged from 0.0 to 1.67 (average, 0.77). Most toxin-sensitive lines were susceptible, with lesion types ranging from 2.0 to 4.5. Six toxin-sensitive lines had lesion types less than 2.0, of which lines 242 and 248 were as resistant as ‘BR34’ (Fig. 2). These 2 lines were further investigated for toxin and disease reaction, and no segregation was found within either line (data not shown).

Identification of QTLs for resistance to SNB

Three QTLs associated with SNB seedling resistance were

identified, and all resistance effects were derived from ‘BR34’. Simple linear regression of the genotypic marker scores against the mean lesion types indicated that *tsn1* on chromosome 5B was the marker most significantly associated with resistance ($P < 0.000001$). Simple interval regression revealed that *tsn1* defined the peak of the QTL on chromosome 5B, with a LOD value of 24.62, and explained 62% of the phenotypic variation (Table 2 and Fig. 3). This QTL was designated *QSnb.fcu-5BL.1*, based on the recommendation by McIntosh et al. (1998). Composite interval mapping revealed a 2nd QTL near the distal end of the long arm of chromosome 5B, which was designated *QSnb.fcu-5BL.2*. This QTL explained 6% of the phenotypic variation, and peaked between markers *Xbarc234* and *Xfcp273*, at a distance of 63 cM distal to *Tsn1* (Fig. 3).

There was a significant epistatic interaction detected between *tsn1* and *Xfcp273* (Table 3). Among toxin-insensitive lines, no significant difference in lesion-type means was observed, whether the locus *Xfcp273* carried the ‘BR34’ (0.70) or ‘Grandin’ allele (0.93). But lesion-type means were significantly different if *Xfcp273* had a different allele in the toxin-sensitive lines (3.25 vs. 2.37). The interaction between *tsn1* and *Xfcp273* accounted for 4% of phenotypic variation.

A 3rd QTL was identified on chromosome 1BS, designated *QSnb.fcu-1BS*. Composite interval mapping revealed that *QSnb.fcu-1BS* was located at the distal region of chromosome 1BS, peaked between markers *Xgdm33* and *Xgdm125*; it explained 10% of the phenotypic variation (Table 2 and Fig. 3). Simple linear regression indicated that

Table 2. QTLs for *S. nodorum* blotch seedling resistance in the BG recombinant inbred population detected by simple or composite interval mapping. Chromosomal locations, associated markers, peak positions, confidence intervals, R^2 , and LOD values are given.

Chromosome	Detected QTLs	Marker or marker interval	QTL peak (cM)	CI (cM)*	R^2 †	LOD
1BS	<i>QSnb.fcu-1BS</i>	<i>Xgdm33-Xgdm125</i>	8.0	6.0–14.0	10.0	7.92
5BL	<i>QSnb.fcu-5BL.1</i>	<i>tsn1</i>	102.0	99.0–102.0	62.0	24.62
5BL	<i>QSnb.fcu-5BL.2</i>	<i>Xbarc234-Xfcp273</i>	165.0	159.0–166.0	6.0	4.25

Note: QTLs, quantitative trait loci; LOD, logarithmic odds score.

*Centimorgan position of the 95% confidence interval for the QTL.

†The R^2 value of the multiple regression model containing *QSnb.fcu-5BL.1*, *QSnb.fcu-5BL.2*, *QSnb.fcu-1BS*, and the interaction between *QSnb.fcu-5BL.1* and *QSnb.fcu-5BL.2* = 77.0.

Fig. 3. Composite interval regression analysis revealed 1 major quantitative trait locus (QTL) (*QSnb.fcu-5BL.1*, black line) and a minor QTL (*QSnb.fcu-5BL.2*, grey line) on chromosome 5B, and a minor QTL (*QSnb.fcu-1BS*) on chromosome 1B in the BG population associated with resistance to Sn2000 conidial inoculations. The dotted line represents the logarithmic odds score (LOD) significance threshold of 3.0. A centimorgan (cM) scale is indicated on the y axis; the maximum LOD value for each QTL is indicated on the x axis.

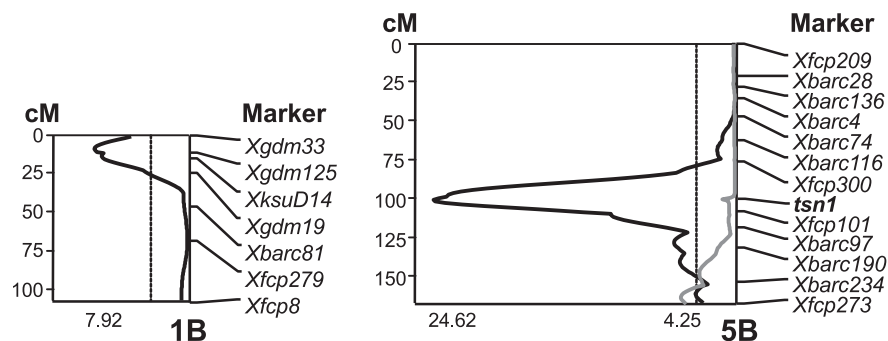


Table 3. Lesion-type means of the 4 possible classifications of recombinant inbred lines (RILs) for the allelic state at *Tsn1* and *Xfcp273* in the BG population inoculated with the *S. nodorum* isolate Sn2000.

<i>Tsn1</i>	<i>Xfcp273</i>	No. RILs	Lesion-type means*
'Grandin'	'Grandin'	27	3.25a
'BR34'	'Grandin'	23	0.93b
'Grandin'	'BR34'	24	2.37c
'BR34'	'BR34'	34	0.70b

*Numbers followed by the same letter are not significantly different at $P = 0.05$ level.

marker *Xfcp231* on the long arm of chromosome 5A and marker *Xbarc201* on the long arm of chromosome 6A were significantly ($P = 0.0004$) associated with resistance, and explained 11% and 10%, of the phenotypic variation, respectively. However, these 2 genomic regions were not significant in simple interval regression mapping or composite interval mapping analyses. The markers *tsn1*, *Xfcp273*, and *Xgdm125*, which were most closely associated with *QSnb.fcu-5BL.1*, *QSnb.fcu-5BL.2*, and *QSnb.fcu-1BL*, respectively, and the interaction between *tsn1* and *Xfcp273* were assembled into a multiple regression model. Together, these markers explained 77.0% of the total variation in resistance to SNB caused by isolate Sn2000.

Discussion

It has been shown in previous work that SnTox1 produced

by isolate Sn2000 interacts with *Snn1*, inducing sensitivity in genotypes such as 'Chinese Spring' and W-7984 wheat, which carry the dominant *Snn1* allele on the short arm of chromosome 1B (Liu et al. 2004a). Recently, it was found that a functional *ToxA*-like gene is present in many isolates of *S. nodorum*, including Sn2000; the toxin produced by this gene was designated SnToxA (Friesen et al. 2006). Therefore, there are at least 2 host-selective toxins, SnTox1 and SnToxA, in the Sn2000 partially purified toxin preparation. Whereas the ITMI population segregated only for sensitivity to SnTox1 when infiltrated with semipurified culture filtrate of isolate Sn2000, the BG population segregated for only SnToxA sensitivity, which cosegregated with sensitivity to Ptr ToxA at the *Tsn1* locus on chromosome 5BL. Here, we demonstrated, by analyzing *Tsn1*-disrupted mutants and molecular mapping, that Ptr ToxA and SnToxA, either directly or indirectly, interact with the product of the *Tsn1* gene to induce necrosis. Furthermore, SnToxA was shown to be a major factor in disease development, because the *Tsn1* locus accounted for 62% of the phenotypic variation. Therefore, the *Tsn1*-ToxA interaction should no longer be associated with the wheat-tan spot pathosystem only, but with the wheat-*S. nodorum* system as well.

The *Tsn1*-SnToxA interaction in the wheat-*S. nodorum* pathosystem parallels that of the *Tsn1*-Ptr ToxA interaction in the wheat-tan spot pathosystem. In the wheat-tan spot system, *Tsn1* has been shown to account for as much as 60% of the phenotypic variation in the development of tan spot (Cheong et al. 2004). Others have suggested that Ptr ToxA is a pathogenicity factor, and that the development of tan spot is entirely dependent on a compatible *Tsn1*-

Ptr ToxA interaction (Lamari and Bernier 1989; Ciuffetti et al. 1997). In contrast, Friesen et al. (2003) conducted tan spot conidial inoculation experiments, using 'Kulm' *Tsn1*-disrupted mutants, and showed that the elimination of the *Tsn1*-ToxA interaction did not preclude the development of disease. In the BG population, the *Tsn1* locus was not significantly associated with the development of tan spot caused by Ptr ToxA-producing isolates (Faris and Friesen 2005). Therefore, the significance of the *Tsn1*-Ptr ToxA interaction in the development of tan spot is dependent on the host genetic background; the same might be true for the *Tsn1*-SnToxA interaction in the development of SNB.

Originally, it was concluded that there was no clear gene-for-gene relationship within the wheat-*S. nodorum* interaction, and that host specificity was not distinct (Shipton et al. 1971; Eyal 1999). The results from this research, together with those from Liu et al. (2004a), strongly suggest that an inverse gene-for-gene interaction exists in the wheat-*S. nodorum* pathosystem, similar to that of the wheat-tan spot system. Worldwide, *S. nodorum* isolates have been collected and found capable of producing multiple different toxins; these toxins can recognize different sensitivity loci in the host (T.L. Friesen and J.D. Faris, USDA-ARS, Fargo, N.D., unpublished data).

Most studies have indicated that resistance to SNB is quantitatively inherited (Bostwick et al. 1993; Du et al. 1999; Czembor et al. 2003; Liu et al. 2004b). This study indicates that complete resistance is controlled by multiple QTLs, but that a single major SNB-resistance QTL, defined by the toxin-insensitivity locus (*tsn1*) on chromosome 5BL, explains most of the variation in disease. This suggests that toxin insensitivity is largely responsible for resistance to fungal infection, and it supports the previous conclusion that toxins play a role in disease development of SNB (Liu et al. 2004b).

Schnurbusch et al. (2003) identified a QTL on the long arm of chromosome 5B for *S. nodorum* glume blotch resistance in Swiss winter wheat that accounted for 7.5% to 14.4% of the phenotypic variation. Using interval mapping, it was found that the genomic region harboring this QTL appeared to be in the vicinity of *tsn1*, which could be responsible for the resistance effects. We showed that the *tsn1* locus underlies a major QTL for seedling resistance to leaf blotch, but its association with resistance to leaf and glume blotch on adult plants needs to be investigated further. SnToxA-producing isolates have been identified worldwide, and it is possible that the natural *S. nodorum* population contains *ToxA*, which led to the identification of the QTL on chromosome 5B by Schnurbusch et al. (2003).

The minor QTL *QSnb.fcu-1BS* identified in the BG population lies in a genomic region that corresponds to the location of the *snn1* gene mapped in the ITMI population (Liu et al. 2004b). Neither 'BR34' nor 'Grandin' carry *Snn1* (SnTox1 sensitivity), but it is possible that multiple alleles exist. Although only the *Snn1* allele can condition sensitivity to SnTox1, 'BR34' might possess different insensitivity alleles with variability in function. The 'BR34' allele might confer a higher level of partial resistance than the 'Grandin' allele. It is interesting to note that Kleijer et al. (1977) reported that a single dominant gene on chromosome 1B conferred a high level of SNB resistance in the cultivar

'Atlas 66', which we found to be insensitive to SnTox1 and highly resistant to Sn2000 (Table 1). The gene in 'Atlas 66' might be allelic to *snn1* and (or) *QSnb.fcu-1BS*. It is also possible that *snn1* and the gene underlying *QSnb.fcu-1BS* are closely linked but different genes. The genomic region containing *snn1* and *QSnb.fcu-1BS* is known to be extremely gene-rich, and harbors many agronomically important genes (Erayman et al. 2004). Analysis and fine-mapping of this region in a large population segregating for both *Snn1* and *QSnb.fcu-1BS* could be used to resolve this issue, and to elucidate the relationship between *snn1/QSnb.fcu-1BS* and the resistance gene in 'Atlas 66'.

Toxin-insensitivity loci in both the ITMI and BG populations are major QTLs for resistance, and several cultivars, such as 'BR34', ND688, and 'Erik', with high levels of resistance are insensitive to both toxins. This suggests that selection for toxin insensitivity can result in a high level of resistance in cultivars. Insensitivity to SnTox1 and SnToxA are highly correlated with resistance to SNB, but toxin insensitivity is recessively inherited. Therefore, selection for the *tsn1* and (or) *snn1* alleles for toxin insensitivity and enhanced SNB resistance would be more efficiently accomplished with a marker-assisted selection scheme. Selection based on phenotypes alone will not distinguish between sensitive homozygotes and heterozygotes in progeny derived from backcrosses to sensitive recurrent parents. Appropriate molecular markers will allow the heterozygotes to be identified and selected for in subsequent rounds of backcrossing, while retaining the desired insensitivity allele. We are in the process of developing SSR markers tightly linked to the *Snn1* gene, and user-friendly SSR markers for the *Tsn1* gene are already publicly available (<http://maswheat.ucdavis.edu/protocols/Tsn1/index.htm>) (Lu et al. 2006).

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