Theor Appl Genet (2009) 118:1489–1497
DOI 10.1007/s00122-009-0997-2

ORIGINAL PAPER

Host-selective toxins produced by *Stagonospora nodorum* confer disease susceptibility in adult wheat plants under field conditions

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Received: 17 November 2008 / Accepted: 13 February 2009 / Published online: 6 March 2009

Abstract *Stagonospora nodorum,* causal agent of Stagonospora nodorum blotch (SNB), is a destructive pathogen of wheat worldwide. As is true for many necrotrophic host–pathogen systems, the wheat-*S. nodorum* system is complex and resistance to SNB is usually quantitatively inherited. We recently showed that *S. nodorum* produces at least four proteinaceous host-selective toxins that interact with dominant host sensitivity/susceptibility gene products to induce SNB in seedlings. Here, we evaluated a population of wheat recombinant inbred lines that segregates for *Tsn1*, *Snn2*, and *Snn3*, which confer sensitivity to the toxins SnToxA, SnTox2, and SnTox3, respectively, to determine if compatible host–toxin interactions are associated with adult plant susceptibility to SNB foliar disease under field conditions. Artificial inoculation of the population in 2 years and two locations with a fungal isolate known to produce SnToxA and SnTox2 indicated that compatible SnToxA–*Tsn1* and SnTox2–*Snn2* interactions accounted for as much as 18 and 15% of the variation in disease severity on the flag leaf, respectively. As previously reported for seedlings, the effects of these two interactions in conferring adult plant susceptibility were largely additive. Additional adult plant resistance QTLs were identified on chromosomes 1B, 4B, and 5A, of which, the 1B and 5A QTLs were previously reported to be associated with seedling resistance to SNB. Therefore, in this population, some of the same QTLs are responsible for seedling and adult plant resistance/susceptibility. This is the first report showing that host-selective toxins confer susceptibility of adult plants to SNB, further substantiating the importance of compatible toxin–host interactions in the wheat-*S. nodorum* pathosystem.

Communicated by X. Xia.

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Introduction

Stagonospora nodorum blotch (SNB) of wheat (*Triticum aestivum*, $2n = 6x = 42$), caused by *Stagonospora nodorum* (teleomorph: *Phaeosphaeria nodorum*), is an economically important disease in most wheat growing regions of the world. This disease has been shown to cause yield losses as high as 31% in areas where susceptible varieties are grown under favorable environmental conditions (Bhathal et al. 2003). In most areas where SNB is a problem, *S. nodorum* has a sexual stage that produces pseudothecia which serves as the over-seasoning structure for this fungus. Pseudothecia eject airborne ascospores which serve as primary inoculum early in the growing season (reviewed in Solomon et al. 2006b). The asexual stage is a repeating cycle consisting of

the production of lesions on leaf tissue followed by the production of pycnidia within the lesions (Solomon et al. 2006b). Pycnidia produce conidia (pycnidial spores) that are splash-dispersed up the plant foliage throughout the growing season (Solomon et al. 2006a). Considering this life cycle, the combination of both seedling and adult plant resistance in the same cultivar is important in stopping or slowing the progress of this fungal pathogen.

SNB resistance has been reported to be quantitatively inherited in some instances and qualitatively inherited in others. Frecha (1973) identified monogenic SNB resistance in the cultivar 'Atlas 66' and located the gene on chromosome 1B. Other monogenic resistance has also been reported, but the majority of recent work has shown SNB resistance on both the leaf and glume to be quantitatively controlled (Xu et al. 2004; Friesen et al. 2008b). Several field evaluations of SNB on the leaf and glume have been performed (Schnurbusch et al. 2003; Toubia-Rahme and Buerstmayr 2003; Czembor et al. 2003; Arseniuk et al. 2004; Aguilar et al. 2005) and each study indicated that adult plant resistance was quantitatively inherited with no single QTL accounting for more than 37% of the variation in disease severity and the total number of QTL ranged from 2 to 11 (reviewed in Friesen et al. 2008b).

The wheat-*S. nodorum* pathosystem involves multiple host-selective toxins (HSTs) that are recognized by corresponding dominant host sensitivity genes in an inverse gene-for-gene manner to confer susceptibility to the pathogen (Liu et al. 2004a, b, 2006; Friesen et al. 2006, 2007, 2008a). Sensitivity to these HSTs is highly correlated with the development of SNB in seedlings, but it has not been shown whether compatible host–toxin interactions are associated with susceptibility of adult plants under field conditions. The objective of the current work was to determine if compatible *Tsn1*–SnToxA and *Snn2*–SnTox2 interactions are associated with the development of SNB on adult plants in field environments.

Materials and methods

Biological materials

A population of 118 $F_{7:9}$ recombinant inbred lines (RILs) from a cross between the Brazilian hard red spring wheat (HRSW) variety 'BR34' and the North Dakota HRSW variety 'Grandin' (hereafter referred to as the BG population) was developed by and obtained from Dr. James A. Anderson, University of Minnesota, St. Paul, MN. This population has been shown to segregate for SNB susceptibility and sensitivity to three HSTs including SnToxA (Liu et al. 2006; Friesen et al. 2006), SnTox2 (Friesen et al. 2007), and SnTox3 (Friesen et al. 2008a).

The *S. nodorum* isolate BBCSn5 was collected from a wheat field near Moorhead, MN and was used for all field and seedling inoculations. BBCSn5 was chosen due to its production of both SnToxA and SnTox2 in culture (data not shown). BR34 is highly resistant to the majority of North American *S. nodorum* isolates and Grandin is susceptible to most North American isolates (Liu et al. 2006; Friesen et al. 2006, 2007, 2008a). The QTL analysis of seedling inoculations using BBCSn5 has shown that this isolate induced susceptibility of approximately equal significance at both *Tsn1* and *Snn2*, the loci that confer sensitivity to SnToxA and SnTox2, respectively, and was therefore used in field inoculations to evaluate the significance of these two loci in adult plant field reactions.

Seedling disease evaluations

Three replications were used for disease evaluation. For each replication, nine plants of each line were grown in cones with three individuals per cone. The cones were placed into racks of 98 bordered by the susceptible parent Grandin. All the plants were grown in the greenhouse at an average temperature of 21°C with a 16-h photoperiod. The plants were inoculated at the two- to three-leaf stage. Inoculum preparation and inoculations were done as described in Liu et al. (2004b) with minor modification. Briefly, isolate BBCSn5 was grown on V8-PDA agar (150 ml V8 juice, 3 g $CaCo_3$, 30 g sucrose, 10 g Difco PDA, 10 g agar in 1,000 ml water) by spreading 200 µl of a pycnidial spore suspension onto a V8PDA plate using a sterile inoculating loop. Cultures were then grown for 5–7 days and plates were washed with sterile distilled water and spores were further diluted to 1×10^6 ml^{-1} for inoculations. One drop of Tween 20 per 100 ml solution was added to the inoculum before inoculation, which was conducted by spraying the prepared inoculum with a hand-held pressurized sprayer. Following inoculations, plants were placed in a mist chamber at 100% relative humidity at room temperature for 24 h followed by incubation in growth chambers with a 12-h photoperiod and constant temperature of 21°C.

After inoculation, plants were placed in a mist chamber with relative humidity at 100% for 24 h followed by 6 days in a controlled growth chamber at 21°C under a 12-h photoperiod using fluorescent lights. For disease evaluations, lesion types were scored on the second leaf using the 0–5 scale described in Liu et al. (2004b) where 0 is highly resistant and 5 is highly susceptible. All seedling disease ratings were done 7 days post-inoculation.

Field evaluation methods

The BG population was evaluated for SNB in 2004 and 2005 in Fargo, ND and in 2004 in Langdon, ND. Nursery

planting dates were May 4, 2004 and April 22, 2005 for Fargo and May 27, 2004 for Langdon. Nurseries were planted in a randomized complete block design with three replicates planted in hill plots of 15 seeds per replicate. Nurseries were planted four hills wide with 30 cm spacing between hills in both directions.

Inoculum for *S. nodorum* isolate BBCSn5 was produced in the lab as described in Liu et al. (2004b). Spore concentrations were adjusted to 1×10^6 spores per ml for inoculation. Approximately 2 L of inoculum was sprayed onto each replicate in a nursery using a pump style sprayer (Chapin International Inc, Batavia, NY, USA). Inoculum was applied when the majority of the plants were at least in the boot stage (Zadoks growth stage 49) (Zadoks et al. 1974). Nurseries were supplemented with misting as needed in order to keep the leaves of the plants wet for 72 h after inoculation. Nurseries were evaluated when acceptable levels of disease appeared on the susceptible parent Grandin. Time from inoculation to disease evaluation was dependant on environmental conditions and ranged from 10 to 14 days post-inoculation.

Three main stems were sampled and evaluated collectively for disease reaction type using the 0–5 scale of Liu et al. (2004b) where 0 = absence of visible lesions (highly resistant) 1 = few penetration points with lesions consisting of small dark spots (resistant); 2 = lesions consisting of dark spots with little surrounding necrosis/chlorosis (moderately resistant); 3 = dark lesions completely surrounded by necrosis or chlorosis, lesions 2–3 mm (moderately susceptible); 4 = larger necrotic or chlorotic lesions 4 mm or greater with little coalescence (susceptible); and 5 = large coalescing lesions with very little green tissue remaining (highly susceptible).

The three flag and the three penultimate leaves (flag-1) were evaluated and a visual average of disease reaction type (Liu et al. 2004b) was recorded. Lines showing approximately equal percentages of two lesion types were given an intermediate reaction type (e.g. lines showing equal percent of 1 and 2 type reactions were given a 1.5 reaction type). Disease reaction types were used because this is the best method to measure necrotic lesion size which is a result of the toxin–host gene interaction. Percent leaf area showed similar QTL patterns and were therefore not included in the analysis.

Statistical and QTL analysis

Bartlett's χ^2 was calculated to test the homogeneity of variances among different experiments using the SAS program (SAS Institute 2006), and data from homogeneous experiments were combined for both statistical and QTL analysis. Mean reaction types of the groups of lines categorized based on sensitivities to SnToxA and SnTox2 were compared using Fisher's protected least significant difference (LSD) at $\alpha = 0.05$ (SAS Institute 2006).

Methods for QTL detection and analysis in the BG population were the same as those described previously (Faris and Friesen 2005; Liu et al. 2006; Friesen et al. 2007; 2008a). Briefly, simple linear regression was conducted to identify markers significantly associated with susceptibility at the 0.001 level of probability. Simple and composite interval mapping (SIM and CIM) were performed using a subset of 324 markers (Liu et al. 2005) giving complete genome coverage to identify marker interval associated with susceptibility. The critical LOD threshold of 3.0 was calculated by executing 1,000 permutations. Single markers with significant main effects were assembled into multiple regression models to determine the total amount of variation explained by the model. All calculations were performed using either the Map Manager QTX software package (Manly et al. 2001) or qGene v3.06 (Nelson 1997).

Results

Association of *Tsn1* and *Snn2* with SNB in seedlings

Seedling reactions to *S. nodorum* isolate BBCSn5 ranged from 0.17 to 4.67 with a population mean of 2.3. Significant differences were identified between genotypic groups *Tsn1* (SnToxA sensitive allele) and *tsn1* (SnToxA insensitive allele) as well as for *Snn2* (SnTox2 sensitive allele) and *snn2* (SnTox2 insensitive allele) (Table 1). When considering both the *Tsn1* and *Snn2* genotypic classes, significant differences were identified when either *Tsn1* or *Snn2* was

Table 1 LSD analysis of reaction type differences based on single toxin sensitivity genes (i.e. *Tsn1* or *Snn2*)

Data set[A]	Mean (+)[B]	Mean (–)[B]	$LSD_{0.05}$
Flag leaf F05 + L04 for *Tsn1*	2.11a	1.58b	0.13
Flag leaf F05 + L04 for *Snn2*	2.01a	1.65b	0.14
Flag leaf F04 for *Tsn1*	1.97a	1.60b	0.15
Flag leaf F04 for *Snn2*	1.89a	1.66b	0.16
Flag-1 leaf F04 + F05 for *Tsn1*	2.72a	2.28b	0.12
Flag-1 leaf F04 + F05 for *Snn2*	2.65a	2.32b	0.12
Flag-1 leaf L04 for *Tsn1*	2.85a	2.49b	0.16
Flag-1 leaf L04 for *Snn2*	2.73a	2.60a	0.16
Seedling for *Tsn1*	2.90a	1.69b	0.18
Seedling for *Snn2*	2.70a	1.80b	0.20

[A] F04, F05 and L04 represent the data collected from the experiments conducted in Fargo in 2004, 2005, and Langdon in 2004, respectively

[B] + and – signifies the presence and absence, respectively, of the corresponding dominant toxin sensitivity gene (i.e. *Tsn1* or *Snn2*). Within a row numbers followed by the same letter are not significantly different from one another at the 0.05 level of probability

Table 2 Reaction type (RT) differences based on all possible allelic combinations of *Tsn1* and *Snn2*

Data set	Mean				$LSD_{0.05}$
	tsn1/snn2	*tsn1/Snn2*	*Tsn1/snn2*	*Tsn1/Snn2*	
Flag leaf F05 + L04	1.46a	1.71b	1.87b	2.34c	0.18
Flag leaf F04	1.61a	1.60a	1.72a	2.21b	0.21
Flag-1 leaf F04 + F05	2.17a	2.39b	2.50b	2.93c	0.16
Flag-1 leaf L04	2.55a	2.44a	2.66ab	3.04c	0.22
Flag leaf combined	1.78a	1.89ab	2.00b	2.46c	0.12
Flag-1 leaf combined	2.74a	2.75a	2.97b	3.29c	0.15
Seedling	1.35a	2.04b	2.34b	3.41c	0.22

Within the same row, numbers followed by the same letter are not significantly different from one another at the 0.05 level of probability

present compared to the classes where both were absent (*tsn1/snn2*) and where both were present (*Tsn1/Snn2*). However, no significant difference between the *tsn1/Snn2* and *Tsn1/snn2* was observed (Table 2) indicating that compatible *Tsn1*–SnToxA and *Snn2*–SnTox2 interactions contribute similarly to SNB and that their effects are additive.

In QTL analysis, the *Tsn1*–ToxA interaction was the most important factor in seedling disease accounting for 37% of the variation (Table 3; Fig. 1). *Snn2* was also highly important in seedling disease accounting for 24% of the variation. The QTLs associated with *Tsn1* and *Snn2* were given the designations *QSnb.fcu-5BL* and *QSnb.fcu-2DS*, respectively.

An additional QTL on chromosome 5AL designated *QSnb.fcu-5AL*, which is not associated with a known toxin sensitivity locus, accounted for 11% of the variation. Each of these loci was shown to have additive effects (Table 3; Fig. 1), which is reflected in a multiple regression model which accounts for 66% of the total variation in disease (Table 4) similar to what was previously shown for other isolates of *S. nodorum* (Friesen et al. 2007; 2008a). All three of these resistance QTL (or lack of susceptibility) were contributed by the resistant parent BR34.

Association of *Tsn1* and *Snn2* with SNB susceptibility of adult plants in the field

Reaction type data were collected for adult plant field reactions on both the flag leaf and the flag-1 at Fargo, ND 2004 (F04), Fargo, ND 2005 (F05), and Langdon, ND 2004 (L04). Tests for homogeneity of variances indicated that, for data collected on the flag leaf, the L04 and the F05 experiments were homogeneous but F04 was heterogeneous. For the flag-1 data, F04 and F05 were homogeneous and L04 was heterogeneous. Therefore, the L04 and F05 flag leaf data were combined, and the F04 and F05 flag-1 data were combined for QTL analysis but the F04 flag leaf data and the L04 flag-1 data were analyzed separately. For comparative purposes, QTL analysis was also conducted on all flag leaf data combined, and on all flag-1 data combined.

Average adult plant disease reactions of the genotypic classes involving *Tsn1* and *Snn2* are reported in Tables 1 and 2. Significant differences in average disease reaction types were observed between RI lines carrying the *Tsn1* (SnToxA sensitive) allele compared to RI lines carrying the *tsn1* (SnToxA insensitive) allele for all individual locations and combinations analyzed (Table 1). Significant differences in average disease reaction types were also observed between the *Snn2* class compared to the *snn2* class for all individual locations and combinations analyzed except for the flag-1 reaction type at L04 (Table 1).

Analysis of the *Tsn1* and *Snn2* allelic combinations indicated that significant differences occurred between the *tsn1/snn2* and *Tsn1/Snn2* classes, *tsn1/Snn2* and *Tsn1/Snn2* classes, and between the *Tsn1/snn2* and *Tsn1/Snn2* classes in all field experiments or combinations thereof (Table 2). Differences were observed between the *tsn1/snn2* and *tsn1/Snn2* classes in the F05 + L04 flag leaf data and in the F04 + F05 flag-1 data (Table 2). No significant differences were seen between those lines that carried only SnToxA sensitivity (*Tsn1/snn2*) and those that carried only SnTox2 sensitivity (*tsn1/Snn2*) except for flag-1 combined, indicating that in this population, similar levels of disease were conferred by compatible SnToxA–*Tsn1* and SnTox2–*Snn2* interactions.

QTL analysis of SNB in adult plants

Where significant, *QSnb.fcu-2DS* (*Snn2)* accounted for 8–15% of the variation in disease across the three environments on the flag leaf and on the flag-1 compared to 24% association with disease in the seedling stage (Table 3; Fig. 1). However, *QSnb.fcu-2DS* was not significantly associated with disease in the flag-1 F04 + F05 data or in the flag-1 combined average (Table 3; Fig. 1).

The QTL *QSnb.fcu-5BL* (*Tsn1*) was significantly associated with disease in all environments including both the flag leaf and the flag-1 data, and it explained from 11 to 20% of the variation in SNB on adult plants compared to 37% in the seedling stage (Table 3; Fig. 1). The importance

Table 3 Detected QTLs for Stagonospora nodorum blotch resistance/susceptibility in the population of recombinant inbred lines derived from BR34 × Grandin

Chr. arm	QTL[a]	Marker Interval	Leaf	Environment[b]	Peak	LOD	R^2	Add.
1BS	*QSnb.fcu-1BS* (BR34)	*Xfcp267-Xbarc240*	Seedling	Greenhouse	–	NS	–	–
			Flag	F04	–	NS	–	–
			Flag	L04 + F05	–	NS	–	–
			Flag	Combined	4	3.3	0.10	0.14
			Flag-1	L04	4	4.2	0.12	0.17
			Flag-1	F04 + F05	4	4.1	0.13	0.19
			Flag-1	Combined	4	5.0	0.13	0.18
2DS	*QSnb.fcu-2DS* (BR34)	*Xgwm614-Xcfd53*	Seedling	Greenhouse	5	13.1	0.24	0.50
			Flag	F04	13	3.3	0.08	0.16
			Flag	L04 + F05	14	6.0	0.15	0.20
			Flag	Combined	14	6.3	0.13	0.17
			Flag-1	L04	11	4.9	0.12	0.19
			Flag-1	F04 + F05	–	NS	–	–
			Flag-1	Combined	–	NS	–	–
4BS	*QSnb.fcu-4BS* (Grandin)	*Xwmc47-Xfcp301*	Seedling	Greenhouse	–	NS	–	–
			Flag	F04	–	NS	–	–
			Flag	L04 + F05	34	3.4	0.07	0.14
			Flag	Combined	–	NS	–	–
			Flag-1	L04	–	NS	–	–
			Flag-1	F04 + F05	–	NS	–	–
			Flag-1	Combined	33	3.6	0.07	0.14
5AL	*QSnb.fcu-5AL* (BR34)	*Xbarc151-Xfcp13*	Seedling	Greenhouse	162	4.0	0.11	0.33
			Flag	F04	159	4.3	0.12	0.21
			Flag	L04 + F05	161	4.2	0.14	0.18
			Flag	Combined	161	5.8	0.18	0.20
			Flag-1	L04	161	4.5	0.15	0.20
			Flag-1	F04 + F05	161	3.6	0.09	0.18
			Flag-1	Combined	160	6.0	0.18	0.22
5BL	*QSnb.fcu-5BL* (BR34)	*Xbarc1116-Xbarc43*	Seedling	Greenhouse	107	18.0	0.37	0.61
			Flag	F04	106	3.7	0.11	0.17
			Flag	L04 + F05	103	6.0	0.17	0.20
			Flag	Combined	104	6.7	0.18	0.20
			Flag-1	L04	105	7.7	0.20	0.22
			Flag-1	F04 + F05	101	4.4	0.12	0.20
			Flag-1	Combined	102	6.6	0.17	0.21

[a] The parent contributing effects for resistance is shown in parentheses

[b] F04, F05 and L04 represent the data collected from the experiments conducted in Fargo in 2004, 2005, and Langdon in 2004, respectively

The chromosome arm, QTL designation, and marker interval are presented, and when significant, the leaf, environment, peak position, LOD value, R^2, and additive effects are also presented. The 2DS and 5BL QTL correspond to *Snn2* (SnTox2 sensitivity) and *Tsn1* (SnToxA sensitivity), respectively

of the *QSnb.fcu-5BL* (*Tsn1)* and *QSnb.fcu-2DS* (*Snn2)* QTL was consistent between seedling and adult plant reactions where the effects of a compatible SnToxA–*Tsn1* interaction were slightly more significant in disease than were the effects of a compatible SnTox2–*Snn2* interaction.

Similar to that observed in seedlings, the effects of *Tsn1* and *Snn2* were largely additive. Additive gene action of the two toxin sensitivity loci was evidenced by a multiple regression model using only *Tsn1* and *Snn2* associated markers. This analysis showed that together, *Tsn1* and *Snn2* accounted for 29% of the variation in SNB on the flag leaf compared to individual effects of 18 and 13%, respectively.

Additional QTL not known to be associated with toxin sensitivity loci were also identified in the adult plant

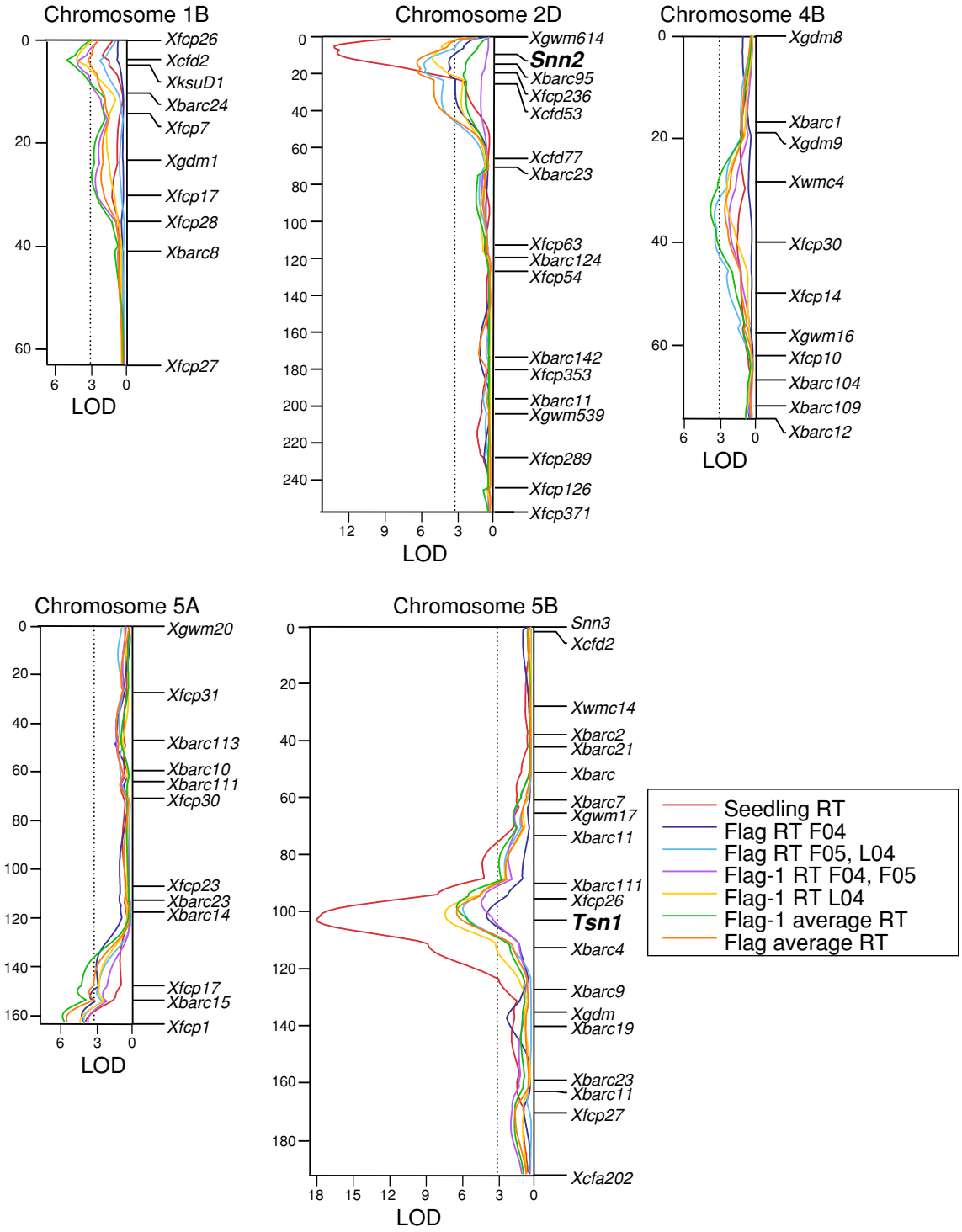

Fig. 1 Composite interval mapping of QTL associated with Stagonospora nodorum blotch on chromosomes 1B, 2D (*Snn2*), 4B, 5A, and 5B (*Tsn1*) in the population of recombinant inbred lines derived from BR34 × Grandin. *Markers* are shown along the right of the graphs and centiMorgan distances are along the left. The *dotted line* represents the critical LOD threshold of 3.0. Different *colored lines* represent different data sets, which are indicated in the *boxed legend* to the lower right

experiments. *QSnb.fcu-5AL*, which was associated with seedling resistance, was also associated with adult plant resistance in all experiments, and it accounted for 9–18% of the variation in disease. QTL on chromosome arms 1BS and 4BS, designated *QSnb.fcu-1BS* and *QSnb.fcu-4BS*, were also associated with susceptibility in some experiments accounting for as much as 13 and 7% of the variation, respectively, (Table 3; Fig. 1).

Table 4 Molecular markers used in multiple regression models for Stagonospora nodorum blotch in seedling data, combined flag leaf data, and combined flag-1 data, along with the *P* and R^2 values of the models

Data	Markers in model (chromosome arm)	*P* value	R^2
Seedling	*Snn2* (2DS), *Xfcp13* (5AL), *Tsn1* (5BL)	≤0.0001	0.66
Flag combined	*Xcfd21* (1BS), *Xfcp236* (2DS), *Xfcp13* (5AL), *Tsn1* (5BL)	≤0.0001	0.49
Flag-1 combined	*Xcfd21* (1BS), *Xfcp301* (4BS), *Xfcp13* (5AL), *Tsn1* (5BL)	≤0.0001	0.44

Multiple regression analysis of the combined means of the flag leaf reaction types was done using the markers most closely associated with *QSnb.fcu-1BS (Xcfd21), QSnb.fcu-2DS (Snn2), QSnb.fcu-5AL (Xfcp13)*, and *QSnlb.fcu-5BL (Tsn1)*. The multiple regression model for the flag leaf reaction type data accounted for 49% of the disease variation (Table 4). Multiple regression analysis was also done for the flag-1 disease reaction type data and included the markers associated with *QSnb.fcu-1BS, QSnb.fcu-4BS, QSnb.fcu-5AL*, and *QSnb.fcu-5BL*. The resulting multiple regression model explained 44% of the variation in SNB that occurred on the flag-1 leaf. *QSnb.fcu-2DS* (*Snn2)* was not significant in the combined mean and was therefore not used in the flag-1 multiple regression analysis.

Discussion

Knowing the mechanism of spread and the polycyclic, splash-dispersed nature of this disease, it is important that both seedling and adult plant resistance be deployed in popular cultivars. The current work shows that toxin sensitivity genes such as *Tsn1* and *Snn2*, present in the host, are important in disease development and therefore in the repeating cycle of the pathogen. Elimination of these host sensitivity/susceptibility genes is critical to being able to control SNB.

We previously showed that *S. nodorum* HSTs are important in development of SNB in seedlings with individual toxin sensitivity loci accounting for as much as 62% (Liu et al. 2006) of the disease variation. Several studies have been conducted to evaluate seedling resistance, adult plant foliar resistance, and glume blotch resistance independently (reviewed in Xu et al. 2004 and Friesen et al. 2008b), but little correlation has been shown for resistance for the different growth stages or different plant parts. In this study, we found that *Tsn1*, *Snn2* and a QTL on chromosome arm 5AL were significantly associated with SNB development in both the adult plant and seedling stages whereas QTL on chromosome arms 1BS and 4BS were significantly associated with SNB in adult plants only with 4BS only being significant in the F04 flag and combined flag-1 reaction.

To date, four *S. nodorum* HSTs and their corresponding host sensitivity genes have been identified and characterized (Liu et al. 2004a; Friesen et al. 2006, 2007, 2008a). However, until this study, no association between a toxin–host gene interaction and adult plant resistance/susceptibility had been identified. This study shows that *S. nodorum* HSTs are not only important in conferring susceptibility at the seedling stage, but that they are also highly important in the adult plant interaction as well. This work indicates that toxins can be important in the early stages of seedling infection making them important in the secondary spread of the fungus all the way to the flag leaf where grain size and quality can be affected. The current study should influence the way we perceive the epidemiology of this disease and should underscore the importance of breeding to eliminate HST sensitivity genes such as *Tsn1* and *Snn2* from elite germplasm and cultivars.

In seedling, flag, and flag-1 reactions, no significant differences were identified between lines carrying only *Tsn1* (SnToxA sensitivity) and those carrying only *Snn2* (SnTox2 sensitivity), however, both of these classes were significantly different from lines carrying both *Tsn1* and *Snn2*. This result indicates that when using the BBCSn5 isolate, compatible *Tsn1*–SnToxA and *Snn2*–SnTox2 interactions have similar, if not equal, impact on disease and that the effects of these two interactions are additive. In QTL analysis, the *Snn2* locus showed less importance in disease relative to *Tsn1*. This difference is likely an isolate-dependant relative difference. Friesen et al. (2007) used the *S. nodorum* isolate Sn6 in a greenhouse study to show that a compatible SnTox2–*Snn2* interaction accounted for 47% of the variation in disease whereas the SnToxA-*Tsn1* interaction accounted for only 20% of the variation. This indicates that some isolate-dependant mechanism of disease is present even though both toxins are produced. This difference could be due to several things including, but not limited to, gene regulation or variation in recognition specificity due to differences in the protein sequence of the toxin or the host interacting protein (Friesen et al. 2006; Stukenbrock and McDonald 2007).

Even though the *Tsn1* and *Snn2* loci are significant in both the seedling and adult plant interactions, the level of significance in the field experiments is much less than that of the seedling experiments. This difference could be due to a plant age effect where the sensitivity to host-selective toxins has a greater impact on seedlings than it does on

adult plants. However, it is also likely that this is due to there being less control over outside effects in the field than in the greenhouse. These outside effects include but are not limited to plant growth stage differences, susceptibility to other diseases segregating in the BG population, and environmental effects. This is reflected in the fact that the multiple regression model for the average seedling reaction accounted for 66% of the phenotypic variation whereas the multiple regression model for the combined flag and flag-1 reactions accounted for only 49 and 44%, respectively.

In addition to SnToxA and SnTox2, the isolate used in this study (BBCSn5) is known to produce the host-selective toxin SnTox3. The BG population used in this study is also known to segregate for *Snn3* (SnTox3 sensitivity) (Friesen et al. 2008a, b), however, no disease significance was identified at the *Snn3* locus on wheat chromosome arm 5BS. Friesen et al. (2008a) showed that the SnToxA–*Tsn1* interaction is epistatic to the SnTox3–*Snn3* interaction in the seedling stage. Friesen et al. (2008a) also used an SnToxA-disruption mutant to show that when the SnToxA–*Tsn1* interaction was eliminated, the SnTox3–*Snn3* interaction became significant. Epistasis may also be present in the adult plant stage. It is also possible that due to the increased error in data collection in field experiments and variation in heading and plant height, combined with the relatively small size of the BG population, the SnTox3–*Snn3* interaction was not detected.

Other studies to evaluate resistance to SNB in adult plants under field conditions have identified multiple QTLs significantly associated with adult plant resistance (Arseniuk et al. 2004; Czembor et al. 2003; Aguilar et al. 2005; Schnurbusch et al. 2003; reviewed in Friesen et al. 2008b). The results of our current work indicate that the effects of some of the reported adult plant resistance/susceptibility QTL could potentially be due to underlying, yet unidentified, HST sensitivity genes. Further analysis of these mapping populations using pathogen culture filtrates or purified toxins could identify whether this is the case.

Given the significance of compatible *Tsn1*–SnToxA and *Snn2*–SnTox2 interactions in causing SNB in wheat, it would be highly desirable to eliminate the toxin sensitivity genes *Tsn1* and *Snn2* from elite germplasm and commercially grown cultivars. The most efficient means of doing this is through marker-assisted selection. We have recently reported the development and validation of PCR-based markers suitable for high-throughput genotyping and marker-assisted selection against the dominant *Tsn1* and *Snn2* alleles (Zhang et al. 2008). The use of these markers for removal of the dominant *Tsn1* and *Snn2* alleles from desirable genotypes should lead to the development of new cultivars with improved seedling and adult plant resistance to SNB.

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