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ORIGINAL PAPER

Genetic analysis of disease susceptibility contributed by the compatible *Tsn1*–SnToxA and *Snn1*–SnTox1 interactions in the wheat-*Stagonospora nodorum* pathosystem

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Abstract *Stagonospora nodorum* is a foliar pathogen of wheat that produces several host-selective toxins (HSTs) and causes the disease Stagonospora nodorum blotch (SNB). The wheat genes *Snn1* and *Tsn1* confer sensitivity to the HSTs SnTox1 and SnToxA, respectively. The objectives of this study were to dissect, quantify, and compare the effects of compatible *Snn1*–SnTox1 and *Tsn1*–SnToxA interactions on susceptibility in the wheat-*S. nodorum* pathosystem. Inoculation of a wheat doubled haploid population that segregates for both *Snn1* and *Tsn1* with an *S. nodorum* isolate that produces both SnTox1 and SnToxA indicated that both interactions were strongly associated with SNB susceptibility. The *Snn1*–SnTox1 and *Tsn1*–SnToxA interactions explained 22 and 28% of the variation in disease, respectively, and together they explained 48% indicating that their effects are largely additive. The *Snn1*–SnTox1 interaction accounted for 50% of the variation when the population was inoculated with an *S. nodorum* strain where the *SnToxA* gene had been mutated, eliminating the *Tsn1*–SnToxA interaction. These results support the theory that the wheat-*S. nodorum* pathosystem is largely based on multiple host–toxin interactions that follow an inverse gene-for-gene scenario at the host–toxin interface, but disease exhibits quantitative variation due to the mainly additive nature of compatible interactions. The elimination of either *Snn1* or *Tsn1* toxin sensitivity alleles resulted in decreased susceptibility, but the elimination of both interactions was required to obtain high levels of resistance. We propose the use of molecular markers to select against *Snn1*, *Tsn1*, and other toxin sensitivity alleles to develop wheat varieties with high levels of SNB resistance.

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Introduction

Stagonospora nodorum blotch (SNB) [caused by *Stagonospora nodorum* (Berk.) Castellani & E. G. Germano (Teleomorph: *Phaeosphaeria nodorum* (E. Muller) Hedjaroude)] is a destructive foliar disease of both common wheat (*Triticum aestivum* L.) and durum wheat (*T. turgidum* L. var. *durum*). The disease occurs in all major wheat-growing areas of the world (Leath et al. 1993) and can cause significant yield loss (Fried and Meister 1987) and negatively impact grain quality (Eyal et al. 1987). Incidence of the disease has recently become more common in many wheat production areas (DePauw 1995).

The majority of the studies reporting SNB resistance have shown resistance exhibit quantitative variation (Bostwick et al. 1993; Du et al. 1999; Ecker et al. 1989; Fried and Meister 1987; Wicki et al. 1999; Wilkinson et al. 1990), and loci associated with resistance have been identified on numerous chromosomes (see Xu et al. 2004a and Friesen et al. 2008a for review). Molecular markers linked to SNB resistance genes have been detected in both bread wheat (Aguilar et al. 2005; Czembor et al. 2003;

Schnurbusch et al. 2003) and durum wheat (Cao et al. 2001).

Recently, compatible host–toxin interactions have been shown to be important in SNB development. In these interactions, a pathogen-produced host-selective toxin (HST) and a corresponding single dominant host gene allele are both required for a compatible interaction to take place, and this leads to disease susceptibility. When either the toxin or the dominant allele of the host gene is absent, incompatibility occurs and the result is resistance (if no other host–toxin interaction is present). Therefore, this system is essentially the inverse of a classic gene-for-gene system (Flor 1956) at the host–toxin interface. However, the overall response of the host to the pathogen may not necessarily follow the inverse gene-for-gene model if multiple host–toxin interactions are involved because the interactions may show various effects such as additivity and epistasis (see below), or other genes with minor effects may influence the overall response.

To date, five proteinaceous necrosis-inducing HSTs produced by *S. nodorum* and their corresponding host sensitivity genes have been reported (Friesen et al. 2006, 2007, 2008b; Liu et al. 2004a; Abeysekara et al. 2009). SnTox1, produced by isolate Sn2000 was the first HST identified in *S. nodorum*. The *Snn1* locus, located on the distal end of chromosome arm 1BS (Liu et al. 2004a; Reddy et al. 2008), conferred SnTox1 sensitivity and accounted for up to 58% of the phenotypic variation for reaction to SNB (Liu et al. 2004b).

The second toxin identified in *S. nodorum* was SnToxA (Friesen et al. 2006). The SnToxA gene has an identity of >99% to that of Ptr ToxA, a toxin previously identified in *Pyrenophora tritici-repentis* (Ballance et al. 1996; Ciuffetti et al. 1997) causal agent of tan spot of wheat. The *Tsn1* locus, which confers Ptr ToxA sensitivity in wheat, has been mapped to chromosome arm 5BL (Faris et al. 1996; Lu and Faris 2006). Liu et al. (2006) showed that *Tsn1* confers sensitivity to both Ptr ToxA and SnToxA, and susceptibility to both tan spot and SNB. Further studies have demonstrated that a compatible *Tsn1*–SnToxA interaction was the major factor causing SNB in both hexaploid and tetraploid wheat genotypes that carry the *Tsn1* gene (Friesen et al. 2006; Liu et al. 2006; Faris and Friesen 2009).

The other HSTs identified in *S. nodorum* were SnTox2 (Friesen et al. 2007), SnTox3 (Friesen et al. 2008b; Liu et al. 2009), and SnTox4 (Abeysekara et al. 2009). The sensitivity-conferring loci, designated *Snn2*, *Snn3*, and *Snn4* were located on chromosome arms 2DS, 5BS, and 1AS, respectively. Compatible *Snn2*–SnTox2, *Snn3*–SnTox3, and *Snn4*–SnTox4 interactions were also found to be important in SNB development (Friesen et al. 2007, 2008b; Abeysekara et al. 2009). Therefore, all compatible host gene–HST interactions reported to date have been highly associated with disease caused by *S. nodorum*, indicating that the wheat-*S. nodorum* pathosystem is largely based on multiple host–toxin interactions (Friesen et al. 2008a).

Friesen et al. (2008b) showed that the effects of the *Tsn1*–SnToxA and the *Snn2*–SnTox2 interactions were mainly additive, whereas *Snn2* (sensitivity to SnTox2) was epistatic to *Snn3* (sensitivity to SnTox3), and the disease significance of a compatible *Snn3*–SnTox3 interaction was undetectable when a compatible *Tsn1*–SnToxA interaction was present. Therefore, even though each HST alone plays a significant role in causing disease, interactions may exist among different host gene–HST interactions. Investigation of these interactions could provide additional information about the SNB disease mechanism as well as useful information for developing germplasm with enhanced SNB resistance through negative selection of toxin sensitivity genes.

In the current study, we evaluated a wheat doubled haploid (DH) population for reaction to SNB, and we used quantitative trait loci (QTL) analysis to compare the effects of compatible *Tsn1*–SnToxA and *Snn1*–SnTox1 interactions in the development of SNB.

Materials and methods

Plant materials

A DH-mapping population referred to as the NC60 population consists of 120 lines derived from a cross of the synthetic hexaploid wheat (SHW) (*Aegilotriticum* spp., $2n = 6x = 42$, AABBDD) line TA4152-60 and the North Dakota hard red spring wheat breeding line ND495. This population has been used for developing whole genome linkage maps (Chu et al. 2008). TA4152-60 was developed at the International Maize and Wheat Improvement Center (CIMMYT) from a cross between the durum wheat variety Scoop 1 and the *Aegilops tauschii* accession WPI358 (TA2516). ND495 is a selection from 'Justin*2/3/ND 259/Conley//ND 112'. TA4152-60 is sensitive to SnTox1 but insensitive to SnToxA and moderately resistant to the isolate Sn2000. In contrast, ND495 is insensitive to SnTox1 but sensitive to SnToxA and highly susceptible to the isolate Sn2000 (Chu et al. 2008; Xu et al. 2004b). The NC60 DH population was previously screened with SnTox1 and SnToxA to determine the map positions of *Snn1* and *Tsn1* (Chu et al. 2008). For reaction to SnToxA, the population segregated in a ratio of 57 insensitive:63 sensitive, and segregation for reaction to SnTox1 was 52 insensitive:68 sensitive, with both reactions fitting the expected 1:1 segregation ratio.

Fungal isolates, inoculation, and disease rating

The *S. nodorum* isolate Sn2000 and a derivative of Sn2000 with a disrupted *SnToxA* gene designated Sn2000KO6-1 (Friesen et al. 2006) were used to produce conidia for preparing inoculum. Sn2000 was collected from a North Dakota wheat field in 1980 and was chosen because it has been used to screen North Dakota germplasm and breeding lines, and has been shown to be an aggressive isolate that produces both SnTox1 (Liu et al. 2004a, b) and SnToxA (Friesen et al. 2006; Liu et al. 2006). Friesen et al. (2008b) found that the *Tsn1*–SnToxA interaction, or potential epistatic interactions between *Tsn1* and other loci, reduced the ability to detect other SNB resistance QTL. Therefore, the use of the strain Sn2000KO6-1 has the potential to reveal significant QTL not detectable by Sn2000 inoculation since the effects of a compatible *Tsn1*–SnToxA interaction are eliminated.

For evaluation of disease reaction, the entire population was inoculated with conidia of Sn2000 and Sn2000KO6-1, respectively. Inoculations were conducted in three replicated experiments under controlled conditions using the procedure described by Liu et al. (2004b) except that the lines within the population were randomized for each experiment. Each DH line was planted in three super-cell cones (Stuewe and Sons, Inc., Corvallis, OR) with three seeds per cone in each experiment, and cones were then placed in RL98 trays (Stuewe and Sons, Inc., Corvallis, OR). To counteract possible edge effects, the susceptible cultivar 'Grandin' was planted in all the border cones of each RL98 tray except for six cones which were used for planting the parental lines. Therefore, for each fungal strain, three experimental units were used. Fungi were grown and conidia were harvested as described by Liu et al. (2004b). Spore inoculum was adjusted to 1×10^6 spores ml^{-1}, and two drops of Tween-20 (polyoxyethylene sorbitan monolaurate) were added per 100 ml of inoculum. Plants were inoculated until runoff and placed in 100% relative humidity in the dark at 21°C for 24 h, and then placed in a growth chamber under a 12-h photoperiod at 21°C. The disease reactions were scored using a 0–5 numerical scale based on reaction type as described by Liu et al. (2004b) where 0 equals highly resistant; 1 resistant; 2 moderately resistant; 3 moderately susceptible; 4 susceptible; and 5 highly susceptible. Plants showing equal numbers of two different reaction types were given an intermediate value (e.g., reaction types 1 and 2 equals 1.5).

Statistical and QTL analysis

Homogeneity of error variances among disease rating data from each replicated experiment was tested by Bartlett's χ^2-test using command PROC GLM (SAS Institute 2008), and data lacking significant heterogeneity for error variances were then combined for QTL analysis. Mean reaction types of the categories based on sensitivities to SnTox1 and SnToxA were compared using Fisher's protected least significant difference (LSD) at $\alpha = 0.05$, and analysis of variance (ANOVA) was performed to test the interaction between HST sensitivity loci *Tsn1* and *Snn1* by partitioning the sum squares into components of *Tsn1*, *Snn1*, *Tsn1* × *Snn1,* and error (SAS Institute 2008). QTL analysis was further used to evaluate the effects of compatible *Tsn1*–SnToxA and *Snn1*–SnTox1 interactions on SNB susceptibility. The linkage maps developed for the DH population were previously reported (Chu et al. 2008). A subset of 449 markers spaced approximately 5–20 cM apart and giving the most complete genome coverage was selected and used for QTL detection. The computer program Map Manager QTX (Manly et al. 2001) was used to perform composite interval mapping (CIM) to evaluate marker intervals putatively associated with trait phenotypes. A permutation test with 1,000 permutations was conducted to determine that the critical logarithm of odds (LOD) threshold of 3.0 in this DH population yields an experiment-wise significance level of 0.05. Genetic loci with the most significant effect for each QTL were assembled into multiple regression models to determine the total amount of the phenotypic variation explained (Nelson 1997).

Results

Reaction to Sn2000 and Sn2000KO6-1 inoculations in the DH population

Homogeneity tests indicated that the error variance of spore inoculation data for both Sn2000 and Sn2000KO6-1 were homogeneous (Bartlett's $\chi^2_{df=2} = 0.53$ and 0.14, and $P = 0.77$ and 0.93 for Sn2000 and Sn2000KO6-1, respectively) in different experiments, and data for each strain were thus combined and used for analysis. TA4152-60 was resistant or moderately resistant to both Sn2000KO6-1 and Sn2000, whereas ND495 was moderately susceptible to Sn2000KO6-1 and highly susceptible to Sn2000 (Fig. 1, Table 1).

For reaction to Sn2000, mean reaction types of DH lines ranged from 0 to 5.0 with an overall average of 2.88. SNB susceptibility in the DH population was highly associated with sensitivity to both SnTox1 and SnToxA, i.e., DH lines that harbored *Snn1* and/or *Tsn1* alleles were more susceptible to SNB than those that harbored *snn1* and *tsn1* alleles (Table 1, Figs. 1, 2). The DH lines that were insensitive to both toxins (i.e. harboring *snn1* and *tsn1* alleles) were significantly more resistant than DH lines

Fig. 1 Disease reaction to inoculation of *S. nodorum* isolates Sn2000 (SnToxA+, SnTox1+) (**a**) and Sn2000KO6-1 (SnToxA−, SnTox1+) (**b**) on parental lines ND495 (*snn1/Tsn1*), TA4152-60 (*Snn1/tsn1*), four *Snn1/Tsn1,* and four *snn1/tsn1* DH lines. Line names are shown on the *left side* and the corresponding genotype for loci *Snn1* and *Tsn1* are shown on the *right side*

sensitive to either or both toxins (i.e. harboring *Snn1* and/or *Tsn1* alleles). Lines that were sensitive to only SnTox1 (*Snn1*) or only SnToxA (*Tsn1*) were moderately susceptible and lines that were sensitive to both toxins (i.e. harboring *Snn1* and *Tsn1* alleles) were significantly more susceptible than those with sensitivity to either SnTox1 or SnToxA or those that were insensitive to both toxins (Table 1). Therefore, both compatible *Snn1*–SnTox1 and *Tsn1*–SnToxA interactions alone play significant roles in disease caused by Sn2000 in the DH population and a significant increase in susceptibility was caused when both were present, which indicated that the effects of these interactions were mostly additive.

Mean reaction types of the DH lines following inoculation with Sn2000KO6-1 were significantly less than that found for the wild-type Sn2000 isolate that harbored functional SnToxA and SnTox1 (Fig. 1, Table 1). As expected, sensitivity to SnTox1 (*Snn1*) was highly associated with disease caused by Sn2000KO6-1, whereas no significant association between SnToxA sensitivity (*Tsn1*) and disease were observed (Table 1, Fig. 2). Comparison of the reaction types among the groups of lines based on *Snn1* allelic constitutions indicated that sensitivity to SnTox1 alone greatly increased the severity of the disease regardless of the allelic constitution of the *Tsn1* locus (Table 1). In addition, the mean reaction types of greater than 4.5 for lines sensitive to both SnTox1 and SnToxA that were present in Sn2000 inoculation, were not observed in the Sn2000KO6-1 inoculation (Table 1, Fig. 2). Furthermore, the DH lines with both *Snn1* and *Tsn1* alleles (sensitive to both toxins) had significantly lower reaction types when inoculated with Sn2000KO6-1 compared with the same lines inoculated with Sn2000 (Table 1). Therefore, reaction to Sn2000KO6-1

Table 1 Reaction type means of TA4152-60, ND495, DH population, and lines grouped based on *Snn1* and *Tsn1* alleles for reaction to conidial inoculation using strains Sn2000 and Sn2000KO6-1 of *Stagonospora nodorum*

Lines	No. of lines	Sn2000 inoculation		Sn2000KO6-1 inoculation	
		Reaction type means[a]	Range	Reaction type means[a]	Range
ND495		4.33		3.00	
TA4152-60		1.75		1.50	
Population	120	2.88 ± 1.23	0–5.0	1.99 ± 1.15	0.5–4.5
snn1/tsn1 lines	27	1.31 ± 0.50a	0–3.2	1.04 ± 0.74a	0.5–2.8
snn1/Tsn1 lines	25	3.16 ± 0.88b	1.0–4.5	1.13 ± 0.73a	0.5–2.7
Snn1/tsn1 lines	30	2.99 ± 0.75b	1.7–4.3	2.62 ± 0.85b	1.2–4.3
Snn1/Tsn1 lines	38	3.71 ± 0.81c	2.0–5.0	2.75 ± 0.89b	1.0–4.5
LSD (0.05)		0.25		0.25	

[a] Different letters following the reaction type means indicate they are significantly different at $P < 0.05$ level. The numerical scale used for disease reaction was based on a 0–5 system described in Liu et al. (2004b), where reaction type of 0 equals highly resistant; 1 resistant; 2 moderately resistant; 3 moderately susceptible; 4 susceptible; and 5 highly susceptible

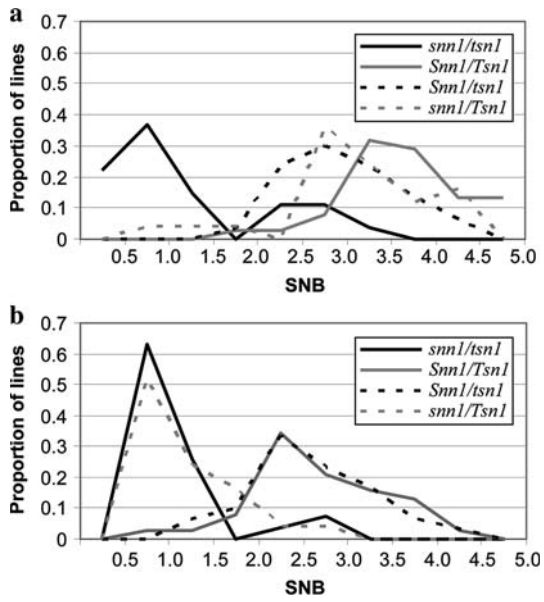

Fig. 2 Frequency polygons showing the average disease reaction type distribution in the NC60 population of the four genotypic classes (*snn1/tsn1*, *snn1/Tsn1*, *Snn1/tsn1*, and *Snn1/Tsn1*) following inoculation with *S. nodorum* strains Sn2000 wild type (**a**) and Sn2000KO6-1 (**b**)

in the DH population further demonstrated the impact of the *Tsn1*–SnToxA interaction and the most probably additive effects between *Snn1* and *Tsn1* on host reaction to SNB.

Variance analysis of reaction type data for reaction to strains Sn2000 and Sn2000KO6-1 in the DH population found that *Snn1* and *Tsn1*, as well as the *Snn1–Tsn1* interaction, were all significant for reaction to Sn2000, whereas *Snn1* was the only component significant for reaction to Sn2000KO6-1 (Table 2) due to the absence of SnToxA. The mean square of the disease rating for the Sn2000 inoculation that partitioned into components of *Snn1* and *Tsn1* were 106.06 and 151.51, respectively. Both were much greater than the mean square partitioned into the *Snn1–Tsn1* interaction component (28.01). Therefore, variance analysis indicated that the effects of *Snn1* and *Tsn1* were mainly additive for the reaction to Sn2000, and the non-additive interaction between the two loci likely exists but showed minor effects on disease severity.

QTL analysis of the reaction to spore inoculation

The combined reaction type data for reaction to Sn2000 and Sn2000KO6-1 from the three experiments were used in QTL CIM analysis to determine the amount of disease variation that could be attributed to *Snn1* and *Tsn1*. The Sn2000 inoculation data indicated two major QTL, one peaking at the *Snn1* locus and the other at the *Tsn1* locus. This analysis revealed that the *Snn1* and *Tsn1* accounted for approximately equal portions of disease variation at 22 and 28%, respectively, with additive effects of 1.19 and 1.30, respectively (Table 3). Together, *Snn1* and *Tsn1* explained 48% of the variation in disease.

Table 2 Variance analysis of disease rating data in the TA4152-60 × ND495 derived DH population for reaction to conidial inoculation using strains Sn2000 and Sn2000KO6-1 of *S. nodorum*

Variance component	*df*	Mean square	*F* test
Sn2000 inoculation			
Snn1	1	106.06	$P < 0.01$
Tsn1	1	151.51	$P < 0.01$
Snn1 × *Tsn1*	1	28.01	$P < 0.01$
Error	356	0.72	
Sn2000KO6-1 inoculation			
Snn1	1	223.95	$P < 0.01$
Tsn1	1	3.06	NS
Snn1 × *Tsn1*	1	0.02	NS
Error	356	0.69	

df degrees of freedom, *NS* non-significant

Analysis of the Sn2000KO6-1 data indicated one major QTL that peaked at the *Snn1* locus and, as expected, no significant QTL was present at the *Tsn1* locus (Fig. 3). Here, *Snn1* itself explained 50% of the disease variation (Table 3).

In addition to the effects observed at the *Snn1* and *Tsn1* loci, additional QTL with minor effects were detected (Table 3). The Sn2000 inoculation data revealed minor QTL on chromosome arms 3DL (designated *QSnb.fcu-3D.2*) and 5AS (designated *QSnb.fcu-5A.1*), which explained 9 and 8% of the variation, respectively. Alleles for resistance at these two QTL were contributed by TA4152-60. Together, *Snn1*, *Tsn1*, *QSnb.fcu-3D.2,* and *QSnb.fcu-5A.1* explained 64% of the total phenotypic variation in response to isolate Sn2000 (Table 3). *QSnb.fcu-3D.2* and *QSnb.fcu-5A.1* were also significantly associated with disease conferred by Sn2000KO6-1 and explained 4 and 5% of the variation, respectively. Three additional QTL with minor effects associated with resistance to Sn2000KO6-1 were identified on chromosome arms 3DS (*QSnb.fcu-3D.1*), 4AL (*QSnb.fcu-4A*), and 5AL (*QSnb.fcu-5A.2*) and explained 8, 6, and 6% of the variation, respectively, and resistance alleles for these three QTL were contributed by TA4152-60. Together, the six QTL associated with reaction to Sn2000KO6-1 explained a total of 69% of the phenotypic variation (Table 3).

Discussion

Our results agree with previous studies indicating that the inheritance of SNB resistance can be affected by multiple loci (Bostwick et al. 1993; Du et al. 1999; Ecker et al. 1989; Fried and Meister 1987; Wicki et al. 1999;

Table 3 Composite interval mapping analysis of QTL associated with reaction to *S. nodorum* in the TA4152-60 × ND495 derived DH population

Gene or QTL	Marker interval	R^2 Value[a]		LOD Score[b]		Additive effect[c]	
		Sn2000	KO6-1[d]	Sn2000	KO6-1	Sn2000	KO6-1
Snn1	*Xfcp566-Xfcp596*	0.22	0.50	9.98	20.26	1.19 N	1.57 N
Tsn1	*Xgwm260-Xbarc73*	0.28	–	12.61	NS	1.30 T	–
QSnb.fcu-3D.1	*Xgwm161-Xbarc6*	–	0.08	NS	4.57	–	0.45 T
QSnb.fcu-3D.2	*Xcfa2134-Xfcp597*	0.09	0.04	3.39	2.52	0.74 T	0.37 T
QSnb.fcu-4A	*Xcfa2121-Xcfa2173*	–	0.06	NS	3.04	–	0.39 T
QSnb.fcu-5A.1	*Xcfa2250-Xfcp497*	0.08	0.05	3.09	2.83	0.78 T	0.47 T
QSnb.fcu-5A.2	*Xbarc1061-Xcfa2185*	–	0.06	NS	3.04	–	0.42 T

[a] The multiple regression model based on *Snn1* and *Tsn1* found the two loci explained a total of 48% of the phenotypic variation for reaction to Sn2000, whereas the multiple regression model based on all significant markers explained a total trait variation of 64 and 69% for reaction to Sn2000 and Sn2000KO6-1, respectively

[b] NS indicates non-significant

[c] Additive effect indicates the amount of mean disease reaction type increased or decreased by the gene or QTL. Letters following indicate the corresponding parent (N = ND495 and T = TA4152-60) that contributed the resistance effects

[d] KO6-1 represents Sn2000KO6-1, the ToxA mutant of Sn2000

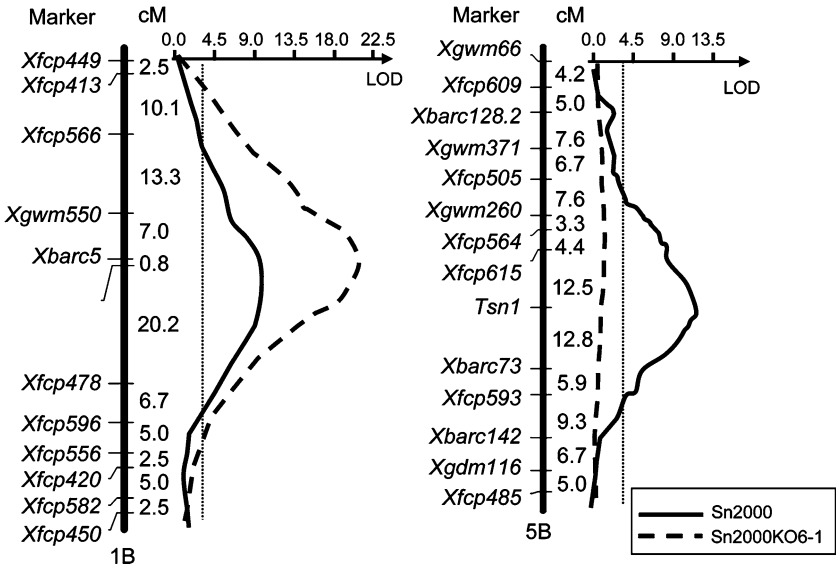

Fig. 3 Composite interval regression maps of major QTL associated with resistance to *S. nodorum* in the NC60 DH population. Positions of marker loci are shown to the left of the linkage groups and centiMorgan (cM) distances between loci are shown along the right. *Black* and *red lines* indicate QTL for resistance to inoculation of Sn2000 and Sn2000KO6-1, respectively. The *vertical dotted line* represents the logarithm of the odds (LOD) significance threshold of 3.0. The LOD and R^2 values for each QTL are listed in Table 3

Wilkinson et al. 1990). In the wheat-*S. nodorum* pathosystem, we can dissect the quantitative nature of resistance into qualitative components involving individual pathogen-produced HSTs which interact directly or indirectly with host sensitivity gene products in an inverse gene-for-gene manner, which is effectively pathogen effector-driven susceptibility. In this work, we dissected, quantified, and compared the effects of two compatible host–toxin interactions with large effects.

Inoculation of the DH population with Sn2000 allowed us to observe the effects of compatible *Snn1*–SnTox1 and *Tsn1*–SnToxA together in the same background. Here, the *Snn1*–SnTox1 interaction accounted for 22% of the variation and the *Tsn1*–SnToxA interaction accounted for 28%. This result and the fact that the DH lines with sensitivity to only SnTox1 (*Snn1/tsn1* lines) or sensitivity to only SnToxA (*snn1/Tsn1* lines) had approximately equal reaction types (2.99 and 3.16, respectively), indicates that the two interactions contribute equally to the development of SNB using this isolate in this population. Furthermore, variance analysis of reaction type data along with the fact that together *Snn1* and *Tsn1* explained 48% of the disease variation and that DH lines with sensitivity to both toxins (*Snn1/Tsn1* lines) had an average reaction type of 3.71 demonstrates that the effects of these host–toxin interactions are mainly additive. These results are similar to those of Friesen et al. (2007, 2008b), who evaluated the effects of compatible *Snn2*–SnTox2 and *Tsn1*–SnToxA interactions

and showed them to be largely additive. However, the results of this work differ somewhat from those of Friesen et al. (2007, 2008b) in that we found the effects of the *Snn1*–SnTox1 and *Tsn1*–SnToxA interactions to contribute equally to disease, whereas Friesen et al. (2007) showed that the *Snn2*–SnTox2 interaction contributed significantly more to disease (47%) than did the *Tsn1*–SnToxA interaction (20%) when using the *S. nodorum* isolate Sn6. In addition, Friesen et al. (2008b) showed that the *Snn2*–SnTox2 interaction contributed significantly less to disease (17%) than did the *Tsn1*–SnToxA interaction (35%) when using isolate SN15. Therefore, isolates may express different toxins at different levels, which may lead to variation in the amount of disease variation attributed to compatible interactions (Z. Zhang, T. Friesen, J. Faris, unpublished). In any case, the mainly additive effects of *Snn1* and *Tsn1* identified in this research indicate that a higher level of host resistance could be reached by removing both toxin sensitivity alleles compared to only one or the other. This differs from a gene-for-gene type interaction, in which the introgression of a single effective resistance gene confers similar levels of resistance as that of multiple resistance genes.

As expected, reaction to the strain Sn2000KO6-1 showed no association of susceptibility with the *Tsn1* locus, because a compatible *Tsn1*–SnToxA interaction was absent due to the disrupted *ToxA* gene in this strain. Hence, *Snn1* explained 50% of the phenotypic variation for reaction to Sn2000KO6-1. In addition, disease caused by Sn2000 in the NC60 population had an overall mean reaction type of 2.88, which is significantly higher than the average reaction of 1.99 caused by Sn2000KO6-1 (Table 1). This indicates that elimination of the compatible *Tsn1*–SnToxA interaction would lead to less susceptibility, but it also demonstrates the independent role of the *Snn1*–SnTox1 interaction and reinforces the conclusion that both *Snn1* and *Tsn1* sensitivity alleles must be eliminated in order to obtain the highest level of SNB resistance.

Besides *Snn1* and *Tsn1*, QTL with minor effects associated with Sn2000 and Sn2000KO6-1 were also identified. The size of our mapping population is too small to reliably detect loci with minor effects, and therefore, the QTLs reported in this work should be considered putative until they can be verified in a much larger population. However, comparisons with QTL reported in other populations can provide some indications of their validity. Based on the position of common markers in the maps of Erayman et al. (2004) and Sourdille et al. (2004), *QSnb.fcu-3D.2* and the one reported in Liu et al. (2004b) co-located to deletion bin 3DL-3, suggesting the two are possibly the same. Likewise, *QSnb.fcu-4A* may be the same as the 4A QTL reported by Liu et al. (2004b) based on marker positions on the maps of Sourdille et al. (2004), Liu et al. (2004b) and ours. Liu et al. (2006) reported a QTL associated with resistance to Sn2000 on chromosome 5AL, which could potentially be the same as *QSnb.fcu-5A.2*. However, the position of the common marker *Xcfa2163* suggests that *QSnb.fcu-5A.2* is in a position slightly different from the one reported in Liu et al. (2006). SNB QTL have not previously been reported on chromosome arms 3DS or 5AS. Therefore, *QSnb.fcu-3D.1* and *QSnb.fcu-5A.1* could possibly represent novel SNB resistance loci.

The putative minor QTL were not found to be associated with any known toxin sensitivity in the current or previous studies. It is possible that these QTL regions also confer insensitivity to toxins that have yet to be identified. Isolate Sn2000 likely produces toxins other than those identified in this and previous work (data not shown). The aforementioned genomic regions could condition sensitivity to these potentially unidentified toxins, and these toxins may have relatively minor effects compared with SnTox1 and SnToxA. It is also possible that these genomic regions are non-toxin associated loci, and that other mechanisms associated with SNB resistance are involved. Finally, combinations of these scenarios may exist as well. It is interesting to note that two peaks are present in the reaction type distribution of the *tsn1*/*snn1* genotypic class when inoculated with Sn2000 (Fig. 2a), and to a lesser degree with Sn2000 KO6-1 indicating that factors other than *Tsn1* and *Snn1* are playing a significant role in disease.

The degree of significance of some putative QTL varied in different inoculations. As mentioned earlier, *QSnb.fcu-3D.1*, *QSnb.fcu-4A*, and *QSnb.fcu-5A.2* were only significant for reaction to Sn2000KO6-1. Since the only difference between the two pathogen strains was the mutation in the *ToxA* gene, undetectable effects of those QTL for reaction to Sn2000 might be due to the overwhelming effects of the *Tsn1*–SnToxA interaction in this population. A compatible *Tsn1*–SnToxA interaction leads to extensive necrosis in lines that possess the *Tsn1* allele, which would greatly reduce or eliminate the ability to detect disease reaction differences among lines that carried minor resistance QTL(s) if they harbor the *Tsn1* allele. Friesen et al. (2008a, b) observed that the *Tsn1*–SnToxA interaction likely decreases the significance of the QTL corresponding to the *Snn3* locus (sensitivity to SnTox3). Additionally, the relatively small size of the population limits the ability to detect QTL with minor effects as mentioned previously.

In conclusion, by dissecting, quantifying, and comparing the effects of compatible *Snn1*–SnTox1 and *Tsn1*–SnToxA interactions, we showed that both play highly significant roles in conferring SNB susceptibility in wheat. The two interactions contribute equally to disease development, and their effects are largely additive. This work provides further strength to the theory that individual host–toxin interaction in the wheat-*S. nodorum* pathosystem follows

an inverse gene-for-gene scenario, but resistance to SNB is quantitatively inherited particularly when multiple host–toxin interactions are present. Based on current terminology, *S. nodorum* induces effector-triggered susceptibility (ETS) rather than effector-triggered immunity (ETI) (e.g. gene-for-gene) as defined for the biotrophic and bacterial systems (Chisholm et al. 2006; Jones and Dangl 2006). This research also indicates that breeding should focus largely on the elimination of host genes conferring sensitivity to the known HSTs produced by *S. nodorum*. This can be accomplished most efficiently using the molecular markers described in Reddy et al. (2008) and Zhang et al. (2009) to select against the *Snn1* and *Tsn1* alleles, respectively.

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