Whole-Genome QTL Analysis of *Stagonospora nodorum* Blotch Resistance and Validation of the SnTox4–*Snn4* Interaction in Hexaploid Wheat

Nilwala S. Abeysekara, Justin D. Faris, Shiaoman Chao, Phillip E. McClean, and Timothy L. Friesen

First and fourth authors: Department of Plant Sciences, North Dakota State University, Fargo 58105; and second, third, and fifth authors: United States Department of Agriculture–Agricultural Research Service Red River Valley Research Center, Cereal Crops Research Unit, 1307 18th Street North, Fargo, ND 58102-2765.
Accepted for publication 11 August 2011.

ABSTRACT

Abeysekara, N. S., Faris, J. D., Chao, S., McClean, P. E., and Friesen, T. L. 2012. Whole-genome QTL analysis of *Stagonospora nodorum* blotch resistance and validation of the SnTox4–*Snn4* interaction in hexaploid wheat. *Phytopathology* 102:94–104.

Necrotrophic effectors (also known as host-selective toxins) are important determinants of disease in the wheat–*Stagonospora nodorum* pathosystem. To date, five necrotrophic effector–host gene interactions have been identified in this system. Most of these interactions have additive effects while some are epistatic. The *Snn4*–SnTox4 interaction was originally identified in a recombinant-inbred population derived from a cross between the Swiss winter wheat cultivars ‘Arina’ and ‘Forno’ using the *S. nodorum* isolate Sn99CH 1A7a. Here, we used a recombinant-inbred population consisting of 121 lines developed from a cross between the hexaploid land race Salamouni and the hexaploid wheat ‘Katepwa’ (SK population). The SK population was used for the construction of linkage maps and quantitative trait loci (QTL) detection using the Swiss *S. nodorum* isolate Sn99CH 1A7a. The linkage maps developed in the SK population spanned 3,228 centimorgans (cM) and

consisted of 441 simple-sequence repeats, 9 restriction fragment length polymorphisms, 29 expressed sequence tag sequence-tagged site markers, and 5 phenotypic markers. The average marker density was 6.7 cM/marker. Two QTL, designated *QSnbfcu-1A* and *QSnbfcu-7A* on chromosome arms 1AS and 7AS, respectively, were associated with disease caused by the *S. nodorum* isolate Sn99CH 1A7a. The effects of *QSnbfcu-1A* were determined by the *Snn4*–SnTox4 interaction and accounted for 23.5% of the phenotypic variation in this population, whereas *QSnbfcu-7A* accounted for 16.4% of the phenotypic variation for disease but was not associated with any known effector sensitivity locus. The effects of both QTL were largely additive and collectively accounted for 35.7% of the total phenotypic variation. The results of this research validate the effects of a compatible *Snn4*–SnTox4 interaction in a different genetic background, and it provides knowledge regarding genomic regions and molecular markers that can be used to improve *Stagonospora nodorum* blotch resistance in wheat germplasm.

Additional keywords: *Triticum aestivum*.

Wheat (*Triticum aestivum* L.) is the third most-produced cereal and ranks second among food crop species in the world (FAOSTAT 2007, <http://faostat.fao.org>). Like any other crop species, wheat is prone to diseases caused by a number of pathogens, the majority of which are caused by fungi. *Stagonospora nodorum* (Berk.) Castell. & Germano (teleomorph: *Phaeosphaeria nodorum* (E. Müll.) Hedjar.) is a necrotrophic fungal pathogen that causes *Stagonospora nodorum* blotch (SNB), a major disease in wheat. SNB causes yield losses and reduction in grain quality across the globe (7,8,20). This filamentous ascomycete produces multiple necrotrophic effectors, also known as host-selective toxins (HSTs), which are major disease determinants. *S. nodorum* is pathogenic on wheat, barley, and a wide range of wild grasses (34), and has the ability to infect both the glume and the leaf, causing glume blotch and leaf blotch, respectively.

Necrotrophic effectors are a special class of pathogen effectors which range from low-molecular-weight metabolites to proteins, and induce cell death, resulting in disease in specific genotypes of the host (14,41). Sensitivity to these effectors is more often a dominant trait and the effectors can reproduce the symptoms of the disease in part or in whole when introduced into plants at appropriate concentrations (30,40). In necrotrophic systems such as those involving *S. nodorum* or *Pyrenophora tritici-repentis* (the fungal pathogen that causes tan spot of wheat), the effectors produced by the pathogen are known to cause disease in the presence of corresponding host gene products required for susceptibility (41).

In recent years, resistance to wheat diseases such as SNB and tan spot, which are caused by fungal pathogens that produce necrotrophic effectors, has been characterized in host mapping populations derived from diverse wheat genotypes. Whole-genome maps developed in such mapping populations have become an important tool, not only for the identification of quantitative trait loci (QTL) associated with agronomically important quantitative traits but also for map-based cloning and marker-assisted selection. Genetic map construction requires a good mapping population and high-throughput, user-friendly molecular markers such as simple-sequence repeats (SSRs) or microsatellites. However, detecting polymorphism in wheat populations derived from intraspecific or intervarietal crosses can be difficult because wheat has a narrow genetic base (29).

Corresponding author: T. L. Friesen
E-mail address: Timothy.Friesen@ars.usda.gov

*The e-Xtra logo stands for “electronic extra” and indicates that Figures 1 to 5 appear in color online.

<http://dx.doi.org/10.1094/PHTO-02-11-0040>

This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. The American Phytopathological Society, 2012.

Wheat mapping populations that segregate for multiple effector sensitivities have been used in comprehensive QTL studies of the wheat–*S. nodorum* pathosystem. These studies have used genetic maps comprising a combination of molecular marker types to add to our understanding of the multigenic inheritance of resistance to *S. nodorum*. QTL associated with SNB expression have been detected in almost all of the hexaploid wheat chromosomes. However, no necrotrophic effector–host gene interactions were reported for the majority of these studies (14). To date, five necrotrophic effectors produced by *S. nodorum* (SnToxA, SnTox1, SnTox2, SnTox3, and SnTox4), and the corresponding host sensitivity genes (*Tsn1*, *Snn1*, *Snn2*, *Snn3*, and *Snn4*), which are all dominant, have been documented (1,13,15–17,25,26). The results of these studies indicate that each of the effectors plays a significant role in the development of SNB. Several other uncharacterized necrotrophic effectors are also known to be produced by *S. nodorum* (T. L. Friesen, unpublished).

The ToxA–*Tsn1* interaction is the most thoroughly characterized of the five necrotrophic effector–host gene interactions in the wheat–*S. nodorum* pathosystem. Because ToxA was first discovered in *P. tritici-repentis*, the majority of the early work on the ToxA–*Tsn1* interaction was done in relation to the *P. tritici-repentis*–wheat interaction. Approximately two decades after its discovery in *P. tritici-repentis*, ToxA was identified in *S. nodorum* and was shown to have been laterally transferred from *S. nodorum* to *P. tritici-repentis* (16).

The compatible wheat–*S. nodorum* effector interactions can have additive (12,15,17) or epistatic (17) effects, making this system complex. Evaluation of the BR34 × ‘Grandin’ (BG) recombinant-inbred line (RIL) population with the *S. nodorum* isolate Sn6 revealed that both the SnToxA–*Tsn1* and SnTox2–*Snn2* interactions can be additive and result in higher susceptibility to disease than when just one interaction is present (15). However, analysis of the same population with different *S. nodorum* isolates showed that both the SnToxA–*Tsn1* and SnTox2–*Snn2* interactions are epistatic to the SnTox3–*Snn3* interaction (17).

The latest addition to the wheat–*S. nodorum* pathosystem, the SnTox4–*Snn4* interaction, was studied by evaluating a RIL population derived from a cross between the Swiss winter wheat cultivars ‘Arina’ and ‘Forno’ (AF population) with the Swiss *S. nodorum* isolate Sn99CH 1A7a (1). SnTox4 was estimated to be 10 to 30 kDa in size and proteinaceous in nature. Arina was sensitive to SnTox4 while Forno was insensitive. Infiltration of culture filtrates containing SnTox4 resulted in mottled necrosis in the susceptible wheat Arina as opposed to the severe necrosis induced by the other necrotrophic effector–host interactions. *Snn4*, a single dominant gene which governs sensitivity to SnTox4, was mapped to the short arm of wheat chromosome 1A and accounted for 41% of the phenotypic variation. The effects of this major QTL and two additional minor QTL detected on the short arms of chromosomes 2A and 3A were largely additive, explaining 50% of the total phenotypic variation.

Our preliminary research indicated that the wheat hexaploid landrace Salamouni was resistant to numerous isolates of *S. nodorum*, whereas ‘Katepwa’ was susceptible. Therefore, the objectives of this research were to develop a RIL population derived from Salamouni and Katepwa (the SK population) and construct whole-genome genetic linkage maps in the RIL population, to show that QTL or host–effector interactions are associated with SNB caused by the *S. nodorum* isolate Sn99CH 1A7a.

MATERIALS AND METHODS

Plant materials. A RIL population, the SK population, consisting of 121 F_{6:8} plants developed from a cross between the hexaploid wheat Katepwa and the hexaploid landrace Salamouni, was used for whole-genome linkage map construction and pheno-

typic analysis. Details regarding the development of the SK population are described elsewhere (2).

Molecular markers. Genomic DNA was isolated from the plant tissues as described by Faris et al. (10). Initially, 1,500 SSR markers scattered throughout the wheat genome were selected and used to screen the parental lines (Salamouni and Katepwa) for polymorphism. Selected SSR markers were amplified with the primer sets Ac (<http://wheat.pw.usda.gov/GG2/index.shtml>), BARC (36), CFD (37), DuPw (<http://wheat.pw.usda.gov/GG2/index.shtml>), GDM (32), GWM (33), KSUM (42), and WMC (35) (<http://wheat.pw.usda.gov/ggpages/SSR/WMC>). In total, 405 polymorphic SSR markers were selected to genotype the entire SK population. Genotyping of the parental lines and the 121 RILs of the SK population was carried out at the United States Department of Agriculture–Agricultural Research Service Small Grains Genotyping Lab, Fargo, ND. Polymerase chain reaction (PCR) conditions and multiplexing of the PCR products, amplified with four dyes, to a final volume of 10 µl were as described by Tsilo et al. (39). An ABI 3130XL Genetic Analyzer (Applied Biosystems, Foster City, CA) was used to separate the amplified PCR products by capillary electrophoresis after denaturing the multiplexed PCR products at 95°C for 5 min and cooling on ice. Separated fragments were analyzed using the software GeneMapper v3.7 (Applied Biosystems) according to the instructions in the user manual. Each data point was also manually checked for errors in allele calling (identification of alleles).

SSR and expressed sequence tag sequence-tagged site (EST-STS) markers were developed for chromosome arm 2BS using previously published genetic and physical maps of wheat, NSF–wheat bin-mapped EST sequences from bin 2BS3-0.84-1.00, and based on colinearity with the genomes of rice and *Brachypodium* spp. (2). The EST-STS markers developed using the 2DS5-0.47-1.00 deletion bin-mapped ESTs in a previous study (43) were also used to survey Salamouni and Katepwa for polymorphism. The 121 RILs were genotyped with the polymorphic 2BS SSR and 2BS/2DS EST-STS markers using PCR conditions described by Lu et al. (27). Amplified PCR products were separated on 6% polyacrylamide gels and visualized using a Typhoon 6410 variable mode imager (GE Healthcare, Waukesha, WI) after staining with SYBR Green II (Sigma-Aldrich, St. Louis). Corresponding EST clones of the monomorphic 2BS EST-STS markers were used as probes for restriction fragment length polymorphism (RFLP) analysis using procedures described by Faris et al. (10).

Disease evaluations and statistical analysis. The Swiss *S. nodorum* isolate Sn99CH 1A7a (hereafter referred to as 1A7a) which produces SnTox4 was obtained from Bruce McDonald (ETH Zürich, Switzerland) and was used to screen the SK population. Inoculum for disease evaluations was prepared as described by Liu et al. (26) using mycelial plugs of 1A7a grown in V8-potato dextrose agar (Difco PDA; Becton, Dickinson and Company, Sparks, MD) plates for 5 to 7 days under cool fluorescent light with a 24-h photoperiod. Parents and the SK population planted in a completely randomized design in three replicates were used for 1A7a conidial inoculations. Three seeds of each SK progeny line were planted in single conetainers (Stuewe and Sons, Inc., Corvallis, OR). Cones were placed in racks capable of holding 98 conetainers, with the outside cones being planted to the susceptible Grandin to reduce any edge effect. Two racks of 98 cones made up one replicate. Therefore, the experimental unit consisted of three plants per line. Plants were inoculated until runoff, with the conidial suspensions containing 1×10^6 spores ml⁻¹ at the two- to three-leaf stage. Two drops of Tween 20 (polyoxyethylene sorbitan monolaurate; J. T. Baker Chemical Co., Phillipsburg, NJ) per 100 ml of inoculum were added as a surfactant prior to inoculation. Inoculated plants were subjected to 6 days of incubation in the growth chamber at 21°C under a 12-h photoperiod after 24 h at 21°C in a mist chamber with 100% relative humidity. Secondary leaves of the inoculated plants were scored on a 0 to 5 lesion type scale (26) 7 days postinoculation.

Bartlett's χ^2 test using SAS (version 9.1; SAS Institute Inc., Cary, NC) was carried out to determine the homogeneity of variances among the three replicates. The computer program Graphpad (<http://www.graphpad.com/quickcalcs>) and Fischer's protected least significant difference at an α level of 0.05 was used to conduct the χ^2 tests and to determine the separation of the genotypic means, respectively. Analysis of variance and multiple regression analysis were conducted using Minitab (version 16.0; Minitab Inc.).

Necrotrophic effector (HST) evaluations. Culture filtrates (CFs) containing SnTox4 were prepared using isolate 1A7a as described by Liu et al. (26). All of the culture filtrates were tested for toxin activity prior to infiltration using the SnTox4 differential line AF89. Plants were kept in the growth chamber at 21°C under a 12-h photoperiod after infiltrating the fully expanded secondary leaf of each plant with 1A7a CF. Infiltrated plants were evaluated 4 days post infiltration and scored as sensitive or insensitive based on the presence or absence of necrosis.

Additionally, the SK population and the parents were infiltrated with Ptr ToxA, SnTox1, and SnTox3. Necrotrophic effectors were produced following the same procedure described for SnTox3 production (24) using the commercial kit developed for the constitutive expression and purification of recombinant proteins (Invitrogen, Carlsbad, CA). The necrotrophic effector genes were cloned into the pGAPZA vector and transformed into the wild-type yeast strain *Pichia pastoris* X33. Preparation and transformation of the competent *P. pastoris* cells using the *Pichia* EasyComp kit (Invitrogen) were as described in the user manual. The parents and the 121 individuals of the SK population were screened for reaction to necrotrophic effector sensitivities three times.

Whole-genome map construction and QTL analysis. Genetic linkage maps were constructed using the computer program MAPMAKER V2.0 for Macintosh (22) using a logarithm of odds (LOD) value of 3.0, as described by Liu et al. (23). The Kosambi mapping function (21) was used to calculate centimorgan distances. Marker order was validated using the "RIPPLE" (LOD > 3.0) command. Previously published consensus and physical

maps of wheat (37) were surveyed to compare the markers on each chromosome and to estimate the centromere positions on the maps, respectively.

QTL analysis was performed using composite interval-regression mapping (CIM) with the computer programs QGene (19) and MapManager QTX (28). A permutation test with 1,000 iterations was executed to determine the critical LOD threshold, which was found to be 3.23.

RESULTS

Marker analysis and linkage map construction. Parental lines of the SK population, Salamouni and Katepwa, were screened with 1,500 SSR primer pairs, of which 618 (41.2%) detected polymorphism between the two lines. In total, 405 polymorphic markers, including 98 BARC, 143 GWM, 54 CFD, 13 GDM, 85 WMC, 7 KSUM, 4 DuPw, and 1 Ac, were further selected to be mapped in the SK population, and resulted in the detection of 472 SSR marker loci. Eight 2D EST-STS primer pairs that detected polymorphism between the two parental lines were also mapped.

The linkage maps constructed in the SK population consisted of 484 markers, which included 441 SSR, 9 RFLP, 29 EST-STS, and 5 phenotypic markers spanning a total genetic distance of 3,228 centimorgan (cM) with an average marker density of 6.7 cM/marker (Table 1). The average marker density of the A, B, and D genomes was 7.2, 5.3, and 7.9 cM/marker, respectively. The A-genome map consisted of 146 markers and spanned 1,049.2 cM. The B-genome map was more dense than those for the A or D genome and contained 193 markers that spanned 1,027.5 cM. The D-genome map contained 145 markers and spanned 1,151.3 cM (Table 1). Chromosome 7D was the longest linkage group (291.0 cM) and chromosome 6B was the shortest (43.8 cM). The number of markers found on each chromosome ranged from 10 (1D, 6A) to 52 (2B) (Fig. 1; Table 1). The A- and D-genome maps contained similar numbers of SSRs (135 and 139, respectively) while the B-genome map contained the most SSRs (167). Twenty-two EST-derived (RFLP and STS) markers were mapped in the B genome (Fig. 1; Table 2).

TABLE 1. Summary of markers mapped in each wheat chromosome/genome in the Salamouni $\times$ Katepwa recombinant-inbred population

Chromosome	Marker ^y				Total	Length (cM) ^z	Marker density (cM/marker)
	SSR	RFLP	EST-STS	Phenotypic			
1A	22	1	3	1	27	138.6	5.1
1B	32	...	1	1	34	202.5	6.0
1D	10	...	...	...	10	98.5	9.9
2A	26	2	1	...	29	190.7	6.6
2B	34	2	15	1	52	200.9	3.9
2D	32	2	2	...	36	212.7	5.9
3A	23	...	...	...	23	170.1	7.4
3B	30	1	1	...	32	223.8	7.0
3D	16	...	2	...	18	152.6	8.5
4A	19	...	...	...	19	167.0	8.8
4B	12	...	1	...	13	62.0	4.8
4D	13	...	...	...	13	52.7	4.1
5A	17	...	1	...	18	94.6	5.3
5B	27	...	...	2	29	141.2	4.9
5D	28	...	...	...	28	228.8	8.2
6A	9	1	...	...	10	166.1	16.6
6B	12	...	...	...	12	43.8	3.7
6D	13	...	...	...	13	115.0	8.8
7A	19	...	1	...	20	122.1	6.1
7B	20	...	1	...	21	153.3	7.3
7D	27	...	...	...	27	291.0	10.8
A genome	135	4	6	1	146	1,049.2	7.2
B genome	167	3	19	4	193	1,027.5	5.3
D genome	139	2	4	0	145	1,151.3	7.9
Total	441	9	29	5	484	3,228.0	6.7

^y SSR = simple-sequence repeat, RFLP = restriction fragment length polymorphism, and EST-STS = expressed sequence tag sequence-tagged site.

^z Length in centimorgans (cM).

(Continued on following page)

Fig. 1. Genetic linkage maps generated in the 'Salamouni' × 'Katepwa' population with 441 simple-sequence repeats (SSRs), 9 restriction fragment length polymorphisms (RFLPs), 29 expressed sequence tag sequence-tagged sites (EST-STS), and 5 phenotypic markers. Markers that mapped at logarithm of odds (LOD) < 3.0 are shown in their most likely linkage positions along the maps. Black regions in the chromosomes indicate the approximate positions of centromeres. Groupings at an LOD < 3.0 are indicated by double diagonal lines drawn across the chromosomes.

Fig. 1. (Continued from previous page)

Fig. 1. (Continued from previous page)

Analysis of *S. nodorum* effector sensitivity genes in the SK population. Infiltration analyses indicated that Salamouni was sensitive to SnTox4, exhibiting a mottled necrotic reaction similar to that observed in Arina (1), whereas Katepwa was insensitive to SnTox4, with no visible symptoms (Fig. 2). Segregation of the SK population for reaction to the SnTox4 culture filtrates indicated that a single gene governed sensitivity (Table 3), and molecular mapping of response to SnTox4 as a phenotypic marker placed the locus on the short arm of chromosome 1A (Fig. 1) in a position that closely agreed with that in the AF population (1). These results strongly indicated that the *Snn4*–SnTox4 interaction was the one observed.

Salamouni was also sensitive to SnTox1 and insensitive to SnToxA and SnTox3, whereas Katepwa was insensitive to SnTox1 and sensitive to SnToxA and SnTox3 (data not shown). Therefore, Salamouni carried the dominant alleles *Snn4* and *Snn1* and the recessive alleles *tsn1* and *snn3*, whereas Katepwa carried the *snn4*, *snn1*, *Tsn1*, and *Snn3* alleles. Segregation analysis confirmed that each of these effector sensitivities was governed by a single gene (Table 3), and genomic mapping of the effector sensitivity loci in the SK population (Fig. 1) corroborated their known chromosomal locations determined by previous research (13). There were no visible reactions in either parental line when infiltrated with culture filtrates containing SnTox2 (data not shown), suggesting that the SK population does not segregate for SnTox2 sensitivity.

Identification of QTL associated with SNB caused by isolate 1A7a in the SK population. Average disease reaction types for

each RIL were used for the QTL analysis, because all three replicates of the SK population inoculated with *S. nodorum* isolate 1A7a were homogeneous (Bartlett's χ^2 for homogeneity: df = 2, 4.436, P = 0.109). Salamouni was moderately resistant to 1A7a, whereas Katepwa was moderately susceptible (Fig. 3). The average disease reaction types of Salamouni and Katepwa were 2.2

Fig. 2. Secondary leaves inoculated with conidia of the Swiss *Stagonospora nodorum* isolate Sn99CH 1A7a or infiltrated with the Sn99CH 1A7a culture filtrate containing SnTox4. Disease reaction of **A**, 'Salamouni' (average disease reaction type 2.20) and **B**, 'Katepwa' (average disease reaction type 3.1) to conidial inoculations with Sn99CH 1A7a. Reaction of **C**, Salamouni and **D**, Katepwa infiltrated with Sn99CH 1A7a culture filtrate containing SnTox4. Boundaries of the infiltrated regions are marked in black.

TABLE 2. Expressed sequence tags (ESTs) mapped in the recombinant-inbred population derived from 'Salamouni' and 'Katepwa'

Chromosome	Marker ^y	Marker type (enzyme) ^z	F primer	R primer	Annealing temperature (°C)
1A	<i>XBE590632*</i>	EST-STS	AACGATGATCCATCCGTCTT	TCCATCCTTCAACCACAACA	53
1A	<i>XBG262975*</i>	EST-STS	TCCAGTCAACAGCAACCATC	CCAAACAGTGAAGCTGCAAA	54
1A	<i>XBG262267*</i>	EST-STS	CGTTACAACGATTGGTGCAT	TCATCCAACCTCACCAACCA	53
1A	<i>XBF293581</i>	RFLP (<i>Dra</i> I)	AAACGAAAAGTTGGATCGTG	GATTGGCAGAGAATTGTGCTT	51
1B	<i>XBG274643</i>	EST-STS	TGAAGACCTGCCTGAGTTGT	CTCCGTCCGTTTGTGCTGT	55
2A	<i>XBE446068</i>	RFLP (<i>Hind</i> III)	ATGGCTTGGTTTCCCTTTTT	TCTTTGTAGGTTATTTGTCAGTCGTC	53
2A	<i>XBG263347</i>	RFLP (<i>Dra</i> I)	TGAGCATTGGAGGGATTAG	AGCCTGGTTTCTTGCTTCTC	50
2A	<i>XBF201235</i>	EST-STS	ATGCCCGCTGTTTATTGTAG	TGTGATTTTCCTTGCTGTCC	53
2D	<i>XTC253803</i>	EST-STS	TGCTTTTGTGCCAGATGATG	CCACCGGGACAAGTCAGATA	53
2D	<i>XTC240114</i>	EST-STS	GGAATTAACCGAGCTGCGTA	GCGGCCTCTGTATCTTGATT	54
2D	<i>XBF474028</i>	RFLP (<i>Bgl</i> II)	GAAAATTACCGCAAATCGTG	ACTTGCCTGAATATCGCTTG	50
2D	<i>XBF484266</i>	RFLP (<i>Dra</i> I)	GCAGGTTAGCTGTCCAAAAA	TTCTTCCAGAGCAAGGACAC	53
3B	<i>XBE604911</i>	EST-STS	ACCACGGAAGGTATCAGCAT	CGCAAGTCCATTCTTTTCTC	50
3B	<i>XBE489227</i>	RFLP (<i>Bgl</i> II)	TGAAACCTGGCTTGTCATTT	TGGAATAGGCCAAAGAGATG	52
3D	<i>XTC274745</i>	EST-STS	TACCTCCAGATGGGTTCCA	CGACAGTGACCACAGTCAGC	54
3D	<i>XBE470848</i>	EST-STS	GGAAATGCAATGGAAGTGTG	AGCAGCAACTTGGATCTCTG	50
4B	<i>XTC262751</i>	EST-STS	AACAGACCATTGCCCAATC	AGATTTGCATCCCTCCGTTG	55
5A	<i>XBM138119</i>	EST-STS	ACTCACCTAGGCGGTCTCT	TATTCTCATCCGTGCAGTCG	54
6A	<i>XBE604879</i>	RFLP (<i>Eco</i> RI)	CATGAACAAATGCAACAAGC	TGACAAGAACAACCAACTGC	50
7A	<i>XBF293121</i>	EST-STS	TATCTTAGCCACAGGCATC	TGAAGCCGAAGTCATTGTAT	50
7B	<i>XTC270455</i>	EST-STS	CGACATCGAGCTGTGAAGC	AGTAAGGTGCGACCCGATGT	55
2B	<i>XBM140322**</i>	EST-STS	AAGGCTCACCAACAACCTCC	CTCGGAACAGCAACATTACC	50
2B	<i>XBF200812**</i>	EST-STS	GCATTCCCTCTAACGACTGA	TTCTTTGATAACCATCCGTAGG	50
2B	<i>XBE604773**</i>	EST-STS	GAGAGGCGCAAACAAAATAA	AACATCCTCATAGCCATCCA	50
2B	<i>XBE444541**</i>	EST-STS	TGGACCATGATGAGA	TTCTGGAGGATGTTGAGCAC	55
2B	<i>XBE517745**</i>	EST-STS	CTCACCTCCATTGCCTCTAA	AGAACCGAAGATTGCTGATG	50
2B	<i>XBF202540**</i>	EST-STS	TGGTTATGGACAAGAGAAGAAA	AGGTAGAAATGAGGCGAGTG	50
2B	<i>XBF485266**</i>	EST-STS	CAGCAAAGAACACGGAAAGT	GCAAGCACAGTAAAAGAAGCA	50
2B	<i>XBE606912**</i>	RFLP (<i>Hind</i> III)	GTCCAGAAGACCTGCAAGA	AGACAAGGTGAACCTCCACA	54
2B	<i>XBF483211**</i>	RFLP (<i>Eco</i> RI)	GATACACCAATGACGGAACAA	GCCAAGAAGGCTCCAATAAG	50
2B	<i>XTC313504**</i>	EST-STS	CTCGTCATGGGGTGACTTTT	TGGATCTGCTCGTCAAAGTG	50
2B	<i>XTC317202**</i>	EST-STS	AGGCATTTGGTCATTTTGG	GAAGGCATACTCAACAGAAATCA	50
2B	<i>XCK208937.2**</i>	EST-STS	TCTCATTAATGGCGCTCTCC	GAGGTTTCTTCTTGCGCTTG	52
2B	<i>XTX307686**</i>	EST-STS	AAGTCGCGTTGATGCAATTA	TTGACGTTTGCAACAGTTGAT	54
2B	<i>XTX339813**</i>	EST-STS	CGTTCCTTGCACATCACTAA	TGGAAATGCCACATCACTGT	50
2B	<i>XTX236919</i>	EST-STS	GCTCATATCGGAGGCTAGTTC	AAGTGCCCAACACTGGAAAG	50
2B	<i>XTX266385</i>	EST-STS	GCCGATTCATACCCACTCAA	ACTGCCTCTCCGCACTTACA	50
2B	<i>XTX266064</i>	EST-STS	GATCAACACCAGGGAGCACA	TTCTTGGAACCGGTGAGTGA	50

^y Asterisks (*) and (**) indicate ESTs mapped to the wheat chromosome arm 1AS in the Arina × Forno population, initially (1), and those mapped to the wheat chromosome 2B in the Salamouni × Katepwa population in a different study (2), respectively.

^z EST-STS = expressed sequence tag sequence-tagged site and RFLP = restriction fragment length polymorphism.

and 3.1, respectively (Table 4; Fig. 4), and those of the SK population were 1.0 to 4.2, with an overall mean of 2.6.

CIM identified significant QTL on the short arms of chromosomes 1AS and 7AS, designated *QSnbf.cu-1A* and *QSnbf.cu-7A*, respectively (Fig. 3). *QSnbf.cu-1A* was defined by the *Snn4* locus with an LOD of 7.03 (Fig. 3; Table 5) and *QSnbf.cu-7A* peaked at the EST-STS marker *XBF293121* with an LOD of 4.64 (Fig. 3; Table 5). Resistance effects at both QTL were contributed by Katepwa. *QSnbf.cu-1A* and *QSnbf.cu-7A* accounted for 23.5 and 16.4%, respectively, of the phenotypic variation in disease in this population (Table 5). Whereas the effects of *QSnbf.cu-1A* were due to a compatible *Snn4*–SnTox4 interaction, *QSnbf.cu-7A* was not associated with any of the known effector sensitivity loci. There were no significant QTL–QTL interactions observed for reaction to isolate 1A7a.

The average disease reaction types of the SnTox4-sensitive RILs were 2.0 to 4.2 with a mean of 3.0, and those of the SnTox4-insensitive RILs were 1.0 to 4.2 with a mean of 2.3 (Table 4; Fig. 4). RILs harboring the *Snn4* and *XBF293121* alleles from Salamouni (*Snn4*^S/*XBF293121*^S) showed relatively higher susceptibility (average disease reaction type of 3.2) to SNB compared with those harboring *snn4*^K/*XBF293121*^K, which had an average disease reaction type of 2.0 (Table 4; Fig. 5). The average disease reaction types in the presence of either *Snn4*^S or *XBF293121*^S were similar (2.7 and 2.5, respectively) but were less than the

values observed for *Snn4*^S/*XBF293121*^S and higher than those for *snn4*^K/*XBF293121*^K (Table 4; Fig. 5). Hence, the RILs harboring both *Snn4*^S and *XBF293121*^S were more susceptible to SNB than

TABLE 4. Phenotypic ratings of ‘Salamouni’ and ‘Katepwa’ and the 121 recombinant inbred lines of the Salamouni × Katepwa population for the allelic state combinations for *Snn4*, *XBF293121*, and *Snn4*/*XBF293121* after inoculation with conidia of *Stagonospora nodorum* isolate Sn99CH 1A7a

Genotype ^y	Average disease reaction type ^z	Reaction type range
Katepwa	3.1	3.0–3.5
Salamouni	2.2	2.0–2.5
<i>Snn4</i> ^S	3.0 a	2.0–4.2
<i>snn4</i> ^K	2.3 b	1.0–4.2
LSD = 0.17	...	...
<i>Snn4</i> ^S / <i>XBF293121</i> ^S	3.2 a	1.8–4.2
<i>Snn4</i> ^S / <i>XBF293121</i> ^K	2.7 b	2.0–3.3
<i>snn4</i> ^K / <i>XBF293121</i> ^K	2.5 b	1.3–4.2
<i>snn4</i> ^K / <i>XBF293121</i> ^S	2.0 c	1.0–3.0
LSD = 0.24	...	...
<i>XBF293121</i> ^S	2.9 a	1.3–4.2
<i>XBF293121</i> ^K	2.3 b	1.0–3.3
LSD = 0.18	...	...

^y Superscript S and K indicate alleles derived from Salamouni and Katepwa, respectively. LSD = least significant difference.

^z Numbers followed by the same letter in each section are not significantly different at the 0.05 level of probability.

TABLE 3. Analysis of the necrotrophic effector sensitivities segregating in the Salamouni × Katepwa recombinant-inbred population^y

Effector	Host gene	Chromosome	Markers	Sensitive RILs	Insensitive RILs	$\chi^2_{(df=1)}$ ^z	P
Ptr ToxA	<i>Tsn1</i>	5BL	<i>Xgwm499</i> , <i>Xbarc346.2</i>	64	57	0.405	0.5245
SnTox1	<i>Snn1</i>	1BS	<i>Xgwm33.1</i>	63	58	0.207	0.6494
SnTox3	<i>Snn3</i>	5BS	<i>Xcfd20.1</i>	69	52	2.388	0.1222
SnTox4	<i>Snn4</i>	1AS	<i>XBG262267</i> , <i>Xksum182.1</i>	54	67	1.397	0.2373

^y RILs = recombinant-inbred lines.

^z For a 1:1 segregation.

Fig. 3. Composite interval mapping of quantitative trait loci associated with Stagonospora nodorum blotch on chromosomes A, 1A and B, 7A in the ‘Salamouni’ × ‘Katepwa’ recombinant-inbred population. Genetic maps of the chromosomes with the positions of the marker loci and the centimorgan (cM) distances between loci are shown on the left. The logarithm of odds (LOD) threshold of 3.23 is indicated by the dotted line.

those harboring just one allele. This suggests that the *Snn4*–SnTox4 interaction as well as the interaction at the locus defined by the marker *XBF293121* contribute individually to disease caused by 1A7a in this population and effects of the two loci appear to be largely additive. Multiple regression analysis using the markers *Snn4* and *XBF293121* showed that, combined, the interactions accounted for 35.7% of the total variation in SNB disease in the SK population. The correlation between SNB disease and *Snn4* is slightly higher than that between SNB disease and *XBF293121* (Pearson's product moment correlation = 0.485 and 0.405 respectively).

Fig. 4. Frequency of the average *Stagonospora nodorum* blotch disease reaction types of the ‘Salamouni’ × ‘Katepwa’ recombinant-inbred population.

TABLE 5. Composite interval-regression mapping analysis of significant quantitative trait loci (QTL) associated with resistance to the Swiss *Stagonospora nodorum* isolate Sn99CH 1A7a in the ‘Salamouni’ × ‘Katepwa’ recombinant-inbred population

Chromosome arm	QTL	Marker/marker interval	R ²	LOD ^y	Additive effects ^z
1AS	<i>QSnb.fcu-1AS</i>	<i>Snn4</i>	0.23	7.03	–0.36
7AS	<i>QSnb.fcu-7AS</i>	<i>XBF293121</i>	0.16	4.64	–0.30

^y Logarithm of the odds.

^z Negative additive effects indicate that Katepwa contributed the alleles for resistance.

Fig. 5. Frequency polygon of the average *Stagonospora nodorum* blotch disease reaction types of the recombinant-inbred lines of the ‘Salamouni’ × ‘Katepwa’ population for the allelic state combinations of *Snn4*/*XBF293121* (K = Katepwa alleles and S = Salamouni alleles) after inoculation with conidia of the *Stagonospora nodorum* isolate Sn99CH 1A7a.

DISCUSSION

Evaluation of the SK population with the SnTox4-producing Swiss *S. nodorum* isolate 1A7a, which was also used in the study by Abeysekara et al. (1) to characterize the *Snn4*–SnTox4 interaction, revealed QTL on chromosome arms 1AS and 7AS. In agreement with the results of Abeysekara et al. (1), the *Snn4*–SnTox4 interaction was responsible for the effects observed for QTL *QSnb.fcu-1A*. The compatible *Snn4*–SnTox4 interaction caused mottled necrosis in the SnTox4-sensitive parents in both the AF and SK populations. There was moderately high correlation between the average SNB disease and the SnTox4 reaction in the AF population whereas that of the SK population was relatively low. Therefore, the *Snn4*–SnTox4 interaction seems to play a more significant role in causing SNB in the AF population compared with the SK population. Nevertheless, the QTL defined by the *Snn4*–SnTox4 interaction had the largest effect in both of these populations.

QTL *QSnb.fcu-7A* detected on the short arm of chromosome 7A was not associated with any of the currently known effector-sensitivity loci. Even though infiltration of the SK population with the culture filtrate of 1A7a only identifies SnTox4, it is possible that the 7AS locus has a corresponding virulence factor, which could be an as-yet-unidentified or uncharacterized necrotrophic effector. Either the effects at the 7AS locus are not due to a necrotrophic effector or the concentration of the effector produced in culture was insufficient to induce a visible phenotype. No SNB-resistant QTL have been previously identified on chromosome 7A, suggesting that this resistance QTL is novel and may be useful for the development of wheat varieties with improved SNB resistance.

Results of the infiltration and inoculation experiments of the parental lines of the SK population revealed what appeared to be

contradictory results. Salamouni was sensitive to the culture filtrate of 1A7a but moderately resistant to SNB caused by 1A7a, whereas Katepwa was insensitive to the culture filtrate but moderately susceptible to SNB caused by 1A7a, with the difference between the reaction types of the two parental lines being 0.90. The range of the average disease reaction types of the SK population inoculated with 1A7a showed transgressive segregation. Susceptibility alleles for both the *QSnb.fcu-1A* and *QSnb.fcu-7A* QTL were contributed by the SnTox4-sensitive parent Salamouni. Therefore, one would expect Salamouni to be more susceptible to SNB than Katepwa but the opposite was observed and, therefore, additional disease determinants must exist. Multiple susceptibility factors in Katepwa or resistance factors in Salamouni with minor effects may have gone undetected due to the relatively small population size, which could explain the apparent contradiction. Good evidence for this is provided by the fact that Salamouni and Katepwa had average disease reaction types of 2.2 and 3.1, respectively, whereas RILs having Salamouni alleles at both QTL had an average reaction type of 3.2 and those having Katepwa alleles at both QTL had an average reaction type of 2.0.

Additionally, there could be epistatic interactions, which are also difficult to detect in small populations. As mentioned before, it has been shown that host-effector interactions in the wheat-*S. nodorum* system can have additive or epistatic effects (14). Friesen et al. (17) showed that the presence of a compatible SnToxA-Tsn1 interaction could mask the effects of a compatible SnTox3-Snn3 interaction. Previous studies conducted on the wheat-tan spot system have also indicated that certain genotypes insensitive to known tan spot effectors can demonstrate susceptibility to disease and vice versa (5,9). Similar results have been reported in the wheat-SNB system. Liu et al. (26) showed that the parental lines W-7984 and 'Opata 85' differ in their responses to SnTox1 and disease development, whereas W-7984 is sensitive to SnTox1 but is the more resistant parent. Our results indicate that the same could be true in the SK population.

The two EST markers *XBG262267* and *XBE590632*, closely linked to *Snn4*, detect the same marker alleles in both Salamouni and Arina. Therefore, these two EST loci can potentially be useful as markers for marker-assisted selection and genotyping of lines carrying *Snn4*. Once SnTox4 has been purified, more robust screening of diverse wheat genotypes can be conducted to determine the efficacy of these markers for detecting *Snn4* alleles.

Previously published hexaploid wheat maps have varying genetic lengths (4,6,18,23,31,35,36,38). If the genetic length of the entire hexaploid wheat genome is $\approx 4,000$ cM, the genetic linkage map constructed in the SK population has a genome coverage of $\approx 80\%$. The markers were not evenly distributed on chromosomes and we observed 17 gaps, which were ≈ 30 to 50 cM in size. These gaps were found in the distal ends of the chromosomes. To obtain more complete genome coverage, these gaps between SSR markers will need to be filled and the linkage groups should be extended into telomeric regions where the frequency of recombination is relatively high. This objective can be achieved by targeting the gaps with additional SSRs or employing high-throughput marker systems such as target region amplified polymorphism (6,23) or Diversity Arrays Technology (3) markers. The SK population segregates for reaction to other *S. nodorum* and *Pyrenophora tritici-repentis* isolates and various agronomic characters and, therefore, will be useful for the genetic analysis and exploitation of genes for wheat improvement.

There is mounting evidence that necrotrophic pathogens exploit biological pathways in plants to trigger responses that create environments favorable for their survival and sporulation. Interestingly, these pathways appear to involve classical resistance-like genes and, therefore, likely are the same pathways which are detrimental to the survival of a biotrophic pathogen (11). Hence, the role of resistance genes in conditioning not only resistance but

also susceptibility could have repercussions when trying to pyramid resistance genes in crops.

ACKNOWLEDGMENTS

This research was supported by United States Department of Agriculture-Agricultural Research Service CRIS projects 5442-22000-037-00D and 5442-22000-030-00D. We thank C. Chu for assistance with statistical analyses and Z. Liu for providing the necrotrophic effectors used in this study.

LITERATURE CITED

1. Abeysekara, N. S., Friesen, T. L., Keller, B., and Faris, J. D. 2009. Identification and characterization of a novel host-toxin interaction in the wheat-*Stagonospora nodorum* pathosystem. *Theor. Appl. Genet.* 120:117-126.
2. Abeysekara, N. S., Friesen, T. L., Liu, Z. H., McClean, P. E., and Faris, J. D. 2010. Marker development and saturation mapping of the tan spot Ptr ToxB sensitivity locus *Tsc2* in hexaploid wheat. *Plant Genome* 3:179-189.
3. Akbari, M., Wenzl, P., Craig, V., Carling, J., Xia, L., Yang, S., Uszynski, G., Mohler, V., Lehmensiek, A., Kuchel, H., Hayden, M. J., Howes, N., Sharp, P., Vaughan, P., Rathmell, B., Huttner, E., and Kilian, A. 2006. Diversity arrays technology (DArT) for high-throughput profiling of the hexaploid wheat genome. *Theor. Appl. Genet.* 113:1409-1420.
4. Chalmers, K. J., Cambell, A. W., Krestschmer, J., Karakousis, A., Henschke, P. H., Pierens, S., Harker, N., Pallotta, M., Cornish, G. B., Shariflou, M. R., Rampling, L. R., McLanchlan, A., Daggard, G., Sharp, P. J., Holton, T. A., Sutherland, M. W., Apples, R., and Langridge, P. 2001. Construction of three linkage maps in bread wheat, *Triticum aestivum*. *Aust. J. Agric. Res.* 52:1089-1119.
5. Chu, C.-G., Friesen, T. L., Xu, S. S., and Faris, J. D. 2008. Identification of novel tan spot resistance loci beyond the known host-selective toxin insensitivity genes in wheat. *Theor. Appl. Genet.* 117:873-881.
6. Chu, C.-G., Xu, S. S., Friesen, T. L., and Faris, J. D. 2008. Whole-genome mapping in a wheat doubled haploid population using SSRs and TRAPs and the identification of QTL for agronomic traits. *Mol. Breed.* 22:251-266.
7. Eyal, Z. 1981. Integrated control of *Septoria* diseases of wheat. *Plant Dis.* 65:763-768.
8. Eyal, Z., Scharen, A. L., Prescott, J. M., and van Ginkel, M. 1987. The *Septoria* Disease of Wheat: Concepts and Methods of Disease Management. CIMMYT, Mexico.
9. Faris, J. D., and Friesen, T. L. 2005. Identification of quantitative trait loci for race-nonspecific resistance to tan spot in wheat. *Theor. Appl. Genet.* 111:386-392.
10. Faris, J. D., Haen, K. M., and Gill, B. S. 2000. Saturation mapping of a gene-rich recombination hotspot region in wheat. *Genetics* 154:823-835.
11. Faris, J. D., Zhang, Z., Lu, H. J., Lu, S. W., Reddy, L., Cloutier, S., Fellers, J. P., Meinhardt, S. W., Rasmussen, J. B., Xu, S. S., Oliver, R. P., Simons, K. J., and Friesen, T. L. 2010. A unique wheat disease resistance-like gene governs effector-triggered susceptibility to necrotrophic pathogens. *Proc. Natl. Acad. Sci. USA* 107:13544-13549.
12. Friesen, T. L., Chu, C.-G., Liu, Z. H., Xu, S. S., Halley, S., and Faris, J. D. 2009. Host-selective toxins produced by *Stagonospora nodorum* confer disease susceptibility in adult wheat plants under field conditions. *Theor. Appl. Genet.* 118:1489-1497.
13. Friesen, T. L., and Faris, J. D. 2010. Characterization of the wheat-*Stagonospora nodorum* disease system: What is the molecular basis of this quantitative necrotrophic disease interaction? *Can. J. Plant Pathol.* 32:20-28.
14. Friesen, T. L., Faris, J. D., Solomon, P. S., and Oliver, R. P. 2008. Host-specific toxins: Effectors of necrotrophic pathogenicity. *Cell Microbiol.* 10:1421-1428.
15. Friesen, T. L., Meinhardt, S. W., and Faris, J. D. 2007. The *Stagonospora nodorum*-wheat pathosystem involves multiple proteinaceous host-selective toxins and corresponding host sensitivity genes that interact in an inverse gene-for-gene manner. *Plant J.* 51:681-692.
16. Friesen, T. L., Stukenbrock, E. H., Liu, Z. H., Meinhardt, S. W., Ling, H., Faris, J. D., Rasmussen, J. B., Solomon, P. S., McDonald, B. A., and Oliver, R. P. 2006. Emergence of a new disease as a result of interspecific virulence gene transfer. *Nat. Genet.* 38:953-956.
17. Friesen, T. L., Zhang, Z., Solomon, P. S., Oliver, R. P., and Faris, J. D. 2008. Characterization of the interaction of a novel *Stagonospora nodorum* host-selective toxin with a wheat susceptibility gene. *Plant Physiol.* 146:682-693.
18. Groos, C., Gay, G., Perretant, M. R., Gervais, L., Bernard, M., Dedryver, F., and Charmet, G. 2002. Study of the relation between pre-harvest

- sprouting and grain color by quantitative trait loci analysis in a white-red grain bread-wheat cross. *Theor. Appl. Genet.* 104:39-47.
19. Joeheanes, R., and Nelson, J. C. 2008. QGene 4.0, an extensible Java QTL-analysis platform. *Bioinformatics* 24:2788-2789.
20. King, J. E., Cook, R. J., and Melville, S. C. 1983. A review of *Septoria* diseases of wheat and barley. *Ann. Appl. Biol.* 103:345-373.
21. Kosambi, D. D. 1944. The estimation of map distances from recombination values. *Ann. Eugen.* 12:172-175.
22. Lander, E. S., Green, P., Abrahamson, J., Barlow, A., Daly, M. J., Lincoln, S. E., and Newburg, L. 1987. MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* 1:174-181.
23. Liu, Z. H., Anderson, J. A., Hu, J., Friesen, T. L., Rasmussen, J. B., and Faris, J. D. 2005. A wheat intervarietal genetic linkage map based on microsatellite and target region amplified polymorphism markers and its utility for detecting quantitative trait loci. *Theor. Appl. Genet.* 111:782-794.
24. Liu, Z. H., Faris, J. D., Oliver, R. P., Tan, K.-C., Solomon, P. S., McDonald, M. C., McDonald, B. A., Nunez, A., Lu, S., Rasmussen, J. B., and Friesen, T. L. 2009. SnTox3 acts in effector-triggered susceptibility to induce disease on wheat carrying the *Snn3* gene. *PLoS. Pathog.* 5:e10000581.
25. Liu, Z. H., Friesen, T. L., Ling, H., Meinhardt, S. W., Oliver, R. P., Rasmussen, J. B., and Faris, J. D. 2006. The *Tsn1*-ToxA interaction in the wheat-*Stagonospora nodorum* pathosystem parallels that of the wheat-tan spot system. *Genome* 49:1265-1273.
26. Liu, Z. H., Friesen, T. L., Rasmussen, J. B., Ali, S., Meinhardt, S. W., and Faris, J. D. 2004. Quantitative trait loci analysis and mapping of seedling resistance to *Stagonospora nodorum* leaf blotch in wheat. *Phytopathology* 94:1061-1067.
27. Lu, H. J., Fellers, J. P., Friesen, T. L., Meinhardt, S. W., and Faris, J. D. 2006. Genomic analysis and marker development for the *Tsn1* locus in wheat using bin-mapped ESTs and flanking BAC contigs. *Theor. Appl. Genet.* 112:1132-1142.
28. Manly, K. F., Cudmore, R. H., Jr., and Meer, J. M. 2001. MAP MANAGER QTX, cross-platform software for genetic mapping. *Mamm. Genome* 12:930-932.
29. Messmer, M., Keller, M., Zanetti, S., and Keller, B. 1999. Genetic linkage map of a wheat spelt cross. *Theor. Appl. Genet.* 98:1163-1170.
30. Oliver, R. P., and Solomon, P. S. 2010. New developments in pathogenicity and virulence of necrotrophs. *Curr. Opin. Plant Biol.* 13:415-419.
31. Paillard, S., Schnurbusch, T., Winzeler, M., Messmer, M., Sourdille, P., Abderhalden, O., Keller, B., and Schachermayr, G. 2003. An integrative genetic linkage map of winter wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* 107:1235-1242.
32. Pestsova, E., Ganal, M. W., and Röder, M. S. 2000. Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. *Genome* 43:689-697.
33. Röder, M. S., Korzun, V., Wendehake, K., Plaschke, J., Tixier, M.-H., Leroy, P., and Ganal, M. W. 1998. A microsatellite map of wheat. *Genetics* 149:2007-2023.
34. Solomon, P. S., Lowe, R. G. T., Tan, K.-C., Waters, O. D. C., and Oliver, R. P. 2006. *Stagonospora nodorum*: Cause of *Stagonospora nodorum* blotch of wheat. *Mol. Plant Pathol.* 7:147-156.
35. Somers, D. J., Isaac, P., and Edwards, K. 2004. A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* 109:1105-1114.
36. Song, Q. J., Shi, J. R., Singh, S., Fickus, E. W., Costa, J. M., Lewis, J., Gill, B. S., Ward, R., and Cregan, P. B. 2005. Development and mapping of microsatellite (SSR) markers in wheat. *Theor. Appl. Genet.* 110:550-560.
37. Sourdille, P., Singh, S., Cadalen, T., Brown-Guedira, G. L., Gay, G., Qi, L., Gill, B. S., Dufour, P., Murigneux, A., and Bernard, M. 2004. Microsatellite-based deletion bin system for the establishment of genetic-physical map relationships in wheat (*Triticum aestivum* L.). *Funct. Integr. Genomics* 4:12-25.
38. Torada, A., Koike, M., Mochida, K., and Ogiwara, Y. 2006. SSR-based linkage map with new markers using an interspecific population of common wheat. *Theor. Appl. Genet.* 112:1042-1051.
39. Tsilo, T. J., Hareland, G. A., Simsek, S., Chao, S., and Anderson, J. A. 2010. Genome mapping of kernel characteristics in hard red spring wheat breeding lines. *Theor. Appl. Genet.* 121:717-730.
40. Walton, J. D. 1996. Host-selective toxins: Agents of compatibility. *Plant Cell* 8:1723-1733.
41. Wolpert, T. J., Dunkle, L. D., and Ciuffetti, L. M. 2002. Host-selective toxins and avirulence determinants: What's in a name? *Annu. Rev. Phytopathol.* 40:251-285.
42. Yu, J.-K., Dake, T. M., Singh, S., Benscher, D., Li, W., Gill, B., and Sorrells, M. E. 2004. Development and mapping of EST-derived simple sequence repeat markers for hexaploid wheat. *Genome* 47:805-818.
43. Zhang, Z., Friesen, T. L., Simons, K. J., Xu, S. S., and Faris, J. D. 2009. Development, identification, and validation of markers for marker assisted selection against *Stagonospora nodorum* toxin sensitivity genes *Tsn1* and *Snn2* in wheat. *Mol. Breed.* 23:35-49.