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Genetic Analysis and Mapping of Seedling Resistance to Septoria Tritici Blotch in 'Steele-ND'/'ND 735' Bread Wheat Population

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Septoria tritici blotch (STB) caused by *Mycosphaerella graminicola,* is one of the most destructive foliar diseases of wheat (*Triticum aestivum* L.) especially in temperate and humid regions across the world. The susceptibility of recently released varieties, evolution of resistance to fungicides and increasing incidence of STB disease emphasizes the need to understand the genetics of resistance to this disease and to incorporate host resistance into adapted cultivars. This study aimed to decipher the genetics and map the resistance to STB using a recombinant inbred line (RIL) mapping population derived from 'Steele-ND' (susceptible parent) and 'ND 735' (resistant parent). The RILs were evaluated in three greenhouse experiments, using a North Dakota (ND) isolate of STB pathogen. The mean disease severity of parental genotypes, 'ND 735' (11.96%) and 'Steele-ND' (66.67%) showed significant differences ($p < 0.05$). The population segregated for STB and the frequency distribution of RILs indicated quantitative inheritance for resistance. The mean disease severity in RILs ranged from 0 to 71.55% with a mean of 21.98%. The genome map of this population was developed using diversity array technology (DArT) and simple sequence repeat (SSR) markers. The framework linkage map of this population was developed using 469 molecular markers. This map spanned a total distance of 1,789.3 cM and consisted of 17 linkage groups. QTL mapping using phenotypic data and the framework linkage maps detected three QTL through composite interval mapping. One QTL was consistently detected in all experiments on the long arm of chromosome 5B, and explained up to 10.2% phenotypic variation. The other two QTLs, detected in single environments, were mapped to 1D and 7A and explain 13% and 5.5% of the phenotypic variation, respectively. The map position of the consistent QTL on 5BL coincides with the map position of durable resistance gene *Stb1* suggesting the importance of this region of 'ND 735' as a source of durable STB resistance for the wheat germplasm.

Keywords: wheat, *Triticum aestivum* L., Septoria tritici blotch, QTL analysis

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Introduction

Septoria tritici blotch (STB), a major leaf spotting disease of durum (*Triticum turgidum* L.) and bread wheat (*T. aestivum* L.), is incited by *Mycosphaerella graminicola* (Fückel) Schröter in Cohn (anamorph: *Septoria tritici* Desm.). It is one of the most destructive foliar diseases of wheat in temperate and humid regions (Eyal et al. 1987). In North America, STB occurs in association with other leaf spotting diseases including Stagonospora nodorum blotch (SNB), caused by *Phaeosphaeria nodorum* and tan spot caused by *Pyrenophora tritici-repentis* (Gilbert and Woods 2001; Singh et al. 2006). STB reduces the photosynthetic area of the leaves, causing a reduction in the amount of carbohydrates assimilation during grain filling that results in lower number and weight of kernels. Losses can be very high and may reach 50% under conditions favourable for disease development (King et al. 1983; Eyal et al. 1987). Further, STB causes significant losses in grain quality by black point, red smudge, and shriveled grain that causes reduced flour yield.

In recent decades, STB has increased in importance worldwide because of the intensified wheat production, growing of cultivars susceptible to STB, and changes in cultural practices including adoption of conservation agriculture practices that have been gaining popularity for reasons ranging from the economics of production to protection of the environment (Eyal et al. 1987; Ghaffary et al. 2011). It is anticipated that due to climate change, further increase in incidence and severity of STB will occur. The most widely grown hard red spring wheat (HRSW) cultivars are susceptible to STB (Singh et al. 2006; Mergoum et al. 2007). Fungicides offer control, but when grain prices are low, their use is not cost-effective and detrimental to the environment. Additionally, the recent discovery of resistance to fungicide has reduced the efficiency of these fungicides over time (Gisi et al. 2000, 2005) and further increased interest in breeding and growing STB resistant cultivars. Therefore, the incorporation of host resistance and development and cultivation of durable resistant cultivars is the most effective, economical, and environmentally friendly strategy for mitigating the threat of STB.

Understanding the mode of inheritance and mapping genes for STB resistance is paramount for breeders to develop breeding strategies for incorporation of STB resistance into adapted germplasm. The deployment of host resistance will prolong the latent period of STB infection as well as reduce the number and size of chlorotic and necrotic lesion formed in the leaf (Eyal et al. 1987; Kema et al. 1996; Somasco et al. 1996). The qualitative mode of inheritance was reported in studies of Arraianio et al. (2007), Brading et al. (2002), McCartney et al. (2002) and Somasco et al. (1996), wherein a gene-for-gene interaction has been suggested in the wheat-*M. graminicola* pathosystem based on significant cultivar-isolate interactions. To date, eighteen major genes conferring STB resistance (Brading et al. 2002; McCartney et al. 2002; Adhikari et al. 2003, 2004a, b, c; Chartrain et al. 2004, 2005; Arraianio et al. 2007; Ghaffary et al. 2011) have been reported and majority of them have been mapped using molecular markers. However, quantitative inheritance was reported in other studies (Jlibene et al. 1994; Simon and Cordo 1997; Zhang et al. 2001; Chartrain et al. 2004; Arraianio and Brown 2006). Quantitative resistance is generally isolate non-specific and provides resistance to wide variety of isolates. Partial

resistance and tolerance mechanism to STB has also been observed. Additionally, QTL mapping studies by Eriksen et al. (2003) reported QTL associated with both seedling and adult plant resistance for STB in a double-haploid population from 'Savannah' and 'Senat'. They mapped six QTL including QTL associated with seedling resistance on chromosome 3A near the *Stb6* locus and QTL for adult plant resistance on chromosomes 3A, 6B and 7B. Simon et al. (2004) using two different Argentinean isolates, mapped three QTL for seedling resistance to STB on chromosomes 1D, 2D and 6B and two QTL for adult plant resistance on 3D and 7B. This study also concluded that the position of the major QTL located on chromosome 7B is near or coincides with previously mapped gene *Stb8* by Adhikari et al. (2003).

Breeding for host resistance for STB disease is difficult due to the low heritability of non-specific resistance, lack of knowledge on the pathogen virulence spectrum, and scarcity of durable resistant source (Eyal 1999). Under field conditions, evaluation of wheat for resistance to STB is challenged by the variation in the environment, amount of natural inoculum, and competition between pathogen causing foliar diseases for healthy tissue to infect and colonize the plant (Gilchrist et al. 1999). Additionally, STB disease is seen as mixed infection with other leaf spotting diseases, making phenotypic evaluation difficult and erroneous. Studies have reported that evaluation of seedling for resistance to Septoria diseases under controlled conditions is accurate and reproducible, due to uniform disease pressure (Eyal 1999). Many studies have also reported a positive correlation between assessments of resistance at seedling level and adult plants under natural infection in the field conditions (Somasco et al. 1996).

Several studies have shown that a particular trait could have different genetic control among different segregating populations, making it important to study the genetic control of a trait of interest in different backgrounds and to have a more complete knowledge of the genetic architecture of the trait of interest. This objective of the present study was to determine the genetic control of resistance to STB under greenhouse condition and to map genes/QTL in a RIL population derived from HRSW wheat cultivar 'Steele-ND' (Mergoum et al. 2005) and HRSW line 'ND 735' (Mergoum et al. 2006). The identified molecular markers associated with QTL for resistance to STB may prove useful in HRSW breeding programs aimed at developing cultivars resistant to STB through marker assisted selection (MAS).

Materials and Methods

Plant material

One hundred and twenty nine RILs were developed from a cross between HRSW wheat cultivar 'Steele-ND' (Mergoum et al. 2005) and HRSW line 'ND 735' (Mergoum et al. 2006). North Dakota State University (NDSU), Fargo, ND, released this F_{2-8} RIL population in 2008 (Mergoum et al. 2009). The wheat cultivar 'Steele-ND' released in 2004 has the pedigree of 'Parshall' (PI 613587)/ND706, while line 'ND 735' is derived from the cross of ND 2709/3/'Grandin' (PI 531005)*3//'Ramsey' (CItr 13246)/ND 622/ND 2809.

The cultivar 'Steele-ND' and line 'ND 735' show susceptible and resistant reaction, respectively, to major leaf spotting diseases including STB, SNB, all virulent races of tan spot found in ND, and to toxins Ptr Tox A (produced by races 1 and 2 of *Pyrenophora tritici-repentis*) and Ptr Tox B (produced by race 5) (Singh et al. 2006; Mergoum et al. 2007).

Greenhouse experiments

To evaluate the 129 RILs and their parents, three independent experiments were conducted in 2009 under similar greenhouse conditions at NDSU, Fargo, ND. Each line was planted in plastic cones (3.8 cm in diameter and 20 cm long) filled with Sunshine mix blend #1(Fison Horticulture, Vancouver, B.C.). Fertilization was done using slow releasing fertilizer (3 g/l 15-30-15 Miracle Gro, Scotts, Pot Washington, NY). Watering of plants was done as required. Greenhouse temperatures were maintained in the range of 21–25ºC with 16 h photoperiod. Each experiment was conducted in a randomized complete block design (RCBD) with two replicates and four plants per cone formed an experimental unit. Additionally, 'Salamouni' as a resistant check and 'Glenlea' as a susceptible check were included in each experiment.

Inoculum production

Inoculums were produced using yeast sucrose liquid medium. The medium was prepared by mixing 10 g of yeast extract and 10 g of sucrose in 1 L of distilled water. From this solution, 100 ml medium was transferred to 250 ml capacity Erlenmeyer flasks and autoclaved for 20 min. After cooling, 200 µl of kanamycin sulphate was added to each flask. Approximately 1 ml of liquid culture of STB isolate Ma04-94 was transferred to each flask. Flasks were covered with cotton plugs and kept in an orbit shaker (Barnstead/Thermolyne, Dubuque, IA) at 150 rpm for 3–5 days depending up on growth of culture. After shaking, mycelia were removed by filtering the inoculum through 2–3 layers of cheesecloth. The spore suspension was counted using a haemocytometer and adjusted to 1.0×10^7 spores/ml before inoculation. Two drops of Tween 20 (polyxyethlene sorbitan monolaurate) was added per 100 ml of spore suspension before inoculation.

Inoculation procedure

Plants were inoculated with spores of *M. graminicola* nineteen days after planting using a CO_2-pressurized hand sprayer until runoff. Inoculated plants were allowed to dry off before transferring them to the mist chamber under continuous leaf wetness for 60 h. The mist chamber conditions were maintained at temperature 20–23ºC, humidity 85–100%, and a photoperiod of 16 h. The mist chamber was kept open for 1 h every 24 h to allow proper ventilation and avoid yellowing of leaf. Subsequently, the plants were transferred to greenhouse benches at a temperature of 21–25ºC until disease symptoms were observed.

Disease scoring

Plants were assessed for STB symptoms three weeks after inoculation. One infected leaf from each plant was scored for disease symptoms. Reactions were scored visually for the disease severity on a scale of 0 to 100 percentage by estimating the percentage leaf area covered with necrotic lesions with or without pycnidia (Gaunt et al. 1986; Saadaoui 1987).

Framework linkage map

In the present study, 469 molecular marker data on 118 RILs was used for construction of linkage maps. These include 429 DArT (Akbari et al. 2006) and 10 SSR markers described previously (Singh et al. 2010). Additionally, 30 more polymorphic SSRs were used as anchoring markers to reconstruct the linkage map for this population. DNA isolation and SSR analysis was performed as described earlier (Singh et al. 2010).

Statistical analysis

The analysis of variance was done using the general linear model (PROC GLM) considering genotypes and environment as random effects (Statistical Analysis System version 8.2, SAS Institute 1999). Error homogeneity was tested using a factor of 10 test. The broad sense heritability was calculated based on the ANOVA analysis. The linkage maps were constructed using MAPMAKER v3.0 (Lander et al. 1987) with a minimum LOD score of 3.0 and using Kosambi mapping function (Kosambi 1994). The QTL analysis was conducted on individual experimental data with composite interval mapping (CIM), using Windows QTL Cartographer v2.0 software (Wang et al. 2004). In this method, model 6 with forward and backward step-wise regression with five markers as cofactors to control genetic background effects and a 10 cM genome-wide scan window, was used for the detection of QTL. A LOD score of 2.0 was used for determining the presence of a putative QTL.

Results

Disease phenotyping

The parental genotypes of the RIL population showed contrasting reaction to STB and the RIL population was segregating for this trait. The susceptible parent ‘Steele-ND’ showed (Fig. 1, Table 1) a mean disease severity of 66.67% (range of 60% to 80%), statistically different ($p < 0.05$) from the resistant parent ‘ND 735’ which has a mean disease severity of 11.96% (range of 6.25 to 15.63%). The resistant check cultivar ‘Salamouni’ had mean disease severity of 2.17% (range of 1.25 to 2.75%) while, the susceptible check cultivar ‘Glenlea’ had a mean disease severity of 89.58% with a range varying from 83.75 to 98.75%. The mean disease severity of first experiment was low (14.01%) compared to other experiments. The highest mean disease severity (28.93%) and high pycnidial density was observed in the second experiment. The combined ANOVA for the three greenhouse experiments including the129 RILs, parents, the resistant and susceptible checks indicated

significant differences ($p < 0.0001$) among the means of genotypes (RILs) for STB resistance (Table 2). The significant effect of genotype by location interaction indicates the high variability for this disease. The majority of lines showed transgressive segregation in the direction of resistant parent 'ND 735' and the frequency distribution in each greenhouse season indicated the quantitative nature of disease resistance (Fig. 1). The broad sense heritability was calculated to be 0.88 based on the estimation of the components of variance. Such higher heritability estimates are common in greenhouse based disease resistance studies. Collectively these results indicated significant variations for the STB resistance in the parental genotypes and the RILs of the mapping population used during the present study, thus, suggesting its suitability for conducting QTL analysis.

Table 1. Septoria tritici blotch severity means (%) and range of parental genotypes, checks and RILs in three greenhouse experiments

Experiment	Parental mean		Check mean			RILs	
	'Steele-ND'	'ND 735'	Salamouni	Glenlea	Mean	Min	Max
1	60.00	15.63	2.50	83.75	14.01	0.00	69.38
2	80.00	6.25	1.25	98.75	28.93	0.00	90.00
3	60.00	14.00	2.75	86.25	23.00	0.00	56.25
Mean	66.67	11.96	2.17	89.58	21.98	0.00	71.88

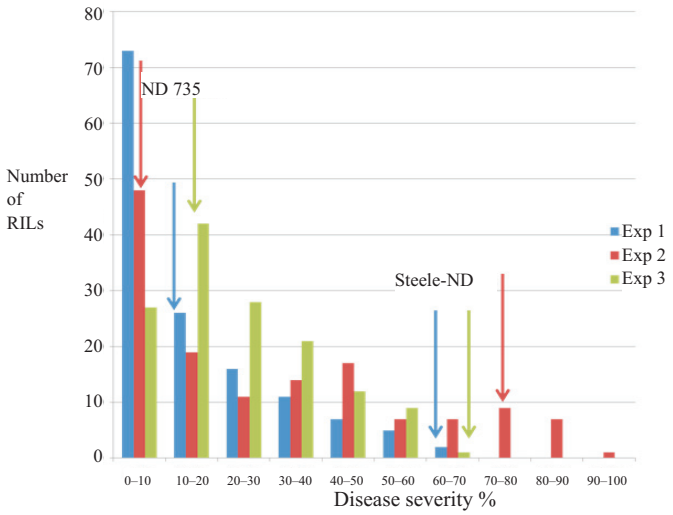

Figure 1. Histogram showing the frequency distribution of RILs and parents 'Steele-ND' and 'ND 735' reactions to Septoria tritici blotch in three greenhouse experiments based on disease severity percentage

Linkage map

A total of 469 polymorphic marker (429 DArT and 40 SSR) data on 118 RILs was used for the construction of linkage maps. Linkage maps, containing at least 3 marker loci, were

Table 2. Analysis of variance for Septoria tritici blotch resistance in 'Steele-ND'/'ND 735' RIL population studied in three different greenhouse locations with two replications during spring and fall of 2009

Source of variation	DF	SS	MSS	F-value
Environment	2	30905.5	15452.7	29.55***
Rep (Environment)	3	7319.6	2439.9	
Genotypes	132	211939.2	1605.6	3.07***
Genotypes × Environment	264	138045.6	522.9	1.39***
Error	396	149212.8	376.8	

*** Significant at 0.001

obtained for 14 chromosomes (Fig. S1*). The final linkage map contain 392 markers (364 DArT and 28 SSR) assigned to 277 unique loci. The total genetic length of the linkage map was 1,789.3 cM; with an average density of one marker per 4.57 cM (Fig. S1). The B genome had the highest number of mapped loci, while the D genome had the minimum loci. The map lengths were 840.3 cM, 788 cM and 161 cM for the A, B and D genomes, respectively. The B genome had 245 markers spread over 788 cM covering whole B genome with average marker density of one marker per 3.22 cM. The A genome had a total of 138 markers covering most of the A genome except chromosome 2A. The A genome had a map length of 840.3 cM with average marker density of one marker per 6.09 cM. The D genome had the least marker density (one marker/17.89 cM) and least map coverage (9 markers covering 161 cM map length only on chromosome 1D). The individual map lengths range from 42.60 cM on 5B to 201.60 cM on 7B and the number of markers in individual chromosomes range from 9 on 1D to 59 in 2B. The order of SSR markers and the DArT markers was mostly in agreement with the other published maps (Semagn et al., 2006; Francki et al., 2009).

QTL mapping

Results of QTL mapping conducted on individual experiment data as well as mean data is given in Table 3 and Figure 2. Composite interval mapping identified one stable QTL on chromosome 5BL that was mapped in all individual experimental data. This QTL on 5BL explained up to 10.20% of phenotypic variation. The other two QTLs were detected one each on 1D and 7A. These QTLs explained 13% and 5.5% of phenotypic variation, re-

Table 3. QTL for STB detected using composite interval mapping in 'Steele-ND'/'ND 735' RIL mapping population

Environment	Chromosome	Flanking markers	Position (cM)	LOD	$R^2 \times 100$ (%)	Additive effect
1	5B	XwPt7101/X377410	27.00	2.20	7.10	4.35
2	5B	XwPt-250/X377410	23.00	2.90	8.75	9.93
3	1D	XwPt1799	147.90	3.50	13.00	5.40
	5B	XwPt7101/X377410	23.00	3.80	10.20	4.80
	7A	XwPt8043	103.60	2.10	5.50	–3.50

* Further details about the Electronic Supplementary Material (ESM) can be found at the end of the article.

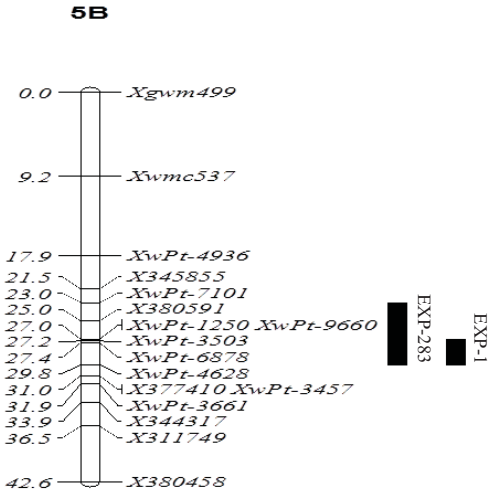

Figure 2. QTL map showing position of STB resistance QTL on 5BL chromosome of 'Steele-ND'/'ND 735' RIL mapping population.The length of bar indicate one LOD interval. EXP: Experiment

spectively. The QTLs on 1D and 7A were detected only in the third experimental data. The position of QTL detected on 5BL was similar in all experimental data. This stable QTL was mapped on long arm of 5B flanked by two DArT markers *(XwPt-7101/X377410)*. As the stable QTL only explained a maximum of 10.20% of the phenotypic variation, the rest of the variation could possibly be explained by the other QTLs and the interaction that may be present in unmapped linkage groups.

Discussions

The genomic constraints of the large hexaploid wheat genome as well as the lower level of polymorphism exhibited by common wheat compared to other cereals (Langridge et al. 2001) makes the molecular mapping efforts in common wheat very complex. Therefore, genetically diverse parents or inter-specific crosses have been utilized to create the mapping populations. However, these populations lack desirable trait variation that can be readily used in breeding program for commercial cultivars. Molecular mapping on populations derived from inter-varietal crosses were suggested to make the marker-trait association more relevant to the objectives of the breeding program (Varshney et al. 1998; Somers et al. 2004). This study involved a population developed from an inter-varietal cross, similar to their inter-varietal populations utilized by Liu et al. (2005) and Paillard et al. (2003) for developing genetic maps. The length of the linkage map (1,789.7 cM) developed during the present study was less than other published wheat maps, which could be attributed to low level of polymorphism for the D genome. The largest linkage group in our study was in chromosome 7B (201.60 cM), which is slightly larger than other published maps of Liu et al. (2005) and Paillard et al. (2003).

The line 'ND 735' showed a high level of resistance to STB. Additionally, Singh et al. (2010) studying the same RIL mapping population mapped resistance genes *Tsr1* and *Tsr6* for resistance to races 2 and 5 of *Pyrenophora tritici-repentis,* cause of tan spot of wheat. The gene *Tsr1* was mapped in 5BL and *Tsr6* was mapped in 2BS, with DArT markers *wPt-3049* (2.9 cM) and *wPt-0289* (4.6 cM), linked to both genes, respectively. The same mapping population was used by Singh et al. (2011) for mapping *Tsn1* locus in 5BL, which confers resistance to spore suspension and culture filtrate for *Phaeosphaeria nodorum* isolate Sn2000, the causal agent of Stagonospora nodorum blotch (SNB). The line 'ND 735' is also resistant to Fusarium head blight (Mergoum et al. 2007). The presence of the tetraploid wheat cultivar Ramsey as well as FHB-resistant Chinese wheat cultivar 'Sumai 3' in its pedigree makes 'ND 735' resistant to Septoria diseases and FHB (unpublished data). The cultivar 'Steele-ND' shows susceptible reaction to the STB, SNB, tan spot and FHB (Mergoum et al. 2005; Singh et al. 2010, 2011), but is superior in many agronomic and quality traits. This shows that this RIL population segregates for a lot of traits, meaning that it is useful for identifying QTLs associated with disease resistance, agronomy and quality traits.

The accurate phenotyping of STB is difficult in field conditions of ND, due to the mixed infection of various leaf spotting diseases as well as less favorable environmental conditions for disease development during the spring wheat-growing season. Therefore, most of the STB studies in ND (Mergoum et al. 2007; Ali et al. 2008), were conducted in greenhouse conditions at seedling stage. Previous QTL mapping experiments on STB (Eriksen et al. 2003; Simon et al. 2004; Simon et al. 2010) involved testing at seedling and adult plant stages. Simon et al. (2010) mapped two-linked isolate specific QTLs for seedling and adult plant resistance to STB using an Argentinean isolate. This further confirmed the positive correlation found in the studies of Somasco et al. (1996) and the efficacy of doing seedling stage evaluation for resistance to STB. However, seedling resistance is not always strongly associated with adult plant resistance for STB hence additional efforts are needed to identify and incorporate adult plant resistance into commercial cultivars.

The majority of molecular mapping studies in STB confirmed the existence of major gene resistance following the gene-for gene-model. The QTL mapping studies of Eriksen et al. (2003), Simon et al. (2004), and Simon et al. (2010) found the position of major QTL coinciding with the position of already mapped major genes. During our study, the position of stable QTL was mapped to the long arm of chromosome 5B. Previously, Adhikari et al. (2003), mapped resistance gene *Stb1* on the long arm of chromosome 5B using RAPD and microsatellite markers. The gene *Stb1,* originated from the wheat cultivar 'Bulgaria 88' was bred into the soft red winter cultivars 'Oasis' and 'Sullivan' in Indiana and is among the few genes that have provided durable resistance to STB in the central USA since its deployment in the early 1970s (Adhikari et al. 2003). Therefore, it seems the QTL found in this study is the same as *Stb1,* a durable resistance gene, which is more likely the major source of resistance to STB in the 'Steele-ND'/'ND 735' RIL mapping population. The identification of a QTL on 5BL at the same location where a major gene/QTL for STB had been earlier reported suggests the importance of this region in a variety of wheat germplasm and environments, but probably with various levels of expression in different

genetic backgrounds. Previous mapping of several other disease resistance genes, like *Tsr1* for resistance to race 2 *Pyrenophora tritici-repentis,* causing tan spot (Singh et al. 2010) and *Tsn1* conferring resistance to spore suspension and culture filtrate for *Phaeosphaeria nodorum* isolate Sn2000, cause of Stagonospora nodorum blotch (Mergoum et al. 2009) in the same region suggests the importance of the this region of chromosome 5B.

This study, utilizing 469 molecular markers observed 17 linkage groups across 14 chromosomes. The study identified one consistent QTL on long arm of chromosome 5B across environments and two QTL on 1D and 7A in only specific environments. This indicates that genotype × environmental interaction influence on QTL detection. Previous studies have identified genes contributing to STB resistance located on chromosomes 1D (Chartrain et al. 2005) and 7A (Goodwin 2007), however, further studies are needed to confirm the relationship. Additionally, since in this study involved markers located on 14 chromosomes hence there may be more loci present in the rest of the genome that may be contributing to STB resistance.

The current study as well as previous studies by our research team (Singh et al. 2010, 2011) confirms the presence of multiple leaf spot resistance in 'ND 735' and the effectiveness of the combination of DArT and SSR markers for molecular mapping of disease resistance genes. The line 'ND 735', in all likelihood possess durable resistance, a resistance effective over time and space, for tan spot, Stagonospora nodorum blotch and Septoria tritici blotch that can be easily utilized as a source for incorporating multiple leaf spot resistance into varietal development. Additionally, since 'ND 735' possesses good quality and agronomic characteristics, utilizing 'ND 735' in breeding programs will not bring undesired traits, due to linkage drag when using it as a donor parent.

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Electronic Supplementary Material (ESM)

Electronic Supplementary Material (ESM) associated with this article can be found at the website of CRC at http://www.akademiai.com/content/120427/

Electronic Supplementary *Figure S1.* Genetic linkage maps constructed in the 'Steele-ND'/'ND 735' RIL mapping population