GENETICS AND QUANTITATIVE TRAIT LOCI MAPPING OF SEPTORIA TRITICI BLOTCH RESISTANCE, AGRONOMIC, AND QUALITY TRAITS IN WHEAT

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Genetics and Quantitative Trait Loci Mapping of Septoria Tritici

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ABSTRACT

Most breeding programs aim at developing superior germplasm and better cultivars that combine high yield, disease and pest resistance, and end-use quality to satisfy the requirements of the growers as well as industry. A population, consisting of 138 F_{2-8} recombinant inbred lines (RILs) derived from a cross between 'Steele-ND' and ND 735, was evaluated to study the inheritance pattern of the septoria tritici blotch (STB)-resistant genes, agronomic and quality traits. The framework map made of 392 markers, including 28 simple sequence repeat (SSR) markers and 364 DArT markers, spanned a total distance of 1789.3 cM and consisted of 17 linkage groups. The map position of quantitative trait loci (QTL) found in this study coincided with the map position of durable STB resistance genes, Stb1. Thirteen QTL were detected for agronomic and quality traits. More saturation of the current map is needed to explore more QTL for this population.

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GENERAL INTRODUCTION

Wheat (Triticum spp.) is one of the most important grain crops in the world, and based on cultivated area, it ranks along with maize (Zea mays) and rice (Oryza sativa) as one of the world's three major crops (Smith, 2001). The estimated demand for wheat is expected to be 40% greater than its current level by 2020 (Rosegrant et al., 1997). Wheat production is required to rise at the rate of 2% annually to meet the predicted demand of about 800 million metric tons by 2020; current production is 600 million metric tons (Singh et al., 2008).

As a principal cereal crop in the United States (U.S.), wheat covers more than 20 million hectares of cultivated area, with an average production of 3 metric tons per hectare (United States Department of Agriculture-Foreign Agricultural Service [USDA- FAS], 2008). In 2009, US harvested 20.18 million hectares of wheat, producing 60.31 million metric tons of grain and making it the fourth-largest producer of wheat in the world behind China, India, and Russia. This harvest includes 9.77 million hectares of hard red winter wheat with a production of 24.83 million metric tons, 4.98 million hectares of hard red spring wheat with a production of 14.79 million metric tons, and 0.98 million hectares of durum wheat with a production of 2.94 million metric tons. Approximately 2.91 million hectares of soft red winter wheat and 1.53 million hectares of white wheat have a production of 10.89 million metric tons and 6.38 million metric tons, respectively.

According to the North Dakota Wheat Commission (NDWC, 2006), North Dakota (ND) produces 44% of all U.S. hard red spring wheat, making it the national leader. North Dakota produces 6.4 metric tons of hard red spring wheat from an average of 2.7 million hectares of cultivated area (NDWC, 2006). Hard red spring wheat is known for its superior quality; in

particular, high protein content combined with high gluten strength make it suitable for blending to get the desired protein level in other low-protein wheat classes for good-quality bread making.

Reduced contributions of new wheat-growing areas and a declining trend in existing wheat-producing areas put the responsibility for increasing production to meet the requirements of population growth entirely under our ability to explore genetic diversity and to release higher-yielding cultivars. It is important to study the genetics of yield and the associated agronomic traits to achieve this goal. Additionally, genetic studies help breeders understand the inheritance of traits and give ideas about potential genetic gain for each trait.

LITERATURE REVIEW

Septoria Tritici Blotch

Septoria tritici blotch (STB) of wheat is caused by the ascomycete fungus *Mycosphaerella graminicola* (Fückel) J. Schröt. (Anamorph: *Septoria tritici* Desm.), and it is grouped under the kingdom fungi, phylum *ascomycota*, class *loculoascomycetes,* order *dothideales*, genus *Mycosphaerella*, and species *graminicola*. Septoria tritici blotch is presently considered the most serious foliar fungal disease of wheat-growing areas in Europe, South America, North Africa, and central Asia, where the climatic conditions of high humidity and a temperature range of 15-20 ^{o}C coincide with the wheat-growing season and favor disease development (Duveiller et al., 2007). Historical evidence points toward the occurrence of this pathogen in wild species of wheat (Eyal, 1974), but the evolutionary history of this pathogen suggests the recent origin of this new pathogen along with the domestication of wheat in the Fertile Crescent (Stukenbrock et al., 2007).

The importance of STB as a disease, and the emergence of this pathogen as a serious pest in wheat-producing areas, was attributed to the widespread growth of high-yielding, susceptible, semi-dwarf spring wheat cultivars with an increased rate of nitrogen fertilizer top-dressing in late 1960s and early 1970s (Bayles, 1991; Eyal et al., 1987; Lovell et al., 1997). Additionally, the poor practices for stubble and residue management in a high-frequency crop rotation system helped the regular incidence of this disease due to the stubble-borne nature of this pathogen (Brokenshire, 1975). Simon et al. (2003) observed the faster disease progression and higher disease severity of STB in nitrogen-fertilized treatments under environmental conditions that were favorable for disease development, verifying the earlier reports that green-revolution practices contribute to the increased incidence of STB in wheat.

As early as 1929, Mackie reported the first incidence of STB in the United States; subsequently, the disease was reported in the Pacific Northwest (Camacho-Casas et al., 1995), northern great plains (Zhang et al., 2001), and eastern soft-wheat regions (Shaner and Finney, 1982). Even in the absence of recent outbreaks of this disease in North Dakota, the importance of this disease as part of wheat leaf-spotting complex, which includes STB; stagnospora nodorum blotch (SNB), caused by *Phaeosphaeria nodorum* [anamorph: *Stagnospora nodorum* (Berk.) Castellani & Germano.]; and tan spot, caused by *Pyrenophora tritici-repentis* [anamorph: *Drechslera tritici-repentis* (Died.) Shoem.], is considered an important factor for yield reduction (Gilbert and Woods 2001; Singh et al., 2006). This disease-complex formation also prevents disease testing and scoring for resistance in the field condition.

The impact of septoria diseases on yield reduction is in the range of 10-15%; however, under favorable conditions of disease development, the disease outbreaks cause yield reductions up to 50% (Eyal et al., 1987; King et al., 1983). As a disease affecting the flag leaf, reduction in the photosynthetic area caused by STB creates significant economic loss for the farmer through yield reduction and a subsequent reduction in grain quality. The quality reduction is seen as grain shriveling; red smudge; black point of grains; and a reduction in grain volume weight, milling, and baking quality (Eyal, 1999; McKendry et al., 1995). There are contradictory reports regarding the effect of STB on flour protein; a reduction in flour protein was reported in the studies of Arabi et al. (2007), however, McKendry et al. (1995) reported an increase for flour protein.

Infection Process

The STB infection starts with the penetration of hyphae, and depending on the nature of the host (susceptible vs. resistant), a high or low biomass of fungal hyphae is produced. Even

though the fungal colonization happens, the symptoms of this infection are not seen until 10 d later (Palmer and Skinner, 2002). An initial symptom of STB starts with chlorotic specks on the inoculated leaf (Eyal et al., 1987). The formation of small, tan- colored lesions of necrotic tissue is seen following the chlorotic spots within 14 d after inoculation (Goodwin, 2007). The necrosis is delimited by leaf veins and looks rectangular, but under favorable conditions, these necrotic lesions invade the leaf vein and appear as irregular or lens shaped (Goodwin, 2007). The rapid formation of chlorosis and necrosis indicates the collapse of mesophyll tissue and points towards the role of toxic compounds in this host-pathogen interaction (Kema et al., 1996c). However, scientists could not isolate any biologically active toxic compound produced by fungi (Eyal, 1999).

The formation of pycnidia begins 14 d after inoculation, seen as a black dot within the necrotic lesions (Eyal et al., 1987), and it exudes pinkish-brown, column-shaped, jelly-like masses of spores upon wetting by rain or dew. The production of extensive necrosis is sometimes seen with restricted pycnidial development (Cohen and Eyal, 1993), and pycnidia may develop in green leaf tissue without necrosis (Rosielle, 1972). Further research made Kema and co-workers (1996a) suggest that the production of necrosis and pycnidia seems closely related, but it may be under different genetic controls.

Even though an ascospore produces similar symptoms as pycnidiospore infection, the number of pycnidia produced during the ascospore infection is less due to the longer latent-period spores. A decrease in the number of infection units and the longer time needed for the ascospores to produce pycnidiospore for infection lowers the disease severity resulting from ascospore infection (Garcia and Marshall, 1992; Hunter et al., 1999). The effect of STB disease is seen as a reduction in green tissue, early leaf senescence, a slow leaf expansion, and a reduced

size for emerging leaves (Gaunt et al., 1986). Yield reduction happens because of a reduction in the number of grains per spikelet and a reduction in grain weight (Eyal and Ziv, 1974 Thomson and Gaunt, 1986). The yield loss is higher when the infection reaches the flag leaf.

Factors Affecting Disease Development

Among the factors affecting the disease development and severity of STB in field conditions, the amount of natural inoculum, the temperature, and the duration of leaf wetness are very important. There are several reports about environmental conditions, moisture and temperature (Hess and Shaner, 1987), and light and leaf wetness (Shaw, 1991) associated with the infection process. Hess and Shaner (1987) found a positive correlation among the post-inoculation leaf moisture, temperature after fungal colonization, and STB development. Shaw (1991) studied the effect of intermittent moisture and light period on STB development for winter wheat and observed a reduction in disease due to the reduction or break in the 100% humid condition. The light reduced the infection in early stages but enhanced later stages of infection.

Chungu et al. (2001) studied the effects of moisture, temperature, and inoculum concentration on the STB-infection process and disease severity for two durum and bread wheat cultivars in greenhouse conditions. They found that the optimal temperatures for disease screening of spring wheat were 18 ^{0}C day/15 ^{0}C night, starting from the mist chamber incubation, and 22 ^{0}C day/15 ^{0}C nights after 15 d of inoculation. Additionally, the higher temperatures resulted in an increased percentage of pycnidial coverage. Even though the pycnidial density of leaf increases with a higher temperature, the host cultivar has more of an effect on pycnidial density than temperature (Hess and Shaner, 1987; Shaner and Finney, 1982). The latent period of the pathogen is also prolonged with an increase in temperature, and the

minimal latent period is observed at 17 ^{0}C (Hess and Shaner, 1987; Shaw, 1991). Moisture is considered essential for all stages of disease development (Shaner et al., 1975). A leaf wetness period up to 96 h is required for the ascospores to generate pycnidia production (Garcia and Marshall, 1992). Chungu et al. (2001) suggest 48-72 h as an optimal period for greenhouse testing with an inoculation concentration of 1×10^{7} spores' ml^{-1}. Besides macro-environmental factors, a micro-environmental factor, such as temperature of the crop canopy, is considered important in disease development. A higher temperature for the crop canopy increases the disease severity and causes a proportionate decrease in green leaf tissue (Eyal and Blum, 1989).

In addition to environmental factors, some phenotypic traits of cultivars affect disease progression. The host resistance that is often found in the tall, late-maturing cultivars is considered an escape mechanism (Camacho-Casas et al., 1995) or is due to the microclimate differences in crop canopy (Eyal et al., 1987). Baltazar and co-workers (1990) found that lines carrying the '*Rht 2* gene' are more resistant when compared to lines containing the '*Rht 1* gene'. Conversely, Arama et al. (1999) proposed that host resistance is not dependent on heading date and that STB resistance and heading date are genetically different. Additionally, Simon et al. (2004b) studied near isogenic lines differing in dwarfing genes and found that the positive correlation between days to heading and STB development was caused by environmental conditions.

Disease Control

The disease control of STB can be achieved by different means: via cultural practices to reduce the disease incidence, chemical control in the case of wheat growing in areas with high disease pressure, biological control as a more sustainable strategy using suitable bio-agents, and developing cultivars tolerant or resistant to this disease.

The cultural practices include the destruction of stubble and plant debris from infected fields, and the practice of several crop rotations with non-host crops. The viability and pathogenicity of spores can be greatly reduced by following a tillage system that can bury the crop stubble under the soil (Brokenshire, 1975). Eyal et al. (1987) suggests that a three- to five-year crop rotation followed by proper management of stubble is necessary to reduce the viability of a pathogen in the field. Even in properly managed fields, STB infection is possible by means of wind-borne ascospores from neighboring fields.

Chemical control of STB is recommended when the crop is growing under high disease pressure where STB is a common disease. Depending on the environmental conditions, the application of foliar fungicides at the time of spike emergence prevents yield and quality loss (Eyal, et al., 1987; Shaner and Buechley, 1995). The main group of fungicides used to control STB is the Azoles and Quinone outside inhibitor (QoI) types. However, the development of fungicide resistance reduces the efficiency of these fungicides over time (Fraaije et al., 2001). Although Triazoles (Azoles) have been extensively used for over 20 years without any resistance developing (Gisi et al., 2000), high levels of resistance are reported in populations of *M. graminicola* after the introduction of Strobilurins (QoI) in 1996 (Gisi et al., 2005). Even though Azoles are still used in controlling STB, the reduced efficiency of a fungicide makes it less cost effective for the farmer due to the increased application rate, reduced yield potential, and price fluctuations in market.

Biological control methods offer a more sustainable use of bio agents for controlling STB disease. Additionally, slow development and a longer latent period associated with disease development favors biological control by providing sufficient time for STB disruption using a microorganism (Nolan and Cooke, 2000). *Trichoderma spp*. is widely used in Argentina for

controlling leaf-spotting diseases in wheat and is found to be effective against septoria diseases (Perello et al., 2006). The other potential bio-agents include *Bacillus megaterium*, which controls STB without affecting yield (Kildea et al., 2008). The main disadvantage of biological control is its reduced efficacy in field conditions. Additionally, the efficacy of this method is also affected by soil type, host-plant cultivar, pathogen strains, and inoculation method (Perello et al., 2006).

Tolerance to STB has been reported in several high-yielding cultivars of both spring wheat and winter wheat (Parker et al., 2004; Ziv and Eyal, 1978; Zuckerman et al., 1997). The crop canopy density, stem reserve capacity, and leaf photosynthetic rate are major candidate traits which can affect tolerance (Parker et al., 2004). Zukerman et al. (1997) found that tolerance resulted from the most effective fixation of CO_2 per unit of infected leaf area, per tiller, and per unit of chlorophyll. The disease-tolerant cultivars are able to endure the high disease level without affecting the yield and exerting selection pressure on the pathogen when compared to the other susceptible cultivars. However, developing host resistance and deploying resistant germplasm are considered eco-friendly and are an economical method for disease control (Eyal, 1999; Fraaije et al., 2001). The fact that more than 70% of fungicides applied to wheat in Europe are to prevent this disease (Goodwin, 2007) signifies the need for host resistance to combat the disease.

Genetics of Host Resistance

The efficiency of disease-resistant breeding for developing STB-resistant cultivars improves with knowledge about the inheritance of host resistance and sources of resistant genes. The most widely used sources for STB resistance include germplasm from South American, Chinese origin, winter wheat, and wild relatives of wheat. The germplasm Kavkaz-K4500 L.6.A.4, obtained by combining the resistant genes from Russian winter wheat ‘Kavkaz’ and a

Brazilian cultivar, Frontana, was one of the earliest sources used in the breeding program at the International Maize and Wheat Improvement Center (CIMMYT) in Mexico (Eyal, 1999). The other widely used sources for STB-resistant genes include 'Arina' (Switzerland), 'Bulgaria 88' (Bulgaria), 'Chinese Spring' (China), 'Courtot' (France), 'Catbird' and 'Milan' (CIMMYT), 'Olaf' (USA), 'Israel 493' and 'Shafir' (Israel), 'Flame' and 'Tonic' (UK), 'Senat' (Denmark), 'Veranopolis' (Brazil), and TE9111 (Portugal); Chartrain et al., 2004a). The wild species of wheat that are useful for disease-resistant breeding include accessions of *T. monococcum* L. (Jing et al., 2008; Singh et al., 2006), *T. dicoccum* (Gilchrist and Skovmand, 1995; Rosielle,1972; Singh et al., 2006), *T. timopheevii* (Singh et al., 2006), *T. pyramidale*, *T. carthelium*, *T. polonicum* (Rosielle, 1972), *T. speltoides* (McKendry and Henke, 1994b), *T. tauschii* (May and Lagudah, 1992; McKendry and Henke, 1994a), and *Lophopyrum elongatum* (Anderson et al., 2010).

Genetic resistance to STB is seen as a reduced chlorotic and necrotic area, a reduction in the size and number of necrotic lesions, and a reduced density of pycnidia produced (Eyal et al., 1987; Kema et al., 1996a; Somasco et al., 1996). The inheritance of STB resistance may follow dominant, partially dominant, epistatistic, recessive, additive, and non-additive gene action (Eyal, 1999; Jilbene et al., 1994; Rosielle, 1972; Somasco et al., 1996). The cultivar resistance to STB may be either isolate-specific or isolate non-specific. The isolate-specific resistance may be monogenic or oligogenic, and the near-complete nature of resistance follows the gene-for-gene model (Arraiano and Brown, 2006; Brading et al., 2002; McCartney et al., 2002a, 2002b; Somasco et al., 1996). Additionally, the breakdown of the recently deployed genes verifies the isolate-specific nature. The isolate non-specific resistance is polygenic and provides partial resistance to a wide variety of isolates (Arraiano and Brown, 2006; Chartrain et al., 2004b;

Jilbene et al., 1994; Simon and Cordo, 1997; Zhang et al., 2001). The partial resistance is durable under field conditions and is expressed as a reduction in epidemic development (Chartrain et al., 2004b).

Until now, 15 major genes for STB have been identified and mapped in wheat. The *Stb1, Stb2*, *Stb3*, *Stb4,* and *Stb8* mapped, respectively, on 5BL, 3BS, 7AS, 7DS and 7BL (Adhikari et al., 2003, 2004a, 2004b, 2004c), and *Stb5* and *Stb15* mapped, respectively, on 7DS and 6AS (Arraiano et al., 2001, 2007). The STB genes *Stb9*, *Stb10*, *Stb11*, and *Stb1*2 mapped, respectively, on 2BL, 1D, 1BS, and 4AL (Chartrain et al., 2009, 2005a, 2005b). The other mapped genes include *Stb6* on 3AS, *Stb7* on 4AL, *Stb13* on 7BL, and *Stb14* on 3BS (Brading et al., 2002; Brule-Babel, 2007; Cowling, 2006; McCartney et al., 2002a).

The first QTL mapping study for STB resistance was done by Eriksen et al. (2003) using a double haploid population of a cross between the susceptible winter wheat cultivar, Savannah, and the resistant cultivar, Senat, in a growth-chamber and field conditions with two different isolates. They mapped six major QTL on 2BL, 3AS, 3BL, 3B, 6B, and 7B; they concluded that the QTL on 3A is linked to STB resistance in the field and growth-chamber studies. Simon and co-workers (2004a), using two different Argentinean isolates, found QTL controlling the trait in a mapping population where *Stb*8 was previously mapped. However, the comparable map location of QTL and *Stb8* shows the presence of additional genes on the same loci, or it may be the same locus controlling the trait. The difference may arise due to the various isolates used, eroding of resistance, and disease testing in the natural environment. They mapped three QTL on 1D, 2D, and 6B for seedling resistance and two QTL on 3D and7B for adult plant resistance. Additionally, they showed that different QTL controlled the trait in the seedling and adult plant stage.

Breeding for host resistance septoria diseases is hampered by irregular, sporadic epidemics; continuous selection pressure; lower heritability of non-specific resistance; a lack of knowledge about the pathogen's virulence spectrum; and a scarcity of durable resistant sources (Eyal, 1999). Additionally, the nature of disease development, the high influence of environment on the disease severity, and the cost involved with field disease screening force breeders to screen wheat lines in controlled environmental conditions with uniform disease pressure to find the susceptible lines and to further evaluate resistant lines in field conditions (Chungu et al., 2001).

Quantitative Trait Loci Mapping

The increased availability of molecular markers for genetic studies led to the development of detailed genetic and physical chromosome maps for bread wheat and enhanced the efficacy of molecular technology in plant breeding by carrying out indirect selection through molecular markers linked to the traits of interest (Gupta et al., 1999). QTL analysis involves selecting and hybridizing parental lines that differ for one or more quantitative traits and then analyzing the segregating progeny to link the QTL to known DNA markers. These DNA tags can be used by plant breeders to improve the breeding efforts and to speed up the creation of cultivars through marker-assisted selection (MAS).

Among the QTL mapping methods of single marker analysis (SMA), interval mapping (IM), and composite interval mapping (CIM), the CIM approach is widely used. The CIM is a modified IM method where the power of QTL detection is improved by using the markers as cofactors in the regression model; CIM permits the reduction of likely bias in the assessment of QTL positions and effects (Zeng, 1994). However, population size and trait heritability (Lander and Thomson, 1990) affect QTL detection. A larger population size is required for QTL mapping

of traits with low heritability; however, considering the time and cost needed for QTL mapping, a population size of fewer than 250 individuals was used in most studies (Bernardo, 2002). A multi-environment, replicated trial for phenotyping the quantitative trait is essential when mapping. Additionally, the proper experimental design and the use of replicates improve the power of QTL detection by enabling the researcher to investigate environmental influences on QTL affecting the traits of interest (Hittalmani et al., 2003).

In wheat, molecular maps have been prepared using restriction fragmented length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), simple sequence repeats (SSR), amplified fragmented length polymorphism (AFLP), sequence related amplified polymorphism (SRAP), and target region amplified polymorphism (TRAP) markers. The first linkage maps in hexaploid wheat were constructed using RFLP markers; however, RFLPs were not efficient in finding the polymorphism in wheat (Chao et al., 1989; Devos et al., 1992, 1992; Liu and Tsunewaki, 1991; Marino et al., 1996; Nelson et al., 1995a, 1995b, 1995c; Van Deynze et al., 1995; Xie et al., 1993). In comparison to RFLP markers, the PCR-based markers, SSRs (Plaschke et al., 1995; Röder et al., 1995; Ma et al., 1996; Bryan et al., 1997), RAPDs (Suenaga et al., 2005), AFLPs (Quarrie et al., 2005), TRAP (Liu et al., 2005), and SRAP (Li and Quiros, 2001), were able to detect higher polymorphism levels in wheat.

Bread wheat is an allohexploid containing three distinct, but genetically related, genomes, A, B, and D, where 80% of the genome consists of repetitive DNA (Röder et al., 1998). According to Arumuganathan and Earle (1991), the haploid DNA content of the bread wheat genome is approximately 1.7×10^{10} bp with an average of 810 Mb bp per chromosome. When comparing the size of the wheat genome with the genome size of maize and rice, three wheat chromosomes are equal to the haploid maize genome, and one-half of an average wheat

chromosome equals a haploid rice genome (Gill and Gill, 1994). Despite the low level of polymorphism exhibited by bread wheat (Langridge et al., 2001) and the complexity of the large genome size, success was achieved in characterizing the important agronomic and quality traits for bread wheat.

Molecular Mapping of Agronomy Traits

Wheat is one among the three most important cereals that are widely cultivated around the world. As the demand for high-yield and high-quality grain increases, the responsibility of producing high-yield and high-quality grain rests with the ability of the plant breeder to explore the genetic diversity and to achieve the maximum genetic gain. The important agronomic traits include heading date, plant height, spike and kernel characteristics, test weight (TW), grain yield, yield components, and maturity. Traits such as spike density, number of kernel per spike, and thousand-kernel weight (TKW) are included in the yield components needed to predict the total grain yield. The interaction between cultivars and the environment makes several traits, such as grain yield, complex to breed when selected in isolation. Thereby, suggested the need to select component traits when selecting for yield (Choudhry et al., 1986).

In the past, studies using chromosome substitution lines, as well as RIL, were employed to dissect the complex quantitative traits (Law, 1965, 1966). The addition of genetic-marker technology resulted in assigning yield-related loci to various wheat chromosomes: 3A (Campbell et al., 2003; Shah et al., 1999), 4A (Araki et al., 1999), 5A (Kato et al., 2000; Snape et al., 1985), 5B (Miura et al., 1992), and 7B (Law, 1967). With the advent of molecular markers and the availability of high-density linkage maps, the complete study of complex quantitative traits was possible with gene/QTL mapping. The availability of RFLP markers led to the development of genetic maps in individual homoeologous chromosomes of wheat (Chao et al., 1989; Devos et

al., 1992; Jia et al., 1996; Marino et al., 1996; Nelson et al., 1995a, 1995b, 1995c; Van Deynze et al., 1995; Xie et al., 1993). A combination of RFLP and AFLP markers resulted in complete genetic maps (Cadalen et al., 1997; Gale et al., 1995; Liu and Tsunewaki, 1991; Messmer et al., 1999). Among these different molecular maps, the map of Cadalen et al. (1997) was the first inter-varietal genetic map of wheat developed by crossing the French cultivar 'Curtot' and Chinese spring.

Earlier mapping efforts revealed a number of agronomically important genes: height reducing (*Rht*), response to photoperiod (*Ppd*), response to vernalization (*Vrn*), and earliness (*Eps*); (Worland et al., 1998). Cadalen et al. (1997) conducted more studies on plant height QTL for first inter-varietal map they published. In hexaploid wheat, the International Triticeae Mapping Initiative (ITMI) population was developed by crossing synthetic wheat 'W7984' and Mexican cultivar 'Opata85'. Researchers around the world used the ITMI mapping population to study agronomic and quality traits in wheat. Wheat yield QTLs are normally associated with the yield component QTLs of grain weight, spike length, number of grains per spike, and test weight. Thus, the distribution of yield QTL is seen all over the wheat genome. However, majority of studies with the ITMI population (Ayala et al., 2002; Borner, et al., 2002; Kulwal et al., 2003; Kumar et al., 2007) identified major yield QTL on chromosomes 2DS, 4 AL, and 4 DL. Borner et al. (2002) mapped major QTLs for various traits: ear emergence on 2DL and 5DL; plant height on 1AS, 2DS, 4AL, and 6AS; spike length on 4AL, 4 AS, and 5AL; number of grain per spikes on 2DS and 4AL; and grain weight on3AS, 5AL, and 6BS. They found yield QTLs associated with grain number per spike and spike length. Whereas, the QTL mapping study of Araki et al. (1999) found yield QTLs on chromosome 4A associated with grain weight. A number of mapping studies for agronomic traits utilized a wide cross-mapping population, and

numerous QTLs for yield and related traits were identified (Huang et al., 2004; Keller et al., 1998; Peng et al., 2003).

An inter-varietal mapping population was utilized by Cadalen et al. (1997), Groos et al. (2003), Sourdille et al. (2003), and McCartney et al. (2006) for mapping important agronomic traits in bread wheat. Sourdille et al. (2003) saturated the inter-varietal map made by Cadalen et al. (1997). They mapped QTLs for various agronomic traits: spike length (1AL, 2BS, 2DS, 4AS, and 5AL), number of spikelet (2AS, 2BS, and 5AL), heading time (2BS, 5AL, and 7BS), plant height (4BS, 4DL, 7AL, and 7BL), and ear compactness (1AL, 2AL, 2BS, 2DS, 4AS, 5AL, 5BL, and 6DL). They also reported the importance of clustering QTLs in yield-related traits on 2BS. The clustering of QTL may be due to the presence of gene clusters in linkages or the pleiotropic effect of one gene present in that region. Groos et al. (2003), using an RIL population, mapped one QTL for yield on 7D and three QTLs for TKW on chromosomes 2B, 5B, and 7A. During their study, the yield QTL only explained 15.6% of phenotypic variation, and minor QTL accounted for 10-15% of the variation. McCartney et al. (2006) studied most of the agronomy traits in a double-haploid population generated from a cross between RL 4452 and 'AC Domain'. They mapped six QTL for plant height; among these QTL, the QTL on the 4B and 4D chromosomes coincided with the *Rht-B1* and *Rht-D1*, respectively. Among two important yield QTLs mapped in this study, the QTL on 2B was either linked or pleotropic with the QTL for test weight. They mapped 10 QTLs for TW and 6 QTLs for TKW; an increased yield QTL on 2A is mapped in a similar position with a decreased TKW-QTL on 2A. Recently, Zhang et al. (2008) reviewed the distribution of yield and yield-related QTL published in wheat. Among 541 yield-related QTL reported, 33% of the QTL were reported for yield, followed by 21% of the QTL reported for TKW and 16% of the QTL reported for plant height. Among wheat genomes, the

majority of QTL (41%) were reported on the A genome, followed by 30% each on the B and D genomes.

Molecular Mapping of Quality Traits

The definition for the end-use quality of wheat varies with the end users, including the wheat industry as well as consumer preferences, however, characteristics such as grain protein content(GPC), milling yield, water absorption, mixing time and tolerance, loaf volume, and crumb grain appearance are very important (Delwiche et al., 1998). The selection for key quality attributes is difficult; therefore, the genetic improvement for protein content, flour yield, and loaf volume is not significant (Gutteri et al., 2000). Genetically, these traits are polygenic with additive, epistatic, and genotype by environment interactions, resulting in low heritability for these traits (Zhang et al., 2008). Therefore, it is important for breeders to get information about the QTL controlling these traits.

Grain protein content is an important quality trait for the producer as well as consumers. A premium price is paid by the industry for high-protein wheat, and protein is an important element for the nutritive value of grain. Grain protein content also determines the rheological properties of flour that are essential for superior bread-making quality. Gluten is the complex protein that is essential for the CO_2 retention capacity of dough, producing leavened bread that rises. Chemically, glutens consist of gliadins and glutenins proteins. Gladins are important for dough viscosity and extensibility while glutenins are important for dough strength and elasticity. Improving GPC by using conventional breeding methods has been slow because of the (i) high influence of the environment on GPC, (ii) negative correlation between GPC and grain yield (Groos et al., 2003), and (iii) quantitative genetic control of GPC involving a large number of genes/QTL with small effects and low heritability.

Past wheat studies suggested that the genes/QTL for GPC are distributed on all 14 chromosomes in tetraploid wheat (Blanco et al., 1996; Joppa et al.,1997; Khan et al., 2000; Mesfin et al., 1999) and on 21 chromosomes in hexaploid wheat (Boner et al., 2002; Groos et al., 2003; Prasad et al., 1999, 2003). A major QTL/gene (*Gpc-B1*) controlling GPC was detected in a survey of wild populations of tetraploid wheat, *Triticum turgidum* var. *dicoccoide*s accession FA-15-3, from Israel (Avivi, 1978). The *Gpc-B1* gene was mapped on chromosome arm 6BS (Joppa et al., 1997) and showed consistent improvement in GPC in both tetraploid and hexaploid wheat. A number of QTL-controlling GPCs have been mapped on chromosomes 2A, 2D, 3A, 3D, 4A, 7A, and 7D (Groos et al., 2003) Nelson et al., 2006; Prasad et al., 1999, 2003); 6B (Mesfin et al.,1999); 2B and 4D (McCarteny et al., 2006); and 3A, 3B, 5D, and 6D (Zhao et al., 2010). However, an increase in GPC also depends greatly upon applying higher doses of nitrogen fertilizers.

High milling yield is an important trait for profitability in the milling industry. This trait is affected by several factors, such as the size of germ; thickness of bran; size of grain; depth of crease; and, most importantly, hardness of kernel (Marshall et al., 1984; Stenvert, 1972). Increased kernel hardness results in higher flour yield, as well as better flowing and sifting properties during milling, however, it increases the energy required for milling (Basset et al., 1989; Pomeranz and Williams, 1990). The hardness locus on chromosome arm 5DS carries two tightly linked purindoline genes: *PinB,* which gives harder and higher flour yield, and *PinA*, which leads to a softer kernel (Martin et al., 2001). Higher starch damage of hard kernels while milling increases the water absorption and hydrolysis of starch into fermentable sugar, thereby increasing the loaf volume and bread-making quality (Pomeranz and Williams, 1990).

Quantitative trait loci analysis has been used to study traits such as milling yield (McCartney et al., 2006; Nelson et al., 2006; Parker et al., 1999), grain hardness (Sourdille et al., 2003; Turner et al., 2004), kernel size and shape (Dholakia et al., 2003), grain length (Campbell et al., 1999; Sun et al., 2009), mixograph traits (Huang et al., 2006; McCartney et al., 2006; Nelson et al., 2006), flour color (Parker et al., 1999), sedimentation volume (Huang et al., 2006; McCartney et al., 2006; Zanetti et al., 2001), and baking properties (Campbell et al., 2001; McCartney et al., 2006). Thus, the objectives of this research were to utilize the 'Steele-ND'/ND735 (Mergoum et al., 2009) RIL population and the DArT marker date to study:

1) Genetics of STB resistance in wheat from the data collected.

2) Genetics and QTL mapping for important agronomic and quality traits.

References

Adhikari, T.B., J.M. Anderson, and S.B. Goodwin. 2003. Identification and molecular mapping of a gene in wheat conferring resistance to *Mycosphaerella graminicola*. Phytopathology 93:1158-1164.

Adhikari, T.B., J.R. Cavaletto, J. Dubcovsky, J.O. Gieco, A.R. Schlatter, and S.B. Goodwin. 2004a. Molecular mapping of the *Stb4* gene for resistance to Septoria tritici blotch in wheat. Phytopathology 94:1198-1206.

Adhikari, T.B., X. Yang, J.R. Cavaletto, X. Hu, G. Buechley, H.W. Ohm, G. Shaner, and S.B. Goodwin. 2004b. Molecular mapping of the *Stb1*, a potentially durable gene for resistance to Septoria tritici blotch in wheat. Theor. Appl. Genet. 109:944-953.

Adhikari, T.B., H. Wallwork, and S.B. Goodwin. 2004c. Microsatellite markers linked to the *Stb2* and S*tb3* genes for resistance to septoria tritici blotch. Crop Sci. 44:1403-1411.

Anderson, J.M., D.L. Bucholtz, N. Sardesai, J.B. Santini, G. Gyulai, C.E. Williams, and S.B. Goodwin. 2010. Potential new genes for resistance to *Mycosphaerella graminicola* identified in *Triticum aestivum* x *lophopyrum elongatum* disomic substitution lines. Euphytica 172:251-162.

Arabi, M.I.E., M. Jawhar, and N. Mirali. 2007. The effects of *Mycosphaerella graminicola* infection on wheat protein content and quality. Cereal Res. Comm. 35:81-88.

Araki, E., H. Miura, and S. Sawada. 1999. Identification of genetic loci affecting amylose content and agronomic traits on chromosome 4A of wheat. Theor. Appl. Genet. 98:977–984.

Arama, P.F., J.E. Parlevliet, and C.H. Van Silfhout. 1999. Heading date and resistance to septoria tritici blotch in wheat are genetically associated. Euphytica 103:63-68.

Arraiano, L.S., and J.K.M. Brown. 2006. Identification of isolate-specific and partial resistance to septoria tritici blotch in 238 European wheat cultivars and breeding lines. Plant Pathol. 55:726-738.

Arraiano, L.S., L. Chartrain, E. Bossolini, H.N. Slatter, B. Keller, and J.K.M. Brown. 2007. A gene in European wheat cultivars for resistance to an African isolate of *Mycosphaerella graminicola*. Plant Pathol. 56:73-78.

Arraiano, L.S., A.J. Worland, C. Ellerbrook, and J.K.M. Brown. 2001. Chromosomal location of a gene for resistance to septoria tritici blotch (*Mycosphaerella graminicola*) in the hexaploid wheat 'Synthetic 6x'. Theor. Appl. Genet. 103:758-764.

Arumuganathan, E., and E.D. Earle. 1991. Nuclear DNA content of some important plant species. Plant Mol. Biol. 9:208-218.

Avivi, L. 1978. High protein content in wild tetraploid Triticum dicoccoides Korn. In S. Ramanujam (Ed) 5th Int. Wheat Genet Symp. New Delhi, India. p. 372-380.

Ayala, L., M. Henry, M. Van Ginkel, R. Singh, B. Keller, and M. Khairallah. 2002. Identification of QTL for BYDV tolerance in bread wheat. Euphytica 128:249-259.

Baltazar, B.M., A.L. Scharen, and W.E. Kronstad. 1990. Association between dwarfing genes '*Rht1*' and '*Rht2*' and resistance to septoria tritici blotch in winter wheat (*Triticum aestivum* L.em Thell). Theor. Appl. Gent. 79:422-426.

Basset, L.M., R.E. Allan, and G.L. Rubenthaler. 1989. Genotype by environment interaction on soft white winter wheat quality. Agron. J. 81:955-960.

Bayles, R.A. 1991. Research note: varietal resistance as a factor contributing to the increased importance of *Septoria tritici* Rob. & Desm. in the UK wheat crop. Plant Varieties and Seeds 4:177-185.

Bernardo, R. 2002. Breeding for quantitative traits in plants. Woodbury, MN. Stemma Press.

Blanco, A., C.D. Giovanni, B. Laddomada, A. Sciancalepore, R. Simeone, K.M. Devos, and M.D. Gale. 1996 Quantitative trait loci influencing grain protein content in tetraploid wheat. Plant Breeding 115:310-316.

Borner, A., E. Schumann, A. Furste, H. Coster, B. Leithold, S. Roder, and E. Weber. 2002. Mapping of quantitative trait loci determining agronomic important characters in hexaploid wheat (*Triticum aestivum* L.). Theor. Appl. Genet. 105:921-936.

Brading, P.A., E.C.P. Verstappen, G.H.J. Kema, and J.K.M. Brown. 2002. A gene-for-gene relationship between wheat and *Mycosphaerella graminicola*, the septoria tritici blotch pathogen. Phytopathology 92:439-445.

Brokenshire, T. 1975. Wheat debris as an inoculum source for seedling infection by *Septoria tritici*. Plant Pathol. 24:202-207.

Brule-Babel, A.L. 2007. Catalogue of gene symbols for wheat. Annual Wheat Newsletters 53:171.

Bryan, G. J., A. J. Collins, P. Stephenson, A. Orry, J. B. Smith, and M. D. Gale. 1997. Isolation and characterization of microsatellites from hexaploid bread wheat. Theor. Appl. Genet.94: 557-563.

Cadalen, T., C. Boeuf, S. Bernard, and M. Bernard. 1997. An intervarietal molecular marker map in *Triticum aestivum* L. Em. Thell. and comparison with a map from a wide cross. Theor. Appl. Gent. 94:367-377.

Camacho-Casas, M. A., W.E. Kronstad, and A.L. Scharen. 1995. Septoria tritici resistance and associations with agronomic traits in a wheat cross. Crop Sci. 35:971-976.

Campbell, B.T., P.S. Baenziger, K.S. Gill, K.M. Eskridge, H. Budak, M. Erayman, I. Dweikat, and Y. Yen. 2003. Identification of QTLs and environmental interactions associated with agronomic traits on chromosome 3A of wheat. Crop Sci. 43:1493-1505.

Campbell, K. G., C.J. Bergman, D.G. Gualberto, J. A. Anderson, M. J. Giroux, G. Hareland, R. G. Fulcher, M. E. Sorrells, and P. L. Finney. 1999. Quantitative trait loci associated with kernel traits in soft × hard wheat cross. Crop Sci. 39:1184-1195.

Campbell, K.G., P.L. Finney, C.J. Bergman, D.G. Gualberto, J.A. Anderson, M.J. Giroux, D. Siritunga, J. Zhu, F. Gendre, C. Roue, A. Verel, and M.E. Sorrells. 2001. Quantitative trait loci associated with milling and baking quality in soft/hard red wheat cross. Crop Sci.41:1275-1285

Chao, S., P.J. Sharp, A.J. Worland, E.J. Warham, R.M.D. Koebner, and M.D. Gale. 1989. RFLP-based genetic maps of wheat homoeologous group 7 chromosomes. Theor. Appl. Genet. 78:495-504.

Chartrain, L., S.T. Berry, and J.K.M. Brown. 2005a. Resistance of wheat line Kavkaz-K4500 L. 6.A.4 to septoria tritici blotch controlled by isolate-specific resistance genes. Phytopathology 95:664-671.

Chartrain, L., P.A. Brading, J.C. Makepeace, and J.K.M. Brown. 2004a. Sources of resistance to septoria tritici blotch and implications for wheat breeding. Plant Pathol. 53:454-460.

Chartrain, L., P.A. Brading, J.P. Widdowson, and J.K.M. Brown. 2004b. Partial resistance to septoria tritici blotch (*Mycosphaerella graminicola*) in wheat cultivars Arina and Riband. Phytopathology 94:497-504.

Chartrain, L., P. Joaquin, S.T. Berry, L.S. Arraiano, F. Azanza, and J.K.M. Brown. 2005b. Genetics of resistance to septoria tritici blotch in the Portuguese wheat breeding line TE9111. Theor. Appl. Genet. 110:1138-1144.

Chartrain, L., P. Sourdille, M. Bernard, and J.K.M. Brown. 2009. Identification of Stb9, a gene for resistance to septoria tritici blotch in wheat cultivars Courtot and Tonic. Plant Pathol. 58:547-555.

Choudhry, A.R., A.H. Shah, L. Ali, and M. Bashir. 1986. Path coefficient analysis of yield and yield components in wheat. Pak. J. Agri. Res. 7:71-75.

Chungu, C., J. Gilbert, and F.T. Smith. 2001. Septoria tritici blotch development affected by temperature, duration of leaf wetness, inoculum concentrations and host. Plant Dis. 85:430-435.

Cohen, L., and Z. Eyal. 1993. Histology of process associated with the infection resistant and susceptible cultivars with septoria tritici. Plant Pathol. 42:737-743.

Cowling, S.G. 2006. M.S. thesis. Department of Plant Science, University of Manitoba. Winnipeg, Canada. p. 129-130.

Devos, K.M., M.D. Atkinson, C.N. Chinoy, C. Liu, and M.D. Gale. 1992. RFLP-based genetic map of the homoeologous group-3 chromosomes of wheat and rye. Theor. Appl. Genet. 83:931-939.

Delwiche, S.R., R.A. Graybosch, and C.J. Peterson. 1998. Predicting protein composition, biochemical properties, and dough handling properties of hard red winter wheat flour by near-infrared reflectance. Cereal Chem. 75:412-416.

Dholakia, B.B., J.S.S. Ammiraju, H. Singh, M.D. Lagu, M.S. Roder, V.S. Rao, H.S. Dhaliwal, P.K. Ranjekar, and V.S. Gupta. 2003. Molecular marker analysis of kernel size and shape in bread wheat. Plant Breeding 122:392-395.

Duveiller, E., R.P. Singh, and J.M. Nicol. 2007. The challenges of maintaining wheat productivity: pests, diseases, and potential epidemics. Euphytica 157:417-430.

Eriksen, L., F. Borum, and A. Jahoor. 2003. Inheritance and localization of resistance to *Mycosphaerella graminicola* causing septoria tritici blotch and plant height in wheat (*Triticum aestivum* L.) genome with DNA markers. Theor. Appl. Genet. 107:515-527.

Eyal, Z. 1976. Research on septoria leaf blotch of wheat caused by *Septoria tritici* in Israel. p. 49-53. In Proc. Septoria Diseases of Wheat Workshop.. Georgia Agric. Exp Stn. Special Publ. 4. 69.

Eyal, Z. 1999. The septoria tritici and stagnospora nodorum blotch diseases of wheat. Eur. J. Plant Pathol. 105:629-641.

Eyal, Z., and A. Blum. 1989. Canopy temperature as a correlative measure for assessing host response to septoria tritici blotch of wheat. Plant Dis. 73:468-471.

Eyal, Z., A.L. Scharen, J.M. Prescott, and M. Van Ginkel. 1987. The septoria diseases of wheat: the concepts and methods of disease management. International Maize and Wheat Improvement Center (CIMMYT). Mexico, DF. p. 55-60.

Eyal, Z., and O. Ziv. 1974. The relationship between epidemics of septoria leaf blotch and yield losses in spring wheat. Phytopathology 66:11-14

Fraaije, B.A., D.J. Lovell, J.M. Coelho, S. Baldwin, and D.W. Hollowmon. 2001. PCR-based assays to assess wheat varietal resistance to blotch (*Septoria tritici* and *Stagonospora nodorum*) and rust (*Puccinia striiformis* and *Puccinia recondita*) disease. Eur. J. Plant Pathol. 107:905-917.

Gale, M.D., M.D. Atkinson, C.N. Chinoy, R.L. Harcourt, J. Jiu, Q.Y. Li, and K.M. Devos. 1995. Genetic maps of hexaploid wheat. In Z.S. Li and Z.Y. Xin (Eds) Proc 8th Int. Wheat Genet. Symp. China Agric Sci. Tech. Press, Beijing, China. p. 29-40.

Garcia, C., and D. Marshall. 1992. Observations on the acrogenous stage of *Septoria tritici* in Texas. Mycological Research 96:65-70.

Gaunt, R.E., W.J. Thomson, and J. Suctcliffe. 1986. The assessment of speckled leaf blotch in winter wheat in New Zealand. Ann Bot. 58:33-38.

Gilchrist, L., and B. Skovmand. 1995. Evaluation of emmer wheat (*Triticum dicoccum*) for resistance to *Septoria tritici*. In: Proceedings of a *Septoria tritici* Workshop. L. Gilchrist, M. van Ginkel, A. McNab, and G.H.J. Kema (Eds). International Maize and Wheat Improvement Center (CIMMYT). Mexico, DF. p. 130-134.

Gill, K.S., and B.S. Gill. 1994. Mapping in the realm of polyploidy: the wheat model. Bioassays 16:841-846.

Gisi, U., K.M. Chin, G. Knbapova, R.K. Farber, U. Mohr, S. Parisi, H. Sierotzki, and U. Steinfeld. 2000. Recent developments in elucidating modes of resistance to phenyl-amide, DMI and strobilurins fungicides. Crop Protection 19:863-872.

Gisi, U., L. Pavic, C. Stanger, U. Hugelshofer, and H. Sierotzki. 2005. Dynamics of *Mycosphaerella graminicola* population in response to selection by different fungicides In H.W. Dehne, U. Gisi, K. H. Kuck, P.E. Russell, H. Lyr.(Eds). BCPC: Alton, UK. p. 89-101.

Goodwin, S.B. 2007. Back to basics and beyond: increasing the level of resistance to septoria tritici blotch in wheat. Aust. J. Plant Pathol. 36:532-538.

Groos, C., N. Robert, E. Bervas, and G. Charmet. 2003. Genetic analysis of grain-protein content, grain yield and thousand kernel weight in bread wheat. Theor. Appl. Genet. 106:1032-1040.

Gupta, P.K., R.K. Varshney, P.C. Sharma, and B. Ramesh. 1999. Molecular markers and their application in wheat breeding. Plant Breeding 118:369-390.

Gutteri, M.J., R. Ahmad, J.C. Stark, and E. Souza. 2000. End-use quality of six hard red spring wheat cultivars at different irrigation levels. Crop Sci. 40:631-635.

Hess, D.E., and G. Shaner. 1987. Effect of moisture and temperature on development of septoria tritici blotch in wheat. Phytopathology 77:215-219.

Hittalmani, S., H.E. Shashidhar, P.G. Bagali, N. Huang, J.S. Sidhu, V.P. Singh, and G.S. Khush. 2003. Molecular mapping of quantitative trait loci for plant growth, yield, and yield related traits across three diverse locations in a double haploid rice population. Euphytica 125:207-214.

Huang, X.Q., S. Cloutier, L. Lycar, N. Radovanovic, D.G. Humphreys, J.S. Noll, D.J. Somers, and P.D. Brown. 2006. Molecular detection of QTLs for agronomic and Quality traits in a double haploid population derived from two Canadian wheats. (*Triticum aestivum*). Theor. Appl. Genet. 113:753-766.

Huang, X.Q., H. Kempf, M.W. Ganal, and M.S. Röder. 2004. Advanced backcross QTL analysis in progenies derived from a cross between a German elite winter wheat variety and synthetic wheat (*Triticum aestivum L.*). Theor. Appl. Genet. 109:933-943.

Hunter, T., R.R. Coker, and D.J. Royale. 1999. The telomorph stage, *Mycosphaerella graminicola,* in epidemics of septoria tritici blotch on winter wheat in UK. Plant Pathol. 48:51-57.

Jia, J., K.M. Devos, S. Chao, T.E. Miller, S.M. Reader, and M.D. Gale. 1996. RFLP-based as of homoeologous group-6 chromosomes of wheat and their application in the tagging of Pm12, a powdery mildew resistance gene transferred from *Aegilops speltoids* to wheat. Theor. Appl. Genet. 92:559-565.

Jilbene, M., J.P. Gustafson, and S. Rajaram. 1994. Inheritance of resistance to *Mycosphaerella graminicola* in hexaploid wheat. Plant Breeding 112:301-310.

Jing, H.C., D. Lovell, R. Gutteridge, D. Jenk, D. Kornyukhin, O.P. Mitrofanova, G.H.J. Kema, and K.E. Hammond-Kosack. 2008. Phenotypic and genetic analysis of the *Triticum monococcum-Mycosphaerella graminicola* interaction. New Phytologist 179:1121-1132.

Joppa, L.R., C. Du, G.E. Hart, and G.A. Hareland. 1997. Mapping genes for grain protein in tetraploid wheat (*Triticum turgidum* L.) using a population of recombinant inbred chromosome line. Crop Sci. 37:1586-1589.

Kato, K., H. Miura, and S. Sawada. 2000. Mapping QTLs controlling grain yield and its components on chromosome 5A of wheat. Theor. Appl. Genet. 101:1114-1121.

Keller, M., C. Karutz, J. E. Schmid, P. Stamp, M. Winzeler, B. Keller, and M. M. Messmer.1998. Quantitative trait loci for lodging resistance in a segregating wheat× spelt population. Theor. Appl. Genet. 6:1171-1182.

Kema, G.H.J., J.G. Annone, R. Sayoud, C.H. Van Silfhout, J.Van Ginkel, and I. de Bree. 1996a. Genetic variation for virulence and resistance in wheat-*Mycosphaerella graminicola* pathosystem. 1, Interactions between pathogen isolates and host cultivars-Phytopathology 200:2-12.

Kema, G.H.J., D.Z. Yu, F.H.J. Rijkenberg, M.W Shaw, and R.P. Baayen. 1996b. Histology of the pathogenesis of *Mycosphaerella graminicola* in wheat. Phytopathology 86:777-786.

Khan, I.A., J.D. Procunier, D.G. Humphreys, G. Tranquilli, A.R. Schlatter, S. Marcucci-Poltri, R. Frohberg, and J. Dubcovsky. 2000. Development of PCR-based markers for a high grain protein content gene from *Triticum turgidum* ssp. *dicoccoides* transferred to bread wheat. Crop Sci. 40:518-524.

Kildea, S., V. Ransbotyn, M.R. Khan, B. Fagan, G. Leonard, E. Mullins, and M. Doohan. 2008. *Bacillum megaterium* shows potential for the bio-control of septoria tritici blotch of wheat. Bio. Cont. 47:37-45.

King, J.E., R.J. Cook, and S.C. Melville. 1983. A review of septoria diseases of wheat and barley. An. App. Biol. 103:354-373.

Kulwal, P.L., J.K. Roy, H.S. Balyan, and P.K. Gupta. 2003. QTL mapping for growth and ear characters in bread wheat. Plant Sci. 164:267-277.

Kumar, N., P.L. Kulwal, H.S. Balyan, and P.K. Gupta. 2007. QTL mapping for yield and yield contributing traits in two mapping populations of bread wheat. Mol. Breed. 19:163-177.

Langridge, P., E. S. Lagudah, T. A. Holton, R. Appels, P. J. Sharp, and K.J. Chalmers. 2001. Trends in genetic and genome analyses in wheat: a review. Aust. J. Agric Res. 52: 1043-1077.

Lander, R., and R. Thomson. 1990. Efficiency of marker-assisted selection in the improvement of quantitative traits. Genetics 124:743-756.

Law, C.N. 1965. Biometrical analysis using chromosome substitutions within a species. In R. Riley and K.R. Lewis (Eds) Chromosome manipulations in plant genetics. Suppl. Heredity. 20. p. 59-85.

Law, C.N. 1966. The location of genetic factors controlling a quantitative character in wheat. Genetics 53:487-498.

Law, C.N. 1967. The location of genetic factors controlling a number of quantitative characters in wheat. Genetics 56:445-461.

Liu, Y.G., and K. Tsunewaki. 1991. Restriction fragment length polymorphism (RFLP) analysis in wheat. II. Linkage maps of the RFLP sites in common wheat. Genetics 66: 617-633.

Li, G., and C. F. Quiros. 2001. Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in Brassica. Theor. Appl. Genet. 103:455-461.

Liu, Z. H., J. A. Anderson, J. Hu, T. L. Friesen, J. B. Rasmussen, and J. D. Faris. 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.

Lovell, D.J, S.R. Parker, T. Hunter, D.J. Royle, and R.R. Coker. 1997. Influence of crop growth and structure on the risk of epidemics by *Mycosphaerella graminicola* (*Septoria tritici*) in winter wheat. Plant Pathol. 40:126-138.

Mackie, W. W. 1929. Resistance to *Septoria tritici* in wheat. (Abstr.) Phytopathology 19:1139-1140.

Marino, C.L., J.C. Nelson, Y.H. Liu, M.E. Sorrells, P. Leroy, N.A. Tuleen, C.R. Lopes, and G.E. Hart. 1996. RFLP-based linkage maps of the homoeologous group six chromosomes of wheat (*Triticum aestivum* L.em. Thell). Genome 39:359-366.

Marshall, D. R., D. J. Mares, H. J. Moss, and F. W. Ellison. 1986. Effects of grain shape and size on milling yields in wheat. II. Experimental studies. Crop and Pasture Sci. 37:331-342.

Martin, J.M., R.C. Frohberg, C.F. Morris, L.E. Talbert, and M.J. Giroux. 2001. Milling and bread baking traits associated with puroindoline sequence type in hard red spring wheat. Crop Sci. 41:228-234.

May, C.E., and E.S. Lagudah. 1992. Inheritance in hexaploid wheat of septoria tritici blotch resistance and other characteristics derived from *Triticum tauschii*. Aust. J. Agric. Res. 43:433-442.

Ma, Z. Q., M.S. Röder, and M. E. Sorrells. 1996. Frequencies and sequence characteristics of di-, tri-, and tetra-nucleotide microsatellites in wheat. Genome 39:123-130.

McCartney, C.A., A.L. Brule-Babel, and L. Lamari. 2002a. Inheritance of race-specific resistance to *Mycosphaerella graminicola* in the spring wheat cultivar ST6. Theor. Appl. Gent. 107:1181-1186.

McCartney, C.A., A.L. Brule-Babel, and L. Lamari. 2002b. Inheritance of race specific resistance to *Mycosphaerella graminicola* in wheat. Phytopathology 92:138-144.

McCartney, C.A., D.J. Somers, O. Lukow, N. Ames, J. Noll, S. Cloutier, D.G. Humphreys, and B.D. McCallum. 2006. QTL analysis of quality traits in the spring wheat cross RL4452/AC Domain. Plant Breeding 125:565-575.

McKendry, A.L., and G.E. Henke. 1994a. Evaluation of wheat wild relatives for resistance to septoria tritici blotch. Crop Sci. 34:1080-1084.

McKendry, A.L., and G.E. Henke. 1994b. Tolerance to septoria tritici blotch in soft red winter wheat. Cereal Res. Comm. 22:353-359.

McKendry, A.L., G.E. Henke, and P.L. Finney. 1995. Effect of septoria blotch on soft red winter wheat milling and baking quality. Cereal Chem. 72:142-146.

Mergoum, M., P.K. Singh, R.C. Frohberg, S.F. Kianian, F. Ghavami, K. Hussain, T.B. Adhikari, V.E. Harilal, and S. Simsek. 2009. Registration of Steele-ND/ND 735 wheat recombinant inbred lines mapping population. J. Plant Reg. 3:300-306.

Mesfin, A., R.C. Frohberg, and J.A. Anderson. 1999. RFLP markers associated with high protein from *Triticum turgidum* L. var. *dicoccoides* introgressed into hard red spring wheat. Crop Sci. 39:508-513.

Messmer, M.M., M. Keller, S. Zanetti, and B. Keller.1999. Genetic linkage map of wheat and spelt cross. Theor. Appl. Genet. 98:1163-1170.

Miura, H., B.B. Parker, and J.W. Snape. 1992. The location of major genes and associated quantitative trait loci on chromosome arm 5BL of wheat. Theor. Appl. Genet. 85:197-204.

Nelson, J.C., C. Andreescu, F. Breseghello, P.L. Finney, D.G. Gualberto, C.J. Bergman, R.J. Pena, M.R. Perretant, P. Leroy, C.O. Qualset, and M.E. Sorrells. 2006. Quantitative trait locus analysis of wheat quality traits. Euphytica 149:145-159.

Nelson, J.C., M.E. Sorrells, A.E. Van-Deynze, Y.H. Lu, M. Atkinson, M. Bernard, P. Leroy, J.D. Faris, and J.A. Anderson. 1995a. Molecular mapping of wheat: major genes and rearrangements in homoeologous group 4, 5 and 7. Genetics 141:721-731.

Nelson, J.C., A.E. Van-Deynze, E. Autrique, M.E. Sorrells, Y.H. Lu, S. Negre, M. Bernard, and P. Leroy. 1995b. Molecular mapping of wheat homoeologous group two. Genome 38:516-524.

Nelson, J.C., A.E. Van-Deynze, E. Autrique, M.E. Sorrells, Y.H. Lu, S. Negre, M. Bernard, and P. Leroy. 1995c. Molecular mapping of wheat homoeologous group three. Genome 38:525-533.

Nolan, S., and B.M. Cooke. 2000. Control of stagnospora nodorum and septoria tritici in wheat by pre-treatment with Drechslera teres, a non-host pathogen. Eur. J. Plant Pathol. 106:203-207.

North Dakota Wheat Commission. 2006. Wheat information [Online]. Available at http://www.ndwheat.com/uploads/resources/565/smgrainstruthagwheat.pdf (accessed 12 Mar. 2008; verified 15 Apr. 2008). North Dakota Wheat Commission, Bismarck, ND.

Palmer, C.L., and W. Skinner. 2002. *Mycosphaerella graminicola*: latent infection, crop devastation and genomics. Molecular Plant Pathol. 3:63-70.

Parker, G.D., K.J. Chalmers, A.J. Rathjen, and P. Langridge. 1999. Mapping loci associated with milling yield in wheat (*Triticum aestivum* L.). Mol. Breed. 5:561-568.

Parker, S.R., S. Welham, N.D. Paveley, J. Foulkes, and R.K. Scott. 2004. Tolerance of septoria leaf blotch in wheat. Plant Pathol. 53:1-10.

Peng, J.H., Y. Ronin, T. Fahima, M.S. Roder, Y.C. Lin, E. Nevo, and A. Korol. 2003. Domestication quantitative trait loci in Triticum dicoccoides, progenitor of wheat. Proc. Natl. Acad. Sci. 100:2489-2494.

Perello, A.E., C.I. Monaco, M.V. Moreno, C.A. Cordo, and M.R. Simon. 2006. The effect of *Trichoderma harzianum* and *T-koningii* on the control of tan spot (*Pyrenophora tritici-repentis*) and leaf blotch (*Mycosphaerella graminicola*) of wheat under field conditions in Argentina. Bio-control Sci. and Tech. 16:803-813.

Plaschke, J., M. W. Ganal, and M. S. Röder.1995. Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theor. Appl. Genet. 6:1001-1007.

Pomeranz, Y., and P. C. Williams.1990. Wheat hardness: Its genetic, structural, and biochemical background, measurement, and significance. Advances in cereal sci. and tech. 10:471-544.

Prasad, M., N. Kumar, P.L. Kulwal, M.S. Roder, H.S. Balyan, H.S. Dhaliwal, and P.K. Gupta. 2003. QTL analysis for grain protein content using SSR markers and validation using NILs in bread wheat. Theor. Appl. Genet. 106:659-667.

Prasad, M., R.K. Varshney, A. Kumar, H.S. Balyan, P.C. Sharma, K.J. Edwards, H. Singh, H.S. Dhaliwal, J.K. Roy, and P.K. Gupta. 1999. A microsatellite marker associated with a QTL

for grain protein content on chromosome arm 2DL of bread wheat. Theor. Appl. Genet. 99:341-345.

Quarrie, S. A., A. Steed, C. Calestani, A. Semikhodskii, C. Lebreton, C. Chinoy, and N. Steele. 2005. A high-density genetic map of hexaploid wheat (Triticum aestivum L.) from the cross Chinese Spring× SQ1 and its use to compare QTLs for grain yield across a range of environments. Theor. Appl. Genet. 110:865-880.

Roder, M.S., J. Plaschke, S.U. König, A. Börner, M.E. Sorrells, S.D. Tanksley, and M.W. Ganal. 1995. Abundance, variability and chromosomal location of microsatellites in wheat. Mol. Gen. Genet. 246:327-333.

Roder, M.S., V. Korzun, K. Wendehake, J. Plaschke, M.H. Tixier, P. Leroy, and M. W. Ganal. 1998. A microsatellite map of wheat. Genetics 149:2007-2023.

Rosegrant, M.W., M.A. Sombilla, R.V. Gerpacio, and C. Ringler. 1997. Global food markets and US exports in the twenty-first century. Paper presented at World Food and Sustainable Agriculture Program Conf. Meeting the Demand for food in the 21st Century: Challenges and Opportunities for Illinois agriculture. International Food Policy Research Institute. Memo. Washington DC.

Rosielle, A.A. 1972. Sources of resistance in wheat to speckled leaf blotch caused by *Septoria tritici*. Euphytica 21:152-161.

Shah, M.M., K.S. Gill, P.S. Baenziger, Y. Yen, S.M. Kaeppler, and H.M. Ariyarathne. 1999. Molecular mapping of loci for agronomic traits on chromosome 3A of bread wheat. Crop Sci. 39:1728-1732.

Shaner, G., and G. Buechley. 1995. Epidemiology of leaf blotch of soft red winter wheat caused by *Septoria tntici* and *Stagnospora nodorurn.* Plant Dis. 79:928-938.

Shaner, G., and R.E. Finney. 1982. Resistance in soft red winter wheat to *Mycosphaerella graminicola*. Phytopathology 72:154-158.

Shaner, G., R.E. Finney, and F.L. Patterson. 1975. Expression and effectiveness of resistance in wheat to septoria leaf blotch. Phytopathology 65:761-766.

Shaw, M.W. 1991. Interacting effects of interrupted humid periods and light on infection of wheat leaves by *Mycosphaerella graminicola* (Septoria tritici). Plant Pathol. 40:595-607.

Simon, M.R., F.M. Ayala, C.A. Cordo, M.S. Roder, and A. Borner. 2004a. Molecular mapping of quantitative trait loci determining resistance to septoria tritici blotch caused by *Mycosphaerella graminicola* in wheat. Euphytica 138:41-48.

Simon, M.R., C. A. Cordo, A.E. Perello, and P.C. Struik. 2003. Influence of nitrogen supply on the susceptibility of Wheat to *Septoria tritici*. Phytopathology 151:283-289.

Simon, M.R., A.J. Worland, and P.C. Struik. 2004b. Influence of plant height and heading date on the expression of the resistance to septoria tritici blotch in near isogenic lines of wheat. Crop Sci. 44:2078-2085.

Singh, P.K., M. Mergoum, S. Ali, T.B. Adhikari, E.M. Elias, and G.R. Hughes, 2006. Identification of new sources of resistance to tan spot, stagnospora nodorum blotch, and septoria tritici blotch of wheat. Crop Sci. 46:2047-2053.

Singh, R.K., D.P. Hodson, J.H. Espino, Y. Jin, P. Njau, R. Wanyera, S.H. Foessil, and R.W. Ward. 2008. Will stem rust will destroy the world's wheat crop. Advances in Agronomy 98:271-309.

Smith, G.P. 2001. Production trends and developments in wheat markets. Aspects of Applied Biol. 64:7-14.

Snape, J.W., C.N. Law, B.B. Parker, and A.J. Worland. 1985. Genetical analysis of chromosome 5A of wheat and its influence on important agronomic characters. Theor. Appl. Genet. 71:518-526.

Somasco, O.A., C.O. Qualset, and D.G. Gilchrist. 1996. Single-gene resistance to septoria tritici blotch in spring wheat cultivar Tadina. Plant Breeding 115:261-267.

Sourdille, P., T. Cadalen, H. Guyomarch, J. Snape, M. Perretant, G. Charmet, C. Boeuf, S. Bernard, and M. Bernard. 2003. An update of the Courtot × Chinese Spring intervarietal molecular marker linkage map for the QTL detection of agronomic traits in wheat. Theor. Appl. Genet. 106:530-538.

Stenvert, N. L. 1972. The measurement of wheat hardness and its effect on milling characteristics. Animal Production Sci. 55:159-164.

Stukenbrock, E.H., S. Banke, M.J. Nikkhah, and B.A. McDonald. 2007. Origin and domestication of the fungal wheat pathogen *Mycosphaerella graminicola* via sympatric speciation. Mol. Biol. Evol. 24:398-411.

Suenaga, K., M. Khairallah, H. M. William, and David A. Hoisington. 2005. A new intervarietal linkage map and its application for quantitative trait locus analysis of" gigas" features in bread wheat. Genome 48:65-75.

Thomson, W.J., and R. E. Gaunt. 1986. The effect of speckled leaf blotch on apical development and yield in winter wheat in New Zealand. Ann. Bot. 58:39-48.

Turner, A. S., R. P. Bradburne, L. Fish, and J. W. Snape. 2004. New quantitative trait loci influencing grain texture and protein content in bread wheat. J. Cere Sci. 40:51-60.

USDA-Foreign Agricultural Service. 2008. World agricultural production [Online]. Available at http://www.fas.usda.gov/wap/circular/2008/08-05/productionfull05-08.pdf (accessed 11 Mar. 2008; verified 15 Apr. 2008). USDA-FAS, Washington, DC.

Van Deynze, A.E., J. Dubcovsky, K.S. Gill, J.C. Nelson, M.E. Sorrells, J. Dvorak, B.S. Gill, E.S. Lagudah, S.R. McCouch, and R. Appels. 1995. Molecular genetic maps for chromosome 1 in Triticeae species and their relation to chromosome in rice and oats. Genome 38:47-59.

Worland, A. J., V. Korzun, M. S. Röder, M. W. Ganal, and C. N. Law. 1998. Genetic analysis of the dwarfing gene Rht8 in wheat. Part II. The distribution and adaptive significance of allelic variants at the Rht8 locus of wheat as revealed by microsatellite screening. Theor. Appl. Genet. 96:1110-1120.

Xie, D.X., K.M. Devos, G. Moore, and M.D. Gale.1993. RFLP-based genetic maps of the homoeologous group 5 chromosome of bread wheat (*Triticum aestivum* L.). Theor. Appl. Genet. 87:70-74.

Zanetti, S., M. Winzeler, C. Feuillet, B. Keller, and M. Messmer. 2001. Genetic analysis of bread making quality in wheat and spelt. Plant Breeding 120:13-19.

Zeng, Z.B. 1994. Precision mapping of quantitative trait loci. Genetics 136:1457-1468.

Zhang, L.Y., D.C. Liu, X.L. Guo, W.L. Yang, J.Z. Sun, D. Wang, and A. Zhang. 2008. Distribution in genome of quantitative trait loci (QTL) for yield and yield-related traits in common wheat (*Triticum aestivum* L.). Available at http://www.jipb.net/file/12916137426593-500.pdf. (Verified 05 Jan. 2011).

Zhang, X., S.D. Haley, and Y. Jin. 2001. Inheritance of *Septoria tritici* blotch resistance in winter wheat. Crop Sci. 41:323-326.

Zhao, L. K.P. Zhang, B. Liu, Z.Y. Deng, H.L. Qu, and J.C. Tian. 2010. A comparison of grain protein content QTLs and flour protein content QTLs across environments in cultivated wheat. Euphytica 174:325-335.

Ziv, O., and Z. Eyal. 1978. Assessment of yield component losses caused in spring wheat cultivars by selected isolates of septoria tritici. Phytopathology 68:791-796.

Zukerman, E., A. Eshel, and Z. Eyal. 1997. Physiological aspects related to tolerance of spring wheat cultivars to septoria tritici blotch. Phytopathology 87:60-65.

PAPER 1. GENOME AND QUANTITATIVE TRAIT LOCI MAPPING OF SEPTORIA TRITICI BLOTCH RESISTANCE IN WHEAT

Abstract

Septoria tritici blotch (STB), caused by ascomycete fungus *Mycosphaerella graminicola* (Fückel) J. Schröt. in Cohn (anamorph: *Septoria tritici* Desm.), is one of the important destructive foliar diseases of wheat- (*Triticum aestivum* L.) . The susceptibility of recently released and mostly grown cultivars to this disease increases the importance of understanding the genetics of this disease and efforts to incorporate host resistance into adapted germplasm. Therefore, this study aimed to identify the nature and inheritance of STB and to map the quantitative trait loci (QTL) for STB resistance in the 'Steele-ND'/ND 735 recombinant inbred line (RIL) mapping population. The genome map of this population was constructed using diversity array technology (DArT) markers. The RILs were evaluated in three greenhouse experiments using a North Dakota (ND) isolate of STB. The mean disease severity of ND 735 (11.96%) and 'Steele-ND' (66.67%), parents of the RIL, showed significant differences ($p<0.05$). The mean disease severities of RILs were 21.98%. However, the range of disease severity varied from 0 to 71.55%. The framework map spanned a total distance of 1789.3 cM and consisted of 17 linkage groups. Among three QTL detected through composite interval mapping, one QTL consistently detected in all experiments, on long arm chromosome 5B, explained 7.1-10.2% phenotypic variation. The other two QTL were mapped to 1D and 7A, contributing to phenotypic variation by 13% and 5.5%, respectively, based on one experiment's data. The map position of consistent QTLs coincides with the map position of durable resistance

gene *Stb1*, confirming the importance of ND 735 as a source of STB resistance to the wheat germplasm.

Introduction

Septoria tritici blotch (STB), caused by ascomycete fungus *Mycosphaerella graminicola* (Fückel) J. Schröt. in Cohn (anamorph: *Septoria tritici* Desm.), is one of the most destructive foliar diseases of wheat- (*Triticum aestivum* L. 2n=6x =42, AABBDD) growing areas worldwide (Eyal et al., 1987). In North America, STB, along with other leaf spotting diseases, including Stagonospora nodorum blotch (SNB), which is caused by *Phaeosphaeria nodorum* [anamorph: *Stagonospora nodorum* (Berk.) Castellani & Germano.], and tan spot, which is caused by *Pyrenophora tritici-repen*tis [anamorph: *Drechslera tritici-repentis* (Died.) Shoem.], is considered an important factor for yield reduction (Gilbert and Woods, 2001; Singh et al., 2006). The yield reduction due to septoria diseases is in the range of 10-15%; however, under the favorable conditions of disease development, the disease outbreaks cause a yield reduction up to 50% (Eyal et al., 1987; King et al., 1983).

The most widely grown hard red spring wheat (HRSW) cultivars are susceptible to STB (Mergoum et al., 2007, 2008; Singh et al., 2006). The main group of fungicides used to control STB includes Azoles and Quinone outside inhibitor (QoI) types. However, the development of the fungicide resistance reduces the fungicide efficiency over time (Gisi et al., 2000, 2005). Additionally, their application is not cost effective in low-yielding environments. Therefore, the incorporation of host resistance is considered as an environment friendly and cost-effective approach to combat this disease.

Understanding the mode inheritance and mapping of resistant genes is very important for the breeder to develop strategies to incorporate resistant genes into adapted germplasm. The

deployment of host resistance prolongs the latent period of STB infection as well as reducing the number and size of chlorotic and necrotic lesions formed in the leaf (Eyal et al., 1987; Kema et al., 1996; Somasco et al., 1996). The qualitative mode of inheritance was reported in the studies of Arraiano and Brown (2006), Brading et al. (2002), McCartney et al. (2002), and Somasco et al. (1996), which followed the gene-for gene model and noticeable by the isolate specificity of disease reaction. However, quantitative inheritance was reported in several studies (Arraiano and Brown, 2006; Chartrain et al., 2004; Jilbene et al., 1994; Simon and Cordo, 1997; Zhang et al., 2001). This type of resistance is isolate nonspecific and provides resistance for a wide variety of isolates. To date, 15 major genes conferring STB resistance (Adhikari et al., 2003, 2004a, 2004b, 2004c; Arraiano et al., 2001, 2007; Brading et al., 2002; Brule-Babel., 2007; Chartrain et al., 2004, 2005a, 2005b; Cowling; 2006, McCartney et al., 2002) have been reported and mapped using molecular markers in wheat. Additionally, Erikson et al. (2003) reported QTLs associated with both seedling and adult plant resistance for STB in a double-haploid population from 'Savannah' and 'Senat'. They mapped six QTLs and found QTLs associated with seedling resistance on or near the *Stb6* locus on chromosome 3A as well as adult plant resistance on chromosomes 3AS, 6B, and 7B. Simon and co-workers (2004), using two different Argentinean isolates, mapped three QTL for in 1D, 2D, and 6B for seedling resistance and two QTLs on 3D and7B for adult plant resistance; they concluded that the major QTL position is near or coincides with the previously mapped location of the *Stb8* gene by Adhikari et al. (2004c).

Breeding for host resistance to septoria diseases is difficult due to the low heritability of non-specific resistance, the lack of knowledge about the pathogen virulence spectrum, and the scarcity of a durable resistant source (Eyal, 1999). Under field conditions, evaluation of wheat for field resistance to STB is challenged by variation in the environment, the 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. Eyal (1999) reported the evaluation of seedlings for resistance to septoria diseases under controlled conditions to obtain accurate and reproducible results from the examination of a large number of genotypes under uniform disease pressure. Many studies reported the positive correlation found between resistance assessments at the seedling level and the rating of adult plants under natural infection in field conditions (Somasco et al., 1996). Thus, the main objective of this study was to evaluate the Steele-ND/ND 735 RIL for STB resistance and to map the gene/QTL controlling the trait using DArT marker data.

Materials and Methods

Plant Material

One hundred and thirty-eight 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 ND 735 is derived from the cross of ND 2709/3/Grandin*3// 'Ramsey' (CItr 13246)/ND 622/ND 2809. The cultivar Steele-ND shows a susceptible reaction to major leaf-spotting diseases, including STB, SNB, all virulent races of tan spot found in North Dakota, and to toxins Ptr Tox A and Ptr Tox B; produced by races 1 and 2, and 5, respectively. However, ND 735 is highly resistant to major leaf spotting diseases including STB, SNB, tan spot races 1, 2, 3, 5, and toxins Ptr Tox A and Ptr Tox B produced by races 1 and 2, and 5, respectively (Mergoum et al.,2007; Singh et al.,2006).

Greenhouse Experiments

To evaluate the 138 RILs and their parents, three independent experiments were conducted under greenhouse conditions during the spring and fall of 2009 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, Port Washington, NY). Greenhouse temperatures were maintained in the range of 21-25 ^{0}C with a 16-h photoperiod. Each experiment was conducted in a randomized complete block design (RCBD) with two replicates and four plants per cone forming an experimental unit. Additional checks, 'Salamouni' as a resistant check as well as 'Glenlea' and ND495 as susceptible checks, were included in each experiment.

Inoculum Production

Inoculums were produced using a 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 of medium were transferred to 250-ml capacity Erlenmeyer flasks and autoclaved for 20 min. After cooling, 200 µl of kanamycin sulphate were added to each flask. Approximately 1 ml of a 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 d depending upon the culture growth. After shaking, mycelia were removed by filtering the inoculum through 2-3 layers of cheesecloth. The spore suspension was counted using a hemacytometer and adjusted to 1.0×10^{7} spores $^{-ml}$ before inoculation. Two drops of Tween 20 (Polyoxyehtylene-sorbitan monolaurate) were added per 100- ml of spore suspension before inoculation.

Inoculation Procedure

Plants were inoculated with balstospores of *M. graminicola* 19 d after planting using a CO_2-pressurized hand sprayer until runoff. Inoculated plants were allowed to dry before transferring them to the mist chamber under continuous leaf wetness for 60 h. The mist chamber conditions were maintained at a temperature of 20-23 ^{0}C, humidity of 85-100%, and a photoperiod of 15 h. The mist chamber was kept open for 1 h every 24 h to allow proper ventilation and to avoid leaf yellowing. Subsequently, the plants were transferred to greenhouse benches at an average temperature of 20-23 ^{0}C until initial symptoms were observed. After initial chlorotic symptoms appeared on the plants, a higher temperature was allowed. Plants were fertilized with a dilute solution of N-P-K fertilizer before inoculation and immediately after taking them from the mist chamber.

Disease Scoring

Plants were assessed for STB symptoms 22 d after inoculation. One infected leaf from each plant was scored for disease symptoms. Reactions were scored visually for the disease severity (DS) scale of 0-100% by estimating the percentage of leaf area covered with necrotic lesions irrespective of pycnidia formation (Gaunt et al., 1986; Saadaoui, 1987).

DNA Extraction and Genome Mapping

The DNA extraction was carried out by bulking ten plantlets per RIL following the modified method of Guidet et al. (1991). The leaves were frozen in liquid nitrogen, and frozen tissue was ground with a mortar and pestle. The powdered tissue was transferred to a 50-ml polypropylene tube, and 20-25 ml extraction buffer (0.5 M NaCl, 0.1 M Tris-HCl (pH 8.0), 50 nM EDTA, 0.84% w/v SDS, 3.8 g sodium bisulfate/L, and pH was adjusted to 8.0 with 0.8 M NaCl) was added quickly and incubated at 65 ^{0}C for 30 min. An equal volume of a mixture of

chloroform and isoamyl alcohol (24:1) was added, mixed vigorously, and centrifuged at 2800 rpm for 15 min. The top phase was transferred to a new tube, and the DNA was precipitated with two volumes of cold 95% v/v ethanol. The final concentration of DNA was measured using a Nano Drop 100 spectrophotometer (Nano Drop Technologies, Inc., Wilmington, DE, USA). Of 138 RILs, DNA samples from 118 RILs were sent to Triticarte Pvt Ltd. (Canberra, Australia; http://www.triticarte.com.au) for DArT marker analysis. Among the 2300 DArT markers screened, 423 were found polymorphic on parents, were screened on entire RIL population. Forty simple sequence repeat (SSR) markers were run to be used as anchors for linkage map construction.

Statistical Analysis

Each experiment was conducted in a randomized complete block design with two replicates. The analysis of variance was done using the general linear model (PROC GLM) (Statistical Analysis System version 8.2; SAS Institute, 1999). Error homogeneity was tested using a factor of 10 test. A combined ANOVA was performed with the genotypes having fixed effects and the three greenhouse experiments having random effects. The broad-sense heritability was calculated with an entry mean basis. The linkage map was constructed using MAPMAKER version3.0 (Lander et al., 1987) with logarithm (base 10) of odds (LOD) 3.0 and the Kosambi map function (Kosambi, 1994). The QTL analysis was conducted on individual experimental data with composite interval mapping (CIM) using Windows QTL Cartographer version2.0 software (Wang et al., 2004).

Results

Linkage Map

For the linkage map construction, 463 polymorphic markers (423 DArT and 40 SSRs) were run on RIL s. The final linkage map was made using 392 markers (364 DArT and 28 SSRs) excluding the unlinked ones as well as markers with a similar map position. The total genetic distance of the linkage map was 1789.7 cM; an average density of one marker per 4.57 cM was spread on 17 linkage groups (Table 1.1 and Fig. 1.1). The marker distribution was not even for all genomes, and density on the B genome was more compared to the A and D genomes. The map lengths were 848.70 cM, 788 cM, and 161 cM for the A, B, and D genomes, respectively (Table 1.1). The B genome had 245 markers spread over 788 cM, covering the entire B genome with an average marker density of one marker per 3.22cM. The A genome had a total of 138 markers covering most of the chromosomes, except 2A which had a map length of 848.70 cM with an average marker density of one marker per 6.09 cM. The D genome had the least marker density (one marker per 17.89 cM), with nine markers covering a 161-cM map length only on 1D. The range for individual map length was from 42.60 cM on 5B to 201.60 cM on 7B, and the number of markers on individual chromosomes ranged from 9 on 1D to 59 in 2B (Table 1.1). A marker gap of more than 50 cM was observed on five chromosomes (2B, 3B, 4A, 6A, 6B, and 7A). The order of SSR markers was verified and was in agreement with other published maps.

STB Phenotyping

The parents of RILs showed contrasting reactions to STB, and the population was segregating for this trait. The susceptible parent, ‘Steele-ND’, showed (Table 1.1 and Fig. 1.1), a mean disease severity of 66.66% was statistically different ($p<0.05$) from disease severity of resistant parent ND 735 (11.96%). The range of disease severity for ‘Steele-ND’ varied from 60-

80%, whereas the range of ND 735 varied from 6.25-15.23%. The resistant check cultivar Salamouni had a mean disease severity of 2.17%, with a range of 1.25-2.75% and the susceptible check cultivar Glenlea had a mean disease severity of 89.58%, with a range varying from 83.75-98.75%.

Table 1.1. Chromosome assignments, number of markers, genetic length, and marker density for the linkage map of the Steele-ND/ND 735 RIL mapping population

Chromosome	Number of markers	Length (cM)	Marker density (cM/Marker)
1A	23	192.70	8.38
1B	53	60.20	1.14
1D	9	161.00	17.89
2A	0	0.00	0.00
2B	59	119.70	2.03
2D	0	0.00	0.00
3A	20	103.50	5.18
3B	20	132.50	6.63
3D	0	0.00	0.00
4A	29	96.40	3.32
4B	12	121.90	10.16
4D	0	0.00	0.00
5A	17	180.40	10.61
5B	17	42.60	2.51
5D	0	0.00	0.00
6A	23	75.90	3.30
6B	32	109.50	3.42
6D	0	0.00	0.00
7A	26	191.80	7.38
7B	52	201.60	3.88
7D	0	0.00	0.00
A genome	138	840.70	6.09
B genome	245	788.00	3.22
D genome	9	161.00	17.89
Total	392	1789.70	4.57

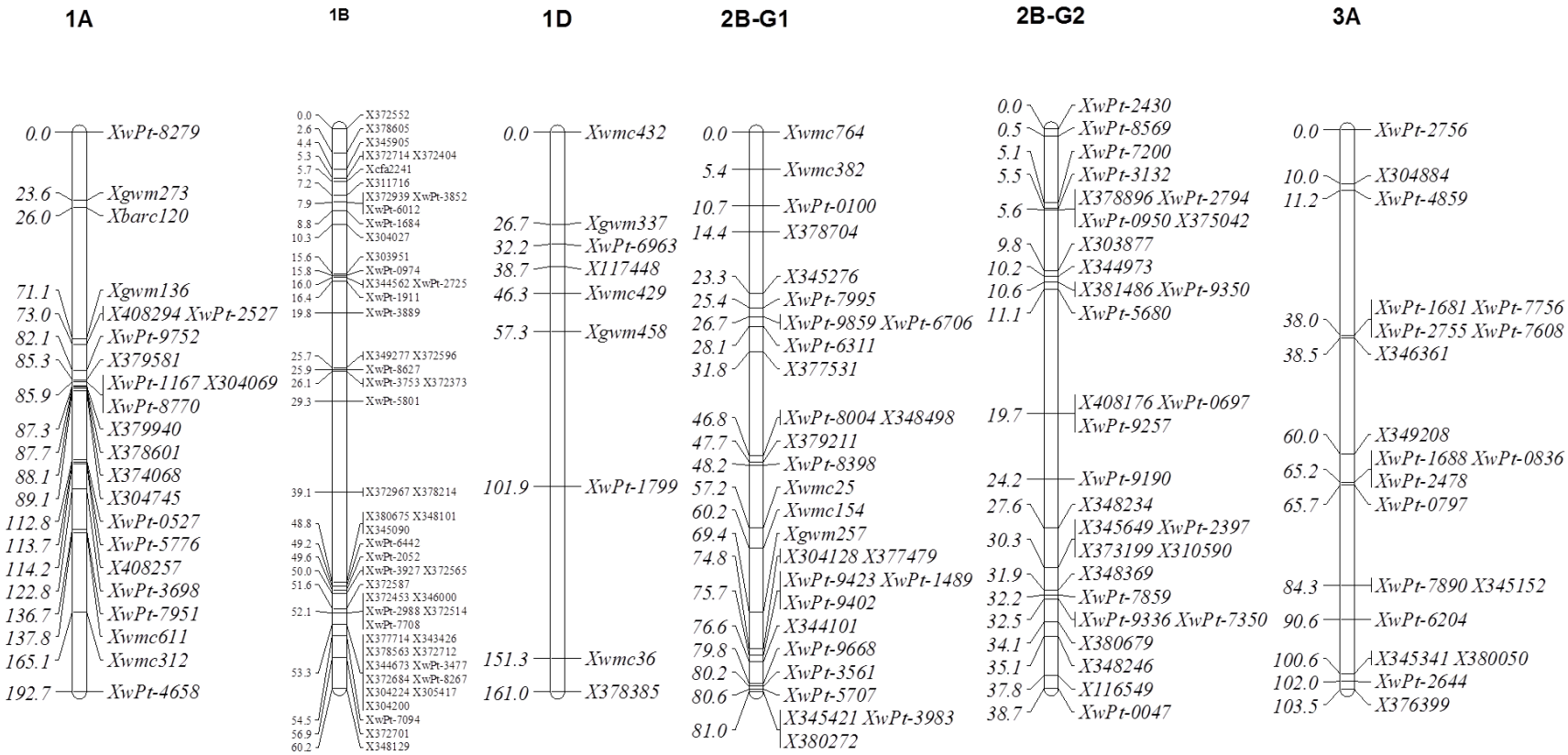


Fig. 1.1. Genetic linkage maps constructed in the ‘Steele-ND’/ND 735 RIL mapping population. The positions of the marker loci are given on the right, and the corresponding centiMorgan (cM) distances are given on the left. The group 1 and group 2 separations indicate the marker interval distance exceeding 50 cM.

(Continued)

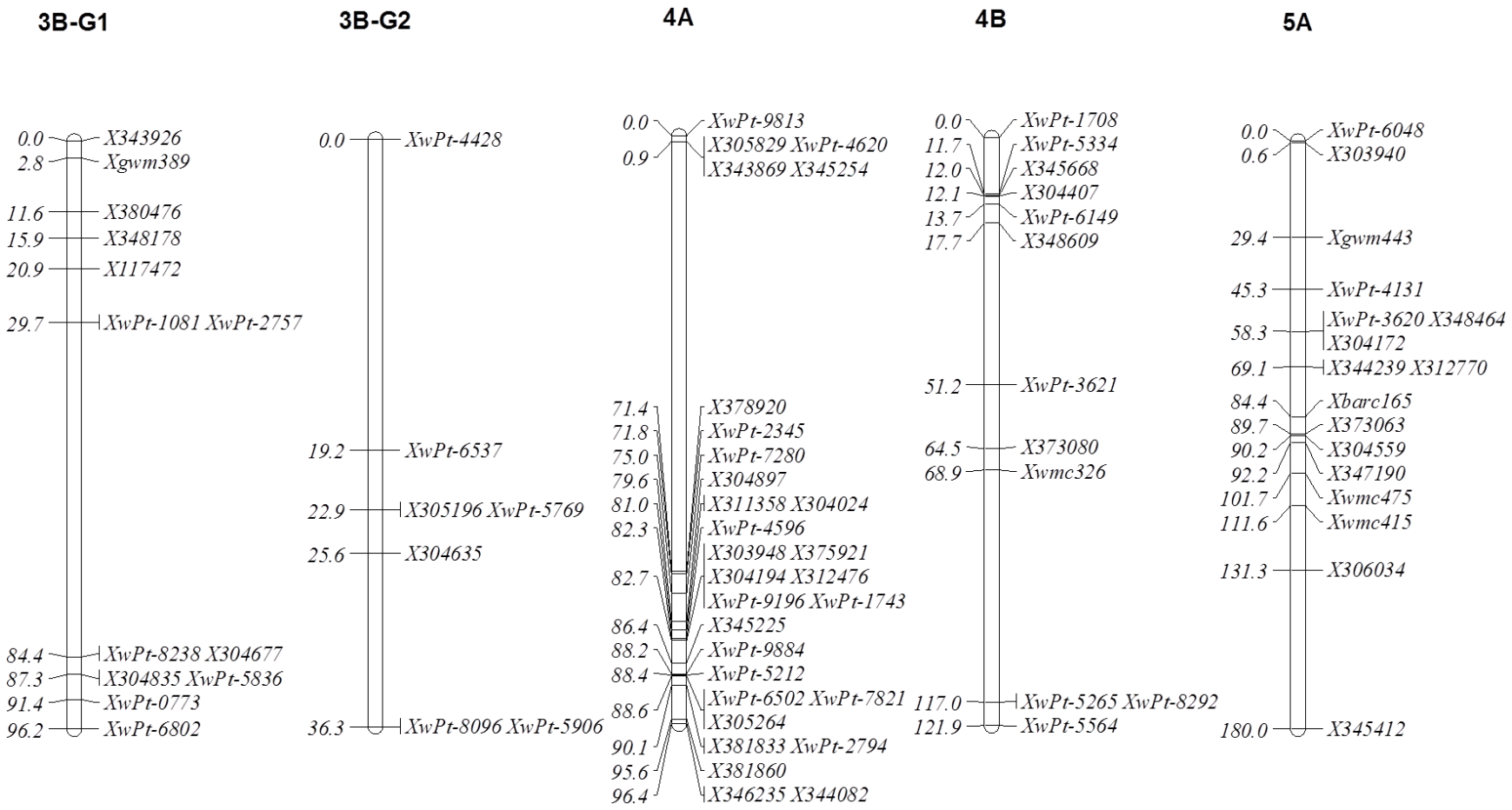


Fig. 1.1. Genetic linkage maps constructed in the 'Steele-ND'/ND 735 RIL mapping population. The positions of the marker loci are given on the right, and the corresponding centiMorgan (cM) distances are given on the left. The group 1 and group 2 separations indicate the marker interval distance exceeding 50 cM (Continued)

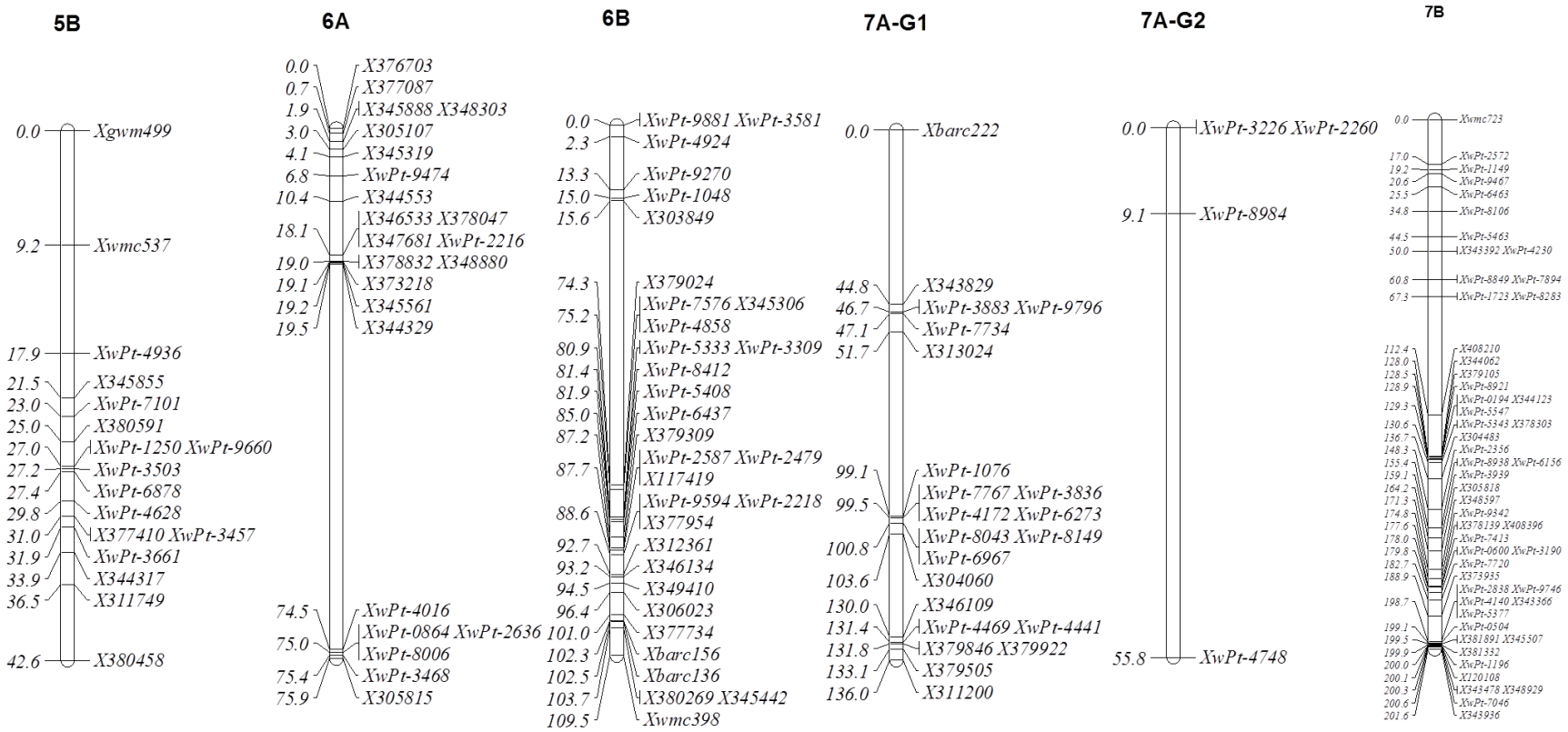


Fig. 1.1. Genetic linkage maps constructed in the ‘Steele-ND’/ND 735 RIL mapping population. The positions of the marker loci are given on the right, and the corresponding centiMorgan (cM) distances are given on the left. The group 1 and group 2 separations indicate the marker interval distance exceeding 50 cM (Continued)

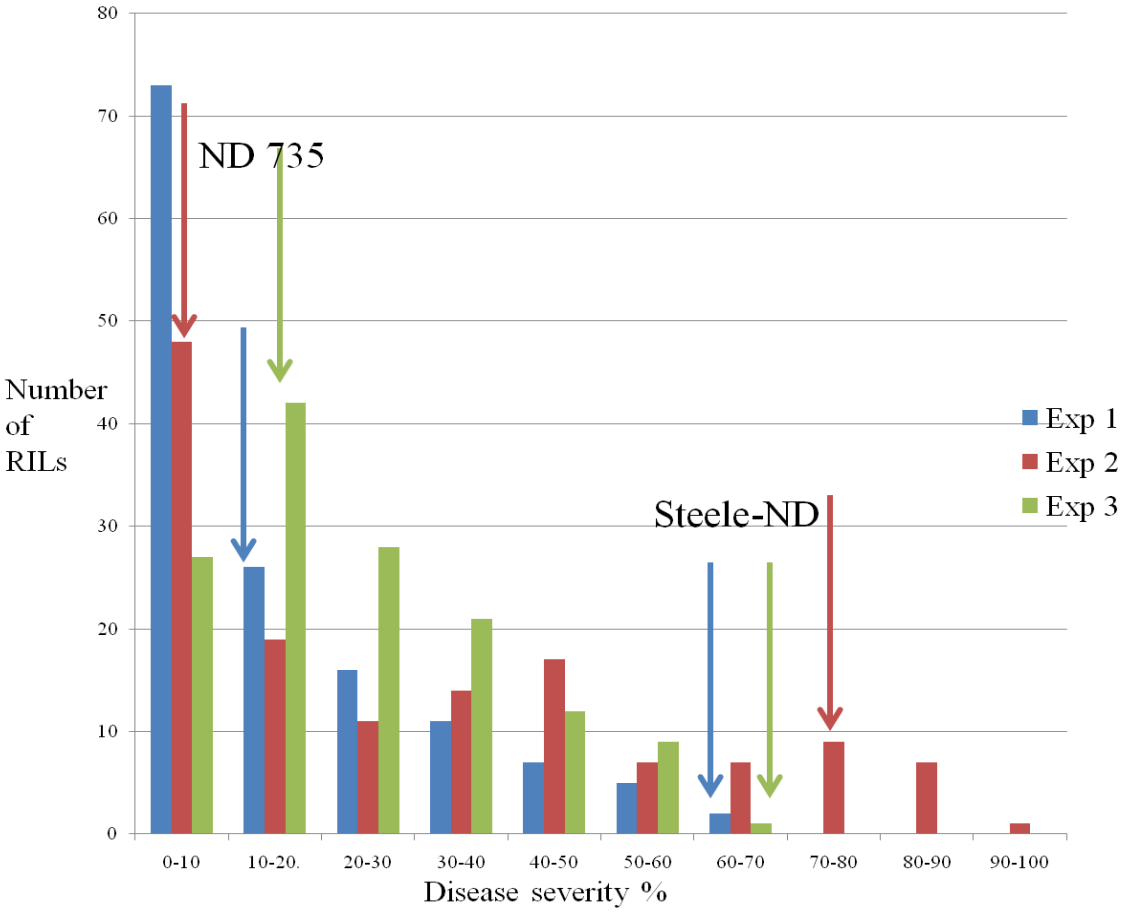


Fig. 1.2. Histogram showing the frequency distribution of RILs and the parents', Steele-ND and ND 735, reactions to septoria tritici blotch in three greenhouse experiments based on the disease severity percentage.

Table 1.2. 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

The mean disease severity of the first experiment was low (14.01%) compared to other experiments. The highest mean disease severity (28.93%) and a high pycnidial density were observed in the second experiment. The ANOVA for the combined analysis indicated significant differences ($p<0.05$) among the means of the RILs for STB resistance; the graph of the residual distribution indicated a normal distribution of the phenotypic data. The significant effect of location and genotype interaction points out the high variability of disease, even in controlled conditions. The majority of lines showed transgressive segregation in the direction of resistant parent ND 735, and the frequency distribution in each greenhouse season presented in Fig1.2 indicated the quantitative nature of disease resistance. Collectively, these results indicated significant and differing levels of variation for STB resistance in the parental genotypes and the RILs of the mapping population used during the present study, thus suggesting the suitability of this data for conducting QTL analysis.

QTL Mapping

Results of QTL mapping conducted on individual experimental data as well as mean data are given in Table 1.3 and Fig. 1.3. The broad-sense heritability estimation based on an entry mean basis was 0.68, and such high heritability estimates are common in disease-resistance studies. The QTL mapping resulted in finding one QTL on chromosome 5BL that was mapped in all individual experimental data. The QTL on 5BL explained a maximum of 10.20 % and a minimum of 7.10% of the phenotypic variation The other three QTLs detected on 1D, 7A, and 3A explained 13% (LOD 3.5), 5.6% (LOD 2.1), and 6% (LOD 2.1) of the phenotypic variation, respectively.

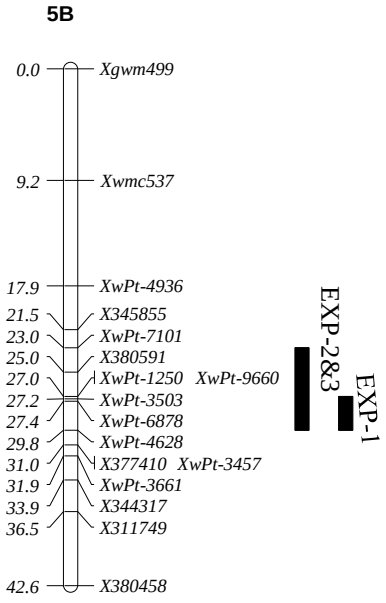


Fig. 1.3. QTL map showing the position of STB resistance QTL on the 5BL chromosome of the Steele-ND/ND 735 RIL mapping population. The length of the bar indicates one LOD interval. Abbreviation, EXP: Experiment

Table 1.3. QTL for STB detected using composite interval mapping in the Steele- ND/ ND 735 RIL mapping population

Environment	Chromosome	Flanking markers	Position cM	LOD	$R^2\times 100$ %	
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

The QTL on 1D and 7A was detected only in the third set of experimental data. The position of the QTL detected on 5BL was similar for all the experimental data. DArT markers XwPt-7101/X 377410 were flanking the QTL, which was mapped approximately 23 cM away in the long arm of 5B. Because the major QTL only explained a maximum of

10.20% of the genotypic variation, the rest of the variation should possibly be explained by the other QTLs and their interactions that may be present in the unmapped linkage groups.

Discussion

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) make the molecular-mapping efforts in common wheat very complex. Therefore, genetically diverse parents or inter-specific crosses were utilized to create the mapping populations. However, these populations lacked desirable trait variation that can be readily used in a breeding program for commercial cultivars. Molecular mapping on a population derived from inter-varietal crosses was suggested to make the marker-trait association more relevant to the objectives of the breeding program (Somers et al., 2004; Varshney et al., 1998). However, these populations often showed a low level of DNA polymorphism. This study involved a population developed from an inter-varietal cross, similar inter-varietal populations were utilized by Chalmers et al. (2001), Liu et al. (2005), and Paillard et al. (2003) for developing genetic maps. The length of the linkage map (1789.7 cM) developed during this study was lower compared to other published maps due to the low level of polymorphism present in the D genome. The largest linkage group in our study was in chromosome 7B (201.60 cM), which was slightly larger than the published maps of Liu et al. (2005) and Paillard et al. (2003). An additional marker should be run on this population to get an entire genome map with a good saturation of markers, and it will allow a better comparison with other published maps.

The ND 735 line shows a high level of resistance to STB in our experiment. Additionally, Singh et al. (2010), studying the same RIL mapping population, mapped

previously designated resistance genes *Tsr1* and *Tsr6* for resistance to races 2 and 5 of *Pyrenophora tritici-repentis*. The gene *Tsr1* was mapped in 5BL, and *Tsr6* was mapped in 2BS, with DArT markers *wPt-3049* (2.9cM) and *wPt-0289* (4.6cM) linked to both genes, respectively. They also verified the marker order and distance between SSR markers of this population and, by comparing the results with the published linkage maps of Somers et al. (2004), verified the accurate genotyping. The same mapping population was used by Singh et al. (2011) to map the *Tsn1* locus in 5BL, conferring resistance to spore suspension and culture filtrate for *Phaeosphaeria nodorum* isolate Sn2000. The presence of the tetraploid wheat cultivar Ramsey, as well as the FHB- resistant Chinese wheat cultivar Sumai3 in its pedigree, makes ND 735 resistant to septoria diseases (Gilchrist et al., 1999; M. Mergoum, personal communication). The Steele-ND cultivar shows a susceptible reaction to the STB, but is superior in many agronomy and quality traits, making this RIL population with a large amount of variation useful for identifying QTLs associated with disease resistance, agronomy, and quality traits. The initial population had 250 lines, and half of the lines were lost during the development. Even though 135 lines were sufficient to detect the major QTL (Lynch and Walsh 1989), it is possible to detect QTLs with minor effects using the larger population.

The accurate phenotyping of STB is difficult in ND field conditions 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 STB studies in North Dakota (Ali et al., 2008; Mergoum et al., 2007) were conducted in greenhouse conditions at the seedling stage. Previous QTL mapping experiments on STB (Eriksen et al., 2003; Simon et al., 2004, 2010) involved testing on seedlings and the

adult plant stage. Simon et al. (2010) mapped two linked, isolate-specific QTLs for seedling and adult plant resistance to STB using an Argentinean isolate. This study confirmed the positive correlation found in the studies of Somasco et al. (1996) and the efficacy of seedling-stage evaluation for STB resistance.

The majority of molecular mapping studies for 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 that the position of the major QTL coincided with the position of the already-mapped major gene. The variation might be attributed to the erosion of major gene resistance or the differences in the isolate and environment used during the study. During our study, the position of the major 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 *Stb1* gene is one among the few genes that provided durable resistance to STB since its deployment in the early 1970s (Adhikari et al., 2003). It may be possible that the *Stb1* gene is providing resistance to STB in the Steele-ND/ND 735 RIL mapping population, but further studies are needed to verify this conclusion.

The current study, as well as previous studies by Singh et al. (2010, 2011), confirms the presence of multiple leaf-spot resistance in ND 735 as well as the utility of the combination of DArT and SSR markers for molecular mapping of disease-resistance genes. Additionally, ND 735 can be used as a source for incorporating multiple leaf-spot resistance to existing germplasm. Additional genes for STB resistance may be present in this population, and it could be mapped if the entire genome map is available. Once validated, QTL identified in the current study can be useful for MAS for resistance to STB.

Conclusion

The current study used a modified protocol for phenotyping septoria blotch in greenhouse conditions. However, more studies are needed to optimize the temperature requirements for disease evaluation in greenhouse conditions. The confirmation of STB resistance in this mapping population, as well as ND 735 as a source of resistance in this mapping population, was verified. The position of the QTL on 5BL may be attributed to the presence of the durable STB resistance gene *Stb1* present in this germplasm. Current study reiterates the importance of ND 735 as a durable source for incorporating STB resistance. Additional QTL discoveries may be possible in unmapped linkage groups for this RIL population because the major QTL only explains a maximum of 10.20% of the variation.

References

Adhikari, T.B., J.M. Anderson, and S.B. Goodwin. 2003. Identification and molecular mapping of a gene in wheat conferring resistance to *Mycosphaerella graminicola*. Phytopathology 93:1158-1164.

Adhikari, T.B., J.R. Cavaletto, J. Dubcovsky, J.O. Gieco, A.R. Schlatter, and S.B. Goodwin. 2004a. Molecular mapping of the *Stb4* gene for resistance to Septoria tritici blotch in wheat. Phytopathology 94:1198-1206.

Adhikari, T.B., X. Yang, J.R. Cavaletto, X. Hu, G. Buechley, H.W. Ohm, G. Shaner, and S.B. Goodwin. 2004b. Molecular mapping of the *Stb1*, a potentially durable gene for resistance to Septoria tritici blotch in wheat. Theor. Appl. Genet. 109:944-953.

Adhikari, T.B., H. Wallwork, and S.B. Goodwin. 2004c. Microsatellite markers linked to the *Stb2* and S*tb3* genes for resistance to septoria tritici blotch. Crop Sci. 44:1403-1411.

Ali, S., P.K. Singh, M.P. McMullen, M. Mergoum, and T.B. Adhikari. 2008. Resistance to multiple leaf spot diseases in wheat. Euphytica 159:167-179.

Arraiano, L.S., and J.K.M. Brown. 2006. Identification of isolate-specific and partial resistance to septoria tritici blotch in 238 European wheat cultivars and breeding lines. Plant Pathol. 55:726-738.

Arraiano, L.S., L. Chartrain, E. Bossolini, H.N. Slatter, B. Keller, and J.K.M. Brown. 2007. A gene in European wheat cultivars for resistance to an African isolate of *Mycosphaerella graminicola*. Plant Pathol. 56:73-78.

Arraiano, L.S., A.J. Worland, C. Ellerbrook, and J.K.M. Brown. 2001. Chromosomal location of a gene for resistance to septoria tritici blotch (*Mycosphaerella graminicola*) in the hexaploid wheat 'Synthetic 6x'. Theor. Appl. Genet. 103:758-764.

Brading, P.A., E.C.P. Verstappen, G.H.J. Kema, and J.K.M. Brown. 2002. A gene-for-gene relationship between wheat and *Mycosphaerella graminicola*, the septoria tritici blotch pathogen. Phytopathology 92:439-445.

Brule-Babel. A.L. 2007.Catalogue of gene symbols for wheat. Annual Wheat Newsletters 53:171.

Chalmers, K.J., A.W. Cambell, J. Kretschmer, A. Karakousis, H. Henschke, S. Pierens, N. Harker, M. Pallotta, G.B. Cornish, M.R. Shariflou, L.R. Rampling, A. McLauchalan, G. Daggard, P.J. Sharp, T.A. Holten, M.W. Sunderland, R. Appels, and P. Langridge. 2001. Construction of three linkage maps in bread wheat, *Triticum aestivum*. Aust. J. Agri. Res. 52:1089-1119.

Chartrain, L., S.T. Berry, and J.K.M. Brown. 2005. Resistance of wheat line kavkaz-k4500 l.6.a.4 to septoria tritici blotch controlled by isolate-specific resistance genes. Phytopathology 95:664-671.

Chartrain, L., P.A. Brading, J.P. Widdowson, and J.K.M. Brown. 2004. Partial resistance to septoria tritici blotch (*Mycosphaerella graminicola*) in wheat cultivars Arina and Riband. Phytopathology 94:497-504.

Chartrain, L., P. Joaquin, S.T. Berry, L.S. Arraiano, F. Azanza, and J.K.M. Brown. 2005b. Genetics of resistance to septoria tritici blotch in the Portuguese wheat breeding line TE9111. Theor. Appl. Genet. 110:1138-1144.

Cowling, S.G. 2006. M.S. thesis. Department of Plant Science, University of Manitoba, Winnipeg, Canada. p. 129.

Eriksen, L., F. Borum, and A. Jahoor. 2003. Inheritance and localization of resistance to *Mycosphaerella graminicola* causing septoria tritici blotch and plant height in wheat (*Triticum aestivum* L.) genome with DNA markers. Theor. Appl. Genet. 107:515-527.

Eyal, Z. 1999. The septoria tritici and stagnospora nodorum blotch diseases of wheat. Eur. J. Plant Pathol. 105:629-641.

Eyal, Z., A.L. Scharen, J.M. Prescott, and M. Van Ginkel. 1987. The septoria diseases of wheat: the concepts and methods of disease management. International Maize and Wheat Improvement Center (CIMMYT). Mexico, DF. p. 55-60.

Gaunt, R.E., W.J. Thomson, and J. Suctcliffe. 1986. The assessment of speckled leaf blotch in winter wheat in New Zealand. Ann. Bot. 58:33-38.

Gilbert, J., and S.M. Woods. 2001. Leaf spot diseases of spring wheat in southern Manitoba farm fields under conventional and conservation tillage. Can. J. Plant Sci. 81:551-559.

Gilchrist, L., B. Gomez, R. Gonzalez, S. Fuentes, A. Mujeeb-Kazi, W. Pfeiffer, S. Rajaram, B. Rodriguez, B. Skovmand, M. Van Ginkel, and C. Velazquez. 1999. *Septoria tritici* resistance sources and breeding progress at CIMMYT, 1970–99. In M. van Ginkel, A. McNab, and J. Krupinsky (Eds) Septoria and Stagonospora of Cereals. Texcoco, Mexico: CIMMYT, DF. p. 134-139.

Gisi, U., K.M. Chin, G. Knbapova, R.K. Farber, U. Mohr, S. Parisi, H. Sierotzki, and U. Steinfeld. 2000. Recent developments in elucidating modes of resistance to phenylamide, DMI and strobilurins fungicides. Crop Protection 19:863-872.

Gisi, U., L. Pavic, C. Stanger, U. Hugelshofer, and H. Sierotzki. 2005. Dynamics of *Mycosphaerella graminicola* population in response to selection by different fungicides. In H.W. Dehne, U. Gisi, K.H. Kuck, P.E. Russel and H. Lyr (Eds) modern fungicides and antifungal compounds IV. BCPC, Alton, UK. p.73-80.

Guidet, F., R. Rogowsky, C. Taylor, W. Song, and P. Langridge. 1991. Cloning and characterization of a new rye-specific repeat sequence. Genome 34:81-87.

Jilbene, M., J.P. Gustafson, and S. Rajaram.1994. Inheritance of resistance to *Mycosphaerella graminicola* in hexaploid wheat. Plant Breeding 112:301-310.

Kema, G.H.J., J.G. Annone, R. Sayoud, C.H. Van Silfhout, J.Van Ginkel, and I. de Bree. 1996. Genetic variation for virulence and resistance in wheat-*Mycosphaerella graminicola* pathosystem. 1, Interactions between pathogen isolates and host cultivars. Phytopathology 200:2-12.

King, J.E., R.J. Cook, and S.C. Melville. 1983. A review of septoria diseases of wheat and barley. Ann. App. Biol. 103:354-373.

Kosambi, D.D. 1994 The estimation of map distances from recombination values. Ann. Eugen. 12:172-175.

Lander, E.S., P. Green, J. Abrahamson, A. Barlow, M.J. Daly, S.E. Lincoln, and L. Newburg. 1987. Mapmaker: an interactive computer package for constructing primary linkage map for experimental and natural populations. Genomics 1:174-181.

Langridge. P., E.S. Lagudah, T.A. Holton, R. Appels, P.J. Sharp, and K.J. Chalmers. 2001. Trends in genetic and genomic analyses in wheat: a review. Aust. J. Agri. Res. 53:1043-1077.

Liu, Y.G., J.A. Anderson, J. Hu, T.L. Friesen, J.B. Rasmussen, and J.D. Faris. 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.

Lynch, M., and B. Walsh. 1989. Genetic analysis of quantitative traits. Sinauer Associates, Sunderland, MA.

McCartney, C.A., A.L. Brule-Babel, and L. Lamari. 2002. Inheritance of race-specific resistance to *Mycosphaerella graminicola* in the spring wheat cultivar ST6. Theor. Appl. Gent. 107:1181-1186.

Mergoum, M., R.C. Frohberg, J.D. Miller, and R.W. Stack. 2005. Registration of 'Steele-ND' wheat. Crop Sci. 45:1163-1164.

Mergoum, M., R.C. Frohberg, P.K Singh, S. Ali, J.B. Rasmussen, and J.D. Miller. 2006. Registration of spring wheat germplasm ND 735. Crop Sci. 46:1003-1004.

Mergoum, M., R.C. Frohberg, R.W. Stack. J.K. Rasmussen, and T.L. Frisen. 2008. Registration of 'Faller' spring wheat. J. Plant Reg. 2:224-229.

Mergoum, M., P.K. Singh, S. Ali, E.M. Elias, J.A. Anderson, K.D. Glover, and T.B. Adhikari. 2007. Evaluation of spring wheat germplasm for septoria diseases. Plant Dis. 91:1310-1315.

Mergoum, M., P.K. Singh, R.C. Frohberg, S.F. Kianian, F. Ghavami, K. Hussain, T.B. Adhikari, V.E. Harilal, and S. Simsek. 2009. Registration of Steele-ND/ND 735 wheat recombinant inbred lines mapping population. J. Plant Reg. 3:300-306.

Paillard, S., T. Schnurbusch, M. Weinzeler, M. Messmer, P. Sourdille, O. Abderhalden, B. Keller, and G. Schachermayr. 2003. An integrative genetic linkage map of winter wheat (Triticum *aestivum* L.). Theor. Appl. Genet. 107:1235-1242.

Saadaoui, E.M. 1987. Physiologic specialization of *Septoria tritici* in Morocco. Plant Dis.. 71:153-155.

SAS Institute. 1999. SAS/STAT User's guide, release 8.2, 8.1, and 8.0. SAA Institute, Cary, NY.

Simon, M.R., F.M. Ayala, C.A. Cordo, M.S. Roder, and A. Borner. 2004. Molecular mapping of quantitative trait loci determining resistance to septoria tritici blotch caused by *Mycosphaerella graminicola* in wheat. Euphytica 138:41-48.

Simon, M.R., and C.A. Cordo. 1997. Inheritance of partial resistance to septoria tritici in wheat (*Triticum aestivum* L.): limitation of pycnidia number and spore production. Agronomy 17:343-37.

Simon, M.R., E.K. Khlestkina, N.S. Castillo, and A. Börner. 2010. Mapping quantitative resistance to septoria tritici blotch in spelt wheat. Eur. J. Plant Pathol. 128:317-324.

Singh, P.K., M. Mergoum, T.B. Adhikari, F. Gavami, and S.F. Kianian. 2011. Genetics and mapping of resistance to spore inoculum and culture filtrate of *Phaeosphaeria nodorum* in spring wheat line ND 735. Crop Protection 30:141-146.

Singh, P.K., M. Mergoum, T.B. Adhikari, T. Shah, F. Gavami, and S.F. Kianian. 2010. Genetic and molecular analysis of wheat tan spot resistance effective against *Pyrenophora tritici-repentis* races 2 and 5. Mol. Breeding 25:369-379.

Singh, P.K., M. Mergoum, S. Ali, T.B. Adhikari, E.M. Elias, and G.R. Hughes. 2006. Identification of new sources of resistance to tan spot, stagnospora nodorum blotch, and Septoria tritici blotch of wheat. Crop Sci. 46:2047-2053.

Somasco, O.A., C.O. Qualset, and D.G. Gilchrist. 1996. Single-gene resistance to septoria tritici blotch in spring wheat cultivar 'Tadina'. Plant Breeding 115:261-267.

Somers, D.G., P. Isaac, and K. Edwards. 2004. A high-density microsatellite consensus map of bread wheat (*Triticum aestivum* L.). Theor. Appl. Genet. 109:1105-1114.

Varshney, R.K., P.C. Sharma, P.K. Gupta, H.S. Balyan, B. Ramesh, J.K. Roy, A. Kumar, and A. Sen. 1998. Low level of polymorphism detected by SSR probes in bread wheat. Plant Breeding 117:182-184.

Wang, S., C.J. Basten, and B. Zeng. 2004. Windows QTL cartographer 2.0. Department of Statistics, North Carolina State University, Raleigh.

Zhang, X., S.D. Haley, and Y. Jin. 2001. Inheritance of *Septoria tritici* blotch resistance in winter wheat. Crop Sci. 41:323-326.

PAPER 2. QUANTITATIVE TRAIT LOCI MAPPING OF AGRONOMIC AND QULITY TRAITS IN 'STEELE-ND'/ND 735 WHEAT RECOMBINANT INBRED LINES

Abstract

Development of high-yielding wheat cultivars with good end-use quality has been a major aim for the wheat-breeding programs around the world. To study the genetics and dissect quantitative trait loci (QTL) for complex traits, including yield, yield-related agronomy, and quality traits, a population of 138 recombinant inbred lines (RILs) was developed from a cross between cultivar Steele-ND and ND 735. A linkage map was constructed based on 392 markers, consisting of 364 DArT and 28 microsatellite markers. Phenotypic data were collected from five North Dakota environments for heading date, plant height, spike density, leaf score, spike length, yield, grain volume weight, thousand kernel weight, kernels per spike, kernel size distribution, protein, flour extraction, and mixograph. For kernel hardness and kernel diameter, data were collected from four environments. Quantitative trait loci analysis was performed using composite interval mapping. Thirteen QTLs were detected for three agronomic traits and three quality-related traits. Two QTL clusters on 5A and 6B were found to control the yield and related traits in this population. Yield QTL on 5A and 6B were larger in effect in terms of R-value (27 and 22%, respectively) and additive effects (252.0 and 243.1 kg ha $^{-1}$, respectively). The map position of the QTL cluster was comparable to the previously mapped yield QTL and contained important genes for plant-growth characteristics. Molecular-mapping studies on inter-varietal population are beneficial for marker-assisted selection, however the

inconsistency of QTLs makes it challenging. More saturation of the current map is needed to explore more QTLs in this population.

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important crops in the world. The demand for a high-yielding cultivar with good end-use quality increased in recent years in response to increased consumption of wheat products by the growing world population. Main objective for the majority of the wheat breeding programs is to improve yield, yield-related agronomy, and quality traits. Yield-related agronomic traits, such as plant height, spike length, kernel number per spike, thousand kernel weight, and maturity, and quality traits, such as protein content, milling yield, and baking quality, are mostly polygenic traits with environmental factors; an interaction of the genotype and the environment has a high influence on deciding the trait value. Nearly 85 years ago, Sax (1923) developed the concepts for detecting quantitative trait loci (QTL) or genomic regions controlling the quantitative traits. In the past, chromosome substitution lines as well as RILs were used to dissect the complex quantitative traits (Law, 1965, 1966). However, with the availability of DNA markers and the development of powerful statistical tools in recent years, considerable progress has been made towards QTL mapping in plants.

The first linkage maps in hexaploid wheat were constructed using RFLP markers; however, RFLPs were not efficient in finding polymorphism in wheat, especially in narrow crosses between cultivars where the RFLP showed a low level of polymorphism (Chao et al., 1989). Inbred lines developed from genetically diverse parents, crosses between an adapted and wild wheat relative species, and synthetic wheat lines were used in many mapping studies in wheat. For instance, researchers around the world extensively used the

International Triticeae Mapping Initiative (ITMI) population (Synthetic W7984 × 'Opata') for genetic mapping using map RFLP, AFLP, and SSR markers (Nelson et al. 1995a, 1995b, 1995c; Van Deynze et al., 1995; Marino et al. 1996; Roder et al. 1998; Song et al. 2005). A number of mapping studies for agronomic traits utilized a wide cross-mapping population and numerous QTL for yield and related traits were identified (Huang et al., 2004; Peng et al., 2003). In comparison to RFLP markers, the PCR-based markers, SSRs (Bryan et al., 1997; Ma et al., 1996; Plaschke et al., 1995; Roder et al., 1995), RAPDs (Suenaga et al., 2005), AFLPs (Quarrie et al., 2005), TRAPs (Chu et al., 2008; Li et al., 2007; Liu et al., 2005), and SRAPs (Li et al., 2007), were able to detect higher levels of polymorphism in wheat. Advancement in PCR based marked technologies resulted in the utilization of an inter-varietal mapping population to map important agronomy and quality traits in wheat (Cadalen et al., 1997; Groos et al., 2003; McCartney et al., 2005; Sourdille et al., 2003).

In a recent paper, Zhang et al. (2008) reviewed the distribution of yield and yield-related QTL published for wheat. Among 541 yield-related QTLs reported, 33% of the QTL were reported for yield, 21% of the QTL for thousand-kernel weight (TKW), and 16% for plant height. Among wheat genomes, the majority of the QTL (41%) were reported on the A genome, followed by 30% each on the B and D genomes. Molecular-mapping efforts on the ITMI population (Ayala et al., 2002; Borner et al., 2002; Kulwal et al., 2003; Kumar et al., 2007) identified major yield QTL on chromosomes 2DS, 4 AL, and 4 DL. However, it is believed that yield QTL is distributed all over the wheat genome due to its association with other traits, such as the yield component QTL of grain weight, spike length, number of grains per spike, and test weight. A similar distribution of QTL for

protein content (GPC) was observed in hexaploid and tetraploid wheat (Blanco et al., 1996; Borner et al., 2002; Groos et al., 2003; Joppa et al., 1997; Khan et al., 2000; Mesfin et al., 1999; Prasad et al., 1999, 2003). A number of QTL controlling GPC have been mapped on chromosomes 2A, 2D, 3A, 3D, 4A, 7A, and 7D (Groos et al., 2003; Nelson et al., 2006; Prasad et al., 1999, 2003); 6B (Mesfin et al.,1999); 2B and 4D (McCarteny et al., 2006); and 3A, 3B, 5D, and 6D (Zhao et al., 2010). Quantitative trait analysis was used to study various wheat-quality traits, including the milling yield (McCartney et al., 2006; Nelson et al., 2006; Parker et al., 1999), grain hardness (Sourdille et al., 2003; Sun et al., 2010), kernel size and shape (Dholakia et al.,2003), grain length (Campbell et al., 1999; Sun et al., 2010), mixograph traits (Huang et al., 2006; McCartney et al., 2006; Nelson et al., 2006), flour color (Parker et al., 1998), sedimentation volume (Huang et al., 2006; McCartney et al., 2006; Zanetti et al., 2001), and baking properties (Campbell et al., 2001; McCartney et al., 2006). All these studies indicated the importance of QTL analysis in elucidating the complex wheat traits.

The recent development of Diversity Array Technology (DArT) provide a valuable tool for doing cost-effective whole-genome fingerprinting even in plant species without any DNA sequencing information (Jaccoud et al., 2001). This technology utilizes the principle of hybridizing a genomic representation of an individual to a microarray containing copies of DArT markers to detect the polymorphism. In combination with other marker systems, DArT markers have been used to produce genetic maps for a range of crop species, including rice (Jaccoud et al., 2001), wheat (Akbari et al., 2006; Semagn et al., 2006), barley (Hearnden et al., 2007; Wenzl et al., 2004), and sorghum (Mace et al., 2008). The objective of this study was to identify QTL controlling important agronomic

and quality traits in adapted spring wheat using a RIL population developed from the cross 'Steele-ND'/ND 735 using DArT and SSR marker data.

Materials and Methods

Plant Material

One hundred and thirty-eight RILs were developed from a cross between HRSW cultivar Steele-ND (Mergoum et al., 2005) and HRSW line ND 735. NDSU, Fargo, ND, released this F_{2-8} RIL population in 2008 (Mergoum et al., 2009). The wheat variety Steele-ND, released in 2004 (Mergoum et al., 2005), has the pedigree of 'Parshall' (PI 613587)/ND706 while the pedigree of the NDSU-released wheat cultivar Parshall is 'Keene'//'Grandin (PI 531005)'*2/ 'Glupro' (PI 592759) and that of the line ND 706 is (Grandin/3/IAS20*4/H567.71// 'Amidon' (PI 527682), /4/Glupro). In the pedigree, Parshall shows moderate resistance to tan spot (caused by *Pyrenophora tritici* repentis) and STB. ND 706 is highly resistant to leaf rust (caused by *Puccinia triticina* Eriks.) and stem rust (caused by *P. graminis* Pers. f. sp. *tritici* Eriks. & E. Henn). Steele-ND shows a resistant reaction to stem and leaf rust, and moderate resistance to fusarium head blight (FHB) caused by *Fusarium graminearum* Schwabe. However, it is highly susceptible to major leaf-spotting diseases, including STB; SNB; all virulent races of tan spot found in North Dakota; and two toxins, Ptr ToxA and Ptr ToxB (Mergoum et al.,2007; Singh et al., 2006). Steele-ND does not have 'Sumai 3' (PI 481542) in its pedigree, and the source of moderate FHB resistance is believed to be from *Triticum dicoccoides*. ND 735 is derived from the cross ND 2709/3/Grandin*3// 'Ramsey (CItr 13246)/ND 622/ND 2809. It is one of the early ND lines with combined leaf-spotting disease resistance, yield, and quality. It is highly resistant to leaf rust; stem rust; and major leaf spotting, including STB, SNB, tan

spot races 1, 2, 3, and 5, as well as toxins Ptr ToxA and Ptr ToxB produced by races 1 and 2, and 5, respectively (Mergoum et al., 2006; Singh et al., 2011. Additionally, it is moderately resistant to FHB due to the presence of 'Sumai3' in its pedigree.

Field Experiments

To evaluate the 138 RIL lines and their parents, the experiments were conducted in a randomized complete block design (RCBD) with two replicates. Experimental locations, Prosper and Carrington, ND, in 2008 and 2009 as well as Casselton, ND, in 2008. At Prosper and Carrington, the experiments were conducted in seven-row plots with a dimension of 1.37 m x 2.44 m long with an inter-row space of 15.2 cm. At Casselton, the experiment was conducted in two 2.44-m long row plots. The Carrington location was irrigated; it represented the East Central region of North Dakota, with a soil type of Heimdal-Emrick series (loamy, mixed, superactive, and Calcic/Hapludolls). The Casselton and Prosper locations were non-irrigated and represented the Eastern region of North Dakota with a soil type of Beardon series (fine silty, mixed, superactive, frigid aeric Calciaquolls).

Agronomic and Quality Data Collection

Field data collection included days to heading, plant height, spike density, and leaf disease score. The lodging was absent in most of the environment, except in 2009 at the Prosper location. Ten spikes were randomly picked from each plot to determine the spike length and kernel number per spike. The grains harvested from the plots were cleaned with clipper grain cleaner, and agronomic traits, including grain yield and grain volume weight (GVW), were calculated. A sample of 200 gm of grain cleaned on a Carter dockage tester

(Carter-day Co., Minneapolis, MN), in Cereal Quality Lab at NDSU, and generated following wheat quality data.

Days to Heading (HD): Heading data (days) were recorded when the inflorescence fully emerged in at least 50% of the spikes in each plot. Heading data were the number of days between planting and heading.

Plant Height (PH): Average plant height (cm) was recorded for each plot by measuring the length of the plant from the soil surface to the top of the spikes, excluding the awn length.

Spike m^{-2} (SD): Spike m^{-2} was calculated based on the number of spikes in a 0.5-m length in two individual rows. The average number of spikes in a 0.5 m^2 area was calculated.

Lodging Score (LS): Lodging was determined on a scale of 1 to 5, where a score of 1 is no lodging and a 5 is entire plot lodged. There was no significant lodging at all three locations in 2008, but in 2009, lodging at Proper was severe due to heavy rains. The lodging data were not included in the analysis.

Grain Yield (GY): Grain yield (Kg ha^{-1}) was determined based on weighing the cleaned seeds from each plot.

Grain Volume Weight (GVW): Grain volume weight (Kg m^{-3}) was calculated according to American Association of Cereal Chemists (AACC) standard method 55-10 (AACC, 2000).

Spike Length (SL): Spike length (cm) was calculated by averaging the length of 10 individual spikes collected at random places from each plot.

Kernel Number Spike $^{-1}$ (KPN): Ten randomly collected spikes were threshed and the kernel number spike^{-1} was calculated by averaging the number of kernels from the 10 spikes. The electronic seed counter (Seedburo Equipment Co., Chicago, IL) was used to count the kernels.

Thousand Kernel Weight (TKW): Thousand-kernel weight (gm) was calculated by counting the number of kernels in 10 g of sample using an electronic seed counter (Seedburo Equipment Co., Chicago, IL).

Kernel Size Distribution (KSD): This test was performed using a mechanical shaker, using 100 gm of seed with a shaking time of 2 minutes. The kernels remaining in the top sieve (Taylor No. 7, 2.92 mm) were classified as "large"; kernels passing through the top sieve and in the middle sieve (Taylor No. 9, 2.24 mm) were considered "medium"-sized kernels. Kernels passing through the middle sieve were considered as "small." Because small-sized kernels are negligible (<1%), only large- and medium-sized kernels were reported. Kernel size was reported as a percentage of large, medium, and small kernels in 100 g of seed that were used for evaluation.

Grain Protein Concentration (GPC): Grain protein concentration (in %) was measured according to AACC standard method 46-30 (AACC, 2000) using an Infratec 1226 Cold Grain Analyzer.

Kernel Hardness (KH): Kernel hardness was measured using the Single Kernel Characterization System (SKCS), and the hardness was expressed as an index of 20 to 120. The SKCS system analyzed 300 individual kernels from approximately 12-20 g of a cleaned sample.

Kernel Diameter (KD) Kernel diameter (mm) was measured as an average diameter of 300 kernels analyzed in the SKCS system.

Flour Extraction (FE): All samples were cleaned using a Carter-Day dockage tester before milling. One 50-g sample was tempered to 15.5% moisture for 16 hr before being milled on a Barbender Quadromat Junior Mill according to the standard procedures of the Cereal Quality Lab at NDSU. The final weight of the sample was fixed to 150 gm before milling. The percentage of flour extraction was calculated by dividing the flour weight by the total grain weight milled.

Mixograph: The mixograph test was done using the National Manufacturing Mixogram with a 10-g mixing bowl (National Manufacturing, TMCO Division, Lincoln NE). Mixograph water absorption was based on GPC at each location following the formula of Finney (1945). Mixograph data were collected using the Mixsmart software program.

DNA Extraction and Genome Mapping

The DNA extraction was carried out on a bulk of 10 plants per RIL following the modified method of Guidet et al. (1991). Of 138 RILs, DNA samples from 118 RILs were sent to Triticarte Pvt Ltd. (Canberra, Australia; http: //www. triticarte.com.au) for DArT marker analysis. Among 2300 DArT markers screened, 423 markers were found polymorphic on parental lines, were used to screen the entire RIL population. The final linkage map was made using 392 markers (364 DArTs and 28 SSRs) excluding the unlinked markers as well as markers with a similar map position.

Statistical Analysis

The analysis of variance for the data was done using the general linear model (PROC GLM; Statistical Analysis System version 8.2; SAS Institute, 1999). Error homogeneity was tested using a factor of 10 test. A combined ANOVA was performed by considering genotypes as having fixed effects and the environments as having random effects. The linkage map was constructed using Mapmaker version3.0 (Lander et al., 1987) with a LOD of 3.0 and the Kosambi map function (Kosambi, 1994). The QTL analysis was conducted for individual experimental data with composite interval mapping (CIM) using Windows QTL Cartographer version 2.0 software (Wang et al., 2004). A standard model Zmapqtl 6 with a window size of 10 cM and automatic cofactor selection was conducted with forward and backward regression method with the CIM procedure. The walking speed chosen for the CIM was 1 cM. The empirical LOD threshold at 5% was determined by a 1000-permutation test. The position of the QTL was automatically detected using a one LOD interval.

Results

Agronomic and Quality Data

Considerable variation among the Steele-ND/ND 735 RILs was observed for the agronomic and quality traits evaluated. The phenotypic values of the parents, checks, and RILs for all the traits are summarized in Table 2.1. The combined analysis of data was conducted for all traits (Appendix B, Tables B.1-B.27) in four environments. The data from Casselton 2008 were analyzed separately (Appendix B, Tables B.1-B.27). The combined analysis showed significant differences ($P < 0.05$) between RILs for all traits. However, in Casselton 2008, SL and TKW showed no difference among RILs. The

combined analysis in four environments showed a significant interaction between the RIL and environment for a majority of traits except HD, SD, and KH.

The HD for RILs varied from 48.8 to 61.3 d while 'Steele-ND' and ND 735 headed at 58.8 and 59.5 d, respectively. Similarly, the mean PH for 'Steele-ND' and ND 735 was 86.4 cm and 93.6 cm, respectively. However, the PH for RILs ranged from 80.0 to 102.6 with a mean PH of 90.3. The SD of RILs ranged from 326.3 to 488.2, with the highest mean SD observed in Casselton during 2008. The SLs were 7.8 and 8.6 cm, respectively, for 'Steele-ND' and ND 735, and the mean SLs for RILs were 8.1 cm. However, the SL for RILs ranged from 7.9 to 8.9 cm. Similarly, the KPN mean for RILs (24.1) was less than ND 735 (25.9) and greater than Steele-ND (23.0). The GY mean of RILs (4493.0 Kg ha^{-1}) is lower than the parental lines, Steele-ND (4510.8 Kg ha^{-1}) and ND 735 (4507.2 Kg ha^{-1}), and the check cultivar Faller (Mergoum et al., 2008; 5111.6 Kg ha^{-1}), but it was higher than the check cultivar Glenn (Mergoum et al., 2006; 4163.6 Kg ha^{-1}). The upper range for the GY of RILs exceeded the GY of parental lines as well as checks in all experiment. The highest GY for RILs was recorded at Casselton in 2008, followed by Carrington in 2009 and the lowest GY was recorded at Prosper in 2009. The lowest GY at Prosper in 2009 may be attributed to the severe lodging witnessed during the growing season.

The mean GVW of 'Steele-ND' and ND 735 was 776.4 and 786.8 kg m^{-3}, respectively. The ND 735 line had a lower TKW (31.4 gm) and GPC (14.8%) than 'Steele-ND'. The range of RILs for GPC was from 14.0-16.1%. The highest GVW was observed for check cultivar Glenn (810.6 kg m^{-3}) in all experiments, but regarding GPC, the upper range for RILs was better than the Glenn cultivar.

Table 2.1. Mean, range, and standard deviation (SD) for the recombinant inbred lines (RILs), parents Steele-ND and ND735, and check cultivars Glenn and Faller for 15 traits

Trait	Env‡	Parents		Checks		RILs		
		Steele-ND	ND 735	Glenn	Faller	Mean	Range	SD
Heading date (d)	CR08	73.5	73.0	70.0	75.0	73.5	70.0-76.0	0.9
	CS08	52.7	53.5	51.0	54.0	53.0	51.5-55.0	0.7
	PS08	60.5	60.5	57.0	60.5	59.5	57.5-61.5	0.7
	CR09	57.0	58.5	55.5	59.50	58.1	56.5-60.5	0.9
	PS09	50.5	52.0	47.0	52.5	51.3	48.5-53.0	0.9
	Mean	58.8	59.5	56.1	60.3	59.1	48.8-61.3	1.2
Plant height (cm)	CR08	78.7	93.9	72.3	81.2	80.6	66.0-95.3	5.2
	CS08	95.8	97.1	92.7	88.9	94.9	86.4-104.1	3.3
	PS08	91.4	99.0	93.9	95.2	97.2	85.1-109.2	3.7
	CR09	77.4	80.0	81.2	78.7	86.6	73.7-102-9	5.0
	PS09	88.9	97.7	91.4	88.9	92.4	82.6-106.7	3.9
	Mean	86.4	93.6	86.3	86.6	90.3	80.0-102.6	3.3
Spike m^{-2}	CR08	342.1	325.7	279.6	250.0	291.4	225.3-340.5	24.3
	CS08	644.7	588.8	580.6	562.5	642.8	398.0-759.9	58.9
	PS08	458.9	500.0	348.7	419.4	459.7	340.5-608.6	48.8
	CR09	273.3	255.0	333.3	296.7	332.3	273.3-431.7	30.6
	PS09	386.7	471.7	395.0	391.7	449.1	368.3-558.3	38.6
	Mean	421.1	428.2	387.4	384.0	431.1	326.3-488.2	27.8

(Continued)

Table 2.1. Mean, range, and standard deviation (SD) for the recombinant inbred lines (RILs), parents Steele-ND and ND735, and check cultivars Glenn and Faller for 15 traits (continued)

Trait	Env.	Parents		Checks		RILs		
		Steele-ND	ND 735	Glenn	Faller	Mean	Range	SD
Yield (Kg ha^{-1})	CR08	4280.8	4138.5	3471.3	4439.9	4148.2	2693.8-5260.9	473.7
	CS08	5414.0	5558.3	4942.8	6187.2	5325.3	4083.8-6331.5	456.1
	PS08	4608.9	4637.5	4475.2	5400.1	4466.9	2389.0-5719.9	622.9
	CR09	4105.5	4006.9	4484.9	5298.2	5020.9	3394.0-6027.4	487.3
	PS09	4144.6	4194.9	3443.8	4232.6	3599.7	2700.6-4620.3	442.7
	Mean	4510.8	4507.2	4163.6	5111.6	4493.1	3246.2-5163.9	343.1
Grain volume weight (kg m^{-3})	CR08	747.9	760.7	789.5	750.3	762.3	724.9-792.8	12.3
	CS08	795.9	809.4	833.6	796.9	802.1	769.9-824.4	9.3
	PS08	760.4	783.9	803.4	770.3	775.7	728.3-805.6	14.6
	CR09	805.0	796.1	826.8	789.3	798.4	798.4-822.2	39.9
	PS09	772.8	783.9	799.5	767.1	776.1	745.4-803.6	12.1
	Mean	776.4	786.8	810.6	774.8	782.6	701.5-801.1	11.6
Kernel number per spike	CR08	18.7	22.2	16.6	23.8	19.6	13.5-26.9	2.2
	CS08	25.6	28.6	28.9	34.1	25.6	14.9-31.7	2.8
	PS08	22.6	29.7	26.4	28.5	25.0	19.9-33.1	2.6
	CR09	27.5	25.4	27.4	32.5	26.5	21.3-31.9	2.4
	PS09	20.7	23.5	22.4	27.9	23.8	17.6-30.4	2.6
	Mean	23.0	25.9	24.3	29.3	24.1	21.2-26.9	1.2
Protein	CR08	15.9	15.7	15.9	14.6	15.5	14.7-16.9	0.4
	CS08	14.6	14.3	14.7	14.1	14.5	13.6-16.1	0.5
	PS08	15.3	14.9	15.8	14.7	14.9	13.8-16.1	0.5
	CR09	15.6	14.4	15.9	14.7	15.4	13.5-17.1	0.6
	PS09	15.1	14.6	14.7	14.2	14.8	13.9-15.8	0.4
	Mean	15.3	14.8	15.4	14.4	15.1	14.0-16.1	0.4

Table 2.1. Mean, range, and standard deviation (SD) for the recombinant inbred lines (RILs), parents Steele-ND and ND735, and check cultivars Glenn and Faller for 15 traits (continued)

Trait	Env.	Parents		Checks		RILs		
		Steele-ND	ND 735	Glenn	Faller	Mean	Range	SD
Thousand kernel Weight (gm)	CR08	29.7	30.4	29.7	33.6	30.9	26.7-35.1	1.8
	CS08	33.4	32.9	34.7	37.9	34.1	28.8-38.3	1.9
	PS08	28.6	29.3	30.0	28.3	28.6	23.9-32.9	1.9
	CR09	35.5	31.9	32.9	37.6	34.5	29.1-38.6	2.1
	PS09	32.1	32.2	31.7	38.5	32.1	28.2-36.7	1.8
	Mean	31.8	31.4	31.8	35.2	31.9	28.1-35.4	1.6
Large size kernels (%)	CR08	45.5	50.0	36.0	61.5	53.5	33.5-79.5	9.2
	CS08	72.3	69.5	68.5	79.0	70.3	51.5-81.0	6.3
	PS08	56.9	47.9	54.9	71.3	55.8	28.0-76.4	9.8
	CR09	64.4	46.5	56.1	74.5	63.3	37.0-80.9	9.3
	PS09	52.5	50.8	47.9	65.7	51.4	27.9-79.2	8.9
	Mean	58.3	52.9	52.7	70.4	58.6	36.3-74.5	7.2
Medium size Kernels (%)	CR08	52.0	48.5	63.0	37.5	44.9	18.5-64.0	8.8
	CS08	26.5	29.3	30.5	19.5	28.4	18.0-46.0	6.0
	PS08	42.0	51.6	44.6	27.9	43.1	24.0-68.9	9.2
	CR09	34.8	52.9	43.9	24.6	36.2	18.8-60.6	8.9
	PS09	44.7	46.4	49.9	31.9	45.9	20.5-66.6	7.7
	Mean	40.0	45.7	46.4	28.3	39.9	24.9-60.1	6.7
Flour Extraction (%)	CR08	63.4	61.8	63.9	65.5	61.8	55.6-66.7	2.1
	CS08	63.3	62.8	63.5	67.3	63.4	42.2-68.9	2.8
	PS08	58.9	58.2	56.2	64.9	57.8	48.4-64.1	3.3
	CR09	58.3	55.2	62.0	65.1	57.1	28.8-63.7	4.3
	PS09	61.4	55.6	59.3	62.9	57.6	30.3-64.0	4.3
	Mean	61.1	58.7	60.9	65.1	59.5	52.6-63.5	2.1

Table 2.1. Mean, range, and standard deviation (SD) for the recombinant inbred lines (RILs), parents Steele-ND and ND735, and check cultivars Glenn and Faller for 15 traits".

Trait	Env.	Parents		Checks		RILs		
		Steele-ND	ND 735	Glenn	Faller	Mean	Range	SD
Kernel	CR08	2.7	2.7	2.8	2.7	2.7	2.5-2.8	0.1
Diameter	PS08	2.8	2.7	2.7	2.8	2.7	2.5-2.8	0.1
(mm)	CR09	3.0	2.8	2.9	3.0	2.9	2.7-3.0	0.1
	PS09	2.9	2.8	2.9	3.1	2.8	2.5-3.0	0.1
	Mean	2.8	2.7	2.8	2.9	2.8	2.6-2.9	0.1
Kernel	CR08	81.0	73.5	81.7	73.1	75.7	66.8-88.2	4.8
Hardness	PS08	86.8	78.9	77.6	82.2	80.2	71.7-92.3	4.3
index	CR09	76.3	69.9	77.9	73.9	72.5	62.3-87.2	5.6
	PS09	83.5	76.6	82.7	74.1	76.9	65.5-86.6	4.5
	Mean	81.9	74.7	79.9	75.9	76.4	68.3-85.6	4.2
Mixograph	CR08	5.8	6.8	6.8	6.5	6.8	4.5-11.4	1.3
Peak time	PS08	5.5	8.1	7.4	5.2	6.7	3.7-10.7	0.9
(Minute)	CR09	4.1	7.7	5.1	4.8	4.9	3.1-7.6	1.3
	PS09	4.9	10.1	9.4	5.7	7.1	4.1-11.5	1.5
	Mean	5.1	8.2	7.2	5.4	6.4	4.0-9.9	1.1
Spike	CR08	7.0	8.0	7.0	7.2	7.1	6.1-8.1	0.4
length	CS08	8.1	8.6	8.2	7.8	8.2	7.3-9.0	0.3
(cm)	PS08	8.1	8.9	8.4	8.9	8.4	7.6-9.8	0.4
	CR09	8.2	8.8	7.8	7.9	8.5	7.7-9.5	0.3
	PS09	7.6	8.5	8.1	7.6	8.1	7.2-9.1	0.4
	Mean	7.8	8.6	7.9	7.7	8.1	7.9-8.9	0.3

‡Environment, CR, Carrington; PS, Prosper; CS, Casselton. 08, 2008; 09, 2009

The GVW and TKW for RILs ranged from 701.5-801.1 kg m^{-3} and from 28.1-35.4 gm, respectively. The check Faller had the highest percentage of large kernels (70.4%) and flour extraction (65.1%). The mean of RILs for the large-size kernels and flour extraction was 58.6% and 59.5%, respectively. The range of RILs for the percentage of large-size kernels (36.3-74.5%) and flour extraction (52.6-63.5%) showed the segregation for these traits. KD, KH, and mixograph peak time (MPT) were recorded in four environments, and the mean RIL values for these traits were 2.8 mm, 76.4, and 6.4 min, respectively. Transgressive segregation was observed in the 'Steele-ND'/ND 735 RIL mapping population for a majority of the traits.

Linkage Map and QTL Mapping

The linkage map for the Steele-ND/ND 735 RIL population was constructed using 364 DArT and 28 SSR markers. The linkage map spanned 17 linkage groups, with a total map distance of 1789.7 cM and an average density of one marker per 4.57 cM. The lowest marker density was observed in the D genome (one marker per 17.89 cM), with nine markers covering the 161-cM map length on 1D. However, on the B genome, where the highest marker density was observed, 245 markers were spread over 788 cM, covering the entire B genome with an average marker density of one marker per 3.22 cM. Details about the linkage map construction, the average marker density of each sub genome, and the length of each linkage group is reported in Paper 1.

The QTL mapping results are given in Table 2.2 and Fig. 2.1. QTL mapping was conducted for ten agronomic and five quality traits. The CIM resulted in 13 QTL for six traits: GY, TKW, KD, KH, KPN, and mixograph peak time. No significant QTL was observed for HD, PH, GPC, SL, SD, kernel size distribution, and flour extraction. Two major QTL clusters were detected in CIM, chromosome 5A between DArT markers

Xwpt4131 and *X344239*, and chromosome 6B between DArT markers *Xwpt9881* and *Xwpt9270*. Six QTLs were detected for three (GY, TKW, and KPN) agronomic traits. Similarly, QTL analysis revealed seven QTL in three (KD, KH, and MPT) quality traits. Details of the QTL mapping are given in Table 2.2.

The GY QTL was located on chromosome 5A and 6B between DArT markers *Xwpt4131*, *X344239*, *Xwpt9881*, and *Xwpt9270*, respectively. The grain yield QTL on 5A was found using the date from environment Prosper 2008, explained 27% of phenotypic variation for yield. Similarly, the QTL on chromosome 6B, found in the same environmental data, contributed 22% of the phenotypic variation. The ND 735 alleles, at the 5A and 6B yield QTL, increased the grain yield relative to the Steele-ND alleles with an additive effect of 252.04 kg ha^{-1} and 243.14 kg ha^{-1}, respectively. These QTL were larger based on the R^2 and estimated additive effect. The QTL for TKW and KPN from the Prosper 2009 environmental data coincided with the GY QTL in 5A, explaining 16% and 27% of the phenotypic variation, respectively. Similarly, the GY QTL at 6B coincided with the QTL at TKW and KPN for the Prosper 2009 environment's data. Each of these QTL explained 19% and 20% of phenotypic variation, respectively. Among the four QTL detected for KH, the QTL on 7A explained the maximum (26%) phenotypic variation. The ND 735 alleles, on 5A and 1A, reduced the KH and 'Steele-ND' alleles on 7B and 7A increased the KH. The KH QTL on 5A coincided with the QTL for TKW found in Prosper during 2008.

Table 2.2. Summary of QTLs for six traits detected in a RIL population derived from the Steele-ND/ND 735 cross in multiple environments in North Dakota using composite interval mapping

.

Trait	Chromo some	QTL peak position (cM)	Flanking markers	LOD	R^2 (%)	Additive	Significant dataset
Grain yield	5A	58.5	Xwpt4131/X344239	3.53	0.27	-252.0	PR 08
(kg ha $^{-1}$)	6B	0	Xwpt9881/Xwpt9270	2.79	0.22	-243.1	PR 08
Thousand kernel	5A	58.4	Xwpt4131/X344239	2.53	0.16	-3.1	PR 09
weight (g)	6B	2.1	Xwpt9881/Xwpt9270	3.35	0.19	-3.4	PR 09
kernel spike^{-1}	5A	58.1	Xwpt4131/X344239	2.81	0.27	-3.6	PR 09
	6B	2.70	Xwpt9881/Xwpt9270	4.04	0.20	-3.2	PR 09
Kernel diameter (mm)	3BG1	17.5	X343926/Xwpt 1081	2.50	0.23	0.1	CR 08
Kernel hardness	5A	131.8	Xwmc475/X345412	2.67	0.23	3.5	CR 08
index	7B	5.90	Xwmc723/Xwpt5463	2.57	0.23	-3.3	CR 09
	1A	136.5	Xwpt3698/Xwmc312	2.79	0.25	3.3	PR 09
	7AG1	44.5	Xbarc222/Xwpt1076	2.56	0.26	-3.2	PR 09
Mixograph peak	7B	15.6	Xwmc723/Xwpt8106	3.40	0.44	1.3	CR 09
time (minutes)	2BG1	23.3	Xwmc382/Xwpt8004	2.80	0.26	0.9	PR 08

CR, Carrington; PR, Prosper; 08, 2008; and 09, 2009.

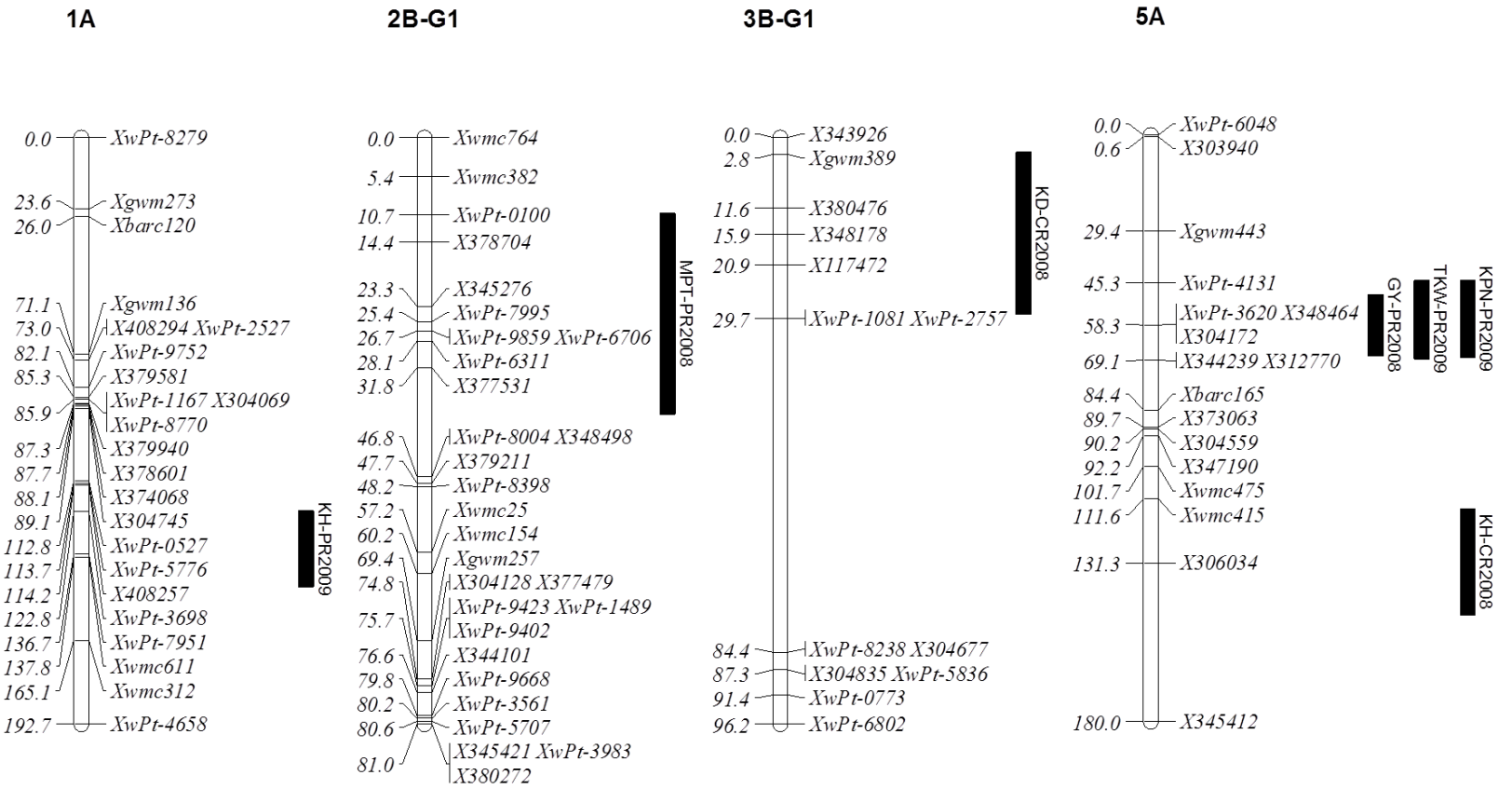
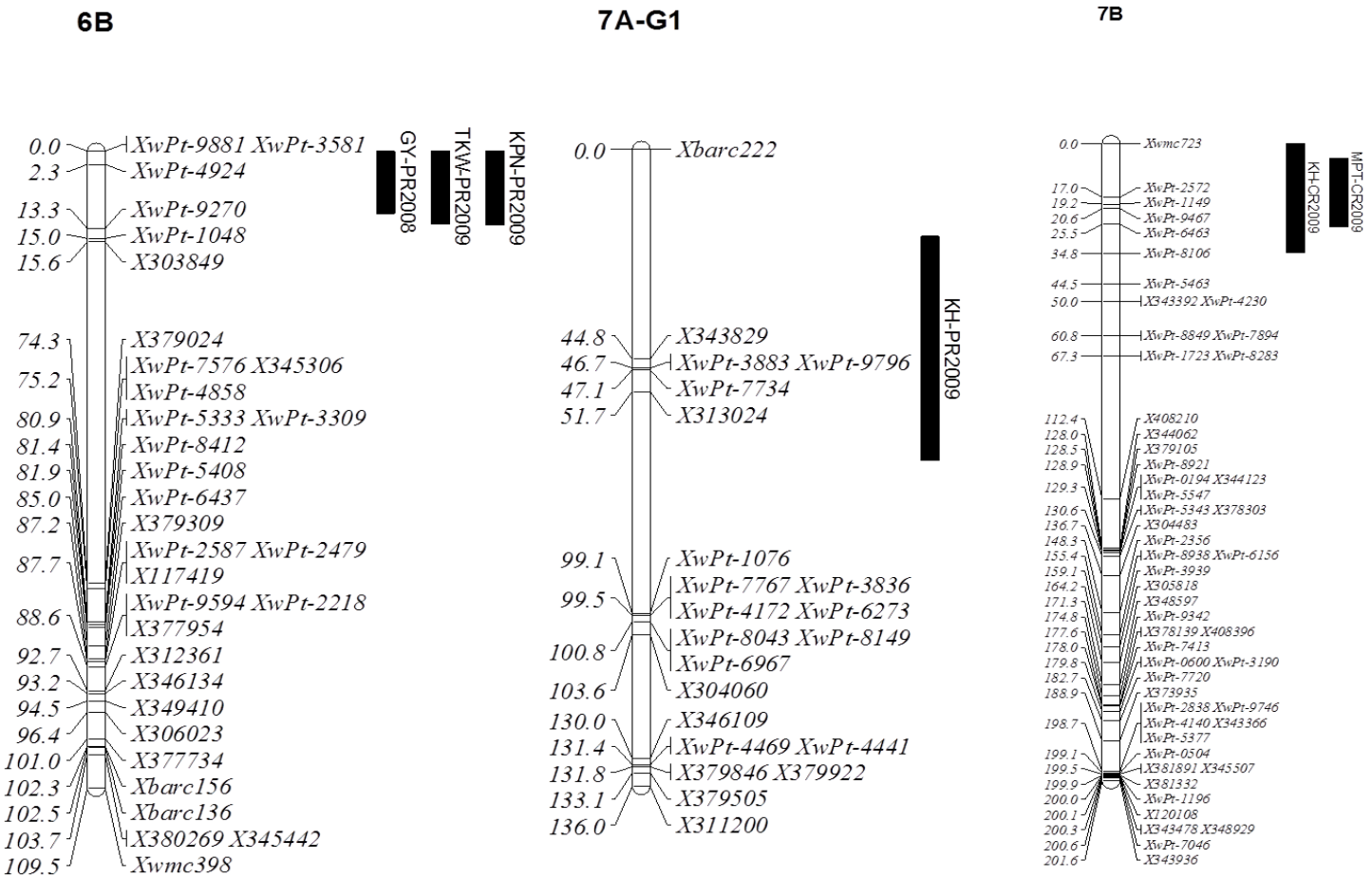


Fig 2.1. Locations of QTLs identified by the composite interval mapping for six traits evaluated in multiple environments in North Dakota using the RIL population derived from the Steele-ND/ND 735 cross. The length of the bar indicates one LOD interval. Abbreviations for traits are KD, kernel diameter; KH, kernel hardness; MPT, mixograph peak time; TKW, thousand-kernel weight; KPN, kernel spike^{-1}; and GY, grain yield. Abbreviations for environment are CR, Carrington, and PR, Prosper

(Continued)

Fig 2.1. Locations of QTLs identified by the composite interval mapping for six traits evaluated in multiple environments in North Dakota using the RIL population derived from the Steele-ND/ND 735 cross. The length of the bar indicates one LOD interval. Abbreviations for traits are KD, kernel diameter; KH, kernel hardness; MPT, mixograph peak time; TKW, thousand-kernel weight; KPN, kernel spike^{-1}; and GY, grain yield. Abbreviations for environment are CR, Carrington, and PR, Prosper (Continued)



The only QTL detected for KD was mapped to chromosome 3B, which was flanked by DArT markers *X343926* and *Xwpt 1081*, explained 23% of the phenotypic variation. This QTL was only mapped in the 2008 Carrington environment's data. The additive effect of this QTL contributed by Steele-ND alleles increased the kernel diameter. Two QTL were mapped for mixograph peak time on chromosomes 2B and 7B, respectively. The QTL on 7B, contributed by alleles from Steele-ND, explained 44% of the phenotypic variation in the Carrington 2009 environment. This QTL with an additive effect of 1.29 min increased the mixograph peak time in this environment.

Discussion

This study identified 13 QTLs for six traits and the detection of QTLs was highly inconsistent among environments. This inconsistency was attributed to the highly significant genotype by environment interaction for a majority of the GY, GY component, and quality traits. The majority of QTL identified in this study had a LOD value in the range of 2.5 to 4, with a significant effect of each QTL ranging from 16-30% for QTLs in KPN and KH identified on 6B and 3BG2, respectively. The reduced number of QTL identified in this study may be due to the incomplete linkage map and the low-level polymorphism found in this RIL population. Chalmers et al. (2001), Liu et al. (2005), Li et al. (2007), McCartney et al. (2006), and Paillard et al. (2003) utilized inter-varietal populations for developing genetic maps. When compared to our study, a higher D genome marker density and consistent QTL were reported in these studies. Molecular mapping using inter-varietal crosses makes the marker-trait association more relevant to the objectives of the breeding program (Somers et al., 2004; Varshney et al., 1998). Among the QTL found for GY on 5A and 6B, the alleles from ND 735 increased the GY compared

to the Steele-ND alleles. Similarly, the GY QTL in 5A was reported in the studies of Huang et al. (2006), Kato et al. (2000), and Marza et al. (2006). The GY QTL on 5A mapped in our study, which contributed 27% and 22% of the phenotypic variation.

In the Huang et al. (2006) and Marza et al. (2006) studies, the phenotypic variation explained by the 5A QTL ranged from 8.1-18.5%. However, the study of Kato et al. (2000) reported similar results as ours with the phenotypic variation explained by the 5A QTL up to 27%. The GY QTL on 6B was reported in studies of Huang et al. (2004) and Marza et al. (2006). Huang et al. (2004) used a BC_2F_1 population developed from a cross between the elite German cultivar Flair and the synthetic wheat line XX86 to map nine GY QTL among which the QTL on 6B explained 11.8% of the phenotypic variation. The studies of Marza et al. (2006) mapped consistent QTL on 6B for all three environments with the maximum phenotypic variation explained by the QTL up to 7.3%. However, the additive effect of the QTL was lesser (175 kg ha^{-1}) compared to our study.

The QTL for TKW and KPN coincides with the yield QTL on 5A and 6B, indicating the clustering of yield and yield-related QTL. The QTL cluster may be the result of the pleiotropic effect of a single gene or the presence of a linkage between two or more genes affecting the related traits. Chromosome 5A carries a number of important genes that influence the anthesis date, frost and drought tolerance (Sourdille et al., 2002; Toth et al., 2003), productivity, and adaptability (Huang et al., 2004; Kato et al., 2000). Similarly, chromosome 6B carries major genes for awn length (Sourdille et al., 2002), GPC (Khan et al., 2000), and pre-harvest sprouting tolerance (Roy et al., 1999). The clustering of QTL for important agronomic traits was observed in the studies of Borner et al. (2002). In their study of 20 agronomic traits in wheat, QTL for 11 traits were mapped in the same genomic

region. Clustering of QTL on 5A was observed for GY and GVW by Huang et al. (2006), GY, SL, shattering score, and lodging score (Marza et al., 2006).

Our study identified one QTL on 3B for KD. The QTL on 3B explained 23% of the phenotypic variation and was only mapped in one environment. The studies of Sun et al. (2010) reported six QTL mapped for KD using an RIL population derived from hard and soft wheat crosses. The identified QTLs were mapped on chromosomes 4AL, 5AL, 5AS, and 6AS, which were different from our QTL mapping study. The QTL found in our study was reported for the first time and is worth exploration in future studies.

KH is an important trait for wheat quality and affects the milling yield by increasing flour yield (Basset et al., 1989.). The major locus controlling hardness is the *Ha* locus on the 5DS chromosome arm. This locus contains two tightly linked puroindoline genes, *PinA* and *PinB*, which control the hardness and softness of kernels (Martin et al., 2001). Therefore, QTL mapping studies for KH (Nelson et al., 2006; Sun et al., 2010) reported QTL on 5DS. Our study reported five QTL for KH on chromosomes 5A, 7B, 1A, 7A, and 3B-G2. The QTL were different from the previously reported studies of Campbell et al. (1999) and Nelson et al. (2006). However, Sun et al. (2010) reported minor QTL affecting the KH apart from the major hardness locus on 5D. This may be attributed to the different genetic background of the population studied as well as the influence of environmental effects on QTL.

Two QTL for mixograph peak time were mapped to the B genome (2B and 7B). The QTL on 7B overlapped with the KH QTL. The studies of Campbell et al. (2001), McCartney et al. (2006), and Zanetti et al. (2001) mapped a cluster of QTLs related to grain quality near the *Glu-B1* locus. The current study could not map any QTLs near the

high-molecular glutenin locus *Glu-B1* on the B genome. Therefore, the QTL mapped in our study is worth exploring to increase the mixograph peak time and, thereby, to improve bread-making quality.

The present study utilized the DArT as well SSR markers for linkage map construction and QTL mapping. Despite the high level of polymorphism obtained for the A and B genome, DArT markers did not reveal sufficient polymorphism for the D genome. This may be attributed to the lack of diversity for the D genome between the two parents, Steele-ND and ND 735. In the future, additional SSR markers could be run on this population to develop the complete genetic map for this population. This will help to reveal more QTL on unmapped chromosomes.

Conclusion

The robust molecular markers can be cost efficient compared to phenotyping; however, the low diversity of the D genome makes mapping efforts difficult for inter-varietal mapping populations. The combination of DArT and SSR markers can result in a better linkage map. The inconsistency of QTL among environments makes MAS challenging for complex traits such as yield and quality. However, the information on QTL clusters helps the breeders understand the genetic correlations among traits. Additionally, the lines having desired allele can be selected and used for stacking of different traits. The agronomic QTL cluster found in this study certainly provides good information for the breeders and opens new opportunities for further genetic and mapping studies of the complex traits.

References

Akbari, M., P. Wenzl, V. Caig, J. Carling, L. Xia, S.Y. Yang, G. Uszynski, V. Mohler, A. Lehmensiek, H. Kuchel, M.J. Hayden, N. Howes, P. Sharp, P. Vaughan, B. Rathmell, E. Huttner, and A. Kilian. 2006. Diversity array technology (DArT) for high throughput profiling of the hexaploid wheat genome. Theor. Appl. Genet. 113:1409-1420.

American Association of Cereal Chemists. 2000. Approved methods of the AACC 10th Ed, Method 55-10, 46-30, 39-70A, 56-81B, 56-70. The Associations, St. Paul, MN.

Ayala, L., M. Henry, M. Van Ginkel, R. Singh, B. Keller, and M. Khairallah. 2002. Identification of QTL for BYDV tolerance in bread wheat. Euphytica 128:249-259.

Basset, L.M., R.E. Allan, and G.L. Rubenthaler. 1989. Genotype by environment interaction on soft white winter wheat quality. Agron. J. 81:955-960.

Blanco, A., C.D. Giovanni, B. Laddomada, A. Sciancalepore, R. Simeone, K.M. Devos, and M.D. Gale.1996. Quantitative trait loci influencing grain protein content in tetraploid wheats. Plant Breeding 115:310-316.

Borner, A., E. Schumann, A. Furste, H. Coster, B. Leithold, S. Roder, and E. Weber. 2002. Mapping of quantitative trait loci determining agronomic important characters in hexaploid wheat (*Triticum aestivum* L.). Theor. Appl. Genet. 105:921-936.

Bryan, G.J., A.J. Collins, P. Stephenson, A. Orry, J.B. Smith, and M.D. Gale. 1997. Isolation and characterization of microsatellites from hexaploid bread wheat. Theor. Appl. Genet. 94:557-563.

Cadalen, T., C. Boeuf, S. Bernard, and M. Bernard. 1997. An intervarietal molecular marker map in Triticum aestivum L. Em. Thell. and comparison with a map from a wide cross. Theor. Appl. Genet. 94:367-377.

Campbell, K. G., C.J. Bergman, D.G. Gualberto, J. A. Anderson, M. J. Giroux, G. Hareland, R. G. Fulcher, M. E. Sorrells, and P. L. Finney. 1999. Quantitative trait loci associated with kernel traits in a soft× hard wheat cross. Crop Sci. 39:1184-1195.

Campbell, K.G., P.L. Finney, C.J. Bergman, D.G. Gualberto, J.A. Anderson, M.J. Giroux, D. Siritunga, J. Zhu, F. Gendre, C. Roue, A. Verel, and M.E. Sorrells. 2001. Quantitative trait loci associated with milling and baking quality in soft/hard red wheat cross. Crop Sci.41:1275-1285.

Chalmers, K.J., A.W. Cambell, J. Kretschmer, A. Karakousis, H. Henschke, S. Pierens, N. Harker, M. Pallotta, G.B. Cornish, M.R. Shariflou, L.R. Rampling, A. McLauchalan, G. Daggard, P.J. Sharp, T.A. Holten, M.W. Sunderland, R. Appels, and P. Langridge.

2001. Construction of three linkage maps in bread wheat, *Triticum aestivum*. Aust. J. Agric .Res. 52:1089-1119.

Chao, S., P.J. Sharp, A.J. Worland, E.J. Warham, R.M.D. Koebner, and M.D. Gale. 1989. RFLP-based genetic maps of wheat homoeologous group 7 chromosomes. Theor. Appl. Genet.78:495-504.

Chu, C.G., S. Xu, T.L. Friesen, and J.D. Faris. 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.

Dholakia, B.B., J.S.S. Ammiraju, H. Singh, M.D. Lagu, M.S. Roder, V.S. Rao, H.S. Dhaliwal, P.K. Ranjekar, and V.S. Gupta. 2003. Molecular marker analysis of kernel size and shape in bread wheat. Plant Breeding 122:392-395.

Finney, K.F. 1945. Methods of estimating and effects of variety and protein content level on the baking quality of flour. Cereal Chem. 22:149-160.

Groos, C., N. Robert, E. Bervas, and G. Charmet. 2003. Genetic analysis of grain-protein content, grain yield and thousand kernel weight in bread wheat. Theor. Appl. Genet. 106:1032-1040.

Guidet, F., R. Rogowsky, C. Taylor, W. Song, and P. Langridge. 1991. Cloning and characterization of a new rye-specific repeat sequence. Genome 34: 81-87.

Hearnden, P.R., P.J. Eckermann, G.L. McMichael, M.J. Hayden, J.K. Elington, and K.J. Chalmers. 2007. A genetic map of 1,000 SSR and DArT markers in a wide barley cross. Theor. Appl. Genet. 115:383-391.

Huang, X.Q., S. Cloutier, L. Lycar, N. Radovanovic, D.G. Humphreys, J.S. Noll, D.J. Somers, and P.D. Brown. 2006. Molecular detection of QTLs for agronomic and quality traits in a double haploid population derived from two Canadian wheats (*Triticum aestivum*). Theor. Appl. Genet. 113:753-766.

Huang, X.Q., H. Kempf, M.W. Ganal, and M.S. Röder. 2004. Advanced backcross QTL analysis in progenies derived from a cross between a German elite winter wheat variety and synthetic wheat (*Triticum aestivum L.*). Theor. Appl. Genet. 109: 933-943.

Jaccoud, D., K. Peng, D. Feintein, and A. Kilian. 2001. Diversity arrays: a solid-state technology for sequence information independent genotyping. Nucleic Acids Res. 29:425.

Joppa, L.R., C. Du, G.E. Hart, and G.A. Hareland. 1997. Mapping genes for grain protein in tetraploid wheat (*Triticum turgidium L.*) using a population of recombinant inbred chromosome line. Crop Sci. 37:1586-1589.

Kato, K., H. Miura, and S. Sawada. 2000. Mapping QTLs controlling grain yield and its components on chromosome 5A of wheat. Theor Appl Genet. 101:1114–1121.

Khan, I.A., J.D. Procunier, D.G. Humphreys, G. Tranquilli, A.R. Schlatter, S. Marcucci-Poltri, R. Frohberg, and J. Dubcovsky. 2000. Development of PCR-based markers for a high grain protein content gene from Triticum turgidum ssp. dicoccoides transferred to bread wheat. Crop Sci. 40:518-524.

Kosambi, D.D. 1994 The estimation of map distances from recombination values. Ann. Eugen. 12:172-175.

Kulwal, P.L., J.K. Roy, H.S. Balyan, and P.K. Gupta. 2003. QTL mapping for growth and ear characters in bread wheat. Plant Sci. 164:267-277.

Kumar, N., P.L. Kulwal, H.S. Balyan, and P.K. Gupta. 2007. QTL mapping for yield and yield contributing traits in two mapping populations of bread wheat. Mol. Breed. 19:163-177.

Lander, E.S., P. Green, J. Abrahamson, A. Barlow, M.J. Daly, S.E. Lincoln, and L. Newburg. 1987. Mapmaker: an interactive computer package for constructing primary linkage map for experimental and natural populations. Genomics.1:174-181.

Law, C.N. 1965. Biometrical analysis using chromosome substitutions within a species. In: Riley R, Lewis KR (Eds) Chromosome manipulations in plant genetics. Suppl Heredity 20:59–85.

Law, C.N. 1966. The location of genetic factors controlling a quantitative character in wheat. Genetics 53:487-498.

Li, S., J. Jia, X. Wei, X. Zhang, L. Li, H. Chen, Y. Fan, H. Sun, X. Zhao, T. Lei, Y. Xu, F. Jiang, H. Wang, and L. Li 2007. An intervarietal genetic map and QTL analysis for yield traits in wheat. Mol. Breed. 20:167-178.

Liu, Z.H., J.A. Anderson, J. Hu, T.L. Friesen, J.B. Rasmussen, and J.D. Faris. 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.

Ma, Z.Q., M.S. Röder, and M.E. Sorrells. 1996. Frequencies and sequence characteristics of di-, tri-, and tetra-nucleotide microsatellites in wheat. Genome 39:123-130.

Mace, E., L. Xia, D. Jordan, K. Halloran, D. Parh, E. Hutner, P. Wenzl, and A. Kilian. 2008. DArT markers: diversity analyses and mapping in *Sorghum bicolor*. BMC Genomics 9:26.

Marino, C.L., J.C. Nelson, Y.H. Liu, M.E. Sorrells, P. Leroy, N.A. Tuleen, C.R. Lopes, and G.E. Hart. 1996. RFLP-based linkage maps of the homoeologous group 6 chromosomes of wheat (*Triticum aestivum* L.em. Thell). Genome 39:359-366.

Martin, J.M., R.C. Frohberg, C.F. Morris, L.E. Talbert, and M.J. Giroux. 2001. Milling and bread baking traits associated with puroindoline sequence type in hard red spring wheat. Crop Sci. 41:228-234.

Marza, F., G.H. Bai, B. F. Carver, and W.C. Zhou. 2006. Quantitative trait loci for yield and related traits in the wheat population Ning7840× Clark. Theor. Appl. Genet 112: 688-698.

McCartney, C.A., D.J. Somers, D.G. Humphreys, O. Lukow, N. Ames, J. Noll, S. Clouther, and B.D. McCallum. 2005. Mapping quantitative trait loci in the spring wheat cross RL4452 × 'AC Domain'. Genome 48:870-883.

McCartney, C.A., D.J. Somers, O. Lukow, N. Ames, J. Noll, S. Cloutier, D.G. Humphreys, and B.D. McCallum. 2006. QTL analysis of quality traits in the spring wheat cross RL4452/AC Domain. Plant Breeding 125:565-575.

Mergoum, M., R.C. Frohberg, J.D. Miller, and R.W. Stack. 2005. Registration of 'Steele-ND' wheat. Crop Sci. 45:1163-1164.

Mergoum, M., R.C. Frohberg, P.K. Singh, S. Ali, J.B. Rasmussen, and J.D. Miller. 2006. Registration of spring wheat germplasm ND 735. Crop. Sci. 46:1003-1004.

Mergoum, M., R. C. Frohberg, R.W. Stack, J.W. Rasmussen, and T.L. Friesen. 2008 Registration of 'Faller' spring wheat. J. Plant Reg.3:224-229.

Mergoum, M., P.K. Singh, S. Ali, E.M. Elias, J.A. Anderson, K.D. Glover, and T.B. Adhikari. 2007. Evaluation of spring wheat germplasm for septoria diseases. Plant Dis.. 91:1310-1315.

Mergoum, M., P.K. Singh, R.C. Frohberg, S.F. Kianian, F. Ghavami, K. Hussain, T.B. Adhikari, V.E. Harilal, and S. Simsek. 2009. Registration of Steele-ND/ND 735 wheat recombinant inbred lines mapping population. J. Plant Reg. 3:300-306.

Mesfin, A., R.C. Frohberg, and J.A. Anderson. 1999. RFLP markers associated with high protein from *Triticum turgidum* L. var. *dicoccoides* introgressed into hard red spring wheat. Crop Sci. 39:508-513.

Nelson, J.C., C. Andreescu, F. Breseghello, P.L. Finney, D.G. Gualberto, C.J. Bergman, R.J. Pena, M.R. Perretant, P. Leroy, C.O. Qualset, and M.E. Sorrells. 2006. Quantitative trait locus analysis of wheat quality traits. Euphytica 149:145-159.

Nelson, J.C., M.E. Sorrells, A.E. Van-Deynze, Y.H. Lu, M. Atkinson, M. Bernard, P. Leroy, J.D. Faris, and J.A. Anderson. 1995a. Molecular mapping of wheat: major genes and rearrangements in homoeologous group 4, 5 and 7. Genetics 141:721-731.

Nelson, J.C., A.E. Van-Deynze, E. Autrique, M.E. Sorrells, Y.H. Lu, S. Negre, M. Bernard, and P. Leroy. 1995b. Molecular mapping of wheat. Homoeologous group 2. Genome 38:516-524.

Nelson, J.C., A.E. Van-Deynze, E. Autrique, M.E. Sorrells, Y.H. Lu, S. Negre, M. Bernard, and P. Leroy. 1995c. Molecular mapping of wheat. Homoeologous group 3. Genome 38:525-533.

Paillard, S., T. Schnurbusch, M. Weinzeler, M. Messmer, P. Sourdille, O. Abderhalden, B. Keller, and G. Schachermayr. 2003. An integrative genetic linkage map of winter wheat (Triticum *aestivum* L.). Theor. Appl. Genet. 107:1235-1242.

Parker, G. D., K. J. Chalmers, A. J. Rathjen, and P. Langridge. 1998. Mapping loci associated with flour color in wheat (Triticum aestivum L Theor. Appl. Genet. 97:238-245.

Parker, G.D., K.J. Chalmers, A.J. Rathjen, and P. Langridge. 1999. Mapping loci associated with milling yield in wheat (*Triticum aestivum* L.). Mol. Breed. 5:561-568.

Peng, J.H., Y. Ronin, T. Fahima, M.S. Roder, Y.C. Lin, E. Nevo, and A. Korol. 2003. Domestication quantitative trait loci in Triticum dicoccoides, progenitor of wheat. Proc. Natl. Acad. Sci. 100:2489-2494.

Plaschke, J., M. Ganal, and M.S. Roder. 1995. Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theor. Appl. Genet. 91:1001-1007.

Prasad, M., N. Kumar, P.L. Kulwal, M.S. Roder, H.S. Balyan, H.S. Dhaliwal, and P.K. Gupta. 2003. QTL analysis for grain protein content using SSR markers and validation using NILs in bread wheat. Theor. Appl. Genet. 106:659-667.

Prasad, M., R.K. Varshney, A. Kumar, H.S. Balyan, P.C. Sharma, K.J. Edwards, H. Singh, H.S. Dhaliwal, J.K. Roy, and P.K. Gupta. 1999. A microsatellite marker associated with a QTL for grain protein content on chromosome arm 2DL of bread wheat. Theor. Appl. Genet. 99:341-345.

Quarrie, S.A., A. Steed, C. Calestani, A. Semikhodskii, C. Lebreton, C. Chinoy, N. Steele, D. Pljevljakusic, E. Waterman, J. Weyen, J. Schondelmaier, D.Z. Habash, P. Farmer, L. Saker, D.T. Clarkson, A. Abugalieva, M. Yessimbekova, Y. Turuspekov, S. Abugalieva, R. Tuberosa, M.C. Sanguineti, P.A. Hollington, R. Aragues, A. Royo, and D. Dodig. 2005. A high-density genetic map of hexaploid wheat (*Triticum aestivum* L.) from the cross Chinese Spring × SQ1 and its use to compare QTLs for grain yield across a range of environments. Theor. Appl. Genet. 110:865-880.

Roder, M.S., J. Plaschke, S.U. König, A. Börner, M.E. Sorrells, and M.W. Ganal. 1995. Abundance, variability and chromosomal location of microsatellites in wheat. Mol. Gen. Genet. 246:327-333.

Roder, M.S., V. Korzun, K. Wendehake, J. Plaschke, M.H. Tixer, P. Leroy, and M.W. Ganal. 1998. A microsatellite map of wheat. Genetics 149:2007-2023.

Roy, J. K., M. Prasad, R. K. Varshney, H. S. Balyan, T. K. Blake, H. S. Dhaliwal, K. J. Edwards, and P. K. Gupta. 1999. Identification of a microsatellite on chromosomes 6B and a STS on 7D of bread wheat showing an association with preharvest sprouting tolerance. Theor. Appl. Genet. 99:336-340.

SAS Institute. 1999. SAS/STAT User's guide, release 8.2, 8.1, and 8.0. SAS Institute, Cary, NY.

Sax, K. 1923. Association of size differences with seed-coat pattern and pigmentation in Phaseolus vulgaris. Genetics 8: 552-560.

Semagn, K., A. Bjorstad, H. Skinnes, A.G. Maroy, Y. Tarkegne, and M. William. 2006. Distribution of DArT, AFLP, and SSR markers in a genetic linkage map of a double haploid hexaploid wheat population. Genome 49:545-555.

Singh, P.K., M. Mergoum, S. Ali, T.B. Adhikari, E.M. Elias, and G.R. Hughes, 2006. Identification of new sources of resistance to tan spot, stagnospora nodorum blotch, and septoria tritici blotch of wheat. Crop Sci. 46:2047-2053.

Singh, P.K., M. Mergoum, T.B. Adhikari, F. Gavami, and S.F. Kianian. 2011. Genetics and mapping of resistance to spore inoculum and culture filtrate of *Phaeosphaeria nodorum* in spring wheat line ND 735. Crop Protection 30:141-146.

Somers, D.G., P. Isaac, and K. Edwards. 2004. A high-density microsatellite consensus map of bread wheat (*Triticum aestivum* L.). Theor. Appl. Genet. 109:1105-1114.

Song, Q.J., J.R. Shi, S. Singh, E.W. Fickus, J.M. Costa, J. Lewis, B.S. Bill, R. Ward, and P.B. Cregan. 2005. Development and mapping of microsatellite (SSR) markers in wheat. Theor. Appl. Genet. 110:550-560.

Sourdille, P., T. Cadalen, G. Gay, B. Gill, and M. Bernard. 2002. Molecular and physical mapping of genes affecting awning in wheat. Plant Breed. 121:320-324.

Sourdille, P., T. Cadalen, H. Guyomarch, J. Snape, M. Perretant, G. Charmet, C. Boeuf, S. Bernard, and M. Bernard. 2003. An update of the Courtot × Chinese Spring intervarietal molecular marker linkage map for the QTL detection of agronomic traits in wheat. Theor. Appl. Genet. 106:530-538.

Suenaga, K., M. Khairallah, H.M. William, and D.A. Hoisington. 2005. A new intervarietal linkage map and its application for quantitative trait locus analysis of ‘‘gigas’’ features in bread wheat. Genome 48:65-75.

Sun, X., F. Marza, H. Ma, B.F. Carver, and G. Bai. 2010. Mapping quantitative trait loci for quality factors in an inter-class cross of US and Chinese wheat. Theor. Appl. Genet. 120:1041-1051.

Toth, B., G. Galiba, E. Feher, J. Sutka, and J.W. Snape. 2003. Mapping genes affecting flowering time and frost resistance on chromosome 5B of wheat. Theor. Appl. Genet. 107:509-514.

Van Deynze, A.E., J. Dubcovsky, K.S. Gill, J.C. Nelson, M.E. Sorrells, J. Dvorak, B.S. Gill, E.S. Lagudah, S.R. McCouch, and R. Appels. 1995. Molecular genetic maps for chromosome 1 in Triticeae species and their relation to chromosome in rice and oats. Genome 38:47-59.

Varshney, R.K., P.C. Sharma, P.K. Gupta, H.S. Balyan, B. Ramesh, J.K. Roy, A. Kumar, and A. Sen. 1998. Low level of polymorphism detected by SSR probes in bread wheat. Plant Breeding 117:182-184.

Wang, S., C.J. Basten, and B. Zeng. 2004. Windows QTL cartographer 2.0. Department of Statistics, North Carolina State University, Raleigh.

Wenzl, P., J. Carling, D. Kudrna, D. Jaccoud, E. Huttner, A. Kleinhofs, and A. Killian 2004. Diversity arrays technology (DArT) for whole-genome profiling of barley. Proc. Natl. Acad.

Zanetti, S., M. Winzeler, C. Feuillet, B. Keller, and M. Messmer. 2001. Genetic analysis of bread making quality in wheat and spelt. Plant Breeding 120:13-19.

Zhang, L.Y., D.C. Liu, X.L. Guo, W.L. Yang, J.Z. Sun, D. Wang, and A. Zhang. 2008. Distribution in genome of quantitative trait loci (QTL) for yield and yield-related traits in common wheat (*Triticum aestivum* L.). Available at http://www.jipb.net/file/12916137426593-500.pdf. (Verified 05 Jan. 2011).

Zhao, L. K.P. Zhang, B. Liu, Z.Y. Deng, H.L. Qu, and J.C. Tian. 2010. A comparison of grain protein content QTLs and flour protein content QTLs across environments in cultivated wheat. Euphytica 174:325-335.

APPENDIX A

Table A.1. Combined ANOVA results for septoria tritici blotch resistance for Steele-ND/ND 735 RIL population in three greenhouse experiments.

Source of variation	DF	SS	MSS	F-value
Environment	2	30905.5	15452.7	
Rep (Environment)	3	7319.6	2439.9	
Genotypes	142	227988.6	1605.6	4.3***
Genotypes × Environment	284	148510.8	522.9	1.4***
Error	426	160501.8	376.8	
Total	857	575226.2		

*** Significant at 0.001.

APPENDIX B

Table B.1. Combined ANOVA for days to heading for the Steele-ND/ND 735 RIL population across four environments in North Dakota during 2008-2009

Source of variation	DF	SS	MSS	F-value
Environment	3	70916.0	23638.7	
Rep (Environment)	4	147.2	36.8	
Genotypes	134	1956.6	14.6	1.5***
Genotypes × Environment	402	4028.4	10.0	1.0
Error	536	5071.3	9.5	
Total	1079	82119.5		

*** Significant at 0.001.

Table B.2. Combined ANOVA result for plant height for the Steele-ND/ND 735 RIL population across four environments in North Dakota during 2008-2009.

Source of variation	DF	SS	MSS	F-value
Environment	3	42215.6	14071.9	
Rep (Environment)	4	3224.6	806.2	
Genotypes	134	13519.5	100.9	4.0***
Genotypes × Environment	402	10027.9	24.9	1.2*
Error	536	11536.7	21.5	
Total	1079	80524.2		

* and *** Significant at 0.05 and 0.001,respectively

Table B.3. Combined ANOVA result of yield for the Steele-ND/ND 735 RIL population across four environments in North Dakota during 2008-2009.

Source of variation	DF	SS	MSS	F-value
Environment	3	265002704.3	88334234.8	
Rep (Environment)	4	47691934.6	11922983.7	
Genotypes	134	139024849.5	1037498.9	2.7***
Genotypes × Environment	402	152222294.9	378662.4	1.5***
Error	531	134340715.2	252995.7	
Total	1074	738282498.5		

*** Significant at 0.001.

Table B.4. Combined ANOVA result for spike m^{-2} for the Steele-ND/ND 735 RIL population across four environments in North Dakota during 2008-2009.

Source of variation	DF	SS	MSS	F-value
Environment	3	5517263.5	1839087.9	
Rep (Environment)	4	117176.7	29294.2	
Genotypes	134	554781.5	4140.2	1.8***
Genotypes × Environment	402	935708.2	2327.6	0.9
Error	536	1411164.4	2632.8	
Total	1079	8536094.3		

*** Significant at 0.001.

Table B.5. Combined ANOVA result for spike length for the Steele-ND/ND 735 RIL population across four environments in North Dakota during 2008-2009.

Source of variation	DF	SS	MSS	F-value
Environment	3	336.6	112.2	
Rep (Environment)	4	4.0	1.0	
Genotypes	134	115.3	0.9	6.6***
Genotypes × Environment	402	52.3	0.1	1.5***
Error	536	48.2	0.1	
Total	1079	556.4		

*** Significant at 0.001.

Table B.6. Combined ANOVA results for leaf score for the Steele-ND/ND 735 RIL population across for environments in North Dakota during 2008-2009.

Source of variation	DF	SS	MSS	F-value
Environment	3	25.1	8.4	
Rep (Environment)	4	3.5	0.9	
Genotypes	134	123.8	0.9	2.3***
Genotypes × Environment	402	166.8	0.4	2.0***
Error	536	110.6	0.2	
Total	1079	429.9		

*** Significant at 0.001.

Table B.7. Combined ANOVA results for kernel number spike^{-1} for the Steele-ND/ND-735 RIL population across four environments in North Dakota 2008-2009.

Source of variation	DF	SS	MSS	F-value
Environment	3	7314.7	2438.2	
Rep (Environment)	4	464.8	116.2	
Genotypes	134	2199.8	16.42	1.5***
Genotypes × Environment	402	4376.8	10.9	1.5***
Error	536	3871.4	7.2	
Total	1079	18227.5		

*** Significant at 0.001.

Table B.8. Combined ANOVA result for grain volume weight for the Steele-ND/ND 735 RIL population across four environments in North Dakota during 2008-2009.

Source of variation	DF	SS	MSS	F-value
Environment	3	189265.7	63088.6	
Rep (Environment)	4	7130.6	1782.7	
Genotypes	134	172529.4	1287.5	1.6***
Genotypes × Environment	402	317633.6	790.1	1.2*
Error	536	361251.2	674.0	
Total	1079	1047810.5		

* and *** Significant at 0.05 and 0.001, respectively.

Table B.9. Combined ANOVA results for thousand-kernel weight for the Steele-ND/ND 735 RIL population across four environments in North Dakota during 2008-2009.

Source of variation	DF	SS	MSS	F-value
Environment	3	4856.4	1618.80	
Rep (Environment)	4	259.9	64.98	
Genotypes	134	2525.9	18.85	5.8***
Genotypes × Environment	402	1308.0	3.25	1.5***
Error	536	1155.9	2.16	
Total	1079	10106.2		

*** Significant at 0.001.

Table B.10. Combined ANOVA for grain protein content for the Steele-ND/ND 735 RIL population across four environments in North Dakota during 2008-2009.

Source of variation	DF	SS	MSS	F-value
Environment	3	97.6	32.5	
Rep (Environment)	4	5.9	1.5	
Genotypes	134	179.6	1.3	5.1***
Genotypes × Environment	402	106.4	0.3	2.1***
Error	536	66.2	0.1	
Total	1079	455.7		

*** Significant at 0.001.

Table B.11. Combined ANOVA results for kernel hardness for the Steele-ND/ND 735 RIL population across four environments in North Dakota during 2008-2009.

Source of variation	DF	SS	MSS	F-value
Environment	3	7562.7	2520.9	
Rep (Environment)	4	1395.0	348.8	
Genotypes	134	18502.5	138.1	9.1***
Genotypes × Environment	402	6100.5	15.2	1.1
Error	536	7545.5	14.1	
Total	1079	41106.2		

*** Significant at 0.001.

Table B.12. Combined ANOVA results for kernel diameter for the Steele-ND/ND 735 RIL population across four environments in North Dakota during 2008-2009.

Source of variation	DF	SS	MSS	F-value
Environment	3	8.7	2.9	
Rep (Environment)	4	0.3	0.1	
Genotypes	134	3.1	0.0	5.0***
Genotypes × Environment	402	1.9	0.0	1.3**
Error	536	1.9	0.0	
Total	1079	15.9		

** and *** Significant at 0.01 and 0.001, respectively.

Table B.13. Combined ANOVA results for large size kernel for the Steele-ND/ND 735 RIL population across four environments in North Dakota during 2008-2009.

Source of variation	DF	SS	MSS	F-value
Environment	3	20018.9	6673.0	
Rep (Environment)	4	7083.7	1770.9	
Genotypes	134	58468.4	436.3	5.4***
Genotypes × Environment	402	32744.7	81.5	1.7***
Error	536	25879.7	48.3	
Total	1079	144195.4		

*** Significant at 0.001.

Table B.14. Combined ANOVA results for medium-size kernels for the Steele-ND/ND 735 RIL population across four environments in North Dakota during 2008-2009.

Source of variation	DF	SS	MSS	F-value
Environment	3	14780.1	4926.7	
Rep (Environment)	4	5230.6	1307.6	
Genotypes	134	50836.9	379.4	5.2***
Genotypes × Environment	402	29293.7	72.9	1.4**
Error	536	28295.4	52.8	
Total	1079	128436.8		

** and *** Significant at 0.01 and 0.001, respectively.

Table B.15. Combined ANOVA results for flour extraction for the Steele-ND/ND 735 RIL population across four environments in North Dakota during 2008-2009.

Source of variation	DF	SS	MSS	F-value
Environment	3	3949.9	1316.6	
Rep (Environment)	4	1825.2	456.3	
Genotypes	134	6440.8	48.1	2.4***
Genotypes × Environment	402	8119.5	20.2	1.0
Error	536	10920.3	20.4	
Total	1079	31255.6		

*** Significant at 0.001.

Table B.16. Combined ANOVA results for mixograph peak time for the Steele-ND/ ND735 RIL population across four environments in North Dakota during 2008-2009.

Source of variation	DF	SS	MSS	F-value
Environment	3	718.1	239.4	
Rep (Environment)	4	19.8	5.0	
Genotypes	134	1270.3	9.5	6.8***
Genotypes × Environment	402	562.9	1.4	1.5***
Error	536	487.8	0.9	
Total	1079	3058.8		

*** Significant at 0.001.

Table B.17. ANOVA results for days to heading for the Steele-ND/ND 735 RIL population in Casselton, ND, during 2009.

Source of variation	DF	SS	MSS	F-value
Replication	1	15.8	15.8	
Genotypes	129	140.6	1.1	2.4***
Error	129	50.4	0.5	
Total	259	206.8		

*** Significant at 0.001.

Table B.18. ANOVA results for plant height for the Steele-ND/ND 735 RIL population in Casselton, ND, during 2009.

Source of variation	DF	SS	MSS	F-value
Replication	1	77.8	77.8	
Genotypes	129	3534.6	27.4	4***
Error	129	883.7	6.8	
Total	259	4379.6		

*** Significant at 0.001.

Table B.19. ANOVA results for grain yield for the Steele-ND/ND 735 RIL population in Casselton, ND, during 2009.

Source of variation	DF	SS	MSS	F-value
Replication	1	1065491.6	1065491.6	
Genotypes	129	58486765.6	453385.8	2.5***
Error	112	20519849.3	183212.9	
Total	242			

*** Significant at 0.001.

Table B.20. ANOVA results for grain volume weight for the Steele-ND/ND 735 RIL population in Casselton, ND, during 2009.

Source of variation	DF	SS	MSS	F-value
Replication	1	223.2	223.2	
Genotypes	129	26060.6	202.0	6.0***
Error	129	4346.0	33.7	
Total	259	30629.8		

*** Significant at 0.001.

Table B.21. ANOVA results for spike m^{-2} for the Steele-ND/ND 735 RIL population in Casselton, ND, during 2009.

Source of variation	DF	SS	MSS	F-value
Replication	1	58836.0	58836.0	
Genotypes	129	859042.0	6659.2	1.4*
Error	129	624623.2	4842.0	
Total	259	1542501.1		

* Significant at 0.05.

Table B.22. ANOVA results for spike length for the Steele-ND/ND 735 RIL population in Casselton, ND, during 2009.

Source of variation	DF	SS	MSS	F-value
Replication	1	0.1	0.1	
Genotypes	129	32.3	0.3	1.2
Error	129	27.1	0.2	
Total	259	59.4		

Table B.23. ANOVA results for leaf score for the Steele-ND/ND 735 RIL population in Casselton, ND, during 2009.

Source of variation	DF	SS	MSS	F-value
Replication	1	38.4	38.5	
Genotypes	129	50.3	0.4	1.7*
Error	129	29.7	0.2	
Total	259	118.4		

* Significant at 0.05.

Table B.24. ANOVA results for kernel number spike^{-1}for the Steele-ND/ND 735 RIL population in Casselton, ND, during 2009.

Source of variation	DF	SS	MSS	F-value
Replication	1	395.6	395.6	
Genotypes	129	2296.2	17.8	1.4*
Error	129	1673.1	13.0	
Total	259	4364.9		

* Significant at 0.05.

Table B.25. ANOVA results for thousand-kernel weight for the Steele-ND/ND 735 RIL population in Casselton, ND, during 2009.

Source of variation	DF	SS	MSS	F-value
Replication	1	6.7	6.7	
Genotypes	129	45.2	0.4	0.4^{ns}
Error	129	118.7	0.9	
Total	259	170.6		

Ns, Non-significant.

Table B.26. ANOVA results for grain protein content for the Steele-ND/ND 735 RIL population in Casselton, ND, during 2009.

Source of variation	DF	SS	MSS	F-value
Replication	1	11.1	11.1	
Genotypes	129	63.2	0.5	2.0***
Error	129	32.3	0.3	
Total	259	106.6		

*** Significant at 0.001.

Table B.27. ANOVA results for large kernel size for the Steele-ND/ND 735 RIL population in Casselton, ND, during 2009.

Source of variation	DF	SS	MSS	F-value
Replication	1	10.0	10.0	
Genotypes	129	11691.3	90.6	4.0***
Error	129	2887.0	22.4	
Total	259	14588.3		

*** Significant at 0.001.

Table B.28. ANOVA results for medium kernel size for the Steele-ND/ND 735 RIL population in Casselton, ND, during 2009.

Source of variation	DF	SS	MSS	F-value
Replication	1	15.4	15.4	
Genotypes	129	10593.5	82.1	4.6***
Error	129	2310.4	17.9	
Total	259	12919.3		

*** Significant at 0.001.

Table B.29. ANOVA results for flour extraction for the Steele-ND/ND 735 RIL population in Casselton, ND, during 2009.

Source of variation	DF	SS	MSS	F-value
Replication	1	480.9	480.9	
Genotypes	129	1929.8	15.0	1.5*
Error	129	1305.5	10.1	
Total	259	3716.2		

* Significant at 0.05.