Molecular Mapping of Quantitative Trait Loci Controlling Yield and Yield Components in Spring Wheat (*Triticum aestivum* L.)

BY

JANICE LOUISE CUTHBERT

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
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University of Manitoba
Winnipeg, Manitoba

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ABSTRACT

Cuthbert, Janice Louise, PhD. The University of Manitoba, 2008. Molecular mapping of quantitative trait loci (QTL) controlling yield and yield components in a spring wheat cross. Major Professors: Dr. D.J. Somers, Cereal Research Centre, Agriculture and Agri-Food Canada; Dr. A. L. Brûlé-Babel, Department of Plant Science, University of Manitoba.

Identifying the location of genes controlling economically important traits such as grain yield in spring wheat (Triticum aestivum L.) is very important to plant breeders. An F_1 derived doubled haploid population from the spring wheat cross Superb (high yielding) / BW278 (low yielding) was developed to identify quantitative trait loci (QTL) associated with yield and yield components. A genetic map was constructed with 268 microsatellite marker loci and composite interval mapping was conducted to estimate the location and effect of QTL associated with the evaluated traits. A total of 53 QTL were identified on 12 chromosomes for the nine traits with the coefficient of determination ranging from 0.03 to 0.21 of the total phenotypic variation. The increase in yield and yield components ranged from 4.5 to 17.1% over the population mean. The five grain yield QTL were detected on chromosomes 1A, 2D, 3B, and 5A and showed a combined increase of 34.4% over the population mean. This study identified potential chromosome segments for use in marker-assisted selection to improve yield in spring wheat.

The validation of identified QTL is essential prior to implementing a marker assisted selection strategy for cultivar improvement. The objective of this study was to verify the usefulness of the identified grain yield QTL in an independent breeding population where Superb was a parent. A population of 83 F_3 derived F_6 individuals from the spring wheat cross Superb/RL4831 was evaluated for grain yield in western

Canada. Chromosome regions previously associated with grain yield were mapped with four to eight microsatellite markers each and single marker analysis was used to determine if any of these markers had significant ($P \leq 0.05$) associations with grain yield. Interval mapping was then used to estimate the effects and location of the yield QTL. Four of the five previously described QTL were validated in this new genetic cross and explained 0.07 to 0.20 of the phenotypic variation in grain yield. The fifth QTL on chromosome 3B was not validated. This study validated four grain yield QTL in spring wheat and showed their potential usefulness in identifying high yielding breeding lines using marker-assisted selection.

Foreword

This thesis follows the manuscript style outlined by the Department of Plant Science, University of Manitoba. The manuscripts follow the style recommended by Theoretical and Applied Genetics. The thesis is presented as three manuscripts, each containing an abstract, introduction, materials and methods, results, and discussion sections. A literature review precedes the manuscripts and a general discussion follows the manuscripts.

1.0 Introduction

Wheat (*Triticum aestivum* L.) is an important crop grown world wide for food, feed and fuel. Since its introduction in 1812 to western Canada, the acreage of wheat has steadily increased making wheat one of the top two crops produced on the Prairies (DePauw and Hunt 2001). Currently, annual production in western Canada has been estimated to be 28 million tonnes with a value over two billion dollars (Statistics Canada 2007). The average yield per hectare was calculated to be 2400 kg or 35.6 bushels per acre.

Grain yield, defined as the mass or weight of product produced per unit land area, has been an important focus of wheat breeding problems. Yield is a complex, quantitative trait controlled by several genes and is significantly influenced by the environment (Falconer and MacKay 1981). Heritability of yield is considered to be low. Grain yield in wheat is the product of three main yield components: spike number area^{-1}, kernel number spike^{-1}, and kernel weight (Woodworth 1931). Since wheat breeders have little information on the number, location, and contribution of each gene to the final expression of the trait, it has been difficult to make genetic improvements in grain yield (Koebner and Snape 1999; Mohan et al. 1997). From 1882 to 1985, Hucl and Baker (1987) determined the genetic gain for Canadian cultivars was 0.5% per year.

Genetic advance in wheat breeding has been largely dependent on the variation created by intraspecific hybridization. Breeding material is usually screened in large-scale breeding programs where desirable traits are identified and selection is based on phenotype. Early generation selection has generally not been successful for quantitative traits (Shebeski 1967; Knott 1972; DePauw and Shebeski 1973). Selection for grain yield

could be more efficient at the genotypic level without the interference of the other interactions such as the environment, however, it is necessary to identify and understand the genes controlling the trait.

The principles of quantitative trait loci (QTL) analysis were developed more than 75 years ago when Sax (1923) reported the first linkage of a trait, seed weight in beans (*Phaseolus vulgaris*), to a major gene for seed pigmentation. Wide scale application of QTL analysis was not possible at the time due to the lack of available genetic markers. In the last 50 years, the availability of DNA markers and statistical methods has lead to considerable progress in QTL mapping in plants (Mohan et al. 1997). The genomic organization and structure of wheat makes it one of the most complex crops for genetic analysis, therefore QTL analysis of grain yield, its components and agronomic traits are limited (Liu 1998; Gupta et al. 1999; Roder et al. 1998; Langridge et al. 2001).

Previously reported QTL for yield, its components and agronomic traits have been associated with nearly all the chromosomes in the wheat genome. While there is some agreement between the studies on the location of the QTL, the observed effects of the detected QTL were very different. A number of different methods and population structures have been used to detect QTL. Often QTL were identified in populations derived from wide crosses that are ideal for genetic analysis but are of limited value to breeding programs.

Wheat breeders can use information from QTL studies to design and implement marker assisted breeding strategies for quantitative traits only if the results can be reproduced. Identified QTL and the estimation of their effects are subject to experimental error and bias, therefore putative QTL should be independently confirmed

or validated (Lander and Kruglyak 1995). Validation studies should include independent populations developed from the same parental genotypes or genotypes closely related to those used in the primary QTL study. The QTL analysis could detect a false QTL or fail to detect a real QTL, over- or under-estimate the true effects of a QTL or provide inaccurate estimates of QTL position. To date a limited number of these studies have been completed in wheat, barley, soybeans and maize with inconsistent results.

The objectives of this study were to identify and estimate the effects of QTL controlling yield, yield components, and agronomic traits in a spring wheat cross between two adapted parents and validate the associated QTL in an independent breeding population.

2.0 Literature Review

2.1 Spring Wheat

The Triticeae family contains several important crop species including *Triticum aestivum* L. (bread wheat). Grown primarily as a food source, spring wheat is one of the most valuable crops to producers. In western Canada, 20.0 million tonnes of spring wheat were produced on 8.8 million hectares in 2007 with a value of 2.4 billion dollars (Statistics Canada 2007).

2.1.1 Wheat Breeding

The goal of wheat breeding has been to develop genetically uniform cultivars with excellent agronomic, disease resistance and quality characteristics. To date, the success in wheat breeding has been largely from application of traditional plant breeding methods. These methods include: pedigree, bulk population, single seed descent, doubled haploid, backcross, and recurrent selection (Poehlman and Sleper 1995). Pedigree, bulk population, and single seed descent are common procedures used to identify desirable genotypes from segregating populations following hybridization, while recurrent selection is a population improvement procedure designed to increase the frequency of desired genes by repeated cycles of selection (Poehlman and Sleper 1995). Backcross breeding allows the transfer of a simply inherited trait from a poorly adapted donor line into a well-adapted cultivar (Buzza 1995). Regardless of the breeding method, selection is primarily based on phenotypic evaluation of desirable traits.

In western Canada, spring wheat is divided into six classes with the predominant acreage being the Canada Western Red Spring (CWRS) wheat class. This class is bred to meet stringent quality requirements including milling properties as well as a kernel visual distinguishability characteristics (medium size with an ovate to oval shape) for cultivar registration (Hunt and DePauw 2001). Minimum standards for disease resistance and agronomic performance are also required. As a result genetic variability is narrow and it is difficult to make significant gains in yield.

2.1.2 Wheat Genetics

The genomic organization and structure of wheat makes it one of the most complex crops for genetic analysis (Liu 1998; Gupta et al. 1999; Röder et al. 1998). Unlike some other cereal crops, bread wheat is a polyploid species and contains the genomes of *Triticum monococcum* (AA), an unknown progenitor believed to be an *Aegilops speltoides* (BB), and *Triticum tauchsii* (DD). The haploid chromosome number of this hexaploid crop is 21. Each genome has seven chromosomes identified by a number from one to seven and genome designation A, B, or D. Chromosomes with the same number are considered to be homeologous and frequently contain common loci for a particular trait (Poehlman and Sleper 1995). Wheat has a large genome with over 80% of the genome consisting of repetitive DNA as a result of polyploidy and extensive duplications. The haploid DNA content of bread wheat is approximately 1.7×10^{10} bp with an average of 810 Mb per chromosome (Arumuganathan and Earle 1991 as cited by Gupta et al. 1999). The average wheat chromosome is 25 times longer than an average rice chromosome (Moore et al. 1995).

2.2 Grain Yield

Grain yield, defined as the mass or weight of product produced per unit land area, has been an important focus in plant breeding programs around the world. It is a complex quantitative trait controlled by a number of genes each believed to have a small effect on the final product and is highly influenced by the environment (Falconer and Mackay 1981). Grain yield in wheat is the product of three main yield components: spike number area^{-1}, kernel number spike^{-1}, and kernel weight (Woodworth 1931).

2.2.1 Genetic Improvement in Yield

It has been suggested that the progress in yield results from the accumulation of genes conferring higher yields and/or the elimination of unfavorable genes through the breeding process (Evans and Fisher 1999). Genetic gain in yield has been studied in several countries and researchers have determined wheat breeding has played a significant role in increasing grain yield. Over the last one hundred years the progress in improving grain yield appears to be approximately linear, however when shorter periods of time are observed, the progress is quite irregular. The greatest gains in grain yield were observed in the last 60 years when dwarfing genes and resistance genes for several diseases were introduced (Slafer and Andrale 1991). The documented magnitude of the relative genetic gains (grain yield increases relative to the average grain yield of the experiment) ranged from 0.35 to 0.55% per year for the countries studied. The relative genetic gain in Canada between 1882 and 1985 was 0.5% (Hucl and Baker 1987).

The most notable increase in grain yield was observed when dwarfing genes were introduced (Gale et al. 1985). *Rht-B1b* and *Rht-D1b* alleles, previously known as *Rht1* and *Rht2* have been the most widely used dwarfing genes in plant breeding during the last 60 years (Gale and Youssefian 1985). The decrease in plant height immediately reduced lodging and increased partitioning of assimilates to developing grain (Evans 1993). Significant increases in grain yield, spike number m^{-2}, and kernel number $spike^{-1}$ have been documented throughout the world in semidwarf wheats while significant decreases in kernel weight and plant biomass have been observed (Law et al. 1978; Gale and Youssefian 1985; Gale et al. 1985; Brandle and Knott 1986; Uddin and Marshall 1989; Allan 1989). Knott (1986) also observed that semidwarf alleles were associated with earliness.

2.2.2 Yield Components

The three primary yield components of wheat are kernel weight, kernel number $spike^{-1}$, and number of spikes $area^{-1}$. Analyzing these components should allow the source of variation for grain yield to be explained (Stoskopf and Reinberg 1965). Engledow and Wadham (1923) attempted to divide the yield of cereals into components. They suggested it should be possible to produce high yielding wheat by selecting parents for crossing with the optimum combination of yield components.

Harvest index is a secondary yield component and is defined by Donald (1962) as the ratio of dry grain weight to the total aboveground weight at maturity of the crop. Harvest index has been described as a useful means of determining yield efficiency. Fischer and Kertesz (1976) found harvest index to be a predictor of yielding ability.

Knott and Talukar (1971) determined kernel weight was positively correlated with yield and negatively correlated with kernel number plot^{-1} while the kernel number spike^{-1} showed a highly significant negative correlation with spike number plot^{-1}. Hucl and Baker (1987) indicated a close association between grain yield, yield spike^{-1} and kernel number spike^{-1}. They also found that harvest index had increased over the hundred year period while spike number area^{-1} remained unchanged. Wang et al. (2002) reported similar findings. Nass (1987) indicated harvest index, kernel number spike^{-1}, and seed weight spike^{-1} were associated with grain yield. Kernel number and seed weight spike^{-1} were also negatively correlated. Increased kernel number was correlated more with kernel number spike^{-1}, rather than spike number area^{-1} in studies from around the world (Slafer and Andrale 1989).

2.2.3 Breeding for Increased Yield

Due to the low heritability of grain yield, early generation selection is generally not successful (Shebeski 1967; Knott 1972; DePauw and Shebeski 1973). It is also ineffective to select for any polygenic character on a single plant basis (Shebeski and Evans 1973). High yielding cultivars have largely been identified through multilocation, multiyear yield testing.

It has been suggested that indirect selection based on one or more yield components may be more effective than direct selection for grain yield. Improvement in grain yield by selecting one or more yield components should be superior to selection for yield per se when the component trait has a higher heritability than yield and when the

correlation between the two traits is high (Falconer 1960; Johnson et al. 1966; Smith 1976; Woodworth 1931).

Sidwell (1975) found indirect selection for grain yield based on kernel weight to be more effective than either direct selection for grain yield or indirect selection based on spike number or kernel number spike^{-1}. Whereas McNeal et al. (1978) found indirect selection based on kernel weight and kernel number spike^{-1} to be productive. Several researchers have also suggested indirect selection for kernel weight as a means to identify high yielding lines (Keltata et al. 1976; Sharma and Knott 1964; Sharma and Baghel 1972; Sidwell et al. 1976).

Stoskopf and Reinbergs (1965) determined kernel number spike^{-1} was the most reliable component to use in estimating yield while grain size was found to be of limited use. However, they also indicated that one component alone cannot predict yield, and all three yield components should be used to describe yield.

McCaig and DePauw (1995) determined the yield advances made within the Canada Western Red Spring wheat class in a 90 year period resulted from an increase in the kernel number spike^{-1} rather than an increase in the kernel size. The researchers suggested that bread wheat grown on the prairies has been sink limited during grain filling. McCaig and DePauw (1995) also noted considerable genetic variation still exists for both kernel weight and number, and potential gains in yield may be possible by increasing kernel number and/or kernel weight.

Perry and D'Antuonon (1989) also observed the improvement in grain yield of new spring wheat cultivars in Australia was the result of a significant increase in kernel number. Similar findings have been reported for spring and winter wheat cultivars in the

United States, northwest Mexico, and Argentina (Cox et al. 1988; Waddington et al. 1986; Slafer and Andrale 1991).

Increases in grain yield have been observed in several studies however, the physiological basis for the genetic improvements is unknown. Wang et al. (2002) compared yield components for four new CWRS wheat cultivars with two older cultivars, Neepawa and Marquis. The new cultivars significantly increased kernel weight and kernels spike^{-1} compared to the older cultivars. This indicated that the new CWRS cultivars have increased the sink size of each tiller to increase yield. The strong association between grain yield and increased kernel number spike^{-1} rather than an increased number of spikes plant^{-1} has long been recognized as an important characteristic of wheat ideotypes (Donald 1968). Larger spikes have been found to compete more efficiently than a smaller spike for assimilates with other sinks, such as new tillers (Cook and Evans 1983). Wang et al. (2002) suggests that genetically increasing the sink size spike^{-1} has contributed to the yield increase in new cultivars.

Further investigation of the relationship between yield and yield components will improve understanding of yield and help to identify the genes responsible for increased grain yield.

2.3 Molecular Mapping

2.3.1 Molecular Markers

To be an effective genetic marker, the marker locus has to detect variation at different levels. The variation could be a simple heritable phenotype or a difference in the nucleotide sequence (Liu 1998; Mohan et al. 1997). This detectable and heritable

variation at a locus is referred to as a polymorphism and is essential to identify desirable traits. A number of genetic marker systems have been developed for use in different plant species however some systems may not be suitable for all purposes. In general, the desirable characteristics of a marker system are to detect a high level of polymorphism, detect specific loci, provide clear, highly repeatable, genetic information in a short period of time, and are easily automated (Liu 1998). The marker systems available for any species will depend on the amount of pre-existing genome information.

The first available molecular markers used were allozymes, protein variants detected by differences in migration on starch gels in an electric field. Since the late 1960s, protein markers were used extensively and were relatively inexpensive to score in large numbers but there was often insufficient protein variation for high-resolution mapping. During the mid 1980s, methods became available to evaluate genetic variation directly at the DNA level and lead to allozymes being replaced with DNA based markers in mapping studies (Tanksley 1993; Liu 1998). The advent of molecular DNA technology has made it possible to map and characterize the genes controlling economically important traits in crop species. DNA-based molecular markers are used in genomic analysis and provide the foundation for marker-assisted selection.

There are two basic approaches, hybridization or amplification, used to detect variation in DNA. Detection of variation through random fragment length polymorphisms (RFLPs) is hybridization based, while amplification based technologies use the polymerase chain reaction (PCR) and include random amplified polymorphic DNA (RAPDs), amplified fragment length polymorphism (AFLPs), and microsatellite markers also known as simple segment repeats (SSRs) (Mohan et al. 1997; Gupta et al.

1999; Liu 1998). Molecular markers may exhibit either codominance or dominance. Codominant markers distinguish between homozygous and heterozygous genotypes while dominant markers are scored as present or absent and cannot distinguish heterozygous from homozygous individuals.

The main applications of molecular markers in cereals and other field crops can be divided into three categories a) assessment of genetic variability and characterization of germplasm; b) identification and characterization of genomic regions controlling quantitative traits and c) marker assisted selection following the identification of specific genomic regions (Ribaut et al. 2002).

2.3.1.1 RFLP – Restriction Fragment Length Polymorphism

RFLPs are highly reproducible, codominant markers that can identify unique loci (Mohan et al. 1997; Gupta et al. 1999; Liu 1998). These markers have been successfully used for mapping plant genomes. In this procedure, DNA is digested with restriction enzymes and gel electrophoresis is used to separate the resulting fragments according to molecular weight. The fragments are then transferred to a membrane by Southern blotting and identified by hybridization to a complementary radioactively labeled cDNA probe. Genetic variation is revealed by differences in restriction patterns that can arise from base substitutions within restriction sites or from insertions, deletions, or sequence rearrangements between restriction sites (He et al. 1999).

RFLP analysis has limitations since the procedures are very labor intensive, require technical skill, and are expensive for the quantity of information obtained (Gupta et al. 1999; D'Ovidio et al. 1990). This method also requires a large amount of high

quality DNA and is therefore not suitable when a limited amount of plant material or preserved tissue is available. The probe can also hybridize to repeated sequences at multiple locations in the genome. In these cases, allelic and non-allelic variations cannot be distinguished. When cDNA with known gene function are used as probes, the chromosomal position of the specific gene or genes can be identified on the chromosome (Gupta et al. 1999). Duplicate/multiple loci can also be distinguished when the size of the band of interest is known. The greatest barrier to the use of RFLPs in marker assisted selection (MAS) is the low level of polymorphism in a number of important crops including wheat (He et al. 1999). The low frequency is sometimes attributable to the polyploid nature of these crops, the high proportion of repetitive DNA, and large genome size (Gupta et al. 1999). Efforts have been made to develop RFLP markers for MAS in wheat, however, the level of polymorphism is low and breeders can only use a small portion of available markers. Röder et al. (1998) reported RFLP markers are of limited use because usually less than 10% of all RFLP loci are polymorphic in wheat.

RFLPs were the first DNA-based markers and were initially used for human genome mapping (Botstein et al. 1980). Since then these markers have been used to construct linkage maps for several crop species including maize (Helentjaris et al. 1986), tomato (Paterson et al. 1988) and rice (McCouch et al. 1988). To date, many RFLP markers have been linked to genes controlling economically important traits in various crops.

2.3.1.2 PCR Based Markers

Amplification based markers use the polymerase chain reaction (PCR). Developed in 1985, PCR uses two oligonucleotide primers of a known sequence to amplify specific regions of DNA (Liu 1998). PCR based markers have increased the possibilities for genome studies since they require only a small amount of tissue, small amount of template DNA and can be adapted to handling large numbers of samples (Ribaut and Hoisington 1998; Gupta et al. 1999). The techniques are robust and amenable to automation and are widely applied to large scale marker development or implementation.

2.3.1.2.1 RAPD - Randomly Amplified Polymorphic DNA

RAPD markers are produced by amplifying random DNA segments with short oligonucleotides primers typically 10 bp long of an arbitrary sequence (Gupta et al. 1999; Liu 1998; Devos et al. 1992). Amplified fragments are those regions of the genome that contain two sequences complementary to the primer on the opposite strands of DNA (Williams et al. 1990). If polymorphism exists at the binding sites among genotypes or fragment length differs at the same site from genotype to genotype, then a RAPD marker is obtained (Liu 1998). Since the primers used in this marker system are relatively short, there is a greater likelihood they will anneal to several regions of the genome and identify multiple loci as well as other regions that display some degree of sequence homology thereby producing unwanted bands. The use of longer primers will allow specific loci to be detected.

RAPD markers have several advantages over RFLP. Genetic variation at many loci from different regions of the genome can be examined quickly. Very small amounts of DNA are required with no prior template DNA sequence information. RAPDs are simpler, less costly and less time consuming than RFLP because the procedure does not require probes, Southern blotting, or hybridization (Liu 1998).

There are also limitations to RAPDs. RAPD markers are dominant and therefore they are scored as present or absent and do not identify heterozygous individuals. This needs to be accounted for when mapping or using marker-assisted selection where backcross/recombinant inbred populations or doubled haploids should be used. RAPD technology has proven useful in wheat studies; however they have not been widely used due to low levels of detectable polymorphism and lack of reproducible results (Gupta et al. 1999). Because the primers are short relative to other marker systems, template DNA quality and quantity along with amplification conditions (concentration of MgCl, TAQ polymerase, and annealing/denaturing temperature) must be carefully controlled to ensure reproducible results, especially among different labs (Devos and Gale 1992; D'Ovidio et al. 1990).

2.3.1.2.2 AFLPs – Amplified Fragment Length Polymorphisms

AFLPs are a relatively new type of marker system developed by combining the RFLP marker system and the PCR method. Genomic DNA is digested with two different restriction enzymes and double stranded oligonucleotide adapters are added to the ends of the resulting restriction fragments (Vos et al. 1995). Adapted fragments are selectively amplified using primers that extend into the restriction fragments, usually one to three

arbitrarily chosen bases beyond the restriction site. Those fragments with nucleotides flanking the restriction site that match primer extensions are amplified. The amplified products are then radioactively or fluorescently labeled and separated on sequencing gels. Polymorphisms are detected by differences in the length of the amplified fragment.

AFLPs can be generated for any organism without prior knowledge of the DNA sequence and requires only a small amount of DNA. AFLPs are reproducible and can resolve extremely small genetic differences. AFLPs are more reproducible than RAPDs and provide more genomic coverage. AFLPs have been limited in use since they are a dominant marker. Studies have observed that use of AFLP is a more efficient mapping technique to detect polymorphisms when compared to RFLPs and RAPDs (Powell et al. 1996; Lin et al.1998).

2.3.1.2.3 Microsatellites

Microsatellites or simple sequence repeats (SSR) are highly informative, codominant markers composed of tandemly repeated di- to tetra-nucleotide sequence motifs flanked by unique sequences in the genome (Röder et al. 1998; Primrose 1995; Powell et al. 1996). The most common form of these repeats is simple di-nucleotide repeats; tri- and tetra-nucleotide repeats are also found in the genome but are less frequent (Hearne et al. 1992). PCR primers for the regions flanking the microsatellite are developed, and the target region is amplified followed by high-resolution electrophoresis of microsatellite PCR products. Microsatellites are locus specific and evenly distributed along chromosomes (Röder et al. 1998; Ribaut et al. 2002).

Allelic variation attributable to differences in as few as two base pairs can be resolved with this method (Liu 1998). Microsatellites detect a high level of polymorphism because they target highly variable regions of the genome. They have been widely used: to tag resistance genes (Peng et al. 1999), enable marker assisted selection in wheat (Huang et al. 2000), and assess genetic diversity in closely related bread wheat and collections of wheat accessions (Plasche et al. 1995; Huang et al. 2002). Unlike RFLPs, microsatellites are genome specific and will amplify a specific locus containing the microsatellite in the A, B, or D genome. In some cases however, the primer pairs will amplify fragments located in two different genomes and the loci map to apparently homoeologous sites on chromosomes. The use of deletion lines allows researchers to assign the markers to defined chromosome regions (Röder et al. 1998). The major disadvantage of microsatellites is that the discovery of primers requires some molecular skills for cloning, and sequencing is costly, labor intensive and time consuming. Abundance, locus specificity, and the high level of polymorphism associated with microsatellites make them an ideal marker system to construct genetic maps, mapping quantitative traits and marker assisted selection.

2.3.2 Genetic Maps

One of the main uses of DNA markers has been to construct genetic or linkage maps for a number of crop species. The maps have been used to identify chromosome regions that contain genes controlling simple traits (controlled by a single gene) and complex traits (controlled by several genes) (Langridge et al. 2001). Linkage maps indicate the position and relative genetic distances between the markers along the

chromosome. Mapping is based on the principle that genes and markers segregate via chromosome recombination (crossing over) during meiosis. Genes or markers that are in close proximity or are tightly linked will be transmitted together from parent to progeny more frequently than genes or markers that are located further apart. In a segregating population there is a mixture of parental and recombinant genotypes. The frequency of recombinant genotypes can be used to calculate recombination fractions that may be used to infer the genetic distances between markers. By analyzing the segregation of markers the relative order and distance between markers can be determined. Linkage maps are constructed from the analysis of many segregating markers.

Construction of linkage maps is completed with computer software. MapMaker/EXP (Lander et al. 1987; Lincoln et al. 1993) and JoinMap (Stam 1993) are two commonly used programs. Linked markers are grouped together into linkage groups, which represent chromosomal segments or entire chromosomes. Markers are usually not distributed over the chromosome but clustered in some regions and absent in others (Patterson 1996). In addition to the non-random distribution of markers the frequency of recombination is not equal along the chromosomes. The ability to accurately measure the genetic distance and determine marker order is directly related to the number of individuals in the mapping population. Ideally, the population should consist of at least 50 individuals (Young 1994).

The first genetic maps were constructed with RFLPs in maize, tomato, and soybeans (Paterson et al.1991). The majority of the problems in genetic map construction have been with the available markers types, the lack of informative markers, and the polyploid nature of many crop genomes. Genetic maps are currently available for

all chromosomes and homoeologous groups in wheat (Langridge et al. 2001). The first maps of the wheat genome were constructed with RFLP markers (Chao et al. 1989; Devos and Gale 1993; Nelson 1995a, b, c). There is generally a lower level of polymorphism in wheat relative to other cereals and requires a large number of molecular markers to provide adequate genome coverage. In addition, the level of polymorphism is not consistent across the genomes or between crosses (Roder et al. 1998, Mohan et al. 1997). The D genome tends to be more conserved and is usually more difficult to map. These features introduce a level of complexity to genome analysis and can create technical difficulties. Recently, a high-density microsatellite consensus map was constructed by combining four independent genetic maps of bread wheat (Somers et al. 2004).

2.3.3 Marker Assisted Selection

Marker assisted selection (MAS) is a valuable plant breeding tool which uses molecular markers to select the genomic regions involved in the expression of desirable traits (Ribault and Hoisington 1998). MAS holds great promise, especially for quantitative traits, which have been previously difficult to incorporate and analyze using traditional plant breeding methods. However, in order for MAS to be effective, the following requirements must be met: the identified marker(s) should co-segregate or be closely linked (1cM or less) to the desirable trait; there should be an efficient means to screen large populations for the marker(s) with high repeatability between samples and labs; the marker should be cost effective, and easy to use (Mohan et al. 1997).

When compared to traditional breeding methods, MAS offers a number of advantages. Plant breeders can make selections during the very early stages of a plant-breeding program reducing the reliance on multi-location, multi-year field trials to identify superior individuals. Young plants or individual seeds can be evaluated for the presence of the desirable trait, as a marker is present and detectable at all stages of plant growth (Mohan et al. 1997; Ribault and Hoisington 1998). Unlike phenotypic or product-based markers, selection for the trait of interest is carried on the molecular marker(s) linked to that trait not directly on the trait. Environment and genotype x environment interactions, which can be large in a conventional breeding program, are irrelevant in MAS since molecular markers are unaffected by the conditions in which the plants are grown (Koebner and Snape 1998; Mohan et al. 1997).

2.4 Quantitative Trait Loci (QTL)

2.4.1 Quantitative traits

A major limitation in the genetic improvement of wheat is the lack of information about the genes controlling quantitative traits such as grain yield and its components. Quantitative traits are controlled by a number of genes with small effects, are significantly influenced by the environment, and vary in degree rather than kind (Falconer and MacKay 1981). These traits are difficult to study because the continuous phenotype distribution does not provide any insight into the genotype of the trait. The lack of discrete phenotypic categories also does not allow the use of phenotypic ratios or inheritance patterns to describe quantitative traits. Until the 1980's, the study of quantitative traits was limited to means, variances, covariances of relatives, and

heritabilities (Tanksley 1993; Falconer and MacKay 1981). These statistics allowed a number of parameters to be estimated including the approximate number of loci affecting the trait of interest, the average gene action, and the degree to which the various genes interact to determine the phenotype (Tanksley 1993). However, it is difficult to determine the magnitude of effect, inheritance, or gene action of any specific locus affecting the trait of interest. As a result, the term quantitative trait loci (QTL) was coined to describe a region of a chromosome that has a significant effect on a quantitative trait.

2.4.2 QTL Analysis

The principles of QTL analysis were developed more than 75 years ago when Sax (1923) reported the first linkage of a trait, seed weight in beans (*Phaseolus vulgaris*), to a major gene for seed pigmentation. Wide scale application of QTL analysis was not possible at the time due to the lack of available genetic markers. The identification of QTL followed 50 years later when the first class of molecular markers (RFLPs) successfully produced complete genetic maps in many crop species (Botstein et al. 1980).

The goal of QTL analysis is to estimate the number, location, and effect of QTL controlling a quantitative trait. QTL analysis is based on the principle of detecting an association between phenotype and the genotype of the marker. QTL can only be identified for traits that segregate between the parents used to develop the mapping population. A number of methods have been developed to model the effects of either single QTL or multiple QTL. The three most common methods of QTL analysis are:

single factor analysis, interval mapping, and composite interval mapping (Liu 1998; Tanksley 1993).

2.4.2.1 Single–Factor Analysis

Single-factor analysis (also called single-marker or single-point analysis) refers to the detection of QTL by considering one marker at a time. Differences among genotype means are tested for significance at each marker locus using an analysis of variance (ANOVA), *t*-test, or linear regression (Liu 1998). Each marker trait association is performed independent of information from all other markers and does not require a complete genetic map. This is the simplest method to identify QTL; however, the analysis is limited by two factors. Separate estimates are not provided in this analysis for the location of the QTL relative to the marker and its effects. Location of the QTL can be inferred from the markers with the greatest differences between genotype means. It is also possible, two or more adjacent markers could detect the same or different QTL. Secondly, as the distance between the QTL and markers increases, the power to detect QTL decreases due to crossing over events between the marker and the QTL. Single factor analysis is a good method to detect QTL rather than estimate its position and effects. Single-factor analysis was used in the first molecular marker/quantitative genetic studies (Edward et al. 1987; Tanksley 1993; Liu 1998).

2.4.2.2 Interval Mapping

To overcome the disadvantages of single factor analysis Lander and Botstein (1989) developed interval mapping (also referred to as simple interval mapping). This

method requires a complete genetic map and like single-factor analysis assumes only a single QTL is present. The location of a QTL is determined relative to adjacent pairs of flanking markers instead of using single markers. Using a maximum likelihood approach, interval mapping evaluates the likelihood that a QTL is located at a specific position. The procedure involves calculating a logarithm of odds (LOD) score, which is equal to the logarithm of the likelihood ratio. The likelihood ratio is a function of the likelihood that the data arose from a linked QTL, divided by the likelihood that the data did not arise from a linked QTL. The conventional threshold for declaring the presence of a QTL is a LOD score of 3.0, which corresponds to odds of 1000:1 (Lander and Botstein 1989). Significance thresholds are more widely determined using permutation tests (Churchill and Doerge 1994). The LOD threshold will depend on population size, genome size, marker density, population type, and marker used (Hackett and Broadfoot 2002). The LOD score is then plotted against genome location and is compared to a genome wide threshold. Whenever the LOD score exceeds the threshold, the presence of a QTL is inferred. The point at which the LOD is maximized (the peak) is used as the estimate of the QTL location. A one- or two-LOD interval around the inferred QTL is used as an estimate for QTL location.

Interval mapping can also be performed using a regression approach, known as regression mapping. A series of regression analyses are performed at all positions between a pair of adjacent markers. A QTL is declared at the position where the residual sums of squares are minimized. Regression mapping is computationally simpler than interval mapping by maximum likelihood (Haley and Knott 1992; Martinez and Curnow 1992).

An accurate estimate of the QTL location is not always provided with interval mapping, especially when two or more QTL are present in a small chromosome region. Martinez and Curnow (1992) found that interval mapping could lead to the detection of 'ghost' or non-existent QTL between two pairs of flanking markers. They recommended that information from three or more nearby markers are used to map the QTL. By using linked markers in the analysis, interval mapping can compensate for the recombination between the markers and the QTL, increasing the possibility of statistically detecting the QTL and also providing an unbiased estimate of the QTL effect on the character. Interval mapping was first used on an interspecific backcross of tomato (Paterson et al. 1988) and has subsequently been used in several quantitative trait studies. A number of software packages have implemented interval mapping including MAPMAKER/QTL (Lander and Botstein 1989) and QTL Cartographer (Basten et al. 1994).

When interval mapping and single-factor analysis are compared, interval mapping gives a more precise estimate of the location and effect of a QTL but does not give an increase in the power to detect QTL and requires a great deal more computational effort than single-factor analysis (Lander and Botstein 1989; Liu 1998). Interval mapping should be used when the linked markers are relatively far apart (greater than 20 cM) since there are likely to be a number of crossovers between the marker and QTL, which can be compensated for with interval mapping. When the marker density is less than 15 cM apart, single-factor analysis and interval mapping are identical. However, when the marker loci are very far apart (greater than 35 cM), interval mapping is inefficient in detecting QTL in the interval between the loci (Tanksley 1993; Knott and Haley 1992; Hyne et al. 1995).

2.4.2.3 Composite Interval Mapping

In the last two decades, efforts have been made to develop methods to model multiple QTL in an attempt to improve the sensitivity of QTL analysis and separate linked QTL. Utz and Melchinger (1994) found estimates of QTL locations and effects can be biased if the effects of other QTL are not taken into account. Jensen and Zeng independently developed a method, which combines interval mapping and linear regression to reduce the multi-dimensional search for identifying multiple QTL to a one-dimensional search (Jansen 1993; Jansen and Stam 1994; Zeng 1993, 1994). Jansen 1993 referred to this method as MQM (multiple QTL mapping) while Zeng called this method composite interval mapping (CIM).

The location of a QTL between a pair of markers is estimated by interval mapping, while the effects of QTL located in other intervals of the genome are accounted for by regression analysis. Additional markers are incorporated as cofactors in the regression to control the effects of QTL in other intervals while improving the power of detecting and estimating QTL effects more precisely (Liu 1998). The selection of cofactors is determined by regression analysis (forwards, backwards, or stepwise) in QTL mapping software such as QTL Cartographer (Basten et al. 1994).

Forward stepwise regression with backward elimination is a common method of stepwise regression used in QTL Cartographer (Basten et al. 1994). This method ranks the markers for their effect on the quantitative trait as well as determines whether adding or deleting a marker makes a significant difference to the fit of the model. The model tests each marker in turn for its effect on the quantitative trait using linear regression but

only adds markers to the model while the p-value of the partial F-statistic is below a defined threshold, $p(F_{in})$. When a step is reached where no more markers can be added, all of the markers are retested to determine whether they are still significant. Each marker is in turn deleted from the model, a p-value is calculated for the partial F-statistic, and if the p-value is greater than a specified level $p(F_{out})$, it is deleted. The in and out value of 0.1 is considered to have low stringency in searching for QTLs while the in and out value of 0.01 are for greater stringency. CIM uses the results of the stepwise regression analysis to estimate the location and effect of the chromosome regions associated with the trait(s) of interest. As in interval mapping, a LOD score is calculated at each locus and is plotted as a function of genome position and is compared to a genome wide threshold. When the LOD curve exceeds the threshold, a QTL is said to be present in that area of the genome. Empirical significance thresholds are usually determined with permutation tests (Churchill and Doerge 1994).

2.5 QTL Studies in Wheat

2.5.1 Grain Yield and Yield Component QTL

In early studies of crop plants, grain yield was found to be heritable and under polygenic control (Aamodt and Torrie. 1935; Clark 1924; Torrie 1936). The study of quantitative traits in wheat has a long history and is based on the use of morphological and isozyme markers with cytogenetically derived single chromosome substitution lines (Law 1966; 1967; Berke et al. 1992; Snape et al. 1985; Langridge et al. 2001).

The development of chromosome substitution lines provided researchers with a valuable tool to determine the chromosomes involved in quantitative traits. Chromosome

substitution lines were produced by replacing 'Chinese Spring' chromosomes with homologous chromosomes of other varieties (Law 1966; Law and Worland 1973). This usually requires five to six backcrosses to derive the substitution line. Each substituted chromosome is present in a uniform genetic background of Chinese Spring to allow the effect of genes present on the substituted chromosome to be studied. With this tool, researchers are limited to the individual chromosome being investigated to locate QTL for the trait of interest (Law 1966; Law and Worland 1973).

Kuspira and Unrau (1957) produced three sets of chromosome substitution lines from the donor varieties Thatcher, Hope, and Timstein to study the genetics of thousand grain weight (TGW) and yield. The lines were studied in replicated field trials over three years. Both TGW and yield were observed to be affected by several genes on many chromosomes. The effects of the individual genes were found to be small and were usually not equal. Seven chromosomes (1A, 2A, 2B, 2D, 4A, 6A, 7A) were determined to carry genes that affect TGW. Chromosome 1A was observed to have the greatest effect on TGW. Chromosomes 4B, 5D, and 7D were determined to carry no differential genes affecting yield while the other chromosomes of the three donors out yielded Chinese Spring by 180 to 200%. This yield increase was assumed to be the result of genes for yield introduced by the transferred chromosomes.

Using monosomic analysis, Giura and Saulescu (1996) indicated grain weight in wheat was increased by the presence of chromosomes 4A and 6D and decreased by 5B and 5D.

Berke et al. (1992) studied QTL controlling grain yield and yield components in reciprocal sets of chromosome substitution lines between two hard red winter wheat

cultivars. The researchers observed yield and its components were influenced by one or more QTL on relatively few chromosomes. Only three (3A, 3B, and 6A) had a major effect on yield. They concluded the increase in grain yield was due to the production of heavier seeds since approximately the same number of seeds spike^{-1} and spikes meter^{-2} were observed for the lines evaluated. These findings also suggested it would be possible to increase grain yield.

Chromosome substitution lines were developed and RFLP markers were used to study agronomic traits on chromosome 3A in bread wheat (Shah et al. 1999). QTL were identified for TGW, kernel number spike^{-1} and spike number m^{-2} using single factor analysis. Araki et al. (1999) also developed chromosome substitution lines for chromosome 4A and used RFLP markers to study agronomic traits on chromosome 4A. This chromosome is known to carry the *Wx-B1* gene encoding the granule-bound starch synthase involved in amylose synthesis in the endosperm. QTL were detected on chromosome 4A for plant yield, grain weight spike^{-1}, spikes plant^{-1}, and 50-grain weight. The identified QTL did not map to the *Wx-B1* gene or to an adjacent region.

Kato et al. (2000) constructed an RFLP map from 118 single-chromosome recombinant lines derived from F_1 plants of a cross between substitution lines for chromosome 5A from a spring accession of *Triticum spelta* and a French winter wheat cultivar 'Capelle-Desprez'. The chromosome lines and parental lines were evaluated in replicated field trials over three years at one location to locate QTL controlling yield and its components. Each plot was a single 1-m row of 11 plants and each row was spaced 30-cm apart. The study identified five regions of chromosome 5A including the vernalization requirement gene, *Vrn-A1,* and the ear morphology gene, *Q*, controlling

grain yield and its components. Increases in yield, grain weight, and spikelet number spike^{-1} were determined by complementary QTL alleles from both parents. Grain yield QTL were correlated with QTL for yield components and located at the same map position.

The lack of polymorphic marker loci hampered the analysis of quantitative traits until molecular markers were developed (Tanksley 1993). Molecular markers enabled researchers to construct genetic maps of the entire genome and map QTL to specific chromosome regions. In hexaploid wheat, QTL analyses of grain yield, yield components, and other important agronomic traits using molecular markers are limited because RFLP and RAPD markers show a low level of polymorphism in wheat and it is difficult to construct high-resolution genetic maps (Hyne et al. 1995; Choa et al. 1989; Devos and Gale 1992).

Hyne et al. (1995) conducted a partial genome assay for QTL in wheat using four different analytical techniques. Genetic maps of chromosomes 6B, 7A, 7B, and 7D were constructed using a range of morphological, isozyme, RFLP marker loci and a DH population of 114 lines using European spring wheat varieties. Phenotypic data were collected from five single row microplot replicates per genotype over two years. QTL analysis was completed using four analytical approaches: model fitting, analysis of variance, Mapmaker /QTL (Lander and Botstein 1989) and regression mapping (Haley and Knott 1992; Martinez and Curnow 1992). QTL were located on 6B for plant height and spike weight; 7A for heading date, grain weight and yield; 7B for plant height, spike weight, and yield and 7D for heading date. The four techniques used in the analysis were consistent in the detection and estimation of the size of the QTL effects.

Seventy-eight recombinant inbred lines (RILs) from a soft by hard wheat cross were genotyped with 313 RFLP markers and a map was constructed displaying all chromosomes to study kernel traits including TGW (Campbell et al. 1999). Four QTL were detected for TGW on chromosomes 1A, 1B, 3B, and 7A that explained 5.8 to 12.2% of the phenotypic variation observed.

Börner et al. (2002) constructed a high density RFLP map of the International Triticeae Mapping Initiative (ITMI) population to identify QTL associated with 20 morphological, agronomic, and disease resistance traits studied under a range of conditions. In total, 210 individual QTL were detected for the traits evaluated however only 64 of the identified QTL were considered major QTL with LOD scores >3.0. Three major QTL were detected for TGW on chromosomes 3A, 5A, and 6B with 11 minor QTL detected on chromosomes 1B, 2D, 3A, 3B, 6A, 6B, 7B, 7D. The detected QTL were only observed in one environment. In total, 6 major and 11 minor QTL were identified for grain weight spike^{-1}. The major QTL were detected in 2D, 4A, and 6B while the minor QTL were detected on 1A, 1B, 2A, 2B, 2D, 4A, 5A, 6A, and 7A. The most repeatable QTL was observed on 2D since it was detected in four of the environments. A major QTL for grain filling time (GFT) was detected on 5A with a minor QTL on 5BL. Börner et al. (2002) indicated an increase in GFT results in an increase in grain yield however there are no other reports of GFT QTL in the literature.

Groos et al. (2003) evaluated 194 (RILs) derived from a cross between two wheat varieties and were grown at six locations in France to detect QTL for grain yield and TGW. A genetic map of 254 RFLP, AFLP, and microsatellite loci was constructed. Using marker regression seven QTL for yield were detected on chromosomes 2B, 3B,

4A, 4B, 5A, 5B, and 7D. However, only the QTL on 7D was considered to be stable since it was observed in four of the six environments evaluated. The other yield QTL were observed in less than three environments. Nine QTL were identified for TGW on chromosomes 1D, 2B, 2D, 3A, 5B, 6A, 6D, 7A, and 7D while only the QTL on 2B, 5B, and 7A were considered to be stable. QTL for TGW and yield co-located on 5B and 7D. In most cases the favorable alleles came from the same parental line for yield and TGW. The identified QTL differed from the previous studies by Campbell et al. (1999) and Varshney et al. (1998).

Huang et al. (2003) used QTL analysis to identify QTL for yield and yield components in a genotyped BC_2F_2 population of 72 plants derived from a cross between a German winter wheat and a synthetic wheat line. A genetic map was constructed with 210 microsatellite markers and the BC_2F_3 families were grown at four locations in Germany to collect the phenotypic data. Using single marker regression analysis, and interval mapping, Huang et al. (2003) identified 40 QTL with LOD scores that ranged from 1.5 to 5.5 and explained 9.2 to 29.5% of the phenotypic variation. Eleven QTL were associated with yield while 16 QTL were associated with the yield components, spikes m^{-2} and TGW. No significant correlations between yield and its components were observed and in most cases the QTL for these traits mapped independently. The yield QTL were identified on chromosomes 1A, 1B, 2A, 2B, 2D, 3B, 4D, and 5B while the QTL for TGW were detected on chromosomes 2A, 2D, 4D, 5B, 7A, 7B, 7D. The TGW QTL on 7B and 7D mapped in positions similar to Börner (2002). Spikes m^{-2} were only measured at two of the four locations where eight QTL were detected on chromosomes 1B, 2A, 2D, 3B, 4D, 5D, 6D, and 7A.

Huang et al. (2004) studied seven agronomic traits in a 111 BC_2F_3 families derived from a cross between a German winter wheat and a Japanese synthetic wheat line at six sites in Germany. The individuals were genotyped with 197 microsatellite markers. In total, 57 QTL were detected for yield, TGW, spikes m^{-2}, and grain weight $spike^{-1}$. Nine QTL were identified for yield on chromosomes 1A, 3D, 4D, 5A.1, 5A.2, 5B, 6B, and 6D while 14 QTL were identified for TGW on chromosomes 1B, 1D, 2A, 2D, 3A, 3B, 3D, 4B, 6A, 7A, and 7D. QTL for spikes m^{-2} were detected on chromosomes 1B and 7A while five QTL were identified for grain weight ear^{-1} on 3B, 6A, 6D and 7D. No significant correlations were observed for grain yield and yield components. Börner et al. (2002) and Huang et al. (2003) observed similar results. Grain weight $spike^{-1}$ and TGW were positively correlated while grain weight $spike^{-1}$ and spikes m^{-2} was negatively correlated. When the studies from 2003 and 2004 are compared, 88 QTL were identified for the five traits measured in both studies, however, only eight of the QTL are potentially orthologous between the two synthetic lines. It is likely that the large difference in the results was caused by the different backgrounds of the synthetic lines. The source of the synthetic line, W7984, in the initial studied was CIMMYT while the synthetic line, in the second study, XX86, was from Japan.

Four QTL for TGW on chromosome 3A, 3B, 6A, and 7D along with the QTL for grain weight $spike^{-1}$ on chromosome on 7A mapped to similar positions as the QTL reported by Börner et al. (2002).

Using a population of RIL derived from a cross between a Chinese facultative wheat, 'Ning 7840', and an American soft red winter wheat, 'Clark', Marza et al. (2005) identified 206 putative QTL for the 15 yield, yield components, and agronomic traits

evaluated when each environment was analyzed separately. Grain yield and yield component QTL were detected on chromosomes 1A, 1B, 2B, 3B, 4A, 4B, 5A, 5B, 6B, 7A, and 7D. The QTL explained between 7 and 23% of the phenotypic variation observed for the evaluated traits. Grain yield and yield components were correlated and yield components mapped to similar positions as grain yield.

The studies completed by Börner et al. (2002), Huang et al. (2003; 2004) and Marza et al. (2005) provide insight on the number, location, and an estimate of the effects of grain yield and yield component QTL while considering the influence of the other chromosomes. However, each of these studies reported the results for each environment separately and it is difficult to determine the repeatability of the effects of the identified QTL.

2.5.2 Heading Date

For more than eighty years, the genetic control of heading date in wheat has been investigated. Time to heading is regulated by three groups of genes that control the duration of the life in wheat: vernalization response genes, photoperiod response genes, and '*earliness per se*' or developmental rate genes. The ability of wheat to adapt to the diverse environmental conditions under which it is grown worldwide is determined by these groups of genes and ultimately affects crop performance. Vernalization and photoperiod responses interact with the environment while the '*earliness per se*' genes control the developmental rate of the crop independent of environment stimuli (Worland 1996; Snape et al. 2001; Börner 2002). The use of chromosome substitution lines has

determined that chromosomes of nearly all homoeologous groups are involved in the genetic control of heading (Worland et al. 1987; Law et al. 1991; Snape et al. 2001).

2.5.2.1 Vernalization Genes

Wheat is generally classified into two types: winter and spring depending on the vernalization requirement. Winter wheats require an extended period of vernalization (exposure to temperatures between 0 and 10 °C) to initiate floral primordia and convert from the vegetative to reproductive growth stage while spring wheats do not have a vernalization requirement or have a reduced vernalization requirement. In response to vernalization, heading is primarily determined by three major genes in wheat, *Vrn-A1*, *Vrn-B1*, and *Vrn-D1* (previously known as *Vrn1, Vrn4,* and *Vrn3)* located on the homoeologous group 5 chromosomes and a fourth gene, *Vrn-B4*, located on chromosome 7B. (Law et al. 1976; Worland et al. 1987; MacIntosh et al. 1998; Snape et al. 2001; Börner 2002). The dominant alleles usually confer a lack of vernalization requirement (spring types) while the recessive alleles confer a vernalization requirement (winter types).

2.5.2.2 Photoperiod Genes

Wheat is usually a long-day plant, sensitive to day length and some varieties require a period of long days to allow heading to proceed (Sourdille et al. 2000). Major genes determining heading time in response to photoperiod (*Ppd*) in wheat were located on the short arms of the wheat homoeologous group 2 chromosomes (Welsch et al. 1973; Law et al. 1978; Scarth and Law 1983, 1984; Worland and Sayers 1995). The

photoperiod response genes are *Ppd-A1*, *Ppd-B1*, and *Ppd-D1* (previously *Ppd3, Ppd2,* and *Ppd1)* located on chromosomes 2A, 2B, and 2D, respectively (MacIntosh et al. 1998; Worland et al. 1998; Snape et al. 2001; Sourdille et al. 2000; Börner 2002). The dominant alleles confer photoperiod insensitivity whereas the recessive alleles confer photoperiod sensitivity. These genes have been shown to play an important role in speeding up or slowing down flowering time in the spring following the vernalization requirement in field studies (Snape et al. 2001).

2.5.2.3 Earliness per se Genes

The genetic control of the *earliness per se* (*Eps*) genes in wheat is not well characterized (Snape et al. 2001; Sourdille et al. 2000). *Eps* genes have been located on several chromosomes including the homoeologous group 2 chromosomes (Scarth and Law 1993). Studies completed in barley indicate that by homoeology all homoeologous groups should carry *Eps* genes (Laurie et al. 1995). Law (1987) observed an *Eps* gene on 6D while Miura and Worland (1994) and Shah et al. (1999) reported an *Eps gene* on 3A. Hoogendoorn (1985) detected genes involved in earliness per se on chromosomes 3A, 4A, 4D, 6B, and 7B. While other genes have been predicted, only two genes have been mapped in wheat, *eps-2B* and *eps-2D* on chromosomes 2B and 2D, respectively (Scarth and Law 1993; Snape et al. 2001). *Eps* genes were identified in crosses made to study the segregation of major vernalization and photoperiod genes therefore the *Eps* genes are frequently obscured by the effect of the vernalization and photoperiod genes.

While the effects of each group of genes have been studied individually, little information is available on the combined effects of this complex group of genes. Further

work is required to describe these genes and determine their pleiotrophic effects on other aspects of plant growth and development.

2.5.2.4 Heading date QTL Studies

Kato et al. (1999) constructed a RFLP map from 120 recombinant substitution lines to determine if genetic variation for spike emergence time is affected by the vernalization loci (*Vrn-A1*) or spike morphology loci (*Q*) known to exist on chromosome 5A. A major effect on spike emergence time was observed from the *Vrn-A1* gene. In addition, a QTL, *QEet.ocs-5A.1*, was identified approximately 10 cM from the spike morphology loci.

Börner et al. (2002) detected two major QTL (LOD score >3.0) on chromosomes 2BS and 2DS where the major genes *Ppd-B1* and *Ppd-D1* are located as well as a QTL on 5DL, which may correspond, with *Vrn-D1*. Sordille et al. (2002) also detected a QTL for flowering time in comparable positions on 2BS and 5DL using a recombinant DH population derived from a cross between a winter wheat, 'Courtot' and a spring wheat, 'Chinese Spring'. A QTL for heading was detected by Miura et al. (1999) on chromosome 3AL, which may correspond to the *earliness per se* gene, *Eps-A1*.

Eight QTL were associated for spike emergence time in a BC_2F_3 families developed from a German winter wheat and a synthetic hexaploid wheat line (Huang et al. 2003). The QTL were detected on chromosomes 2A, 2D, 3B, 5A, 5B, 6A, and 7B. The QTL on 7S could be the same as the genetic factor located by Law and Wolfe (1966) for ear emergence time while the QTL on 2D is likely the QTL, *QEet.ipk.2D,* identified by Börner et al. (2002) in the ITMI population. The position of the QTL on 5A is similar

to the QTL, *QEet.ocs-5A*.1, reported by Kato et al. (1999). Huang et al. (2003) did not observe any correlation between grain yield and spike emergence time. In a subsequent study, Huang et al. (2004) identified five QTL for spike emergence time on chromosomes 2D, 3A, 4A, 7A, and 7D.

2.6 QTL Validation

The detection and estimation of QTL locations and effects are subject to experimental error and bias, therefore putative QTL should be independently confirmed or validated (Lander and Kruglyak 1995). The QTL analysis could detect a false QTL or fail to detect a real QTL, over or underestimate the true effects of a QTL or provide inaccurate estimates of QTL position. Validation studies should include independent populations developed from the same parental genotypes or genotypes closely related to those used in the primary QTL study. Melchinger et al. (1998) and Utz et al. (2000) suggested that QTL positions and effects should be evaluated in an independent population because QTL mapping results in low power of QTL detection and a large bias of QTL effects. To date a limited number of these studies have been completed in wheat, barley, soybeans and maize with inconsistent results.

2.6.1 Validation Studies

2.6.1.1 Wheat

A major QTL for seed dormancy in wheat was detected in a doubled haploid population on the long arm of 4A using a genetic map constructed with microsatellite markers (Torada et al. 2005). The QTL explained 43.3% of the phenotypic variation

under greenhouse conditions and 13.4% of the phenotypic variation under field conditions. Since pre-harvest sprouting, controlled by seed dormancy, often causes severe damage to grain quality and yield in wheat production regions that are humid during grain ripening, researchers attempted to validate the detected QTL on 4A using the markers flanking the QTL in two independent populations under greenhouse and field conditions. Validation Population A consisted of 96 DH lines and was evaluated twice in the field and once in the greenhouse. The seed dormancy QTL was detected in greenhouse experiment explaining 28.5% of the variation but was only detected in one of the two field trials. The detected QTL explained only 8.5% of the variation. Validation Population B consisted of 67 BC_1F_2 lines and the QTL was detected in the greenhouse experiment explaining 39.0% of the variation. This population was not evaluated under field conditions. Based on these results, the researchers were unsure if this QTL would be detected in the wheat production areas due to the large differences in QTL effects between the greenhouse and the field.

2.6.1.2 Barley

In barley, there have been several attempts to validate the presence, location, and position of putative QTL. Larson et al. (1996) used marker assisted selection backcross to transfer alleles at two yield QTL from Steptoe into Morex, both six-row cultivars. Significant effects were detected for only one QTL. Using the same population, Ramagosa et al. (1999) verified the effects of four yield QTL and found the effects were more consistent for some QTL than for others.

2.6.1.3 Soybeans

Diers et al. (1992) reported none of the QTL identified for iron deficiency chlorosis in soybean could be detected in lines from the same cross not used in the original mapping study. In another study Li et al. (2001) reported that MAS at two QTL conferring resistance to southern root-knot nematode, (*Meloidogyne incognita* (Kofoid and White) Chitwood, was successful in identifying resistant lines in an independently derived soybean population.

2.6.1.4 Maize

In maize, three independent experiments in the same genetic background revealed that the identified QTL were not consistent (Beavis et al. 1994; Beavis 1994). Ajmon-Marsan et al. (1996) evaluated previously identified QTL for grain yield in an independent sample drawn from the same maize population. They found two QTL were consistent with those detected in the previous experiment but two QTL identified in the first sample remained undetected in the independent sample. Two independent samples of a F_2 population were genotyped with RFLP markers to identify QTL for grain yield and other agronomically important traits (Melchinger et al. 1998). In total, 107 QTL were detected from the first sample while only 39 QTL were detected from the second sample. Of the QTL identified, only 20 QTL were common between the two populations. Possible causes for the inconsistencies in the results include population structure, source of parental lines, different sets of environments, and sampling (Beavis 1994).

2.6.1.5 QTL Validation Summary

These inconsistent results demonstrate that while some of the QTL detected in the mapping experiments have repeatable and verifiable effects, others may be spurious, poorly mapped or subject to strong interaction with environmental conditions or other loci. It is therefore important to validate detected QTL prior to designing and implementing marker assisted breeding strategies.

3.0 Molecular mapping of quantitative trait loci for yield and yield components in spring wheat (*Triticum aestivum* L.).

3.1 Abstract

An F_1 derived doubled haploid (DH) population of 402 lines from the adapted spring wheat cross Superb (high yielding) / BW278 (low yielding) was developed to identify quantitative trait loci (QTL) associated with yield and yield components. A subset of the population (186 lines) was evaluated in replicated field trials in 2001 and 2002 at six locations in Manitoba and Saskatchewan, Canada. Agronomic parameters, grain yield and yield components including thousand grain weight, harvest index, average seed weight spike^{-1}, seed number spike^{-1} and spikes number m^{-2} were measured. A genetic map was constructed with 268 microsatellite marker loci and included two morphological genes, reduced plant height, *Rht-B1b,* and the presence/absence of awns, *B1.* Composite interval mapping was conducted to estimate the location and effect of QTL associated with the evaluated traits. A total of 53 QTL were identified on 12 chromosomes for the nine evaluated traits with the coefficient of determination ranging from 0.03 to 0.21 of the total phenotypic variation. The increase in yield and yield components ranged from 4.5 to 17.1% over the population mean. The five grain yield QTL were detected on chromosomes 1A, 2D, 3B, and 5A and showed a combined increase of 34.4%, over the population mean. The alleles from Superb were associated with increased yield for four of the five QTL. This study identified potential chromosome segments for use in marker-assisted selection to improve yield and yield components in spring wheat.

3.2 Introduction

Selection for grain yield has been an important focus of wheat (*Triticum aestivum* L.) breeding programs for decades. Yield is a complex, quantitative trait controlled by a number of genes with low heritability and is significantly influenced by the environment. Quantitative traits provide the greatest challenge for making genetic improvement because plant breeders have little information on the number, location, and contribution of each gene to the final expression of the trait (Koebner and Snape 1999; Mohan et al. 1997). Grain yield can be divided into a number of components including spike number m^{-2}, seed number $spike^{-1}$, and thousand grain weight. Several genes also control yield components however some components are less environmentally sensitive and have higher heritabilities than grain yield (Bezant et al. 1997). Therefore, it is useful to examine yield components when evaluating grain yield to provide specific information about the genetic control and relationship between yield and its components.

Genetic advance in wheat breeding is largely dependent on the variation created by intervarietal hybridization and historically has lead to small, incremental increases in yield of 0.5% per year (Hucl and Baker 1987). Early generation selection is generally not successful for quantitative traits such as grain yield due to low heritabilities and genotype by environment interactions (Bernardo 2002). Progress in molecular marker technology and the development of quantitative trait analysis software have permitted researchers to construct genetic maps in wheat to identify and estimate the effects of quantitative trait loci (QTL) associated with important agronomic traits including yield and its components.

In diploid crop species such as maize, rice, and barley, yield and yield components have been shown to map to coincident chromosome regions within a species (Abler et al. 1991; Xiao et al. 1996; Tinker et al. 1996). Wheat, however, is hexaploid with a large genome, which makes it one of the most complex crops for genetic analysis. As a result, QTL analyses of grain yield and its components in wheat are limited and have generally focused on single chromosomes (Hyne and Snape 1991; Berke et al. 1992). Using recombinant chromosome substitution lines, QTL for yield and yield components have been reported on chromosomes 3A (Shah et al. 1999), 4A (Araki et al. 1999), and 5A (Kato et al. 1999; 2000). Börner et al. (2002) used 114 recombinant inbred lines (RIL) of the International Triticeae Mapping Initiative population and a restriction fragment length polymorphism (RFLP) based map to detect QTL under field and greenhouse conditions for two agronomic and three yield component traits across the entire wheat genome. In total, 56 QTL were identified for the traits of interest when each environment was analyzed separately. Seventeen yield component QTL mapped to chromosomes 2D, 3A, 4A, 5A, and 6B. Börner et al. (2002) also observed coincident QTL for heading, grain number spike^{-1}, thousand grain weight, and grain weight spike^{-1}. Huang et al. (2003) studied seven agronomic, yield and yield component traits in a BC_2F_1 population derived from a cross between a winter wheat and a synthetic wheat line. Using advanced backcross QTL (AB-QTL) analysis, Huang et al. (2003) identified a total of 35 QTL for yield and yield components mapped to chromosomes 1A, 1B, 2A, 2B, 2D, 3B, 4D, 5A, 5B, 5D, 6A, 7A, 7B, and 7D. Coincident QTL were observed for all traits and only six of the reported QTL had LOD scores greater than 3.0. Using a population of RIL derived from a cross between a Chinese wheat, Ning 7840, and an American soft red

winter wheat, Clark, Marza et al. (2005) identified 206 putative QTL for the 15 yield, yield components, and agronomic traits evaluated when each environment was analyzed separately. Grain yield and yield component QTL were detected on chromosomes 1A, 1B, 2B, 3B, 4A, 4B, 5A, 5B, 6B, 7A, and 7D. These three studies implicate 18 chromosomes controlling yield and yield components.

The objectives of this study were to construct a genetic map of the adapted spring wheat cross Superb (high yielding) / BW278 (low yielding) with microsatellite markers and combined with extensive replicated phenotypic data locate and estimate the effects of QTL controlling grain yield. In addition, the association between yield component (thousand grain weight, seed number spike^{-1}, spikes m^{-2}) and agronomic trait (average seed weight spike^{-1}, harvest index, grain filling time, days to heading and days to maturity) QTL that are coincident with each of the grain yield QTL was determined.

3.3 Materials and Methods

3.3.1 Plant Material

A population of 402 doubled haploid (DH) lines was derived from the spring wheat cross Superb/BW278 using the wheat-maize pollination method (Fedak et al. 1997). Superb [Grandin*2/AC Domain] is a high yielding hard red spring wheat cultivar registered by the Agriculture and Agri-Food Canada (AAFC) – Cereal Research Centre in 2000 and has been used extensively as a parent in the spring wheat breeding program at AAFC. BW278 [AC Domain*2/Sumai 3] is a low yielding breeding line with Fusarium head blight (FHB) resistance incorporated from Sumai 3. The population was selected because it was known to be segregating widely for yield, plant height, presence/absense

of awns, leaf spot diseases and FHB. The population of 402 DH lines was divided into two subsets. One subset, referred to as "Mapping Population", consisted of 186 DH lines. These DH lines came from the two F1 plants producing the largest number of DH lines and were used for detailed mapping of QTL associated with grain yield and yield components. The second subset referred to as the "Interval Mapping Population", consisted of the remaining 216 DH lines derived from five F_1's and was used to provide additional grain yield data.

3.3.2 Field trials

The Mapping Population was evaluated in replicated field trials at four locations in Manitoba (Brandon, Portage la Prairie, Morden, and Winnipeg) and two locations in Saskatchewan (Melfort and Scott) in 2001 and 2002. The parents, Superb and BW278, and the hard red spring cultivars Roblin, Katepwa and AC Barrie were also included as checks. The field trials were arranged in a 14x14 lattice design with two replicates. A common seed source was used for all trials in both years. This seed source was derived by forming a seed composite from trials conducted in 2000 at Morden and Portage la Prairie. Plant height (Ht), days to heading (Hdg), days to Maturity (Mat), grain yield (Yld), and thousand grain weight (Tgw) were measured at all locations with the exception of Hdg at Melfort in 2002 and presence/absence of awns was noted at Winnipeg only. Grain filling time was calculated by subtracting days to heading from the days to physiological maturity.

The plots in Winnipeg and Portage la Prairie consisted of 5 rows, 4.27m long, spaced 15.2 cm apart. In Brandon, the plots consisted of 4 rows, 4m long, spaced 22.3

cm apart. The plots in Morden consisted of 5 rows, 4m long and spaced 17.8 cm apart. The plots in Melfort, SK consisted of 7 rows, 4.2m long and spaced 17.8 cm apart while the plots in Scott, SK, consisted on 4 rows, 5m long, and spaced 23 cm apart. All plots were seeded at a rate of 250 seeds m^{-2}. All plots were bordered with a row of fall rye or winter wheat to minimize border effects from neighboring plots. The grain yield from plots was converted to kg ha^{-1} for analysis. Since the Mapping Population segregated for FHB resistance and susceptibility to leaf spot diseases, Tilt® (propiconazole, Syngenta Crop Production) was applied at the flag leaf stage (Zadoks = 39) for leaf spot disease control and Bravo® (chlorothalonil, Syngenta Crop Production) was applied as close to anthesis as possible (Zadoks = 65-69) for FHB control to eliminate any effects these diseases may have on yield and its components. Fungicides were applied at the recommended field rate. Prior to whole-plot harvest, the above-ground biomass of two, 50 cm row segments was hand harvested from the center rows of each plot at the four Manitoba locations, to measure the yield components: harvest index (Hi), average seed weight $spike^{-1}$ (Asw), seed number $spike^{-1}$ (Sns), and spikes $meter^{-2}$ (Sm2) (Table 3.1). Two trials in Saskatchewan were lost due to extreme drought (Melfort, 2001) and an infestation of wheat stem sawfly (*Cephus cinctus*) (Scott, 2002) therefore only 10 site-years of data were available for analysis.

The Interval Mapping Population was grown in a single replicate trial at Brandon, Morden, Portage la Prairie, and Winnipeg, Manitoba in 2001 and 2002. The parents, Superb and BW278, and the hard red spring cultivars Roblin, Katepwa, and AC Barrie

Table 3.1. Summary of the agronomic traits and yield and yield components measured for the Superb/BW278 Mapping Population in 2001 and 2002.

Trait[a]	Abbreviation	Environments Evaluated[b]	Method of Measurement
Plant Height	Ht	B01, B02, Me02, M01, M02, P01, P02, S01, W01, W02	Average plant height measured from the soil surface to tip of spike, excluding awns (cm)
Days to Heading	Hdg	B01, B02, M01, M02, P01, P02, S01, W01, W02	Assessed as the number of days from planting until emergence of 50% of the inflorescences in each plot (days)
Days to Maturity	Mat	B01, B02, Me02, M01, M02, P02, S01, W01, W02	Assessed as the number of days from planting to physiological maturity (days)
Grain Yield	Yld	B01, B02, Me02 , M01, M02, P01, P02, S01, W01, W02	Weight of grain harvested per unit area (kg ha^{-1})
Thousand Grain Weight	Tgw	B01, B02, Me02, M01, M02, P01, P02, S01, W01, W02	Weight of a 1000 grain sample (g)
Harvest Index	Hi	B01, B02, M01, M02, P01, P02, W01, W02	Grain weight from a one meter row / total aboveground biomass harvested from a one meter row
Average Seed Weight $Spike^{-1}$	Asw	B01, B02, M01, M02, P01, P02, W01, W02	Grain weight from a one meter row / number of spikes harvested from a one meter row (g)
Spikes m^{-2}	Sm2	B01, B02, M01, M02, P01, P02, W01, W02	Number of spikes per meter square
Seed Number $Spike^{-1}$	Sns	B01, B02, M01, M02, P01, P02, W01, W02	Seed weight per spike / individual grain weight (no.)
Grain Filling Time	Gft	B01, B02, M01, M02, P01, P02, S01, W01, W02	Period of time between date of heading and date of maturity (days)

[a]Traits Hi, Asw, Sm2, Sns were calculated from the 8 Manitoba locations only.
[b]Environment codes: B = Brandon, MB, M = Morden, MB, Me = Melfort, SK, P = Portage la Prairie, MB, S = Scott, SK, W= Winnipeg, MB, 01 = 2001, 02 = 2002.

were included as checks. The lines were evaluated in yield plots of the same dimension as described above for each location. Plant height and grain yield were the only measurements collected. Presence/absence of awns was noted at Winnipeg only. The 2002 trial in Brandon suffered from excessive moisture and did not emerge uniformly therefore only seven site-years of data were available for analysis.

3.3.3 Statistical Analysis

All traits were analyzed using the procedure MIXED with all effects in the model considered random (site-year, DH line, block, and replicate) (SAS v8.2 SAS Institute Inc., Cary, N.C.). Each site-year was analyzed individually as a separate environment. Further analyses were conducted by combining all years for each site, by combining all sites for each year, and by combining all sites and years. Variance components and best linear unbiased predictors (BLUPs) were obtained by the method of restricted maximum likelihood for all traits and datasets (Littell et al. 1996). The BLUP trait values for each DH line evaluated from each dataset were used for QTL analysis. The broad sense heritability of each trait was estimated from the variance components derived in the PROC MIXED determined as the ratio of genotypic variance to the sum of the genotypic and environmental variance. Pearson correlation coefficients were used to determine the degree of association among the traits of interest.

3.3.4 Construction of the genetic map

Leaf tissue was harvested from a single plant for each DH line and lyophilized for DNA extraction with the Qiagen DNeasy 96 Plant Kit (Qiagen, Mississauga, ON). DNA

was quantified by fluorimetry using Hoechst 33258 stain. Approximately 1,000 microsatellite markers were screened to detect polymorphisms between Superb and BW278. The parents of Superb (Grandin and AC Domain) were also included in the screening to determine the allelic composition of Superb. Genotyping data were obtained using M13 tailing (Schuelke 2000) and fluorescent capillary electrophoresis on an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, Calif., USA) (Somers et al. 2004). Data generated by the ABI3100 was converted to a gel-like image using Genographer (available at http://hordeum.oscs.montana.edu/genographer). Primer sequences for the GWM and GDM microsatellite markers were obtained from Röder et al. (1998) and Pestova et al. (2000). The BARC microsatellite marker sequences were obtained from the USA wheat and barley scab initiative website (http://www.scabusa.org) while the sequences for the WMC, CFA, and CFD microsatellites were obtained from the Grain Genes website (http://wheat.pw.usda.gov).

The polymorphic markers were used to genotype the Mapping Population. The initial map was constructed using MAPMAKER/EXP version 3.0b (Lincoln et al. 1993; Lander et al. 1987) with a minimum LOD of 3.0 and maximum recombination fraction of 0.35. Marker order was tested using the "compare" and "ripple" commands. The map was verified using JoinMap® 3.0 (Stam 1993).

3.3.5 QTL Analysis

Composite interval mapping (CIM) analysis (Zeng 1993, 1994) was performed using Windows QTL Cartographer v2.0 (Wang 2004 http://statgen.ncsu.edu/qtlcart/WQTLCart.htm). Forward stepwise regression with

backward elimination was used to search for QTL and identify cofactors for CIM analysis. The threshold for $p(F_{in})$ and $p(F_{out})$ were set at 0.05, based on the ability to repeatedly detect the same QTL with similar effects across all environments. CIM analysis was completed using the standard model (Model 6). Window size was set at 10 cM and the maximum number of cofactors was used to control the genetic background for each trait. Empirical LOD thresholds were estimated using 1,000 permutations (Churchill and Doerge 1994). A QTL was declared for a trait when the LOD score was greater than the threshold LOD score in a minimum of four environments and was also detected in the site, year, and overall data sets.

QTL analysis for grain yield and yield components revealed major effects from two morphological genes including *Rht-B1b* and the presence/absence of awns, *B1* (Table 3.2). Therefore the phenotypic data for all traits in the Mapping Population was adjusted before CIM analysis using the results of a least squares ANOVA to determine the contribution of awns and height on the final phenotype of the trait and reveal other important QTL.

3.3.6 Interval Mapping

The Interval Mapping Population was genotyped with six to twelve markers from each interval identified in the QTL analysis as being associated with grain yield. BLUP estimates were also obtained for the 216 DH lines using the MIXED procedure with a random effects model. Since these lines were only evaluated in single replicate trials, the BLUP trait values for each line from the seven site-years was averaged to determine the line performance.

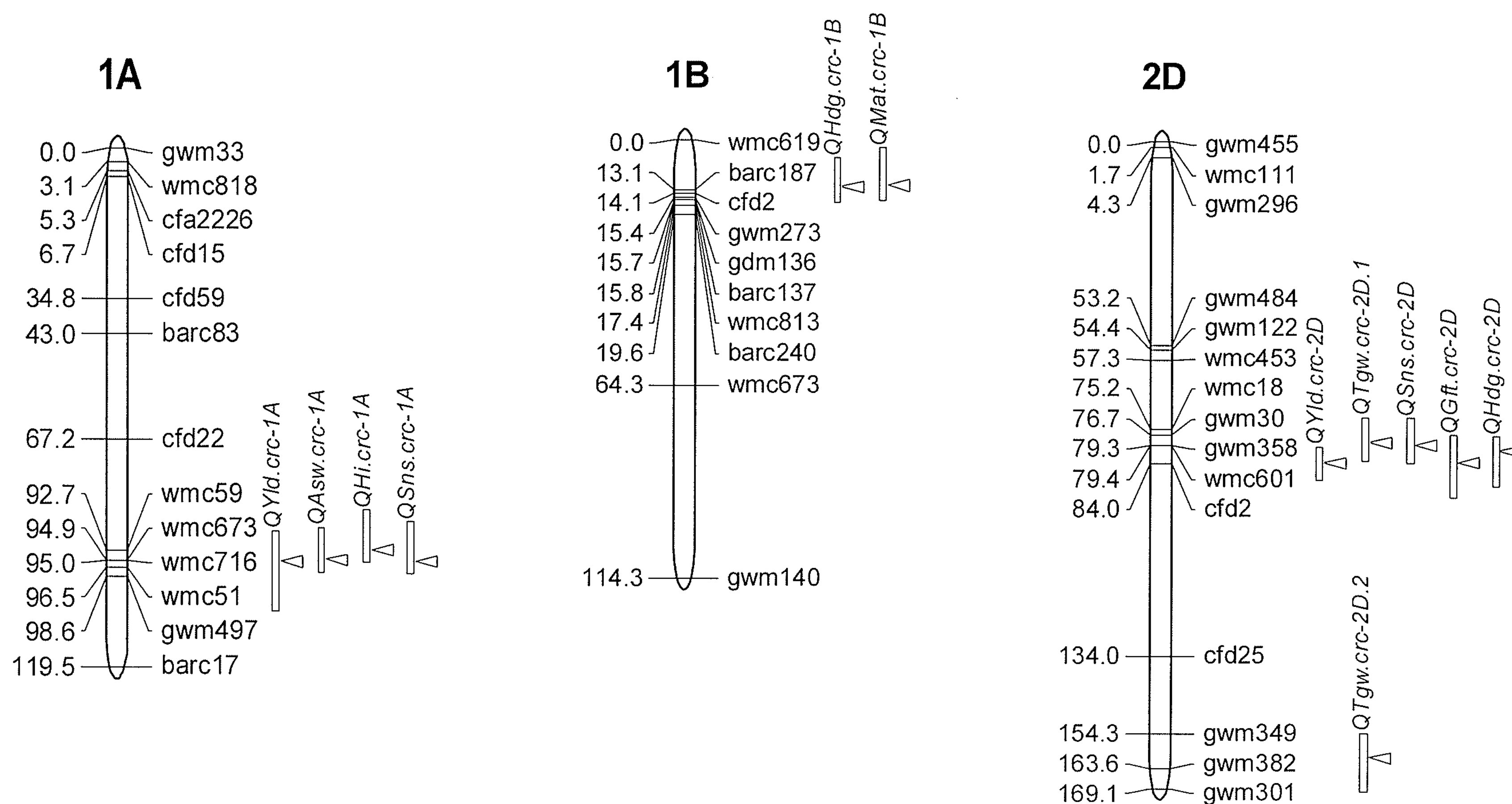
3.4 Results

3.4.1 Quantitative Traits

Frequency distributions were generated for all traits evaluated in the field trials (data not shown). Normal distributions were observed for all traits except for plant height, which showed a bimodal segregation pattern (ratio=1:1, $\chi^2 = 0.36$, $p<0.05$) and was mapped as a Mendelian gene to chromosome 4BS (*Rht-B1b*) (Figure 3.1). The Mapping Population size was reduced to 178 DH lines since plant height for eight lines was intermediate and could not be categorically determined. This population is hereafter referred to as the Mapping Population(178). The effect of *Rht-B1b* and *B1* on all traits evaluated was additive and there were no instances of epitasis between the genes.

To be thorough and independently show the data adjustment was effective, the Mapping Population(178) was divided into eight subpopulations to remove the effects of *Rht-B1b* and *B1* including: short plants; tall plants; awned plants; awnless plants; short-awned; short-awnless; tall-awned; and tall-awnless and QTL analysis was completed for all traits on these subpopulations. When the results of the short and tall subsets were examined, a major effect was still observed from the *B1* locus for all traits ranging from $R^2 = 0.060$ for Asw to 0.295 for Yld. Similarly, when the results of the awned and awnless subsets were examined, a major effect was observed from the *Rht-B1b* locus ranging from $R^2 = 0.07$ for Hdg to 0.24 for Sm2. The subpopulations where both genes were fixed identified the same QTL that were detected for all traits when the Mapping Population(178) was analyzed and none of these QTL were coincident with *Rht-B1b* or *B1* (i.e. effects were removed). These findings provided independent evidence that the data adjustment by the least squares ANOVA was successful in removing the major

Figure 3.1. Genetic linkage map of the thirteen chromosomes and QTL for yield, yield components, and agronomic traits in the Superb/BW278 spring wheat cross. The QTL with solid bars on chromosomes 4B and 5A were detected in the Mapping Population. QTL indicated with open bars were detected in the Mapping Population(178) by accounting for the effects of the morphological genes controlling the presence/absence of awns, *B1,* and plant height, *Rht-B1b.* The LOD peak of each QTL is indicated by an arrowhead and the length of the bars indicate a 1.0 LOD drop in the QTL confidence interval. Map distances are indicated on the left of each chromosome in Kosambi centimorgan.



3A

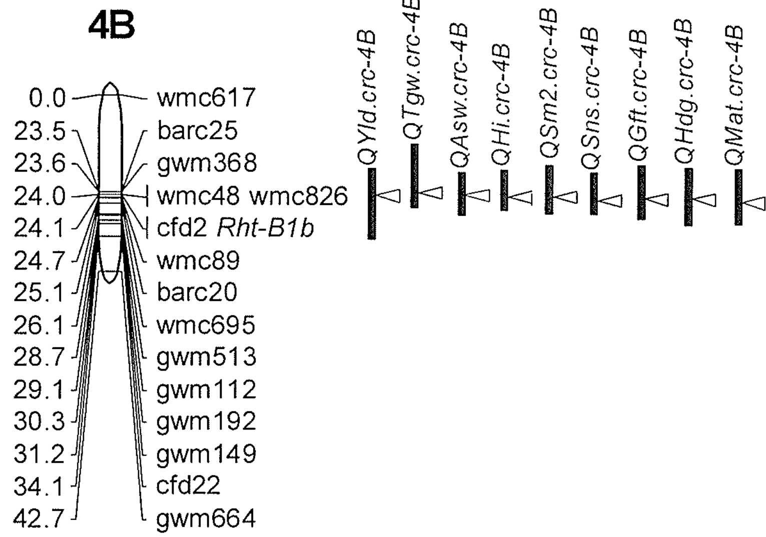
cM	Marker
0.0	wmc388
2.6	gwm2
4.9	wmc505
8.5	gwm666
9.5	wmc664
10.4	wmc640
12.4	gwm133
15.8	cfa2134
17.9	wmc695

QTLs: *QHi.crc-3A*, *QGft.crc-3A*, *QHdg.crc-3A*

3B

cM	Marker
0.0	barc75
0.3	barc180
3.1	wmc430
6.2	gwm533
56.2	cfd28
63.4	wmc679
70.4	wmc51
70.5	wmc43
75.5	barc173
77.2	gwm284
77.9	wmc505
78.2	barc68
79.2	cfd6
80.0	wmc625
80.3	gwm274
80.5	barc73
82.0	wmc544
87.4	cfa2134
94.1	barc164
96.5	wmc827
97.1	wmc418
98.9	barc145
103.6	gwm4
106.8	cfd283
111.9	barc229
117.5	cfa2170
130.0	wmc326
133.5	barc77
183.5	gwm247 gwm340
185.2	gdm132

QTLs: *QYld.crc-3B*, *QTgw.crc-3B.2*, *QTgw.crc-3B.1*, *QAsw.crc-3B*, *QHi.crc-3B*, *QSm2.crc-3B*, *QSns.crc-3B*, *QMat.crc-3B*


4B
0.0
23.5
23.6
24.0
24.1
24.7
25.1
26.1
28.7
29.1
30.3
31.2
34.1
42.7
wmc617
barc25
gwm368
wmc48 wmc826
cfd2 Rht-B1b
wmc89
barc20
wmc695
gwm513
gwm112
gwm192
gwm149
cfd22
gwm664
QYld.crc-4B
QTgw.crc-4B
QAsw.crc-4B
QHi.crc-4B
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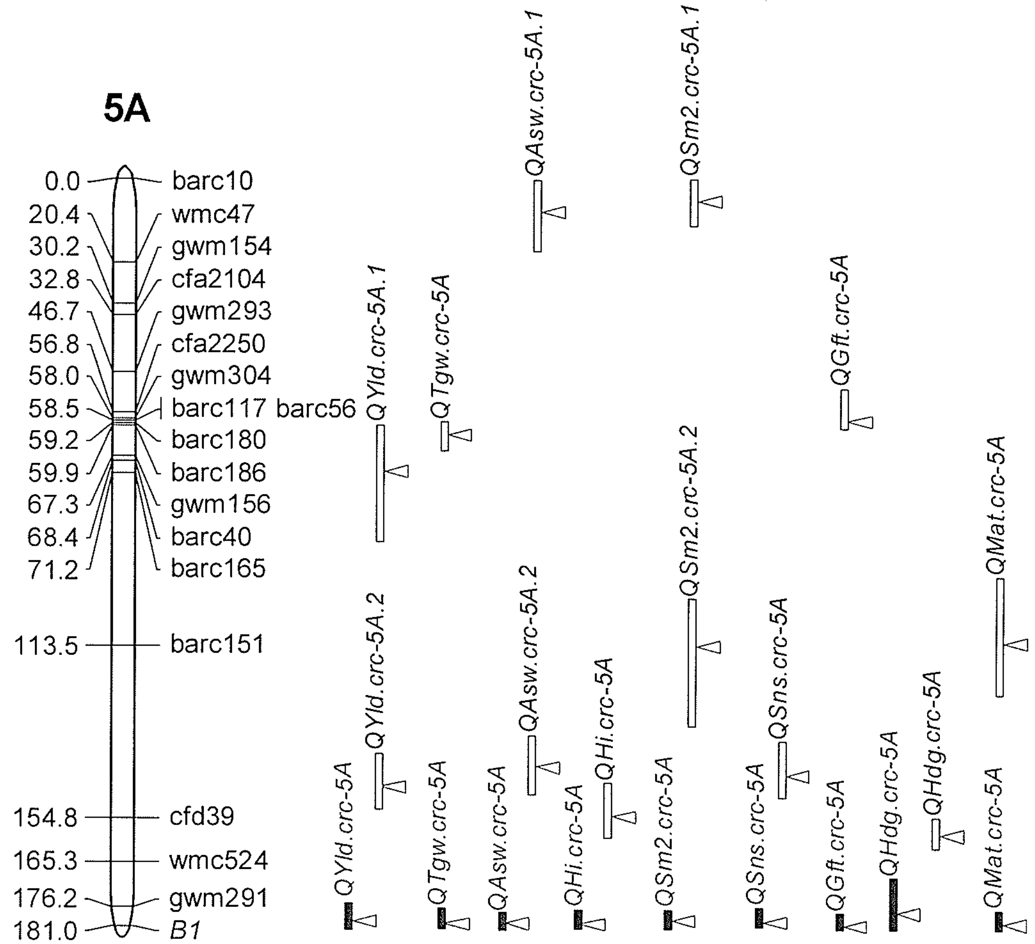
QGft.crc-4B
QHdg.crc-4B
QMat.crc-4B


5A
0.0
20.4
30.2
32.8
46.7
56.8
58.0
58.5
59.2
59.9
67.3
68.4
71.2
113.5
154.8
165.3
176.2
181.0
barc10
wmc47
gwm154
cfa2104
gwm293
cfa2250
gwm304
barc117 barc56
barc180
barc186
gwm156
barc40
barc165
barc151
cfd39
wmc524
gwm291
B1
QAsw.crc-5A.1
QSm2.crc-5A.1
QYld.crc-5A.1
QTgw.crc-5A
QGft.crc-5A
QSm2.crc-5A.2
QMat.crc-5A
QYld.crc-5A.2
QAsw.crc-5A.2
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QHdg.crc-5A
QYld.crc-5A
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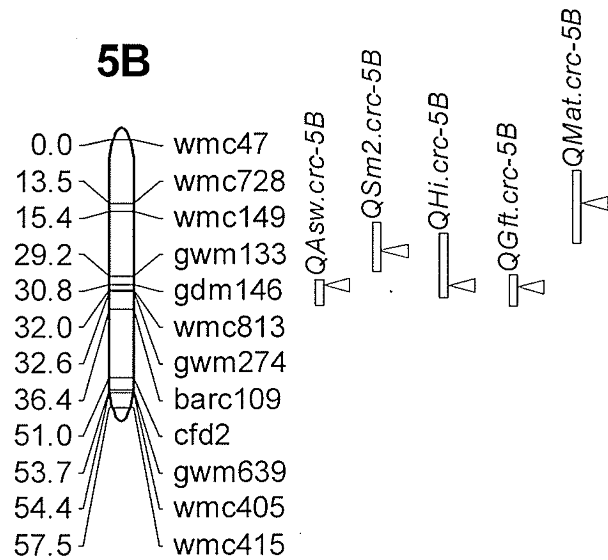
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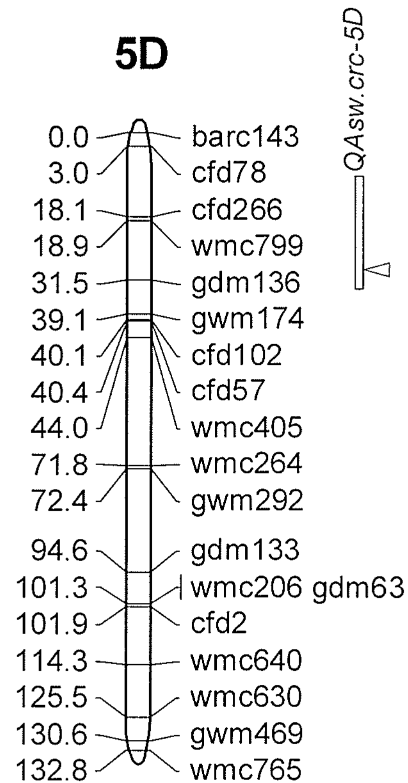
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71.8 wmc264
72.4 gwm292
94.6 gdm133
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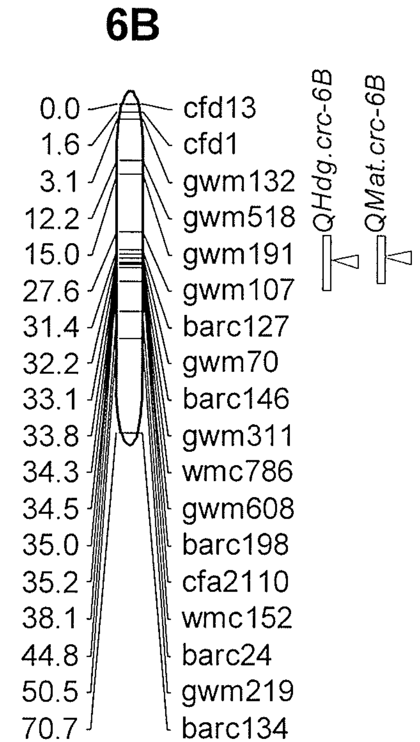
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33.1 barc146
33.8 gwm311
34.3 wmc786
34.5 gwm608
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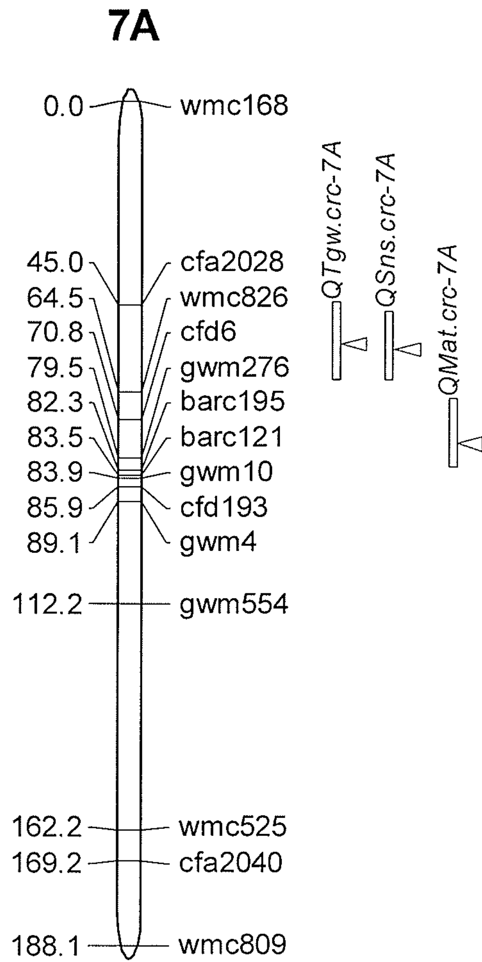
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85.9 cfd193
89.1 gwm4
112.2 gwm554
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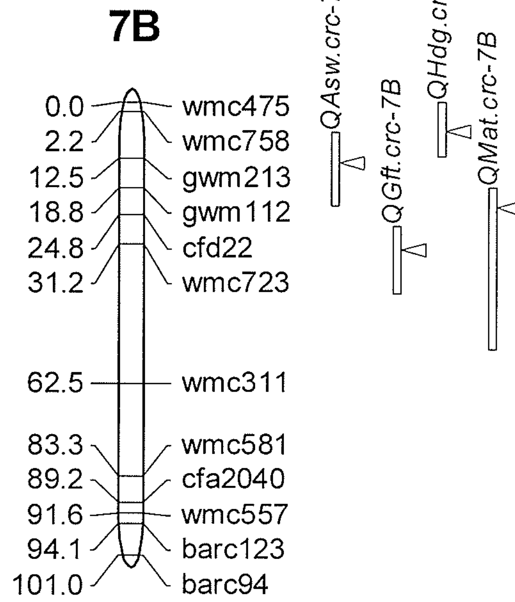
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83.3 wmc581
89.2 cfa2040
91.6 wmc557
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QHdg.crc-7B
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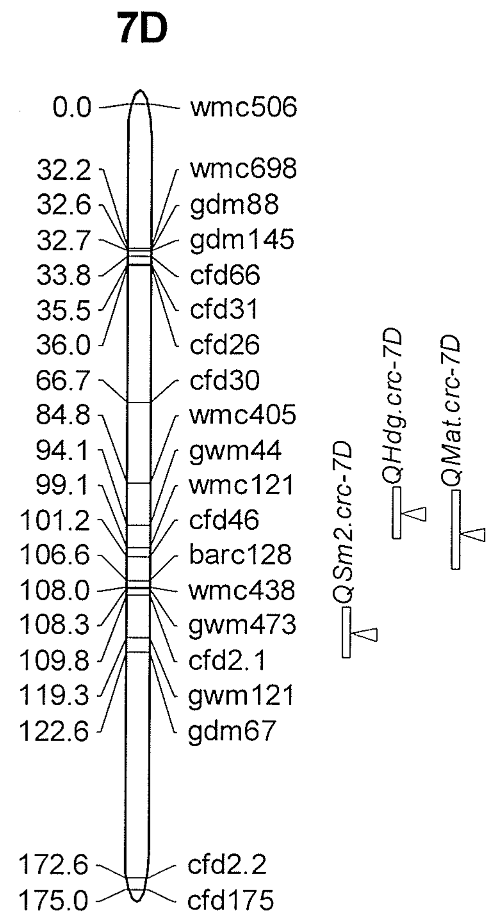
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66.7 cfd30
84.8 wmc405
94.1 gwm44
99.1 wmc121
101.2 cfd46
106.6 barc128
108.0 wmc438
108.3 gwm473
109.8 cfd2.1
119.3 gwm121
122.6 gdm67
172.6 cfd2.2
175.0 cfd175
QSm2.crc-7D
QHdg.crc-7D
QMat.crc-7D

effects from the *Rht-B1b* and *B1* loci and permitted the identification of QTL for all traits in this study.

The phenotypic data indicated there was significant genetic variation for all traits in the Superb/BW278 population as well as considerable differences between the parents of the cross (Table 3.3). Transgressive segregants on both ends of the distribution were observed for all traits except Tgw and Asw in the Mapping Population(178). Estimates of broad sense heritability across environments and years were intermediate (H^2 = 0.48-0.58) for Yld, Hi, Sns, Gft, Hdg and Mat while the estimates for Tgw, Asw, and Sm2 were high (H^2 = 0.77-0.98). Correlations were calculated for all traits evaluated in the Superb/BW278 population (Table 3.4) and the association between yield and most of the yield components was highly significant (P<0.001).

3.4.2 Genetic Map

The genetic map was constructed with 268 microsatellite loci and spanned approximately 1,822 cM across 20 chromosomes. Chromosome 4A was monomorphic at all markers tested (data not shown). Figure 3.1 shows only the 13 chromosomes carrying QTL associated with yield and yield components and the *Rht-B1b* (Börner et al. 1997) and *B1* (Kato et al. 2000) loci. See Appendix 1 for the genetic maps of the remaining seven chromosomes.

3.4.3 Effect of *Rht-B1b* and *B1* genes

Superb alleles for reduced plant height and the presence of awns resulted in an increase in yield, most of the yield components, and agronomic traits. Exceptions to this

Table 3.2. Effects of the *Rht-B1b* and *B1* genes on yield, yield component and agronomic traits measured in the Superb/BW278 Mapping Population based on BLUP trait values across all environments.

Trait [a]	Gene	LOD	R^2	Additive [b]	Positive Allele	Increase [c] (%)	Environments observed / total
Yld	*Rht-B1b*	9.6	0.076	110.6 kg ha^{-1}	Superb	4.0	8/10
Yld	*B1*	19.2	0.186	178.4 kg ha^{-1}	Superb	6.0	9/10
Tgw	*Rht-B1b*	6.3	0.177	1.3 g	BW278	4.6	8/10
Tgw	*B1*	4.0	0.068	1.1 g	Superb	4.0	8/10
Asw	*Rht-B1b*	6.9	0.075	0.026 g	BW278	4.8	5/8
Asw	*B1*	12.2	0.123	0.039 g	Superb	6.2	7/8
Hi	*Rht-B1b*	14.2	0.118	0.016 g	Superb	4.9	6/8
Hi	*B1*	10.8	0.089	0.007	BW278	2.1	5/8
Sm2	*Rht-B1b*	19.4	0.172	43.0	Superb	6.3	8/8
Sm2	*B1*	8.4	0.083	31.7	BW278	4.6	4/8
Sns	*Rht-B1b*	3.1	0.052	1.0	BW278	5.3	6/8
Sns	*B1*	10.5	0.158	1.3	Superb	6.9	6/8
Gft	*Rht-B1b*	11.4	0.110	0.68 days	Superb	1.8	4/7
Gft	*B1*	12.3	0.121	0.69 days	Superb	2.0	6/7
Hdg	*Rht-B1b*	5.5	0.072	0.8 days	BW278	1.4	7/9
Hdg	*B1*	8.7	0.132	1.1 days	Superb	2.0	8/9
Mat	*Rht-B1b*	14.0	0.115	1.0 days	BW278	1.1	7/9
Mat	*B1*	9.2	0.078	0.7 days	Superb	0.8	7/9

[a]Yld=yield, Tgw=thousand grain weight, Asw=average seed weight $spike^{-1}$, Hi=harvest index, Sm2= spikes m^{-2}, Sns= seed number $spike^{-1}$,Gft=grain filling time, Hdg=days to heading, Mat=days to maturity.
[b]Increase (%) represents the improvement in the trait over the mean of the Mapping Population.
[c]Additive effect of allele substitution.

Table 3.3. Mean and range of the adjusted BLUP trait values across all environments for the nine yield, yield components, and agronomic traits measured on the Superb/BW278 Mapping Population(178).

	Parents		DH Population			
Trait[a]	Superb	BW278	Mean	Min	Max	Heritability[b]
Yld	3756.7	3178.8	3348.1	2291.1	4285.8	0.48
Tgw	39.0	24.6	28.4	20.0	36.4	0.77
Asw	0.730	0.311	0.530	0.253	0.723	0.97
Hi	0.360	0.321	0.333	0.233	0.406	0.50
Sm2	637.0	817.8	682.3	445.3	972.6	0.98
Sns	18.7	12.6	18.7	8.3	25.3	0.58
Gft	33.7	32.5	33.6	29.8	36.3	0.52
Hdg	51.9	54.3	53.3	46.8	57.8	0.49
Mat	86.8	87.3	87.8	80.8	93.3	0.48

[a]Yld=yield, Tgw=thousand grain weight, Asw=average seed weight spike^{-1}, Hi=harvest index, Sm2= spikes m^{-2}, Sns= seed number spike^{-1},Gft=grain filling time, Hdg=days to heading, Mat=days to maturity.
[b]Heritability was estimated from the variance components for the 186 DH lines of the Superb/BW278 Mapping Population ($H^2 = \sigma^2_g$ / $\sigma^2_g + \sigma^2_{ge/e} + \sigma^2_{e/re}$).

Table 3.4. Correlations coefficients of adjusted BLUP trait values across all environments between yield, yield components, and agronomic traits measured on the Superb/BW278 Mapping Population(178).

Trait[a]	Yld	Tgw	Asw	Hi	Sm2	Sns	Gft	Hdg
Tgw	0.30***							
Asw	0.62***	0.50***						
Hi	0.59***	0.14ns	0.68***					
Sm2	-0.05ns	-0.49***	-0.61***	0.08ns				
Sns	0.60***	-0.08ns	0.82***	0.66***	-0.35***			
Gft	-0.16**	0.01ns	-0.10*	0.11ns	0.04ns	-0.01ns		
Hdg	-0.39***	-0.06ns	-0.30***	-0.44***	0.04ns	-0.38***	0.03ns	
Mat	-0.23**	-0.11ns	-0.34***	-0.31***	0.23***	-0.30***	-0.04ns	0.69***

[a]Yld=yield, Tgw=thousand grain weight, Asw=average seed weight spike^{-1}, Hi=harvest index, Sm2= spikes m^{-2}, Sns= seed number spike^{-1},Gft=grain filling time, Hdg=days to heading, Mat=days to maturity.
*, **, ***, and ns = significant at P <0.05, P <0.01, P <0.001, and not significant, respectively.

were observed for Tgw and Asw at the *Rht-B1b* locus and Hi, Sm2, and Sns at the *B1* locus (Table 3.2). The LOD scores for yield and yield components ranged from 3.1 to 19.4 at the *Rht-B1b* locus and explained 5.2 to 17.7% of the phenotypic variation in the Mapping Population. The LOD scores for yield and yield components at the *B1* locus in the Mapping Population ranged from 4.0 to 19.2 and explained 6.8 to 18.6% of the phenotypic variation.

When the Interval Mapping Population was analyzed, significant effects were also observed at the *Rht-B1b* and *B1* loci explaining 6.0 and 16.4 % of the phenotypic variation in yield at the *Rht-B1b* and *B1* loci, respectively (data not shown). The phenotypic data for grain yield in the Interval Mapping Population was also adjusted using the results of a least squares ANOVA. The Interval Mapping Population size was reduced from 216 to 193 DH lines since plant height for 16 lines was intermediate and could not be categorically determined as well the presence/absence of awns data was missing for 7 DH lines. This population is hereafter referred to as the Interval Mapping Population(193). The Mapping Population and Interval Mapping Population were combined to determine the effects of *Rht-B1b* and *B1* loci on grain. The *Rht-B1b* locus explained 6.7% of the phenotypic variation for yield while the *B1* locus explained 15.3% of the phenotypic variation for yield. A yield increase of 3.4% over the population mean for genotypes carrying the Superb *Rht-B1b* allele was observed while a 6.4% increase in yield was observed over the population mean for genotypes carrying the Superb *B1* allele. Comparable results are shown in Table 3.2 for the Mapping Population.

3.4.4 QTL Mapping

QTL analysis of the Mapping Population(178) detected 53 repeatable QTL across environments for grain yield, yield components and the agronomic traits that were not coincident with either *Rht-B1b* or *B1*. (Table 3.5, Figure 3.1). Five QTL were identified on four chromosomes, 1A, 2D, 3B and 5A, for grain yield with each QTL explaining between 4.1 and 20.4% of the phenotypic variation. LOD scores for the observed yield QTL ranged from 3.7 to 12.6 while the mean increase in yield of the genotypes carrying the positive allele ranged from 5.3 to 10.1% over the population mean. These five QTL were also measured in the Interval Mapping Population(193) with LOD scores between 3.1 and 9.9 and accounted for 3.9 to 17.4% of the phenotypic variation observed. The DH lines from the Mapping Population(178) and Interval Mapping Population(193) were combined for QTL analysis. The same five yield QTL on chromosomes 1A, 2D, 3B, and 5A, were observed. LOD scores for yield in the combined populations ranged between 3.1 and 9.9 while each QTL explained between 3.5 and 20.4% of the phenotypic variation (data not shown).

Superb alleles were associated with an increase in yield at four QTL identified on chromosomes 2D, 3B, and 5A while BW278 was the positive allele at the yield QTL on chromosome 1A (Figure 3.1, Table 3.5). The allele present at each of the yield QTL was determined for the top ten highest yielding lines in the entire population (Table 3.6). Eight of the 10 highest yielding lines had Superb alleles at the four QTL on chromosomes 2D, 3B, and 5A and BW278 alleles were observed at the yield QTL on chromosome 1A. The other two lines had BW278 alleles instead of Superb at the QTL on chromosome 3B (Table 3.6).

Table 3.5. Summary of QTL detected in the Superb/BW278 Mapping Population(178) for yield, yield components, and agronomic traits based on adjusted BLUP trait values across all environments.

QTL (LOD Threshold[a])	Marker	Allele Size (bp)		LOD	R^2	Additive[c]	Positive Allele	%Increase[d]	Environments Observed / total
		Superb	BW278						
Yld[b] (3.00)									
QYld.crc-1A	WMC716	137	165	6.1	0.066	219.9 kg ha^{-1}	BW278	6.6	7/10
QYld.crc-2D	CFD2	231	229	3.7	0.042	178.4 kg ha^{-1}	Superb	5.3	8/10
QYld.crc-3B	WMC544	126	null	4.5	0.041	197.6 kg ha^{-1}	Superb	5.9	5/10
QYld.crc-5A.1	GWM156	336	339	5.1	0.050	197.8 kg ha^{-1}	Superb	5.9	9/10
QYld.crc-5A.2	CFD39	190	171	12.6	0.204	340.2 kg ha^{-1}	Superb	10.1	9/10
Tgw (3.30)									
QTgw.crc-2D.1	WMC601	223	211	5.1	0.047	1.3 g	Superb	4.6	9/10
QTgw.crc-2D.2	GWM382	85	null	5.6	0.055	1.4 g	Superb	4.9	9/10
QTgw.crc-3B.1	GWM284	116	122	7.5	0.076	1.8 g	Superb	6.3	10/10
QTgw.crc-3B.2	CFA2170	153	158	5.0	0.047	1.4 g	Superb	4.9	10/10
QTgw.crc-5A	BARC186	196	211	10.2	0.107	2.1 g	Superb	7.4	9/10
QTgw.crc-7A	WMC826	262	258	3.5	0.050	1.5 g	Superb	5.3	8/10
Asw (3.18)									
QAsw.crc-1A	WMC716	137	165	7.0	0.067	0.051 g	BW278	9.7	4/8
QAsw.crc-3B	CFD6	null	323	7.9	0.096	0.063 g	Superb	11.9	8/8
QAsw.crc-5A.1	BARC10	299	null	6.0	0.080	0.055 g	Superb	10.4	7/8
QAsw.crc-5A.2	CFD39	190	171	12.5	0.209	0.091 g	Superb	17.1	7/8
QAsw.crc-5B	WMC813	247	243	4.4	0.058	0.046 g	Superb	8.7	6/8
QAsw.crc-5D	GDM136	160	169	3.0	0.029	0.029 g	Superb	5.5	4/8
QAsw.crc-7B	GWM213	null	117	3.1	0.030	0.032 g	Superb	6.0	4/8

Hi (3.08)									
QHi.crc-1A	WMC673	null	124	8.9	0.081	0.019	BW278	5.7	5/8
QHi.crc-3A	GWM666	101	99	7.8	0.070	0.017	Superb	5.1	5/8
QHi.crc-3B	BARC173	null	348	5.0	0.042	0.015	Superb	4.5	4/8
QHi.crc-5A	CFD39	190	171	12.1	0.119	0.028	Superb	8.4	6/8
QHi.crc-5B	GDM146	178	208	9.9	0.112	0.022	Superb	6.6	5/8
Sm2 (3.01)									
QSm2.crc-3B	CFD283	null	268	10.3	0.102	69.1	BW278	10.0	6/8
QSm2.crc-5A.1	BARC10	299	null	6.2	0.079	55.3	BW278	8.8	4/8
QSm2.crc-5A.2	BARC151	224	237	4.6	0.049	41.7	BW278	6.6	4/8
QSm2.crc-5B	GWM133	191	150	3.3	0.027	34.9	BW278	5.6	5/8
QSm2.crc-7D	GWM121	326	324	6.5	0.060	47.5	Superb	7.0	5/8
Sns (3.03)									
QSns.crc-1A	WMC673	null	124	6.8	0.071	1.7	BW278	9.0	5/8
QSns.crc-2D	WMC601	223	211	5.1	0.054	1.7	BW278	9.0	5/8
QSns.crc-3B	BARC173	null	348	4.3	0.042	1.3	Superb	7.0	4/8
QSns.crc-5A	CFD39	190	171	8.6	0.160	2.4	Superb	12.8	7/8
QSns.crc-7A	WMC826	262	258	7.4	0.076	1.7	BW278	9.0	5/8
Gft (3.11)									
QGft.crc-2D	CFD2	231	229	3.4	0.039	0.70 days	Superb	2.0	5/7
QGft.crc-3A	GWM666	101	99	4.8	0.053	0.79 days	BW278	2.3	5/7
QGft.crc-5A	BARC180	192	203	4.6	0.051	0.77 days	BW278	2.2	5/7
QGft.crc-5B	GDM146	178	208	4.9	0.054	0.81 days	Superb	2.3	5/7
QGft.crc-7B	WMC723	209	197	6.3	0.072	1.01 days	Superb	2.9	6/7
Hdg (3.09)									
QHdg.crc-1B	BARC187	258	253	5.2	0.045	0.8 days	BW278	1.4	5/9
QHdg.crc-2D	WMC601	223	211	8.2	0.070	1.0 days	BW278	1.7	6/9

QHdg.crc-3A	GWM133	null	87	10.6	0.095	1.1 days	Superb	2.0	7/9
QHdg.crc-5A	CFD39	190	171	13.7	0.146	1.4 days	Superb	2.6	9/9
QHdg.crc-6B	BARC146	132	128	8.5	0.073	1.0 days	Superb	1.7	9/9
QHdg.crc-7B	WMC758	225	233	3.3	0.031	0.6 days	Superb	1.1	5/9
QHdg.crc-7D	CFD46	185	182	5.9	0.058	0.9 days	Superb	1.6	5/9
Mat (2.50)									
QMat.crc-1B	BARC187	258	253	3.2	0.028	0.8 days	BW278	0.9	4/9
QMat.crc-3B	WMC827	222	208	5.1	0.045	1.0 days	Superb	1.1	4/9
QMat.crc-5A	BARC151	224	237	5.8	0.055	1.2 days	BW278	1.3	7/9
QMat.crc-5B	WMC149	175	178	11.6	0.118	1.7 days	Superb	1.8	8/9
QMat.crc-6B	BARC146	132	128	14.9	0.168	2.0 days	Superb	2.2	8/9
QMat.crc-7A	GWM276	83	132	2.6	0.028	0.8 days	BW278	0.9	4/9
QMat.crc-7B	CFD22	237	239	9.5	0.119	1.6 days	Superb	1.8	4/9
QMat.crc-7D	GWM44	175	181	3.6	0.035	0.9 days	Superb	1.0	4/9
Yld[e]									
QYld.crc-1A	WMC716	137	165	5.1	0.059	187.92 kg ha^{-1}	BW278	5.5	n/a
QYld.crc-2D	CFD2	231	229	3.1	0.039	138.43 kg ha^{-1}	Superb	4.0	n/a
QYld.crc-3B	WMC544	126	null	3.5	0.044	157.13 kg ha^{-1}	Superb	4.6	n/a
QYld.crc-5A.1	GWM156	336	339	4.2	0.048	168.63 kg ha^{-1}	Superb	4.9	n/a
QYld.crc-5A.2	CFD39	190	171	9.9	0.174	317.42 kg ha^{-1}	Superb	9.3	n/a

[a]LOD thresholds were estimated in QTL Cartographer v2.0 using 1000 permutations.
[b]Yld=yield, Tgw=thousand grain weight, Asw=average seed weight spike^{-1}, Hi=harvest index, Sm2= spikes m^{-2}, Sns= seed numberspike^{-1},Gft=grain filling time, Hdg=days to heading, Mat=days to maturity.
[c]Additive effect of allele substitution.
[d]Increase (%) represents the improvement in the trait over the mean of the population
[e]Yld QTL detected in the combined mapping population(178) + interval mapping population(193) using interval mapping

Table 3.6. Alleles present at the five yield QTL of the top ten yielding DH lines based on BLUP trait values across all environments in the Superb/BW278 Mapping Population.

DH Line	Yield (kg ha^{-1})	*QYld.crc-1A* (BW278)[a]	*QYld.crc-2D* (Superb)	*QYld.crc-3B* (Superb)	*QYld.crc-5A.1* (Superb)	*QYld.crc-5A.2* (Superb)	*B1* (Superb)	*Rht-B1b*	%Increase[b]
A75	4285.75	BW278	Superb	Superb	Superb	Superb	BW278	Superb	28.0
B73	4268.27	BW278	Superb	Superb	Superb	Superb	Superb	Superb	27.5
B143	4102.58	BW278	Superb	Superb	Superb	Superb	BW278	Superb	22.5
A45	4101.37	BW278	Superb	Superb	Superb	Superb	BW278	Superb	22.5
B66	4018.45	BW278	Superb	BW278	Superb	Superb	Superb	Superb	20.0
A44	3959.73	BW278	Superb	Superb	Superb	Superb	Superb	Superb	18.3
B99	3942.19	BW278	Superb	Superb	Superb	Superb	BW278	Superb	17.7
B139	3918.55	BW278	Superb	Superb	Superb	Superb	BW278	Superb	17.0
B25	3914.69	BW278	Superb	Superb	Superb	Superb	Superb	Superb	16.9
B28	3913.75	BW278	Superb	BW278	Superb	Superb	Superb	Superb	16.9

[a]Allele associated with increased yield at each identified QTL
[b]Increase (%) represents the observed improvement in yield over the mean of the population.

For the yield components examined in this study, six QTL were detected for Tgw and five QTL were detected for Sm2 and Sns (Table 3.5, Figure 3.1). The LOD scores for the yield component QTL ranged between 3.3 and 10.3 with each QTL explaining between 2.7 and 16.0% of the phenotypic variation. Seven QTL were identified for Asw and five QTL were observed for Hi that explained between 3.0 and 20.9% of the observed phenotypic variation. Seven, eight, and five QTL were detected for Hdg, Mat, and Gft, respectively (Table 3.5, Figure 3.1).

3.5 Discussion

The present study identified QTL controlling yield, yield components, and agronomic traits in a spring wheat DH population. Unlike other studies, the traits examined were evaluated in disease free conditions to accurately assess the yield potential of each line in the DH population. In similar studies conducted by Börner et al. (2002), Marza et al. (2005), and Kuchel et al. (2007) the RIL and DH populations were affected by leaf rust, yellow rust, stripe rust, FHB, powdery mildew, and barley yellow dwarf virus. The occurrence of these diseases facilitated identification of QTL for disease resistance in each population, however, the yield potential of each line was likely compromised and the estimates for the yield and yield component QTL detected in these studies may have been affected.

There are very few reports of QTL controlling grain yield, yield components, and agronomic traits covering the whole genome of hexaploid wheat. QTL shown to be associated with grain yield across multiple environments and/or years are less common. Previous yield QTL studies have evaluated crosses between synthetic wheat (Börner et al.

2002; Huang et al. 2003, 2004; Kumar et al. 2007), Chinese x American wheat (Marza et al. 2005), Canadian wheat (Huang et al. 2006) and Australian cultivars (Kuchel et al. 2007) in order to map the effects of yield QTL. The DH population evaluated in this study was suitable to identify and estimate the effects of QTL for yield, yield components, and agronomic traits since it was derived from a cross between locally bred, adapted (i.e. acceptable maturity) lines differing in yield. The performance of the parents as well as the range of values observed for the DH population indicated there was substantial genetic variation for all traits evaluated (Table 3.3).

In the Superb/BW278 DH population, two morphological genes, *Rht-B1b* and *B1*, segregated and had significant effects on all traits evaluated in this study (Table 3.2). *Rht-B1b* maps to 4BS and is a gibberellic acid insensitive dwarfing gene derived from 'Norin10' (Gale and Youssefian 1985). The source of the *Rht-B1b* allele in Superb is from the Grandin parent and was confirmed by PCR (Ellis et al. 2002). These findings are supported by Kato et al. (2000) where AC Domain had tall alleles at the *Rht-B1b* locus in the cross AC Domain/Haruyutaka.

Significant increases in Yld, Sm2, Sns and significant decreases in grain weight and plant biomass have been observed in semidwarf wheats (Law et al. 1978; Gale and Youssefian 1985; Brandle and Knott 1986; Uddin and Marshall 1989; Allan 1989; Kuchel et al. 2007). Chapman et al. (2007) compared the grain yield of six near isogenic pairs of semidwarf and tall spring wheat lines and reported the average yield advantage of the semidwarf lines was 6%. The current study also observed an increase in Yld and Sm2 as well as a decrease in kernel weight associated with the dwarfing alleles at *Rht-B1*

(Table 3.2). Knott (1986) observed that semidwarf alleles were associated with earliness as was observed in this study.

In other studies, the presence of awns increased yield and grain weight (Goulden and Neatby 1929; Atkins and Norris 1955; Patterson et al. 1962; Weyhrich et al. 1994). The current study also observed significant increases in Yld and Tgw when awns were present (Table 3.2). McNeal et al. (1969) reported a yield increase of nearly 7% at one location in awned wheat lines compared to awnless. This result is similar to the 6% yield increase over all site-years observed in this study and is in agreement with data reported by Suneson and Ramage (1962) and with the response predicted by Grundbacher (1963). Grain yield differences between awned and awnletted lines of hard red spring wheat were inconsistent among locations in Canada; however, kernel weight of the awned lines was significantly higher at all locations (Knott, 1986). In contrast, McKenzie (1972) observed lower yields and kernel weights of awned spring wheat when compared with awnless spring wheats in southern Alberta. McNeal et al. (1969) reported an awned population took significantly longer to head than the awnless populations at all locations evaluated. The current study observed Hdg increased one day in the awned DH lines and Mat was 0.7 days earlier than the awnless DH lines. Knott (1986) also observed awned lines were earlier to head and mature than awnletted lines.

The present study identified five major Yld QTL on four chromosomes and in all instances the Yld QTL were consistent across the environments evaluated and coincident with QTL for at least one yield component. Significant correlations were observed between Yld and most of the yield components suggesting pleiotropy and /or coincidence with these QTL (Table 3.4; Figure 3.1). The yield components, Asw and Sns, were most

frequently associated with the grain yield QTL while Sm2 was the least coincident yield component. This was consistent with the highly significant correlations observed between Yld, Asw, and Sns and no observed correlation between Yld and Sm2 (Table 3.4). Quarrie et al. (2005) also observed similar relationships between yield, grain number spike^{-1}, and number of spikes plant^{-1} in a cross between Chinese Spring and an experimental breeding line. Huang et al. (2003; 2004) did not observe any positive correlations between yield and its components and in most cases, the QTL identified for yield and yield components mapped independently. In other studies, significant correlations and coincident QTL were observed between grain yield and the evaluated yield components (Kato et al. 2000; Borner et al. 2002; Quarrie et al. 2005; Huang et al. 2006; Kuchel et al. 2007; Kumar et al. 2007).

In previous studies, grain yield QTL are reported on all chromosomes with the exception of chromosomes 3D and 5D (Borner et al. 2002; Huang et al. 2003; 2004; 2006; Marza et al. 2005; McCartney et al. 2005; Quarrie et al. 2005; Kuchel et al. 2007; Kumar et al. 2007). Most of these studies have identified a large number of grain yield QTL however, the majority of these QTL were only detected in a single environment. When a QTL was detected in more than one environment, variation in the magnitude of its effects was typically observed (Huang et al. 2003; 2004; Kumar et al. 2007; Kuchel et al. 2007).

The most significant QTL identified for grain yield in the current study was located on chromosome 5AL proximal to *B1* (Figure 1). Chromosome 5A is known to carry a number of major genes affecting productivity and adaptability and several studies have reported the most repeatable Yld QTL are located on chromosome 5AL in a similar

position to *QYld.crc-5A.2* (Kato et al. 2000; Huang et al. 2004; Marza et al. 2005; Quarrie et al. 2005). The presence of the Superb allele at *QYld.crc-5A.2* increased Yld, Asw, Hi, Sns and decreased Sm2 suggesting the increase in Yld contributed by *QYld.crc-5A.2* is the result of an increased number of heavier seeds spike^{-1} produced on fewer spikes m^{-2} (Table 3.5). Quarrie et al. (2005) also observed this Yld QTL was coincident with grain number spike^{-1}, number of spikes plant^{-1}, and grain weight.

There were 17 recombinants identified as awnless (BW278 allele) and carrying Superb alleles at *QYld.crc-5A.2.* These recombinants had an average yield of 3585 kg ha^{-1} with yields ranging between 2792 to 4285 kg ha^{-1} compared to the yield of Superb at 3756.7 kg ha^{-1}. These recombinants show that the *B1* locus and *QYld.crc-5A.2* were independent loci and the recombinants are useful to develop awnless, high yielding cultivars of spring wheat. The *QYld.crc-3B* interval is approximately 70 cM from the recently mapped *Fhb1* gene conferring *Fusarium* resistance on the distal end of chromosome 3B (Cuthbert et al. 2006). Given the large distance between these loci, potential yield penalties associated with *Fhb1* derived from exotic sources would be avoided by selection of recombinants with *QYld.crc-3B.*

The presence of Superb alleles at *QYld.crc-3B* was associated with an increase in Tgw, Asw, Hi, and Sns and a decrease in Sm2 suggesting that the increase in Yld at *QYld.crc-3B* is the result of an increased number of heavier seeds being produced on fewer spikes (Table 3.5). A grain yield QTL on chromosome 3B was observed in one of the RIL populations evaluated by Kumar et al. (2007) only in one environment with no coincident yield components QTL observed. *QYld.crc-3B* was the weakest of the five

grain yield QTL identified in the current study and would likely not be a target for MAS in spring wheat breeding programs.

Yield was also significantly correlated with the agronomic traits: Hdg, Mat, and Gft however the correlations were very low and would have little biological significance (Table 3.4). QTL for Hdg, Mat and Gft were coincident with a maximum of two of the five Yld QTL and never with *QYld.crc-1A* (Table 3.5, Fig. 3.1). A limited number of QTL studies have evaluated agronomic traits such as Hdg, Mat, and Gft and there are no consistent relationships between these traits and Yld (Borner et al. 2002; Marza et al. 2005). Photoperiod, vernalization, and 'earliness per se' are groups of major genes known to control heading in hexaploid wheat (Shah et al. 1999). 'Earliness per se' genes are known to map to chromosome groups 2 and 4, and to chromosomes 3A, 6B, and 7B (Shah et al. 1999). Four of the seven QTL identified for Hdg in the current study map to these same chromosomes and were coincident with QTL for Mat.

Superb alleles were associated with increased yield at four of the five identified grain yield QTL. Superb carries a Grandin allele at the four yield QTL whereas the Sumai 3 allele from BW278 was responsible for increasing yield at *QYld.crc.1A*. Sumai 3 is an adapted Chinese spring wheat cultivar with known resistance genes for Fusarium head blight (Bai et al. 1999) that has been widely used in spring wheat breeding programs. This finding suggests that there is a deficiency in Superb for grain yield at *QYld.crc-1A*.

Traditionally, breeding high yielding spring wheat cultivars has been accomplished by making direct selections for grain yield. Since yield is a complex trait with low heritability, early generation selection has generally not been effective and

breeders usually maintain large breeding population for a number of generations before selecting for grain yield. In the current study, the grain yield QTL were coincident with an increase in at least one yield component suggesting that selecting for a yield component could efficiently increase grain yield. High heritabilities were also observed for the yield components (Table 3.3). The results of the analysis indicated the potential improvement marker assisted selection could make in yield and yield components with the identified QTL. With future validation work, the identified grain yield and yield components QTL should allow marker assisted breeding strategies to be developed and implemented in spring wheat breeding programs.

4.0 Validation of grain yield quantitative trait loci in a spring wheat cross (*Triticum aestivum* L.)

4.1 Abstract

The validation of quantitative trait loci (QTL) is recommended prior to implementing a marker assisted selection (MAS) strategy for cultivar improvement. Previously, a QTL mapping study of yield and yield components in a spring wheat cross between a high yielding cultivar, Superb [Grandin*2/AC Domain] and an adapted low yielding breeding line, BW278 [AC Domain*2/Sumai3] identified five chromosome regions that were associated with increased grain yield on chromosomes 1A, 2D, 3B, and 5A. The objective of this study was to validate the grain yield QTL in an independent breeding population where Superb was a parent. A population of 83 F_3 derived F_6 individuals from the spring wheat cross Superb/RL4831 was evaluated for grain yield in single replicate trials at four locations in Manitoba, Canada in 2001. Chromosome regions previously associated with grain yield were mapped with four to eight microsatellite markers each and single marker analysis was used to determine if any of these markers had significant ($P \leq 0.05$) associations with grain yield. Interval mapping was then used to estimate the effects and location of the yield QTL. Four of the five yield QTL were validated in this new genetic cross and explained 6.7 to 20.2% of the phenotypic variation in grain yield in the new population. The fifth yield QTL on chromosome 3B was not validated. The results of this study validated four grain yield

QTL on chromosomes 1A, 2D and 5A in spring wheat and showed their potential usefulness in identifying high yielding breeding lines using marker assisted selection.

4.2 Introduction

Genomic regions underlying quantitative trait loci (QTL) controlling a number of agronomic and economically important traits including grain yield in wheat (*Triticum aestivum* L.) have been studied for more than fifty years. Grain yield is one of the most important traits in plant breeding programs, yet it is the least understood. Yield is a complex, quantitative trait controlled by many genes with low heritability and is significantly influenced by the environment (Falconer and MacKay 1981). Genetic improvement has been limited since breeders have little information on the location and number of genes controlling grain yield (Koebner and Snape 1999; Mohan et al. 1997).

Initially, chromosome substitution lines were used to identify chromosome regions associated with the control of grain yield. With this method, results were limited to the chromosome studied in isolation and did not account for the influence of the genetic background of the entire genome (Law 1966). The advent of molecular markers offered a different approach to aneuploid analysis. Complete genetic maps of the wheat genome can be constructed with markers allowing more than one chromosome to be studied at one time.

In wheat, more than 500 QTL have been reported for grain yield, agronomic traits, and disease resistance but there is little information on the validation or confirmation of these previously reported QTL (Gupta et al. 1999; Langridge et al. 2001). Using chromosome substitution lines, grain yield QTL have been reported in wheat on

chromosomes 3A (Shah et al. 1999), 4A (Araki et al. 1999), and 5A (Kato et al. 2000). Using complete genetic maps and QTL analysis, grain yield QTL have been identified on all wheat chromosomes except 3D and 5D (Börner et al. 2002; Huang et al. 2003; 2004; 2006; Marza et al. 2005; McCartney et al. 2005; Kuchel et al. 2007).

High yielding spring wheat cultivars are generally developed by crossing parents with superior traits from different, adapted genetic backgrounds to produce segregating populations where individuals with desirable traits can be identified. Selections are usually based on phenotype in replicated, multi-location, multi-year field trials. For traits with low heritability such as grain yield, early generation selection is generally not effective (Knott 1972); therefore breeders are usually required to maintain large breeding populations for several generations before selections can be performed. Improvements in yield have been realized in plant breeding programs however it is unknown whether the source of the high yielding parental alleles will be expressed in the new genetic background.

Validation studies for agronomic traits including grain yield have been completed in maize with inconsistent results. Three independent experiments in the same genetic background could not confirm grain yield QTL in all experiments (Beavis et al. 1994; Beavis 1994). Ajmon-Marsan et al. (1996) evaluated QTL for grain yield in an independent sample drawn from the same maize population and found two QTL were consistent with those detected in a previous experiment, however the other two QTL identified in the first sample remained undetected in the independent sample.

Recently, an extensive multi-year, multi-location study of QTL controlling yield and yield components in a doubled haploid (DH) population derived from the spring

wheat cross, Superb (a high yielding hard red spring wheat cultivar) / BW278 (an adapted, low yielding spring wheat breeding line) identified five major grain yield QTL, *QYld.crc-1A*, *QYld.crc-2D, QYld.crc-3B, QYld.crc-5A.1* and *QYld.crc-5A.2,* with a combined additive effect explaining 37.0% of the variation in grain yield (Chapter 3.0; Table 3.5). Superb alleles at *QYld.crc-2D, QYld.crc-3B, QYld.crc-5A.1* and *QYld.crc-5A.2* increased yield and BW278 alleles increased yield at *QYld.crc-1A*. The objective of this study was to validate these five grain yield QTL in an independent population derived from the spring wheat cross Superb (high yielding cultivar) /RL4831 (a high yielding breeding line).

4.3 Materials and Methods

4.3.1 Plant material

A population of 83 F_3 derived F_6 lines from the cross Superb/RL4831 was obtained from the spring wheat breeding program at Agriculture and Agri-Food Canada (AAFC) – Cereal Research Centre (CRC). Superb [Grandin*2/AC Domain] is a high yielding hard red spring wheat cultivar registered by AAFC in 2000 and has been used frequently as a parent in the AAFC wheat breeding program. RL4831 [BW150*2/Erik] (BW150 = Katepwa*6/RL4509) is also an adapted, high yielding, hard red spring wheat breeding line developed at AAFC-CRC. The initial cross was completed in the fall of 1998 at the CRC and the resulting F_1 generation was selfed and advanced to the F_3 in the greenhouse. The F_3 families were grown in hills in New Zealand during the winter of 1999 and from each F_3 hill a single head was selected to produce a row of F_4 plants in Glenlea, Manitoba during the summer of 2000. The seed from the F_4 row was bulked to

produce an F_5 row planted in New Zealand during the winter of 2000. The bulked F_6 seed was planted in yield trials in 2001. No selection for grain yield or yield components was made during the development of the population.

4.3.2 Field trials

The F_3 derived F_6 population was evaluated in a randomized single replicate field trial at Brandon, Glenlea, Portage la Prairie, and Morden, Manitoba in 2001. The plots in Glenlea and Portage la Prairie consisted of 5 rows, 4.27m long, spaced 15.2 cm apart. In Brandon, the plots consisted of 4 rows, 4m long, spaced 22.3 cm apart. The plots in Morden consisted of 5 rows, 4m long and spaced 17.8 cm apart. All plots were seeded at a rate of 250 seeds m^{-2}. All plots were bordered with a row of fall rye or winter wheat to minimize border effects from neighboring plots. The grain yield from plots was converted to kg ha^{-1} for analysis. Hard red spring wheat cultivars Superb, Roblin, McKenzie, and BW259 were also included as checks. Plant height and grain yield were the only measurements collected.

4.3.3 Statistical Analysis

Grain yield was analyzed using the procedure MIXED with a random effects model (SAS v8.2 SAS Institute Inc., Cary, N.C.). Since these lines were only evaluated in single replicate trials, data from each site year were combined to generate an overall estimate of mean line performance. Variance components and best linear unbiased predictors (BLUPs) were obtained by the method of restricted maximum likelihood for grain yield (Littell et al. 1996).

4.3.4 Interval Mapping

Leaf tissue was harvested from each RIL and lyophilized for DNA extraction with the Qiagen DNeasy 96 Plant Kit (Qiagen, Mississauga, ON). Presence / absence of awns was noted when the population was grown for tissue collection. DNA was quantified by fluorimetry using Hoechst 33258 stain. Approximately 140 microsatellite markers on chromosomes 1A, 2D, 3B, 4B, and 5A (Somers et al. 2004) were screened to detect polymorphisms between Superb and RL4831. The parents of Superb (Grandin, AC Domain) and RL4831 (BW150, Erik) were also included in the screening to determine the allelic composition of parental lines. Chromosome 4B which contains the plant height gene, *Rht-B1b,* was also mapped to characterize possible pleiotropic effects from this locus. Markers were selected to cover approximately 50 cM of each chromosome associated with the grain yield QTL (Figure 4.1).

Genotyping data were obtained using M13 tailing and fluorescent capillary electrophoresis on an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, Calif., USA). M13 tailing required adding the M13 sequence (CACGACGTTGTAAAACGAC) to the 5' end of the forward primer during primer synthesis (Schuelke 2000). PCR reactions were preformed in 10 μl volumes and included 24 ng of template DNA, 1X PCR buffer (Applied Biosystems, Foster City, Calif.), 1.5 mM $MgCl_2$, 20 μM forward primer, 0.8mM dNTPs, 2 pmol reverse primer, 0.2 pmol forward primer; 1.8 pmol M13 primer fluorescently labeled with 6-FAM, VIC, NET, or PET (Applied Biosystems), and 0.5 U of *Taq* DNA polymerase (Promega). The reaction mixture was denatured at 94°C for 2 min; followed by 30 cycles of 95°C for 1

min, 49°C/58°C for 50 s, 73°C for 1 min; with a final extension step of 73°C for 5 min. The internal size standard for the ABI3100 was GeneScan-500 LIZ (Applied Biosystems). Data generated by the ABI3100 were converted to a gel-like image using Genographer (available at http://hordeum.oscs.montana.edu/genographer). Primer sequences for the GWM and GDM microsatellite markers were obtained from Röder et al. (1998) and Pestova et al. (2000). The BARC microsatellite marker sequences were obtained from the USA wheat and barley scab initiative website (http://www.scabusa.org) while the sequences for the WMC, CFA, and CFD microsatellites were obtained from the Grain Genes website (http://wheat.pw.usda.gov).

The map was constructed using MAPMAKER/EXP version 3.0b (Lincoln et al. 1993; Lander et al. 1987) with a minimum LOD of 3.0 and maximum recombination fraction of 0.35. The marker order was tested using the "compare" and "ripple" commands. The map was verified using JoinMap® 3.0 (Stam 1993).

4.3.5 QTL Analysis

Single marker analysis of variance was used to determine the significance ($P \leq 0.05$) among the marker genotypic class means using an F-test from the Type III mean squares obtained from the GLM procedure (SAS Institute 1992). Interval mapping (Lander and Botstein 1989) was subsequently performed using Windows QTL Cartographer v2.0 (Wang 2004 http://statgen.ncsu.edu/qtlcart/WQTLCart.htm) to estimate the location and the effects of the significant QTL.

4.4 Results

4.4.1 Validation Population

The phenotypic data indicated there was significant variation for grain yield in the Superb/RL4831 population. Grain yield ranged from 2601 to 3884 kg ha^{-1} and the population mean was 3410 kg ha^{-1}. The yield of Superb was 3772 kg ha^{-1}. RL4831 was not included in the 2001 field trials. However, based on multi-location and multi-year testing in other yield trials such as the 1997 Western Canadian Central Bread Wheat B Test (Table 4.1), RL4831 is considered to be a high yielding line. When the frequency distribution for plant height was generated a normal distribution was observed, suggesting that more than one plant height gene was segregating in the population (plant height ranged from 85 to 96 cm with an average plant height of 90 cm). There was a low, negative correlation ($r = -0.30$) between grain yield and plant height. The Superb/RL4831 population also segregated for the presence of awns controlled by the major awn suppressor locus *B1* on 5AL. The results of an ANOVA indicated variation at the *B1* locus had no significant effect on grain yield (data not shown).

4.4.2 Interval Map Construction

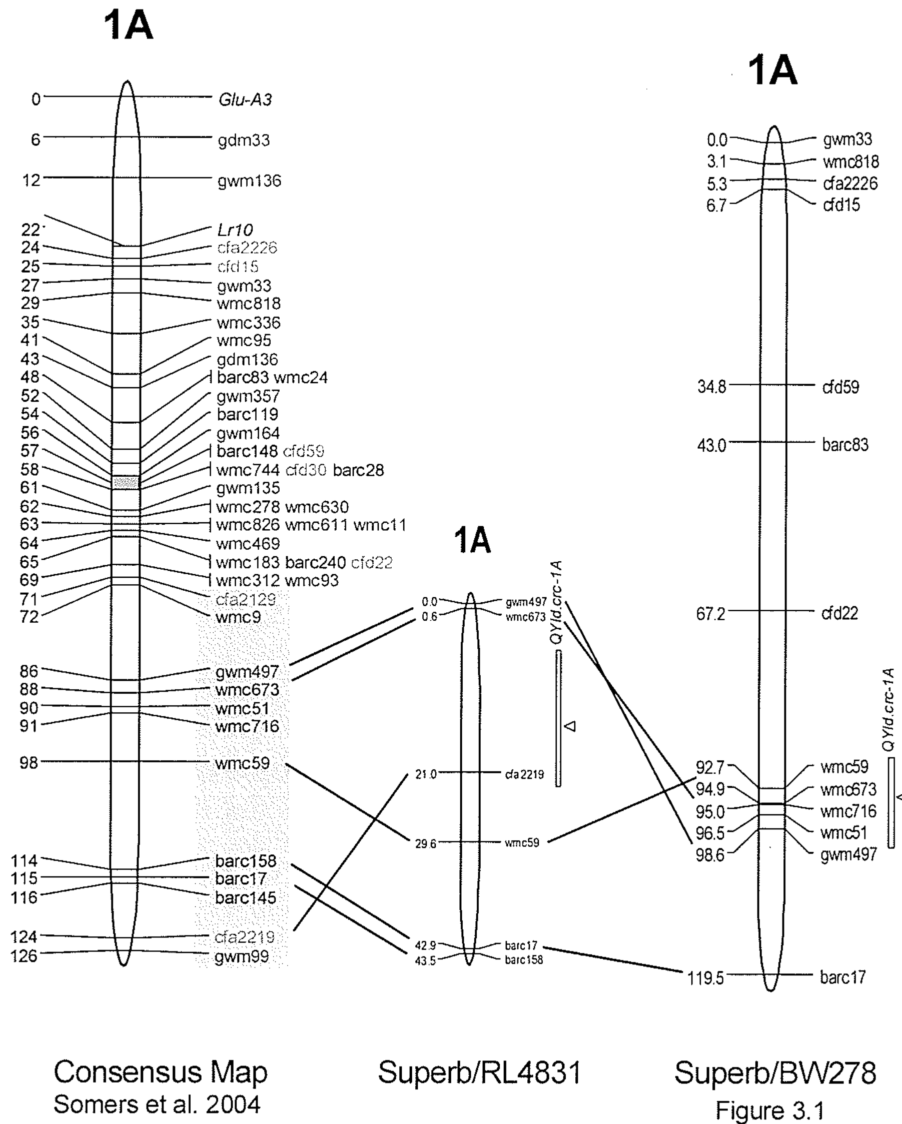
Approximately 140 microsatellite markers were screened for polymorphism between Superb and RL4831. All of the markers amplified on 3B were monomorphic and the chromosome interval examined was expanded in an attempt to find polymorphic markers. The five chromosome regions associated with the yield QTL (Figure 3.1; Table 3.5) were mapped with six to eight microsatellite markers each (Figure 4.1). Each region spanned between 31.6 and 140.6 cM.

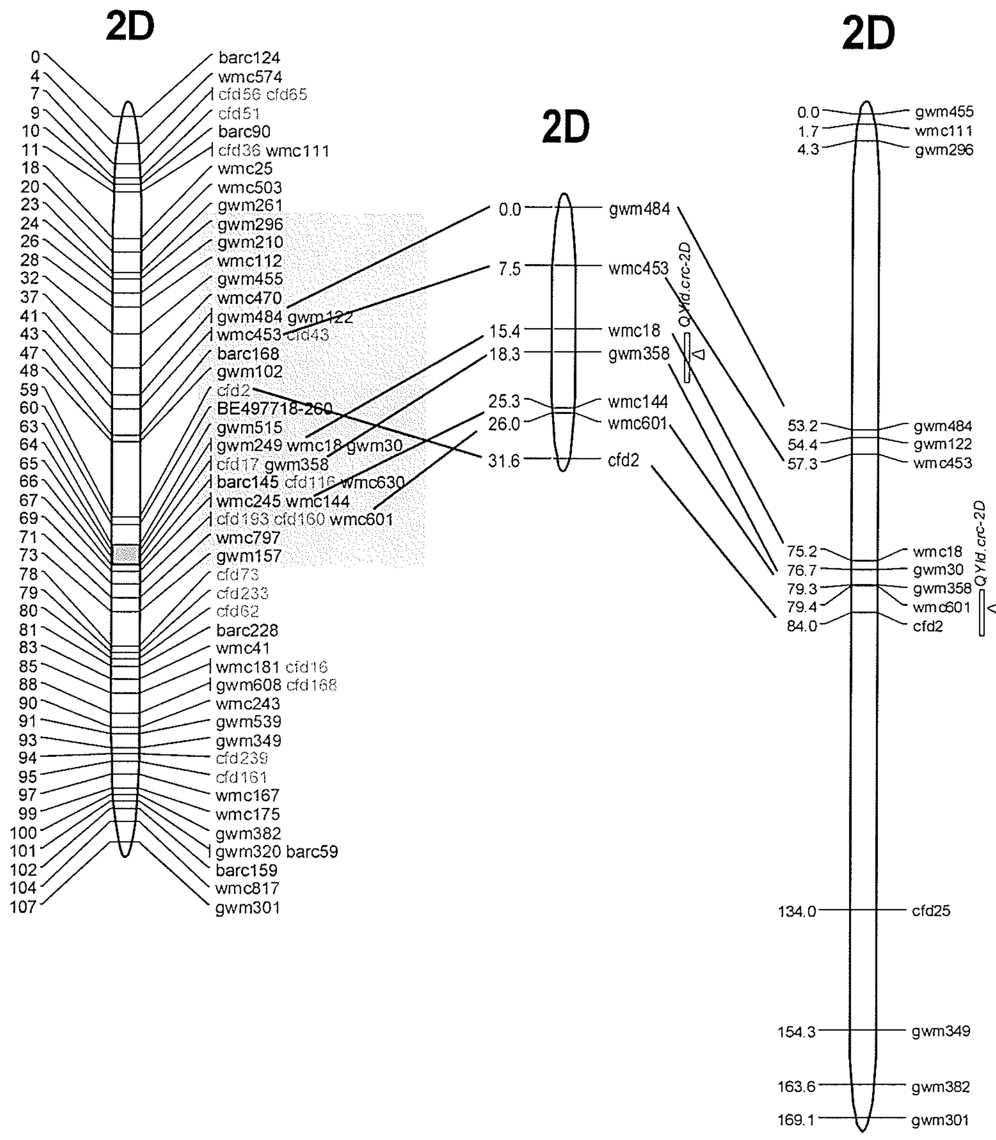
Table 4.1. Comparison of grain yield (kg ha^{-1}) for Superb, RL4831, and check cultivars from the Superb/RL4831 trial, the Superb/BW278 trial and the 1997 Central Bread Wheat B Test.

Parent/ Check cultivar	RL4831/Superb Trial kg ha^{-1}	Superb/BW278 Trial[a] kg ha^{-1}	1997 Central Bread Wheat B Test kg ha^{-1}
Superb	3772	3757	n/a
BW278	n/a	3179	n/a
Katepwa	n/a	3501	3550
Roblin	3160	3151	3220
RL4831	n/a	n/a	3920

[a]Superb/BW278 yield trials conducted in 2001 and 2002 at the same locations in Manitoba (Table 3.3 and Chapter 3.4.1) with either Superb or Katepwa and Roblin, as check varieties

Figure 4.1. Genetic linkage maps of the five chromosome intervals mapped in the spring wheat cross Superb/RL4831. On the left is the chromosome from the wheat microsatellite consensus map (from Somers et al. 2004) with a shaded region to indicate the markers screened in this study. In the center is the chromosome from the Superb/RL4831 cross. An arrowhead indicates the LOD peak of each QTL and the length of the bar indicates a 1.0 LOD drop in the QTL confidence interval. On the right is the chromosome from the Superb/BW278 cross with grain yield QTL indicated (from Figure 3.1). The broken line on chromosome 3B represents the placement of marker WMC612 on the Superb/BW278 chromosome by comparative mapping. Map distances are indicated on the left of each chromosome in Kosambi centimorgans.



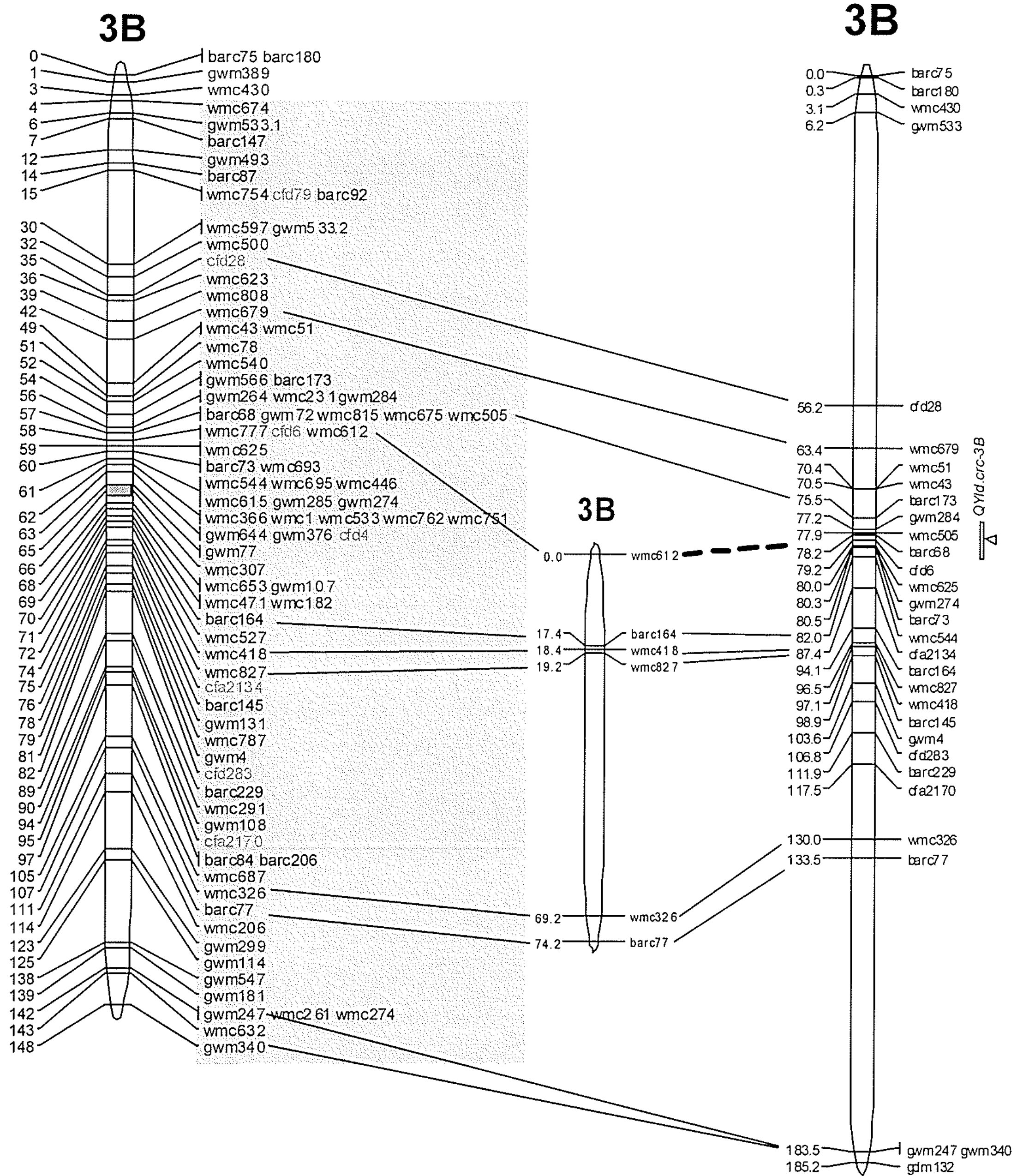


Consensus Map
Somers et al. 2004

Superb/RL4831

Superb/BW278

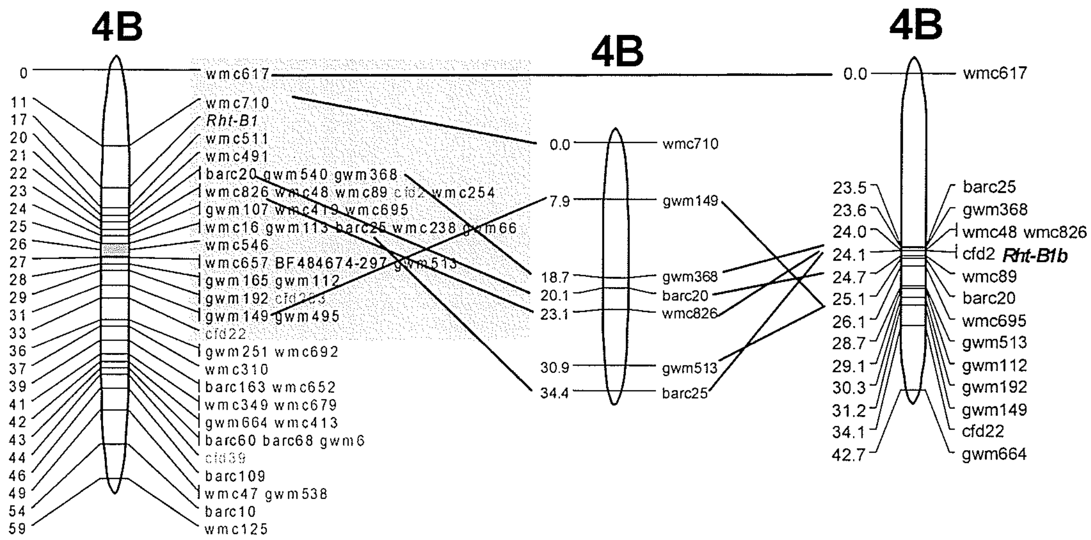
Figure 3.1



Consensus Map
Somers et al. 2004

Superb/RL4831

Superb/BW278
Figure 3.1



Consensus Map
Somers et al. 2004

Superb/RL4831

Superb/BW278
Figure 3.1

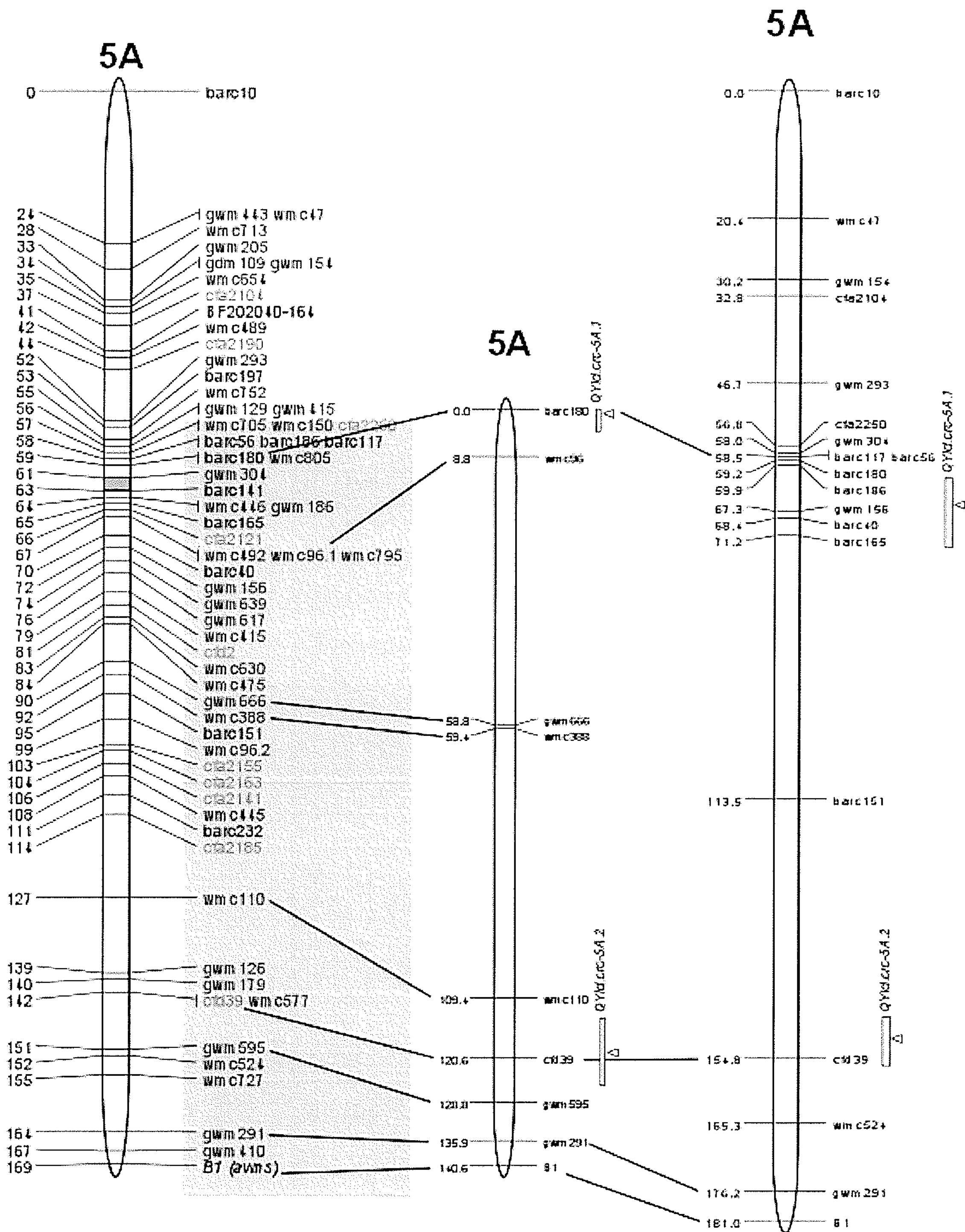


Figure 3.1

A comparison of the Superb/RL4831 map to the consensus map (Somers et al. 2004) and the Superb/BW278 map (Figure 3.1), revealed good colinearity of markers for chromosomes 2D, 3B, and 5A while some minor rearrangement of the microsatellite loci were noted on chromosomes 1A and 4B. The map distances were larger for all chromosomes in the Superb/RL4831 population compared to the consensus map and Superb/BW278.

The results of the single marker analysis for all markers mapped in the Superb/RL4831 population are presented in Table 4.2. Significant effects were observed for at least one marker on chromosomes 1A, 2D, and 5A suggesting that *QYld.crc-1A*, *QYld.crc-2D, QYld.crc-5A.1,* and *QYld.crc-5A.2* affect yield in the Superb/RL4831 population. These four yield QTL explained between 5.3 and 18.2% of the total phenotypic variation. The RL4831 allele was associated with increased yield at *QYld.crc-1A*. No significant effects were observed for any markers on chromosomes 3B therefore the *QYld.crc-3B* QTL could not be validated in this cross. Single marker analysis revealed there were no effects on yield associated with the markers on chromosome 4B near *Rht-B1b*, indicating this plant height gene did not have any pleiotropic effects on grain yield.

The microsatellite loci in the intervals on chromosomes 2D and 5A showed Superb was composed of Grandin alleles and RL4831 was composed of alleles common to both BW150 and Erik, with the exception of WMC110 on chromosome 5A (Table 4.2). These results suggested that the Grandin haplotype was associated with increased yield at *QYld.crc-2D*, *QYld.crc-5A.1* and *QYld.crc-5A.2* and an RL4831 haplotype associates with lower yield. Across these intervals, BW150 and Erik were non-

Table 4.2. Summary of the markers tested with single marker analysis at previously reported grain yield QTL in the F_3 derived F_6 spring wheat Superb/RL4831 population.

Chromosome	Marker	Allele Size (bp)								Allelic Mean Yield	
		Superb	RL4831	BW150	Erik	AC Domain	Grandin	P^a	R^{2b}	Superb	RL4831
										kg ha^{-1}	
1A	GWM497	null	144	144	144	null	null	0.036	0.053	3350	3488
	WMC673	null	133	133	133	null	Null	0.007	0.090	3322	3501
	CFA2219	216	246	246	246	238	216	0.002	0.120	3298	3508
	WMC59	195	175	175	199	195	195	0.056	0.047	3372	3500
	BARC17	282	263	263	272	263	282	0.038	0.056	3347	3483
	BARC158	249	252	252	249	252	249	0.163	0.026	3360	3456
2D	GWM484	152	150	150	150	164	152	0.904	0.000	3376	3385
	WMC453	196	189	189	189	null	196	0.398	0.013	3426	3360
	WMC18	221	243	243	243	241	221	0.470	0.007	3430	3380
	GWM358	154	156	156	156	157	154	0.040	0.053	3471	3333
	WMC144	142	140	140	140	138	142	0.775	0.001	3409	3428
	WMC601	223	228	228	228	211	223	0.514	0.006	3377	3424
	CFD2	230	232	232	232	228	230	0.168	0.026	3374	3478
3B	WMC612	279	281	281	281	283	279	0.700	0.002	3386	3415
	BARC164	188	176	188	176	188	188	0.838	0.001	3415	3429
	WMC418	270	266	270	266	270	270	0.571	0.004	3384	3424
	WMC827	222	206	206	206	208	222	0.863	0.000	3398	3410
	WMC326	188	181	181	181	188	171	0.629	0.003	3376	3419
	BARC77	141	209	209	209	205	141	0.057	0.046	3323	3458
4B	WMC710	109	104	104	127	127	109	0.282	0.015	3460	3389
	GWM149	163	153	153	153	153	163	0.520	0.005	3389	3432

	GWM368	248	244	244	244	244	248	0.601	0.004	3440	3404
	BARC20	187	184	184	184	184	187	0.658	0.003	3422	3391
	WMC826	245	241	241	null	241	245	0.325	0.013	3447	3378
	GWM513	145	148	148	145	143	145	0.808	0.001	3416	3400
	BARC25	169	166	166	171	171	169	0.103	0.034	3486	3373
5A	BARC180	192	196	196	196	195	192	0.001	0.133	3515	3299
	WMC96	281	283	283	283	283	281	0.351	0.011	3445	3383
	WMC666	98	94	94	94	96	98	0.075	0.039	3351	3469
	WMC388	158	155	155	155	156	158	0.054	0.045	3344	3484
	WMC110	170	168	170	168	170	170	0.670	0.002	3396	3424
	CFD39	190	171	171	171	171	190	0.000	0.182	3535	3285
	GWM595	143	194	194	186	180	143	0.185	0.024	3465	3373
	GWM291	150	161	161	161	161	150	0.752	0.001	3400	3422
	B1	awned	awnless	awnless	awned	awnless	awned	0.468	0.006	3389	3434

[a]Probability of an association between the marker and the trait. Marker/trait association significant at $P \leq 0.05$

[b]Coefficient of determination represents the proportion of variability for the trait accounted for by the marker. R^2 calculated using single marker analysis.

informative at all microsatellite loci therefore it was not possible to draw any conclusions about the parental lines of RL4831. It is interesting to note the interval on chromosome 2D contains three unique haplotypes: Superb which is composed entirely of Grandin alleles in this interval, RL4831 is composed of BW150 and Erik alleles, and AC Domain which is composed of completely different alleles (Table 4.2).

QTL analysis estimated the location and effects of *QYld.crc-1A, QYld.crc-2D, QYld.crc-5A.1,* and *QYld.crc-5A.2* (Figure 4.1, Table 4.3). Each yield QTL explained between 6.7 and 20.2% of the total phenotypic variation and the LOD scores ranged from 3.2 to 7.4. The mean increase in yield of the genotypes carrying the positive allele ranged from 4.7 to 8.5% over the population mean. The allelic contribution at each grain yield QTL was determined for the top ten highest yielding lines in the Superb/RL4831 population (Table 4.4). Eight of the ten highest yielding lines had Superb alleles at *QYld.crc-2D, QYld.crc-5A.1,* and *QYld.crc-5A.2* while all ten lines had RL4831 alleles at *QYld.crc-1A*. (Table 4.4).

4.5 Discussion

Five QTL for grain yield were identified in the cross Superb/BW278 cross on chromosomes 1A, 2D, 3B, and 5A (Figure 3.1; Table 3.5). The present study mapped and validated four of the five grain yield QTL in a different genetic background (Superb/RL4831) (Table 4.2, Figure 4.1).

QYld.crc-3B was not associated with yield in the Superb/RL4831 population. Chromosome 3B was largely monomorphic between Superb and RL4831, however it was possible to identify six polymorphic markers that provided marginal coverage of

Table 4.3. Comparison of the grain yield QTL regions identified in the Superb/RL4831 population using interval mapping and the Superb/BW278 population using composite interval mapping.

	Superb/RL4831						Superb/BW278[a]					
Chromosome	Marker[d]	LOD	R^2	Additive[b] (kg ha^{-1})	Increase[c] (%)	Positive Allele	Marker[e]	LOD	R^2	Additive (kg ha^{-1})	Increase (%)	Positive Allele
1A	CFD2219	4.4	0.102	217.7	6.4	RL4831	WMC716	6.1	0.066	219.9	6.6	BW278
2D	GWM358	3.2	0.067	159.8	4.7	Superb	CFD2	3.7	0.042	178.4	5.3	Superb
3B	n/a	n/a	n/a	n/a	n/a	n/a	WMC544	4.5	0.041	197.6	5.9	Superb
5A.1	BARC180	3.7	0.081	183.5	5.4	Superb	BARC180	5.1	0.050	197.8	5.9	Superb
5A.2	CFD39	7.4	0.202	290.7	8.5	Superb	CFD39	12.6	0.204	340.2	10.1	Superb

[a]From Table 3.5.
[b]Additive effect of allele substitution.
[c]Increase (%) represents improvement in grain yield over the population mean (3410 kg ha^{-1}).
[d]Peak marker from interval mapping.
[e]Peak marker from composite interval mapping

Table 4.4. Alleles present at four grain yield QTL of the top ten highest yielding F_3 derived F_6 lines in the Superb/RL4831 population.

Line	Yield (kg ha^{-1})	*QYld.crc-1A* (RL4831)[a]	*QYld.crc-2D* (Superb)	*QYld.crc-5A.1* (Superb)	*QYld.crc-5A.2* (Superb)	*B1* (Superb)	%Increase[b]
DP1	3883	RL4831	Superb	Superb	Superb	RL4831	13.9
CD1	3880	RL4831	Superb	Superb	Superb	Superb	13.8
DW3	3878	RL4831	Superb	Superb	Superb	RL4831	13.7
CX3	3865	RL4831	Superb	Superb	Superb	Superb	13.4
DQ4	3846	RL4831	Superb	Superb	Superb	RL4831	12.8
A3	3830	RL4831	Superb	Superb	Superb	Superb	12.3
CD4	3800	RL4831	Superb	Superb	Superb	Superb	11.4
DN1	3760	RL4831	RL4831	Superb	Superb	RL4831	10.3
FC4	3741	RL4831	RL4831	Superb	Superb	RL4831	9.7
ES3	3719	RL4831	Superb	Superb	Superb	RL4831	9.1

[a]Allele associated with increased yield at each QTL

[b]Increase (%) represents the observed improvement in yield over the mean of the population (3410 kg ha^{-1}).

QYld.crc-3B. The consensus map (Somers et al. 2004), Superb/BW278 map (Figure 3.1; Table 3.5), and Superb/RL4831 maps revealed good colinearity for the markers on chromosome 3B which facilitated placing WMC612 onto the Superb/BW278 population via comparative mapping. In the Superb/BW278 population, the QTL peak marker at *QYld.crc-3B* was WMC544 (Table 4.3). The consensus map (Somers et al. 2004) interval between CFD28 and BARC164 is approximately 45 cM in length while the Superb/BW278 map interval between CFD28 and WMC625 is approximately 40 cM in length. Based on comparative mapping, WMC612 could be placed on the Superb/BW278 map between WMC505 and BARC68 which is within the confidence interval for *QYld.crc-3B* (Figure 4.1). Single marker analysis did not show any significant effects for marker WMC612 therefore *QYld.crc-3B* was not validated in the Superb/RL4831 population (Table 4.2). In the Superb/BW278 population, a QTL was declared when the LOD score was greater than the threshold LOD score in a minimum of four environments and was also detected in the combined data sets (Table 3.5). *QYld.crc-3B* was observed in only five of the ten environments and had the lowest R^2 value of the five grain yield QTL. This QTL could be considered a marginal QTL and would likely not be a breeding target in a spring wheat breeding program.

The Superb/RL4831 population is composed of RILs while the Superb/BW278 population consisted of DH lines and this difference likely accounts for the increase in map distance between some of the microsatellite loci on chromosomes 1A and 4B since RILs have more opportunities for crossovers than a DH population. Also, population size and sampling can lead to differences in the estimated map distances.

In the Superb/BW278 population, two major morphological genes, *Rht-B1b* on chromosome 4B and *B1* on chromosome 5AL segregated and had major pleiotropic effects on yield and yield components. As a result, the phenotypic data for the yield and yield components were adjusted to account for the contribution of plant height and the presence/absence of awns on the final phenotype of the traits. The adjustment allowed other important QTL to be detected (Figure 3.1; Table 3.5). In the present study, the Superb/RL4831 population also segregated for plant height and the presence/absence of awns. The frequency distribution for plant height showed a normal distribution which indicated more than one *Rht* gene was segregating in the population and the genes could not be mapped qualitatively. QTL analysis using the plant height data showed no significant association between plant height and the 4B region suggesting the *Rht-B1b* gene was not segregating in the Superb/RL4831 population and thus could not affect yield (Table 4.2). Plant height was then compared between the Superb/BW278 population and Superb/RL4831 population. The individuals in the Superb/BW278 population ranged from 80 to 105 cm with an average plant height of 92 cm while the individuals in the Superb/RL4831 population ranged from 85 to 96 cm with an average plant height of 90 cm. Therefore the Superb/RL4831 population contained shorter plants with less than half the phenotypic range compared to Superb/BW278 and thus it may be more difficult to observe the effect between plant height and grain yield.

The effect of awns on cereal productivity has been studied for over 80 years with some inconsistent results. In general it is believed awns increase yield but it has been documented that the effect of awns is strongly dependent on genotype (Motzo and Guinta 2002). The effect of awns in awned and awnless bulk populations of two hard red winter

(HRW) wheat backgrounds was extensively studied in 11 environments by Weyrich et al. (1994). Both HRW backgrounds had the same source of awns and were high yielding wheat cultivars currently grown in the United States. Weyrich et al. (1994) observed no effect on grain yield between the awned and awnless bulks. The source of awns in both the Superb/BW278 and Superb/RL4831 populations is from Superb through the Grandin parent. A significant increase in grain yield was observed in awned lines from the Superb/BW278 population (Table 3.2) however there was no observed effect of awns on yield in the Superb/RL4831 population. The difference in the effect of awns between the two populations could be the effect of the genetic background of the crossing parent. BW278, in the Superb/BW278 population, was a low yielding breeding line whereas RL4831 in the Superb/RL4831 population was a high yielding line. The Superb/BW278 population had the wider range in yield when compared to the Superb/RL4831 population however the mean yield of these populations was very similar (Table 3.3; Chapter 4.4.1). This indicates the Superb/BW278 population was a wider cross and exhibited higher genetic variation than the Superb/RL4831 population.

It is also possible that other traits on chromosome 5AL are linked to presence of awns that could be responsible for the significant effects of awns since chromosome 5A is known to carry a number of agronomic and productivity traits (Snape et al. 1985). Beta amylase (*Beta-Amy-B1*) is a quality parameter of wheat that affects baking quality and is closely linked to the *B1* locus (Ainsworth et al. 1983). Since one of the parents of BW278 is Sumai3, an unadapted Chinese spring wheat cultivar with no known desirable quality characteristics, it is possible that there is segregation at the beta amylase locus in the Superb/BW278 population that is not observed in the Superb/RL4831population. All

parents in the Superb/RL4831 population have acceptable quality characteristics for western Canada. This suggests that the effect of awns as it associates with yield may be an artifact of another gene linked to the awn locus that is segregating in the Superb/BW278 population and not the Superb/RL4831 population.

A comparison of the results of the QTL analysis for the Superb/RL4831 population and the composite interval mapping QTL analysis for the Superb/BW278 population are summarized in Table 4.3. Similar effects were observed for *QYld.crc-1A*, *QYld.crc-2D*, *QYld.crc-5A.1*, and *QYld.crc-5A.2* in both populations; however, the coefficients of determination for the Superb/RL4831 population were higher for the four grain yield QTL when compared with the Superb/BW278 population. The Superb/BW278 population contained 178 DH lines while the Superb/RL4831 population contained 83 F_3 derived F_6 lines. It has been reported that smaller populations may lead to over estimation of QTL effects (Liu 1998). The method of QTL analysis can also affect the results of an experiment. The Superb/RL4831 population was analyzed with QTL analysis where the location of a QTL was determined relative to adjacent pairs of flanking markers instead of using single markers. This approach used maximum likelihood and evaluated the likelihood that a QTL was located at a specific position. The Superb/BW278 population was analyzed using composite interval mapping (CIM). This analysis considers variables for the interval being tested as well as variables for other parts of the genome to control genetic background effects. CIM provides a more reliable estimate of QTL effects.

In the current study, phenotypic data were collected on F_3 derived F_6 RILs while genotypic data were collected from a single F_7 seed harvested from each of the F_3 derived

F_6 RILs. Genetically, an individual F_7 plant would be expected to be homozygous at approximately 98.4% of its loci. However, phenotypic data was collected from an F_3 derived F_6 RIL and for each locus, it is expected that 25% of the lines would have been heterozygous in the F_3 and would be a mix of approximately 48.4% homozygotes for each of the two alleles in the F_6 generation. The phenotypic data collected on the F_3 derived F_6 RIL would include the performance of the heterozygous lines that would be somewhere between the phenotype of the Superb and RL4831 alleles. This would likely result in the phenotype of the plot being closer to the mean of the parental alleles and lead to an underestimation of the effects of the identified QTL. Lower LOD scores and estimates of additive effects were observed for the validated QTL in the Superb/RL4831 population when compared to the effects of the identified QTL in the Superb/BW278 population support this observation (Table 4.3).

In the Superb/ BW278 and Superb/RL4831 populations, the increase in grain yield at *QYld.crc.2D, QYld.crc.5A.1 and QYld.crc.5A.2* was associated with the presence of Superb alleles. Closer examination of the haplotype of the parents in the Superb/BW278 and Superb/RL4831 populations revealed Superb carried the haplotype from its parental line Grandin, a high yielding American spring wheat cultivar (Table 4.2). RL4831 and its parents, BW150 and Erik, all have the same haplotype in the 2D and 5A intervals with the exception of marker locus WMC110 and the haplotype differs from Superb. These findings suggest two different yield haplotypes exist at these chromosome intervals. BW150 and Erik are not different at any of the marker loci on chromosomes 2D and 5A, therefore they are considered to be non-informative and it is not possible to determine which of the two parental lines was higher yielding. *QYld.crc.2D* appears to

contain three unique haplotypes including Superb which carries the Grandin haplotype associated with increased yield, RL4831 which carries the haplotype of BW150 and Erik and was associated with lower yield in this cross, while AC Domain, the other parental line of Superb carries a haplotype that differs from Superb and RL4831. A similar pattern was observed on chromosome 5A however the haplotype segregation is less evident. The increase of yield at *QYld.crc.1A* was associated with the presence of RL4831 alleles. This is similar to the Superb/BW278 population where the presence of the BW278 alleles at *QYld.crc.1A* was associated with higher yield. At this interval, it could be suggested that BW150 could be the source of the high yielding alleles in the Superb/RL4831 population, however three of the marker loci are not informative.

Strong similarity in the magnitude of the effects of *QYld.crc-5A.2* between the Superb/BW278 and the Superb/RL4831 populations was also noted. In total, 13 recombinants in the Superb/RL4831 population were identified as being awnless and carrying Superb alleles at *QYld.crc-5A.2.* These recombinants had an average yield of 3633 kg ha^{-1} with yields ranging between 3107 to 3884 kg ha^{-1} compared to the yield of Superb at 3772 kg ha^{-1}. These recombinants are useful for producing awnless, high yielding cultivars of spring wheat.

The overall improvement for each of the top ten yielding lines in the Superb/RL4831 population ranged from 9.1 to 13.9 % over the population mean indicating that even in a cross between high yielding parents it is possible to make significant increases in yield. It should also be noted that seven of these top ten yielding lines yielded better than Superb and the remaining three lines were within 60 kg ha^{-1} of Superb.

Since Superb alleles positively influenced yield at three of the four validated yield QTL and RL4831 is high yielding, these findings suggested there are other regions or haplotypes in the genome that control grain yield. The results also indicated the validated QTL could be successfully used in a marker assisted breeding strategy to identify the high yielding lines in the early generations of breeding. Further mapping of yield and yield component QTL in RL4831 is also recommended.

In the current study, the validation population had one of the parents, Superb, in common with the Superb/BW278 mapping population. If the validation study had been conducted with an unrelated population, it is possible previously described QTL would be identified especially given the magnitude of the effects of the identified QTL, in particular *QYld.crc-5A.2*. However, it would be necessary to associate the phenotype and genotype of the new population to determine which allele would be associated with increased yield and which allele should be selected. In the current study, we observed unique haplotypes at the validated QTL, which supports the suggestion that previously identified QTL, could be detected in unrelated populations.

5.0 Characterization of Superb spring wheat using microsatellite markers

5.1 Abstract

Superb is a high yielding hard red spring wheat cultivar. Superb (Grandin*2/AC Domain) was developed by combining the high yielding American cultivar, Grandin with the adapted Canadian spring wheat, AC Domain, that possesses good quality characteristics. Since its release in 2000, Superb has been used extensively as a breeding parent and is of interest to study at the genome level. The objective of this study was to complete a whole genome haplotype analysis of Superb and determine the allele composition of the identified grain yield QTL. Superb and the parents, AC Domain and Grandin, were screened with approximately 1,000 microsatellite markers. Graphical images of the 21 Superb chromosomes were created using the graphical genotyping software GGT: Graphical Genotypes and the wheat microsatellite consensus map. The proportion of the genome that was derived from each parent was also determined. The Superb genome was composed of 30% AC Domain and 70% Grandin. The chromosome images also allowed recombination events to be visualized. The number of detectable crossovers ranged from 0.5 to 2.5 for each Superb chromosome per meiosis with an average of 1.2 crossovers per chromosome.

5.2 Introduction

One of the main uses of DNA markers has been to construct genetic maps for a number of crop species to identify chromosome regions containing genes that control simple and complex traits (Langridge et al. 2001). Mapping is based on the principle that genes and markers segregate via chromosome recombination (crossing over) during meiosis. Genes or markers that are in close proximity will be transmitted together from parent to progeny more frequently than genes or markers that are located further apart.

The majority of the problems in genetic map construction have been related to limitations of available markers types, the lack of informative markers, and the polyploid nature of many crop genomes. Genetic maps are currently available for all chromosomes and homoeologous groups in wheat (Langridge et al. 2001). The first maps of the wheat genome were constructed with RFLP markers (Chao et al. 1989; Devos and Gale 1993; Nelson 1995a, b, c). There is generally a lower level of polymorphism in wheat relative to other cereals and wheat requires a large number of molecular markers to provide adequate genome coverage. In addition, the level of polymorphism is not consistent across the genomes or between crosses (Roder et al. 1998, Mohan et al. 1997). The D genome tends to be more conserved and is usually more difficult to map. These features introduce a level of complexity to genome analysis and can create technical difficulties. Recently, a high-density microsatellite consensus map was constructed by combining four independent genetic maps of bread wheat (Somers et al. 2004).

Superb is a high yielding hard red spring wheat cultivar developed and released by Agriculture and Agri-Food Canada (AAFC) – Cereal Research Centre in 2000. Superb was developed by crossing the high yielding American cultivar, Grandin, with the

adapted spring wheat cultivar, AC Domain followed by one generation of backcrossing to Grandin. A doubled haploid population was developed from the BC_1F_1 to produce a segregating population that was evaluated for yield and pre-harvest sprouting. Grandin (PI 531005) was developed and released by North Dakota State University in 1989 (Mergoum et al. 2005) however it could not be registered for commercial production in Canada because it did not meet quality standards for the hard red spring wheat class including unacceptable susceptibility to pre-harvest sprouting. AC Domain (BW148) is a widely grown, adapted hard red spring wheat cultivar with good quality characteristics including pre-harvest sprouting resistance. AC Domain was also developed by AAFC and released in 1990.

The objective of this study was to complete a whole genome haplotype analysis of the high yielding cultivar, Superb and compare identified QTL in other AC Domain populations.

5.3 Materials and Methods

5.3.1 Genotypic Data

The genotypic data for Superb, Grandin and AC Domain was obtained using the procedure described in Chapter 3.3.4. The allelic composition of Superb was determined by examining the gel-like images produced by Genographer (available at http://hordeum.oscs.montana.edu/genographer) for the approximately 1000 microsatellite markers. The Superb allele was compared to the parent alleles and if the parents were polymorphic, the parental source of the Superb allele was recorded. When no difference was observed between Superb and its parents, the locus was recorded as monomorphic. If

the microsatellite failed to amplify or give clear results, the result was recorded as unknown. The wheat microsatellite consensus map developed by Somers et al. (2004) was used as the genetic map in this study to produce the images of Superb and complete the genome analysis.

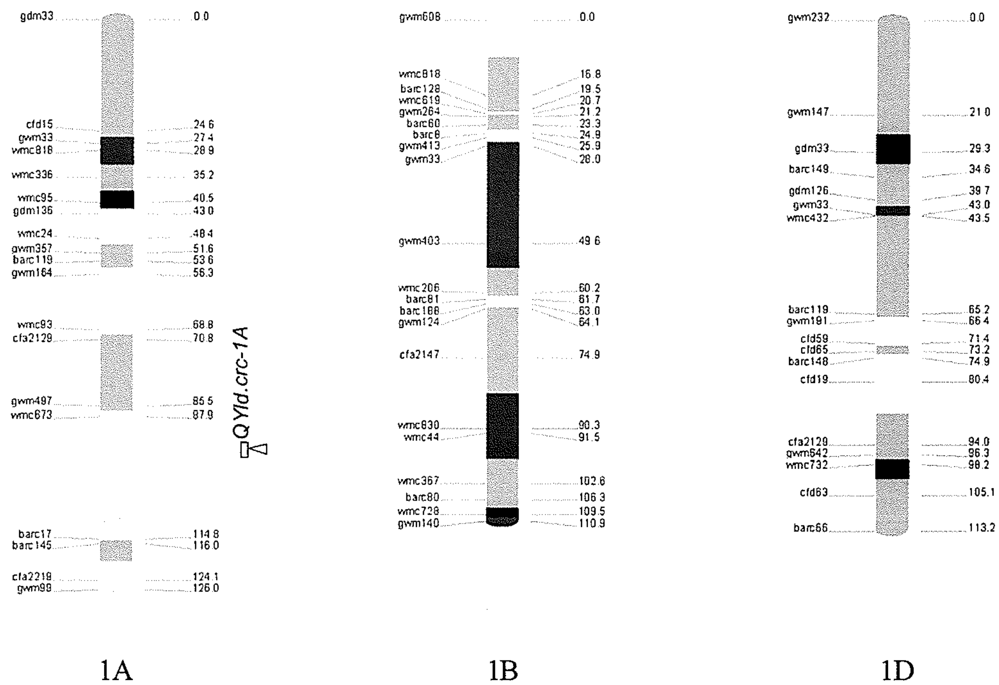
5.3.2 Graphical Images

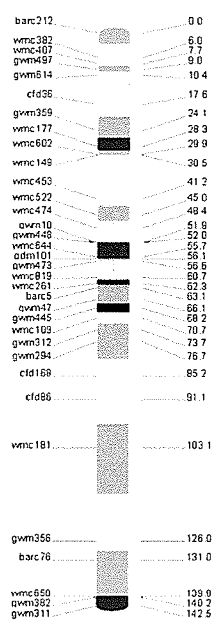
The software package, GGT: Graphical GenoTypes v 2.0 from Ralph van Berloo, Wageningen, University The Netherlands (http://www.dpw.wau.nl/pv/pub/ggt/) produces images that graphically represent molecular data. GGT requires input in the form of two data sources containing marker names and raw marker scores and a linkage map file that specifies marker positions on a linkage map. Marker order was determined using the wheat microsatellite consensus map (Somers et al. 2004) and the genotypic data from the primer screening (Chapter 3.3.4). Based on these data, the GGT software was able to estimate the proportion of the genome that was derived from each parent as well as the monomorphic and unknown portions. The wheat microsatellite consensus map had over 1200 microsatellite loci therefore the chromosome images presented in this chapter only include the first and last microsatellite loci for each parental chromosome segment (Figure 5.1).

5.4 Results

The allelic composition of Superb is presented in Figure 5.1 and Table 5.1. The whole genome haplotyping revealed that approximately 45.6% of Superb's genome was composed of alleles common to both AC Domain and Grandin and approximately 7% of

Figure 5.1. Graphical images of the 21 chromosome of Superb spring wheat. The blue regions indicate the proportion of the genome from the AC Domain parent while the yellow region represents the proportion of the genome from the Grandin parent. The gray regions represent the monomorphic regions while the black region indicate the unknown regions of the Superb genome. Microsatellite loci are indicated on the left of each chromosome. Due to the number of loci per chromosome, only the first and last loci for each region are displayed. Map distances are indicated on the right of each chromosome in Kosambi centimorgans. The five identified Yld QTL are indicated on the respective chromosomes.





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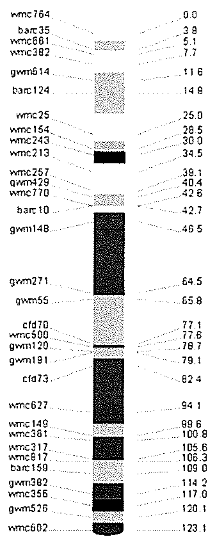

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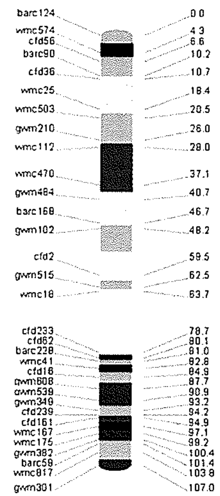

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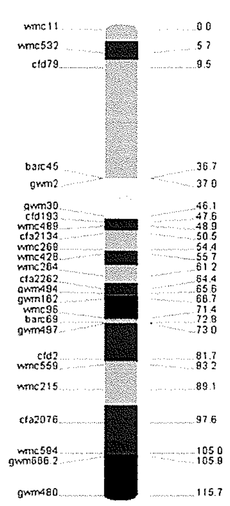

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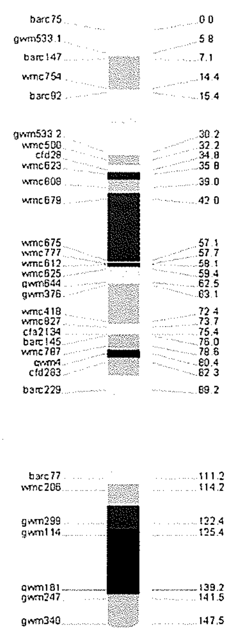

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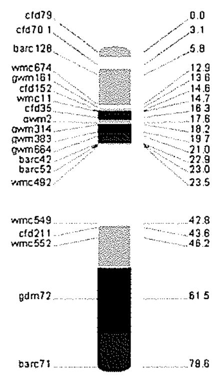

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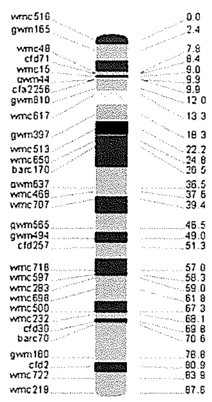
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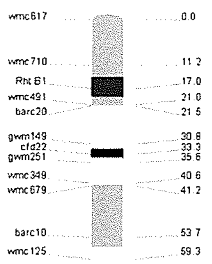
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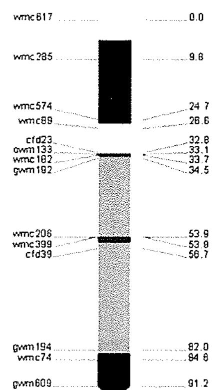
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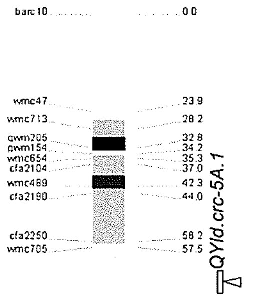
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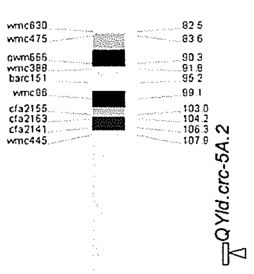
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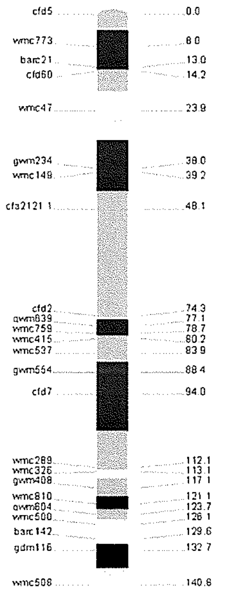
5A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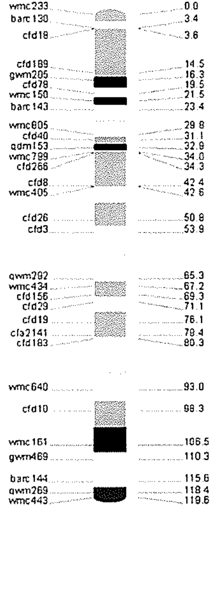
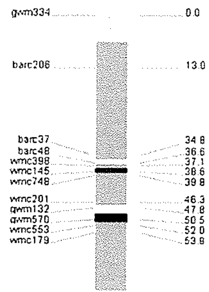
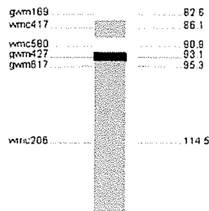
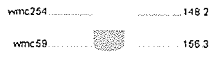

5D

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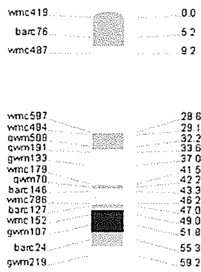

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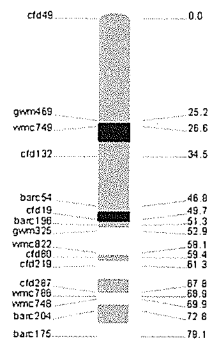

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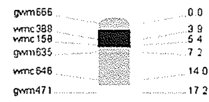
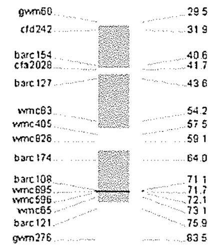
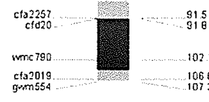
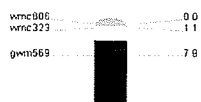
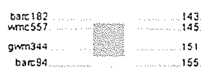
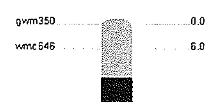
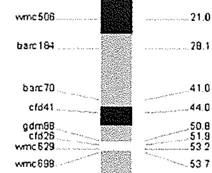
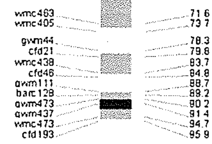




7A





7B

7D

Table 5.1 Summary of the allelic composition of Superb spring wheat.

Chromosome	AC Domain (%)	Grandin (%)	Monomorphic (%)	Unknown (%)	Genetic Length (cM)	Detectable Crossovers[a] (no.)
1A	6.5	44.2	47.1	2.1	126	0.5
1B	44.8	9.9	43.8	1.4	111	1.5
1D	0.4	21.1	67.1	11.4	117	0.5
2A	5.4	46.6	45.7	2.3	143	2.0
2B	37.7	18.2	37.9	6.3	123	0.5
2D	21.0	34.3	38.0	6.6	107	1.5
3A	28.5	9.2	48.6	13.7	116	1.0
3B	14.6	38.0	34.4	13.0	148	1.5
3D	6.2	30.2	38.3	25.3	79	1.5
4A	36.0	5.9	55.0	3.1	88	1.0
4B	5.5	25.9	61.4	7.2	59	0.5
4D	3.9	17.9	52.6	25.6	91	0.5
5A	3.1	73.1	19.6	4.1	184	1.5
5B	26.0	26.0	45.2	2.8	173	1.5
5D	1.0	51.9	38.8	8.3	120	0.5
6A	1.0	18.3	78.2	2.4	156	1.0
6B	7.7	68.7	23.6	0.0	82	1.5
6D	8.8	43.4	47.8	0.0	110	0.5
7A	12.0	47.6	40.4	0.0	132	2.5
7B	11.3	35.4	35.8	17.5	151	2.0
7D	16.6	19.2	62.5	1.7	154	2.0
A genome	11.2	38.0	47.1	3.8	944	9.5
B genome	22.0	30.9	39.4	7.7	847	9.0
D genome	8.8	31.0	50.4	9.8	778	7.0
Group 1	15.9	26.1	53.0	5.1	354	2.5
Group 2	20.5	33.7	40.9	4.8	373	4.0
Group 3	17.4	26.5	40.1	16.1	343	4.0
Group 4	16.1	15.5	55.6	12.7	238	2.0
Group 5	11.2	49.9	34.2	4.7	477	3.5
Group 6	5.1	39.1	55.8	1.1	348	3.0
Group 7	13.3	33.4	46.5	6.8	436	6.5
Total	14.0	33.5	45.6	6.9	2569	25.5

[a]Detectable crossovers per generation

the genome was unknown. Of the proportion of the genome that could be characterized, the parental contribution of AC Domain and Grandin was approximately 30 and 70%, respectively (Table 5.2).

The haplotyping data revealed 15 of the 21 chromosomes were composed of more than 70% Grandin and chromosomes 1B, 2B, 3A, and 4A were composed of more than 65% AC Domain (Table 5.2). Chromosomes 5B and 7D were made-up of approximately equal parental portions. When the chromosome groups were studied, groups 1, 2, and 3 were approximately 40% AC Domain and 60% Grandin. Group 4 was approximately equal portions of AC Domain and 50% Grandin and groups 5, 6, and 7 were composed of approximately 20% AC Domain and 80%Grandin. The results for the three related genomes indicated the A and D genomes were both composed of approximately 25% AC Domain and 75% Grandin while the B genome was spilt equally between AC Domain and Grandin.

When the chromosome images were reviewed, crossovers or recombination events for each Superb chromosome could be visualized (Figure 5.2). The number of detectable crossovers ranged from 0.5 to 2.5 for each Superb chromosome per meiosis with an average of 1.2 crossovers per chromosome. In comparison, in the Superb/BW278 population an average of 1.2 crossover events per chromosome were observed with a range of 0.4 to 2.0 crossovers per chromosome (Table 5.3).

5.5 Discussion

The present study graphically displayed the haplotype of the high yielding spring wheat cultivar, Superb (Grandin*2/AC Domain). As previously reported, the level of

Table 5.2 Summary of the allelic composition of Superb (Grandin*2/AC Domain) spring wheat for the portion of the genome that could be characterized.

Chromosome	AC Domain (%)	Grandin (%)	Genetic Length (cM)	Detectable Crossovers[a] (no.)
1A	12.8	87.2	126	0.5
1B	81.9	18.1	111	1.5
1D	1.9	98.1	117	0.5
2A	10.4	89.6	143	2.0
2B	67.4	32.6	123	0.5
2D	38.0	62.0	107	1.5
3A	75.6	24.4	116	1.0
3B	27.8	72.2	148	1.5
3D	17.0	83.0	79	1.5
4A	85.9	14.1	88	1.0
4B	17.5	82.5	59	0.5
4D	17.9	82.1	91	0.5
5A	4.1	95.9	184	1.5
5B	50.0	50.0	173	1.5
5D	1.9	98.1	120	0.5
6A	5.2	94.8	156	1.0
6B	10.1	89.9	82	1.5
6D	16.9	83.1	110	0.5
7A	20.1	79.9	132	2.5
7B	24.6	75.8	151	2.0
7D	46.4	53.6	154	2.0
A genome	22.9	77.6	944	9.5
B genome	41.6	58.4	847	9.0
D genome	22.1	77.9	778	7.0
Group 1	37.8	62.2	354	2.5
Group 2	37.8	62.2	373	4.0
Group 3	39.6	60.4	343	4.0
Group 4	51.1	48.9	238	2.0
Group 5	18.3	81.7	477	3.5
Group 6	11.8	88.2	348	3.0
Group 7	28.6	71.4	436	6.5
Total	29.5	70.5	2569	25.5

[a]Detectable crossovers per generation

Table 5.3 The number of detectable crossovers per generation and chromosome length for Superb compared to the average number of detectable crossovers and chromosome length for 93 doubled haploid lines from the Superb/BW278 mapping population.

	Superb		**Superb/BW278**	
Chromosome	Detectable Crossovers (no.)	Genetic Length (cM)	Detectable Crossovers (no.)	Genetic Length (cM)
1A	0.5	126	1.3	120
1B	1.5	111	1.2	114
1D	0.5	117	0.4	41
2A	2.0	143	2.0	231
2B	0.5	123	0.4	36
2D	1.5	107	1.6	169
3A	1.0	116	0.5	18
3B	1.5	148	2.0	185
3D	1.5	79	0.4	29
4A	1.0	88	n/a	N/a
4B	0.5	59	0.6	43
4D	0.5	91	0.8	65
5A	1.5	184	2.0	181
5B	1.5	173	0.9	58
5D	0.5	120	1.8	133
6A	1.0	156	1.4	150
6B	1.5	82	1.4	71
6D	0.5	110	0.9	108
7A	2.5	132	1.5	188
7B	2.0	151	1.4	101
7D	2.0	154	1.5	175
A genome	9.5	944	8.6	887
B genome	9.0	847	7.9	608
D genome	7.0	778	7.4	719
Group 1	2.5	354	2.9	275
Group 2	4.0	373	4.0	436
Group 3	4.0	343	2.9	232
Group 4	2.0	238	1.4	107
Group 5	3.5	477	4.7	371
Group 6	3.0	348	3.7	329
Group 7	6.5	436	4.4	464
Total	25.5	2569	23.9	2214

polymorphism in wheat is generally considered to be low and the current study estimated that 45% of the Superb parental alleles from AC Domain and Grandin were monomorphic. The GGT software calculated the monomorphic portion of the genome by determining the number of markers that did not detect any differences between the parental lines (Table 5.1). The large monomorphic chromosome segments observed in Figure 5.1 also support the lack of polymorphism in wheat. When only the regions that were polymorphic between the parents were considered, Superb's genome was composed of approximately 30% AC Domain and 70% Grandin (Table 5.2). This result is very close to the expected allelic contribution of 25% AC Domain and 75% Grandin for one cycle of backcrossing (Poehlman and Sleper 1995). In wheat, the level of polymorphism is also not consistent across the three related genomes and the D genome tends to be more conserved and is usually more difficult to map (Roder et al. 1998, Mohan et al. 1997). The results of the genome haplotyping support this finding and observed the A and B genomes were more polymorphic when the parental contribution, genetic length, and detectable crossovers were compared to the D genome (Table 5.2).

Previously, recombination events along wheat chromosomes have been reported to be inconsistent (Dvorak and Chen 1984; Gill et al. 1996). The graphical images of the chromosomes permitted the recombination events between AC Domain and Grandin to be visualized. The actual number of crossovers was determined by counting the AC Domain and Grandin allele intervals on each chromosome (Figure 5.1). Since Superb underwent two meiotic events during its development, the observed number of crossovers was divided by two to determine the number of crossovers per chromosome per meiosis. An average 1.2 crossovers per chromosome were observed with a range of 0.5 and 2.5

crossovers per chromosome (Table 5.2). The Superb/BW278 population was then investigated to determine the number of crossovers observed per chromosome. This DH population was derived from the F_1 generation therefore it is possible to compare the results of the cultivar Superb and the Superb/BW278 spring wheat cross. The genotypic data from the 93 DH individuals obtained from one F_1 plant was reviewed and the number of detectable crossovers observed per chromosome was recorded for each individual and subsequently averaged (Table 5.3). Overall, 1.2 crossover events per chromosome per meiosis were also detected in the Superb/BW278 cross. The rates of recombination detected in Superb and the Superb/BW278 population agree with a principle of meiosis that one crossover per chromosome is expected per meiotic event (Fu and Sears 1973). The rate of recombination for each chromosome, genome, or chromosome group was not consistent and should be considered by wheat breeders when developing breeding populations or crossing strategies to incorporate new traits or increase genetic variation.

Superb was developed by crossing Grandin with AC Domain to incorporate pre-harvest sprouting resistance while maintaining the high yield of Grandin. Pre-harvest sprouting can significantly impact grain quality and is generally caused by physiologically ripe seeds germinating under moist or humid conditions before harvest (Nielsen et al. 1984). Rasul (2007) recently identified six major QTL associated with resistance to pre-harvest sprouting in a DH population developed from the spring wheat cross RL4452/AC Domain. Three of these QTL were associated with AC Domain alleles on chromosomes 3A, 4A, and 4B. The QTL on chromosome 4B accounted for the largest proportion of phenotypic variation in pre-harvest sprouting. The intervals that

contained the identified QTL were examined in Superb and in all instances Superb was composed of AC Domain alleles. These findings suggest Superb carries the pre-harvest sprouting resistance QTL identified in Rasul (2007). It is also interesting to note chromosome 3A and 4A were two of the chromosomes where the parental contribution was more than 75% of AC Domain (Table 5.2).

In the same population, McCartney et al. (2005) identified QTL for several agronomic and quality traits including glutenins, a major component of the storage proteins in wheat. High molecular weight and low molecular weight glutenins are the two types of glutenins that have been identified and are currently being used in wheat breeding programs around the world (Payne 1987). The RL4452/AC Domain population segregated for one high molecular weight subunit, *Glu-B1,* on chromosome 1BL and three low molecular weight subunits, *Glu*-A3, *Glu*-B3, and *Glu-D3,* on chromosomes 1AS, 1BS, and 1DS, respectively. Superb carried the AC Domain allele at *Glu-B1* and *Glu-B3* and the allelic composition for the remaining subunits was either unknown or composed of the Grandin allele. The presence and absence of these storage proteins in Superb is not surprising since chromosome 1B was composed of more than 81% AC Domain alleles and chromosomes 1A and 1D where the other glutenins have been mapped, were almost entirely composed of Grandin alleles.

The five grain yield QTL, *QYld.crc.1A, QYld.crc.2D, QYld.crc.3B, QYld.crc.5A.1,* and *QYld.crc.5A.2,* identified in the Superb/BW278 population (Chapter 3.0), were included in Figure 5.1 to allow the chromosome intervals containing the yield QTL to be observed. Superb alleles were associated with an increase in yield at the four QTL on chromosomes 2D, 3B, and 5A and a decrease in yield at the QTL on chromosome 1A.

The identified QTL were detected in chromosome regions that contained large regions of Grandin segments. Within these regions, chromosome segments were observed to be monomorphic since there were no detectable differences between the alleles of the parental lines, AC Domain and Grandin (Figure 5.1). It is likely these regions are actually composed of Grandin alleles and these entire segments of Grandin spanned 30 cM for interval that contained *QYld.crc.5A.1* and 83 cM for the interval that contained *QYld.crc.1A*. This would suggest wheat breeders would be able to successfully select Grandin alleles within these chromosome segments in crossing material where Grandin was a parent.

McCartney et al. (2005) identified two grain yield QTL on chromosomes 2A and 4D, five test weight QTL on chromosomes 2D, 4D, 5D, 6B, and 7B, as well as two grain weight QTL on chromosomes 4A and 4D that were associated with AC Domain alleles in the RL4452/AC Domain cross. For all these QTL, the presence of the AC Domain allele resulted in an increase in grain yield, test weight or grain weight. When the results between this study and the Superb/BW278 population were compared, only one QTL for thousand grain weight on chromosome 2D was identical. The other QTL reported by McCartney et al. (2005) were examined in Superb and were composed of Grandin alleles and this is likely why these QTL were not detected in the Superb/BW278 population.

The current study revealed the genome haplotype of the high yielding cultivar, Superb. This analysis allowed recombination events between the parental lines, AC Domain and Grandin, to be visualized and tabulated. The images and statistics produced by the GGT software clearly indicated Superb was composed of large segments of Grandin. This is expected since Superb was developed through doubled haploidy from a

BC_1F_1 and underwent two meiotic events. More genetic recombination would be observed if a recombinant inbred line (RIL) was haplotyped since there would have been more opportunity for meioses. The rate of recombination is important to plant breeders and should be considered when developing breeding strategies for incorporating new traits or increasing genetic variation.

6.0 General Discussion

Grain yield has been an important focus of wheat breeding programs around the world for more than 100 years. Traditionally, breeding high yielding spring wheat cultivars has been accomplished by making direct selections for grain yield. Since yield is a complex, quantitative trait with low heritability, early generation selection has generally not been effective and breeders usually maintain large breeding populations for a number of generations before selecting for grain yield (Falconer and MacKay 1981; Knott 1972; Bernardo 2002). Quantitative traits are also difficult to study because the phenotype does not provide any insight into the genotypes of the traits. Selection for quantitative traits could be more efficient at the genotypic level without the interference of other interactions like environment, however it is necessary to identify and understand the genes controlling the trait.

Up until the 1980s, quantitative traits were primarily described using statistical techniques such as means, variances, and heritabilities rather than individual gene effects. With the advent of molecular markers, the ability to construct complete genetic maps, and the development of statistical methods and software to aid with map construction and quantitative trait analysis, chromosome regions associated with quantitative traits of interest can be identified. The genomic organization and structure of wheat makes it one of the most complex crops for genetic analysis, therefore quantitative trait loci (QTL) analysis of grain yield, its components and agronomic traits are limited (Liu 1998; Langridge 2001). Previously reported QTL for yield, its components and agronomic traits have been associated with almost all of the wheat chromosomes (Huang et al. 2003; 2004; 2006; Borner et al. 2002; Marza et al. 2005; Kuchel 2007. While there is some

agreement between the studies on the location of the QTL, the observed effects of the detected QTL were very different. Due to the lack of repeatability in QTL experiments and significant differences in the effects of the identified QTL, in particular yield QTL, there is limited interest or confidence in identifying QTL controlling yield.

This project was a comprehensive study of QTL controlling grain yield, yield components, and agronomic traits in a spring wheat cross between a high yielding cultivar, Superb, and a low yielding breeding line, BW278. In previous studies, QTL were identified in populations derived from wide crosses that are ideal for genetic analysis but are of limited value to breeding programs. The large double haploid population was extensively phenotyped across western Canada under disease free conditions and the performance of the parents as well as the range of values observed for the DH population indicated there was substantial genetic variation for all traits evaluated. In total, 53 QTL were consistently identified across the environments tested for the nine traits and included five QTL for grain yield. The grain yield QTL were identified on chromosomes 1A, 2D, 3B, and 5A and accounted for approximately 35% of the total phenotypic variation, which supports previous findings that yield, is a complex trait. The grain yield QTL were coincident with an increase in at least one yield component suggesting that selecting for a yield component could efficiently increase grain yield. High heritabilities were also observed for the yield components which is not surprising given the wide range of phenotypes observed for the population. The results of the analysis indicated the potential improvement marker assisted selection could make in yield and yield components with the identified QTL.

Wheat breeders can use information from QTL studies to design and implement marker assisted breeding strategies for quantitative traits such as yield only if the results can be reproduced. Since the identified QTL may be spurious and/or the estimation of their effects are subject to experimental error, putative QTL should be independently confirmed or validated in independent populations developed from the same parental genotypes or genotypes closely related to those used in the primary QTL study. The validation process verifies the legitimacy of a previously identified QTL and allows researchers to determine its potential usefulness in a marker assisted selection program. Failure to validate a QTL could result in a waste of time and resources in a breeding program.

The usefulness of the five identified grain yield QTL on chromosomes 1A, 2D, 3B, and 5A were evaluated in an independent breeding population where Superb was a parent. A population of 83 F_3 derived F_6 individuals from the spring wheat cross Superb (high yielding cultivar) / RL4831 (high yielding breeding line) was evaluated for grain yield in single replicate trials. Four of the five identified QTL were validated and there was strong similarity in the magnitude of the effects of *QYld.crc-1A, QYld.crc-2D, QYld.crc-5A.1,* and *QYld.crc-5A.2* between the Superb/BW278 and the Superb/RL4831 populations. This suggests that the four validated QTL could be successfully used to identify breeding lines carrying alleles for increased yield.

The validation population had one of the parents, Superb, in common with the mapping population. If the validation study had been conducted with an unrelated population, it is possible previously described QTL would be identified especially given the magnitude of the effects of the identified QTL, in particular *QYld.crc-5A.2*.

However, it would be necessary to associate the phenotype and genotype of the new population to determine which allele would be associated with increased yield and which allele should be selected. In the current study, we observed unique haplotypes at the validated QTL, which supports the suggestion that previously identified QTL, could be detected in unrelated populations.

In both the mapping and the validation study, Superb alleles were associated with increased yield at the identified and validated QTL, with the exception of *QYld.crc-1A*. The genome haplotyping of Superb indicated the QTL were identified in large segments of Grandin. Since Superb alleles were also associated with increased yield in the Superb/RL4831 population and RL4831 is high yielding, these findings suggested there are other regions or haplotypes in the genome that control grain yield. Further mapping of yield and yield component QTL in RL4831 is also recommended.

The Superb/BW278 population segregated for two morphological genes, reduced plant height, *Rht-B1b,* and the presence/absence of awns, *B1* which the QTL analysis revealed had major effects on the yield, yield components, and agronomic traits measured in the study. A data adjustment that determined the contribution of plant height and presence/absence of awns on the final phenotype was completed and successfully unmasked 53 QTL controlling the evaluated traits. A significant difference between the mapping study and the validation study was the effect of these morphological genes. When the frequency distribution for plant height was generated in the Superb/RL4831 population, a normal distribution was observed, suggesting that more than one plant height gene was segregating in the population. The interval on chromosome 4B was mapped and with single marker analysis no effect was detected. The validation

population did segregate for the presence/absence of awns however a significant effect from the *B1* locus was not observed. This suggests that the effect of awns as it associates with yield may be an artifact of another gene linked to the awn locus that is segregating in the Superb/BW278 population and not the Superb/RL4831 population.

Increases in knowledge and technology, genomics and molecular biology have opened new possibilities for breeding including the use of genetic rather than phenotypic selection. Molecular markers are now integrated into many breeding programs and are accelerating the development of new varieties or the introduction of new traits since they provide information at an earlier stage of plant breeding and target specifics traits (Dr Tom Frances, Syngenta Seeds Canada, pers comm.).

The current studies identified and validated four grain yield QTL that could be used in marker assisted selection programs to screen early generation breeding material for potential high yielding lines and enriched breeding populations. Unique yield haplotypes were identified for the chromosome intervals where the QTL were located. These haplotypes could be used in breeding programs to target specific regions and increase genetic variation. Breeders could then focus on incorporating other agronomic traits such as disease resistance and high quality in lines already identified as being high yielding. The application of these major grain yield QTL would facilitate the identification of new breeding lines more effectively and efficiently, which will accelerate the release of new varieties.

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8.0 Appendix

8.1. Genetic linkage map of the remaining seven chromosomes in the Superb/BW278 spring wheat cross. Map distances are indicated on the left of each chromosome in Kosambi centimorgans.