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
for the Degree of

DOCTORATE OF PHILOSOPHY

Department of Plant Science
University of Manitoba
Winnipeg, Manitoba

© Janice Louise Cuthbert, 2008

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

*****

COPYRIGHT PERMISSION

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

BY

Janice Louise Cuthbert

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirement of the degree

DOCTOR OF PHILOSOPHY

Janice Louise Cuthbert © 2008

Permission has been granted to the University of Manitoba Libraries to lend a copy of this thesis/practicum, to Library and Archives Canada (LAC) to lend a copy of this thesis/practicum, and to LAC's agent (UMI/ProQuest) to microfilm, sell copies and to publish an abstract of this thesis/practicum.

This reproduction or copy of this thesis has been made available by authority of the copyright owner solely for the purpose of private study and research, and may only be reproduced and copied as permitted by copyright laws or with express written authorization from the copyright owner.

Acknowledgements

I would like to express my sincere appreciation to the following people for their contribution to this study:

Co-advisors, Dr Anita Brûlé-Babel and Dr Daryl Somers for their guidance and advice throughout the course of this study.

Dr Doug Brown and Dr Gary Crow for serving on my advisory committee.

Dr Luther Talbert for serving as my external examiner.

Peter Dyck Fellowship provided by Agriculture and Agri-Food Canada – Cereal Research Centre for financial support.

Agriculture and Agri-Food Canada – Matching Initiative and Western Grains Research Foundation for financial support.

Dr. Fred Townley-Smith for developing the Superb/BW278 and Superb/RL4832 populations used in my studies.

Tony Romanow, Denis Green, and the Cereal Research Centre breeding crew for seeding, maintaining, and harvesting my trials.

Mary Meleshko and the University of Manitoba wheat breeding crew for maintenance of my trials at the Point.

Off Station Coordinators: Gerald Loeppky (Portage la Prairie), Rick Svistovski (Brandon), Glenn Dyck (Morden), Cecil Vera (Melfort) and Arlen Kapiniak (Scott) for seeding, maintaining, and harvesting my trials

The Molecular Genetics Lab (Zlatko Popovic, Mira Popovic, Elsa Reimer, Debbie Miranda, Karen LoVetri, Graham Curry, Celia Bondar, Allison Brown, Monica Eng, Yanfen Zhang, and Leslie Bestze) for their help with hand harvesting and counting/weighing samples.

Syngenta Crop Protection for providing the chemicals used in my trials.

Friends and fellow graduate students for their friendship.

My Mom and Dad, sister Patricia, brother Richard and brother-in-law Kelvin for their love and support. Thank you for everything.

Table of Contents

Acknowledgements i
List of Figures vi
List of Tables vii
List of Appendices ix
Abstract x

Foreword xii

1.0 Introduction 1

2.0 Literature Review 4
2.1 Spring Wheat 4
2.1.1 Wheat Breeding 4
2.1.2 Wheat Genetics 5
2.2 Grain Yield 6
2.2.1 Genetic Improvement in Yield 6
2.2.2 Yield Components 7
2.2.3 Breeding for Increased Yield 8
2.3 Molecular Mapping 10
2.3.1 Molecular Markers 10
2.3.1.1 RFLP – Restriction Fragment Length Polymorphism 12
2.3.1.2 PCR Based Markers 14
2.3.1.2.1 RAPD - Randomly Amplified Polymorphic DNA 14
2.3.1.2.2 AFLPs – Amplified Fragment Length Polymorphisms 15
2.3.1.2.3 Microsatellites 16
2.3.2 Genetic Maps 17
2.3.3 Marker Assisted Selection 19
2.4 Quantitative Trait Loci (QTL) 20
2.4.1 Quantitative traits 20
2.4.2 QTL Analysis 21
2.4.2.1 Single–Factor Analysis 22
2.4.2.2 Interval Mapping 22
2.4.2.3 Composite Interval Mapping 25
2.5 QTL Studies in Wheat 26
2.5.1 Grain Yield and Yield Component QTL 26
2.5.2 Heading Date 33
2.5.2.1 Vernalization Genes 34
2.5.2.2 Photoperiod Genes 34
2.5.2.3 Earliness per se Genes 35
2.5.2.4 Heading date QTL Studies 36
2.6 QTL Validation 37
2.6.1 Validation Studies 37

2.6.1.1 Wheat....37
2.6.1.2 Barley....38
2.6.1.3 Soybeans....39
2.6.1.4 Maize....39
2.6.1.5 QTL Validation Summary....40

3.0 Molecular mapping of quantitative trait loci for yield and yield components in spring wheat (*Triticum aestivum* L.)....41
3.1 Abstract....41
3.2 Introduction....42
3.3 Materials and Methods....44
3.3.1 Plant Material....44
3.3.2 Field trials....45
3.3.3 Statistical Analysis....48
3.3.4 Construction of the genetic map....48
3.3.5 QTL Analysis....49
3.3.6 Interval Mapping....50
3.4 Results....51
3.4.1 Quantitative Traits....51
3.4.2 Genetic Map....57
3.4.3 Effect of *Rht-B1b* and *B1* genes....57
3.5 Discussion....67

4.0 Validation of grain yield quantitative trait loci in a spring wheat cross (*Triticum aestivum* L.)....74
4.1 Abstract....74
4.2 Introduction....75
4.3 Materials and Methods....77
4.3.1 Plant material....77
4.3.2 Field trials....78
4.3.3 Statistical Analysis....78
4.3.4 Interval Mapping....79
4.3.5 QTL Analysis....80
4.4 Results....81
4.4.1 Validation Population....81
4.4.2 Interval Map Construction....81
4.5 Discussion....91

5.0 Characterization of Superb spring wheat using microsatellite markers....101
5.1 Abstract....101
5.2 Introduction....102
5.3 Materials and Methods....103
5.3.1 Genotypic Data....103
5.3.2 Graphical Images....104

5.4 Results 104
5.5 Discussion 110

6.0 General Discussion 118

7.0 Literature Cited 123

8.0 Appendix 137

List of Figures

Figure **Page**

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. 52

4.1 Genetic linkage maps of the five chromosomes interval 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 (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. 83

5.1 Graphical images of the 21 chromosome of Superb spring wheat. The yellow regions indicate the proportion of the genome from the AC Domain parent and the blue region represents the proportion of the genome from the Grandin parent. The gray regions represent the monomorphic regions and 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. 105

List of Tables

Table **Page**

3.1 Summary of the agronomic traits and yield and yield components measured for the Superb/BW278 Mapping Population in 2001 and 2002. 47

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........58

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).........59

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).........60

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........63

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. 66

4.1 Comparison of grain yield (kg ha^{-1}) for Superb, RL4831, and check cultivars from the Superb/RL4831 population, the Superb/BW278 population and the 1997 Central Bread Wheat B Test.........82

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. 89

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.........92

4.3 Alleles present at four grain yield QTL of the top ten highest yielding F_3 derived F_6 lines in the Superb/RL4831 population. 93

5.1 Summary of the allelic composition of Superb spring wheat.........109

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........111

5.2 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..112

List of Appendices

Appendix **Page**

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..137

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.