Marker-assisted backcross breeding in wheat
by John Edward Erpelding

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Crop and Soil Science
Montana State University

Abstract:
DNA marker technology provides a tool for genetic investigations and has potential applications in crop improvement. Genetic linkage maps based on restriction fragment length polymorphisms (RFLPs) have been constructed for many crop species. The development of the polymerase chain reaction (PCR) technology provides an economical alternative to RFLP marker analysis. Mapped low copy RFLP makers can be converted to sequence-tagged-site (STS) markers that are amplified by PCR. The successful application of the approach relies on the ability to emulate RFLP information. The cereals provide a unique system to test the applicability, since a considerable degree of synteny exist between related species and RFLP markers can be transferred between species. STS-PCR amplification products were mapped in wheat using the nullisomic-tetrasomic series and in barley with wheat-barley addition lines. STS amplification products mapped to the same homoeologous group in wheat as the RFLP approximately 70% of the time. Barley STS location corresponded to the RFLP location approximately 60% of the time. RFLP mapping information was transferable 82% of the time between wheat and barley. The same homoeologous chromosomes was identified 74% of the time with the STS-PCR system. Multiple STS locations were generally mapped in wheat. Southern analysis was used to evaluate the homology of the amplification products to the RFLP clone. Homologous sequences were generally amplified, but non-homologous sequences were also amplified. The results indicated that RFLP location could provide an indication of the STS location in both wheat and barley, but amplification of unmapped RFLP locations and non-homologous sequences is a factor to consider in their application.

The potential application of STS markers in a backcross breeding program to introgress Russian wheat aphid resistance into wheat was also investigated. Molecular markers provide a tool in which the genotype of a plant can be evaluated in early generations, allowing for increased selection efficiency. Three recombinant inbred populations were established from crosses between a Russian wheat aphid resistant line, PI372129, and two Montana hard red spring wheat cultivars, Pondera and Newana, in order to evaluated the level of donor genome and potential correlations with phenotypic performance. The percentage of donor genome, based on marker analysis, varied between the lines within the populations. Significant positive correlations between percentage of donor genome with plant height and straw strength were observed for the Pondera single cross population. No significant correlations were observed for the two backcross populations. The backcross population means approached that of the recurrent parent with a decrease in lines significantly different from the recurrent parent. Results suggests that molecular marker could be used to select for the recurrent parent genotype, and provide optimism for marker-assisted selection to recover the recurrent parent phenotype.

MARKER-ASSISTED BACKCROSS BREEDING IN WHEAT

by

John Edward Erpelding

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Doctor of Philosophy

in

Crop and Soil Science

MONTANA STATE UNIVERSITY
Bozeman, Montana

April 1996

APPROVAL

of a thesis submitted by

John Edward Erpelding

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

4/17/96
Date

Chairperson, Graduate Committee

Approved for the Major Department

4/18/96
Date

Head, Major Department

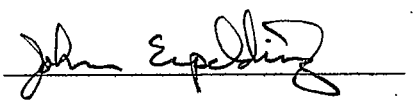
Approved for the College of Graduate Studies

4/25/96
Date

Graduate Dean

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a doctoral degree at Montana State University, I agree that the Library shall make it available to borrowers under rules of the Library. I further agree that copying of this thesis is allowable only for scholarly purposes, consistent with "fair use" as prescribed in the U.S. Copyright Law. Requests for extensive copying or reproduction of this thesis should be referred to University Microfilms International, 300 North Zeeb Road, Ann Arbor, Michigan 48106, to whom I granted "the exclusive right to reproduce and distribute copies of the dissertation in and from microfilm and the right to reproduce and distribute by abstract in any format."

Signature 

Date 4-18-96

TABLE OF CONTENTS

Page

APPROVAL ii
STATEMENT OF PERMISSION iii
TABLE OF CONTENTS iv
LIST OF TABLES vi
LIST OF FIGURES xv
ABSTRACT xvii
CHAPTER 1: INTRODUCTION 1
CHAPTER 2: MAPPING AND EVALUATION OF STS-PCR MARKERS 7
Literature Review 7
Materials and Methods 10
Plant Material 10
STS-PCR Marker Analysis 11
Southern Analysis 13
Results 15
Mapping STS Markers to Chromosomes 15
Correspondence of Chromosomal Locations 22
Southern Analysis of Amplification Products 27
Discussion 40
CHAPTER 3: MARKER ASSISTED SELECTION IN BACKCROSS BREEDING TO INTROGRESS RUSSIAN WHEAT APHID RESISTANCE 44
Literature Review 44
Russian Wheat Aphid 44
Host Plant Resistance 46
Sources and Genetics of Resistance 48
Resistance of PI372129 49
Backcross Breeding 50
Marker-Assisted Selection 52
Wheat Genome 54

TABLE OF CONTENTS - Continued

Page

Materials and Methods 55
Plant Populations 55
Field Evaluation 56
Agronomic Evaluation 57
Quality Evaluation 57
Marker Analysis 58
Results 60
Parental Marker Evaluation 60
Progeny Marker Evaluation 62
Storage Protein Evaluation 66
Donor Genome Estimation 67
Field Evaluation 68
Quality Evaluation 75
Marker Correlations 81
Discussion 84
LITERATURE CITED 91
APPENDIX 101

LIST OF TABLES

Table		Page
1.	RFLP clone sources used to design the STS-PCR primer sets and the number of primer sets selected from each source to evaluate the STS map location in both wheat and barley	12
2.	Total number of STS primer sets from the various clone sources in which mapping data was obtained for the wheat nullisomic-tetrasomic lines and the wheat-barley addition lines, along with the number having available RFLP mapping data for wheat and barley from a total of 97 primer sets analyzed	16
3.	Total number of locations mapped, average number of locations mapped per primer set, and total number mapped to a single chromosome or homoeologous chromosome group determined for 83 of the 97 STS-PCR primer sets designed from various sources mapped using the wheat nullisomic-tetrasomic series	17
4.	Total number of STS locations mapped for each of the wheat chromosomes and homoeologous groups as determined from the nullisomic-tetrasomic analysis of 83 of the 97 primer sets designed from various sources	18
5.	Total number of STS locations mapped for each of the three genomes of wheat as determined by nullisomic-tetrasomic analysis of 83 of the 97 primer sets derived from various sources	19
6.	Total number of locations mapped, average number of locations mapped per primer set, and total number mapped to a single chromosome for 57 of the 97 STS-PCR primer sets derived from various sources and analyzed using the wheat-barley addition lines for mapping in barley	21

LIST OF TABLES - Continued

Table		Page
7.	Total number of mapped STS locations for each barley chromosome as determined by the wheat-barley addition line analysis of 57 of the 97 primer sets derived from various sources	21
8.	RFLP mapping data available in both wheat and barley for the 97 cases evaluated, and the number of clones mapped to corresponding homoeologous chromosome locations in both species along with the occurrence of additional locations being mapped	23
9.	STS-PCR mapping results for both wheat and barley with the 97 primer sets derived from various sources, along with the number mapping to homoeologous chromosome locations in both species and the number in which additional locations were also mapped besides the homoeologous location	24
10.	Total number of markers for wheat with both RFLP and STS mapping data available for the 97 cases evaluated, including the number from various sources identifying the same chromosome locations and the number mapping to additional locations	25
11.	Total number of markers for barley with available RFLP and STS mapping data for the 97 cases evaluated from various sources, including the number having the same chromosome location mapped, along with the number mapping to additional locations	26
12.	The total number of markers with barley RFLP and wheat STS mapping data for the 97 cases evaluated from various sources with the number mapping to the same homoeologous chromosome location, along with the total number of cases where additional locations were mapped	27

LIST OF TABLES - Continued

Table		Page
13.	Results from Southern analysis of the wheat amplification products for 34 primer sets derived from various sources, along with the hybridization results being summarized for the sources	28
14.	Southern analysis results for the products amplified with the barley cDNA-derived primer sets in wheat, including the mapped locations of the RFLP clone and STS products	29
15.	Southern analysis results in wheat for the amplification products of the barley genomic-derived primer sets, along with the RFLP and STS map locations	30
16.	Results of Southern analysis of amplification products in wheat for the primer sets derived from wheat genomic clones and the map locations for the RFLP clones and STS products	31
17.	Results for the Southern analysis of amplification products of primer sets derived from *T. tauschii* genomic clones in wheat, including the RFLP and STS map locations	32
18.	Results of the Southern analysis in barley for the 34 primer sets derived from various sources, including the hybridization results summarized for the primer sources	33
19.	Southern analysis results in barley for the amplification products of the barley cDNA-derived primer sets, plus the RFLP and STS map locations	33
20.	Results of Southern analysis of the amplification products in barley for primer sets derived from the barley genomic clones, along with map locations for the RFLP clones and the STS products	34

LIST OF TABLES - Continued

Table		Page
21.	Results from the Southern analysis in barley of the primers sets derived from wheat genomic clones, including the RFLP and STS map locations	34
22.	Results of Southern analysis in barley for the amplification products of the primer sets derived from genomic clones of *T. tauschii*, and the map locations for the RFLP clones and STS products	35
23.	Total number of primer sets derived from various sources screened on the parental lines, PI372129, Pondera, and Newana, with the number identified as conditioning amplification of polymorphic fragments between the three parents when amplification products were digested with one of four restriction enzymes, *Hin*fI, *Rsa*I, *Dde*I, or *Hha*I	62
24.	Marker analysis of the 14 single cross progeny derived from the cross between PI372129 and Pondera, including the total number of STS restriction fragments and SDS-PAGE protein bands scored relative to the PI372129 parent for each line. The total number of markers for each line in comparison to PI372129 was used to determine an estimate of the level of donor genome in each line	63
25.	Marker results for the 23 Pondera first backcross progeny derived from the cross between PI372129 and Pondera. The results include the total number of STS restriction fragments and SDS-PAGE protein bands scored relative to the PI372129 parent each line. The total number of markers corresponding to PI372129 was used to determine an estimate of the level of donor genome in each line	64

LIST OF TABLES - Continued

Table		Page
26.	Results of the marker analysis of the 21 Newana first backcross progeny derived from the cross between PI372129 and Newana, including the total number of STS restriction fragments and SDS-PAGE Protein bands scored relative to the PI372129 parent for each line. The total number of markers corresponding to PI372129 was used to determine an estimate of the level of donor genome in each line	65
27.	Results for five agronomic traits evaluated in the Pondera single cross population. The numbers represent the means obtained for the two locations with four replications each evaluated over a two year period for the 14 lines and Pondera	72
28.	Results for five agronomic traits evaluated for the Pondera first backcross population. The numbers represent the means for the 23 backcross lines and Pondera obtained for the two locations with four replications each evaluated over a two year period	73
29.	Results for five agronomic traits evaluated for the Newana backcross population. The numbers represent the means obtained for the two locations with four replications each evaluated over a two year period for the 21 backcross lines and Newana	74
30.	Results for three quality traits evaluated in the Pondera single cross population. The numbers represent the means obtained for the two locations with four replications each evaluated over a two year period for the 14 lines and Pondera	78

LIST OF TABLES - Continued

Table		Page
31.	Results for three quality traits evaluated in the Pondera first backcross population. The numbers represent the means obtained for the two locations with four replications each evaluated over a two year period for the 23 lines and Pondera	79
32.	Results for three quality traits evaluated in the Newana first backcross population. The numbers represent the means obtained for the two locations with four replications each evaluated over a two year period for the 21 lines and Newana	80
33.	Correlations between the eight traits and the percentage of donor genome for the Pondera single cross population	82
34.	Correlations between the eight traits and the percentage of donor genome for the Pondera backcross population	82
35.	Correlations between the eight traits and the percentage of donor genome for the Newana backcross population	83
36.	RFLP and STS chromosome locations in wheat and barley. Ninety-seven primer sets derived from various sources were mapped in wheat using the nullisomic-tetrasomic series and in barley using wheat-barley addition lines. Southern analysis was used to determine if STS amplification products for a subset of the primer sets had homology to the RFLP clone used to design the primer pair	102
37.	Results from the evaluation of 310 primer sets from various sources to determined the presence of polymorphic fragments between Pondera, Newana, and PI372129 when the amplified products were digested with *Hin*fI, *Rsa*I, *Dde*I, and *Hha*I	106

LIST OF TABLES - Continued

Table		Page
38.	Subset of 75 primers sets from various sources conditioning amplification of polymorphic fragments between Pondera and PI372129 used to evaluate the level of donor genome in the Pondera single cross population. Chromosome locations for STS products and/or RFLP clones are indicated for wheat and barley	114
39.	Subset of 52 primers sets from various sources directing amplification of polymorphic fragments between Pondera and PI372129 used to evaluate the level of donor genome for the Pondera backcross population. STS and/or RFLP chromosome locations are indicated for wheat and barley	116
40.	Subset of 41 primers sets from various sources and directing amplification of polymorphic fragments between Newana and PI372129 used to evaluated the level of donor genome in the Newana backcross population. STS and/or RFLP chromosome locations are indicated for wheat and barley	118
41.	Analysis of variance for heading date in the Pondera single cross population	119
42.	Analysis of variance for plant height in the Pondera single cross population	119
43.	Analysis of variance for grain yield in the Pondera single cross population	119
44.	Analysis of variance for test weight in the Pondera single cross population	120
45.	Analysis of variance for lodging index in the Pondera single cross population	120

LIST OF TABLES - Continued

Table		Page
46.	Analysis of variance for percent grain protein in the Pondera single cross population	120
47.	Analysis of variance for NIR kernel hardness in the Pondera single cross population	121
48.	Analysis of variance for SDS sedimentation protein quality in the Pondera single cross population	121
49.	Analysis of variance for heading date in the Pondera backcross population	121
50.	Analysis of variance for plant height in the Pondera backcross population	122
51.	Analysis of variance for grain yield in the Pondera backcross population	122
52.	Analysis of variance for test weight in the Pondera backcross population	122
53.	Analysis of variance for lodging index in the Pondera backcross population	123
54.	Analysis of variance for percent grain protein in the Pondera backcross population	123
55.	Analysis of variance for NIR kernel hardness in the Pondera backcross population	123
56.	Analysis of variance for SDS sedimentation protein quality in the Pondera backcross population	124
57.	Analysis of variance for heading date in the Newana backcross population	124

LIST OF TABLES - Continued

Table		Page
58.	Analysis of variance for plant height in the Newana backcross population	124
59.	Analysis of variance for grain yield in the Newana backcross population	125
60.	Analysis of variance for test weight in the Newana backcross population	125
61.	Analysis of variance for lodging index in the Newana backcross population	125
62.	Analysis of variance for percent grain protein in the Newana backcross population	126
63.	Analysis of variance for NIR kernel hardness in the Newana backcross population	126
64.	Analysis of variance for SDS sedimentation protein quality in the Newana backcross population	126

LIST OF FIGURES

Figure Page

1. Mapping results for STS-PCR primer set KSUG44 in wheat and barley, plus the results from the Southern hybridization of amplified products to the corresponding RFLP clone used to design the primer set. Panel A, nullisomic-tetrasomic wheat lines used to map the *Rsa*I digested STS-PCR products of primer set KSUG44 in wheat. Lanes 1A through 7D represent the nullisomic chromosome of the nullisomic-tetrasomic wheat lines. Lane CS represents Chinese Spring wheat. Missing bands in lanes 5A (minor band), 5B, and 5D indicates the map location for the PCR products. Panel B, wheat-barley addition lines used to map the *Rsa*I digested STS-PCR products of primer set KSUG44 in barley. Lanes 2H through 7H represent wheat lines with the addition of a barley chromosome. CS represents Chinese Spring and B represents Betzes barley. Lane 5H contains the diagnostic barley band, indicating that KSUG44 maps to chromosome 5H of barley. Panel C, Southern analysis of the PCR amplified products of KSUG44. Lanes 1, 3, and 5 represent Chinese Spring digested with *Hin*fI, *Rsa*I, and undigested, respectively. Lanes 2, 4, and 6 represent Betzes digested with *Hin*fI, *Rsa*I, and undigested, respectively. Lane 7 represents the hybridization of the probe to the KSUG44 RFLP clone. Hybridization was detected for mapped and unmapped bands in wheat and to the mapped band in barley 38

LIST OF FIGURES - Continued

Figure Page

2. The mapping results for STS-PCR primer set CDO213 in wheat and barley, plus the results from the Southern hybridization of amplified products to the corresponding RFLP clone used to design the primer set. Panel A, nullisomic-tetrasomic lines used to map the *Rsa*I digested STS-PCR products of primer set CDO213 in wheat. Lanes 1A through 7D represent the nullisomic chromosome of the nullisomic-tetrasomic wheat lines. Lane CS represents Chinese Spring wheat. Missing bands in lanes 1A, 1D, 4A, 4B, 5D, and 7D indicates the map locations for the PCR products. Panel B, wheat-barley addition lines used to map the undigested STS-PCR products of primer set CDO213 in barley. Lanes 2H through 7H represent wheat lines with the addition of a barley chromosome. CS represents Chinese Spring and B represents Betzes barley. Lane 7H contains the diagnostic barley band, indicating that CDO213 maps to chromosome 7H of barley. Panel C, Southern analysis of the PCR amplified products of CDO213. Lanes 1, 3, and 5 represent Chinese Spring digested with *Hin*fI, *Rsa*I, and undigested, respectively. Lanes 2, 4, and 6 represent Betzes digested with *Hin*fI, *Rsa*I, and undigested, respectively. Lane 7 represents the hybridization of the probe to the CDO213 RFLP clone. No hybridization was detected for mapped or unmapped bands in wheat or barley 39

ABSTRACT

DNA marker technology provides a tool for genetic investigations and has potential applications in crop improvement. Genetic linkage maps based on restriction fragment length polymorphisms (RFLPs) have been constructed for many crop species. The development of the polymerase chain reaction (PCR) technology provides an economical alternative to RFLP marker analysis. Mapped low copy RFLP makers can be converted to sequence-tagged-site (STS) markers that are amplified by PCR. The successful application of the approach relies on the ability to emulate RFLP information. The cereals provide a unique system to test the applicability, since a considerable degree of synteny exist between related species and RFLP markers can be transferred between species. STS-PCR amplification products were mapped in wheat using the nullisomic-tetrasomic series and in barley with wheat-barley addition lines. STS amplification products mapped to the same homoeologous group in wheat as the RFLP approximately 70% of the time. Barley STS location corresponded to the RFLP location approximately 60% of the time. RFLP mapping information was transferable 82% of the time between wheat and barley. The same homoeologous chromosomes was identified 74% of the time with the STS-PCR system. Multiple STS locations were generally mapped in wheat. Southern analysis was used to evaluate the homology of the amplification products to the RFLP clone. Homologous sequences were generally amplified, but non-homologous sequences were also amplified. The results indicated that RFLP location could provide an indication of the STS location in both wheat and barley, but amplification of unmapped RFLP locations and non-homologous sequences is a factor to consider in their application.

The potential application of STS markers in a backcross breeding program to introgress Russian wheat aphid resistance into wheat was also investigated. Molecular markers provide a tool in which the genotype of a plant can be evaluated in early generations, allowing for increased selection efficiency. Three recombinant inbred populations were established from crosses between a Russian wheat aphid resistant line, PI372129, and two Montana hard red spring wheat cultivars, Pondera and Newana, in order to evaluated the level of donor genome and potential correlations with phenotypic performance. The percentage of donor genome, based on marker analysis, varied between the lines within the populations. Significant positive correlations between percentage of donor genome with plant height and straw strength were observed for the Pondera single cross population. No significant correlations were observed for the two backcross populations. The backcross population means approached that of the recurrent parent with a decrease in lines significantly different from the recurrent parent. Results suggests that molecular marker could be used to select for the recurrent parent genotype, and provide optimism for marker-assisted selection to recover the recurrent parent phenotype.

CHAPTER 1

INTRODUCTION

Wheat has a major impact on the economy of Montana as well as the rest of the world. Montana is ranked third in the United States for total wheat production and second in the production of hard red spring wheat, accounting for 15 to 20% of the nation's total (Montana Agricultural Statistics 1994). Wheat is second only to maize in total acreage and production in the United States (Briggle and Curtis 1987). Approximately 20% of the world's arable land (232 million ha) is sown to wheat, which is more than any of the other crop species, thus making wheat the world's leading cereal crop (Briggle and Curtis 1987; Wiese 1977). Wheat is cultivated under the greatest range of environmental conditions and is the most broadly adapted of the cereal crops (Briggle and Curtis 1987). Approximately 40% of the world's population relies on wheat as a staple food, which supplies 20% of the caloric intake (Wiese 1977). Wheat can be used to make a variety of food products, but breadmaking properties unique to hard wheats have contributed to the economic importance of the crop worldwide. Wheat has been under cultivation for over 10,000 years.

It was the conscious efforts of early man to select mutant plants with desirable phenotypes and save this seed to be replanted the following growing season that altered the genetic information being passed to the next generation. These efforts of man, along with environmental selection pressure, have contributed to the evolution of the crop species (Helbaek 1959). Crop evolutionary development proceeded at a very slow rate, but can be considered one of the greatest accomplishments of mankind. The art of breeding plants to meet human needs has since taken on a more scientific approach with the application of the principles of genetics. Plant breeders are faced with new challenges as agriculture continues to evolve. Recent advances in technology offer new tools to further increase the efficiency of the breeding process.

The major endeavor of the plant breeder is to increase the overall productivity of the crop species while maintaining or improving a desired standard of quality. This objective is achievable by the assemblage of many favorable genes into a single cultivar through hybridization followed by selection. The greatest challenge faced by the plant breeder is the identification of plants with the most favorable combination of genes. The dramatic success that is possible is exemplified in maize, where productivity, as measured by the grain yield, has more than doubled within the past 30 years with the introduction in the 1960's of single cross hybrids (Tollenaar et al. 1994). A similar situation exists for wheat where productivity has increased by 250% since the turn of the century, with the majority of this increase occurring within the last 40 years (Slafer et al. 1994). One of the key factors to the continual success in plant breeding is genetic diversity, but the process

of breeding itself erodes this diversity by placing strong selection pressure on a limited set of desirable traits. The resultant narrowing of the germplasm base is of considerable concern, especially with introduced self-pollinated crops were diversity can be dramatically reduced over a relatively short time frame.

The cultivation of crop species with a narrow germplasm base over a wide geographical range provides an opportunity for pests to cause considerable damage. A classic example is the severe wheat stem rust epidemics affecting wheat productivity at the turn of the century. An estimated 38% of the wheat crop was lost due to stem rust (*Puccinia graminis* Pers. f. sp. *tritici* Eriks. & Henn.) in 1916, with a 66% reduction in yield in the North Central states of Minnesota, North Dakota, and South Dakota (Loegering et al. 1967; Roelfs 1982). These losses were so devastating and had such an economic impact that a federal program was initiated to eliminate the principle alternate host, barberry (*Berberis vulgaris* L.), in 1918 (Roelfs 1982). The elimination of barberry reduced the level of inoculum and disrupted the pathogen's sexual cycle resulting in more sporadic epidemics. Stem rust remained a major threat and in 1935 North Dakota suffered the single greatest statewide loss to be reported, estimated to be 57% (Schafer 1987). A more recent example of the potential threat pests can inflict on crop productivity due to the reliance on a narrow germplasm base was the Southern corn leaf blight epiphytotics in 1969 through 1971 affecting maize production. The leaf blight fungus, *Bipolaris maydis* (Nisik.) Shoem., was estimated to have caused $1 billion in losses in 1970 and in the

process eliminated the wide spread use of Texas male sterile cytoplasm in the hybrid seed corn industry (Smith and White 1988).

Resistance breeding is a major component of many breeding programs, and provides an economical means of controlling crop losses due to pests. The paucity of resistance genes in adapted germplasm has lead to investigations of more diverse sources. The ability to transfer the gene(s) to commercial cultivars determines the usefulness of the resistance. One breeding procedure that is commonly used to facilitate the transfer of genes from nonadapted to adapted germplasm is backcrossing. The procedure involves repeatedly crossing derived progeny to the adapted (recurrent) parent. The goal of the procedure is to eliminate the majority of the nonadapted (donor) genome and recover the recurrent parent genome with the addition of the gene of interest. Approximately 99% of the recurrent parent genome may be recovered after six backcross generations. Eight or more backcrosses may be performed to insure a greater recovery of the recurrent parent genome, making the process time consuming (Young and Tanksley 1989). The success in deriving a suitable variety can also be greatly influenced by the starting material. Recent advancements in DNA marker technology may provide a means to increase the overall efficiency of the procedure.

The development of molecular markers and the construction of genetic linkage maps have a number of potential applications that can aid plant breeding endeavors, and thus make the art of plant breeding a more exact science. Molecular markers have been used to study the complex inheritance of quantitative traits, which are under the control of

many genes with small effects and also significantly influenced by environmental factors. Markers have aided in the dissection of these quantitative traits, thus providing the means to evaluate the characters as simply inherited Mendelian factors. The application of markers has allowed specific chromosomal regions to be identified as having major influence on the genetic variation. These markers can then be used be manipulated the trait more efficiently in the breeding process by providing a means to monitor inheritance. Molecular markers can also serve as linked tags to specific genes of interest. This provides a means to indirectly monitor the transfer of the gene, and allows efficient selection to be practiced without evaluating the phenotype of the gene. Molecular tags allow for early generation screening for the desired gene without being destructive to the plant. Molecular tags for pest resistance genes can reduce the costs associated with screening for the resistant phenotype and reduce the inherent inaccuracies of phenotype selection. Molecular markers can thus be used to monitor the introgression of genes in a backcross breeding program, and based on mathematical models marker-assisted selection should aid in the efficient recovery of the recurrent parent genotype.

We wish to test the potential benefit molecular markers may provide towards increasing the efficiency of backcross breeding for pest resistance. A research project was conducted to evaluate the applicability of marker-assisted selection in the recovery of the recurrent parent phenotype in an effort to introgress Russian wheat aphid resistance into Montana hard red spring wheat varieties.

The wheat and barley projects at Montana State University have initiated an effort to converted mapped low copy restriction fragment length polymorphisms (RFLPs) to sequence-tagged-site polymerase chain reaction (STS-PCR) markers for use in various breeding applications. The conversion of RFLPs to STS-PCR markers involves the sequencing of the ends of the clones followed by the designing of primer sets from the sequence information (Talbert et al. 1994). The primer pairs are then recognized by the polymerase chain reaction (PCR) which initiates the amplification of a specific DNA sequence flanked by the pair. The amplified fragments can be evaluated in a similar fashion as with RFLP markers.

One concern in a number of the genetic analyses is the chromosomal coverage obtained for the STS-PCR markers. A related concern is the correspondence of chromosomal location between species and the ability to predict map location based on RFLP mapping data, since the STS markers have been derived from various RFLP clone sources. A study was conducted to address these concerns and provide information on the applicability of transferring marker data from various species.

CHAPTER 2

MAPPING AND EVALUATION OF STS-PCR MARKERS

Literature Review

Botstein et al. (1980) proposed the use of RFLPs in the development of a genetic linkage map of the human genome. There has been a concerted effort to develop RFLP maps for many of the crop species. RFLP maps have been developed for wheat (Liu and Tsunewaki 1991; Nelson et al. 1995a,b), barley (Graner et al. 1991; Heun et al. 1991; Kleinhofs et al. 1993), rice (Causse et al. 1994; McCouch et al. 1988), rye (Philipp et al. 1994; Wanous et al. 1995) and oat (O'Donoughue et al. 1995). Genetic maps have also been constructed for the diploid progenitors of wheat and oat to serve as starting points for map development in the cultivated polyploid forms (Gill et al. 1991; Kam-Morgan et al 1989; O'Donoughue et al. 1992). Map construction for the cereals has been aided by the fact that RFLP clones isolated and mapped from related species often hybridized to homologous sequences in other species (O'Donoughue et al. 1995). Comparative mapping results have indicated a conservation of marker order suggesting that mapping information may be transferable across species (Ahn et al. 1993; Devos et al. 1993; Kurata et al. 1994). The conservation of RFLP map order among the cereals has shown some

correspondence to the genetics underlying similar phenotypes in related crops (Pereira and Lee 1995). Their results suggest that genomic regions in sorghum controlling plant height produce similar phenotypes to the corresponding homoeologous regions in maize due to similar gene action and function. Syntenous relationships among the cereals have also enabled Laurie et al. (1994) to identify a photoperiod response gene on barley chromosome 2H based on comparative mapping data and the known existence of photoperiod response genes on wheat homoeologous group 2 chromosomes. A similar situation has been identified for the wheat vernalization gene (*Vrn*1), located on wheat chromosome 5A, in that homoeoloci have been identified on the homoeologous barley chromosome 5H, loci *Sh*2, and on the homoeologous rye chromosome 5R, locus *Sp*1 (Galiba et al. 1995). The ability to capitalize on the genetic conservation in cereals has added to the scope of the mapping data. The refinement of RFLP maps along with the development of new technologies has made this information more applicable to many plant breeding endeavors.

Conversion of mapped RFLP markers to a STS-PCR based marker system offers several attractive features to the plant geneticist. These include the relative ease, greater throughput and safety of PCR analysis, convenience of sharing primer sequences, and the relatively small amount and reduced quality of genomic DNA acceptable for use in PCR applications. The STS-PCR system is also less affected by reaction conditions as compared to other PCR-based approaches.

Olson et al. (1989) proposed the use of STSs as a common language for the development and synthesis of a physical map of the human genome. STS technology has since been extended to several cereals, including wheat (Talbert et al. 1994), barley (Tragoonrung et al. 1992), and rice (Inoue et al. 1994; Williams et al. 1991), where mapped RFLP clones have been sequenced and primer sets designed for use in PCR analysis. The resultant codominant nature of the STS amplification products makes the information content comparable to that obtained from RFLP analysis.

Recent experiments have shown that STS-PCR primer sets developed in wheat and barley may be, as with RFLPs, transferable between the two crops (Talbert et al. 1994), thus increasing their potential usefulness. Storlie and Talbert (1993) showed that primer set ST4,6, developed from a barley chromosome 4H genomic clone, also marked wheat homoeologous group 4 chromosomes. Nieto-Lopez and Blake (1994) found that primer set KSUD14, developed from a *Triticum tauschii* genomic clone and mapped on chromosome 1D, was associated with a gene for Russian wheat aphid resistance on the homoeologous barley chromosome 1H. There are also cases whereby STS primer sets do not appear to amplify homologous sequences between wheat and barley. Talbert et al. (1996) found that four primer sets designed from oat and barley RFLP clones and mapped on barley chromosome 4H did not amplify products from wheat homoeologous group 4 chromosomes. These inconsistent results would suggest a potential limitation to the transferability of STS primer sets between related species.

Transferability of STS marker information between related species would enhance the general utility of STS technology. A research project was conducted to assess transferability of STS primer sets between wheat and barley by determining the chromosomal location of STS products in both species.

Materials and Methods

Plant Material

The 21 nullisomic-tetrasomic lines of 'Chinese Spring' wheat (Sears 1954) and wheat-barley addition lines (Shepherd and Islam 1981), with the exception of addition line 1H which was unavailable, were utilized to map the STS-PCR products in wheat and barley, respectively. DNA was extracted from young leaves of greenhouse grown plants using the procedure of Talbert et al. (1992). Approximately 1.0 g of fresh leaf tissue was ground in 15 mL extraction buffer (100 m*M* Tris pH 8.0, 50 m*M* EDTA pH 8.0, 100 m*M* NaCl, 1% SDS, and 10 m*M* 2-mercaptoethanol) using a mortar and pestle. The ground samples were then transferred to 30 mL Oakridge tubes and incubated in a water bath for 10 minutes at 65°C followed by addition of 5 mL of 5 *M* potassium acetate with a 20 minute incubation on ice. The tubes were centrifuged for 20 minutes at 25,000 X g with the resulting supernatants being filtered through miracloth into clean 30 mL Oakridge

tubes containing 10 mL cold isopropanol and 1 mL 5 *M* ammonium acetate. The tubes were then incubated at -20°C for 20 minutes with the DNA being pelleted by centrifugation for 15 minutes at 20,000 X g. The supernatants were discarded and the DNA pellets were resuspended in 0.7 mL TE buffer (10 m*M* Tris-Cl, 1 m*M* EDTA pH 8.0). Resuspended DNAs were then transferred to 1.5 mL microfuge tubes and 75 µL 3 *M* sodium acetate (pH 7.0) plus 0.5 mL cold isopropanol were added followed by a 30 second centrifugation in a microfuge (15,000 rpm). The resulting supernatants were discarded and the DNA pellets were resuspended in 0.1 mL TE buffer. DNAs were quantified by comparison to a DNA standard separated on a 0.7% agarose gel ran in 1X TBE buffer and stained with ethidium bromide. DNA concentrations were adjusted to approximately 100 ng/µL for use is PCR assays.

STS-PCR Marker Analysis

A total of 97 STS-PCR primer sets was evaluated. Primer sets were developed from several sources: (1) 29 were from genomic clones of *T. tauschii* (KSU); (2) 21 were from barley cDNA clones (ABC and BCD); (3) 31 were from barley genomic clones (ABG, BarG10, and MWG); (4) four were from oat cDNA clones (CDO); and (5) 12 were from wheat genomic clones (WG) (Table 1). Primer set BarG10 was designed from a barley PCR product amplified by *T. tauschii*-derived primer set KSUG10. Primer pairs were developed by sequencing both ends of the RFLP clone using the dideoxy chain termination protocol (Sanger et al. 1977). Approximately 200 base pairs (bp) of sequence