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.

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Head, Major Department

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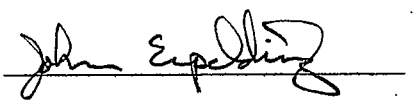
Signature 

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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

information were obtained from each end which was used to design primer pairs consisting of approximately 20 bp each. Concentrations of the synthesized primers were determined spectrometrically and adjusted to 100 ng/μL.

Table 1. RFLP clone sources used to design 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.

Source	Number of Primers Sets Evaluated	Reference
Barley Genomic	31	Heun et al. 1991; Kleinhofs et al. 1993
Barley cDNA	21	Graner et al. 1991; Kleinhofs et al. 1993
Oat cDNA	4	Heun et al. 1991
Wheat Genomic	12	Heun et al. 1991
T. tauschii Genomic	29	Gill et al. 1991
Total	97	

PCR amplification conditions, product digestion, and gel separation were performed as described by Talbert et al. (1994) with an annealing temperature of 45°C or 50°C being used, depending on the optimal temperature for each primer set in the two species. PCR reactions consisted of 50 m*M* KCl, 10 m*M* Tris-HCl, 0.1% Triton X-100, 50 μ*M* of each of the four dNTPs, 1.5 m*M* $MgCl_2$, 400 n*M* of each of left and right primers, 0.8 units of *Taq* DNA polymerase, and 200 ng of genomic DNA in a total volume of 50 μL. Reactions were performed in 0.5 mL microfuge tubes and overlayed with approximately 100 μL of mineral oil. PCR was performed in a MJ (PTC-100) thermocycler (MJ Research, Inc.) using the following protocol; an initial 4 minutes at 94°C

followed by 30 cycles of 1 minute at 94°C, 1 minute at 45°C or 50°C, and 1.2 minutes at 72°C, with a final extension of 7 minutes at 72°C followed by a 4°C hold.

Digestion with restriction enzymes is often necessary to distinguish among PCR products amplified from wheat homoeologous chromosomes (Talbert et al. 1994). PCR products amplified from the nullisomic-tetrasomic wheat lines were therefore digested with two restriction enzymes, *Hin*fI and *Rsa*I. Mapping results were pooled for both enzymes, since digestion with different restriction enzymes can reveal additional locations. Restriction digests were performed in microtiter plates, where 2 units of the restriction enzyme were added to 25 µL of the PCR reaction in individual wells followed by a 1 hour incubation at 37°C. Undigested PCR products for the wheat-barley addition lines were evaluated, and restriction digestions with *Hin*fI and *Rsa*I performed only when inconclusive data were obtained. PCR products were separated on 7% polyacrylamide gels using a 0.5X Tris-borate running buffer (22 m*M* Tris-HCl, 22 m*M* boric acid, and 0.5 m*M* EDTA). Gels were stained with ethidium bromide and the DNA was visualized with *UV* light and photographed. Nullisomic-tetrasomic lines were scored for the absence of bands in comparison with Chinese Spring. Wheat-barley addition lines were scored for the presence of additional bands compared to Chinese Spring.

Southern Analysis

Southern blot analysis was conducted to determine the relationship of the PCR products to the corresponding RFLP clones used to design the primer sets. Aliquots of

PCR products amplified from Chinese Spring and the barley cv. 'Betzes' were digested with *Hin*fI and *Rsa*I, and along with the undigested PCR products separated on a 7% polyacrylamide gel. DNA was transferred to Zeta-Probe (Bio-Rad) nylon membranes using a Trans-Blot SD apparatus (Bio-Rad) following manufacturer's directions. Semi-dry transfers were performed at 400 mA for one hour with the DNA being fixed to the membranes by placing the membranes on blotting paper saturated with 0.4 *M* NaOH for 10 minutes followed by a single rinse in 2X SSC solution for 10 minutes before being baked at 80°C for one hour. PCR-amplified RFLP clone inserts served as probes for hybridization and were labeled with [^{32}P]dCTP by the random hexamer primer reaction (Feinberg and Vogelstein 1983) with unincorporated nucleotide triphosphates being removed by spin dialysis through Sephadex G-50 (Pharmacia) columns. Blots were washed at 60°C in blot wash buffer (0.1X SSC and 0.1% SDS), and in cases where no hybridization was observed, blots were stripped (Sambrook et al. 1989) and reprobed following the same protocol, with the exception that the stringency of the blot wash was lowered to 50°C. Southern blot hybridization is summarized as follows. Blots were washed for 1 hour in blot wash buffer (0.1X SSC and 0.1% SDS) at 65°C followed by a prehybridization for 6 hours at 42°C in hybridization buffer (3X SSC, 5X Denhardt's, 50% formamide, 1% SDS, and 100 μg/mL sheared salmon sperm DNA). Hybridizations, with the labeled denatured probe added to 10 mL of the hybridization buffer, were carried out overnight at 42°C. Blots were then washed for 30 minutes at 42°C in formamide wash buffer (50% formamide, 5X SSC, and 0.2% SDS) followed by three 30 minute washes at

60°C or 50°C in blot wash buffer. Hybridization filters were exposed to X-ray film with a single intensifying screen at -80°C for 1 hour.

Results

Mapping STS Markers to Chromosomes

Chromosome locations of STS products were determined using nullisomic-tetrasomic stocks for mapping in wheat, and wheat-barley addition lines for mapping in barley. Nullisomic-tetrasomic lines were compared to Chinese Spring, which is the genetic background used to create the aneuploid stocks, and scored for the absence of restriction fragments. A missing fragment would indicate that the sequence being amplified was on the nullisomic (missing) chromosome. Nullisomic-tetrasomic data were obtained for 84 of 97 primer sets analyzed (Table 2). STS products for 13 primer sets were not mapped, since no missing fragments were observed with any of the 21 nullisomic-tetrasomic lines when compared to Chinese Spring. All the amplified products, after restriction digestion, for 10 of the 84 primer sets were mapped using the 21 nullisomic-tetrasomic stocks. Mapped and unmapped bands were observed for 74 of the 84 primer sets, with the number of unmapped bands varying from one to more than ten. These unmapped bands can result from the amplification of homoeologous sequences

lacking restriction site differences. Twenty-nine primer sets were characterized as having greater than 10 restriction fragments, many of which corresponded to minor bands. Most primer sets conditioned amplification of one to seven major bands, with an average of three to four major bands, and fewer than 10 minor bands.

Table 2. Total number of STS primer sets designed from 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 cases analyzed.

Source	Wheat Nullisomic-Tetrasomic Lines (STS)	Wheat-Barley Addition Lines (STS)	Wheat RFLP	Barley RFLP
Barley Genomic (31)	27	26	5	31
Barley cDNA (21)	18	16	11	20
Oat cDNA (4)	4	2	4	4
Wheat Genomic (12)	9	5	10	12
T. tauschii Genomic (29)	26	5	29	9
Total (97)	84	54	59	76

The number of locations mapped in wheat for the 84 primer set ranged from one to nine with an average of 3.7 locations (Table 3). Primer sets derived from oat cDNA and *T. tauschii* genomic clones had a greater average number of mapped locations, 4.5 and 4.2, respectively, followed by the primers designed from barley cDNA clones with and average of 3.9 locations. A single chromosome or homoeologous chromosome group was

mapped for 19 of the 84 primer sets. A single chromosome location was mapped for 11 of the 19 primer sets. Restriction fragments amplified from each of the three chromosomes in a homoeologous group was observed for three of the 19 primer sets. Two of the three chromosomes in a homoeologous group were mapped with the remaining five primer sets.

Table 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 for 83 of the 97 STS-PCR primer sets designed from various sources mapped using the wheat nullisomic-tetrasomic series.

Source	Total Number of Locations Mapped	Average Number of Locations Mapped	Number of Primer Sets Mapping to a Single Chromosome or Homoeologous Chromosome Group
Barley Genomic	87	3.2	6
Barley cDNA	70	3.9	4
Oat cDNA	18	4.5	0
Wheat Genomic	29	3.2	3
T. tauschii Genomic	110	4.2	6
Total	314	3.7	19

An average of approximately 15 restriction fragments were mapped to each of the 21 chromosome pairs of wheat, with a minimum of eight fragments being mapped to chromosome 3D and a maximum of 24 fragments for both chromosomes 5B and 6B (Table 4). Chromosomes 4A and 2B were also frequently mapped with 22 and 21 fragments, respectively.

Table 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.

Wheat Chromosome	Source					Total	Homoeologous Group Total
	Barley Genomic	Barley cDNA	Oat cDNA	Wheat Genomic	*T. tauschii* Genomic		
1A	3	3	2	1	4	13	
1B	4	3	0	0	6	13	
1D	0	4	1	1	5	11	37
2A	4	3	0	0	7	14	
2B	7	4	0	2	8	21	
2D	4	2	1	1	3	11	46
3A	3	5	0	1	3	12	
3B	5	8	0	1	3	17	
3D	2	4	1	0	1	8	37
4A	7	2	3	4	6	22	
4B	5	3	1	3	5	17	
4D	1	2	0	3	6	12	51
5A	5	3	0	0	3	11	
5B	6	5	2	2	9	24	
5D	4	0	2	1	4	11	46
6A	4	4	1	0	2	11	
6B	8	4	0	3	9	24	
6D	4	3	0	1	6	14	49
7A	4	2	2	3	4	15	
7B	6	4	0	1	7	18	
7D	1	2	2	1	9	15	48
Total	87	70	18	29	110	314	314

A maximum of 51 restriction fragments were mapped to the homoeologous group 4 chromosomes, which accounted for approximately 16% of the mapped fragments (Table 4). The next most frequently mapped homoeologous chromosome groups were 6

and 7 with 49 and 48 restriction fragments, respectively. Homoeologous chromosome groups 1 and 3 had the fewest mapped restriction fragments with 37 fragments each. Chromosomes of the B genome contained the most mapped restriction fragments with 134, which accounted for approximately 43% of the fragments (Table 5). Chromosomes of the A genome accounted for approximately 31% of the mapped fragments (97) with the remaining 26% (82) mapping to the D genome chromosomes. Similar results were observed for primer sets design from the various clone sources with the exception of the *T. tauschii* genomic-derived primer sets where map locations for the D genome chromosomes were favored over the A genome chromosomes, but the B genome chromosomes remained the most frequently mapped.

Table 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.

Wheat Genome	Source					Total
	Barley Genomic	Barley cDNA	Oat cDNA	Wheat Genomic	*T. tauschii* Genomic	
A	30	22	8	9	29	98
B	41	31	3	12	47	134
D	16	17	7	8	34	82
Total	87	70	18	29	110	314

The map locations in barley were determined by the occurrence of additional bands in the wheat-barley addition lines when compared to Chinese Spring, with the additional

fragments corresponding to the barley chromosomes added to the Chinese Spring background. Data were obtained for 54 of the 97 primer sets analyzed with the wheat-barley addition lines (Table 2). No distinguishable band was identified either for a specific wheat-barley addition line or between wheat and barley with 21 primer sets, and digestion of the samples with the two restriction enzymes did not aid in the production of differentiating bands. It was not possible to test the primer sets with the wheat-barley addition line containing chromosome 1H, which would be expected to account for the lack of some mapping data. A chromosome 1H location was expected with 13 of the 97 primer sets analyzed based on barley and wheat RFLP mapping information. A chromosome 1 location was identified in 36 of 97 cases based on nullisomic-tetrasomic mapping data. No amplification occurred for the barley DNA in the wheat-barley addition lines in 22 cases, which may be due to the divergence in the priming site when *Triticum*-derived primer set were analyzed or suboptimal amplification conditions for barley, since wheat DNA was amplified.

An average of 1.2 locations were mapped for each of the primer sets in barley (Table 6). A maximum of three chromosomes were mapped for three of the 54 primer set. Forty-seven of the 54 primer sets mapped to a single chromosome. The map location for 24 of the 54 primer sets involved only two chromosomes, 4H and 6H, with 11 primer sets mapping to each chromosome and two primers sets mapping to both of the chromosomes (Table 7). Chromosome 3H had the fewest mapped markers with seven followed by chromosome 7H with nine.

Table 6. Total number of locations mapped, average number of locations mapped per primer set, and total number mapped to a single chromosome for 54 of the 97 STS-PCR primer sets derived from various sources and analyzed using the wheat-barley addition lines for mapping in barley.

Source	Total Number of Locations Mapped	Average Number of Locations Mapped	Number of Primer Sets Mapping to a Single Chromosome
Barley Genomic	31	1.2	23
Barley cDNA	17	1.1	15
Oat cDNA	2	1.0	2
Wheat Genomic	6	1.2	4
T. tauschii Genomic	9	1.8	3
Total	65	1.2	47

Table 7. Total number of mapped STS locations for each barley chromosome as determined by the wheat-barley addition line analysis of 54 of the 97 primer sets derived from various sources.

Barley Chromosome	Source					Total
	Barley Genomic	Barley cDNA	Oat cDNA	Wheat Genomic	*T. tauschii* Genomic	
2H	4	4	0	0	3	11
3H	5	2	0	0	0	7
4H	8	3	0	1	1	13
5H	4	2	1	2	2	11
6H	6	4	0	2	1	13
7H	4	2	1	1	1	9
Total	31	17	2	6	8	64

Correspondence of Chromosomal Locations

The GrainGenes (1995) database was accessed to obtain pertinent RFLP information in order to compared RFLP and STS map locations and determine the level of transferability. Wheat and barley RFLP data were available for 59 and 76 markers, respectively, from a total of 97 markers evaluated (Table 2). More RFLP mapping data was available for barley than wheat, since 68 of the 97 primer sets were designed from RFLP clones used in the development of the barley genetic map. The *T. tauschii* genomic clones were used in mapping wheat, so more mapping information from this source was available for wheat, but this source represents a smaller fraction of the total markers evaluated, 29 of 97.

RFLP mapping data were available for both species in 39 of 97 cases (Table 8). Homoeologous chromosome locations were identical for both species in 32 of the 39 cases, thus indicating that a 82% correspondence of RFLP mapping data exists between wheat and barley. Multiple locations were mapped in two cases, which corresponded to an additional location being detected in barley. RFLP locations in wheat and barley corresponded in 12 of 16 cases for barley-derived clones. *Triticum*-derived clones had corresponding RFLP locations in 17 of 19 cases. Three of the four oat cDNA clones also had corresponding locations. Genomic-derived clones had corresponding RFLP locations in 22 of 24 cases for wheat and barley; whereas, cDNA-derived clones had a considerably lower number of location matches, only 10 of 15 cases.

Table 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.

Source	Number of RFLP Clones with Mapping Data for Both Species	Homoeologous Chromosome Locations	Additional Locations Mapped
Barley Genomic	5	5	0
Barley cDNA	11	7	0
Oat cDNA	4	3	0
Wheat Genomic	10	10	1
T. tauschii Genomic	9	7	1
Total	39	32	2

STS mapping data for both wheat and barley were obtained with 48 of the 97 primer sets (Table 9). Homoeologous chromosome locations for STS products in wheat and barley were detected for 36 of the 48 primer sets, with 10 of 36 primer sets mapping only to the homoeologous location. One to six additional locations were mapped for the remaining 26 primer sets. Fifteen mapped to three or more locations, which generally corresponded to additional locations being amplified in wheat. The majority (33 of 48 primer sets) of the STS data was obtained for primers sets derived from genomic clones with homoeologous locations occurring in 25 of 33 cases. Primer sets derived from cDNA clones had homoeologous locations in 11 of 15 cases. More mapping data was available for barley-derived primer sets than *Triticum*-derived primer sets. Homoeologous

locations were mapped for 26 of 36 barley-derived primer sets. Eight of 10 *Triticum*-derived primer sets mapped to homoeologous locations.

Table 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.

Source	Number of STS Markers with Mapping Data for Both Species	Homoeologous Chromosome Locations	Additional Locations Mapped
Barley Genomic	23	17	12
Barley cDNA	13	9	7
Oat cDNA	2	2	2
Wheat Genomic	5	4	3
T. tauschii Genomic	5	4	2
Total	48	36	26

Comparisons between wheat RFLP and wheat STS data sets showed that 34 of 49 STS markers mapped to the same homoeologous chromosome group as did the RFLP (Table 10). Twenty-seven of the 34 STS markers also mapped additional locations. Three or more additional locations were mapped in 13 of 27 cases, which generally corresponded to additional locations being amplified. The occurrence of additional RFLP locations was reported in six cases. Primer sets derived from *T. tauschii* genomic clones supplied the majority of the mapping data, where amplified products for 21 of 26 primer sets mapped to the expected RFLP location. Amplified products from the *Triticum*-derived primer sets mapped to the expected location in 24 of 33 cases. Expected locations

were mapped in seven of 12 cases for the barley-derived primer sets. Nine of 13 cDNA-derived primers sets, including the four from oat, and 25 of 36 genomic-derived primer sets mapped to the expected wheat RFLP location.

Table 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.

Source	Mapping Data for Both RFLP and STS Markers	Same Chromosome Location Mapped	Additional Locations Mapped
Barley Genomic	3	1	1
Barley cDNA	9	6	5
Oat cDNA	4	3	3
Wheat Genomic	7	3	1
T. tauschii Genomic	26	21	17
Total	49	34	27

The published barley RFLP location matched the barley STS location in 28 of 50 cases (Table 11). Six of the 28 primers sets conditioned amplification of products from additional locations. Barley cDNA-derived primer sets mapped to the expected RFLP location in eight of 16 cases. The expected location was mapped in 18 of 26 cases for the barley genomic-derived primer sets. Primer sets derived from wheat and *T. tauschii* genomic clones mapped to the expected RFLP chromosome location in two of six cases. Eight of 18 cDNA and 20 of 32 genomic-derived primer sets mapped to the corresponding barley RFLP chromosome location.

Table 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.

Source	Mapping Data for Both RFLP and STS Markers	Same Chromosome Location Mapped	Additional Locations Mapped
Barley Genomic	26	18	3
Barley cDNA	16	8	2
Oat cDNA	2	0	0
Wheat Genomic	5	2	1
T. tauschii Genomic	1	0	0
Total	50	28	6

The same homoeologous chromosome was mapped in 45 of 65 comparisons between barley RFLP and wheat STS mapping data (Table 12). Additional locations were mapped in 36 cases, which generally corresponded to additional locations being amplified in wheat, but in three cases additional RFLP locations were mapped for barley. *Triticum*-derived primer sets mapped to the expected location in 13 of 17 cases. All eight *T. tauschii*-derived primer sets mapped to the expected homoeologous chromosome location. Wheat STS locations for the barley-derived primers sets matched the barley RFLP locations in 29 of 44 cases. Fourteen of 21 cDNA-derived primer sets mapped to the expected homoeologous wheat chromosomes. The number of location matches was greater for the genomic-derived primer sets, where the wheat STS location corresponded to the barley RFLP location in 31 of 44 cases.

Table 12. Total number of markers with barley RFLP and wheat STS mapping data available 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.

Source	Mapping Data for Both Barley RFLP and Wheat STS Markers	Same Homoeologous Chromosome Location Mapped	Additional Locations Mapped
Barley Genomic	27	18	14
Barley cDNA	17	11	9
Oat cDNA	4	3	3
Wheat Genomic	9	5	4
T. tauschii Genomic	8	8	6
Total	65	45	36

Southern Analysis of Amplification Products

Southern analysis was performed to determine if the products amplified from the STS-PCR primer sets had homology to the RFLP clone from which they were developed. A subset of 34 STS-PCR primer sets was analyzed which included 13 of wheat (4) or *T. tauschii* (9) origins, 19 of barley origin (7 cDNAs and 12 genomic), and two from oat cDNAs (Table 13). Hybridization to amplified wheat sequences was observed in 22 of 34 cases (Table 13). Amplified wheat sequences from the barley-derived primer sets hybridized to the clone in 11 of 19 cases. All mapped fragments in three of 11 cases

hybridized to the clone. Hybridization to unmapped bands was a common occurrence in wheat and observed for all 11 reactions.

Table 13. Results from Southern analysis of the wheat amplification products for 34 primer sets derived from various sources, along with the hybridization results summarized for the sources.

Source	Hybridization			No Hybridization	Total
	Mapped Locations Only	Mapped and Unmapped Locations	Unmapped Locations Only		
Barley Genomic	0	4	3	5	12
Barley cDNA	0	2	2	3	7
Oat cDNA	0	0	0	2	2
Wheat Genomic	1	2	1	0	4
T. tauschii Genomic	2	5	0	2	9
Total	3	13	6	12	34

Hybridization to both mapped and unmapped amplified wheat sequences for the barley cDNA-derived primer sets was detected in two of seven cases (Table 14). Hybridization to unmapped sequences was observed with two additional primer sets. Primer set BCD129, derived from a barley cDNA clone that mapped to wheat chromosome 4A, amplified a product that mapped to chromosome 2B with hybridization being detected for the amplified chromosome 2B sequence. Southern analysis indicated that homologous sequences were amplified even in cases were chromosome locations did not correspond. Primer set BCD175 mapped to chromosomes 2B and 2D, which

corresponded to the RFLP location, but no hybridization was detected for the amplified sequences. The lack of hybridization would indicate non-homologous sequences were amplified.

Table 14. Southern analysis results for the products amplified with the barley cDNA-derived primer sets in wheat, including the mapped locations for the RFLP clone and STS products.

Primer Set	Wheat RFLP Location	Wheat STS Location	Southern Analysis
ABC158	7A	1D,3D,6D	No Hybridization
ABC253		1A,2A,3B,4B,4D,5A	Hybridization to Unmapped Bands
ABC303		3A,3B,4D	Hybridization to Unmapped Bands
ABC309		3D,6A	No Hybridization
ABC602		3B,3D	Hybridization to 3B,3D + Unmapped bands
BCD129	4A	2B	Hybridization to 2B + Unmapped Bands
BCD175	2A,2B,2D	2B,2D	No Hybridization

Hybridization was also detected for mapped and unmapped amplified wheat sequences with four of 12 barley genomic-derived primer sets (Table 15). Hybridization to only unmapped amplified sequences was observed for three additional primer sets. Primer set ABG391, derived from a barley genomic clone mapping to wheat chromosome 5A, amplified products on chromosomes 4A, 5A, 5B, and 5D, with hybridization to amplified sequences on chromosomes 5A, 5B, and 5D plus unmapped sequences. Southern analysis indicated homologous and non-homologous sequences were amplified in wheat.

Table 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.

Primer Set	Wheat RFLP Location	Wheat STS Location	Southern Analysis
ABG058		2B,5B,6A,7B	No Hybridization
ABG317		2D,6B	No Hybridization
ABG358		1A,5A	No Hybridization
ABG378	6D	2B,2D,3B,3D	No Hybridization
ABG391	5A	4A,5A,5B,5D	Hybridization to 5A,5B,5D + Unmapped bands
ABG602		2B,2D,3B	Hybridization to 2B,2D + Unmapped Bands
ABG618		1B,2A,4A,5B,7B,7D	No Hybridization
ABG701		7A,7B	Hybridization to Unmapped Bands
ABG704		4A,7A	Hybridization to 4A,7A + Unmapped Bands
ABG712		1A,2A,2B,2D	Hybridization to Unmapped Bands
ABG715		4A,4B	Hybridization to 4B + Unmapped Bands
BarG10		3B,4B,5D,6D	Hybridization to Unmapped Bands

Eleven of 13 primer sets derived from wheat and *T. tauschii* genomic clones amplified products in wheat that hybridized to the RFLP clone (Table 13). Hybridization to all mapped bands was observed in four cases. Hybridization to unmapped bands was a common feature and occurred for eight of the 11 reactions.

Mapped amplification products for three of four wheat genomic-derived primer sets hybridized to the RFLP clone with hybridization to unmapped bands occurring for the remaining primer set (Table 16). Primer set WG181 mapped to chromosome 4B and the RFLP clone mapped to chromosomes 4A, 4B, and 4D, with hybridization being detected for the chromosome 4B amplified sequence in addition to unmapped sequences. RFLP

mapping information indicated a chromosome 5D location for the wheat genomic clone WG530, with the STS restriction fragments being mapped to chromosomes 4A and 6B, and hybridization being detected only for unmapped sequences. Results indicate that homologous and non-homologous sequences are amplified with the wheat genomic-derived primer sets.

Table 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 clone and STS products.

Primer Set	Wheat RFLP Location	Wheat STS Location	Southern Analysis
WG181	4A,4B,4D	4B	Hybridization to 4B + Unmapped Bands
WG232		4A,4D,6B,7A	Hybridization to 4A,6B,7A + Unmapped Bands
WG530	5D	4A,6B	Hybridization to Unmapped Bands
WG564	5D	2B,2D,4B,4D	Hybridization to 4B

Hybridization was detected for both mapped and unmapped restriction fragments for five of nine *T. tauschii* genomic-derived primer sets (Table 17). Hybridization to only mapped bands occurred in two cases. No hybridization was detected for the remaining two primer sets. RFLP mapping results indicated map locations on homoeologous group 1 chromosomes (1A, 1B, and 1D) for KSUE8. Multiple chromosomal STS locations were mapped, including chromosomes 1D, 2B, 4D, 5D, 6B, and 7D, with hybridization to amplified fragments on chromosomes 4D, 6B, and 7D, along with hybridization to unmapped fragments. RFLP and STS locations were identified on chromosomes 7A, 7B,

and 7D for KSUD2 with hybridization to these mapped amplified fragments plus unmapped bands.

Table 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.

Primer Set	Wheat RFLP Location	Wheat STS Location	Southern Analysis
KSUD2	7A,7B,7D	7A,7B,7D	Hybridization to 7A,7B,7D + Unmapped bands
KSUD15	7A,7B,7D	1D,2A, 5B,5D,6B,7B,7D	Hybridization to 2A,7B,7D + Unmapped bands
KSUD16	1D	1B,4A, 4B,4D,5B,7A	No Hybridization
KSUE8	1A,1B,1D	1D,2B, 4D,5D,6B,7D	Hybridization 4D,6B,7D + Unmapped Bands
KSUF15	2A,2B,2D	1A,2A,2B,3A, 5B,6A,6B,6D,7A	Hybridization to 2A,2B + Unmapped bands
KSUG12	4A,5D,7A,7D	1D,2B,2D,4A,7B	Hybridization to 2B,2D,7B
KSUG44	5A,5B,5D,6D	5A,5B,5D	Hybridization to 5A,5B,5D
KSUG49	2D	4A,7B,7D	Hybridization to 4A,7D + Unmapped Bands
KSUM148	1D	1B,2A,4B,4D,6B	No Hybridization

Hybridization of STS products in barley to the RFLP clone was observed for 16 of 19 barley-derived primer sets (Table 18). Hybridization was observed for the mapped STS fragment with hybridization to unmapped fragments occurring less frequently. Hybridization was detected for amplified sequences with five of seven barley cDNA-derived primer sets, which corresponded to the mapped location in two cases (Table 19). Hybridization was detected for 11 of 12 barley genomic-derived primer sets, and