



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
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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 evaluate 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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APPROVAL

of a thesis submitted by

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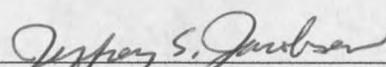
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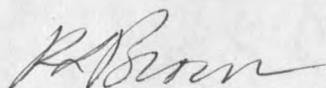
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ABSTRACT

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 evaluate 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 where 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 epiphytotic 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 led 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 to manipulate 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 convert 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'Donoghue 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'Donoghue 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'Donoghue 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 (*Vrn1*), located on wheat chromosome 5A, in that homoeoloci have been identified on the homoeologous barley chromosome 5H, loci *Sh2*, and on the homoeologous rye chromosome 5R, locus *Sp1* (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 mM Tris pH 8.0, 50 mM EDTA pH 8.0, 100 mM NaCl, 1% SDS, and 10 mM 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 mM Tris-Cl, 1 mM 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 in 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/ μ L.

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
<i>T. tauschii</i> 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 mM KCl, 10 mM Tris-HCl, 0.1% Triton X-100, 50 μ M of each of the four dNTPs, 1.5 mM MgCl₂, 400 nM 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 μ L. Reactions were performed in 0.5 mL microfuge tubes and overlaid with approximately 100 μ L 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, *HinfI* and *RsaI*. 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 *HinfI* and *RsaI* performed only when inconclusive data were obtained. PCR products were separated on 7% polyacrylamide gels using a 0.5X Tris-borate running buffer (22 mM Tris-HCl, 22 mM boric acid, and 0.5 mM 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 *Hinf*I 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 [³²P]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 reprobbed 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
<i>T. tauschii</i> 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 an 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
<i>T. tauschii</i> 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	<i>T. tauschii</i> Genomic		
1A	3	3	2	1	4	13	37
1B	4	3	0	0	6	13	
1D	0	4	1	1	5	11	
2A	4	3	0	0	7	14	46
2B	7	4	0	2	8	21	
2D	4	2	1	1	3	11	
3A	3	5	0	1	3	12	37
3B	5	8	0	1	3	17	
3D	2	4	1	0	1	8	
4A	7	2	3	4	6	22	51
4B	5	3	1	3	5	17	
4D	1	2	0	3	6	12	
5A	5	3	0	0	3	11	46
5B	6	5	2	2	9	24	
5D	4	0	2	1	4	11	
6A	4	4	1	0	2	11	49
6B	8	4	0	3	9	24	
6D	4	3	0	1	6	14	
7A	4	2	2	3	4	15	48
7B	6	4	0	1	7	18	
7D	1	2	2	1	9	15	
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	<i>T. tauschii</i> 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
<i>T. tauschii</i> 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	<i>T. tauschii</i> 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 compare 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
<i>T. tauschii</i> 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
<i>T. tauschii</i> 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
<i>T. tauschii</i> 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
<i>T. tauschii</i> 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
<i>T. tauschii</i> 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
<i>T. tauschii</i> 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 where 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

corresponded to the mapped STS location in 10 cases with additional unmapped bands detected for three primer sets (Table 20).

Table 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.

Source	Hybridization			No Hybridization	Hybridization But No Mapping Data	Total
	Mapped Locations Only	Mapped and Unmapped Locations	Unmapped Locations Only			
Barley Genomic	7	3	0	1	1	12
Barley cDNA	1	1	1	2	2	7
Oat cDNA	0	0	0	2	0	2
Wheat Genomic	0	0	0	0	3	4
<i>T. tauschii</i> Genomic	1	0	0	4	4	9
Total	9	4	1	9	10	34

Table 19. Southern analysis results in barley for the amplification products of barley cDNA-derived primer sets, plus the RFLP and STS map locations.

Primer Set	Barley RFLP Location	Barley STS Location	Southern Analysis
ABC158	7H		No Hybridization
ABC253	7H	7H	Hybridization to 7H + Unmapped Bands
ABC303	4H	4H	Hybridization to Unmapped Bands
ABC309	5H		No Hybridization
ABC602			Hybridization but No STS Mapping Data
BCD129	7H		Hybridization but No STS Mapping Data
BCD175	2H	2H	Hybridization to 2H

Table 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 STS products.

Primer Set	Barley RFLP Location	Barley STS Location	Southern Analysis
ABG058	2H	2H	Hybridization to 2H
ABG317	2H	2H	No Hybridization
ABG358	2H		Hybridization but No STS Mapping Data
ABG378	6H	6H	Hybridization to 6H
ABG391	5H	5H	Hybridization to 5H
ABG602	2H	2H,4H,6H	Hybridization to 2H
ABG618	4H	4H	Hybridization to 4H
ABG701	7H	7H	Hybridization to 7H
ABG704	7H	7H	Hybridization to 7H + Unmapped Bands
ABG712	5H	5H	Hybridization to 5H + Unmapped Bands
ABG715	4H	4H	Hybridization to 4H + Unmapped Bands
BarG10	4H	4H	Hybridization to 4H

Less mapping data was obtained for the wheat-barley addition lines with the primer sets derived from wheat and *T. tauschii* genomic clones (Table 18). Hybridization however was observed for eight of 13 primer sets (Tables 21 and 22). No hybridization was detected for either barley or wheat amplified sequences for the two primer sets designed from oat cDNA clones suggesting non-homologous sequences were amplified and mapped.

Table 21. Results from the Southern analysis in barley for the primers sets derived from wheat genomic clones, including the RFLP and STS map locations.

Primer Set	Barley RFLP Location	Barley STS Location	Southern Analysis
WG181	4H		Hybridization but No STS Mapping Data
WG232	4H	6H	No STS Product Amplified
WG530	5H		Hybridization but No STS Mapping Data
WG564	5H		Hybridization but No STS Mapping Data

Table 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.

Primer Set	Wheat RFLP Location	Wheat STS Location	Southern Analysis
KSUD2		7H	No Hybridization
KSUD15			No Hybridization
KSUD16		2H	No Hybridization
KSUE8			Hybridization but No STS Mapping Data
KSUF15	2H		Hybridization but No STS Mapping Data
KSUG12			Hybridization but No STS Mapping Data
KSUG44		5H	Hybridization to 5H
KSUG49			Hybridization to 4A, 7D + Unmapped Bands
KSUM148			No Hybridization

A detailed look at two specific primer sets illustrates the variability in data obtained. Figure 1, panel A, shows the products amplified with primer set KSUG44 in the 21 nullisomic-tetrasomic lines and Chinese Spring. Digestion with *RsaI* resulted in the detection of a 540 bp fragment missing in nullisomic 5B and a 490 bp fragment missing in nullisomic 5D. The missing 220 bp fragment in nullisomic 5A appears as a minor band and as such is not as apparent in the figure. These results indicated that primer set KSUG44 maps to wheat homoeologous group 5 chromosomes, which corresponds to the same locations indicated by the RFLP data (Gill et al. 1991; GrainGenes 1995). Panel B shows the amplification products digested with *RsaI* for the wheat-barley addition lines,

Betzes barley, and Chinese Spring. The 640 bp fragment amplified by primer set KSUG44 mapped to homoeologous chromosome 5H of barley (lane 5H). Southern analysis showed strong hybridization of all the amplified products to the KSUG44 RFLP clone (Panel C). Lane 3, panel C, shows the *RsaI*-digest of amplified products in Chinese Spring and the corresponding hybridization detected for the three homoeologous chromosome 5 specific fragments. Lane 4, panel C, hybridization was detected for the chromosome 5H band of barley. The usefulness of restriction digestion as an aid in distinguishing STS-PCR products is illustrated in lanes 5 and 6. Hybridization is detected for the same size product amplified in both wheat and barley when undigested, but the chromosome 5H band was detected when the same size wheat fragment was digested with *RsaI* to produce the three mapped fragments, lanes 3 and 4 versus lanes 5 and 6. Lane 1, panel C, chromosomes 5B and 5D were not mapped when the products were digested with *HinfI*, but hybridization was detected for unmapped bands on the Southern blot. A 230 bp unmapped fragment (Panel A) is observed in Chinese Spring and the aneuploid stocks with no hybridization of the RFLP clone (Panel C, lane 3) being detected for this band indicating non-homologous sequences are also amplified.

Figure 2 illustrates a different situation, whereby the primer set derived from the oat cDNA clone, CDO213, amplified products that could be mapped after restriction digestion using the 21 nullisomic-tetrasomic lines on chromosomes 1A, 1D, 4A, 4B, 5D, 6A, and 7D of wheat. Bands on chromosomes 1A (550 bp and 500 bp), 4A (340 bp), and 4B (680 bp) were missing in the corresponding nullisomic lines when the PCR

products were digested with *RsaI* (Panel A). The products mapped on chromosomes 1D, 5D, 6A, and 7D were determined from the *HinfI* digest (data not shown). A 320 bp barley specific fragment was detected on chromosome 7H from the undigested PCR products of the wheat-barley addition lines (Panel B, lane 7H). RFLP mapping data (GrainGenes 1995; Heun et al. 1991) indicated that clone CDO213 hybridized to homoeologous group 5 chromosomes in both wheat and barley. Southern analysis revealed no hybridization of the clone to either wheat or barley amplification products, lanes 1 through 6 in panel C. Lane 7, panel C, hybridization was detected with the probe to the RFLP clone. Hybridization results were not changed when the stringency of the wash was lowered to 50°C, indicating a lack of homology between the clone and the STS products in wheat and barley.

Figure 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 *RsaI* 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 indicate the map location for the PCR products. Panel B, wheat-barley addition lines used to map the *RsaI* 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 *HinfI*, *RsaI*, and undigested, respectively. Lanes 2, 4, and 6 represent Betzes digested with *HinfI*, *RsaI*, 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.

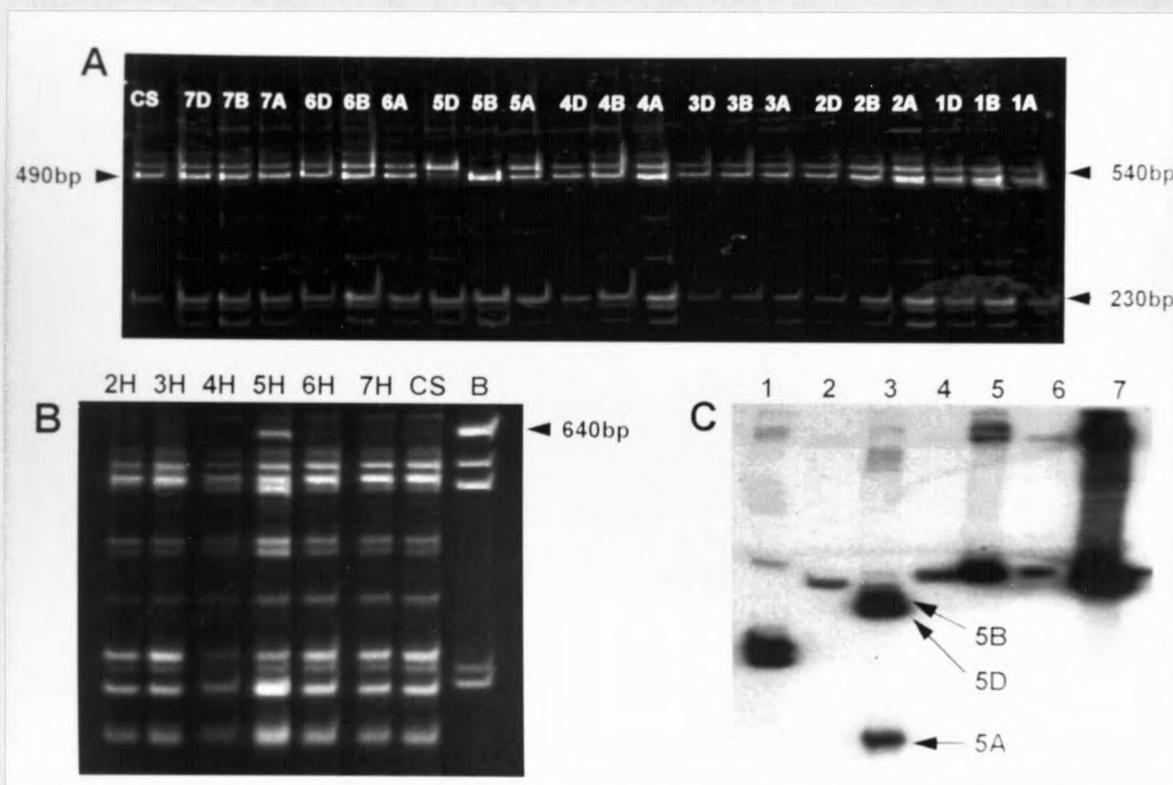
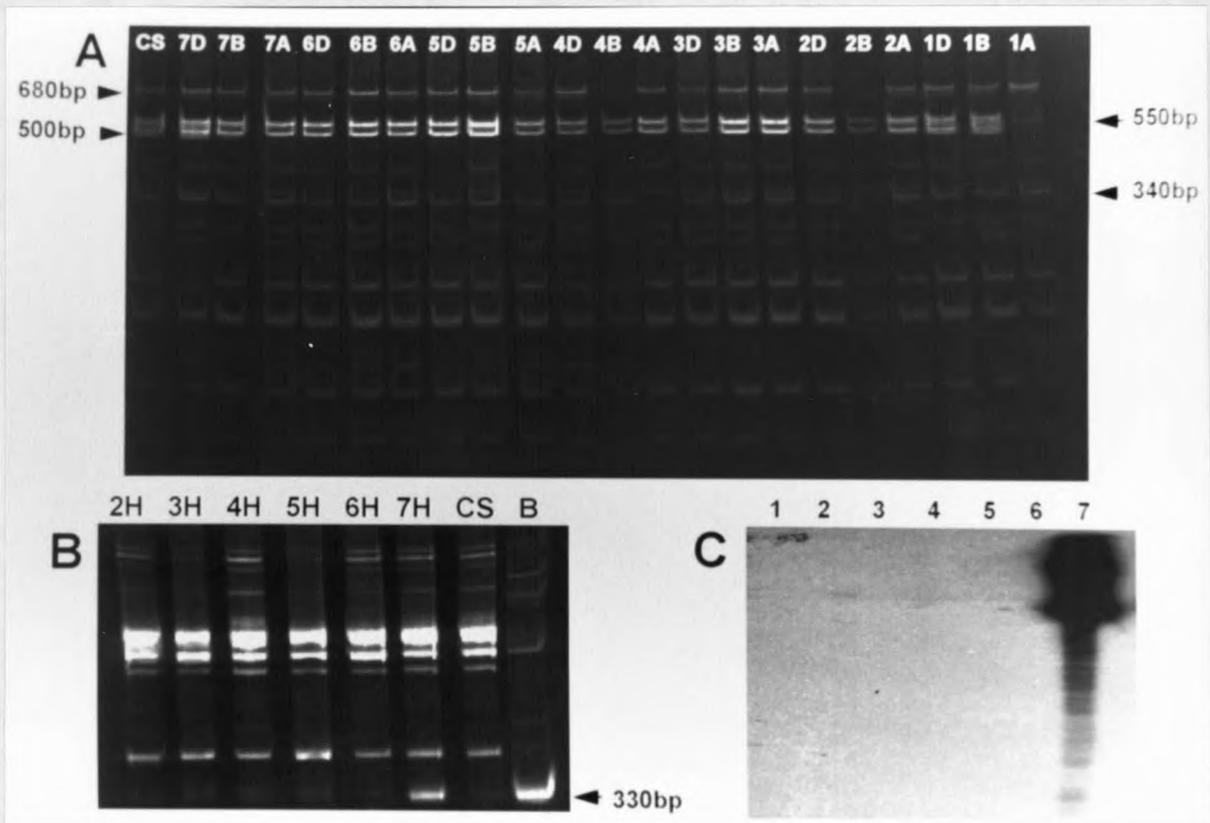


Figure 2. Mapping results for STS-PCR primer set CDO213 in wheat and barley, plus the results from the Southern hybridization of amplified products to the corresponding RFLP clone used to design the primer set. Panel A, nullisomic-tetrasomic lines used to map the *RsaI* digested STS-PCR products of primer 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 *HinfI*, *RsaI*, and undigested, respectively. Lanes 2, 4, and 6 represent Betzes digested with *HinfI*, *RsaI*, 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.



Discussion

The current RFLP maps of wheat and barley each contain over 1000 loci (Nelson et al. 1995a; Kleinhofs et al. 1993; GrainGenes 1995). RFLP probes are usually transferable between wheat and barley, and often reveal patterns of synteny between the two species (Devos et al. 1993). The conversion of RFLP markers into STSs recognized by PCR offers several potential advantages, including the relative ease of conducting PCR analysis. Several experiments using STS-PCR markers have revealed a useful amount of polymorphism within wheat and barley breeding materials, especially when PCR products are digested with restriction enzymes (Talbert et al. 1994; Chen et al. 1994; Tragoonrung et al. 1992). The utility of STS-PCR markers would be enhanced were the primer sets transferable between species. It would also be useful if mapped RFLP locations could be used as a predictor of the chromosomal location identified by STS-PCR.

Available evidence has been inconsistent regarding the transferability of STS-PCR markers and the predictability of STS location based on known RFLP location (Storlie and Talbert 1993; Nieto-Lopez and Blake 1994; Talbert et al. 1996). The present study used 97 STS-PCR primer sets developed from several sources to address the congruence of RFLP and STS locations in wheat and barley. Several reasons prevented making all possible comparisons of the RFLP and STS data for each primer set: (1) RFLP mapping data were lacking for 38 and 21 of the clones in wheat and barley, respectively, with data

available for both species in 39 cases; (2) no nullisomic-tetrasomic data were obtained for 13 of the primer sets evaluated; (3) barley DNA in wheat-barley addition lines was not amplified in 22 cases; and (4) amplified products in the wheat-barley addition lines for 21 of the primer sets could not be distinguished from products amplified in Chinese Spring even with the aid of restriction digestion. Sufficient data was obtained to provide some explanations for the inconsistencies observed in previous studies.

Published RFLP locations (GrainGenes 1995) and STS map locations were not as consistent as seen with comparisons between wheat and barley RFLP locations, where 32 of 39 comparisons identified homoeologous chromosome locations. The same chromosome was identified by both STSs and RFLPs in barley for 28 of 50 cases. This number was somewhat higher (26 of 42 cases) when only barley-derived primer sets were considered. Wheat STS location corresponded to the same homoeologous chromosome group identified by the RFLP in 34 of 49 comparisons. The source of the primer set, whether from cDNA or genomic clones or from different species, had no consistent effect on the mapping results, which was partly due to the limited number of comparisons that could be made in some cases. The assumption that cDNA sequences would be more highly conserved across species was not indicated by the results obtained, since a similar percentage of location matches were obtained when compared to genomic sources for two of the four comparisons. Location matches were favored for the genomic sources rather than the cDNA sources for the other two comparisons. RFLP map location could be used as a predictor of STS location nearly 70% of the time for wheat and 60% of the time for

barley. Barley STS location (36 of 48) was similar to wheat RFLP location (34 of 49) in predicting STS location in wheat.

Several reasons for the lack of correspondence between RFLP and STS locations exist, including the possibility that STS primers did indeed amplify homologous DNA sequences, but that these sequences were not mapped by RFLP due to lack of polymorphism. An RFLP hybridization pattern may reveal multiple bands, only one of which is able to be mapped (Causse et al. 1994). Another possibility is that STS locations may not be mapped in the Chinese Spring aneuploids if the target sequence is amplified on more than one homoeologous chromosome, and no restriction site polymorphisms exist between these sequences. Missing bands would not be observed in any of the aneuploid stocks. This situation was illustrated by the 14 primer sets in which no map location could be identified, since all the nullisomic-tetrasomic lines had similar banding patterns with Chinese Spring. STS primers may also amplify non-homologous sequences, in a manner similar to RAPD primers (Williams et al. 1990).

RFLP clones were hybridized on Southern blots to the cognate STS-PCR products to address these concerns. Sequences amplified from wheat by barley-derived primer sets hybridized to the RFLP probe in only 11 of 19 cases. This may either be due to amplification of non-homologous sequences, or merely divergence between barley and wheat, since hybridization was observed for STS products from barley with 16 of the 19 primer sets evaluated. No additional hybridization was observed when the stringency of

hybridization was lowered. This would suggest that amplification of non-homologous sequences had occurred.

RFLP clones hybridized to amplified wheat sequences in 11 of 13 amplifications with wheat or *T. tauschii*-derived primer sets. Hybridization was usually to the major STS bands. This suggests that sequences homologous to the RFLP clone were often amplified, even in cases where analysis of aneuploid stocks could not confirm the map locations. A similar situation was observed in barley where hybridization to STS products was observed for nine of the 13 primer sets. The results reaffirmed that both homologous and non-homologous sequences were being amplified by the primer sets.

The resulting data suggests that transferability of primer sets between wheat and barley will encounter the same problems seen with the transfer of RFLP markers, such as the occurrence of different mappable polymorphisms in different populations. An additional consideration with the STS primers is that non-homologous and thus non-target sequences may be amplified. Target sequences may not be amplified in some cases. This may be more true when primer sets are transferred between wheat and barley. The mapping data suggest a 70% probability of success in transferring STS-PCR markers between related species. Recombinational map location of STS-PCR products should also be accomplished in both species to provide a more detailed chromosome locational analysis.

CHAPTER 3

MARKER-ASSISTED SELECTION IN BACKCROSS BREEDING
TO INTROGRESS RUSSIAN WHEAT APHID RESISTANCELiterature ReviewRussian Wheat Aphid

It is estimated that a 20% loss is incurred annually due to the destructive nature of pests on wheat production in the United States (Wiese 1977). Losses that can be contributed directly to insects and mites is estimated to be 6% annually (Hatchett et al. 1987). Damage attributed to the Russian wheat aphid (*Diuraphis noxia* Mordvilko) is estimated to be approximately \$100 million yearly for the cereal crops, mainly wheat and barley (Belefant-Miller et al. 1994; Girma et al. 1993).

Wheat serves as the host to more than 30 species of aphids, but only six of these sap-sucking arthropods have been identified as capable of inflicting severe economic losses (Hatchett et al. 1987; Olsen et al. 1993). The Russian wheat aphid, also referred to as the Russian grain aphid or barley aphid, is a new pest in the United States and is considered to be one of the most important pest of small grains in the Great Plains (Girma et al. 1993; Stoetzel 1987). The aphid was first discovered in Texas in March 1986 and by the end of

that year the aphid had spread to eight additional states (Stoetzel 1987; Webster et al. 1987). The aphid can now be found throughout the major wheat producing region of the United States and has been reported in 16 states including Montana (Girma et al. 1993; Kindler and Springer 1989; Quick et al. 1991). The Russian wheat aphid is only capable of short flights with prevailing winds aiding in dispersal of this pest and its introduction into the United States from Mexico (Stoetzel 1987).

The Russian wheat aphid will also colonize barley, oat, rye, rice, triticale, and various forage grasses including annual, perennial, warm season, and cool season species (Kindler and Springer 1989; Kindler et al. 1993; Stoetzel 1987). These alternate hosts provide the aphid with the ability to survive summer and winter by serving as reservoirs or green bridges for the aphid, and thus contributing to increased dispersal to important crop species (Girma et al. 1993; Kindler and Springer 1989; Kindler et al. 1991; Mowry et al. 1995). The wide host range also provides the aphid with an opportunity for establishment in new habitats, thus contributing to its dispersal. Rapid establishment is aided by the ability of the aphid to reproduce by continuous parthenogenesis. The aphid also has been reported as being capable of surviving temperature extremes of -20°C , thus early spring infestations are possible from overwintering (Butts 1992; Girma et al. 1993).

The destructive nature of the Russian wheat aphid in reducing grain yield and quality is considered to be the result of an alteration in the photosynthetic capacity of infested plants (Rafi et al. 1996). Typical symptoms include white, yellow or purple longitudinal chlorotic streaks on leaves and stems, leaf rolling and folding, head trapping,

and stunted growth (Webster et al. 1987). It is believed that the aphid produces a toxin that is introduced into the plant through feeding and is responsible for symptom production (Quisenberry and Schotzko 1994). The aphid induces leaf rolling by preventing the unrolling of newly formed leaves (Burd and Burton 1992). Leaf rolling can prevent proper emergence of the head from the boot resulting in distorted heads and interference with seed set (Smith et al. 1991). Leaf rolling is of considerable significance in that it affects the control measures available to the producer, since tightly rolled leaves will protect the aphid from parasites, predators, and contact insecticides, thus greatly reducing the effectiveness of these control strategies and requiring the use of expensive systemic insecticides for control (Burd et al. 1993; Kindler et al. 1995; Webster et al. 1987). Chemical control also has the hidden costs due to the inconspicuous dangers to human health, the environment, and nontarget beneficial organisms (Kindler et al. 1991). Host resistance therefore provides one of the most economical management strategies for controlling the aphid (Burd et al. 1993; Quisenberry and Schotzko 1994).

Host Plant Resistance

Three modes of plant resistance to the Russian wheat aphid have been identified which include antibiosis, antixenosis, and tolerance (Kindler et al. 1995). Antibiosis refers to the ability of the host plant to affect the lifecycle of the pest, such as reducing the reproductive rate and capacity, growth rate, life span, and fecundity, or increasing the duration to reach reproductive maturity (Kindler et al. 1995; Rafi et al. 1996). This form

of resistance is generally determined by measuring aphid survival and reproductive success (Kindler et al. 1995; Webster et al. 1987). The resistance can be attributed to the ability of the plant to produce some chemical defense compound in response to aphid attack (Belefant-Miller et al. 1994; Rafi et al. 1996).

Antixenosis or nonpreference refers to the inability of the plant to serve as a suitable host and can be determined by measuring the number of aphids per plant when given a choice of host materials (Webster et al. 1987; Kindler et al. 1995). Resistant plants will have significantly lower aphid densities when compared to susceptible controls (Webster et al. 1987). Antixenosis may also involve an altered chemical makeup of the plant or some morphological feature that reduces predation pressures.

Tolerance is the ability of the plant to withstand or recover from insect attack, and is characterized by no significant reduction in aphid density per plant as compared to susceptible controls (Kindler et al. 1995). Tolerance can be determined by measuring the amount of damage incurred or by measuring certain characteristics of the plant, such as height or growth rate (Webster et al. 1987; Kindler et al. 1995). This form of resistance can be considered to be the inherent genetic capacity of the plant to survive aphid attack, since there is no aphid-plant interaction as with the other two forms of resistance.

The development of Russian wheat aphid resistant cultivars in the United States may be aided by the occurrence of only females within the aphid population (Belefant-Miller et al. 1994). The lack of males eliminates sexual recombination as a source of genetic variation, and thus contributes to a more uniform population. Few biotypes

therefore exist, thus increasing the potential longevity of the limited sources of resistance identified (Puterka et al. 1992).

Sources and Genetics of Resistance

The rapid spread and destructive capabilities of the Russian wheat aphid led to intense germplasm searches to identify sources of resistance. Resistance was identified in a variety of different germplasm sources including wild relatives, related species, and cultivated forms of wheat (Du Toit 1989; Kindler et al. 1993; Marais and Du Toit 1993; Marais et al. 1994; Smith et al. 1991). The Russian wheat aphid is indigenous to southern Russia, Asia Minor and the Middle East, and many sources of resistance have been identified from this region (Du Toit 1989; Smith et al. 1991). Five genes for resistance have been identified and characterized (Marais and Du Toit 1993). Four of the genes, *Dn1*, *Dn2*, *Dn4*, and *Dn5*, were identified from hexaploid wheat lines and are inherited as single dominant Mendelian genes. The gene *Dn3*, identified from a *T. tauschii* accession (SQ24), is inherited as a single recessive gene (Du Toit 1989; Marais and Du Toit 1993; Nkongolo et al. 1991a; Nkongolo et al. 1991b). Two genes, *Dn1* from line PI137739 collected from Iran and *Dn5* from line PI294994 collected from Bulgaria, are located on wheat chromosome 7D and may be linked or allelic (Marais and Du Toit 1993). Gene *Dn2* from line PI262660 collected from Russia has been mapped to wheat chromosome group 5 (Ma et al. 1996). Resistance conferred by gene *Dn1* is considered to be antibiosis, while gene *Dn2* confers an antixenosis form of resistance (Du Toit 1989;

Nkongolo et al. 1991b). Knowledge of the type of resistance and its inheritance is of value to the plant breeder, and allows for a more efficient introgression and deployment.

Resistance of PI372129

The plant introduction line PI372129 (Turcikum 57), obtained from Russia, was the first wheat line identified as conditioning resistance to Russian wheat aphid populations within the United States (Quick et al. 1991). The line possess a single dominant resistance gene designated *Dn4* (Nkongolo et al. 1991b). The gene has been determined to be on wheat homoeologous group 1 chromosomes (Ma et al. 1996), which may have some significance in that a resistance gene has been identified in rye associated with the homoeologous chromosome arm 1RS (Marais et al. 1994), thus suggesting the possibility for the occurrence of homoeoloci. A resistance gene has also been identified in barley on the short arm of homoeologous chromosome 1H, which associates with the amplification product of primer set KSUD14 (Nieto-Lopez and Blake 1994).

The resistance of PI372129 is due to tolerance, since there is no reduction in aphid populations when compared to susceptible controls and damage is evident; but, the line has the ability to withstand and recover from aphid attack (Burd et al. 1993; Nkongolo et al. 1991b; Quick et al. 1991). The line has a damage rating of 5.6 on a scale of 1 to 9, where 1 corresponds to no damage with 9 indicating severe damage (GrainGenes, 1995). Tolerance may be a more desirable form of resistance, since no selection pressure would

be placed on the Russian wheat aphid population as with antibiosis and antixenosis forms of resistance (Kindler et al. 1995).

PI372129 is a soft white winter wheat and possesses a number of undesirable characteristics for a hard red spring wheat breeding program, such as late maturity, weak straw, tallness, white grain, and soft endosperm (Quick et al. 1991). The usefulness of this resistance will depend on the ability to transfer the resistance gene to a hard red spring wheat background with the subsequent elimination of the undesirable characteristics associated with the line.

Backcross Breeding

The introgression of specific genes into a desirable background can be efficiently accomplished through a backcross breeding procedure. Briggs (1938) proposed the procedure as an exact method to recreate a variety. The procedure has been commonly used to transfer simply inherited characters, such as insect or disease resistance, from unadapted cultivars or related species into the genetic background of an adapted cultivar. The procedure involves crossing a donor parent, which is the unadapted line carrying the desired gene to be transferred, with the recurrent parent, which is the adapted line having the desired genetic background. This is followed by crossing the recurrent parent back to the F₁ progeny derived from the initial cross, which is then referred to as the first backcross (BC₁) generation. Depending on the inheritance of the donor trait, the progeny produced after the first backcross will segregate for the phenotype of the gene being

transferred. Backcross progeny for a dominantly inherited trait can be selected and once again backcrossed to the recurrent parent to form the second backcross generation. Progeny tests can be conducted for a recessively inherited trait to identify backcross lines with the desired phenotype before being cross back to the recurrent parent.

The F_1 progeny from the initial cross will be composed of 50% donor and 50% recurrent parent DNA. The level of donor genome is reduced in half with each successive backcross to the recurrent parent, so that the progeny in the first backcross generation will be composed of 25% donor and 75% recurrent parent genome. The following equation can be used to predicted the percentage of donor genome in each backcross generation: $[(1/2)^{n+1}]100$, where n refers to the number of backcrosses. The predicted value of donor genome is the mean for the population with the actual level normally distributed around this value. The distribution of donor genome is wider in the initial backcross generations and is substantially reduced as the mean approaches zero with additional backcrosses (Hill 1993; Hillel et al. 1990).

Backcrossing is more efficient at breaking linkage blocks than continual selfing. The degree of linkage removal will depend on the level of recombination, and thus the probability of eliminating undesirable linkages can then be estimated by the following equations: $1-(1-P)^{n+1}$, where P refers to the level of recombination and n the number of backcross generations. The donor parent can also influence the size of the linked genomic segment by affecting the level of recombination (Chetelat et al. 1995; Jena et al. 1992). The degree of chromosomal pairing can be reduced when genetically diverse donor

parents are used, thus large linkage blocks are transferred and are not readily reduced with additional backcrosses. The efficient removal of both linked and unlinked DNA will depend on the intensity and accuracy of the selection process. The potential exists to increase the overall selection efficiency through the use of molecular markers.

Marker-Assisted Selection

Resistant varieties are the most economical means of controlling Russian wheat aphid infestations and provide the producer with a means of stabilizing yields under dramatic shifts in the pest population, thus their availability is a prerequisite for effective management. The backcross breeding procedure is efficient in the use of time, labor, and space, but still requires approximately six years of backcrossing before lines can be derived and a suitable variety is available. The introgression of resistance into suitable cultivars can be monitored by the development or identification of linked molecular markers that serve as tags for the resistance gene (Eastwood et al. 1994; Ishii et al. 1994). These molecular tags provide a valuable means of increasing the efficiency of selection for resistance and provide an important tool to aid in the pyramiding of several sources of resistance in a single cultivar. Molecular markers may have a more important role in backcross breeding by providing a means to enhance the recovery of the recurrent parent genome, and thus reduce the time required for cultivar development.

Computer simulations of whole genome selection in tomato (*Lycopersicon esculentum* Mill.) suggest that the recurrent parent genome could be reconstructed in only

three backcross generations using 30 individuals with markers evenly spaced along the chromosomes and selecting for the recurrent parent genotype (Tanksley et al. 1989). Similar results were obtained by Hospital et al. (1992), which suggested that the gain from marker-assisted selection would correspond to the elimination of approximately two backcross generations. Hillel et al. (1990) using DNA fingerprints suggested that adequate selection for the recurrent parent could be achieved in only two backcross generations. These models are based on a number of assumptions, such as no interference in crossing over, codominant markers with known map locations, the potential to produce large populations, complete selection, even placement of markers or the availability of densely populated linkage maps to aid in marker selection, and a large number of polymorphic markers (Hillel et al. 1990; Hospital et al. 1992). Molecular markers have the major advantage in that they are phenotypically neutral and as such are not effected by environmental conditions, epistatic or pleiotrophic gene action, and the low heritabilities that characterize phenotypic selection.

Phenotypic selection for the trait of interest will influence the size of the introgressed fragment, which can vary greatly due to linkage drag and reduced recombination (Young and Tanksley 1989). The application of molecular markers to select for rare recombinants in such regions has been successful and illustrates the potential utility of molecular markers (Chetelat et al. 1995; Zamir et al. 1994). Results suggest that within two to four backcross generations and with population sizes of 150 to 300 individuals the size of the linked segment can be dramatically reduced to a size that

would only be achievable with 100 backcross generations by conventional means (Tanksley et al. 1989; Chetelat et al. 1995). These results further illustrate the potential application of molecular markers to conventional breeding procedures and the ability to increase selection efficiency, thus affecting potential genetic gain.

Wheat Genome

The potential of marker-assisted selection has been vividly illustrated in tomato, which has a number of advantages when compared to other crop species. Tomato has a relatively small genome size (9×10^8 bp), 12 pairs of chromosomes, and a highly saturated genetic linkage map. Wheat in comparison has a large genome size (17×10^9 bp), nearly twice the chromosomes (21 pairs), and a poorly saturated genetic linkage map. Hexaploid wheat is also composed of three genomes, A, B, and D, each with seven pairs of chromosomes, thus masking of traits is possible. A study was conducted to determine if whole genome marker-assisted selection could also be achievable in wheat to recover the recurrent parent genome in a backcross breeding program to introgress Russian wheat aphid resistance.

Materials and Methods

Plant Populations

Crosses were made between the Russian wheat aphid resistant line, PI372129, and two widely cultivated Montana hard red spring wheat cultivars, 'Pondera' and 'Newana'. Pondera, CI17828, ('Red River 68'/3/'Sheridan'//CI13253/5* 'Centana'), is a semidwarf wheat with lodging resistance, high test weight, and good end-use quality (McNeal and Klindworth 1980). Newana, CI17430, ('Sheridan'/3/'Norin10'/'Brevor14'//5* 'Centana'), is also a semidwarf wheat with superior baking quality and satisfactory milling quality (McNeal and Berg 1977). Three populations were established from these crosses, two populations involved Pondera and one involved Newana as the recurrent parent. The lines within each population were established through a single seed descent breeding procedure with five generations of selfing to produce nearly homozygous inbred lines. The resulting recombinant inbred lines could then be evaluated under field conditions. No selection was practiced for the Russian wheat aphid resistant phenotype, so resistant as well as susceptible lines were included within the populations. The only forms of selection were for maturity under greenhouse conditions and the spring growth habit; therefore, the lines lacked the vernalization requirement needed for the winter growth habit. The two populations developed from crosses with Pondera as the recurrent parent consisted of 14 lines derived from the single cross and 23 lines derived from the first backcross (BC₁F₅)

generation. Five of the 14 single cross lines were classified as resistant, with 10 of the 23 backcross lines being identified as resistant. The one population where Newana was used as the recurrent parent consisted of 21 lines derived from the first backcross (BC₁F₅) generation, with eight of the 21 lines being identified as resistant to the Russian wheat aphid. A total of 58 recombinant inbred lines were evaluated at both the phenotypic and genotypic level.

Field Evaluation

The 58 lines plus the two recurrent hard red spring wheat parents, Pondera and Newana, were planted in a randomized complete block design with four replications. Each plot consisted of four rows with overall plot dimensions of 1.2 m by 3.0 m and a row spacing of 0.3 m. The evaluation was conducted at two locations, a dryland and irrigated site, at the Post Agronomy Research Farm located 9.6 km west of Bozeman, Montana in 1994 and 1995.

For the 1994 growing season, the dryland site was planted on 21 April and harvested on 16 August. The irrigated location was planted 19 April and harvested 25 August. Precipitation during the growing season was 1.1 cm below average (18.4 cm) with temperatures 4°C above average (32.3°C). The irrigated location received an additional 1.8 cm of water during the growing season.

Plantings in 1995 were delayed due to spring rains and wet soil conditions. The dryland site was planted 17 May and harvested 8 September. The irrigated site was

planted 18 May and harvested 25 September. Precipitation was 0.06 cm above average with temperatures 0.7°C cooler than average. The cooler and wetter 1995 growing season delayed maturity and harvest. The irrigated location received an additional 1.3 cm of moisture during the growing season.

Agronomic Evaluation

Heading date was recorded as the number of days after 1 January when 50% of the plants in the plot had heads emerged from the boot. Plant height was determined from the base of the plant to the top of the spikes excluding awns. Lodging was determined for each plot based on a lodging index, which takes into account the percentage as well as the angle of the lodged plants. The index was determined by multiplying the percentage of lodged plants, from 0% to 100%, by the angle of the lodged plants, from 0 to 9, where 0 indicates no deviation from upright and 9 indicating 180° deviation from upright, divided by ten. The larger the lodging index value the more severe the lodging. Total grain yield was determined on a whole plot basis, with the plots being trimmed to 2.4 m before harvest. Test weight was determined on a clean grain sample following standard protocol (AACC 1983).

Quality Evaluation

Total protein was determined by near infrared reflectance of a whole grain sample using a Infratec grain analyzer (model 1225). Kernel hardness was also determined by

near infrared reflectance, but on whole meal flour using a Technicon model 400 InfraAlyzer. The whole meal flour was prepared by grinding a sample of grain in a Udy mill with passage through a 1.0 mm screen. NIR kernel hardness is based on a measurement of particle size; since during the milling process, harder kernels sustain greater damage to the starch granules, which results in a more granular flour that has better breadmaking properties (Carver 1994).

A sample of this whole meal flour was also used in the SDS sedimentation test using a procedure similar to that of Dick and Quick (1983). A 2.5 g sample of the whole meal flour was added to a 25 mm X 200 mm culture tube followed by the addition of 25 mL distilled water. The tubes were vortexed for 5 seconds followed by an additional 5 second vortex after 5 minutes with a final 12 minute rehydration period. The tubes were next vortexed for 5 seconds then 25 mL of the SDS solution (3.3% SDS and 0.85% lactic acid) was added and the tubes were inverted 10 times followed by a 5 minute settling period before being inverted 10 more times. The tubes were allowed to settle for 10 minutes before the sediment height was measured in millimeters.

Marker Analysis

DNA was extracted from individual plants for each of the recombinant inbred lines as described in chapter 2. The three parents, PI372129, Pondera, and Newana, were initially screened with the STS-PCR primer sets and evaluated for polymorphic restriction fragments between the donor and recurrent parents. The procedures for PCR

amplification, product digestion, and polyacrylamide gel separation were the same as described in chapter 2, with the exception that four restriction enzymes, *HinfI*, *RsaI*, *DdeI*, and *HhaI*, were evaluated. Primer sets that were identified as conditioning amplification of suitable polymorphic fragments were then screened on the recombinant inbred lines.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was conducted to analyze the seed storage proteins of the progeny lines and the parents. A single kernel from each line was ground and transferred to a 1.5 mL microfuge tube followed by the addition of 0.5 mL 70% ethanol for protein extraction. The tubes were vortexed and incubated in a water bath at 55°C for 1 hour followed by a 2 minute centrifugation at 10,000 rpm in a microfuge. Next, 250 µL of the supernatant was removed and placed in a new 1.5 mL microfuge tube with a 1 hour incubation at 4°C followed by vacuum centrifugation to remove the ethanol. The protein pellets were then resuspended in 75 µL cracking buffer (19% glycerol, 1.9% SDS, 145 mM Tris-HCl pH 6.8, 1.7 mM bromophenol blue, 2.1 mM xylene cyanole FF, and 30 mM 2-mercaptoethanol) before gel electrophoresis. The proteins were denatured by boiling for 30 minutes before being loaded on a 12% polyacrylamide gel, which was prepared based on the procedure of Laemmli (1970). Electrophoresis was conducted for 16 hours at 80 volts followed by staining with Coomassie Blue (1.2 mM) for 2 hours and destaining in a solution of 25% methanol and 8% acetic acid.

Results

Parental Marker Evaluation

Parental materials were initial screened with 310 primer sets developed from several sources to select primer sets conditioning amplification of polymorphic DNA sequences. Sources included 92 *Triticum* genomic, 88 barley genomic, 54 barley cDNA, and 17 oat cDNA-derived primer sets, with the remaining 59 sets being derived from various cloned barley DNA fragments (Table 23). Polymorphic fragments distinguishing Pondera and PI372129 were revealed for 166 of the 310 primer sets when digested with one of four restriction enzymes. Polymorphic fragments occurred for 168 of 310 primer sets when Newana and PI372129 were compared. Polymorphic fragments were revealed between Pondera and Newana with 133 of the 310 primer sets. A greater portion of cDNA-derived primer sets, both barley and oat, revealed polymorphic fragments between the hard red spring parents and PI372129 than the genomic derived primer sets. Thirty-five of the 54 barley cDNA-derived primer sets and 13 of the 17 oat cDNA-derived primer sets were characterized as producing polymorphic fragments between Pondera and PI372129. Polymorphic fragments were observed with 34 of 54 barley cDNA-derived primer sets and 11 of 17 oat cDNA-derived primer sets when Newana was compared to PI372129. The comparison between Pondera and Newana identified 22 of 54 barley

cDNA and 11 of 17 oat cDNA-derived primer sets that directed amplification of polymorphic fragments. Forty-nine of the 88 barley genomic-derived primer sets directed amplification of polymorphic fragments between PI372129 and both Pondera and Newana. Polymorphic fragments were revealed with 38 of 88 barley genomic-derived primer sets for the comparison between Pondera and Newana.

Detection of polymorphic fragments occurred with a lower frequency for the *Triticum* genomic-derived primer sets (Table 23). Polymorphic fragments between Pondera and PI372129 were amplified for 13 of 21 wheat genomic-derived primer sets. The amplification of polymorphic fragments between Newana and PI372129 occurred for 12 of 21 wheat genomic-derived primer sets. Ten of 21 wheat genomic-derived primer sets revealed polymorphic fragments between Pondera and Newana. The number of primer sets revealing polymorphic fragments was considerably lower for the *T. tauschii* genomic source, where 21 of the 71 primer pairs directed amplification of polymorphic fragments between PI372129 and both Pondera and Newana. Twenty of the 71 *T. tauschii* genomic-derived primer sets revealed polymorphic fragments between Pondera and Newana.

Table 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, *Hinf*I, *Rsa*I, *Dde*I, or *Hha*I.

Primer Source	Number of Primer Sets Evaluated	Number of Primer Sets Polymorphic Between Pondera and PI372129	Number of Primer Sets Polymorphic Between Newana and PI372129	Number of Primer Sets Polymorphic Between Pondera and Newana
Wheat Genomic	21	13	12	10
<i>T. tauschii</i> Genomic	71	21	21	20
Barley Genomic	88	49	49	38
Barley cDNA	54	35	34	22
Oat cDNA	17	13	11	11
Other Barley Sources	59	35	41	32
Total	310	166	168	133

Progeny Marker Evaluation

A subset of the primers identified as conditioning amplification of polymorphic sequences between the parental lines was used to evaluate the recombinant inbred progeny lines. A total of 75 primer sets was evaluated on the Pondera single cross population with 336 polymorphic restriction fragments relative to the PI372129 genotype scored. An average of 4.5 polymorphic fragments was identified for the 75 primer sets. An average of 148 polymorphic fragments in common with the PI372129 genotype was scored for the Pondera single cross lines with a range of 123 to 167 polymorphic fragments scored (Table 24).

Table 24. Marker analysis of the 14 Pondera 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.

Pondera Single Cross Lines	Number of STS Restriction Fragments in Common with PI372129	Number of Protein Bands in Common with PI372129	Estimated Level of Donor Genome (%)
1	167	1	49
2	147	5	44
3	142	0	41
4	152	3	45
5	154	2	45
6	123	3	37
7	155	3	46
8	145	3	43
9	140	3	41
10	134	2	39
11	142	2	42
12	149	3	44
13	167	1	49
14	156	0	45
Mean	148	2.2	44
Range	123-167	0-5	37-49

A subset of 52 primer sets was evaluated for the Pondera backcross population with 285 polymorphic fragments relative to the PI372129 genotype scored. An average of 5.5 polymorphic fragments was detected for the 52 primer sets. Individual lines within the backcross population possessed an average of 68 polymorphic fragments relative to the PI372129 genotype with a range of 36 to 109 fragments scored (Table 25).

Table 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 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.

Pondera Backcross Lines	Number of STS Restriction Fragments in Common with PI372129	Number of Protein Bands in Common with PI372129	Estimated Level of Donor Genome (%)
1	106	0	36
2	57	0	19
3	78	1	27
4	63	2	22
5	64	2	22
6	63	0	21
7	54	7	21
8	66	2	23
9	50	0	17
10	50	0	17
11	55	0	19
12	83	2	29
13	102	2	35
14	76	0	26
15	44	2	16
16	74	2	26
17	109	3	38
18	65	0	22
19	68	1	23
20	67	0	23
21	93	2	32
22	43	2	15
23	36	0	12
Mean	68	1.3	23
Range	36-109	0-7	12-38

A subset of 41 primer sets was screened on the Newana backcross population with 163 polymorphic fragments relative to the PI372129 genotype scored. An average of 4.9 polymorphic fragments was scored for the 41 primer sets. A range of 13 to 69 polymorphic fragments in common with the PI372129 genotype was scored for the backcross population with an average of 37 polymorphic fragments per line (Table 26).

Table 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 for each line.

Newana Backcross Lines	Number of STS Restriction Fragments in Common with PI372129	Number of Protein Bands in Common with PI372129	Estimated Level of Donor Genome (%)
1	37	3	23
2	32	3	20
3	32	3	20
4	29	1	17
5	41	5	26
6	69	3	41
7	13	2	9
8	24	0	14
9	48	2	29
10	46	2	27
11	34	3	21
12	29	4	19
13	29	5	19
14	36	1	21
15	33	3	21
16	29	4	19
17	34	5	22
18	48	1	28
19	51	3	31
20	36	2	22
21	41	1	24
Mean	37	2.7	22
Range	13-69	0-5	9-41

Storage Protein Evaluation

SDS-PAGE was used to evaluate the wheat seed storage proteins, which separates proteins based on size. Nine bands were identified that distinguished Pondera from PI372129. Analysis of the Pondera single cross population resulted in the detection of two lines with the same protein banding pattern as Pondera (Table 24). The remaining 12 lines had one to five distinguishing bands in common with the PI372129 parent. An average of 2.2 protein bands relative to PI372129 was scored for the single cross population.

Ten lines of the Pondera backcross population were characterized as having the Pondera protein banding pattern (Table 25). One line possessed seven distinguishing bands in common with PI372129. The remaining 12 lines of the population had one to three distinguishing bands in common with PI372129. The average line within the backcross population had 1.3 bands relative to the PI372129 parent.

SDS-PAGE analysis of the Newana backcross population revealed 12 distinguishing protein bands in the Newana profile relative to PI372129. Only one line of the population was identified as possessing the protein banding pattern characteristic of Newana (Table 26). The other 20 lines had one to five protein bands in common with PI372129. The average line had 2.7 bands in common with PI372129.

Donor Genome Estimation

Results obtained from the progeny marker analysis of restriction digested STS-PCR amplification products and the SDS-PAGE protein analysis were used to provide an estimate of the level of donor genome retained in each line. The presence of the PI372129 restriction fragment in the progeny lines was given a score of one with the presence of the Pondera or Newana genotype given a score of zero. The total score for each line was divided by the PI372129 score and multiplied by 100 to provide an estimate of percent donor genome. Lines with a larger total marker score would suggest a greater percentage of PI372129 DNA segments in the genome.

The level of donor genome for the Pondera single cross population, based on the number of shared PI372129 markers, averaged 44% with a range of 37% to 49%. (Table 24). The Pondera backcross population had an average of 23% donor markers with the level of donor genome varying from 12% to 38% (Table 25). The results indicate a greater range in donor genome for the lines of the backcross population compared to single cross population. The estimated level of donor genome for the Newana backcross population was similar to that of the Pondera backcross population with a average of 22%. The Newana backcross population however had a greater range in donor genome from 9% to 41% (Table 26). This greater range in donor genome may also be reflected in a greater phenotypic range.

Field Evaluation

Recombinant inbred lines were developed in order to obtain enough seed to conduct a replicated field trial and provide agronomic information to correlate with the marker analysis. Both field performance and end-use quality factors were measured for all three populations. Significant differences were found among the lines within each population for the five agronomic and three quality traits evaluated. Significant genotype by environment interactions were also detected for the traits measured in the three populations.

The mean heading date for Pondera was 184.4 days after 1 January. All the single cross lines were significantly later in heading with an average delay of 4.7 days and a range of 1.1 to 9.5 days (Table 27). Delay in heading was reduced to approximately 1 day for the Pondera backcross population (Table 28). A maximum delay in heading of 5.7 days was observed for one of the backcross lines. No significant delay in heading was observed for seven of the 23 backcross lines when compared to Pondera. Four lines were identified as having a significantly earlier heading date than Pondera with one line being approximately 2 days earlier. A similar range in heading dates, 182 to 190 days (8 days), existed for the backcross population when compared to the single cross population, 186 to 194 days (8 days).

An average heading date of 191 days was observed for the 21 Newana backcross lines compared to 189 days for Newana (Table 29). The maximum delay in heading was 8 days with an average delay of approximately 2 days for the backcross lines. Fifteen of the

21 lines had a significant delay in heading in comparison to Newana. Three lines were observed to have a significantly earlier heading date than Newana with one line being approximately 2.6 days earlier. The Newana backcross lines had a range in heading dates from 186 to 197 days.

Plant height was also significantly increased for the Pondera single cross population compared to Pondera (Table 27). The average plant height for the single cross lines was 101 cm which was approximately 22 cm greater than Pondera. Plant height ranged from 90 to 106 cm, which corresponded to a 11 to 27 cm increase in height compared to Pondera. Eleven of the 14 lines had plant heights over 100 cm. The average height for the Pondera backcross population was 93 cm with only seven of 23 lines greater than 100 cm in height (Table 28). Even though plant height for the majority of the backcross lines approached that of Pondera, two lines were approximately 28 cm greater in height. Four of the 23 lines were not significantly different in height when compared to Pondera. One line had a plant height significantly lower than Pondera by approximately 7 cm.

There was also a tendency towards greater plant height for the Newana backcross population with an average height of 89 cm, which corresponds to an 11 cm increase in plant height compared to Newana (Table 29). Plant height for the backcross lines ranged from 73 to 106 cm. Two lines had plant heights significantly lower than Newana with one line approximately 5 cm shorter. Four of the 21 backcross lines were not significantly

different in height from Newana. Seven of the lines within the population were over 100 cm in height.

The PI372129 line has weak straw and lodging is severe. Lodging is not a significant problem for Pondera and Newana. Severe lodging was common in the Pondera single cross population, where significant lodging occurred for 13 of the 14 lines with an average lodging index value of 44 (Table 27). The lodging index values ranged from 7.1 to 68.9 with six of the 14 lines having lodging index values greater than 50 compared to no lodging for Pondera. The severity of lodging was substantially reduced for the two backcross populations with 13 of 23 Pondera backcross lines (Table 28) and 13 of 21 Newana backcross lines (Table 29) having no significant lodging when compared to their respective recurrent parent. The lodging scores were also reduced with an average lodging index of approximately 12 for both populations, and no lines were identified as having a value greater than 44, the average for the single cross population.

Grain yield is one of the most important factors in a breeding program and for the Pondera single cross population grain yield was reduced an average of 1.0 Mg/ha compared to Pondera (Table 27). One line had a significantly higher grain yield (5.8 Mg/ha) than Pondera (5.4 Mg/ha) by approximately 0.4 Mg/ha. Yields were significantly lower than Pondera for 12 of 14 single cross lines. A 30% reduction in grain yield was observed for one line when compared to Pondera. The additional backcross to Pondera helped to stabilize grain yields in the backcross population with nine of 23 lines having a similar yield to that of Pondera (Table 28). The average grain yield for the

backcross population was 4.8 Mg/ha. Grain yield for the backcross population was reduced by 0.6 Mg/ha, which would correspond to a 12% yield reduction compared to the 18% reduction observed for the single cross population. A 35% reduction in grain yield however was observed for one of the backcross lines when compared to Pondera. No backcross lines were observed to out perform Pondera for grain yield.

The Newana backcross population had an average grain yield reduction of 1.0 Mg/ha when compared to Newana (Table 29). Seventeen of 21 backcross lines yielded significantly lower than Newana with an average grain yield of 4.7 Mg/ha for the population, which corresponded to a 18% yield reduction. One of the backcross lines had an estimated 38% reduction in grain yield when compared to Newana. No backcross lines were identified as out performing Newana for grain yield, and three of the backcross lines were observed to have grain yield reductions exceeding 30%.

Test weight was one of the traits not greatly affected by the presence of PI372129 DNA segments in the Pondera genetic background. Only nine of 14 Pondera single cross lines were significantly different from Pondera, which corresponded to lower test weights (Table 27). No single cross line was observed to have a significantly higher test weight than Pondera. Eight of the 23 Pondera backcross lines were significantly different from Pondera, which also corresponded to lower test weights (Table 28). No backcross line was observed to have a significantly greater test weight than Pondera. The average test weight was 773 kg/m³ for the Pondera single cross population and 778 kg/m³ for the Pondera backcross population compared to 783 kg/m³ for Pondera. The average test

weight was slightly increased (5 kg/m^3) with the additional backcross to Pondera. The range in test weights for the single cross population was 760.6 to 790.2 kg/m^3 with a range of 768.3 to 785.1 kg/m^3 for the backcross population.

Nine of the 21 Newana backcross lines had test weights significantly less than Newana (Table 29). The average test weight for the population was 757 kg/m^3 . Eleven of the lines were not significantly different from Newana for test weight. One line was observed to have a significantly higher test weight than Newana. The range in test weights observed for the Newana backcross population was 728.4 to 782.5 kg/m^3 .

Table 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.

Pondera Single Cross Lines	Heading Date	Plant Height (cm)	Lodging Index	Grain Yield (Mg/ha)	Test Weight (kg/m^3)
1	185.7	89.7	7.1	5.24	769.6
2	188.8	93.2	25.3	5.84	767.1
3	185.5	102.8	26.4	4.41	790.2
4	191.6	102.6	53.0	4.15	765.8
5	188.3	104.7	58.0	4.87	782.5
6	191.4	103.2	62.0	3.74	763.2
7	189.3	100.9	68.9	4.38	764.5
8	188.8	105.6	34.1	4.66	779.9
9	190.1	103.9	39.6	4.20	772.2
10	188.9	103.5	52.4	4.22	782.5
11	188.4	99.1	39.3	4.25	785.1
12	187.9	101.9	49.2	4.21	764.5
13	189.2	105.8	56.7	3.82	773.5
14	193.9	101.8	42.9	4.67	760.6
Pondera	184.4	79.3	0	5.44	782.5
Mean (1-14)	189.1	101.3	43.9	4.48	772.9
Range (1-14)	185.5-193.9	89.7-105.8	7.1-68.9	3.74-5.84	760.6-790.2
LSD _{0.05}	0.7	2.6	13.5	0.4	7.7

Table 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 for the two locations with four replications each evaluated over a two year period.

Pondera Backcross Lines	Heading Date	Plant Height (cm)	Lodging Index	Grain Yield (Mg/ha)	Test Weight (kg/m ³)
1	185.3	107.0	28.3	3.88	785.1
2	186.6	102.1	16.5	3.85	778.6
3	185.4	90.3	16.5	5.42	785.1
4	182.3	100.4	18.1	4.21	778.6
5	185.5	100.1	8.8	5.02	781.2
6	189.0	94.6	19.9	5.52	781.2
7	187.6	97.8	5.0	4.17	782.5
8	184.6	86.1	20.4	5.05	777.3
9	186.1	80.6	2.3	5.66	774.8
10	184.1	85.7	2.6	5.65	779.9
11	184.1	86.4	9.4	5.62	776.1
12	183.4	98.9	17.1	4.22	783.8
13	184.8	79.2	0	5.26	782.5
14	182.9	79.0	0.3	5.54	783.8
15	184.1	71.8	0	5.31	770.9
16	184.8	81.6	0.6	5.56	770.9
17	190.1	104.1	29.7	4.58	768.3
18	185.7	107.3	30.1	3.77	783.8
19	183.7	78.4	3.6	4.98	770.9
20	184.6	97.6	6.1	3.52	769.6
21	185.6	99.8	7.6	4.75	783.8
22	187.7	106.2	18.5	4.47	783.8
23	185.6	95.6	2.6	4.38	773.5
Pondera	184.4	79.3	0	5.44	782.5
Mean (1-23)	185.4	92.6	11.5	4.80	778.5
Range (1-23)	182.4-190.1	71.8-107.3	0.0-30.1	3.77-5.66	768.3-785.1
LSD _{0.05}	0.7	2.0	10.8	0.31	6.4

Table 29. Results for five agronomic traits evaluated for 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 backcross lines and Newana.

Newana Backcross Lines	Heading Date	Plant Height (cm)	Lodging Index	Grain Yield (Mg/ha)	Test Weight (kg/m ³)
1	189.1	82.8	6.4	4.67	751.6
2	190.0	82.5	0	4.82	765.8
3	189.9	101.4	27.8	4.38	782.5
4	191.3	105.4	16.0	4.02	743.9
5	187.8	75.3	1.0	4.84	767.1
6	186.1	73.2	0	5.50	769.6
7	192.6	83.9	2.2	4.99	746.5
8	196.7	83.1	0.4	5.47	728.4
9	194.6	85.4	0	5.38	731.0
10	188.3	99.2	35.3	3.52	764.5
11	190.9	104.1	29.1	4.82	754.2
12	194.6	84.8	0	5.16	731.0
13	190.4	105.5	25.5	4.79	764.5
14	190.9	104.4	32.0	3.94	768.3
15	187.4	79.9	0	5.54	769.6
16	188.7	76.6	0	4.89	764.5
17	189.7	76.4	0	4.40	755.5
18	190.4	79.2	5.2	4.96	772.2
19	190.8	102.8	25.3	4.25	772.2
20	192.1	101.6	41.5	3.66	761.9
21	195.9	82.8	0	4.72	731.0
Newana	188.7	78.0	0	5.70	768.3
Mean (1-21)	190.9	89.0	11.8	4.70	756.9
Range (1-21)	186.1-196.7	73.2-105.5	0.0-41.5	3.52-5.54	728.4-782.5
LSD _{0.05}	0.8	2.2	11.2	0.44	7.7

Quality Evaluation

Several measurements relating to quality were performed to assess the effect genes from PI372129 had on end-use quality. Both the quality as well as the quantity of protein can significantly impact baking performance.

The percentage of grain protein was determined for the three populations based on whole kernel analysis. Protein levels were significantly higher for the majority of the lines in all three populations compared to the recurrent parent. Grain protein averaged 15.3% for the Pondera single cross population, which was a 10% increase compared to Pondera (Table 30). A maximum of 16.4% protein was observed in the single cross population compared to a value of 13.9% for Pondera. Only one line in the population had a protein level not significantly different from Pondera, which was the line that also had a grain yield significantly greater than Pondera. Nine of the 14 lines had protein levels greater than 15%.

An average increase of 8% grain protein was observed for the Pondera backcross population compared to Pondera (Table 31). Only one line in the backcross population was observed to have a significantly lower protein than Pondera. Nineteen of the 23 lines had significantly higher protein levels. The protein level exceeded 15% for 11 of 23 lines. Five of the nine lines having no significant yield reduction relative to Pondera were observed to have significantly higher protein levels with only one of the nine lines having a significantly lower protein level.

The average grain protein level was 14.4% for the Newana backcross population, which was a 5.9% increase over Newana (Table 32). Only one line was observed to have a significantly lower protein level than Newana. Thirteen of the 21 backcross lines had significantly higher grain protein levels than Newana. A maximum of 15.8% protein was observed, which is approximately two percentage points greater than Newana. Only one line in the population with a similar grain yield as Newana had a higher protein level.

The SDS sedimentation procedure was used to evaluate protein quality. The PI372129 parent is a soft wheat and as such has poor breadmaking characteristics (Quick et al. 1991), which was reinforced by the SDS sedimentation test. PI372129 had an approximate sedimentation value of 85 mm based on a grain sample obtained from the 1995 winter wheat nursery grown at the Post Agronomy Research Farm. The two hard wheat parents, Pondera and Newana, had sedimentation values of 124 mm and 119 mm, respectively, thus confirming the superior breadmaking quality of the lines.

Five of the 14 Pondera single cross lines had sedimentation values that were not significantly different from Pondera (Table 30). The average sedimentation value for the 14 single cross lines was 116 mm with all the lines scoring above 100 mm. The sedimentation values ranged from 101 to 125 mm with only three lines scoring below 115 mm. The results would indicate that the lines have fair to good protein quality.

Similar results were obtained for the Pondera backcross population with an average SDS sedimentation value of 119 mm (Table 31). Fourteen of the 23 backcross lines were not significantly different from Pondera. Only one line in the backcross

population could be considered as having poor protein quality with a sedimentation value of 91 mm. The remaining 22 lines have sedimentation values greater than 100 mm with 19 lines having values greater than 115 mm. The range in sedimentation values was from 91 to 128 mm for the Pondera backcross population.

Results obtained from the SDS sedimentation analysis with the Newana backcross population were in contrast to the Pondera single cross and backcross populations. Twelve of the 21 lines scored below 100 mm with an average sedimentation value of 98 mm for the population (Table 32). The values ranged from 75 to 120 mm, with three lines scoring below 85 mm. This indicates poor protein quality for a significant portion of the lines in the population. Only two lines were identified as not being significantly different from Newana.

PI372129 also has soft kernel texture, which affects both baking and milling properties. PI372129 has an approximate NIR hardness score of 16, based on the 1995 grain sample. Pondera and Newana have scores greater than 40, with values of 54 and 47, respectively. Soft wheats generally have NIR hardness scores below 30 (Carver 1994; Norris et al. 1989).

Eleven of the 14 Pondera single cross lines can be classified as hard wheats with hardness scores above 30 (Table 30). Three lines had hardness scores below 10. The average hardness score was 43.8 with a range from 6.1 to 66.4. Results obtained for the Pondera backcross population were similar. Twenty-two of the 23 backcross lines could be classified as hard wheats with hardness values exceeding 30 (Table 31). Only one line

had a hardness score less than 10. The average hardness score was 57.8 for the backcross population. The scores ranged from 7.2 to 69.5 with 21 of the 23 lines having hardness scores greater than 54.

Only six of the 21 Newana backcross lines would be classified as hard wheats with hardness scores above 30 (Table 32). A hardness score below 10 was obtained for 11 of the 21 backcross lines. The average hardness score was 21.7, which is well below the score for Newana. The scores ranged from 3.0 to 54.8 with only two lines significantly harder than Newana.

Table 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.

Pondera Single Cross Lines	Grain Protein (%)	SDS Sedimentation (mm)	NIR Kernel Hardness
1	14.8	109.1	9.9
2	14.1	123.5	6.1
3	15.8	117.4	66.4
4	15.3	117.3	52.7
5	15.0	119.9	49.1
6	16.0	123.7	58.5
7	15.4	118.1	53.7
8	14.7	107.7	56.1
9	15.2	119.7	55.4
10	15.2	117.3	38.9
11	16.4	124.8	51.2
12	15.5	115.4	43.3
13	16.2	116.4	64.4
14	14.4	100.8	7.9
Pondera	13.9	123.6	54.2
Mean (1-14)	15.3	116.5	43.8
Range (1-14)	14.1-16.4	100.8-124.8	6.1-66.4
LSD _{0.05}	0.4	4.2	3.3

Table 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.

Pondera Backcross Lines	Grain Protein (%)	SDS Sedimentation (mm)	NIR Kernel Hardness
1	15.9	121.4	65.4
2	16.2	120.6	69.5
3	15.1	126.6	60.4
4	16.3	125.7	63.9
5	14.9	117.0	60.6
6	14.7	125.6	58.0
7	15.5	119.7	65.0
8	14.8	126.5	61.2
9	13.4	117.7	55.5
10	14.4	127.6	55.4
11	14.7	126.1	56.7
12	16.2	125.7	63.6
13	13.8	115.9	62.2
14	13.8	128.0	57.4
15	13.7	115.7	54.4
16	14.6	123.2	59.8
17	15.5	90.9	7.2
18	15.9	123.4	65.6
19	14.7	120.7	54.9
20	16.3	11.3	41.9
21	15.1	100.6	67.2
22	14.9	105.8	62.2
23	15.5	124.3	60.3
Pondera	13.9	123.6	54.2
Mean (1-23)	15.0	119.1	57.8
Range (1-23)	13.4-16.3	90.9-128.0	7.2-69.5
LSD _{0.05}	0.4	4.4	3.3

Table 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.

Newana Backcross Lines	Grain Protein (%)	SDS Sedimentation (mm)	NIR Kernel Hardness
1	14.3	92.1	3.0
2	14.5	81.1	26.8
3	15.0	96.9	54.8
4	14.6	104.3	26.9
5	14.8	89.2	3.2
6	13.0	120.4	52.4
7	14.4	103.4	25.0
8	14.1	104.8	4.1
9	13.7	93.8	3.2
10	15.3	116.6	48.7
11	15.0	95.4	6.7
12	13.9	97.8	3.8
13	15.1	93.8	5.7
14	14.0	104.1	46.1
15	13.7	105.4	50.5
16	13.8	74.8	20.6
17	13.9	96.6	44.7
18	14.4	103.6	6.3
19	15.5	98.8	8.2
20	15.8	109.4	7.5
21	13.7	78.5	7.1
Newana	13.6	119.2	47.3
Mean (1-21)	14.4	98.1	21.7
Range (1-21)	13.0-15.8	74.8-120.4	3.0-54.8
LSD _{0.05}	0.4	4.7	3.3

Marker Correlations

Molecular markers can be used to screen lines at the DNA level allowing genotypic selection to be practiced. The results of genotypic selection should correspond to phenotypic selection to be the most effective. A highly significant positive correlation was detected between the level of donor genome and plant height for the Pondera single cross population (Table 33). Results suggest that an increase in the percentage of donor genome corresponded to a significant increase in plant height. A significant positive correlation was observed between the level of donor genome and lodging index for the Pondera single cross population. Results indicate a greater severity in lodging with an increased in the level of donor genome. No significant correlations were detected between the level of donor genome and heading date, yield, test weight, grain protein, SDS sedimentation, or kernel hardness. Results were less encouraging with the two backcross populations. No significant correlations were detected for either the Pondera or Newana backcross populations for the level of donor genome and the eight traits evaluated (Tables 34 and 35).

Table 33. Correlations between the eight traits and the percentage of donor genome for the Pondera single cross population.

	Percent Donor Genome
Heading Date	0.44
Plant Height	0.70**
Lodging Index	0.51*
Yield	-0.32
Test Weight	-0.32
Grain Protein	0.41
SDS Sedimentation	-0.37
Kernel Hardness	-0.21

**P<0.01

*P<0.05

Table 34. Correlations between the eight traits and the percentage of donor genome for the Pondera backcross population.

	Percent Donor Genome
Heading Date	0.14
Plant Height	0.28
Lodging Index	0.40
Yield	-0.20
Test Weight	-0.06
Grain Protein	0.27
SDS Sedimentation	-0.35
Kernel Hardness	-0.21

Table 35. Correlations between the eight traits and the percentage of donor genome for the Newana backcross population.

	Percent Donor Genome
Heading Date	-0.22
Plant Height	-0.24
Lodging Index	0.11
Yield	-0.17
Test Weight	0.16
Grain Protein	0.02
SDS Sedimentation	-0.26
Kernel Hardness	-0.07

Discussion

Resistant varieties provide the most economical means of reducing the losses due to pests and provide the producer with a means of stabilizing yields. Resistance varieties also reduce the reliance on chemical control measures with their inherent costs and dangers. The incorporation of resistance into commercially available varieties is therefore a major focus of many plant breeding programs.

The development of PCR and its associated advantages provides an effective means to survey the level of donor genome in lines of a segregating population. The level of donor genome was evaluated using STS-PCR markers for three recombinant inbred populations derived from crosses between a Russian wheat resistant line, PI372129, and two Montana red hard spring wheat cultivars, Pondera and Newana. PI372129 is a soft white winter wheat from Russia and possesses a number of traits undesirable for a hard red spring breeding program. The line however has a single dominant resistance gene (*Dn4*) conditioning tolerance to the Russian wheat aphid. The lack of aphid resistance in commercial cultivars suggests the need to incorporate resistance within a short time frame and molecular markers may provide a method to aid in the process.

A subset of the STS-PCR primer sets identified as amplifying polymorphic fragments were used to evaluate the recombinant inbred populations. The level of donor

genome varied from 9% to 49% for the three populations. The Pondera single cross population averaged approximately 44% donor genome. The theoretical portion of donor genome for the single cross population should average 50%. The average level of donor genome should be reduced in half with each successive backcross generation. The first backcross generation should therefore have an average of approximately 25% donor DNA. Values of 23% and 22% were obtained for the Pondera and Newana backcross populations, respectively, which closely approximates the theoretical expectation. The level of donor genome for the three populations will be lower than the theoretical expectations, since selection for spring types would have reduced the level of donor genome.

The percentage of donor genome for the Pondera single cross lines ranged from 37% to 49%. Theoretical results would suggest that the single cross population should have the greatest range in donor genome normally distributed about the mean, with a range of 25% to 75% (Hillel et al. 1990). The range in donor genome for the single cross population would suggest that the population is skewed in favor of the recurrent parent genome, since all the lines are less than 50% donor genome. This reduction should also affect the phenotypic variability observed within the population. The goal of marker-assisted selection based on whole genome analysis is to identify and select lines near the tail of the distribution corresponding to the lowest level of donor genome, which should favor the recovery of the recurrent parent phenotype.

The ranges in donor genome for the two backcross populations were greater and approximated the theoretical expectations. The expected range in donor genome for the first backcross generation would be approximately 12% to 38% normally distributed around a mean of 25% (Hillel et al. 1990). Ranges were 12% to 38% for the Pondera backcross population and 9% to 41% for the Newana backcross population.

Phenotypic variability of the recombinant inbred populations was evaluated in replicated field trials. The single cross population was significantly more different from the recurrent parent, but the range in variability of this population was reduced when compared to the backcross population. The majority of the single cross lines were phenotypically similar to the PI372129 parent, with late maturity, tallness, and weak straw. There was also a tendency to reflect the characteristics of the donor parent for the backcross lines, but the number of lines significantly different from the recurrent parent were reduced. The backcross population means generally approached that of the respective recurrent parent.

Significant variation for the traits measured existed within the populations, indicating phenotypic selection could be conducted. The Pondera single cross population was 22 cm taller, 5 days later in heading, and severely lodged when compared to Pondera. The Pondera backcross population was characterized as being 14 cm taller, one day later in heading, and no lodging for the majority of the lines in comparison to Pondera. A similar situation was observed for the Newana backcross population with an 11 cm increase in plant height, 2 day delay in heading, and the majority of the lines lacking any

significant lodging in comparison to Newana. All Pondera single cross lines were significantly different from Pondera for these three traits with the exception of one line with a reduce lodging score. The number of lines significantly different from the recurrent parent was reduced in the two backcross populations and the population means approached that of the recurrent parent. Selection of lines with less donor genome should alter the population means in favor of the recurrent parent. The results would offer some optimism for the use of molecular markers to aid in the recovery of the recurrent parent phenotype.

Similar results were obtained for grain yield, even though the performance of the donor parent was unknown. The Pondera single cross population suffered the greatest average yield loss (1.0 Mg/ha), which was substantially reduced for the Pondera backcross population (0.4 Mg/ha). The Newana backcross population had a similar yield reduction (1.0 Mg/ha) as observed for the Pondera single cross population. Two of 14 Pondera single cross lines were not significantly lower in yield than Pondera. Nine of 23 Pondera backcross lines and four of 21 Newana backcross lines not were significantly lower in yield than the recurrent parent. The additional backcross lower the mean reduction in yield and decreased the number of lines significantly different from the recurrent parent, which corresponded to a reduction in donor genome.

Test weight was one the traits least affected by PI372129 in the genetic background of Pondera. Five of 14 single cross and 15 of 23 backcross lines were not significantly different from Pondera. Eleven of 21 Newana backcross lines were not

significantly different from Newana for test weight. The reduction in the number of backcross lines similar to Newana, as compared to the Pondera backcross population, would suggest that different backgrounds may affect the traits differently. The reduction in the number of lines differing significantly for test weight in the Pondera backcross population compared to the single cross population would suggest that marker-assisted selection for reduced donor genome should aid in recovery of recurrent parent phenotype.

Protein quantity and quality are factors affecting baking performance. All three populations were characterized as having significantly higher protein levels relative to the recurrent parent. The increase in grain protein may be related to the reduction in grain yield for the lines. PI372129 may however have a positive effect on grain protein, which would be selected against by selecting for the recurrent parent genotype.

The SDS sedimentation test is an indirect measure of protein quality. The presence of PI372129 genome in the Pondera background appeared to have a limited effect on sedimentation values. Five of 14 single cross and 15 of 23 backcross lines were not significantly lower than Pondera. The lines for the two populations could be considered to have fair to good protein quality with the exception of one backcross line. The single Pondera backcross line with the low sedimentation value also had a greater percentage of donor genome and would be selected against if markers were used as a selection criteria. This would eliminate a line from the backcross population possessing many of the undesirable characteristics associated with the PI372129 line. The elimination of a major

portion of the donor genome with the additional backcross did effectively increase the average sedimentation value.

The presence of PI372129 in the Newana background had a major influence on sedimentation values. Only two of 21 backcross lines were not significantly different from Newana. Twelve of the 21 lines would be classified as having poor protein quality with sedimentation values below 100 mm.

A similar situation was observed for kernel hardness, where the Pondera populations were less affected as compared to the Newana population by the presence of PI372129 in the genetic background. Three of 14 Pondera single cross lines and one of 23 Pondera backcross lines would be classified as having soft kernel texture. Morrison et al. (1989) suggested that chromosome 5D contains a vernalization gene (*Vrn3*) and a gene for grain softness (*Ha*). This may explain the results obtained for the Pondera single cross and backcross populations. The selection for the spring growth habit may have also favored the selection for kernel hardness. The occurrence of a limited number of lines with soft kernel texture may have resulted from recombination in chromosome 5D between the vernalization gene and kernel softness gene. Results for the Newana backcross population would suggest an independent factor controlling kernel hardness, since 15 of the 21 backcross lines would be classified as having soft kernel texture.

The effectiveness of marker-assisted selection could be determined by correlating the phenotypic results with the genotypic results. Selection based on markers would aid in the recovery of lines with reduced plant height and lodging for the Pondera single cross

population. The range in donor genome for the Pondera single cross population may have influenced potential correlations with the other traits.

The results were less encouraging with the Pondera and Newana backcross populations, since no significant correlations were detected between the traits evaluated and percentage of donor markers. One positive observation was that selection for resistance would not favor selection for undesirable factors, especially since the gene controlling resistance is on homoeologous group 1 chromosomes which is the location of important genes controlling seed storage proteins.

Although significant correlations were not obtained for all the traits measured in the three populations, the effect of eliminating half the donor genome with an additional backcross illustrates the potential gain that can be achieved with marker-assisted selection. Selection aided by markers could significantly affect the population means in favor of the recurrent parent. The comparison between the two backcross populations would suggest that the recurrent parent may have a considerable influence on the results obtained for certain traits. Marker-assisted selection would fail to identify transgressive segregants that may occur in the population. However, the goal of backcross breeding is the recovery of the recurrent parent phenotype, and as such, using markers to select for the recurrent parent genotype may offer some potential in achieving this endeavor.

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APPENDIX

Table 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.

Primer Set	Source	Barley RFLP Location	Barley STS Location	Wheat RFLP Location	Wheat STS Location (Major Band)	Wheat STS Location (Minor Band)	Southern Analysis Wheat	Southern Analysis Barley
ABC151	Barley cDNA	7	3	1D	2AD,3B	6A		
ABC156	Barley cDNA	2,3,7	3	1ABD	1A	2B		
ABC158	Barley cDNA	7		7A	1D,3D,6D		No hybridization	No Hybridization
ABC164	Barley cDNA	1,6	4	5B	unmapped			
ABC252	Barley cDNA	2	2,4		unmapped			
ABC253	Barley cDNA	7	7		1A,3B,4BD	2A,5A	Hybridization	Hybridization
ABC302.3	Barley cDNA	5	6		5A,6B,7D	1A,4A		
ABC303	Barley cDNA	4	4			3AB,4D	Hybridization	Hybridization
ABC305	Barley cDNA	7	6		1B,5B,6ABD	3A,7B		
ABC309	Barley cDNA	5			3D,6A		No hybridization	No Hybridization
ABC322	Barley cDNA	1,7			3D,5B	3A,6B		
ABC454	Barley cDNA	2	2		2B			
ABC455	Barley cDNA	7	6	7B	5B,7B			
ABC465	Barley cDNA	7	7	7D	3AB,7D	1B,5B,7A		
ABC483	Barley cDNA	5	5		1D,2A,3B,4AB,5A,7B			
ABC602	Barley cDNA				3BD		Hybridization	Hybridization
ABG002	Barley Genomic	2	3		4D,5B,6B			
ABG003	Barley Genomic	4,5	5		3B,4B,5BD	1B,2B,6BD,7A		
ABG004	Barley Genomic	3	2,4		unmapped			
ABG058	Barley Genomic	2	2		2B,5B,7B	6A	No hybridization	Hybridization
ABG065	Barley Genomic	3	6		6D			
ABG070	Barley Genomic	3	3		3B			
ABG075	Barley Genomic	7	3		2A	3A,4B,7B		

Table 36. Continued.

Primer Set	Source	Barley RFLP Location	Barley STS Location	Wheat RFLP Location	Wheat STS Location (Major Band)	Wheat STS Location (Minor Band)	Southern Analysis Wheat	Southern Analysis Barley
ABG317	Barley Genomic	2	2		2D	6B	No hybridization	No Hybridization
ABG320	Barley Genomic	7	7		5A	6B		
ABG358	Barley Genomic	2			1A	5A	No hybridization	Hybridization
ABG378	Barley Genomic	6	6	6D	3BD	2BD	No hybridization	Hybridization
ABG391	Barley Genomic	5	5	5A	4A,5ABD		Hybridization	Hybridization
ABG394	Barley Genomic	4	4			4A,7B		
ABG396	Barley Genomic	3	3		unmapped			
ABG458	Barley Genomic	6	6		6AB	6D		
ABG460	Barley Genomic	3	7	3A	unmapped			
ABG466	Barley Genomic	6	4,5,6		3A	4B,6B		
ABG471	Barley Genomic	3	6	3AB		2A		
ABG484	Barley Genomic	4		4B	unmapped			
ABG500	Barley Genomic	1,4			1A,6A	6B		
ABG601	Barley Genomic	4			5A	7A		
ABG602	Barley Genomic	2	2,4,6		2BD	3B	Hybridization	Hybridization
ABG608	Barley Genomic	7	3		1B,2B,4A,5A,6B			
ABG618	Barley Genomic	4	4		4A,7D	1B,2A,5B,7B	No hybridization	Hybridization
ABG701	Barley Genomic	7	7		7AB		Hybridization	Hybridization
ABG704	Barley Genomic	7	7		4A,7A		Hybridization	Hybridization
ABG711	Barley Genomic	6	4		3A,4A,5B,6A,7B	3D		
ABG712	Barley Genomic	5	5		1A,2ABD		Hybridization	Hybridization
ABG715	Barley Genomic	4	4		4AB		Hybridization	Hybridization
BarG10	Barley Genomic	4	4		3B,4B	5D,6D	Hybridization	Hybridization
BCD129	Barley cDNA	7		4A	2B		Hybridization	Hybridization
BCD175	Barley cDNA	2	2	2ABD	2BD		No hybridization	Hybridization
BCD304	Barley cDNA	1	2	1AB	1B,5B,6D	1D,3B,7A		
BCD402	Barley cDNA	4	6	4AB	6AB,7B	1D,3AB,4B		

Table 36. Continued.

Primer Set	Source	Barley RFLP Location	Barley STS Location	Wheat RFLP Location	Wheat STS Location (Major Band)	Wheat STS Location (Minor Band)	Southern Analysis Wheat	Southern Analysis Barley
BCD828	Barley cDNA	3	5	3ABD	unmapped			
CDO36	Oat cDNA	7		2BD	2D	7A		
CDO213	Oat cDNA	5	7	5ABD	1A,4B,7D	1D,4A,5D,6A	No hybridization	No Hybridization
CDO506	Oat cDNA	5		5D	5B,7D	4A,7A		
CDO673	Oat cDNA	7	5	7D	1A	3D,4A,5BD	No hybridization	No Hybridization
MWG650	Barley Genomic	5				1B,2B,5D		
WG178	Wheat Genomic	3		3ABD	unmapped			
WG181	Wheat Genomic	4		4ABD	4B		Hybridization	Hybridization
WG232	Wheat Genomic	4	6		4AD,6B,7A		Hybridization	No Amplification
WG464	Wheat Genomic	4	4		2B,4ABD,7A			
WG530	Wheat Genomic	5		5D	4A	6B	Hybridization	Hybridization
WG564-2	Wheat Genomic	5		5D	4BD	2BD	Hybridization	Hybridization
WG669	Wheat Genomic	7		7ABD	3A	3B,5BD		
WG686	Wheat Genomic	7	5,6	7ABD	7AB	1D,4A,6BD,7D		
WG719	Wheat Genomic	7		7D	unmapped			
WG983	Wheat Genomic	1		1ABD	unmapped			
WG996	Wheat Genomic	2	7	2B	1A			
WG1026	Wheat Genomic	4,5	5	5ABD	5B			
KSUA1	<i>T. tauschii</i> Genomic	5,7		5B,7D	7D			
KSUA5	<i>T. tauschii</i> Genomic			7D	7D			
KSUB5	<i>T. tauschii</i> Genomic		2,6	4D		1A,3B,6B		
KSUD2	<i>T. tauschii</i> Genomic		7	7ABD	7ABD		Hybridization	No Hybridization
KSUD7	<i>T. tauschii</i> Genomic			3D	7B	1B,2AB,5AB		
KSUD9	<i>T. tauschii</i> Genomic			4A,7ABD	6D,7B	1B,2A,6A		
KSUD14	<i>T. tauschii</i> Genomic	1,7	2,4,5	1ABD	1B,4B	5A		
KSUD15	<i>T. tauschii</i> Genomic			7ABD	1D,5BD,7BD	2A,6B	Hybridization	No Hybridization

Table 36. Continued.

Primer Set	Source	Barley RFLP Location	Barley STS Location	Wheat RFLP Location	Wheat STS Location (Major Band)	Wheat STS Location (Minor Band)	Southern Analysis Wheat	Southern Analysis Barley
KSUD16	<i>T. tauschii</i> Genomic		2	1D	4A,5B	1B,4BD,7A	No hybridization	No Hybridization
KSUD17	<i>T. tauschii</i> Genomic	6		6D	2B,6D	3AB,4BD		
KSUD18	<i>T. tauschii</i> Genomic	2		2AD	2A			
KSUD22	<i>T. tauschii</i> Genomic	2		2AD	4A	2B,7A		
KSUE8	<i>T. tauschii</i> Genomic			1ABD	2B,4D,6B,7D	1D,5D	Hybridization	Hybridization
KSUE11	<i>T. tauschii</i> Genomic	1		1ABD	1D			
KSUF15	<i>T. tauschii</i> Genomic	2		2ABD	2AB,3A,6ABD	1A,5B,7A	Hybridization	Hybridization
KSUF34	<i>T. tauschii</i> Genomic			3D,7D	2B,3AD,7D	2D		
KSUF37	<i>T. tauschii</i> Genomic			6D	unmapped			
KSUF48	<i>T. tauschii</i> Genomic			7AD	6D	1A,3B,6B		
KSUG12	<i>T. tauschii</i> Genomic			4A,5D,7AD	2BD	1D,4A,7B	Hybridization	Hybridization
KSUG14	<i>T. tauschii</i> Genomic			5D	5D	1AB,4A,5B,6B		
KSUG43	<i>T. tauschii</i> Genomic	2		6D	unmapped			
KSUG44	<i>T. tauschii</i> Genomic		5	5ABD,6D	5BD	5A	Hybridization	Hybridization
KSUG48	<i>T. tauschii</i> Genomic			3ABD,6ABD	6D	5B,7BD		
KSUG49	<i>T. tauschii</i> Genomic			2D	4A,7D	7B	Hybridization	Hybridization
KSUG53	<i>T. tauschii</i> Genomic			3ABD	2A,6B	2B,4A,5B		
KSUH8	<i>T. tauschii</i> Genomic			2D,3D,5D,7D	unmapped			
KSUH9	<i>T. tauschii</i> Genomic			1AD,2D,7B	2D,6B	1D,4BD,5B		
KSUH11	<i>T. tauschii</i> Genomic	4		6D	4D,	6D,7D		
KSUM148	<i>T. tauschii</i> Genomic			1D	1B,2A,4BD,6B		No hybridization	No Hybridization

Table 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 *Hinfl*, *RsaI*, *DdeI*, and *HhaI*.

Primer Set	Source	Pondera vs PI372129	Newana vs PI372129	Pondera vs Newana
AC30	Barley	Polymorphic	Polymorphic	Monomorphic
AC33	Barley	Monomorphic	Monomorphic	Monomorphic
BG123	Barley	No Amplification	No Amplification	No Amplification
BTA1	Barley	No Amplification	No Amplification	No Amplification
BTA2	Barley	Polymorphic	Polymorphic	Polymorphic
DHN1	Barley	Polymorphic	Polymorphic	Polymorphic
DHN1L2R	Barley	Polymorphic	Polymorphic	Polymorphic
DHN2	Barley	Monomorphic	Monomorphic	Monomorphic
KV1&2	Barley	Polymorphic	Polymorphic	Polymorphic
KV1&9	Barley	Monomorphic	Polymorphic	Polymorphic
KV3&4	Barley	Polymorphic	Polymorphic	Monomorphic
KV5&6	Barley	Polymorphic	Polymorphic	Polymorphic
KV7&8	Barley	Polymorphic	Polymorphic	Polymorphic
KV9&10	Barley	Monomorphic	Polymorphic	Polymorphic
KV12&13	Barley	Polymorphic	Monomorphic	Polymorphic
KV12&14	Barley	Polymorphic	Monomorphic	Polymorphic
KV12&24	Barley	Polymorphic	Polymorphic	Polymorphic
KV14&TB14	Barley	Monomorphic	Monomorphic	Monomorphic
KV16&17	Barley	Monomorphic	Polymorphic	Polymorphic
KV22&23	Barley	Polymorphic	Polymorphic	Monomorphic
KV25&26	Barley	Monomorphic	Polymorphic	Polymorphic
KV26&29	Barley	Monomorphic	Polymorphic	Polymorphic
KV27&28	Barley	No Amplification	No Amplification	No Amplification
KV29&30	Barley	Polymorphic	Polymorphic	Polymorphic
KV31&32	Barley	No Amplification	No Amplification	No Amplification
M17&19	Barley	Monomorphic	Monomorphic	Monomorphic
M18&19	Barley	Polymorphic	Polymorphic	Polymorphic
NAR1	Barley	Polymorphic	Polymorphic	Monomorphic
NAR7L1	Barley	No Amplification	No Amplification	No Amplification
NAR7L2	Barley	No Amplification	No Amplification	No Amplification
PST319	Barley	Polymorphic	Polymorphic	Polymorphic
PST321	Barley	Polymorphic	Polymorphic	Polymorphic
PST327	Barley	Monomorphic	Monomorphic	Monomorphic
PST337	Barley	Polymorphic	Polymorphic	Polymorphic
PST340	Barley	Polymorphic	Polymorphic	Polymorphic

Table 37. Continued.

Primer Set	Source	Pondera vs PI372129	Newana vs PI372129	Pondera vs Newana
S1&2	Barley	Polymorphic	Polymorphic	Polymorphic
S2&3	Barley	Polymorphic	Polymorphic	Polymorphic
S2&4	Barley	Polymorphic	Polymorphic	Polymorphic
ST1&2	Barley	Monomorphic	Polymorphic	Polymorphic
ST4&6	Barley	Monomorphic	Polymorphic	Polymorphic
ST7&8	Barley	Polymorphic	Polymorphic	Monomorphic
ST9&10	Barley	Monomorphic	Monomorphic	Monomorphic
ST12&13	Barley	Polymorphic	Polymorphic	Polymorphic
ST14&16	Barley	Monomorphic	Polymorphic	Polymorphic
TB1&2	Barley	Polymorphic	Polymorphic	Monomorphic
TB4&5	Barley	Polymorphic	Polymorphic	Monomorphic
TB10&11	Barley	Polymorphic	Polymorphic	Polymorphic
TB13&14	Barley	Polymorphic	Polymorphic	Monomorphic
TB15&16	Barley	Polymorphic	Polymorphic	Polymorphic
TB17&18	Barley	Polymorphic	Polymorphic	Polymorphic
TB19&20	Barley	Polymorphic	Polymorphic	Monomorphic
TB21&22	Barley	Monomorphic	Monomorphic	Monomorphic
TB33&34	Barley	Polymorphic	Polymorphic	Monomorphic
TB35&36	Barley	Polymorphic	Polymorphic	Polymorphic
TB36&37	Barley	Polymorphic	Polymorphic	Monomorphic
TB38&39	Barley	Monomorphic	Monomorphic	Monomorphic
TB67&68	Barley	Monomorphic	Monomorphic	Monomorphic
TB69&70	Barley	Polymorphic	Polymorphic	Polymorphic
WTA1	Barley	Monomorphic	Monomorphic	Monomorphic
ABC151	Barley cDNA	Polymorphic	Polymorphic	Monomorphic
ABC152	Barley cDNA	Polymorphic	Polymorphic	Polymorphic
ABC153	Barley cDNA	Polymorphic	Polymorphic	Polymorphic
ABC154	Barley cDNA	No Amplification	No Amplification	No Amplification
ABC155	Barley cDNA	No Amplification	No Amplification	No Amplification
ABC156	Barley cDNA	Polymorphic	Polymorphic	Monomorphic
ABC156.2	Barley cDNA	Polymorphic	Polymorphic	Monomorphic
ABC156M	Barley cDNA	Polymorphic	Polymorphic	Polymorphic
ABC158	Barley cDNA	Polymorphic	Polymorphic	Polymorphic
ABC160	Barley cDNA	Polymorphic	Polymorphic	Monomorphic
ABC161	Barley cDNA	Polymorphic	Polymorphic	Polymorphic
ABC164	Barley cDNA	Polymorphic	Polymorphic	Polymorphic
ABC165	Barley cDNA	Monomorphic	Monomorphic	Monomorphic
ABC166	Barley cDNA	No Amplification	No Amplification	No Amplification
ABC167	Barley cDNA	No Amplification	No Amplification	No Amplification
ABC168	Barley cDNA	Monomorphic	Monomorphic	Monomorphic
ABC170	Barley cDNA	Polymorphic	Polymorphic	Polymorphic

Table 37. Continued.

Primer Set	Source	Pondera vs PI372129	Newana vs PI372129	Pondera vs Newana
ABC252	Barley cDNA	Polymorphic	Polymorphic	Polymorphic
ABC253	Barley cDNA	Polymorphic	Polymorphic	Monomorphic
ABC254	Barley cDNA	Polymorphic	Polymorphic	Polymorphic
ABC255	Barley cDNA	No Amplification	No Amplification	No Amplification
ABC257	Barley cDNA	No Amplification	No Amplification	No Amplification
ABC261	Barley cDNA	Polymorphic	Polymorphic	Monomorphic
ABC302	Barley cDNA	Polymorphic	Polymorphic	Polymorphic
ABC302.1	Barley cDNA	Monomorphic	Monomorphic	Monomorphic
ABC302.2	Barley cDNA	Monomorphic	Monomorphic	Monomorphic
ABC303	Barley cDNA	Polymorphic	Polymorphic	Polymorphic
ABC305	Barley cDNA	Polymorphic	Polymorphic	Monomorphic
ABC306	Barley cDNA	Polymorphic	Polymorphic	Monomorphic
ABC308	Barley cDNA	Monomorphic	Monomorphic	Monomorphic
ABC309	Barley cDNA	Polymorphic	Polymorphic	Polymorphic
ABC310	Barley cDNA	No Amplification	No Amplification	No Amplification
ABC311	Barley cDNA	Polymorphic	Polymorphic	Monomorphic
ABC322	Barley cDNA	Monomorphic	Monomorphic	Monomorphic
ABC323	Barley cDNA	Polymorphic	Polymorphic	Polymorphic
ABC451	Barley cDNA	No Amplification	No Amplification	No Amplification
ABC454	Barley cDNA	Polymorphic	Polymorphic	Polymorphic
ABC455	Barley cDNA	Polymorphic	Polymorphic	Polymorphic
ABC465	Barley cDNA	Polymorphic	Polymorphic	Polymorphic
ABC468	Barley cDNA	Monomorphic	Monomorphic	Monomorphic
ABC483	Barley cDNA	Polymorphic	Polymorphic	Polymorphic
ABC601	Barley cDNA	Polymorphic	Polymorphic	Monomorphic
ABC602	Barley cDNA	Polymorphic	Polymorphic	Monomorphic
ABC622	Barley cDNA	Polymorphic	Polymorphic	Polymorphic
ABC717	Barley cDNA	Monomorphic	Monomorphic	Monomorphic
ABC718	Barley cDNA	Polymorphic	Monomorphic	Polymorphic
BCD129	Barley cDNA	Monomorphic	Monomorphic	Monomorphic
BCD175	Barley cDNA	Polymorphic	Polymorphic	Monomorphic
BCD269	Barley cDNA	No Amplification	No Amplification	No Amplification
BCD304	Barley cDNA	Polymorphic	Polymorphic	Monomorphic
BCD327	Barley cDNA	Monomorphic	Monomorphic	Monomorphic
BCD402	Barley cDNA	Polymorphic	Polymorphic	Polymorphic
BCD402A	Barley cDNA	Polymorphic	Polymorphic	Polymorphic
BCD828	Barley cDNA	Polymorphic	Polymorphic	Polymorphic
ABG002	Barley Genomic	Polymorphic	Polymorphic	Polymorphic
ABG003	Barley Genomic	Polymorphic	Polymorphic	Polymorphic
ABG004	Barley Genomic	Polymorphic	Polymorphic	Monomorphic

Table 37. Continued.

Primer Set	Source	Pondera vs PI372129	Newana vs PI372129	Pondera vs Newana
ABG014	Barley Genomic	Monomorphic	Monomorphic	Monomorphic
ABG020	Barley Genomic	Polymorphic	Monomorphic	Polymorphic
ABG035	Barley Genomic	Polymorphic	Polymorphic	Monomorphic
ABG054	Barley Genomic	Monomorphic	Monomorphic	Monomorphic
ABG055	Barley Genomic	Polymorphic	Polymorphic	Monomorphic
ABG057	Barley Genomic	No Amplification	No Amplification	No Amplification
ABG057U1R2	Barley Genomic	Polymorphic	Polymorphic	Monomorphic
ABG057U2R1	Barley Genomic	Monomorphic	Monomorphic	Monomorphic
ABG057U2R2	Barley Genomic	Polymorphic	Polymorphic	Polymorphic
ABG058	Barley Genomic	Monomorphic	Monomorphic	Monomorphic
ABG059	Barley Genomic	No Amplification	No Amplification	No Amplification
ABG062	Barley Genomic	Polymorphic	Polymorphic	Polymorphic
ABG064	Barley Genomic	Polymorphic	Polymorphic	Polymorphic
ABG065	Barley Genomic	Polymorphic	Polymorphic	Polymorphic
ABG070	Barley Genomic	Monomorphic	Monomorphic	Monomorphic
ABG072	Barley Genomic	Monomorphic	Monomorphic	Monomorphic
ABG075	Barley Genomic	Polymorphic	Polymorphic	Polymorphic
ABG077	Barley Genomic	Polymorphic	Polymorphic	Monomorphic
ABG314	Barley Genomic	No Amplification	No Amplification	No Amplification
ABG315	Barley Genomic	No Amplification	No Amplification	No Amplification
ABG316	Barley Genomic	Polymorphic	Polymorphic	Polymorphic
ABG317	Barley Genomic	Monomorphic	Monomorphic	Monomorphic
ABG318	Barley Genomic	Polymorphic	Monomorphic	Polymorphic
ABG319	Barley Genomic	Polymorphic	Polymorphic	Polymorphic
ABG320	Barley Genomic	Polymorphic	Polymorphic	Polymorphic
ABG337	Barley Genomic	Polymorphic	Polymorphic	Polymorphic
ABG356	Barley Genomic	Monomorphic	Monomorphic	Monomorphic
ABG358	Barley Genomic	Polymorphic	Polymorphic	Monomorphic
ABG366	Barley Genomic	Polymorphic	Polymorphic	Polymorphic
ABG373	Barley Genomic	Polymorphic	Polymorphic	Monomorphic
ABG377	Barley Genomic	Monomorphic	Monomorphic	Monomorphic
ABG378	Barley Genomic	Polymorphic	Polymorphic	Polymorphic
ABG379	Barley Genomic	Polymorphic	Polymorphic	Polymorphic
ABG380	Barley Genomic	Monomorphic	Monomorphic	Monomorphic
ABG390	Barley Genomic	No Amplification	No Amplification	No Amplification
ABG391	Barley Genomic	Polymorphic	Polymorphic	Monomorphic
ABG394	Barley Genomic	Monomorphic	Monomorphic	Monomorphic
ABG395	Barley Genomic	Polymorphic	Polymorphic	Polymorphic
ABG396	Barley Genomic	Monomorphic	Monomorphic	Monomorphic
ABG397	Barley Genomic	No Amplification	No Amplification	No Amplification
ABG397.2	Barley Genomic	Monomorphic	Monomorphic	Monomorphic

Table 37. Continued.

Primer Set	Source	Pondera vs PI372129	Newana vs PI372129	Pondera vs Newana
ABG398	Barley Genomic	Monomorphic	Monomorphic	Monomorphic
ABG452	Barley Genomic	Monomorphic	Monomorphic	Monomorphic
ABG458	Barley Genomic	Polymorphic	Polymorphic	Monomorphic
ABG458.1	Barley Genomic	Polymorphic	Polymorphic	Monomorphic
ABG458.2	Barley Genomic	Monomorphic	Monomorphic	Monomorphic
ABG459	Barley Genomic	Polymorphic	Polymorphic	Polymorphic
ABG460	Barley Genomic	Polymorphic	Monomorphic	Polymorphic
ABG461	Barley Genomic	No Amplification	No Amplification	No Amplification
ABG463	Barley Genomic	No Amplification	No Amplification	No Amplification
ABG466	Barley Genomic	Monomorphic	Polymorphic	Polymorphic
ABG468	Barley Genomic	Monomorphic	Monomorphic	Monomorphic
ABG471	Barley Genomic	Polymorphic	Polymorphic	Monomorphic
ABG472	Barley Genomic	No Amplification	No Amplification	No Amplification
ABG473	Barley Genomic	No Amplification	No Amplification	No Amplification
ABG473.2	Barley Genomic	Polymorphic	Polymorphic	Polymorphic
ABG474	Barley Genomic	Polymorphic	Polymorphic	Polymorphic
ABG484	Barley Genomic	Monomorphic	Polymorphic	Polymorphic
ABG494	Barley Genomic	Polymorphic	Polymorphic	Polymorphic
ABG495	Barley Genomic	No Amplification	No Amplification	No Amplification
ABG498	Barley Genomic	Polymorphic	Polymorphic	Monomorphic
ABG499	Barley Genomic	Polymorphic	Polymorphic	Polymorphic
ABG500	Barley Genomic	Polymorphic	Polymorphic	Monomorphic
ABG601	Barley Genomic	Polymorphic	Polymorphic	Polymorphic
ABG602	Barley Genomic	Polymorphic	Polymorphic	Polymorphic
ABG603	Barley Genomic	Monomorphic	Monomorphic	Monomorphic
ABG613	Barley Genomic	No Amplification	No Amplification	No Amplification
ABG616	Barley Genomic	Monomorphic	Polymorphic	Polymorphic
ABG618	Barley Genomic	Polymorphic	Polymorphic	Polymorphic
ABG619	Barley Genomic	Polymorphic	Polymorphic	Monomorphic
ABG654	Barley Genomic	Polymorphic	Polymorphic	Polymorphic
ABG701	Barley Genomic	Monomorphic	Monomorphic	Monomorphic
ABG702	Barley Genomic	Monomorphic	Monomorphic	Monomorphic
ABG704	Barley Genomic	Polymorphic	Polymorphic	Polymorphic
ABG705	Barley Genomic	No Amplification	No Amplification	No Amplification
ABG706	Barley Genomic	No Amplification	No Amplification	No Amplification
ABG707	Barley Genomic	Monomorphic	Polymorphic	Polymorphic
ABG708	Barley Genomic	Monomorphic	Monomorphic	Monomorphic
ABG710	Barley Genomic	Polymorphic	Polymorphic	Polymorphic
ABG711	Barley Genomic	Polymorphic	Polymorphic	Polymorphic
ABG712	Barley Genomic	Polymorphic	Polymorphic	Polymorphic
ABG715	Barley Genomic	Polymorphic	Polymorphic	Monomorphic
ABG716	Barley Genomic	Polymorphic	Polymorphic	Polymorphic

Table 37. Continued.

Primer Set	Source	Pondera vs PI372129	Newana vs PI372129	Pondera vs Newana
BARG10	Barley Genomic	Polymorphic	Monomorphic	Polymorphic
MWG650	Barley Genomic	Polymorphic	Polymorphic	Polymorphic
CDO36	Oat cDNA	Polymorphic	Polymorphic	Polymorphic
CDO63	Oat cDNA	Polymorphic	Polymorphic	Polymorphic
CDO213	Oat cDNA	Polymorphic	Polymorphic	Polymorphic
CDO370	Oat cDNA	Polymorphic	Polymorphic	Monomorphic
CDO395	Oat cDNA	Polymorphic	Polymorphic	Polymorphic
CDO464	Oat cDNA	Monomorphic	Monomorphic	Monomorphic
CDO474	Oat cDNA	Polymorphic	Polymorphic	Polymorphic
CDO475	Oat cDNA	Polymorphic	Monomorphic	Polymorphic
CDO506	Oat cDNA	Polymorphic	Polymorphic	Polymorphic
CDO541	Oat cDNA	No Amplification	No Amplification	No Amplification
CDO545	Oat cDNA	Monomorphic	Monomorphic	Monomorphic
CDO588	Oat cDNA	Polymorphic	Polymorphic	Polymorphic
CDO662	Oat cDNA	Polymorphic	Polymorphic	Polymorphic
CDO673	Oat cDNA	Polymorphic	Polymorphic	Monomorphic
CDO749	Oat cDNA	Polymorphic	Polymorphic	Polymorphic
CDO770	Oat cDNA	Polymorphic	Monomorphic	Polymorphic
CDO1338	Oat cDNA	Monomorphic	Monomorphic	Monomorphic
KSUA1	<i>T. tauschii</i> Genomic	Monomorphic	Polymorphic	Polymorphic
KSUA3	<i>T. tauschii</i> Genomic	No Amplification	No Amplification	No Amplification
KSUA5	<i>T. tauschii</i> Genomic	Polymorphic	Polymorphic	Monomorphic
KSUAB9	<i>T. tauschii</i> Genomic	Monomorphic	Monomorphic	Monomorphic
KSUB5	<i>T. tauschii</i> Genomic	Monomorphic	Monomorphic	Monomorphic
KSUC2	<i>T. tauschii</i> Genomic	Monomorphic	Monomorphic	Monomorphic
KSUD1	<i>T. tauschii</i> Genomic	Monomorphic	Monomorphic	Monomorphic
KSUD2	<i>T. tauschii</i> Genomic	Monomorphic	Monomorphic	Monomorphic
KSUD7	<i>T. tauschii</i> Genomic	Monomorphic	Monomorphic	Monomorphic
KSUD8	<i>T. tauschii</i> Genomic	Polymorphic	Monomorphic	Polymorphic
KSUD9	<i>T. tauschii</i> Genomic	Polymorphic	Polymorphic	Polymorphic
KSUD12	<i>T. tauschii</i> Genomic	Monomorphic	Monomorphic	Monomorphic
KSUD14	<i>T. tauschii</i> Genomic	Polymorphic	Polymorphic	Polymorphic
KSUD15	<i>T. tauschii</i> Genomic	Polymorphic	Monomorphic	Polymorphic
KSUD16	<i>T. tauschii</i> Genomic	Polymorphic	Polymorphic	Polymorphic
KSUD17	<i>T. tauschii</i> Genomic	Polymorphic	Monomorphic	Polymorphic
KSUD18	<i>T. tauschii</i> Genomic	Polymorphic	Polymorphic	Monomorphic
KSUD19	<i>T. tauschii</i> Genomic	No Amplification	No Amplification	No Amplification
KSUD21	<i>T. tauschii</i> Genomic	Monomorphic	Monomorphic	Monomorphic
KSUD22	<i>T. tauschii</i> Genomic	Monomorphic	Polymorphic	Polymorphic
KSUD23	<i>T. tauschii</i> Genomic	Polymorphic	Monomorphic	Polymorphic
KSUD30	<i>T. tauschii</i> Genomic	Polymorphic	Polymorphic	Monomorphic

Table 37. Continued.

Primer Set	Source	Pondera vs PI372129	Newana vs PI372129	Pondera vs Newana
KSUE2	<i>T. tauschii</i> Genomic	No Amplification	No Amplification	No Amplification
KSUE6	<i>T. tauschii</i> Genomic	Polymorphic	Polymorphic	Polymorphic
KSUE6A	<i>T. tauschii</i> Genomic	Monomorphic	Monomorphic	Monomorphic
KSUE8	<i>T. tauschii</i> Genomic	Monomorphic	Monomorphic	Monomorphic
KSUE9	<i>T. tauschii</i> Genomic	No Amplification	No Amplification	No Amplification
KSUE11	<i>T. tauschii</i> Genomic	No Amplification	No Amplification	No Amplification
KSUE11A	<i>T. tauschii</i> Genomic	Polymorphic	Polymorphic	Polymorphic
KSUE14	<i>T. tauschii</i> Genomic	Monomorphic	Monomorphic	Monomorphic
KSUE16	<i>T. tauschii</i> Genomic	Monomorphic	No Amplification	No Amplification
KSUE19	<i>T. tauschii</i> Genomic	Monomorphic	Monomorphic	Monomorphic
KSUF2	<i>T. tauschii</i> Genomic	No Amplification	No Amplification	No Amplification
KSUF2A	<i>T. tauschii</i> Genomic	Monomorphic	Monomorphic	Monomorphic
KSUF8	<i>T. tauschii</i> Genomic	Monomorphic	Monomorphic	Monomorphic
KSUF11	<i>T. tauschii</i> Genomic	No Amplification	No Amplification	No Amplification
KSUF15	<i>T. tauschii</i> Genomic	Monomorphic	Monomorphic	Monomorphic
KSUF19	<i>T. tauschii</i> Genomic	Polymorphic	Polymorphic	Polymorphic
KSUF34	<i>T. tauschii</i> Genomic	Monomorphic	Monomorphic	Monomorphic
KSUF36	<i>T. tauschii</i> Genomic	No Amplification	No Amplification	No Amplification
KSUF37	<i>T. tauschii</i> Genomic	Monomorphic	Monomorphic	Monomorphic
KSUF41	<i>T. tauschii</i> Genomic	Polymorphic	Polymorphic	Polymorphic
KSUF43	<i>T. tauschii</i> Genomic	No Amplification	No Amplification	No Amplification
KSUF48	<i>T. tauschii</i> Genomic	No Amplification	No Amplification	No Amplification
KSUF48A	<i>T. tauschii</i> Genomic	Monomorphic	Polymorphic	Polymorphic
KSUG2	<i>T. tauschii</i> Genomic	No Amplification	No Amplification	No Amplification
KSUG5	<i>T. tauschii</i> Genomic	Monomorphic	Monomorphic	Monomorphic
KSUG8	<i>T. tauschii</i> Genomic	Polymorphic	Polymorphic	Polymorphic
KSUG9	<i>T. tauschii</i> Genomic	No Amplification	No Amplification	No Amplification
KSUG10	<i>T. tauschii</i> Genomic	No Amplification	No Amplification	No Amplification
KSUG10INT	<i>T. tauschii</i> Genomic	Monomorphic	Monomorphic	Monomorphic
KSUG12	<i>T. tauschii</i> Genomic	Monomorphic	Monomorphic	Monomorphic
KSUG13	<i>T. tauschii</i> Genomic	No Amplification	No Amplification	No Amplification
KSUG14	<i>T. tauschii</i> Genomic	Polymorphic	Polymorphic	Polymorphic
KSUG43	<i>T. tauschii</i> Genomic	Monomorphic	Monomorphic	Monomorphic
KSUG44	<i>T. tauschii</i> Genomic	Monomorphic	Monomorphic	Monomorphic
KSUG48	<i>T. tauschii</i> Genomic	Polymorphic	Polymorphic	Polymorphic
KSUG49	<i>T. tauschii</i> Genomic	Polymorphic	Polymorphic	Polymorphic
KSUG53	<i>T. tauschii</i> Genomic	Polymorphic	Polymorphic	Monomorphic
KSUG59	<i>T. tauschii</i> Genomic	Monomorphic	Monomorphic	Monomorphic
KSUG62	<i>T. tauschii</i> Genomic	No Amplification	No Amplification	No Amplification
KSUH7	<i>T. tauschii</i> Genomic	No Amplification	No Amplification	No Amplification
KSUH8	<i>T. tauschii</i> Genomic	Monomorphic	Monomorphic	Monomorphic

Table 37. Continued.

Primer Set	Source	Pondera vs PI372129	Newana vs PI372129	Pondera vs Newana
KSUH9	<i>T. tauschii</i> Genomic	Monomorphic	Polymorphic	Polymorphic
KSUH11	<i>T. tauschii</i> Genomic	Monomorphic	Monomorphic	Monomorphic
KSUH15	<i>T. tauschii</i> Genomic	Polymorphic	Polymorphic	Polymorphic
KSUH16	<i>T. tauschii</i> Genomic	No Amplification	No Amplification	No Amplification
KSUI26	<i>T. tauschii</i> Genomic	Monomorphic	Monomorphic	Monomorphic
KSUI27	<i>T. tauschii</i> Genomic	No Amplification	No Amplification	No Amplification
KSUM148	<i>T. tauschii</i> Genomic	Polymorphic	Polymorphic	Monomorphic
KSUM149	<i>T. tauschii</i> Genomic	No Amplification	No Amplification	No Amplification
G3&A/B19	Wheat Genomic	Polymorphic	Polymorphic	Monomorphic
G3&G9	Wheat Genomic	Polymorphic	Polymorphic	Polymorphic
WG114	Wheat Genomic	No Amplification	No Amplification	No Amplification
WG178	Wheat Genomic	Polymorphic	Polymorphic	Monomorphic
WG178(LP)	Wheat Genomic	Polymorphic	Monomorphic	Polymorphic
WG181	Wheat Genomic	Monomorphic	Monomorphic	Monomorphic
WG232	Wheat Genomic	Polymorphic	Polymorphic	Polymorphic
WG241	Wheat Genomic	Monomorphic	Polymorphic	Polymorphic
WG464	Wheat Genomic	Monomorphic	Monomorphic	Monomorphic
WG530	Wheat Genomic	Polymorphic	Polymorphic	Polymorphic
WG541	Wheat Genomic	Polymorphic	Polymorphic	Polymorphic
WG564	Wheat Genomic	Polymorphic	Polymorphic	Polymorphic
WG564.2	Wheat Genomic	Polymorphic	Polymorphic	Monomorphic
WG662	Wheat Genomic	Monomorphic	Monomorphic	Monomorphic
WG669	Wheat Genomic	Polymorphic	Polymorphic	Polymorphic
WG686	Wheat Genomic	Monomorphic	Monomorphic	Monomorphic
WG719	Wheat Genomic	Monomorphic	Monomorphic	Monomorphic
WG940	Wheat Genomic	No Amplification	No Amplification	No Amplification
WG983	Wheat Genomic	Polymorphic	Polymorphic	Monomorphic
WG996	Wheat Genomic	Polymorphic	Monomorphic	Polymorphic
WG1026	Wheat Genomic	Polymorphic	Polymorphic	Polymorphic

Table 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.

Primer Set	Source	Wheat Location	Barley Location
AC30	Barley		
PST337	Barley		5H
S2&4	Barley		
ST7&8	Barley		2H,7H
TB1&2	Barley		1H,4H,5H
TB10&11	Barley		
TB17&18	Barley		
TB33&34	Barley		2H
TB35&36	Barley		
ABC151	Barley cDNA	2A,2D,3B,6A	3H,7H
ABC153	Barley cDNA		2H
ABC158	Barley cDNA	1D,3D,6D	7H
ABC161	Barley cDNA		3H
ABC164	Barley cDNA		1H,4H,6H
ABC252	Barley cDNA		2H,4H
ABC253	Barley cDNA	1A,2A,3B,4B,4D,5A	7H
ABC261	Barley cDNA		2H,3H,4H
ABC305	Barley cDNA	1B,3A,5B,6A,6B,6D,7B	6H,7H
ABC306	Barley cDNA		2H
ABC309	Barley cDNA	3D,6A	5H
ABC454	Barley cDNA	2B	2H
ABC455	Barley cDNA	5B,7B	6H,7H
ABC601	Barley cDNA		
ABC602	Barley cDNA	3B,3D	
ABC718	Barley cDNA		5H
BCD175	Barley cDNA	2B,2D	2H
BCD402	Barley cDNA	1D,3A,3B,4B,6A,6B,7B	4H,6H
ABC465	Barley Genomic	1B,3A,3B,5B,7A,7D	7H
ABG003	Barley Genomic	1B,2B,3B,4B,5B,5D,6B,6D,7A	4H,5H
ABG004	Barley Genomic		2H,3H,4H
ABG055	Barley Genomic		1H,2H,7H
ABG064	Barley Genomic		6H
ABG065	Barley Genomic	6D	3H,6H
ABG075	Barley Genomic	2A,3A,4B,7B	3H,7H
ABG316	Barley Genomic		3H
ABG320	Barley Genomic	5A,6B	7H

Table 38. Continued.

Primer Set	Source	Wheat Location	Barley Location
ABG358	Barley Genomic	1A,5A	2H
ABG373	Barley Genomic		1H
ABG378	Barley Genomic	2B,2D,3B,3D	6H
ABG379	Barley Genomic		6H
ABG391	Barley Genomic	4A,5A,5B,5D	5H
ABG458	Barley Genomic	6A,6B,6D	6H
ABG459	Barley Genomic		2H,3H
ABG471	Barley Genomic	2A	3H,6H
ABG474	Barley Genomic		2H,6H
ABG494	Barley Genomic		1H
ABG498	Barley Genomic		4H
ABG500	Barley Genomic	1A,6A,6B	1H,4H
ABG601	Barley Genomic	5A,7A	4H
ABG618	Barley Genomic	1B,2A,4A,5B,7B,7D	4H
ABG704	Barley Genomic	4A,7A	7H
ABG711	Barley Genomic	3A,3D,4A,5B,6A,7B	4H,6H
ABG715	Barley Genomic	4A,4B	4H
ABG716	Barley Genomic		5H
BARG10	Barley Genomic	3B,4B,5D,6D	4H
MWG650	Barley Genomic	1B,2B,5D	5H
CDO395	Oat cDNA		3H
CDO588	Oat cDNA		2H
CDO749	Oat cDNA		1H,2H
KSUA5	<i>T. tauschii</i> Genomic	7D	
KSUD8	<i>T. tauschii</i> Genomic	2	
KSUD14	<i>T. tauschii</i> Genomic	1B,4B,5A	1H,2H,4H,5H,7H
KSUD15	<i>T. tauschii</i> Genomic	1D,2A,5B,5D,6B,7B,7D	
KSUD16	<i>T. tauschii</i> Genomic	1B,4A,4B,4D,5B,7A	2H
KSUD17	<i>T. tauschii</i> Genomic	2B,3A,3B,4B,4D,6D	6H
KSUD18	<i>T. tauschii</i> Genomic	2A	2H
KSUD23	<i>T. tauschii</i> Genomic	2	
KSUE6	<i>T. tauschii</i> Genomic	4	
KSUE11	<i>T. tauschii</i> Genomic	1D	1H
KSUG14	<i>T. tauschii</i> Genomic	1A,1B,4A,5B,5D,6B	
WG178	Wheat Genomic		3H
WG232	Wheat Genomic	4A,4D,6B,7A	4H,6H
WG530	Wheat Genomic	4A,6B	5H
WG996	Wheat Genomic	1A	2H,7H
WG1026	Wheat Genomic	5B	4H,5H

Table 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.

Primer Set	Source	Wheat Location	Barley Location
ST7&8 NEW	Barley		2H,7H
ST7&8 OLD	Barley		2H,7H
TB10&11	Barley		
ABC151	Barley cDNA	2A,2D,3B,6A	3H
ABC153	Barley cDNA		
ABC158	Barley cDNA	1D,3D,6D	
ABC161	Barley cDNA		
ABC164	Barley cDNA		4H
ABC252	Barley cDNA		2H,4H
ABC261	Barley cDNA		2H,3H,4H
ABC305	Barley cDNA	1B,3A,5B,6A,6B,6D,7B	6H
ABC309	Barley cDNA	3D,6A	
ABC455	Barley cDNA	5B,7B	6H
ABC465	Barley cDNA	1B,3A,3B,5B,7A,7D	7H
ABC601	Barley cDNA		
ABC602	Barley cDNA	3B,3D	
ABC718	Barley cDNA		5H
ABG003	Barley Genomic	1B,2B,3B,4B,5B,5D,6B,6D,7A	5H
ABG055	Barley Genomic		1H,2H,7H
ABG064	Barley Genomic		6H
ABG065	Barley Genomic	6D	6H
ABG075	Barley Genomic		3H
ABG316	Barley Genomic		
ABG358	Barley Genomic	1A,5A	2H
ABG373	Barley Genomic		
ABG378	Barley Genomic	2B,2D,3B,3D	6H
ABG379	Barley Genomic		6H
ABG391	Barley Genomic	4A,5A,5B,5D	5H
ABG458	Barley Genomic	6A,6B,6D	6H
ABG498	Barley Genomic		
ABG500	Barley Genomic	1A,6A,6B	1H
ABG618	Barley Genomic	1B,2A,5B,4A,7B,7D	4H
ABG704	Barley Genomic	4A,7A	7H
ABG711	Barley Genomic	3A,3D,4A,5B,6A,7B	5H,7H
MWG650	Barley Genomic	1B,2B,5D	
CDO395	Oat cDNA		
CDO749	Oat cDNA		

Table 39. Continued.

Primer Set	Source	Wheat Location	Barley Location
KSUA5	<i>T. tauschii</i> Genomic	7D	
KSUD8	<i>T. tauschii</i> Genomic	2	
KSUD14	<i>T. tauschii</i> Genomic	1B,4B,5A	1H,2H,4H,5H
KSUD15	<i>T. tauschii</i> Genomic	1D,2A,5B,5D,6B,7B,7D	
KSUD16	<i>T. tauschii</i> Genomic	1B,4A,4B,4D,5B,7A	2H
KSUD17	<i>T. tauschii</i> Genomic	2B,3A,3B,4B,4D,6D	
KSUD18	<i>T. tauschii</i> Genomic	2A	
KSUD23	<i>T. tauschii</i> Genomic	2	
KSUE6	<i>T. tauschii</i> Genomic	4	
KSUE11	<i>T. tauschii</i> Genomic	1D	
KSUG14	<i>T. tauschii</i> Genomic	1A,1B,4A,5B,5D,6B	
WG178	Wheat Genomic	3	
WG232	Wheat Genomic	4A,4D,6B,7A	6H
WG996	Wheat Genomic	1A	7H
WG1026	Wheat Genomic	5B	

Table 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.

Primer Set	Source	Wheat Location	Barley Location
BTA2	Barley		
KV5&6	Barley		
KV9&10	Barley		1H
KV29&30	Barley		
PST337	Barley		5H
S1&2	Barley		
S2&4	Barley		
ST7&8	Barley		2H,7H
TB4&5	Barley		
TB15&16	Barley		
TB17&18	Barley		
TB33&34	Barley		2H
TB36&37	Barley		
ABC156	Barley cDNA	1A,2B	2H,3H,7H
ABC454	Barley cDNA	2B	2H
ABC465	Barley cDNA	1B,3A,3B,5B,7A,7D	7H
BCD402	Barley cDNA	1D,3A,3B,4B,6A,6B,7B	4H,6H
BCD828	Barley cDNA	3	3H,5H
ABG358	Barley Genomic	1A,5A	2H
ABG466	Barley Genomic	3A,4B,6B	4H,5H,6H
ABG471	Barley Genomic	2A	3H,6H
CDO36	Oat cDNA	2D,7A	7H
KSUA1	<i>T. tauschii</i> Genomic	7D	5H,7H
KSUA5	<i>T. tauschii</i> Genomic	7D	
KSUD9	<i>T. tauschii</i> Genomic	1B,2A,6A,6D,7B	
KSUD14	<i>T. tauschii</i> Genomic	1B,4B,5A	1H,2H,4H,5H,7H
KSUD16	<i>T. tauschii</i> Genomic	1B,4A,4B,4D,5B,7A	2H
KSUD18	<i>T. tauschii</i> Genomic	2A	2H
KSUD22	<i>T. tauschii</i> Genomic	2B,4A,7A	2H
KSUE6	<i>T. tauschii</i> Genomic	4	
KSUF48	<i>T. tauschii</i> Genomic	1A,3B,6B,6D	
KSUG8	<i>T. tauschii</i> Genomic	6	
KSUG14	<i>T. tauschii</i> Genomic	1A,1B,4A,5B,5D,6B	
KSUG48	<i>T. tauschii</i> Genomic	5B,6D,7B,7D	
KSUG49	<i>T. tauschii</i> Genomic	4A,7B,7D	
KSUH9	<i>T. tauschii</i> Genomic	1D,2D,4B,4D,5B,6B	
KSUH15	<i>T. tauschii</i> Genomic	3	
KSUM148	<i>T. tauschii</i> Genomic	1B,2A,4B,4D,6B	
WG232	Wheat Genomic	4A,4D,6B,7A	4H,6H
WG669	Wheat Genomic	3A,3B,5B,5D	1H,7H
WG1026	Wheat Genomic	5B	4H,5H

Table 41. Analysis of variance for heading date in the Pondera single cross population.

Source	DF	SS	MS	F-Value	P-Value
Environments	3	14604.0	4867.8	2116.4	0.00
Rep. (Env.)	12	27.6	2.3		
Lines	14	1331.1	95.1	256.0	0.00
Env. X Lines	42	75.3	1.8	4.8	0.00
Error	168	62.4	0.4		
Total	239	16100.0			

Table 42. Analysis of variance for plant height in the Pondera single cross population.

Source	DF	SS	MS	F-Value	P-Value
Environments	3	2264.1	754.7	74.7	0.00
Rep. (Env.)	12	121.3	10.1		
Lines	14	11651.0	832.2	97.9	0.00
Env. X Lines	42	1538.6	36.6	4.3	0.00
Error	168	1428.2	8.5		
Total	239	17003.0			

Table 43. Analysis of variance for grain yield in the Pondera single cross population.

Source	DF	SS	MS	F-Value	P-Value
Environments	3	84.2	28.1	58.0	0.00
Rep. (Env.)	12	5.8	0.5		
Lines	14	78.0	5.6	43.3	0.00
Env. X Lines	42	25.1	0.6	4.6	0.00
Error	168	21.6	0.1		
Total	239	214.8			

Table 44. Analysis of variance for test weight in the Pondera single cross population.

Source	DF	SS	MS	F-Value	P-Value
Environments	3	75982.0	25327.0	149.6	0.00
Rep. (Env.)	12	2032.2	169.4		
Lines	14	19873.0	1419.5	21.2	0.00
Env. X Lines	42	13811.0	328.8	4.9	0.00
Error	168	11265.0	67.0		
Total	239	122960.0			

Table 45. Analysis of variance for lodging index in the Pondera single cross population.

Source	DF	SS	MS	F-Value	P-Value
Environments	3	193200.0	64400.0	736.4	0.00
Rep. (Env.)	12	1049.4	87.463.2		
Lines	14	87005.0	6214.6	63.9	0.00
Env. X Lines	42	57559.0	1370.4	13.9	0.00
Error	168	16526.0	98.4		
Total	239	355340.0			

Table 46. Analysis of variance for percent grain protein in the Pondera single cross population.

Source	DF	SS	MS	F-Value	P-Value
Environments	3	65.7	21.9	55.9	0.00
Rep. (Env.)	12	4.7	0.39		
Lines	14	122.8	8.8	95.8	0.00
Env. X Lines	42	23.0	0.54	6.0	0.00
Error	168	15.4	0.09		
Total	239	231.6			

Table 47. Analysis of variance for NIR kernel hardness in the Pondera single cross population.

Source	DF	SS	MS	F-Value	P-Value
Environments	3	755.2	251.7	5.1	0.02
Rep. (Env.)	12	587.2	48.9		
Lines	14	90815.0	6486.8	415.9	0.00
Env. X Lines	42	1528.3	36.4	2.3	0.00
Error	168	2620.3	15.6		
Total	239	96306.0			

Table 48. Analysis of variance for SDS sedimentation protein quality in the Pondera single cross population.

Source	DF	SS	MS	F-Value	P-Value
Environments	3	3633.9	1211.3	23.4	0.00
Rep. (Env.)	12	621.7	51.8		
Lines	14	9997.6	714.1	27.8	0.00
Env. X Lines	42	3060.7	72.9	2.8	0.00
Error	168	4321.8	25.7		
Total	239	21636.0			

Table 49. Analysis of variance for heading date in the Pondera backcross population.

Source	DF	SS	MS	F-Value	P-Value
Environments	3	21579.0	7192.9	2397.6	0.00
Rep. (Env.)	12	36.4	3.0		
Lines	23	1239.0	53.9	124.1	0.00
Env. X Lines	69	88.4	1.3	3.0	0.00
Error	276	119.8	0.43		
Total	383	23062.0			

Table 50. Analysis of variance for plant height in the Pondera backcross population.

Source	DF	SS	MS	F-Value	P-Value
Environments	3	8270.0	2756.7	202.7	0.00
Rep. (Env.)	12	163.2	13.6		
Lines	23	42747	1858.6	322.1	0.00
Env. X Lines	69	1075.0	15.6	2.7	0.00
Error	276	1592.8	5.8		
Total	383	53848.0			

Table 51. Analysis of variance for grain yield in the Pondera backcross population.

Source	DF	SS	MS	F-Value	P-Value
Environments	3	181.3	60.4	137.4	0.00
Rep. (Env.)	12	5.3	0.44		
Lines	23	176.3	7.7	65.3	0.00
Env. X Lines	69	20.0	0.29	2.5	0.00
Error	276	32.4	0.12		
Total	383	415.2			

Table 52. Analysis of variance for test weight in the Pondera backcross population.

Source	DF	SS	MS	F-Value	P-Value
Environments	3	51963.0	17321.0	121.1	0.00
Rep. (Env.)	12	1716.9	143.1		
Lines	23	11039.0	480.0	15.1	0.00
Env. X Lines	69	15730.0	228.0	7.2	0.00
Error	276	8772.9	31.8		
Total	383	89221.0			

Table 53. Analysis of variance for lodging index in the Pondera backcross population.

Source	DF	SS	MS	F-Value	P-Value
Environments	3	48052.0	16017.0	80.6	0.00
Rep. (Env.)	12	2385.1	198.8		
Lines	23	37130.0	1614.3	15.9	0.00
Env. X Lines	69	51616.0	748.0	7.4	0.00
Error	276	27959.0	101.3		
Total	383	167140.0			

Table 54. Analysis of variance for percent grain protein in the Pondera backcross population.

Source	DF	SS	MS	F-Value	P-Value
Environments	3	152.8	50.9	60.6	0.00
Rep. (Env.)	12	10.1	0.84		
Lines	23	291.7	12.7	190.6	0.00
Env. X Lines	69	23.3	0.34	5.1	0.00
Error	276	18.4	0.07		
Total	383	496.2			

Table 55. Analysis of variance for NIR kernel hardness in the Pondera backcross population.

Source	DF	SS	MS	F-Value	P-Value
Environments	3	703.3	234.4	1.8	0.19
Rep. (Env.)	12	1535.1	127.9		
Lines	23	54493.0	2369.2	177.7	0.00
Env. X Lines	69	2332.8	33.8	2.54	0.00
Error	276	3679.1	13.3		
Total	383	62743.0			

Table 56. Analysis of variance for SDS sedimentation protein quality in the Pondera backcross population.

Source	DF	SS	MS	F-Value	P-Value
Environments	3	2685.4	895.2	12.6	0.00
Rep. (Env.)	12	853.2	71.1		
Lines	23	30927.0	1344.6	61.3	0.00
Env. X Lines	69	6899.1	100.0	4.6	0.00
Error	276	6053.5	21.9		
Total	383	47418.0			

Table 57. Analysis of variance for heading date in the Newana backcross population.

Source	DF	SS	MS	F-Value	P-Value
Environments	3	22359.0	7453.1	1414.0	0.00
Rep. (Env.)	12	63.2	5.3		
Lines	21	2512.1	119.6	204.0	0.00
Env. X Lines	63	85.6	1.4	2.3	0.00
Error	252	147.8	0.6		
Total	351	25168.0			

Table 58. Analysis of variance for plant height in the Newana backcross population.

Source	DF	SS	MS	F-Value	P-Value
Environments	3	6225.4	2075.1	107.9	0.00
Rep. (Env.)	12	230.7	19.2		
Lines	21	45862.0	2183.9	340.7	0.00
Env. X Lines	63	1037.8	16.5	2.6	0.00
Error	252	1615.3	6.4		
Total	351	54971.0			

Table 59. Analysis of variance for grain yield in the Newana backcross population.

Source	DF	SS	MS	F-Value	P-Value
Environments	3	407.7	135.9	492.6	0.00
Rep. (Env.)	12	3.3	0.38		
Lines	21	123.7	5.9	58.4	0.00
Env. X Lines	63	45.6	0.72	7.2	0.00
Error	252	25.4	0.10		
Total	351	605.8			

Table 60. Analysis of variance for test weight in the Newana backcross population.

Source	DF	SS	MS	F-Value	P-Value
Environments	3	110350.0	36782.0	82.8	0.00
Rep. (Env.)	12	5331.5	444.3		
Lines	21	84642.0	4030.6	51.5	0.00
Env. X Lines	63	14283.0	226.7	2.9	0.00
Error	252	19708.0	78.2		
Total	351	234310.0			

Table 61. Analysis of variance for lodging index in the Newana backcross population.

Source	DF	SS	MS	F-Value	P-Value
Environments	3	34942.0	11647.0	135.2	0.00
Rep. (Env.)	12	1033.5	86.1		
Lines	21	70931.0	3377.7	34.4	0.00
Env. X Lines	63	58214.0	924.0	9.4	0.00
Error	252	24709.0	98.0		
Total	351	189830.0			

Table 62. Analysis of variance for percent grain protein in the Newana backcross population.

Source	DF	SS	MS	F-Value	P-Value
Environments	3	201.6	67.2	103.2	0.00
Rep. (Env.)	12	7.8	0.65		
Lines	21	165.4	7.9	117.4	0.00
Env. X Lines	63	14.4	0.23	3.4	0.00
Error	252	16.9	0.07		
Total	351	406.1			

Table 63. Analysis of variance for NIR kernel hardness in the Newana backcross population.

Source	DF	SS	MS	F-Value	P-Value
Environments	3	1641.7	547.2	14.3	0.00
Rep. (Env.)	12	458.2	38.2		
Lines	21	134360.0	6398.3	379.5	0.00
Env. X Lines	63	2354.6	37.4	2.2	0.00
Error	252	4248.7	16.9		
Total	351	143070.0			

Table 64. Analysis of variance for SDS sedimentation protein quality in the Newana backcross population.

Source	DF	SS	MS	F-Value	P-Value
Environments	3	22830.0	7609.9	70.4	0.00
Rep. (Env.)	12	1297.9	108.2		
Lines	21	47981.0	2284.8	79.2	0.00
Env. X Lines	63	6624.1	105.1	3.6	0.00
Error	252	7269.4	28.8		
Total	351	86002.0			

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