Genetic analysis of agronomic and malting quality QTLS in barley backcross breeding populations
by Steven Richard Larson

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

Abstract:
Agronomic and cereal quality quantitative trait loci (QTLs) have been identified in barley and other crops using genetic markers (RFLPs). The Steptoe x Morex doubled haploid mapping population, produced by the North American Barley Genome Mapping Project (NABGMP), exemplifies these efforts. These elite varieties have been grown in different production regions of the U.S. for three decades and have distinct utilization characteristics. Several potentially useful QTLs were identified. However, few studies. have explored the reliability or breeding value of the comprehensive information gleaned from these large mapping projects. Using plant genetic materials provided by the NABGMP, efficient genetic tools (STS-PCR) were developed for genetic testing of these potentially complex QTLs. Although the final goal was to achieve practically-oriented plant breeding objectives, the focus of these experiments was to test main QTL effects and examine other possible forms of gene action including epistasis, pleiotropy, and environmental interaction.

Fifty lines were derived from a backcross of Morex and DH72 using the doubled haploid line as donor parent of the Steptoe chromosome 3. The NABGMP experiments suggested that this donor chromosome carries two yield QTLs that lack pleibtropic effects on malt quality. The fifty BC1 lines and parental checks were evaluated in five environments in Montana. Agronomic and grain quality traits were analyzed in all five experiments, and nine malting traits were analyzed using micromalted samples from one experiment. Headshattering and plant lodging QTL(s) reproduced, one of the yield QTLs, first reported by NABGMP, with major effects on yield under certain environmental conditions. The expected backcross gains for malt quality were realized.

A gene with strong effects on barley seed germination was previously identified on chromosome 7. However, the expression of this gene was evidently dependent on genetic background effects (epistasis). The effect of this seed dormancy QTL was investigated using the aPSR128 STS-PCR marker in two Steptoe and three Morex backcross populations. The aPSR128 QTL effects were similar regardless of recurrent parent and therefore lacked epistatic control. Unlike the reciprocal effects of minor QTLs, the aPSR128 QTL showed strong effects on seed germination that persisted during after-ripening.

GENETIC ANALYSIS OF AGRONOMIC AND MALTING QUALITY QTLs IN BARLEY BACKCROSS BREEDING POPULATIONS

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ABSTRACT

Agronomic and cereal quality quantitative trait loci (QTLs) have been identified in barley and other crops using genetic markers (RFLPs). The Steptoe x Morex doubled haploid mapping population, produced by the North American Barley Genome Mapping Project (NABGMP), exemplifies these efforts. These elite varieties have been grown in different production regions of the U.S. for three decades and have distinct utilization characteristics. Several potentially useful QTLs were identified. However, few studies have explored the reliability or breeding value of the comprehensive information gleaned from these large mapping projects. Using plant genetic materials provided by the NABGMP, efficient genetic tools (STS-PCR) were developed for genetic testing of these potentially complex QTLs. Although the final goal was to achieve practically-oriented plant breeding objectives, the focus of these experiments was to test main QTL effects and examine other possible forms of gene action including epistasis, pleiotropy, and environmental interaction.

Fifty lines were derived from a backcross of Morex and DH72 using the doubled haploid line as donor parent of the Steptoe chromosome 3. The NABGMP experiments suggested that this donor chromosome carries two yield QTLs that lack pleiotropic effects on malt quality. The fifty BC_1 lines and parental checks were evaluated in five environments in Montana. Agronomic and grain quality traits were analyzed in all five experiments, and nine malting traits were analyzed using micromalted samples from one experiment. Headshattering and plant lodging QTL(s) reproduced one of the yield QTLs, first reported by NABGMP, with major effects on yield under certain environmental conditions. The expected backcross gains for malt quality were realized.

A gene with strong effects on barley seed germination was previously identified on chromosome 7. However, the expression of this gene was evidently dependent on genetic background effects (epistasis). The effect of this seed dormancy QTL was investigated using the aPSR128 STS-PCR marker in two Steptoe and three Morex backcross populations. The aPSR128 QTL effects were similar regardless of recurrent parent and therefore lacked epistatic control. Unlike the reciprocal effects of minor QTLs, the aPSR128 QTL showed strong effects on seed germination that persisted during after-ripening.

CHAPTER 1

INTRODUCTION

Importance of Barley

Barley (*Hordeum vulgare* L.) is the world's fourth most important cereal crop after wheat, maize, and rice. Barley and wheat have broad ecological adaptations that set them apart from other cereals, such as corn and rice. In climatic areas where both wheat and barley are produced, production is generally determined based on end use. Wheat is utilized for human consumption, whereas barley is primarily used for feed or malt (Poehlman, 1985). Feed and malt barley production is also very important in Montana, second only to North Dakota, in its contribution to U.S. barley production. Montana farmers planted 1.3 million acres of barley in 1995, of which malting varieties accounted for 36 percent of this acreage (Montana Agriculture Statistics, 1995). Barley accounted for 6.3 percent of the total cash marketing receipts from crop and livestock commodities sold in Montana. Most of the malt barley produced in Montana is sold to out-of-state maltsters. However, most of the barley (about 64 percent in 1995) produced in Montana is utilized as a feed supplement, which in turn contributes significantly to the livestock industry which is vital to Montana.

Importance of Plant Breeding

The genetic improvement of crop plants including barley through plant breeding has made a major contribution to the production of food and fiber. Depending on breeding objectives, new cultivars may have higher yields per unit area, better utilization characteristics, expanded areas of adaptation, and reduced losses to environmental stress and other pests (e.g. competitive weeds, disease, and insects). Meeting these objectives is essential to meeting the needs of an increasing human population, and to improve the standard of living in developing countries. Future plant breeding will contribute significantly to local and national economies, and the overall welfare of humankind. Plant breeding will continue to increase food and fiber productivity, and perhaps improve the availability of natural resources such as forest products and fuel (derived from fermented grains). However, it is becoming increasingly clear that many of the expected plant breeding improvements will be the result of carefully planned breeding programs which utilize scientific advances in many fields.

Domestication of Barley

Barley domestication is an interesting story and has relevance to this dissertation in several ways. The wild ancestor of cultivated barley is well known (Harlan and Zohary, 1966). Cultivated barley shows close affinities to a group of wild and weedy barley genotypes which are traditionally grouped in *Hordeum spontaneum* (C. Koch).. However *H. spontaneum* may be classified as *Hordeum vulgare* (L.) subsp. *spontaneum* (C. Koch), a wild race or subspecies of cultivated barley (Zohary and Hopf, 1988). *Spontaneum* is the only wild *Hordeum* species that is cross-compatible and fully interfertile with cultivated barley. These wild barleys are annual, brittle, two-rowed, diploid (2n=14), predominantly self-pollinated barley forms that show seed dormancy characteristics typical of annuals growing in Mediterranean-type or desert climates (Zohary and Hopf, 1988).

Barley was found in several pre-agricultural sites in the Near East (8000-7000 BC). Remains have been found including brittle, two-rowed barley forms that are morphologically indistinguishable from present-day *spontaneum*. The earliest record of what seems to be a wild barley harvest comes from 9000 BC Tell Abu Hureyra in Syria (Hillman, 1975). By 7000 BC brittle *spontaneum* type barley was found in contexts already showing definite signs of cultivation (Zohary and Hopf, 1988). Unmistakable remains of non-brittle barley, i.e. forms that could survive only under cultivation, appear around the 8th and 7th millennium BC.

The morphological similarity between *spontaneum* and two-rowed cultivated barley is striking. They differ mainly in their modes of seed dispersal. *Apontaneum* ears are brittle and at maturity disarticulate into individual arrow-like triplets which are highly specialized structures that ensure plant survival in the wild. During domestication, this specialization broke down because non-brittle mutants were automatically selected for in the man-made system of reaping, threshing and sowing (Zohary and Hopf, 1988). Presumably, selection of the non-brittle mutants dramatically improved the yields of cultivated barley.

The distribution of the brittle rachis genes in some of these barley progenitors have been described (Takahashi, 1955). Several of these headshattering genes including *Bt1*, *Bt2* and possibly *Bt3* have been assigned to chromosome 3 (Sogaard and von Wettstein-Knowles, 1987 and Nilan, 1964).

A second major outcome of domesticating barley is the breakdown of the wild mode of seed germination (Zohary and Hopf, 1988). Most wild grasses, including virtually all of the wild *Hordeum* species (Simpson, 1990), depend on regulation of seed germination for survival through periods of drought. This is especially true for annuals growing in Mediterranean or desert climates, where germination is delayed to avoid periods of drought (Zohary and Hopf, 1988). Seed dormancy adaptations may take several forms. Short term dormancy, over a period of several months, may be used to avoid seasonal periods of cold and/or drought. This short term dormancy probably inhibits germination in a relatively large portion of the seed population. A long term form of dormancy, over a period of one or more years, may help plants survive through

catastrophically bad years of drought. This latter form of dormancy probably occurs in a smaller fraction of the seed population. In these ways, seed dormancy can be adaptive so that germination and plant development correspond with favorable environmental conditions.

A genetic mechanism must exist that can effectively delay germination in a fraction of the seed population. Under cultivation, this wild type regulation of germination is no longer advantageous. Consequently, seed dormancy mutants have been selected automatically during the domestication of barley. Owing perhaps to the value of barley to the malting and brewing industry, seed germination has been studied more extensively in barley than the other major cereal grains. It also follows that seed germination has been a tightly controlled trait in malt barley breeding programs, although few studies make a point of this. Surprisingly however, very few efforts have been made to genetically map seed dormancy genes in any of the cereal, forage, wild, or weedy grass species.

Historical Advances in Barley Breeding

Modern efforts to improve barley by breeding, rather than selection, are seldom mentioned in the literature. 'Horsford,' released about 1880, is the first barley cultivar known to result from planned hybridization in North America (Foster, 1987). Plant breeding efforts have gradually expanded, from the turn of the 20th century, to include large sophisticated cooperative programs (Anderson and Reinbergs, 1985). The predominant type of barley grown in North America is six-rowed, and breeding programs have been in place much longer for six-rowed than for two-rowed barley. It wasn't until about 1940 that introduced two-rowed cultivars began to increase acreage in intermountain areas of Northwestern United States (Foster, 1987) including Montana. Traditionally, specific types of barley have been grown in different regions of North America.

In both the USA and Canada, improvements in six-rowed cultivars have involved many different traits (Schwarz and Horsley, 1995). In the Midwest United States, major improvements in malting quality were achieved with the release of Morex (Rasmusson and Wilcoxson, 1979). Derivations of the Manchuria-Oderbrucker type varieties originally introduced from Germany, such as Morex, continue to be grown in these regions because they have excellent malting quality. These varietal types have six-rows, rough awns, nodding heads, and moderate size kernels.

In California, varieties of the Coast type, originally brought from Africa by the

early Spanish settlers, have predominated. These Coast type varieties, such as Steptoe (Muir and Nilan, 1973), have six-rows, rough awns, and large seeds. Steptoe has exceptional yield potential and is grown as a feed variety, the primary utilization of barley in California.

In Montana, Idaho, Washington, Colorado, and adjacent regions, two-rowed varieties first introduced from western Europe have been grown for feed and malting. Feed utilization of barley is relatively more important in these regions compared to the Midwest, where corn is a more practical feed supplement. However, two-rowed malting cultivars such as Klages (Wesenberg et al. 1974) have replaced introductions from other countries.

The two most commonly grown varieties in Montana comprised over 38 percent of the barley acreage in 1995 (Montana Agriculture Statistics, 1995). The most important widely grown of these is Harrington (Harvey and Rossnagel, 1984), a two-rowed variety recommended by the American Malt Barley Association (AMBA). A recent release from the Montana Agriculture experiment station, Chinook (Blake et al. 1996), has been approved by AMBA and has the potential to replace Harrington acreage. Following Harrington in acreage is Hector (Wells, 1973), a two-rowed feed barley that has been in production many years. Baronesse, a two-rowed feed barley recently introduced from Europe, is rapidly increasing acreage and evidently replacing Hector and Harrington. Less than 5% of Montana barley acreage is six-rowed malt barley.

Advances in the understanding and measurement of the nutritional value of barley utilized as feed will probably contribute significantly to future barley improvements.

Likewise, developing locally adapted varieties with malting characteristics adjusted for export markets is currently of keen interest to barley breeders. Approaches to testing experimental lines for traits related to cereal and forage chemistry are time consuming and expensive. In my opinion, plant breeders can benefit greatly from advances in genetic analysis and genetic testing for these cryptic traits.

Advances in the Genetic Analysis of Complex Traits

Agronomic and cereal quality traits are often affected by one or more genes, environmental conditions, and stochastic error. Likewise, seed dormancy can be dramatically affected by conditions such as temperature, moisture and light (Simpson, 1990). Moreover, seed dormancy shows variable expression in genetically identical seeds. Consequently these complex traits, most relevant to plant improvement, have been least amenable to genetic analysis at the molecular level. Perhaps this explains why so few seed dormancy genes have been mapped in grasses. The advent of molecular genetic markers has greatly facilitated the identification of the genes controlling these quantitative traits (Botstein, 1980), referred to as quantitative trait loci (QTLs). Other advances, such as the ability to produce doubled haploid lines, significantly improve the efficiency of mapping genes with additive effects.

Low copy number RFLP markers, detected using Southern analysis, are excellent tools for generating robust linkage maps (Botstein, 1980) and the study of heterogeneous genetic traits (Lander and Botstein, 1986). Their general applicability, transferability across crosses and among related species, and their efficiency in map construction have contributed to their well-earned status as markers of choice when one wishes to construct a high-quality, medium-density linkage map. This has been illustrated in virtually all the cereal crops including barley (Graner et al., 1991, Heun et al. 1991, and Kleinhofs et al. 1993), rice (McCouch et al., 1988), and corn (Helentjaris et al. 1986). Construction of

these maps has been more difficult in the polyploid crops such as cultivated oat (O'Donoughue et al. 1995). Linkage maps in wheat, which is also hexaploid, have been developed for each chromosome on a group by group basis. For example, wheat linkage maps are available for homoeologous chromosome groups 3 (Devos et al. 1992) and 7 (Chao et al. 1989).

To facilitate genetic mapping of complex traits in barley, the North American Barley Genome Mapping Project (NABGMP) developed doubled haploid mapping populations including one derived from a F_1 cross of Steptoe and Morex varieties (Kleinhofs et al. 1993). These doubled haploid mapping populations have enormous advantages for the detection of quantitative trait loci (QTLs) controlling phenotype. Moreover, analysis of these populations presumably reflects the expected nature of gene action in inbred barley varieties, the type used in commercial production. In a relatively short period of time, NABGMP was able to conduct the first comprehensive genetic analysis for barley traits of agronomic, biological and industrial interest. In part, this is because the data and findings collected from these genetically fixed lines are transferrable across breeding projects and laboratories. As previously discussed, Steptoe and Morex are commercial barley varieties that have been grown in different production regions of the U.S. for three decades. These varieties show widely different adaptation and utilization characteristics. Therefore, the first NABGMP experiment has presented some interesting insights into the genetic differences between these remarkably different and useful varieties.

Application of Genetic Analysis for Barley Improvement

One of the NABGMP goals in constructing the Steptoe x Morex doubled haploid population was to help develop improved cultivars. At least three cultivars, Mingo and Rodeum in Canada and Gwylan in Wales have been developed using the doubled-haploid technique (Foster, 1987). After the initial hybridization in 1974, Ho and Jones (1980) released Mingo only five years later, illustrating the potential benefit of obtaining homozygous lines without repeated selfing. However, as I have discussed, any modern feed or barley breeding program will have highly specific objectives. Most breeding populations are derived from narrow crosses of elite germplasm, to avoid the infusion of many less desirable genes. However, the choice of parents used in any mapping project commonly reflects the efficient use of germplasm that is expected to show the maximum number of detectable genetic differences. From the standpoint of six-rowed malt barley breeding, Steptoe is an exotic, non-elite variety. Consequently, Steptoe would be expected to contribute many undesirable genes affecting malting characteristics. Conversely, the adaptations of the Manchurian-Oderbrucker type varieties are appropriate for the relatively humid Upper Midwest. These adaptations are not useful in the arid, sun-baked West, where bountiful yields of feed grain has been the saving grace of Steptoe. Since Steptoe and Morex have such different adaptation and utilization characteristics, it is not surprising that doubled haploid commercial varieties have not been selected directly from this hybridization. Further breeding work would be required before cultivar

improvements can be achieved based on the NABGMP Steptoe x Morex experiment.

Several of the *a priori* expectations regarding the contributions of agronomic genes from Steptoe and Morex were in fact confirmed by Hayes et al. (1993). Morex contributed favorable QTL alleles for 28 out of 31 genes identified for four malt quality traits. Steptoe contributed high alleles for 5 out of 6 yield QTLs detected, with the two largest yield QTLs reported on chromosome 3. Most fortuitously, Hayes et al. (1993) failed to detect negative pleiotropic effects on malt quality associated with the chromosome 3 yield QTL regions. Based on these new findings it was hypothesized that the agronomic value of the Steptoe chromosome 3 yield QTLs could be combined with the malt quality characteristics of Morex to produce potentially valuable genotypes (Hayes et al. 1993). Few studies have explored the reliability and nature of findings rendered from these enormous data sets combined from diverse growing environments. A number of factors such as QTL x environment or epistatic interactions could add unforeseen complexities to breeding for these putative QTLs. Indeed, Hayes et al. (1993) detected significant QTL x environment interactions for the two largest yield QTL on chromosome 3. Although the largest yield QTL effect could be explained by lodging, other locations also showed yield effects and no lodging, and some locations lacked strong yield effects altogether.

Ullrich et al. (1992) utilized the Steptoe x Morex doubled haploid population to map four QTLs affecting seed dormancy in barley, one of the few studies in the literature to do so for any of the grasses. Steptoe is unusual in that this variety may express high levels of dormancy relatively uncommon among cereals, especially barley. Most

interestingly, they reported a gene on chromosome 7 that accounted for nearly 50 percent of the phenotypic variation for this trait. Oberthur et al. (1995) reported that epistasis among these four putative QTLs was an important genetic phenomenon regulating seed dormancy in this population. Epistasis, if common, could have broad ramifications for the manipulation of potentially valuable QTLs in different breeding populations.

This study will test for the continued effects of the putative Steptoe chromosome 3 yield QTLs and the Steptoe chromosome 7 dormancy gene effects in backcross breeding populations. Several aspects of gene action including epistasis, magnitude of effects, pleiotropy, and genotype x environmental interaction will be examined.

CHAPTER 2

EVALUATION OF BARLEY CHROMOSOME 3 YIELD QTLs IN BACKCROSS BREEDING POPULATIONS

Introduction

Southern analysis of RFLPs was used in the North American Barley Genome Mapping Project to identify the locations of numerous genes with significant effects on yield and malting characteristics (Kleinhofs et al., 1993 and Hayes et al., 1993). Thirty-one QTLs for grain quality traits including grain protein, alpha-amylase, diastatic power and percent malt extract were detected in the analysis of a doubled haploid population derived from a cross between Steptoe and Morex varieties (Hayes et al. 1993). Morex contributed positive alleles for 28 of the 31 QTLs detected for the value-added malt quality traits. These genetic effects on grain quality appeared to be stable across diverse growing conditions of Northwestern United States. Conversely, Steptoe showed positive alleles at five of six grain yield QTLs detected while Morex contributed one positive yield QTL. However, many of these yield QTLs were environmentally conditioned (i.e. the yield QTL effects varied across environments).

The two largest QTLs with positive effects on grain yield were ascribed to the

Steptoe chromosome 3. The larger of these two yield QTLs coincided closely with clearly resolved, single-interval peaks for lodging and plant height (Hayes et al., 1993). Hayes et al. (1993) postulated that plant lodging was a causal factor of the larger yield QTL on chromosome 3. However, this yield component did not explain the beneficial value of the Steptoe allele in the Montana dryland experiment where lodging was not observed. Neither lodging nor heading date factors could be attributed to the second largest yield QTL. Although Hayes et al. (1993) reported that the yield effects of the Steptoe chromosome 3 were consistently positive, significant QTL x environment interaction terms were reported for both QTLs on chromosome 3.

Minor negative effects for grain protein and alpha-amylase were associated with a region near the second largest yield QTL effect of the Steptoe allele. However, no other negative pleiotropic effects on value-added malting characters were apparently associated with the Steptoe chromosome 3 yield QTL. Therefore Hayes et al. (1993) postulated that the Steptoe chromosome 3 yield QTLs may have useful breeding value for developing higher yielding malting varieties. They suggested the use of an offensive breeding strategy such as marker assisted selection for the Steptoe chromosome 3 yield genes, and a defensive strategy such as backcross breeding to recover the Morex alleles at numerous other QTLs for grain quality traits.

Low copy number RFLP markers, detected using Southern analysis, are excellent tools for generating robust linkage maps. Their general applicability, transferability across crosses and among related species, and their efficiency in map construction have contributed to their well-earned status as markers of choice when one wishes to construct

a high-quality medium-density linkage map. With adequate amounts of DNA, several endonucleases, and an abundant supply of clones, sufficient polymorphic information content is generally available to construct complete RFLP linkage maps (Botstein et al. 1980) using Southern analysis (Southern, 1975). The efficiency of this technique is greatly enhanced when blots are repeatedly hybridized with different clones, each identifying one or more loci. Therefore, Southern analysis can be enormously efficient in gathering segregation data when a limited number of individuals are subjected to analysis at a large number of loci. However, this technique can be cumbersome when applied to practically-oriented plant breeding programs. Fast-paced marker-assisted selection in large populations is inefficient using Southern analysis, especially if blots are needed for only one RFLP marker.

RFLPs be detected either by Southern analysis or by following amplification by PCR (Mullis, 1986). The DNA sequences of clones mapped using Southern analysis of RFLPs can be used to design oligonucleotide primers for PCR based analysis (Saiki et al. 1985 and Mullis and Faloona, 1987). The conversion of these sequence tagged sites into polymerase chain reaction based markers (STS-PCR) has the potential to facilitate plant genome mapping experiments in a variety of ways (Tragoonrung et al. 1992 and Talbert et al. 1994). In order to make these markers more practical tools in crop improvement, techniques must be available to develop STS-PCR markers which are informative in the germplasm of interest. As useful tools, these STS-PCR markers must cosegregate with the RFLP locus from which they derive, and should also provide codominant and reliable assays.

The objective of this chapter is to demonstrate the development and use of six such STS-PCR markers. The methods used will test the hypothesis that RFLP clones which were previously mapped to barley chromosome 3 (Kleinhofs et al. 1993) can be efficiently converted to codominant PCR-based markers, allelic to the RFLP markers from which they were derived, and be used effectively for plant breeding. Following that, the second objective was to use these STS-PCR markers to evaluate genetic effects and agronomic components of the putative Steptoe chromosome 3 yield QTLs. This study will investigate and describe the magnitude of effects, genetic map resolution, and possible environmental interactions associated with the putative yield QTLs previously described for chromosome 3 (Hayes et al., 1993).

Materials and Methods

Clones and Genotypic Assay Procedure

The polymerase chain reaction (PCR) was conducted in 50 *u*l volumes of 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 0.1 mM each dNTP, 1.5 mM $MgCl_2$, 330 nM each primer, and 0.6 unit *Taq* polymerase. Genomic DNA samples were obtained using the protocol described by Edwards et al. (1991). For amplification, the GeneAmp PCR System 9600 (Perkin Elmer Cetus, Norwalk, CT) thermocycler was programed for 1 cycle of a 5 minute step at 94° C, then 33 cycles of three 30 second steps of 94°, 50° and 72° C, and finally one cycle of a 5 minute step at 72° C.

The clones used to design STS-PCR primers included four American Barley Genomic (ABG) clones (Table 1) described by Kleinhofs et al. (1993). Primers were also designed using wheat genomic (WG) and oat cDNA (CDO) clones (Table 1) described by Heun et al. (1991). These clones were end sequenced, near the vector insertion points, so that primer sequences (Table 1) could be selected using OLIGO software (National Biosciences, Plymouth MN). Oligonucleotide primers were synthesized using the Model 391 PCR-MATE DNA synthesizer (Applied Biosystems, Foster City, CA). By convention, the RFLP markers, such as ABG070, are distinguished from an "amplifiable" STS-PCR marker using a small case letter "a" in front of the marker locus name (e.g. aABG070).

For all PCR primer pairs shown in Table 1, except aABG070, Steptoe and Morex

amplification products were cloned and sequenced for polymorphism analysis. Barley amplification sequences, using the aCDO113 (oat) and aWG110 (wheat) primers, were also used for designing new barley STS-PCR primers specific to chromosome 3 loci (Table 1). The PCR products were cloned using the Invitrogen TA Cloning Kit (San Diego, CA) and sequenced using the Sequenase Kit (USB, Cleveland, OH) by the dideoxy chain termination method (Sanger et al. 1977). Sequence data from Steptoe and Morex STS-PCR products were analyzed using GENEPRO software (Riverside Scientific, Bainbridge WA) to help identify restriction site polymorphisms.

Polymorphisms among parents and progeny were tested for restriction site presence/absence or ribonuclease cleavage/protection of heteroduplex PCR molecules. Endonuclease digestion of STS-PCR products were typically conducted using several-fold excess of activity units and incubated overnight at temperatures recommended by manufacturers. The Mismatch Detect kit (Ambion, Austin TX) was used to screen for a sequence polymorphism using a ribonuclease protection assay (RPA) at the WG110.2 locus, using the outer and inner nested primer sets, including T7 and SP6 phage promoter sequences overhanging the 5' ends of the inner primer sets (Table 1).

Plant Materials

The 50 BC_1 families were derived from 50 BC_1F_2 plants by bulking seed within families through two and three generations of self pollination. This BC_1 population was constructed using a cross of DH72 (Steptoe chromosome 3 donor) and Morex (recurrent parent). DH72 is a doubled haploid lined derived from a F_1 cross of Steptoe (Muir and

Nilan 1973) and Morex (Rasmusson and Wilcoxson 1979) as described by Kleinhofs et al. (1993). The DH72 line was selected solely because the RFLP genotype, based on the NABGMP data set, indicated that it was fixed for the Steptoe chromosome 3. Otherwise, the RFLP data and recombination analysis suggest that DH72 is 50% Morex.

Experimental Design and Analysis

Irrigated and dryland field trials of BC_1F_4 families were conducted during 1994 at Bozeman. Irrigated and dryland Bozeman BC_1F_5 trials and a BC_1F_5 dryland experiment at Havre were conducted during 1995. Each randomized complete block (RCB) experiment had two replications where blocks contained 53 entries including the 50 BC_1 families plus 3 checks (Steptoe, Morex, and DH72). The Bozeman plots were 1.5 m^2 in 1994 and 3 m^2 in 1995 and the plots in the 1995 Havre experiment were 4.5 m^2. Straw was cut using hand sickles and grain was mechanically threshed (in 1994) or plots were harvested with small plot combines (in 1995).

Seed production from several of the BC_1F_3 families was insufficient to conduct the two replicated (r=2) experiments conducted in 1994. For example BC_1F_3 family #33 produced enough seed to fill one plot in the first rep of the 1994 Bozeman irrigated experiment. Otherwise the 1994 Bozeman irrigated experiment was completely balanced. The BC_1F_4 family #33 and four other lines (#05, #06, #08, and #32) were completely missing from the 1994 Bozeman dryland experiment, for lack of seed. In addition, three other lines (#09, #36, and #50) produced just enough seed, in 1993, to fill two reps in the 1994 Bozeman irrigated experiment and only one rep in the 1994 Bozeman dryland

experiment. These 14 plots were filled with 'Harrington' (Harvey and Rossnagel, 1984), a two-rowed malting variety commonly grown in Montana. The three 1995 experiments were completely balanced, each containing two reps of all 50 BC_1F_5 families. Therefore, the total degrees freedom, over five experiments, in Table 2 was (5 x 100) - 1 - 14 = 485.

Headshattering and lodging traits were measured using a rating scale of 0-9, where 0 was least severe and 9 was most severe. A rating of 9 would indicate that essentially no seed remained after headshatter or that all plants were completely lodged. Lodging was not observed in the 1994 Bozeman dryland trial; therefore, lodging measurements were not taken (Table 4) for that experiment. In 1994 experiments, headshattering was determined by striking three intact heads (per plot) several times, and rating grain loss. In 1995 experiments, headshattering was rated by observation of seed loss from plants and the ground litter within each plot. All headshattering and plant lodging ratings were observed several days before harvest. Average rachis internode lengths were calculated by measuring head length and dividing by the number of internodes on the ear. Analysis of variance for average rachis internode lengths was based on plot means of five plants (ears) per plot. Peduncle angle was visually estimated as the degrees below vertical (0 degrees is erect, whereas 90 degrees is horizontally lax).

MAPMAKER (Lander et al. 1987) was used to genetically map the aCDO113 and aABG070 STS-PCR markers as described below. The ANOVA procedure of MSUSTAT (Version 5.20, Richard E. Lund, Montana State University, Bozeman, MT, 59717) was used for all statistical analyses except Table 2B. PROC GLM (SAS) was used to determine the residual genetic variation nested within marker classes for Table 2B.

Results

STS-PCR

The molecular sizes of STS-PCR products, determined using sequencing or PAGE, were generally close to the expected results (within the margins of estimated insert sizes). However, several non-specific products were obtained from barley using the primers designed from the wheat WG110 clone (see Table 1). However, the aWG110.2 barley product is approximately the same size as the aWG110.2 product obtained using the WG110 clone as the PCR template. Interestingly, the aWG110.2 product from barley shows 83% sequence homology to the WG110 clone. The primary aCDO113 product, from barley, was larger than the 191 product obtained using the CDO113 clone as the PCR template (Table 1 and Figure 2A). However it is not possible to predict with absolute certainty the STS-PCR product size for a cDNA clone (such as CDO113), because introns may be contained within the respective genomic DNA regions.

Table 1. Chromosome 3 STS-PCR clones, primer sequences, predicted and observed products.

Clones	Insert sizes (bp)	Primer sequences (5'>3')	Predicted PCR sizes (bp)	Observed PCR sizes (bp)
ABG070	600*	ggaccaagcaaatatctcag aacacgagtttgaattttac	578*	430*
ABG057	1500*	ttataagcatagactgcggt gcacgagtgagctgagagtg	217	217
CDO113	1800*	ttcgaagctccttctctt catgggaaacagcatagc	191	186 400
CDO113.2 (barley)	186	cattagatcaaaatgctt aggataaggcccatcgta	143	143
ABG396	500*	gggtcacaaagacggaggag aggaaacctatgtaatcatc	388	388
ABG377	700*	gctgctatgaggagagaacc gtgttacagccacctatttc	541*	520*
WG110	900*	tctgatacacacctccagcg acggcgatccgtccacgagc	830	888 + others
WG110.2 (barley)	888	atacaacaggagccactaca cgaggacatcgctgagaaga	409	409
WG110.2 (barley)	409	gataatacgactcactataggg + aattttctacttctc** agatttaggtgacactatagga + ccgggcttgggttcc**	413	413

* estimated by comparison with known size standards using PAGE
** underlined sequences are phage promoter sequences (see text)

STS-PCR RFLP Markers

Restriction fragment length polymorphisms (RFLPs) for the STS-PCR products of the aABG070, aABG057, aABG396, and aABG377 loci are shown in Figure 1. The aABG070, aABG396 and aABG377 primers show specific products that are apparently identical in size for Steptoe and Morex. The aABG070 and aABG377 products from Steptoe and Morex show unmistakable RFLPs when digested with *Taq*I and *Ssp*I endonucleases, respectively. A STS-PCR RFLP, approximately 10 bp in length, was observed between two aABG396 *Mnl*I digestion products. However, both of these Steptoe and Morex RFLPs for aABG396 are less than 100 bp in length and may, in practice, appear as faint bands. Primers for aABG057 will reproducibly amplify a 217 bp product that contains a *Hph*I endonuclease recognition sequence in Steptoe which allows its cleavage into 105 and 112 bp fragments. Depending on reaction conditions, several larger products may be observed using these aABG057 primers. Otherwise, the four STS-PCR RFLPs shown in Figure1 are codominant. The RFLPs, predicted by sequence analysis, for aABG057 and aABG396 (Figure 1) and aCDO113 (Figure 2B) are the only confirmed restriction site polymorphisms for these Steptoe and Morex STS-PCR amplification products. In addition to the *Ssp*I polymorphism shown for aABG377 (Figure 1), a *Hae*III restriction site difference also distinguishes the aABG377 products from Steptoe and Morex. The aABG70 STS-PCR amplification products were not sequenced because a screen of 26 endonucleases was sufficient to reveal seven RFLPs for seven enzymes.