MOLECULAR MARKER ASSISTED ANALYSIS OF GRAIN YIELD AND RELATED TRAITS IN BARLEY

By

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To the Faculty of Washington State University:

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Abstract

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Mapping of quantitative traits is a very active topic in biology. Quantitative trait loci (QTL) data have to be verified and validated for usefulness in plant breeding. The present study aims to determine the usefulness of previously identified yield QTL for barley (*Hordeum vulgare L.*) breeding.

Three previously mapped grain yield QTL on chromosomes 2, 3 and 5 were studied in Steptoe x Morex cross using near isogenic lines (NILs). The chromosome 2 yield QTL region of Morex delayed flowering time of Steptoe by 11 days but did not affect grain yield. The Steptoe chromosome 3 yield QTL, which is the largest yield QTL in Steptoe x Morex cross, reduced lodging and head shattering when introduced into the moderate yielding, high malting quality barley, Morex. Grain yield of Morex, however, was not improved. The chromosome 5 yield QTL transferred from Steptoe to Morex caused slight yield increase in all NILs although differences were not significant.

Two major and a minor QTL were detected on chromosomes 2, 3 and 7 for head shattering. The largest QTL on chromosome 3 was located in a 0.6 cM region using NILs. We detected two flowering time QTL with photoperiod response (*Phr*) on chromosomes 2S and 5L (designated *Phr-2S and Phr-5L*) in positions similar to the previously mapped *PpdH1* and

PpdH2 loci, respectively. NIL analyses located *Phr-2S and Phr-5L* in 0.9 and 6.0 cM intervals. Results suggested the presence of additional loci for flowering time.

NILs developed in this study will be useful to trace additional loci for the respective traits using QTL mapping. The QTL introgressed into the NILs in this study were the major ones. The major QTL make detection of relatively small effect QTL difficult. Minor QTL can be more readily detected if studied in crosses where the major QTL are fixed. Minor QTL could be useful in plant breeding. Discrete phenotype classes created in NILs will be useful in fine mapping for map based cloning of QTL.

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CHAPTER ONE

INTRODUCTION

1. Background

Higher yield is the main target in plant breeding programs of many crop species. In malting barley (*Hordeum vulgare L.*), breeders must consider both grain yield and the malting quality of the grain. This fact necessitates "precision style breeding programs", in which only very limited changes in the makeup of the accepted malting quality of the barley may take place. Molecular marker technology has the advantage of precision that allows introgression of very small genomic fragments from a donor genotype into a commercial variety through marker assisted backcross breeding.

Besides its economic value as the world's fourth crop in acreage (FAO 1996), barley is a candidate model genetic system for related cereal crops such as wheat, oat and rye. The seven pairs of chromosomes in diploid cultivated barley have good homeologous relationships with wheat (*Triticum sp.*) (Nelson *et al.* 1995; Devos *et al.* 1993), rye (Wang *et al.* 1992) and oat chromosomes (Van Deynze *et al.* 1995). Barley research will benefit hexaploid common wheat (*T. aestivum L.*), a major crop with limited application of molecular markers because of high ploidy level (Hart 1994) and low polymorphism (Chao *et al.* 1989). Barley has been a model species for mutation studies and more than 600 genes/mutants have been mapped in barley (Barley Genetic Newsletter Vol:26). Molecular characterization of barley mutants will be useful to identify genes in other cereal species.

A number of barley crosses have been used to develop moderately dense genetic maps (Heun *et al.* 1991; Kleinhofs *et al.* 1993; Graner *et al.* 1991; Bezant *et al.* 1997; Backes *et al.*

1995; Tinker *et al.* 1996; Powell *et al.* 1997). Most of these crosses have been used to detect QTL for grain yield, malting quality and related traits. Verification and detailed characterization of these QTL are essential before their use in plant breeding programs is implemented.

2. QTL analysis

Unlike the qualitative traits with discrete phenotype classes that can be explained by simple genetic principles, quantitative traits with continuous variation are a result of complex systems in which environment and interacting genetic factors play significant roles. Discovery of associations of qualitative traits and quantitative characters as early as the 1920's (Sax 1923) lead to the idea that quantitative traits can be tagged with qualitative trait markers. At the beginning, only a few morphological traits with discrete phenotype classes were used as markers. Molecular markers appeared first in the form of isozymes in the late 1950's and storage protein markers in the early 1970's. The lack of sufficient numbers of markers in morphological, isozyme and protein marker systems was the major limitation for an effort to scan the whole genome for associations with quantitative traits.

Emergence of DNA marker technology introduced practically unlimited numbers of markers. Two types of DNA markers are used; hybridization based (RFLP, in situ hybridization) and PCR based (RAPD, AFLP, STS, microsatellites, etc.). Advantages and limitations of these markers have been reviewed by Kochert (1994), and Burow and Blake (1997).

QTL analysis is basically finding a statistically significant association between a marker and a quantitative trait. The first requirement for such genome-wide analysis is the presence of a genetic map containing reasonably spaced markers every 15-20 cM (Tanksley 1993). If the target is a QTL in an approximately known region, then the development of a full genome map may not be necessary. In this case, markers transferred from available maps (RFLP markers can easily be transferred) or random markers developed by targeting specific regions using bulked segregant analysis (Michelmore *et al.* 1991) can be employed.

QTL mapping can be classified into three categories based on the points of the genome that are considered; a) single marker mapping, which scans the genome at the points represented by markers only, b) interval mapping, which scans points between markers as well as marker points, and c) composite interval mapping, which scans the genome at all marker and interval points and, at the same time, uses other points in the genome (other than the one being evaluated) as cofactors to control genetic background effects . Composite interval mapping may use any marker point, all marker points, or selected marker points that represent the genome with some intervals (Basten *et al.* 1997).

Significance of marker-trait associations can be assessed using different parameter estimation methods such as simple t or F-tests (Stuber *et al.* 1987), least squares method (Haley and Knott 1992), maximum likelihood analysis (Lander and Botstein 1989), and regression analysis (Knapp *et al.* 1990). Many QTL mapping programs were developed employing different combinations of test points (marker, interval or composite interval) and parameter estimation methods (Liu 1998).

An important aspect of QTL mapping is the relatively high threshold levels to declare a QTL because of the high number of hypothesis to be tested (Paterson 1996). A 99.8 % significance level per test is used to ensure that 95 % of the QTL detected throughout a genome are real (Paterson 1998). Although a high threshold level is necessary for the publication, a lower stringency might be acceptable if a "comparative analysis" of previously reported QTL is possible (Paterson 1998). A number of methods are used to set thresholds. F-test, LOD score, and permutation tests are widely used. LOD score is the log (10) of the ratio between the odds of presence of a QTL versus absence of a QTL. Permutation test uses empirical distribution achieved by resampling of the data. Due to multiple test problems and violation of independence principle because of linkage, use of permutation test is favored to set threshold levels for significance (Liu 1998).

QTL mapping is often performed on F_2, recombinant inbred (RI), doubled haploid (DH), and backcross (BC) populations. RI and DH populations have the advantage of being "immortal" i.e. the seed from these lines can be indefinitely increased and evaluations can be replicated. F_2 and BC populations are easy to create. F_2 populations can show dominance effects. Number of recombinations per line is higher in F_2 and RIL populations compared to DH and BC populations.

3. Near isogenic line analysis

QTL mapping identifies the position of the trait only with limited accuracy. Errors with the genetic map and marker data, presence of multiple loci affecting the trait, linkage between QTL especially in repulsion phase, sample size and QTL x environment interactions are the factors that contribute to limited accuracy. Higher resolution mapping may distinguish pleiotropy from close linkage, and separate closely linked QTL. Resolution of QTL to a smaller chromosome region is critical for the precise style plant breeding needed for the maintenance of established malting quality standards and, of course, absolutely essential for map based cloning of quantitative trait genes.

QTL mapping results can be verified by repeating the QTL experiment using a) the same mapping population or a subsample of the population (Bridges and Sobral 1996), b) additional population from the same cross (Spaner *et al.* 1999; Romagosa *et al.* 1999), or c) near isogenic line analysis (Koester *et al.* 1993).

Isogenic line analysis has the advantage of analyzing the QTL in a homogenous background without the effect of segregation of other modifying loci. Development of a series of isogenic lines from the target region also allows high resolution mapping of the QTL. Because of homozygosity, NILs are genetically stable and can be used in replicated experiments. NILs with the QTL of interest can quickly be released as commercial varieties. Linkage drag, the introgression of a genomic fragment from the donor parent around the locus controlling the trait of interest, can be controlled if sufficient markers are available in the chromosome region of interest.

NIL analysis also has some disadvantages. First of all, NIL development requires a relatively long time. Typically six or seven generations are needed. Even with the use of indoor facilities like growth chambers and growth rooms, the whole process takes about two years. If closely and evenly spaced markers are not available, there is a substantial risk of losing the QTL during the generations of NIL development due to double cross-overs in the QTL region. If the QTL needs additional loci for full expression, its function can be partly or completely lost in the new genetic background. Another disadvantage of the NIL system may be in the case of epistatic interactions, which also could be considered an advantage because of information generated about the QTL itself.

Near isogenic lines are commonly developed by backcrossing. A marker, or better flanking markers, tagging the QTL are traced in backcross generations. Using the molecular markers, three or four backcross generations and a selfing generation are usually enough to create near isogenic lines. If high resolution mapping of the QTL is intended, then a relatively large population from the selfing generation may be required. A second selfing generation is also needed to isolate homozygous isogenic lines.

4. Marker assisted selection

Use of molecular markers brings a great deal of precision to backcross breeding programs where one or a few traits are being transferred from a donor genotype to a commercial variety. Selections can be made in an early seedling stage, which reduces the resources needed to maintain large populations. Genotype detection in an early seedling stage also eliminates the number of backcrosses that need to be made compared to classical backcross breeding, where lines are selected after the expression of the trait, commonly in a very late stage of plant life. Depending upon the markers tagging the QTL, linkage drag is decreased compared to conventional backcrossing, which will eliminate unwanted genes coming along with the trait of interest.

Use of marker assisted selection in breeding schemes, other than the backcross breeding, is not practical at the present. There are two major reasons. First, in order to use markers in plant breeding, one needs to determine the association between markers and agronomic traits. Considering the large number of loci affecting crop performance, assessment of these associations is a formidable task. Second, genotyping of the plants with molecular markers is an expensive and labor consuming operation. This expensive technology is not feasible in early generations of plant breeding with hundreds of thousands entries. Development of high output and cost effective automated marker analysis systems will be helpful. In this case, selection for a number of traits could be efficiently conducted using molecular markers resulting in a decrease of progeny that need to be carried to later generations and field testing.

"QTL hot spots" which include QTL for yield and yield related traits such as number of seed per spike, plant height, lodging, kernel weight and fertile tiller number. The *denso* locus on chromosome 3 (Powell *et al.* 1985; Laurie *et al.* 1993), *Eam8* on chromosome 5 (Kjaer *et al.* 1991) and a flowering time QTL region on chromosome 2 (Hayes *et al.* 1996) are such examples. Pleiotropic effects of the flowering genes on plant development are possibly the reason why many QTL for yield related traits have been mapped in the vicinity of flowering genes. Due to the fact that the favorable allele, Steptoe or Morex, changes by the environment, the yield QTL on chromosome 2 short arm may be interesting in breeding of barley for specific environments.

A third grain yield QTL in barley has been reported on the long arm of chromosome 5. In a study with the 150 SM DHLs, this QTL appeared in two of 16 environments and also in combined analysis of environments (Hayes *et al.* 1993a) suggesting that it has small but consistent effects. A short daylength flowering locus, *Ppd-H2* has been reported in this QTL region in Igri x Triumph cross (Laurie *et al.* 1995). At least two short day flowering genes, *Eam8* or *Ea*$_k$ of chromosome 5 (Kjaer *et al.* 1991) and *Eam7* (*Ea7)* of chromosome 6 (Stracke and Borner 1998), have been reported to affect flowering time in long days. The *Eam8* locus was associated with grain yield. Hence, the yield QTL on chromosome 5L may be associated with the short daylength flowering time QTL, *Phr-5L,* also located on chromosome 5. Studies with the SM population showed that the *Phr-5L* region was associated with many malting quality traits. Han and Ullrich (1993) reported QTL for malt β-glucan content, diastatic power, α-amylase activity and malt extract in this QTL region. Since Steptoe confers the undesirable alleles for all of these traits, introgression of this QTL region into Morex may result in malting quality deterioration.

the SM DHL population (Kleinhofs *et al.* 1993; also see Graingenes database at http://probe.nalusda.gov:8000).

QTL studies with the 150 SM DHL mapping population have shown a grain yield QTL in the centromeric region of chromosome 3 (near ABG396) where Steptoe provides the higher yielding allele (Hayes *et al.* 1993a, 1993b; Romagosa *et al.* 1999). Many potential yield related traits such as head shattering, lodging (Larson *et al.* 1996), plant height (Hayes *et al.* 1993a) and kernel weight (Han and Ullrich 1993; Traore 1993) have also been mapped in this region. It has been postulated that the higher grain yield associated with the Steptoe ABG396 region was a result of less head shattering and less lodging (Larson *et al.* 1996). The grain yield QTL region around the chromosome 3 centromere was also studied for malting quality (Larson *et al.* 1997; Han and Ullrich 1993) and is not expected to play a negative role in malting quality when transferred to Morex.

SM QTL mapping showed association of a chromosome 2 region around ABG002 marker with grain yield (Hayes *et al.* 1993b). The higher yielding allele of the chromosome 2 QTL was influenced by the environment and varied between Steptoe and Morex (Hayes *et al.* 1993a; Zhu *et al.* 1999). QTL for some yield related traits have been mapped to this region including flowering time, plant height, lodging (Hayes *et al.* 1993a) and kernel weight (Han and Ullrich 1993). Association of the chromosome 2 region with seed yield has been found in other barley crosses such as Bleinheim x Kym (Bezant *et al.* 1997) and Igri x Triumph (Laurie *et al.* 1994). Plant height (Bezant *et al.* 1996; Karsai *et al.* 1997; Kjaer *et al.* 1995), kernel weight, number of seed on the spike, and flowering time (Laurie *et al.* 1994) have been mapped to a similar region in other crosses. This yield QTL might be associated with a flowering time QTL reported in the region (Hayes *et al.* 1993a) since flowering genes have been associated with some

"QTL hot spots" which include QTL for yield and yield related traits such as number of seed per spike, plant height, lodging, kernel weight and fertile tiller number. The *denso* locus on chromosome 3 (Powell *et al.* 1985; Laurie *et al.* 1993), *Eam8* on chromosome 5 (Kjaer *et al.* 1991) and a flowering time QTL region on chromosome 2 (Hayes *et al.* 1996) are such examples. Pleiotropic effects of the flowering genes on plant development are possibly the reason why many QTL for yield related traits have been mapped in the vicinity of flowering genes. Due to the fact that the favorable allele, Steptoe or Morex, changes by the environment, the yield QTL on chromosome 2 short arm may be interesting in breeding of barley for specific environments.

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6. Head shattering

Head shattering causes serious yield losses in barley throughout the world. Two mechanisms are well known. In the first type, joints in the rachis nodes easily disarticulate (Franckowiak and Konishi 1996) resulting in segmentation of the rachis and loss of seed along with a piece of the rachis. This type of shattering, termed "brittle rachis", is found in *Hordeum vulgare ssp. spontaneum*, and is considered a non-cultivated species adaptive feature for seed dispersal (Takahashi 1955). In the second type, the rachis breaks resulting in the loss of a spike segment (Kaufman and Shebeski 1954). Descriptions in literature indicate the possibility of a third kind of shattering where the kernel itself detaches from the rachis (Platt and Wells 1949; Chapman and Hockett 1976).

Genetics of brittle rachis have been extensively studied. Brittleness is due to two complementary genes *Bt1* and *Bt2* (Takahashi 1955), later named *Btr1* and *Btr2* (Franckowiak and Konishi 1996). Brittle is dominant to non-brittle. *H. vulgare ssp. spontaneum* has brittle rachis as a consequence of the dominant forms of both genes (Takahashi 1964). In a study to determine the distribution of *Btr1/Btr2* genes in cultivated barley, 1267 cultivars were crossed to "tester genotypes" with known genetic composition for the *Btr* genes (Takahashi 1964). Results indicated that every barley cultivar tested had either the *Btr1* or *Btr2* gene in a homozygous recessive form resulting in a non-brittle rachis.

Shattering, as defined by rachis breaking, has not been as well described as brittle rachis. Working with crosses between "weak" and "tough" rachis cultivars, Kaufman and Shebeski (1954) observed that the F_1 plants had a weak rachis that shattered. In the F_2 population, plants with tough, weak and intermediate rachis were observed. These data suggest that the weak rachis may have been mediated by a major dominant gene interacting with some minor genes.

Kaufman and Shebeski (1954) concluded that their observed weak rachis phenotype was different from the brittle rachis phenotype. Platt and Wells (1949) and Clarke (1981) did not clearly distinguish rachis breaking from brittle rachis. Thus, the weak rachis phenotype has not been adequately differentiated from brittle rachis to date.

Using backcross derived homozygous Steptoe x Morex lines, Larson *et al.* (1996) mapped two head shattering QTL to barley chromosome 3 around DNA markers ABG396 and ABG057. These markers are separated by about 44 cM (Kleinhofs *et al.* 1993). The two peaks explained 13.9 and 13.7 % of the variation, respectively. This work also showed an association between spike density and head shattering with dense spikes shattering less.

In another study, using an "Igri" x "Danillo" cross, three head shattering QTL were mapped to intervals of MWG557-MWG769 (50 cM), MWG611-MWG921 (2 cM) and MWG820C-MWG820B (13 cM) on chromosomes 2, 4 and 6, respectively (Backes *et al.* 1995). These three QTL together explained 44 % of the genetic variance. QTL positions of ear breaking, lodging, stalk breaking and physical state of the plants before harvest were quite similar. It was suggested that the stability of the stem tissue was the determining factor for all of the above traits.

7. Flowering time

Flowering time in cereals is under the control of three classes of genes. Vernalization and photoperiod response genes determine flowering time as a response to environmental conditions. The third class of genes controls the flowering time via the developmental rate of the plant and are named "earliness per se" genes (Laurie *et al.* 1995). These three classes of genes and common interactions among them (Karsai *et al.* 1997; Laurie *et al.* 1995; Gallagher *et al.*

1991) determine the adaptation of genotypes to growing environments, which is often associated with production potential of crop plants.

Barley chromosome 2(2H) short arm carries a photoperiod response gene, *Ppd-H1* (Laurie *et al.* 1995), possibly the barley equivalent of wheat *Ppd* gene (reviewed by Laurie *et al.* 1997). Early flowering alleles of *Ppd-H1* from cv. Igri reduced flowering time of DHLs by 12 days in spring sown, seven days in fall sown barley and 10 days in a controlled environment under 16 h light (Laurie *et al.* 1994). QTL studies with the Steptoe x Morex DH mapping population showed a QTL near ABG002 marker on chromosome 2 (cosegregates with MWG858 marker in SM map) for flowering time in 15 out of 16 environments (Hayes *et al.* 1993a). Morex carried the late flowering allele in all environments with additive effects ranging from three to ten days. The ABG002 marker also has been associated with plant height and grain yield. QTL for flowering time have also been reported in a comparable map position of chromosome 2 using T. Prentice x V. Gold (Kjaer *et al.* 1995) and Dicktoo x Morex crosses (Karsai *et al.* 1997).

A QTL for photoperiod response, designated *Ppd-H2*, was detected on barley chromosome 5(1H) long arm (Laurie *et al.* 1995). *Ppd-H2* affects flowering time in controlled short day conditions and winter sowing but not in spring sowing (Laurie *et al.* 1995). Pan *et al.* (1994) reported two intervals (*iPgd*-BCD265C and *HorD*-ABG452) on the long arm of chromosome 5 that affects flowering time under short days. Dicktoo fragments of these intervals delayed the flowering time by 12-17 days in controlled environments (8 h light), and 8 days in fall-sown field experiments.

Besides *Ppd-H1* and *Ppd-H2*, at least four additional photoperiod response genes have been mapped to barley chromosomes using natural or induced mutations. These are *Eam8* (*Ea$_k$*)

of chromosome 5L (Takahashi and Yasuda 1971), *Eam9* (*Ea9*) of chromosome 4L (Yasuda and Hayashi 1980), *Eam10* (*Ea$_{sp}$*) of chromosome 3L (Gallagher *et al.* 1991) and *Eam7* (*Ea7*) of chromosome 6S (Stracke and Borner 1998). Unlike wild type plants, mutant plants reach flowering earlier in short daylength conditions.

Earliness per se (*eps*) genes affect flowering time both in short and long day conditions. These genes tend to have a relatively small effect on flowering time (Laurie 1997). Eight *eps* genes have been mapped as QTL with minor effects on six barley chromosomes (Laurie *et al.* 1995). Among them is a locus associated with the radiation induced semidwarf mutant *denso*. Reports dealing with *eps* genes are not common, possibly because the relatively small effects produced by *eps* genes can not be detected in the presence of segregating photoperiod response loci, which are responsible for the majority of variation in flowering time.

8. The Dissertation

This dissertation research is a part of North American Barley Genome Mapping project (NABGMP). NABGMP is a cooperative, multi-institutional, multidisciplinary project with grower, industry and government support. The NABGMP was initiated in 1990. The first aim of the project was to develop a saturated molecular marker map, which was accomplished by publishing the Steptoe x Morex map with 295 markers located on seven barley chromosomes spanning more than 1,250 cM (Kleinhofs *et al.* 1993). This map now has more than 900 markers (http://barleygenomics.wsu.edu).

1) Goal

This dissertation research is relevant to the second goal of the NABGMP, which deals with identification and mapping of the genes and QTL controlling economically important traits.

The specific goal of the dissertation is to increase grain yield of the Morex cultivar via introgression of favorable Steptoe yield QTL regions without losing its excellent malting quality

2) Objectives

A. Verification of previously reported grain yield QTL of Steptoe x Morex cross

B. Identify multiple components of the head shattering trait

C. High resolution mapping of two photoperiod response genes

The dissertation consists of three manuscripts as chapters 2, 3 and 4. Each manuscript uses the specific format prescribed by the journal. Besides the manuscript chapters, there is an introduction chapter in which background information regarding the research is given and the dissertation is described, and a final chapter in which perspectives and prospects of the dissertation are given. The dissertation concludes with an Appendix, which consists of three figures that are not included in the manuscript chapters. Appendix A shows genomic compositions of three SM DHLs (SM23, SM84 and SM179) used in marker assisted backcrosses along with the markers used to check donor parent background. Appendix B is a representation of the skeletal map used in QTL mapping. Appendix C shows a diagram of molecular marker assisted backcross breeding scheme.

Chapter 2. **Marker assisted analysis of grain yield QTL in barley**. BL Jones, DM Wesenberg, SE Ullrich and A Kleinhofs are also contributors to the manuscript. BL Jones conducted the malting quality analysis. DM Wesenberg maintained two Idaho locations of yield trials. SE Ullrich mediated two Washington locations of yield trials. A Kleinhofs is the advisor for the dissertation. This manuscript was prepared for **Molecular Breeding**.

Chapter 3. **Molecular marker assisted genetic analysis of head shattering in six-rowed barley**. The contributors of this manuscript include DA Kudrna, SE Ullrich and A Kleinhofs. DA Kudrna helped with the molecular marker and crossing work in the early stages of the research. SE Ullrich mediated field experiments. A Kleinhofs is the advisor for the dissertation project. This manuscript was prepared for **Theoretical and Applied Genetics.**

Chapter 4. **Marker assisted analysis of flowering time in a spring barley cross**. The contributors also include DA Kudrna and A Kleinhofs. DA Kudrna assisted with the controlled short day flowering observations and initial part of the molecular marker work. A Kleinhofs is the advisor for the dissertation. This manuscript was prepared for **Theoretical and Applied Genetics.**

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CHAPTER TWO

Marker assisted analysis of grain yield QTL in barley

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ABSTRACT

Three grain yield quantitative trait loci (QTL), previously reported in barley Steptoe x Morex cross, were evaluated for plant breeding. These QTL are on chromosomes 2S(2H), 3(3H) centromeric region and 5L(1H), and designated *QTL-2S*, *QTL-3* and *QTL-5L*, respectively. Our objectives in this study were to identify the mechanism of yield increase, to transfer QTL between different backgrounds. QTL mapping of yield related traits was performed and near isogenic lines were developed. QTL for plant height, head shattering, seed weight and number of rachis nodes/spike were detected in the *QTL-3* region. near isogenic lines developed by introgression of *QTL-3* from the high yielding cultivar Steptoe to superior malting quality, moderate yielding variety Morex acquired reduced height, lodging and head shattering features of Steptoe without major changes in malting quality. The yield of near isogenic lines, measured by minimizing the losses due to lodging and head shattering, did not exceed that of Morex. *QTL-2S* has been reported to be environmentally dependent with either Morex or Steptoe contributing the positive alleles. Steptoe near isogenic lines with Morex *QTL-2S* region flowered 10 days later than Steptoe but the grain yield was not changed. When *QTL-5L* was transferred from Steptoe to Morex, slight but not significant yield increases over Morex were obtained while malting quality traits were not affected. None of the three QTL is likely to play a significant role in exceptional yield level of Steptoe unless they interact with other loci to increase grain yield.

Abbreviations: cM, centimorgan; DHL, doubled haploid line; NIL, near isogenic line; QTL, quantitative trait locus or loci; SM, Steptoe x Morex.

INTRODUCTION

Tagging quantitative trait loci (QTL) with molecular markers facilitates the efficient transfer of QTL between different genetic backgrounds through marker assisted selection. Near isogenic lines (NIL) developed by transferring particular QTL in a new genetic background are useful for high-resolution mapping as well as for verifying the effect of the QTL. In the present study, we focus on three previously reported grain yield QTL [9] in a cross between a feed barley (*Hordeum vulgare L.*), Steptoe, and a malting barley, Morex. The Steptoe x Morex (SM) cross offers the following advantages for detailed analysis of grain yield QTL; a) a good and stable level of grain yield difference between parents [10,17,23], b) availability of a 150 doubled haploid line (DHL) mapping population with grain yield data from 16 environments [9], and c) availability of a moderately dense genetic map based on the SM DHL population [15].

In QTL studies involving the 150 SM DHL mapping population, a grain yield QTL has been identified near the centromeric region of chromosome 3(3H), designated *QTL-3*, where Steptoe provides the higher yielding allele [9-10]. *QTL-3* has been verified using a second set of 92 DHLs from the SM population evaluated in three environments, where Steptoe fragments from this QTL region consistently increased yield by 10-23 % [28]. Several yield related traits, such as head shattering, lodging [17], plant height [9] and kernel weight [8,36] also mapped to this region. Larson *et al.* [17] developed BC_1 derived homozygous lines from the backcross of a SM DHL (SM72), which carries the entire chromosome 3 from Steptoe, to Morex. The Steptoe allele of the ABG396 marker was associated with an increase of 12.2 % grain yield over Morex. Presence of the Steptoe allele of ABG396 marker accounted for nearly 14 % of the variation for both head shattering and lodging. Larson *et al.* [17] concluded that the higher grain yield associated with the ABG396 region from Steptoe was a result of reductions in head shattering and lodging. In another study, Steptoe-like high yields were recovered in DH lines derived from cross of the two low yielding SM DH lines, SM73 and SM145, both of which carried the Steptoe *QTL-3* [37]. These findings imply that the *QTL-3* by itself does not enhance grain yield but is needed in the presence of other Steptoe loci to attain Steptoe-like yields. Other barley crosses have not revealed a yield QTL in the chromosome 3 centromeric region [3,5,22,27,32,33]. The Steptoe *QTL-3* region were associated with higher seed size and kernel plumpness [8], favorable traits for malting quality because of their positive correlations with malt extract [25]. Other studies showed no detrimental effects of Steptoe *QTL-3* fragment on malting quality [9,10,18] indicating that introgression of *QTL-3* is not likely to deteriorate the excellent Morex malting quality.

A grain yield QTL, detected near the ABG002 marker of chromosome 2S [9] was designated *QTL-2S*. The parent conferring the higher yielding allele, Steptoe or Morex, changed with the environment. Several yield related QTL have been mapped to this region including flowering time, plant height, lodging [9] and kernel weight [8]. Association of the *QTL-2S* region with seed yield has been found in other barley crosses such as Bleinheim x Kym [5] and Igri x Triumph [20]. Plant height [4,12,14], kernel weight, number of seed per spike, and flowering time [20] have also been mapped to this QTL region. The map position of this yield QTL and presence of QTL for yield related traits in other crosses suggest that these QTL may be due to the same gene(s). Flowering time genes have been associated with some "QTL hot spots" which include QTL for yield and yield related traits such as number of seed per spike, plant height, lodging, kernel weight and fertile tiller number [11,13,19,26]. Pleiotropic effects of the flowering genes on plant development are possibly the reason why many QTL for yield related traits have been mapped in the vicinity of flowering genes. Presence of the QTL for yield and primary yield components (number of seed per spike and seed weight) in the same region suggests that yield is at least partly determined by primary yield components in specific environments. However, grain yield is not altered over a large range of environments due to a compensation effect among primary yield components [2]. Therefore, yield QTL detected in the vicinity of flowering genes may be interesting only for specific environments.

A third grain yield QTL in barley has been reported on the long arm of chromosome 5 [9]. This QTL, designated *QTL-5L*, appeared in two of 16 environments and also in combined analysis of environments [9], suggesting that it has a small but consistent effect on grain yield. A short daylength flowering locus, *Ppd-H2* has been mapped to this QTL region in the Igri x Triumph cross [21]. Two short day flowering genes, *eam8* or *eak* of chromosome 5L [13] and *ea7* of chromosome 6S [31], have been reported to affect flowering time in long days, and the *eam8* locus was associated with grain yield [13]. Hence, *QTL-5L* may be associated with the short daylength flowering locus mapped in this region. The *QTL-5L* region was associated with a number of malting quality traits such as malt extract, diastatic power, α-amylase activity and malt β-glucan content [8,9]. Thus, transferring the Steptoe *QTL-5L* into Morex may cause deterioration in malting quality.

We aimed to determine the usefulness of three barley yield QTL for plant breeding. The QTL regions were analyzed in detail through the assessment of overlapping QTL effects on grain yield and related traits such as seed weight, plant height, head shattering, flowering time. Near isogenic lines were developed for each QTL region to determine the value of each yield QTL for plant breeding. Our goal is to increase the yield of Morex, while maintaining its excellent malting quality. The objectives of this study were; a) to identify the mechanism of yield increase, and b) to transfer yield QTL between different backgrounds.

MATERIALS AND METHODS

Steptoe and Morex are six-rowed spring barley cultivars grown in the Pacific Northwest (PNW) region of the United States. Steptoe is a high yielding feed barley developed for the PNW whereas Morex is a high quality malting barley developed for the Upper Midwest and not well adapted for production in the PNW. Steptoe and Morex are the parents of a 150 DHL mapping population of North American Barley Genome Mapping Project.

Two approaches were used to characterize and validate grain yield QTL; a) evaluation of overlapping QTL effects on grain yield, as reported by Hayes *et al.* [9,10], and yield related traits such as head shattering, plant height, flowering time, seed weight, and b) analysis of yield and related traits in near isogenic lines, which were developed by transferring QTL regions from one variety into the other.

QTL mapping of yield related traits was performed using agronomic data from field trials of the 150 SM DHLs grown as single rows in 1996 and 1997 at Pullman, WA. The 1997 trial was arranged in two replication blocks while 1996 trial was not replicated. The average of the two replications in 1997 was used in all analyses since there were no block effect for any trait. Phenotypic data were collected for the number of rachis nodes per spike, head shattering, plant height and awn emergence in 1996 and 1997, and for seed weight in 1996. Based on map position and completeness of the marker data set, 149 markers from the SM map were selected to obtain a skeleton map consisting of a marker every 6-8 cM, whenever possible. Marker distances were confirmed using Mapmaker EXP [16]. QTL mapping was performed using MQTL software, which is suitable for multiple location data analyses [34,35]. Linear regression based simple interval mapping was used to detect QTL and QTL x environment interaction [7]. Threshold values for test statistics of QTL main effects and QTL x environment effects to check type 1 error rate were calculated for each trait using permutations replicated 5,000 times for 5 % and 10,000 times for 1 % level of significance [6]. A QTL was declared when the test statistics of QTL main effect and/or QTL x environment effect exceeded the respective threshold level. Additive effects calculated by MQTL are for two alleles per locus, therefore, are twice those reported for other QTL software.

NILs were developed by transferring Steptoe chromosome fragments from the *QTL-3* and *QTL-5L* regions into the Morex genetic background. *QTL-2S* was transferred from Morex into Steptoe background. NILs that carry a combination of different fragments from *QTL-3* and *QTL-5L*, and *QTL-2S* and *QTL-3* regions were also developed (Fig. 1). DHLs SM23 and SM84 were backcrossed to Morex for the development of *QTL-3* and *QTL-5L* NILs. *QTL-2S* NILs were derived by backcrossing the SM179 DHL to Steptoe. Progeny containing large fragments (40-50 cM) from the target region were selected using multiple RFLP markers for each fragment and backcrossed until the BC_4F_1. The Morex background was checked with markers spaced at 20-25 cM intervals in the BC_4F_1. The BC_4F_2 was grown to obtain lines with recombinations in the target area and homozygous lines were selected in the BC_4F_3 using molecular markers. NILs developed for the chromosome 2 QTL region were selected from the BC_3F_3 generation and genomic background was not checked. Only RFLP markers were used in this study except for two morphological markers, short rachilla hair and pubescent leaf. DNA isolation, Southern transfer and hybridization methods, and morphological marker descriptions were as described by Kleinhofs *et al.* [15]. Seed from selected NILs was increased in New Zealand during October 1997-February 1998. The NILs (Fig. 1) were evaluated in four field locations in 1998. Environmental conditions and some characteristics of the trials are summarized in Table 1. Trial plots consisted of eight rows spaced 15 cm apart at Pullman and Fairfield, WA, two rows spaced

35 cm apart at Aberdeen, ID, and one row with 35 cm between rows at Tetonia, ID. Experimental design was a randomized complete block design with four replications. Seeding rate was about 85 kg/ha at Pullman and Fairfield, 95 kg/ha at Aberdeen and 70 kg/ha at Tetonia. The trial at Aberdeen was irrigated whereas the other three locations were dryland. Local growing practices were applied in all trials.

Plant height was measured from ground to the tip of the main spike excluding the awns for 15 plants in each row or plot. The number of rachis nodes per spike was determined on 25 spikes by counting the number of the rachis nodes on both sides of the rachis. Seed weight was determined on 200 seeds from each row or plot. Lodging was measured as a visual estimate of the percentage of lodged plants in each plot. Days to flowering were the number of days from planting to awn emergence for 50 % of the plants in a plot or row. Plots were combine-harvested in Pullman and Fairfield trials with hand picking of lodged plants. Plants in Aberdeen and Tetonia locations were cut, dried and threshed. Harvest was conducted before head shattering in all locations.

Malting quality analyses were performed on a 400 g sample from each plot from two locations (Aberdeen, ID and Fairfield, WA) by the USDA/ARS Cereal Crops Research Unit, Madison, WI following the standard procedures described by American Society of Brewing Chemists [1].

NIL field observations and quality analyses were subjected to analysis of variance. Combined analysis over locations was conducted whenever error variances from environments were homogenous based on Bartlett's homogeneity test [30]. Genotype x environment interactions were calculated when combined analyses were performed. NIL means were grouped using Duncan's multiple comparison test. All statistical analyses were performed using SAS software [29] based on the principles explained by Steel *et al.* [30].

RESULTS

QTL mapping

QTL analyses were performed for five yield related traits (plant height, number of rachis nodes per spike, seed weight, flowering time and head shattering). Results with head shattering and flowering time are presented in Chapters 3 and 4, respectively. Frequency distributions for the SM 150 DHLs and parents averaged over years showed that number of rachis nodes per spike and plant height segregated transgressively while the data for seed weight were ambiguous (Fig. 2). Test statistics calculated by MQTL are graphically displayed for the chromosomes 2, 3 and 5 in Fig. 3. Additive effects, percent variance and QTL x environment test statistics for the genomic regions with significant test statistics are provided in Table 2.

Both *QTL-3* and *QTL-2S* regions carried QTL for plant height, number of rachis nodes and seed weight (Table 3, Fig. 2). Steptoe alleles of both regions conferred shorter stature and heavier seeds. The location of the seed weight QTL peak was shifted from plant height and number of rachis nodes per spike QTL peaks. For the number of rachis nodes per spike, Steptoe conferred higher values at *QTL-3* region but lower in *QTL-2S*. *QTL-5L* region had no QTL for any of the yield related traits studied. Number of rachis nodes per spike and plant height traits each had one additional QTL in chromosomes 6 and 7, respectively (Table 3).

Isogenic Line Evaluations

Agronomic traits

Grain yield, plant height, lodging, flowering time, number of rachis nodes per spike and seed weight data were collected for NILs and parents for four locations. Fairfield and Pullman locations represented typical spring barley production area in the Palouse region of the Pacific Northwest. The irrigated Aberdeen location represented a high yielding environment while Tetonia represented a poor environment because of high altitude and relatively cooler climate (Table 1). Ecological and agronomic features of the trial environments are given in Table 1. Highest grain yield, number of rachis nodes per spike and seed weight values were obtained at the irrigated site in Aberdeen (Table 3). The tallest plants and the highest lodging were obtained at the Fairfield location. The smallest seeds and the lowest number of rachis nodes per spike were recorded at Pullman and Tetonia, respectively.

Significant differences were detected among genotypes for all agronomic traits. Averaging over locations, Steptoe yielded nearly 25 % more grain and flowered about two days earlier than Morex (Table 3). Steptoe plants also were 13 cm shorter and lodged 20 % less than did Morex plants. Morex averaged 2.2 more nodes per spike than Steptoe but the seed weight of Morex was eight mg less than that of Steptoe.

Grain yields of the Morex isogenic lines with the Steptoe chromosome 3 fragment (E19 and F13) were similar to those of Morex (Table 3), even though these lines were nine cm shorter and had 20 % less lodging than Morex. The F13 line had higher number of rachis nodes per spike than Morex in the combined environment analysis.

Introgression of chromosome 5 fragments from Steptoe into Morex in isogenic lines A24, B2, B9, F11, and F15 (Fig. 1) did not greatly alter the Morex-like traits measured. All of these lines had slightly higher seed weight and grain yield averages compared to Morex (Table 3). These NILs had similar plant height and lodging to Morex.

Three NILs carrying both chromosome 3 and chromosome 5 fragments from Steptoe [(A23, A38 and F12 (Fig. 1)] had slightly higher grain yield averages compared to Morex and the two NILs, E19 and F13, which carry only the Steptoe chromosome 3 fragment (Table 3). A23, A38 and F12 NILs were 8-10 cm shorter with 20 % less lodging than Morex. Compared to Morex, A23 had statistically higher number of rachis nodes per spike values in all environments, whereas A38 had higher number of rachis nodes per spike only in the combined analysis.

The Steptoe line C5 with a Morex chromosome 2 fragment (Fig. 1) flowered about 10 days later than Steptoe (Table 3). The C5 plants had 0.8 more rachis nodes per spike and were five cm taller than Steptoe. C5 yielded higher in two low yielding environments (Pullman and Tetonia), whereas Steptoe yielded higher in two high yielding environments (Aberdeen and Fairfield). Another Steptoe line, K5, containing two Morex fragments (one from chromosome 2 and the other from chromosome 3, Fig. 1) flowered nine days later than Steptoe. Compared to Steptoe, K5 was significantly taller in two locations and had lodged more severely in one location (Table 3). K5 had higher number of rachis nodes per spike than both Steptoe and the C5 line that carries only the chromosome 2 fragment from Morex. Seed weight of K5 was significantly lower than Steptoe and C5 at the Aberdeen location. Finally, K5 yielded significantly less than Steptoe or C5 in some locations and also in the combined analysis.

Malting quality traits

Kernel plumpness, malt extract and grain protein percentages, diastatic power, α-amylase activity and malt β-glucan content were measured on seed samples from Aberdeen and Fairfield locations. The irrigated environment at Aberdeen provided plumper seeds that yielded higher malt extract compared to the dryland Fairfield location (Table 4). Protein content of the seed from Aberdeen was slightly higher than that from Fairfield. Diastatic power, α-amylase activity and malt β-glucan content of the seeds from Aberdeen and Fairfield were comparable.

Steptoe and Morex differed significantly for every malting quality character evaluated. As the average of the two environments, Steptoe had about 13 % more plump seeds and 1.5 % less malt extract compared to Morex (Table 4). Morex diastatic power and α-amylase activities were three and two-fold that of Steptoe, respectively. Morex malt β-glucan content was about one-fourth of Steptoe. Steptoe seed contained significantly less protein than Morex.

NILs developed by introgression of Steptoe fragments from two yield QTL regions, *QTL-3* and *QTL-5L*, into Morex had similar values to Morex for kernel plumpness, malt extract, grain protein, diastatic power and malt β-glucan (Table 4). There were some significant differences between NILs and Morex for α- amylase activity, which were not consistent in the two environments.

A Morex chromosome 2 fragment in a Steptoe background (C5 line, Fig. 1) resulted in a slight decrease in kernel plumpness compared to Steptoe (Table 4). C5 had higher malt extract and lower malt β-glucan than Steptoe at Aberdeen and vice versa at Fairfield. Introgression of two Morex fragments from chromosomes 2 and 3 into Steptoe (K5 line, Fig. 1) resulted in significantly higher α-amylase activity but lower malt β-glucan content than Steptoe in both environments. Other malting quality aspects of K5 were similar to C5 and Steptoe.

DISCUSSION

A Steptoe chromosome 3 centromeric region has been associated with grain yield enhancement in several QTL studies [10,17,28]. This region also has been reported to include a QTL for lodging [9]. We mapped a head shattering QTL in this region and successfully transferred non-shattering Steptoe alleles to Morex (Chapter 3). In the present study, we also detected QTL for plant height, number of rachis nodes per spike and seed weight in this region although the QTL peak for the latter was clearly separated from the rest. Steptoe alleles of this region conferred shorter stature, reduced lodging, higher number of rachis nodes per spike and heavier seeds (Table 3). Near isogenic lines (NILs) developed by the transfer of Steptoe fragments from this genomic region into the Morex background did not increase yield over that of Morex. However, the NILs acquired useful Steptoe features, such as shorter height, reduced lodging and head shattering. Based on the Steptoe genomic composition of two chromosome 3 NILs (E19, F13) and three chromosome 3 + 5 NILs (A23, A38 and F12), QTL for plant height and lodging reside in the overlapping three cM area with the outermost borders marked by, but not including, CDO118 and BCD828 (Fig. 1). It is possible that the same gene or tightly linked genes control the plant height, lodging and head shattering traits.

Even though yield losses of Morex were minimized by hand picking lodged plants in plots and harvesting before head shattering, Steptoe still had remarkable yield superiority (13-34 % in different environments) over Morex. It does not appear that lodging and head shattering play significant roles in the yield potential differences of Steptoe and Morex. However, this fact does not eliminate the significance of these traits in determining the harvestable yield differences. Lodging and head shattering most probably play significant roles in the net harvest in environments where these traits are favored.

Lack of a yield increase in Morex isogenic lines containing the Steptoe *QTL-3*, in the absence of lodging and head shattering, suggests that the *QTL-3* yield advantage is a result of less lodging and head shattering, although other explanations exist. First, the environments where the isogenic lines were evaluated may not have promoted the expression of the yield QTL. However, this conclusion would contradict the consistent effects of *QTL-3* detected in previous studies [9,28]. Second, other Steptoe genome regions may be required to interact with *QTL-3* to obtain full expression. This hypothesis is supported by a report by Zhu *et al.* [37], in which crossing two low yielding SM DHLs, both containing the Steptoe *QTL-3* region, produced progeny with Steptoe-like yield levels. These data suggest that other Steptoe loci are required to interact with the chromosome 3 yield QTL to achieve the Steptoe yield level. Comparison of two NILs, C5 and K5, provides additional support for this hypothesis. The Steptoe NIL K5, which carries the Morex chromosome 3 yield QTL fragment in addition to a fragment from chromosome 2 found also in C5, had smaller seed size and lower grain yields than did C5. If this result is due to the presence of the Morex *QTL-3*, then we must conclude that this QTL can depress yield in a Steptoe background, but can not increase yield in a Morex background confirming the need for interaction with additional loci. These findings need to be verified due to the importance of conclusion and the uncertainty of the K5 genetic background purity.

Introgression of the Steptoe *QTL-5L* region into Morex did not result in deterioration of Morex malting quality although some inconsistent effects, usually negative in one environment and positive in another, were detected for α-amylase activity (Table 4). These differences are not likely real because Steptoe has never contributed higher α-amylase activity for any α-amylase QTL in any environment including the irrigated ones [8,9].

Most of the Morex NILs with the Steptoe chromosome 3 fragment had higher number of rachis nodes per spike than Morex confirming the QTL mapping results (Table 3, Fig. 3). Grain yields of these lines, however, were not different from Morex (Table 3). Hence, number of rachis nodes per spike trait is not likely to be a major component of the grain yield effect associated with this QTL region.

Although we hypothesized that a short daylength flowering QTL on chromosome 5 may be associated with the *QTL-5L*, this was probably not the case since the yield of the B2 NIL that did not carry the Steptoe short daylength flowering trait (Chapter 4), was similar to other chromosome 5 NILs. All NILs with the Steptoe chromosome 5 fragment had a slight, but non-significant, yield increase over Morex and Morex NILs that do not carry the Steptoe chromosome 5 fragments (F13 and E19). Most of the chromosome 5 NILs also had slightly heavier seeds than Morex. In conclusion, our results did not verify the *QTL-5L*; however, larger scale experiments might detect minor effects.

QTL mapping with the SM DH population predicted that transferring *QTL-5L* region from Steptoe into Morex could negatively alter malting quality. A number of malting quality QTL have been reported in the *QTL-5L* region with Steptoe conferring unfavorable alleles [8,9]. Morex NILs with Steptoe *QTL-5L* showed no deterioration for malting quality compared to Morex. Either the Morex genetic background or the environments where NILs were grown did not allow the development of unwanted phenotypes conferred by Steptoe alleles from *QTL-5L* region or our NILs simply did not carry the Steptoe malting quality QTL.

QTL-2S is an interesting yield QTL since the favorable parent is changed by the environment [9]. This QTL region was associated with flowering time (Chapter 4), plant height, head shattering (Chapter 3) lodging [9] and seed weight [8]. Such environment dependent QTL effects may be the result of the flowering gene. Transferring *QTL-2S* region from Morex to a Steptoe genetic background resulted in significantly delayed flowering time (Table 3). A long daylength flowering gene, *Ppd-H1*, which affects the awn emergence time by 12 days [20] has been previously reported to reside in this region. Although the two traits appear to be located in a similar region, there are not sufficient common markers mapped to conclude that the two regions are identical. Higher number of rachis nodes per spike values associated with the Morex allele in QTL mapping was verified in the BC_3F_3 line, C5. Higher head shattering associated with Morex alleles from *QTL-2S* region could simply be a result of spikes that carry more seeds. Transfer of Steptoe alleles from *QTL-2S* region into Morex could be useful to reduce head shattering damage of Morex especially in fertile environments where spikes carry more seeds. Transfer of *QTL-2S* from Morex to Steptoe resulted in only minor changes in other agronomic and malting quality aspects. Nevertheless, a ten day difference in flowering time conferred by this QTL region has the potential to affect agronomic and malting quality traits in some environments.

In conclusion, we transferred reduced lodging and head shattering traits from a high yielding barley cultivar, Steptoe, to a high malting quality barley Morex, without altering the good malting quality profile. Grain yield of the NILs, under conditions where yield losses from lodging and head shattering were minimized, however, was not improved over Morex and did not approach Steptoe. The chromosome 3 NILs, with less lodging and head shattering, may be useful to stabilize Morex yield in certain environments while maintaining the excellent Morex malting quality. Two other yield QTL transferred from Steptoe to Morex [chromosome 5(1H)] or from Morex to Steptoe [chromosome 2(2H)] did not result in major changes in the NIL yields. Considering the remarkable yield differences between Steptoe and Morex, finding the loci and interactions responsible for the high Steptoe yield remains an important goal and challenge. Considering the fact that Steptoe has much larger seed size than Morex, tracing the loci associated with seed size might lead to the identification of the yield QTL, which might interact with the chromosome 3 yield QTL, the largest and the most consistent yield QTL in the SM cross.

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Table 1. Ecological and agronomical features of field locations

Location	Soil type	Seasonal[2] rainfall mm	Annual[3] rainfall mm	Irrig. mm	Seasonal[2] av. temp. °C	Sowing date	Harvest area sq-meter	Disease[1]	Lodging
Pullman, WA	Palouse silt loam	181	499	No	15.6	Apr, 16	2.51	SR	Moderate
Fairfield, WA	Palouse silt loam	107	443	No	16.1	Apr, 13	2.51	None	Severe
Aberdeen, ID	Declo silt loam	100	291	330	15.0	Apr, 17	1.74	None	Slight
Tetonia, ID	Tetonia silt loam	300	613	No	11.1	May, 5	0.87	None	None

[1] SR stripe rust, [2] seasonal rainfall and average temperature are from April 98 to August 98, [3] annual rainfall is September 97-August 98.

Table 2. Summary of QTL effects for plant height, NRN and seed weight traits.

Chr.	Marker or interval	Interval size cM	Location of QTL in the interval	Additive[1] effect	Main effect test stat.	% variance explained	QxE effect test stat.
				Plant height			
Threshold values for test statistics				QTL main effect		5 %= 18.8	1 %= 23.4
				QTL x env. effect		5 %= 5.9	1 %= 7.3
2	MWG858	-	-	-8.7	96.5**	27.5	9.8**
2	ABC157-ABG317B	6.7	5.0	-5.7	32.7**	10.4	0.3
3	*Dfr*-cMWG680	5.0	2.0	-4.4	20.0*	6.3	0.7
7	WG530-CDO348B	7.4	6.0	-5.0	25.3**	8.1	0.7
				Number of rachis nodes			
Threshold values for test statistics				QTL main effect		5 %= 19.3	1 %= 24.7
				QTL x env. effect		5 %= 5.1	1 %= 6.5
2	MWG858	-	-	-2.1	91.7**	26.3	1.3
3	*Dfr*	-	-	1.0	19.5*	6.3	1.0
6	MWG798A	-	-	-1.1	21.4*	6.9	1.7
				Seed weight			
Threshold values for test statistics				QTL main effect		5 %= 12.0	1 %= 16.2
2	ABG358-ABG459	4.0	2.0	4.2	62.0**	33.9	
2	ABC152D-MWG865	5.1	2.0	3.4	39.5**	23.2	
3	BCD1532-ABG396	16.5	3.0	2.0	12.3*	7.7	

*, ** $P<0.05$ and 0.01, respectively. [1]Negative additive effects indicate that the Morex allele has higher value.

Table 3. Field evaluations of NILs and parents at four locations

Genotype	Genome	Plant height cm	Number of rachis nodes	Seed weight mg	Lodging %	Days to flowering	Grain yield t ha[-1]
			Aberdeen				
Steptoe	S	93.5 H*	18.0 E*	48.3 A*	7.3 C*	66.0 E*	8.58 A*
Morex	M	105.5 AB	19.0 CDE	38.4 DEF	28.8 A	68.0 D	6.39 DE
E19	M+3S	97.3 E-H	20.0 BCD	38.9 D	9.0 BC	68.8 CD	6.06 E
F13	M+3S	98.0 D-H	20.0 BCD	36.8 G	10.8 BC	68.5 D	6.07 E
A24	M+5S	102.3 A-D	20.0 BC	38.0 D-G	19.5 AB	68.3 D	6.23 DE
B2	M+5S	100.5 C-F	19.0 CDE	37.5 D-G	18.8 AB	70.0 B	6.93 CD
B9	M+5S	106.8 A	19.6 BCD	37.0 FG	18.8 AB	70.0 B	6.39 DE
F11	M+5S	103.0 ABC	19.6 BCD	38.5 DE	26.8 A	68.3 D	6.10 DE
F15	M+5S	101.8 B-E	19.4 CD	37.7 D-G	26.8 A	68.0 D	6.48 DE
A23	M+3S+5S	99.3 C-G	21.6 A	37.3 EFG	9.3 BC	69.8 BC	6.58 DE
A38	M+3S+5S	95.8 FGH	20.6 B	37.3 EFG	6.8 C	68.5 D	6.32 DE
F12	M+3S+5S	95.5 G-H	19.8 BCD	37.0 FG	7.3 BC	69.3 BCD	6.35 DE
C5	S+2M	97.3 E-H	18.2 E	46.7 B	5.3 C	76.5 A	8.07 AB
K5	S+2M+3M	96.0 FGH	18.8 DE	43.3 C	5.0 C	77.0 A	7.64 BC
Average		**99.8**	**19.6 A**	**39.2**	**15.1**	**69.7 A****	**6.61 A****
			Fairfield				
Steptoe	S	94.3 C*	16.6 D*	38.8 A*	51.3	56.8 D*	4.96
Morex	M	113.5 A	19.6 AB	32.3 DE	87.5	59.8 BC	4.40
E19	M+3S	101.8 BC	20.0 AB	31.5 E	62.5	60.8 B	4.53
F13	M+3S	102.8 BC	20.6 A	31.3 E	62.5	60.3 BC	4.72
A24	M+5S	115.8 A	19.6 AB	33.2 DE	66.3	59.5 BC	4.49
B2	M+5S	114.3 A	20.0 AB	34.7 CD	60.0	59.5 BC	3.54
B9	M+5S	115.3 A	19.6 AB	33.1 DE	76.3	60.0 BC	4.71
F11	M+5S	113.0 A	19.2 B	33.9 CDE	66.3	58.8 C	4.03
F15	M+5S	112.5 A	19.8 AB	34.1 CDE	73.8	59.8 BC	4.24
A23	M+3S+5S	102.8 BC	20.6 A	32.7 DE	45.0	60.8 B	4.33
A38	M+3S+5S	98.5 CD	19.6 AB	32.8 DE	65.0	59.8 BC	4.35
F12	M+3S+5S	101.8 BC	19.0 B	32.2 DE	62.5	60.5 B	4.77
C5	S+2M	97.3 BC	17.4 CD	36.2 BC	66.3	68.3 A	4.85
K5	S+2M+3M	104.3 B	17.8 C	38.3 AB	61.3	67.8 A	4.07
Average		**106.6**	**19.2 B**	**33.8**	**64.3**	**60.8 B**	**4.41 B**
			Pullman				
Steptoe	S	76.3 DE*	16.0 E*	28.3 ABC*	18.0 B-E*	54.5 F*	2.87
Morex	M	86.0 B	17.6 D	22.8 EF	44.0 AB	57.8 CDE	2.28
E19	M+3S	74.3 E	18.2 CD	23.8 EF	14.3 B-E	58.5 B-E	2.53
F13	M+3S	77.0 DE	19.8 AB	23.5 EF	6.8 CDE	58.0 CDE	2.54
A24	M+5S	80.5 CD	18.4 BCD	24.2 DEF	42.5 AB	57.3 E	2.73
B2	M+5S	82.5 BC	18.0 CD	27.1 BCD	40.0 ABC	59.3 BCD	3.25
B9	M+5S	82.0 BC	18.8 BCD	25.6 CDE	5.8 DE	58.8 B-E	3.14
F11	M+5S	84.0 BC	18.2 CD	25.1 DEF	36.5 A-D	58.3 B-E	2.81
F15	M+5S	84.5 BC	17.8 D	25.1 DEF	33.8 A-E	57.3 E	2.86
A23	M+3S+5S	76.8 DE	21.0 A	22.2 F	19.0 B-E	60.0 B	2.33
A38	M+3S+5S	73.3 E	19.4 BC	24.8 DEF	5.8 DE	57.5 DE	2.73
F12	M+3S+5S	75.0 E	19.0 BCD	22.7 EF	8.0 CDE	59.5 BC	2.45
C5	S+2M	91.0 A	18.2 CD	31.2 A	2.0 E	67.8 A	3.44
K5	S+2M+3M	86.0 B	18.0 CD	28.6 AB	65.0 A	68.0 A	2.43
Average		**81.0**	**18.4 C**	**25.3**	**23.0**	**59.4 C**	**2.75 D**

Table 3 (Cont'd). Field evaluations of NILs and parents at four locations

Genotype	Genome	Plant height cm	Number of rachis nodes	Seed weight mg.	Lodging %	Days to flowering	Grain yield t ha[-1]
			Tetonia				
Steptoe	S	70.0 DE*	14.0 E*	42.1 A*	No lodging was observed in Tetonia	68.5 DE*	4.24
Morex	M	80.5 AB	17.8 BC	33.0 BC		67.0 F	3.57
E19	M+3S	77.0 ABC	17.6 BCD	33.7 BC		69.0 CDE	3.54
F13	M+3S	72.3 CDE	17.0 BCE	33.1 BC		69.3 CDE	3.43
A24	M+5S	80.3 AB	18.0 BC	34.1 BC		68.8 DE	3.55
B2	M+5S	79.5 AB	16.6 BCD	30.2 C		69.3 CDE	4.43
B9	M+5S	77.5 ABC	18.2 B	34.2 BC		70.0 CD	4.22
F11	M+5S	76.5 ABC	16.4 CD	33.5 BC		68.3 EF	4.00
F15	M+5S	81.3 A	17.8 BC	33.5 BC		69.0 CDE	4.12
A23	M+3S+5S	74.5 BCD	19.6 A	33.9 BC		71.5 B	4.32
A38	M+3S+5S	77.0 ABC	17.0 BCD	35.3 B		68.5 DE	3.77
F12	M+3S+5S	75.0 A-D	16.4 CD	32.2 BC		69.3 CDE	3.45
C5	S+2M	68.0 E	14.0 E	42.0 A		73.8 A	4.63
K5	S+2M+3M	71.3 CDE	16.0 D	40.4 A		70.3 BC	3.34
Average		75.7	16.8 D	34.8		69.4 A	3.83
			Averaged over locations				
Steptoe	S	83.5NC	16.2 H*	39.4NC	27.2NC	61.4 H*	5.16 A*
Morex	M	96.4	18.5 DE	31.6	47.9	63.1 G	4.16 B
E19	M+3S	87.6	18.9 B-E	32.0	29.0	64.3 DE	4.16 B
F13	M+3S	87.5	19.4 B	31.2	28.2	64.0 DEF	4.19 B
A24	M+5S	94.7	19.0 B-E	32.4	40.7	63.4 FG	4.25 B
B2	M+5S	94.2	18.4 E	32.4	38.2	64.5 D	4.54 B
B9	M+5S	95.4	19.1 BCD	32.5	32.6	64.7 D	4.61 B
F11	M+5S	94.1	18.4 E	32.7	40.2	63.4 FG	4.23 B
F15	M+5S	95.0	18.7 B-E	32.5	41.9	63.5 FG	4.44 B
A23	M+3S+5S	88.3	20.7 A	31.5	27.5	65.5 C	4.39 B
A38	M+3S+5S	86.1	19.2 BC	32.5	26.7	63.6 EFG	4.29 B
F12	M+3S+5S	86.8	18.6 CDE	31.0	27.4	64.6 D	4.26 B
C5	S+2M	88.4	17.0 G	39.0	25.7	71.6 A	5.25 A
K5	S+2M+3M	89.1	17.6 F	37.9	42.2	70.8 B	4.35 B

* differences between the means with the same letter are not significant at 5 %. NC combined analysis over locations was not performed.

Table 4. Malting quality characteristics of isogenic lines and parents from two locations

Genotype	Genome	Kernel Plumpness (%)	Malt extract %	Grain protein %	Diastatic power °ASBC	Alpha amylase 20°DU	Beta glucans ppm
				Aberdeen			
Steptoe	S	96.6 A*	75.9 D*	10.25 C*	51.7 F*	22.05 H*	777 A*
Morex	M	86.9 DE	78.1 AB	13.25 A	158.5 A-D	44.92 DEF	192 D
E19	M+3S	88.1 CDE	77.9 AB	13.25 A	166.7 AB	51.35 A	126 E
F13	M+3S	83.1 E	77.7 ABC	13.47 A	163.0 ABC	47.42 BCD	167 DE
A24	M+5S	88.0 CDE	77.8 AB	13.27 A	154.7 BCD	42.10 F	195 D
B2	M+5S	90.2 BCD	77.4 B	13.37 A	168.7 A	46.02 CDE	187 D
B9	M+5S	89.7 BCD	78.1 AB	12.87 A	164.0 ABC	47.87 A-D	192 D
F11	M+5S	86.6 DE	77.8 AB	12.87 A	156.5 A-D	47.30 BCD	195 D
F15	M+5S	87.7 CDE	78.3 A	13.20 A	165.5 AB	49.80 AB	162 DE
A23	M+3S+5S	85.3 DE	77.7 AB	12.76 A	149.2 D	48.30 A-D	207 D
A38	M+3S+5S	84.1 E	77.8 AB	12.96 A	151.7 CD	49.30 ABC	164 DE
F12	M+3S+5S	84.9 DE	77.8 AB	12.80 A	135.7 E	43.30 EF	202 D
C5	S+2M	93.9 AB	76.6 C	10.92 BC	62.7 F	23.85 GH	489 B
K5	S+2M+3M	92.4 ABC	75.8 D	11.10 B	62.3 F	26.50 G	426 C
Average		**87.3**	**77.5**	**12.75**	**139.9**	**43.10 B****	**252**
				Fairfield			
Steptoe	S	78.6 A*	75.4 B*	9.62 C*	51.0 C*	24.42 E*	517 B*
Morex	M	62.7 ABC	76.4 AB	11.65 AB	140.2 AB	52.75 A	170 D
E19	M+3S	57.5 BC	77.5 AB	12.10 AB	150.2 AB	48.12 BC	191 D
F13	M+3S	51.4 C	76.3 AB	12.55 A	155.7 AB	50.15 AB	197 D
A24	M+5S	63.3 ABC	76.7 AB	12.27 AB	150.2 AB	48.87 BC	220 D
B2	M+5S	74.9 AB	78.3 A	11.57 AB	130.5 B	51.52 AB	232 D
B9	M+5S	66.2 ABC	77.9 AB	11.65 AB	137.7 AB	45.45 C	197 D
F11	M+5S	63.5 ABC	77.9 AB	11.47 AB	134.0 AB	49.30 ABC	203 D
F15	M+5S	69.9 ABC	77.8 AB	12.10 AB	142.0 AB	47.83 BC	233 D
A23	M+3S+5S	55.3 C	76.9 AB	12.07 AB	152.7 AB	48.20 BC	171 D
A38	M+3S+5S	59.2 BC	77.2 AB	12.35 AB	163.2 A	49.62 AB	214 D
F12	M+3S+5S	53.6 C	77.1 AB	12.57 A	157.5 AB	45.47 C	158 D
C5	S+2M	64.0 ABC	73.0 C	10.95 BC	64.7 C	26.82 DE	605 A
K5	S+2M+3M	68.2 ABC	72.5 C	10.82 BC	58.5 C	28.65 D	431 C
Average		**61.7**	**76.5**	**11.86**	**131.3**	**44.79 A**	**261**
				Averaged across locations			
Steptoe	S	87.6 NC	75.7 NC	9.94 NC	51.4 NC	23.23 E*	647 NC
Morex	M	74.8	77.2	12.45	149.4	48.83 AB	181
E19	M+3S	72.8	77.7	12.68	158.5	49.73 A	158
F13	M+3S	67.2	77.0	13.01	159.4	48.78 AB	182
A24	M+5S	75.7	77.3	12.78	152.5	45.48 C	207
B2	M+5S	82.6	77.9	12.48	149.6	48.77 AB	210
B9	M+5S	77.9	78.0	12.26	150.9	46.66 BC	194
F11	M+5S	75.0	77.8	12.18	145.3	48.30 AB	199
F15	M+5S	80.1	78.0	12.73	155.4	48.95 AB	192
A23	M+3S+5S	72.0	77.4	12.46	151.0	48.25 AB	189
A38	M+3S+5S	69.9	77.5	12.61	157.5	49.46 A	189
F12	M+3S+5S	69.3	77.4	12.69	146.6	44.38 C	180
C5	S+2M	78.9	74.8	10.94	63.8	25.33 E	547
K5	S+2M+3M	78.6	73.9	10.94	60.1	27.72 D	429

* Differences between means with the same letters are not significant at 5 % level. NC, combined analysis over locations was not performed.

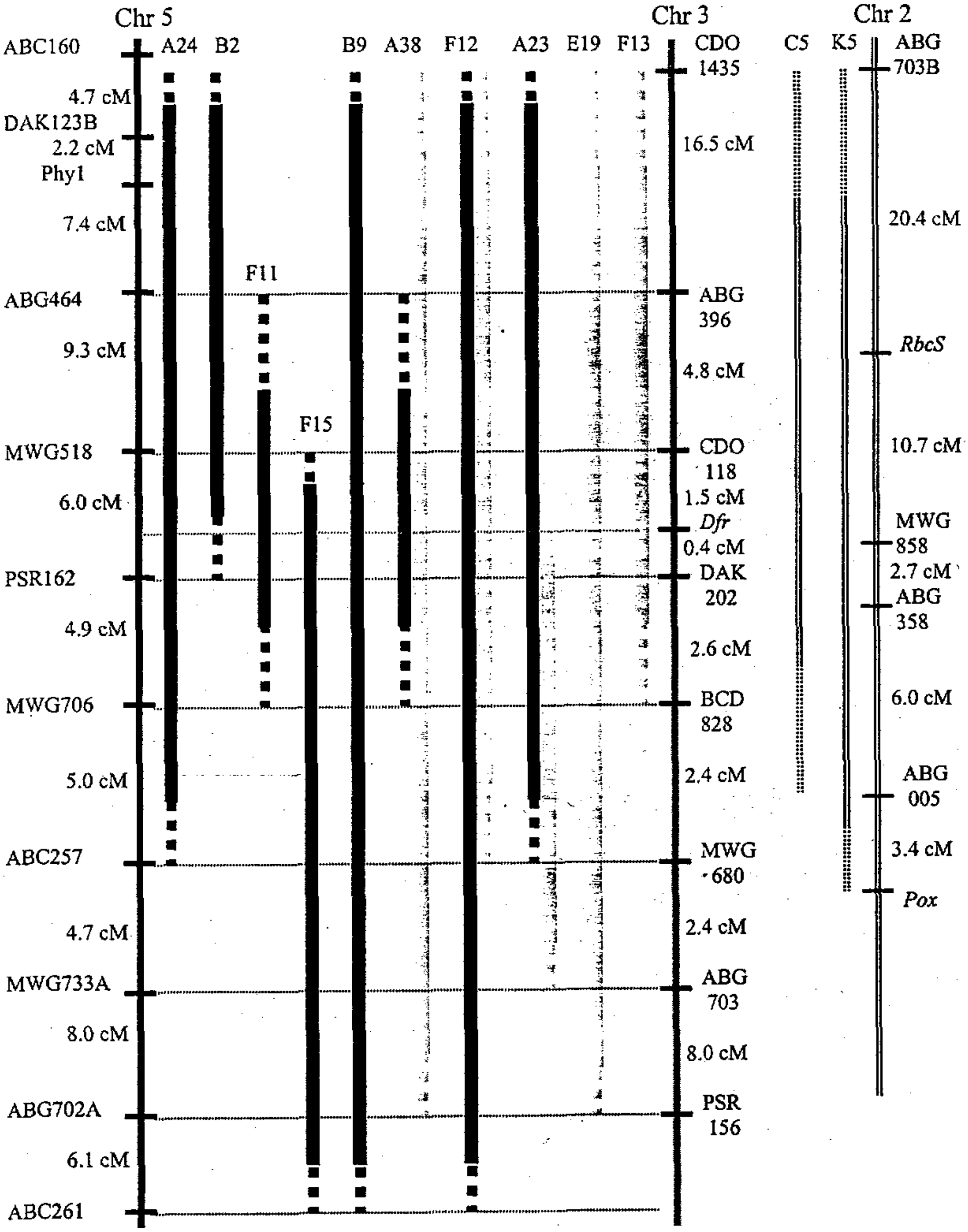

Figure 1. Genomic composition of NILs evaluated in multilocational yield trials. Solid lines show introgressed fragments in near isogenic lines while dotted lines show the recombination region. Dark lines are from chromosome 5, gray lines are from chromosome 3 and double lines are from chromosome 2. K5 line also carries DAK202 region from Morex as determined by lax spike cosegregating with DAK202.

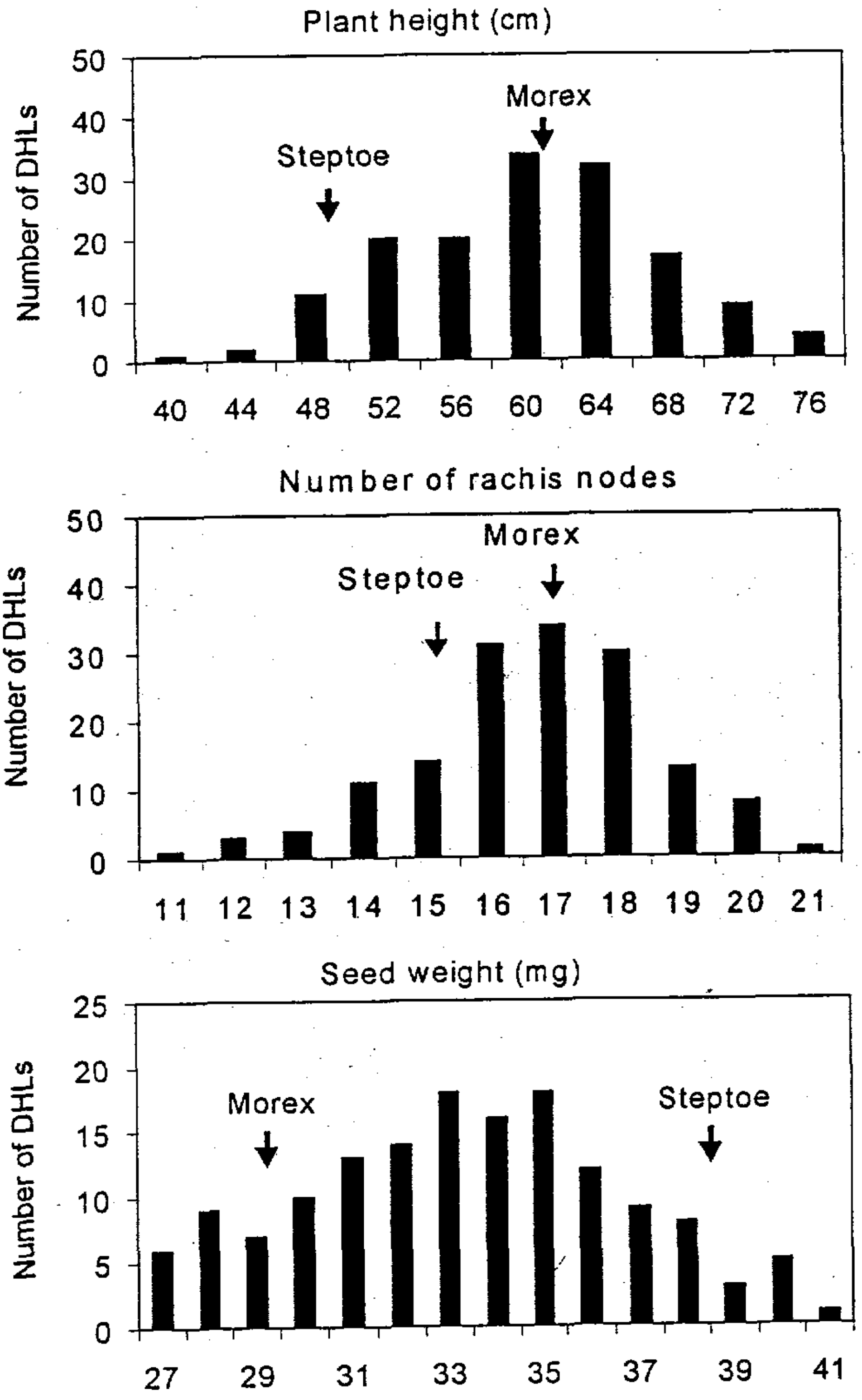

Fig. 2. Frequency distributions of SM DHLs for plant height, number of rachis nodes/spike and seed weight

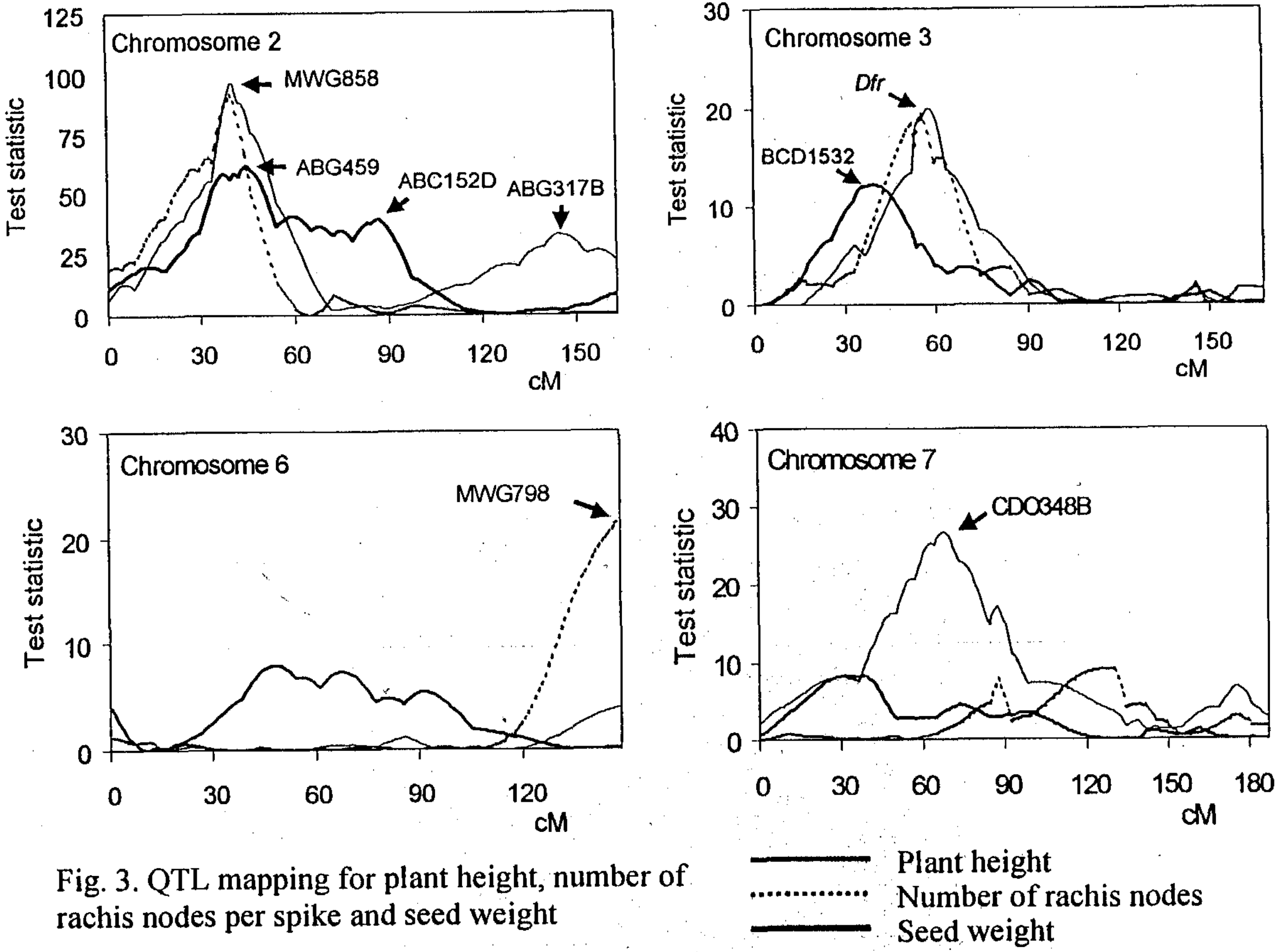

Fig. 3. QTL mapping for plant height, number of rachis nodes per spike and seed weight

CHAPTER THREE

Molecular marker assisted genetic analysis of head shattering in six-rowed barley

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ABSTRACT

Head shattering in barley (*Hordeum vulgare L.*) has two forms; brittle rachis and weak rachis. Brittle rachis is not observed in cultivated barley since all cultivars carry non-brittle alleles at one of the two brittle rachis loci (*Btr1*/*Btr2*). Weak rachis causes head shattering in barley cultivars and is often confused with brittle rachis. Brittle rachis was previously mapped to chromosome 3 short arm while map position(s) of the weak rachis was unknown. Two major and a putative minor QTL for head shattering were mapped using the "Steptoe" x "Morex" doubled haploid population. The largest QTL, designated *Hst-3*, was located on chromosome 3 centromeric region. Steptoe alleles from *Hst-3* region, when transferred into Morex, resulted in a substantial decrease of head shattering. High resolution mapping of *Hst-3* was achieved using isogenic lines. The map position of *Hst-3* is different from that of brittle rachis. The second major QTL, designated *Hst-2S*, was located on chromosome 2S and showed environmental sensitivity. Usefulness of *Hst-2S* in breeding for head shattering resistance is in question due to possible intervention of a yield QTL mapped to the same region.

Key words Head shattering . Brittle rachis . Weak rachis . QTL . Spike density . Peduncle curve

INTRODUCTION

Head shattering causes serious yield losses in barley throughout the world. Two mechanisms are known. In the first type, joints in the rachis nodes easily disarticulate resulting in segmentation of the rachis and loss of seed along with a piece of the rachis (Franckowiak and Konishi 1996). This type of head shattering, termed "brittle rachis", is found in *Hordeum vulgare ssp. spontaneum*, and is considered a non-cultivated species adaptive feature for seed dispersal (Takahashi 1955). In the second type, the rachis breaks resulting in the loss of a spike segment (Kaufman and Shebeski 1954). However, the breaking point of the rachis, node or internode, is not known. Descriptions in literature indicate the possibility of a third kind of head shattering where the kernel itself detaches from the rachis (Platt and Wells 1949; Chapman and Hockett 1976).

Genetics of brittle rachis have been extensively studied. Brittleness is due to two complementary genes (Takahashi 1955), later named *Btr1* and *Btr2* (Franckowiak and Konishi 1996). Brittle is dominant to non-brittle. *H. vulgare ssp. spontaneum* has brittle rachis as a consequence of the dominant forms of both genes (Takahashi 1964). In a study to determine the distribution of *Btr1*/*Btr2* genes in cultivated barley, 1267 cultivars were crossed to "tester genotypes" with known genetic composition for the *Btr* genes (Takahashi 1964). Results indicated that every barley cultivar tested had either the *Btr1* or *Btr2* gene in a homozygous recessive form resulting in a non-brittle rachis. Referring to two reports by Takahashi and Hayashi, Franckowiak and Konishi (1996) noted that two brittle rachis genes were located on chromosome 3 short arm and closely linked. There also have been reports of independent segregation of the two brittle rachis loci (Johnson and Aberg 1943; Turcotte 1957), although the close linkage conclusion is now generally accepted.

Brittle rachis has been reported to be stable and easy to distinguish from non-brittle rachis indicating little environmental effect, but the degree of toughness in the non-brittle group can be variable (Aberg and Wiebe 1948). Segregation ratios that do not fit into the two complementary gene model have been reported suggesting interactions with additional loci (Smith 1951).

Head shattering as caused by rachis breaking has not been as well described as brittle rachis. Kaufman and Shebeski (1954) working with crosses between "weak" and "tough" rachis cultivars observed that the F_1 plants had a weak rachis that shattered. In the F_2 population, plants with tough, weak and intermediate rachis were observed. Because of the difficulty of differentiating weak rachis from intermediate ones, they combined both classes and obtained 3 weak: 1 tough rachis segregation ratio. These data suggest that the weak rachis may be mediated by a major dominant gene interacting with some minor genes. Kaufman and Shebeski (1954) concluded that their observed weak rachis phenotype was different from the brittle rachis phenotype. Platt and Wells (1949) and Clarke (1981) did not clearly distinguish rachis breaking from brittle rachis. Thus, the weak rachis phenotype has not been adequately differentiated from brittle rachis to date.

Platt and Wells (1949) reported that head shattering was promoted by irrigation. Planting time was another factor affecting head shattering where earlier planting resulted in more head shattering losses (Rutger *et al.* 1966). Less head shattering is observed in lodged plants (Kaufman and Shebeski 1954). Turcotte (1957) cited Ubisch's findings that "dense spikes have less tendency to be brittle". Kaufman and Shebeski (1954) speculated that varieties with curved (nodded) heads would have less head shattering because of less weathering damage on the rachis due to rain. Platt and Wells (1949) reported that heads with larger seeds would shatter more than the ones with smaller seeds. These reports indicate that head shattering due to rachis breaking is influenced by environment and associated with some morphological traits.

Using backcross derived homozygous Steptoe x Morex lines, Larson *et al.* (1996) mapped two head shattering QTL to barley chromosome 3 around DNA markers ABG396 and ABG057. These markers are separated by about 44 cM (Kleinhofs *et al.* 1993). The two peaks explained 13.9 and 13.7 % of the variation, respectively. This work also showed an association between head density and head shattering with dense heads shattering less.

In another study, using an "Igri" x "Danillo" cross, three head shattering QTL were mapped to intervals of MWG557-MWG769 (50 cM), MWG611-MWG921 (2 cM) and MWG820C-MWG820B (13 cM) on chromosomes 2, 4 and 6, respectively. These QTL explained 44 % of the genetic variance. Ear breaking, lodging, stalk breaking and "physical state of the plants before harvest" traits had QTL at the same regions. It was suggested that the stability of the stem tissue was the determining factor for all of the above traits (Backes *et al.* 1995).

Genetic control of rachis breakage is complex possibly due to multiple-locus inheritance and to environmental effects. Availability of a genetic map with satisfactory genome coverage facilitates simultaneous detection of multiple loci affecting complex traits. Tagging of hose loci with simply inherited molecular markers is useful to develop isogenic lines, which have uniform genetic backgrounds and segregate only for the QTL of interest. Analysis of complex traits is easier in uniform genetic backgrounds such as near isogenic lines (NILs) since the interactions with modifying loci are standardized (Tanksley 1993). Development of a series of NIL where each NIL has a fragment from the target region also facilitates high resolution mapping of the QTL. In the present study, we analyzed head shattering and related traits via both QTL mapping and NIL analysis. A high-resolution map of a major head shattering QTL on barley chromosome 3 was also developed.

MATERIALS AND METHODS

Head shattering was studied in a Steptoe x Morex cross. Both Steptoe and Morex are six-rowed, spring type cultivars. Morex heads shatter in some environments resulting in serious yield losses (Nedel *et al.* 1993). Steptoe heads generally do not shatter. Steptoe and Morex (SM) are the parents of a 150 doubled haploid line (DHL) mapping population of the North America Barley Genome Mapping Project (http://www.css.orst.edu/barley/nabgmp.htm) which has been extensively mapped with RFLP markers (Kleinhofs *et al.* 1993; http://barleygenomics.wsu.edu).

QTL mapping was performed with the 150 SM DHLs grown in 3.5 m single rows in Pullman, WA, in 1996 and 1997. The 1997 trial was arranged in randomized complete blocks with two replicates. The 1996 trial was not replicated. Based on the map position and completeness of marker data, 149 markers were selected to obtain a skeleton map consisting of markers spaced approximately 6-8 cM apart. Marker distances were confirmed using Mapmaker EXP (Lander *et al.* 1987). QTL analysis was conducted using MQTL software which can handle data repeated over environments (Tinker and Mather 1995a, 1995b). The 1996 and 1997 trials constituted the two environments. Linear regression based simple interval mapping (Haley and Knott 1992) of MQTL was used first. Test statistics for QTL main effects and QTL x environment effects were calculated. Threshold values for test statistics to check type 1 error rate were calculated for both QTL main effects and QTL x environment interaction effects using permutations replicated 5,000 times for 5 % and 10,000 times for 1 % significance levels, respectively (Churchill and Doerge 1994). Using the results of simple interval mapping, simplified composite interval mapping (sCIM) based on partial regression analysis was applied (Zeng 1993; Zeng 1994). For sCIM, markers closest to the peaks of QTL detected by simple interval mapping were used as background markers for each trait. This approach was advocated by QTL Cartographer software (Basten *et al.* 1997), which employs linear regression based interval mapping just like MQTL. Threshold values were not calculated for sCIM since calculation of threshold values using permutations was not advised using multiple environment data (Tinker and Mather 1995b). A strong test statistic value was accepted as an indication of a QTL. The term "secondary QTL" is used for the QTL detected only by sCIM. Additive effects calculated by MQTL are for two alleles at each locus and, therefore, are twice that obtained with other QTL software. Multiple regression analysis was used to calculate interaction between QTL based on principles explained by Allen (1997) using SAS software (SAS Institute 1991). F-test was used to detect significance level of QTL x QTL interaction.

In order to determine the exact location of the head shattering QTL with the largest effect, a series of NILs were developed based on preliminary information about the presence of a major head shattering QTL on barley chromosome 3 (T. Blake, personal communication). The DHL SM23 carried the entire Steptoe chromosome 3 while DHL SM84 had a small piece from Morex at the distal part of the long arm of chromosome 3. The SM23 and SM84 lines were backcrossed to Morex four times and then selfed to produce the BC_4F_2 lines. Molecular markers were used to select the Steptoe allele of chromosome 3 in every generation starting with BC_2F_1. Genetic background of isogenic lines was checked using markers every 20-25 cM on the SM map in the BC_4F_1 generation. Lines with recombination in the target region were grown to BC_4F_3 and homozygous individuals were selected by genotyping. The result was a series of isogenic cv. Morex lines each with a fragment of Steptoe chromosome 3 in the region presumed to contain the head shattering QTL. Only RFLP markers were used in this study except for two morphological markers, short rachilla hair and pubescent leaf. DNA isolation, Southern transfer and hybridization methods, and morphological marker descriptions were as described by Kleinhofs *et al.* (1993). In order to accelerate generation turnover, 20-day old embryos were cultured on a growth medium to start a new generation without waiting for seed maturation. The MS basal medium (Murashige and Skoog 1962) supplemented with vitamins and 30 g/L maltose without any hormones was used. Seedlings were directly transferred to pots when they reached 5-10 cm height.

Head shattering was observed in isogenic lines grown as single rows under field conditions in Pullman, WA in 1997. Evaluation of head shattering phenotypes and marker genotypes of each isogenic line were used to obtain a high-resolution map of the largest head shattering QTL.

The map position of *H. vulgare ssp. spontaneum* brittle rachis locus (*Btr*) was determined using the barley genetic stock GSHO1937. This genetic stock carries the brittle rachis trait from *H. vulgare ssp. spontaneum* introduced into cv. Bowman through BC_9F_4 generations with selection for the brittle rachis trait (Wolfe and Franckowiak 1990). The GSHO1937 line was analyzed with chromosome 3 RFLP markers to determine the location and size of the introgressed *H. vulgare ssp. spontaneum* DNA fragment. The genomic DNA of Bowman was used as a control. Unfortunately, the *H. vulgare ssp. spontaneum* parent of GSHO1937 was not available (JD Franckowiak, personal communication). High polymorphism rate between cultivated barley and *H. vulgare ssp. spontaneum* (Petersen *et al.* 1994) and use of multiple markers provided a reasonable confidence for the results.

Head shattering of DHLs and NILs was observed 2-3 weeks after the crop reached maturity (Platt and Wells 1949). Observations were made based on a visual assessment of the percentage of shattered heads in a plant row. The percentage was converted to a 1-5 scale (1=0%, 2=1-15 %, 3=16-30 %, 4=31-45 % 5>46 %). A spike with a missing piece was considered as shattered.

In addition to head shattering, a number of other traits that may be related to head shattering were analyzed. These traits were the number of rachis nodes (NRN), spike density (nodes/cm) and peduncle curve. The NRN x 3 (three seeds per node in six-rowed barley) is equal to the number of seeds on the spike. Head density was calculated as NRN / spike length (cm). Peduncle curve was scored on a scale of 1 to 3, with 1 being the most curved, 3 being erect, and 2 intermediate. Data for these traits were collected from DHLs grown as single rows in 1996 and 1997. In addition, spike density and head shattering data were collected from NILs grown as single rows at Pullman, WA in 1997.

RESULTS

Head shattering was scored on 150 SM DHLs field grown at Pullman, WA during 1996 and 1997. The head shattering trait, showed a skewed distribution pattern in both years (Fig. 1) where half of the DHLs had no head shattering and the other half had varying degrees of shattering. Only 10 lines in 1996 and 19 lines in 1997 had a score of 5, the highest level of head shattering. Parents, Steptoe and Morex, had the extreme values of 1 and 5, respectively.

Morex and DHLs with high head shattering score had long rachis internodes. These spikes also had a relatively high number of seeds and erect (uncurved) peduncles. The non-shattering parent Steptoe and DHLs, on the other hand, had short rachis internodes, fewer seeds per spike and curved peduncles. Based on these observations, we analyzed spike density (nodes/cm), number of rachis nodes per spike (NRN) and peduncle curvature in the 150 DHLs (Fig. 1). NRN and spike density traits showed transgressive segregation. Average NRN and spike density values were statistically greater ($P<0.01$) in 1997 (18.0 and 2.9) than in 1996 (14.5 and 2.8).

QTL mapping of head shattering and related traits

Head shattering and related traits were mapped as QTL, first using simple interval mapping (SIM) and later simplified composite interval mapping (sCIM). Test statistics calculated by both SIM and sCIM for QTL main effects and QTL x environment interaction effects are summarized in Table 1. Additive effects and per cent variance explained by each QTL are also presented in Table 1. Test statistics calculated by sCIM are presented as graphics in Fig. 2. sCIM results did not show any major deviation in positions of QTL detected by SIM. Magnitude of test statistics was generally similar or higher with sCIM in QTL regions detected by SIM. However, sCIM identified additional QTL. Hence, sCIM test statistic graphics are used to illustrate QTL positions detected by SIM.

Head shattering

Head shattering QTL analysis using simple interval mapping detected two major QTL on chromosomes 2S and 3 centromeric region, designated *Hst-2S* and *Hst-3*, respectively (Fig. 2). *Hst-3* had the peak at the RFLP marker *Dfr* (dihydroflavonol-4- reductase, Kristiansen and Rohde 1991) on chromosome 3. This QTL had a test statistic of 101.2 with SIM (Table 1, $P<0.01$) and explained 28.6 % of the variation. DHLs with the Steptoe *Dfr* allele had 1.3 less head shattering score compared to DHLs with the Morex allele. None of the 19 DHLs with the highest head shattering score in 1996 and 1997 had the Steptoe *Dfr* allele while about 1/3 of the non-shattering group had the Morex *Dfr* allele. *Hst-3* x environment interaction was not significant.

The peak of the *Hst-2S* QTL coincided with the RFLP marker MWG858. The Steptoe MWG858 allele reduced the head shattering by a score of 1.0 compared to the Morex allele (Table 1). This QTL had a test statistic of 48.5 with SIM ($P<0.01$) and explained 14.9 % of the variation for head shattering based on two year data. On the other hand, QTL analysis conducted for each year showed that head shattering variance due to QTL represented by MWG858 is 23.0 % of the total variance in 1997 but only 7.4 % in 1996 (data not shown). This difference is possibly the reason for the significant QTL x environment interaction detected by two-year QTL analysis (Table 1, $P<0.05$).

Hst-3 and *Hst-2S* showed an additive gene effect (Fig. 3). When the Morex alleles of the two QTL were present, the average head shattering score was 3.2. Presence of Steptoe alleles from both QTL regions resulted in a head shattering score of 1.1. Based on multiple regression analysis, the two major head shattering QTL had a highly significant ($P<0.01$) interaction that explained 2.1 % of the variation. Together, *Hst-3*, *Hst-2S* and their interactions explained about 45 % of the variation for head shattering. A major part of the variation remained unexplained and possibly was due to unidentified loci and non-genetic factors including variability caused by environment and experimental error.

sCIM was performed using MWG858 and *Dfr* as background markers. This effort yielded a secondary QTL with strong test statistics on chromosome 7L, designated *Hst-7L* (Fig. 2, Table 1). The QTL profile was broad without a distinct peak, perhaps due to the presence of a 31.2 cM gap between markers in this region. Two markers, CDO057B and CDO504, were used to represent this putative QTL. The CDO057B was the closest marker to the test statistic peak

(17.0 with sCIM) in the interval of CDO057B to mSrh markers. The marker CDO504 on the distal side of the gap had a test statistic of 10.1. CDO057B and CDO504 explained 2.1 and 1.1 % of the variation for head shattering. The Steptoe allele decreased the head shattering score by 0.5 and 0.4, respectively (Table 1). Interactions between *Hst-7L* and *Hst-3* or *Hst-2S* were not calculated due to the uncertainty of the map location of the putative chromosome 7 QTL.

Spike density

The only spike density QTL detected by SIM was on chromosome 3 in the same position and shape as the head shattering QTL *Hst-3* (Fig. 2). The spike density QTL had a very large test statistic of 368.9 with SIM (Table 1, P<0.01) and explained 70.8 % of the variation for spike density. The Steptoe *Dfr* allele was associated with the dense spike trait (an average of 0.7 more nodes/cm spike length than Morex). During the backcross and selfing generations, we observed that the lax spike character is dominant to dense spike. sCIM mapping using the *Dfr* marker as background yielded four secondary QTL for spike density each explaining a relatively small part of the variance (Table 1). Two of these QTL were on chromosome 1, one on chromosome 2 and one on chromosome 4. None of these QTL regions showed coincidence with head shattering.

Number of rachis nodes

The largest NRN QTL coincided with the head shattering QTL *Hst-2S*, also located on chromosome 2 (Fig. 2). This QTL had a test statistic of 91.7 with SIM (Table 1) and explained 26.3 % of the variation for NRN. Morex MWG858 allele resulted in an average of 2.1 more nodes (about six seeds) per spike. The second largest QTL had a peak at the MWG798A marker of chromosome 6 explaining 6.9 % of the variance (Table 1). Morex allele of this QTL conferred 1.1 more nodes/spike. A third NRN QTL explained 6.3 % of the variance and peaked at the *Dfr* marker on chromosome 3 where *Hst-3* was mapped (Table 1, Fig. 2). In this case, it was the Steptoe *Dfr* allele that resulted in increased NRN values (about 1.0 node/spike).

Peduncle curve

The peduncle curve trait was associated with head shattering in the extremely shattering SM DHLs i.e. DHLs with the highest shattering (score of 5) had erect peduncles while the DHLs with the lowest shattering (score of 1) had curved peduncles. Simple interval mapping showed a QTL on chromosomes 2 and 7. The larger QTL marked by CDO504 on chromosome 7, was in the vicinity of a minor head shattering QTL, had a test statistic of 82.9 with SIM (Fig. 2 and Table 1, $P<0.01$) and explained 24.1 % of the variation. The second QTL mapped close to marker MWG858 of chromosome 2, had a test statistic of 33.4, and coincided with the second largest head shattering QTL, *Hst-2S*, and the largest NRN QTL (Fig. 2). MWG858 explained 10.5 % of the variation for the peduncle curve trait. sCIM using CDO504 and MWG858 as background markers, yielded two secondary QTL for peduncle curve both on chromosome 3 and 11 cM apart. These two QTL had test statistic peaks in the MWG571B-ABG377 and ABG398-*Dfr* intervals and explained 3.4 and 4.6 % of the variance, respectively. The Steptoe alleles of the two chromosome 3 peduncle curve QTL resulted in erect peduncles while the Steptoe alleles of the chromosome 2 and 7 QTL resulted in curved peduncles.

High resolution mapping of chromosome 3 head shattering QTL via NILs

It was observed that the breaking of the rachis in shattered heads always happened at the attachment points of rachis internodes. However, this breakage was different from the segmentation or disarticulation of rachis internodes, which in the brittle rachis trait occurs at almost every attachment point. *Hst-3* NILs presented two head shattering phenotypes, either 5 (46 % or higher shattering) or 2 (1-15 % shattering). All lines with a Steptoe segment in the

ABG396-ABG703 region, except E15 and A18, had shattering score of 2 and short rachis internodes (Fig. 4). Since the shortest proximal segment terminated between *Dfr* and DAK202 and the shortest distal segment terminated between DAK202 and DAK160B, we conclude that *Hst-3* resides in 0.6 cM interval between *Dfr* and DAK160B markers. The spike density trait cosegregated with *Hst-3* locus in NILs and was located to the same 0.6 cM interval marked by *Dfr* and DAK160B markers where *Hst-3* was located.

Mapping of the *H. vulgare ssp. spontaneum Btr2* locus

RFLP analysis of the GSHO1937, *H. vulgare ssp. spontaneum* derived brittle rachis line, and its backcross parent line cv. Bowman revealed an *H. vulgare ssp. spontaneum* segment containing markers MWG798B and MWG014, but not including markers ABC171 and ABG396 (Fig. 5). We conclude that the *Btr2* gene resides in this region and does not overlap with *Hst-3* in the *Dfr* to DAK160B interval. Since the Bowman parent is believed to carry *Btr1* (J. Franckowiak, personal communication) it is not possible to determine the location of *Btr1* in this genetic stock.

DISCUSSION

It has been speculated that the head shattering QTL on chromosome 3 may represent weak alleles of the brittle rachis genes *Btr1* and *Btr2*. Our data indicate that the *Btr2* brittle rachis gene, and presumably *Btr1* due to close linkage, are distal from the weak rachis QTL *Hst-3*. These data are in agreement with the different genetic (simple inheritance of brittle rachis vs. quantitative inheritance of weak rachis), mechanism (disarticulation of rachis internodes in every attachment point in brittle rachis vs. one or two breaks in weak rachis) and environmental effects (less environment effect on brittle rachis vs. high environment effect on weak rachis) of the two traits. However, this fact does not exclude the possibility that the traits may be due to diverged duplicated genes.

The *Hst-3* QTL is probably the same as the head shattering QTL reported by Larson *et al.* (1996) although they observed two peaks with the main one at marker ABG396 and the other one is about 44 cM away on the short arm of chromosome 3. We found a single QTL with a peak at the marker *Dfr*, 2.1 cM proximal to ABG396. The *Hst-3* QTL is also probably the same as a previously reported major yield, lodging, plant height and head shattering QTL (Hayes *et al.* 1993a and 1993b; Larson *et al.* 1996).

Hst-3 was associated with a major QTL for spike density. Head shattering-spike density association has been previously reported (Turcotte 1957; Larson *et al.* 1996). Presence of clear transgressive segregation for spike density (Figs. 1 and 6), but not for head shattering, might indicate that head shattering is not a direct result of lax spikes. The spike density locus around the *Dfr* marker must coincide with head shattering due to either pleiotropic effects or tightly linked genes.

Hst-3 explained nearly one-third of the head shattering variation in SM DHL population. Skewed distribution and the presence of intermediate types in the DHL population suggested the involvement of additional loci. QTL mapping uncovered a second major head shattering locus on chromosome 2 (*Hst-2S*) and a putative minor locus on chromosome 7 (*Hst-7L*). Further evidence for multiple head shattering loci was provided by the *Hst-3* NILs. Head shattering in these NILs showed a clear phenotypic separation with scores of either 2 or 5. The Steptoe head shattering score of 1 was not recovered.

The *Hst-2S* QTL is possibly associated with the NRN trait that also has a major QTL with a peak around MWG858. Indeed, considerably less head shattering was observed in DHLs that carry relatively fewer seeds on their spikes. *Hst-2S* had a significant QTL x environment interaction and explained a much higher proportion of head shattering variance in 1997 where NRN values were higher than in 1996. Higher head shattering in spikes that carry more seeds is in accordance with higher head shattering damage under growing conditions that favor development of large spikes e.g. early planting dates (Rutger *et al.* 1966) and irrigated conditions (Platt and Wells 1949). These findings imply that *Hst-2S* might play a significant role in high yielding environments. It is possible that *Hst-2S* is associated with the yield QTL mapped previously on chromosome 2S (Hayes *et al.* 1993a). Since the parent that provides the higher yielding allele changes by environments, transfer of this head shattering QTL from Steptoe into Morex might interfere with Morex yields in some environments. The only previous report indicating the presence of a head shattering QTL on chromosome 2 is from a two-rowed barley cross (Backes *et al.* 1995). The size of the reported QTL interval (50 cM) prevented comparison with our mapping results.

In conclusion, Steptoe allele of *Hst-3* can be used for molecular marker assisted breeding of head shattering resistance in six-rowed barley. Presence of 11 RFLP markers within one cM distance of this locus provides multiple choices for such efforts. Similarly, Steptoe allele of *Hst-2S* can be used to decrease head shattering losses at least in some environments.

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Table 1. Summary of QTL effects for head shattering and related traits.

				SIM				sCIM		
Chr.	Marker or interval	Interval size cM	QTL location	Additive effect[1]	ME[2] TS	% var.	QxE TS	Additive effect	ME TS	QxE TS
				Head shattering						
2	(MWG858)	-	-	-1.0	48.5**	14.9	4.4	-0.9	56.9	5.9
3	(Dfr)	-	-	-1.4	101.2**	28.6	1.9	-1.3	111.3	1.9
7	CDO057B-mSrh	5.4	2.0	-0.4	6.3	2.1	0.4	-0.5	17.0	0.7
7	CDO504	-	-	-0.3	3.3	1.1	0.0	-0.4	10.1	0.1
				Spike density						
1	ABC158	-	-	0.1	9.7	3.2	0.0	0.1	22.7	0.0
1	RZ242-ABC310B	9.2	5.0	-0.1	5.8	1.9	0.1	-0.1	22.5	0.4
2	ABC167B-bBE54D	6.0	4.0	0.0	0.5	0.0	0.7	-0.1	23.7	2.1
3	(Dfr)	-	-	0.7	368.9**	70.8	4.3	0.7	368.9	4.3
4	BCD265B-ABG003A	3.8	3.0	-0.1	6.8	2.2	0.4	-0.1	22.7	1.4
				Number of rachis nodes						
2	(MWG858)	-	-	-2.1	91.7**	26.3	1.3	-2.2	108.9	1.7
3	(Dfr)	-	-	1.0	19.5*	6.3	1.0	1.2	36.2	1.7
6	(MWG798A)	-	-	-1.1	21.4*	6.9	1.7	-0.8	15.7	1.8
				Peduncle curve						
2	ABC156A-(MWG858)	6.0	5.0	-0.5	33.4**	10.5	9.5**	-0.5	40.5	12.8
3	ABG398-Dfr	1.3	1.2	0.3	14.1	4.6	26.9**	0.3	18.3	43.5
3	MWG571B-ABG377	11.7	3.0	0.3	10.4	3.4	20.6**	0.4	23.9	31.6
7	MWG514-(CDO504)	4.4	4.0	-0.8	82.9**	24.1	4.1	-0.8	93.0	4.4

*,** P<0.05 and 0.01, respectively. [1]Negative additive effects indicate that the Morex allele has higher value. Markers in paranthesis are used as background markers for sCIM analysis. [2]ME, main effect; TS, test statistics.

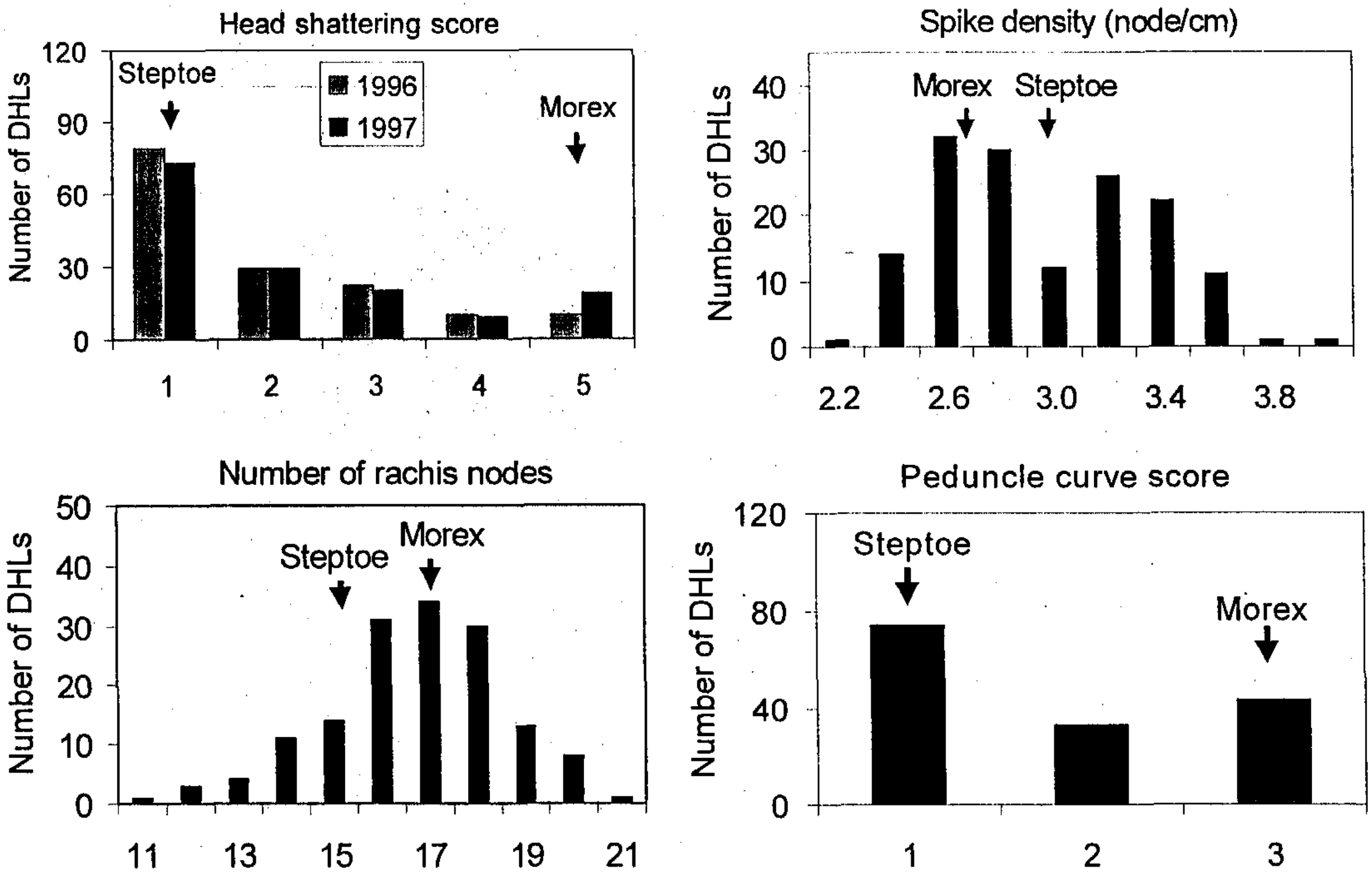

Fig. 1. Frequency distributions of SM DHLs for head shattering, spike density, number of rachis nodes and peduncle curvature.

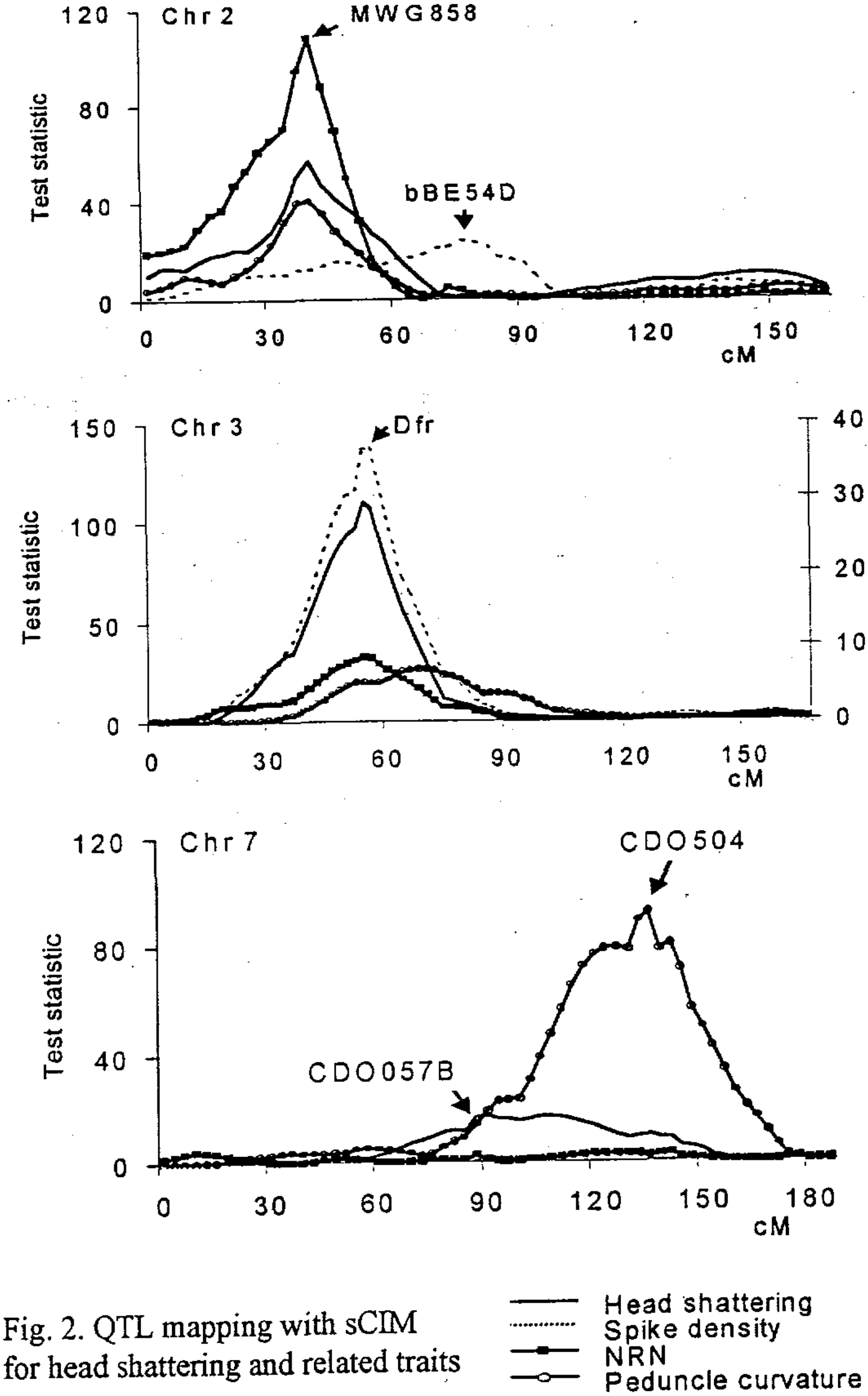

Fig. 2. QTL mapping with sCIM for head shattering and related traits

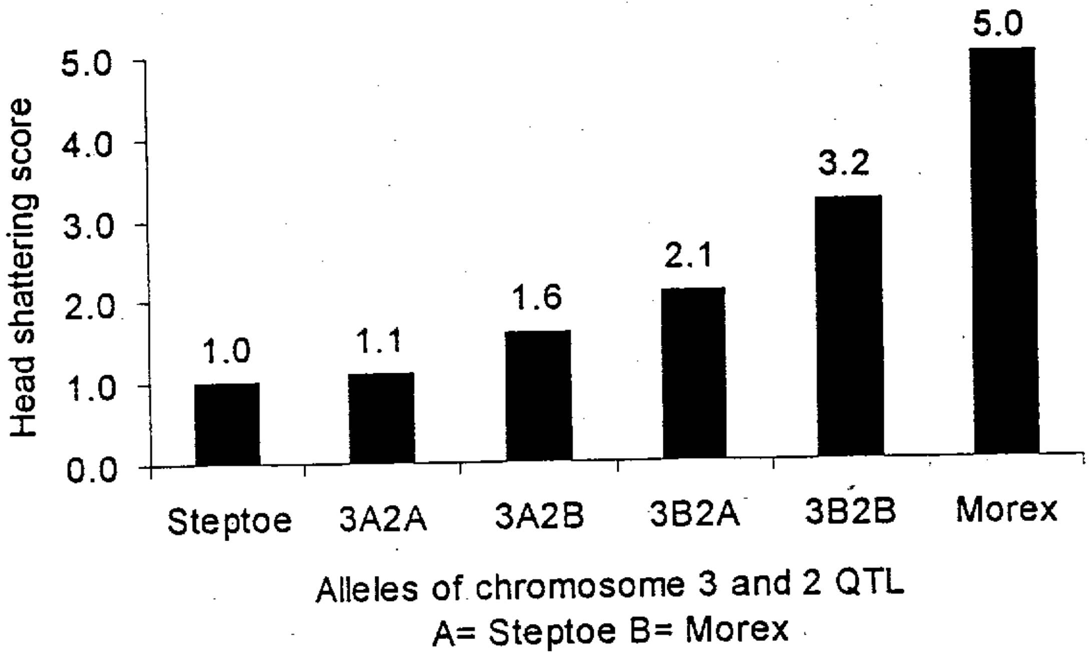

Fig. 3. Interaction between chromosome 3 (*Hst-3*) and 2 (*Hst-2S*) head shattering QTL

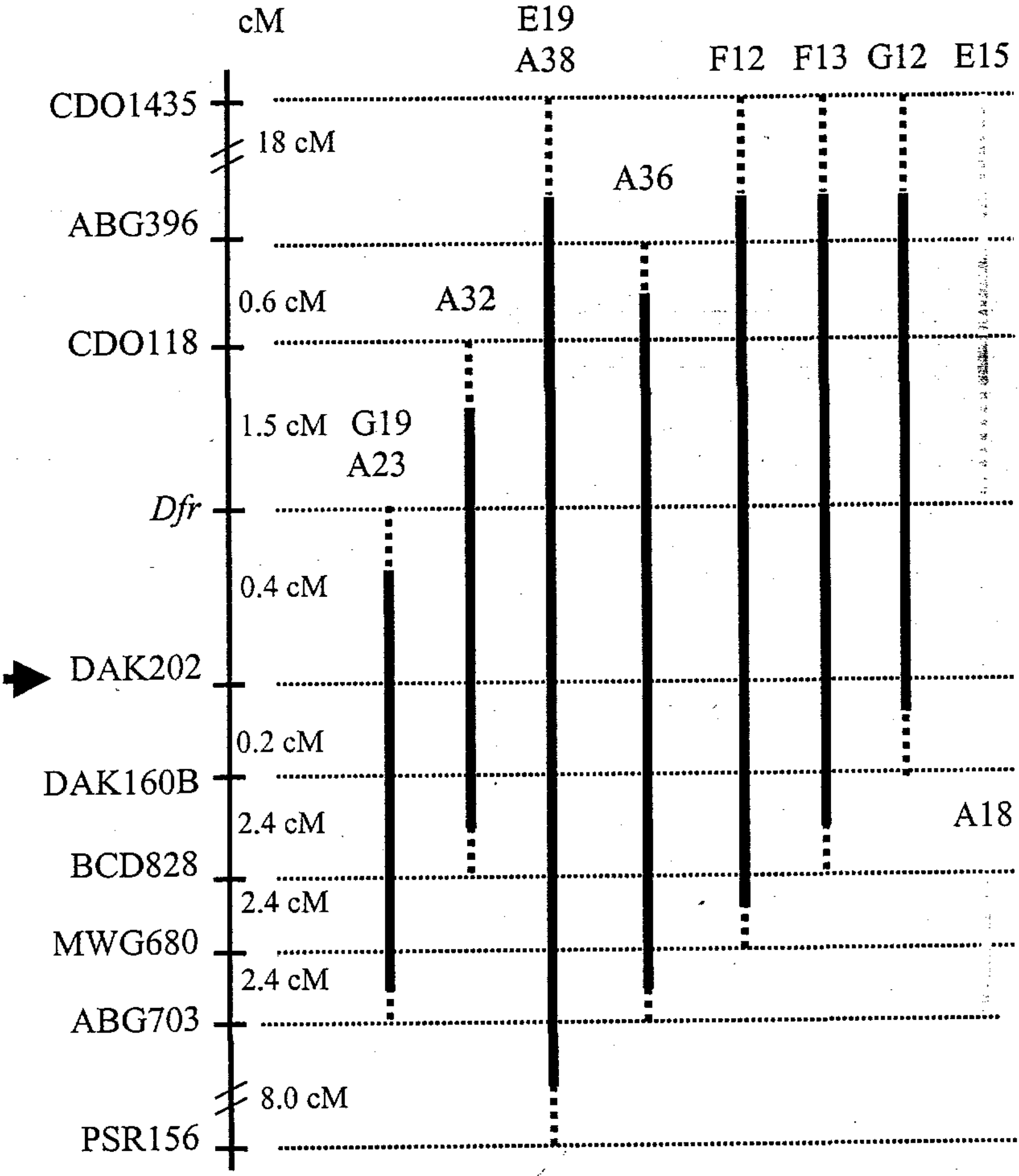

Fig. 4. High resolution mapping of chromosome 3 head shattering locus. Thick lines are Steptoe fragments, dashed lines are cross-over region, dark lines show non-shattering lines while gray lines show shattering ones. Arrow shows head shattering locus. ABG462 and KsuA3C co-segregates with *Dfr*; *Dor*4A and RSB020 co-segregates with DAK202; *Adh*5, KsuF2B, PSR626A and ABC156C co-segregates with DAK160B. *Dfr*, DAK202 and DAK160B cosegregate in original 150 SM DH population

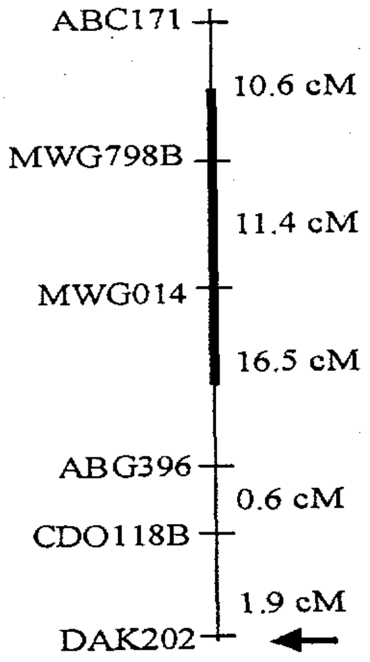

Fig. 5. *H. vulgare ssp. spontaneum* fragment in GSHO1937 (thick line). Arrow shows the location of chromosome 3 head shattering QTL, *Hst-3* detected in SM cross

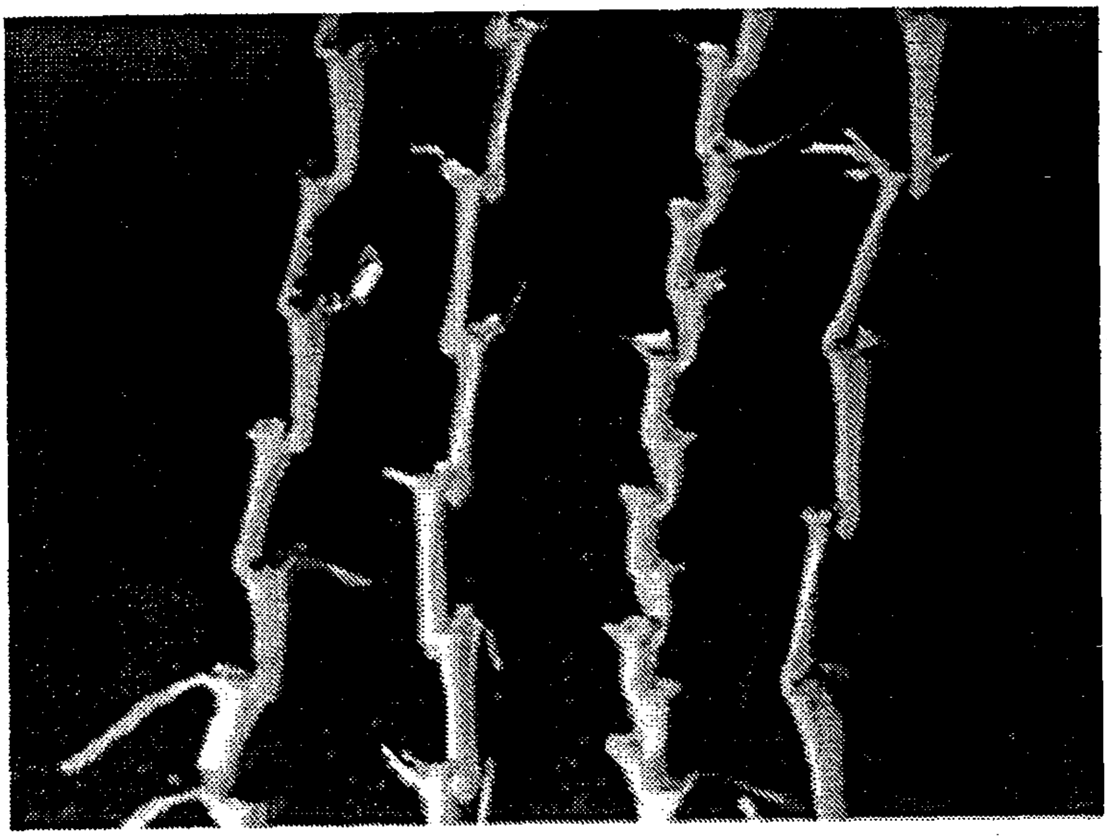

Fig. 6. Spike density in Steptoe x Morex cross. From left to right; Steptoe, Morex, Morex NIL with Steptoe DAK202 region (F13), and Steptoe NIL with Morex DAK202 region (K5). K5 NIL was derived in BC3F2. K5 was not evaluated for head shattering and related traits

CHAPTER FOUR

Marker assisted analysis of flowering time in a spring barley cross

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ABSTRACT

Flowering time is an adaptive trait with a significant role in crop performance. In cereals, flowering time is determined by three classes of genes; vernalization, photoperiod response, and developmental rate (earliness per se). The 150 doubled haploid line mapping population of a Steptoe x Morex spring barley cross was used to identify loci that affect flowering time in short and long day conditions. Two major QTL, each affecting flowering under either long or short daylength conditions, were detected on chromosome 2S(2H) and 5L(1H), and designated *Phr-2S* and *Phr-5L*, respectively. Near isogenic lines developed by transferring late flowering Morex alleles from *Phr-2S* region into Steptoe located the long daylength locus in a 0.9 cM interval flanked by the MWG858 and *Tef4* markers of chromosome 2S. Near isogenic lines developed for *Phr-5L* located the short daylength flowering locus to a 6.0 cM interval flanked by MWG518 and PSR162 markers on chromosome 5L. Map positions of *Phr-2S* and *Phr-5L* suggest that they might be the same as two previously mapped photoperiod response loci, *Ppd-H1* and *Ppd-H2*. Presence of transgressive segregation in the field grown (long daylength) DHLs and failure to quantitatively recover the short daylength late flowering parent phenotype in NILs with *Phr-5L* suggested the presence of additional genes affecting flowering time in both environments.

Key words Photoperiod sensitivity . Flowering time . Marker assisted backcrossing . QTL mapping . Near isogenic lines

INTRODUCTION

Flowering time in cereals is under the control of three classes of genes. Vernalization and photoperiod response genes determine flowering time as a response to environmental conditions. The third class of genes controls the flowering time via the developmental rate of the plant and are named "earliness per se" genes (Laurie *et al.* 1995). These three classes of genes and their interactions determine the adaptation of genotypes to growing environments and are often associated with the production potential of crop plants. Vernalization response genes are not associated with flowering time of spring barley. Earliness per se (*eps*) genes have relatively small effects on flowering time and are expressed both in short and long day conditions (Laurie 1997).

Photoperiod response genes have large effects on flowering time. Most of these genes affect flowering time either in short or long days. A flowering gene, *Eam1*, is located on chromosome 2S(2H) in the barley morphological marker map. The dominant allele confers early flowering under long day conditions (Franckowiak 1996). A major flowering time QTL, *Ppd-H1*, was mapped 1 cM proximal to the MWG858 marker and in a comparable region to *Eam1* (Laurie *et al.* 1995). *Ppd-H1* delays flowering time by 12 days in spring sowing, seven days in fall sowing and 10 days in controlled environments under 16 h light (Laurie *et al.* 1994). *Ppd-H1* has been proposed to be the barley equivalent of the wheat *Ppd* gene and may be the same as *Eam1* (reviewed by Laurie 1997). A number of traits such as plant height, number of seeds per spike and seed weight have been associated with this flowering locus (Laurie *et al.* 1994). Studies with a doubled haploid line (DHL) mapping population of a cross between two spring barley cultivars, Steptoe and Morex, showed a QTL for flowering time in 15 out of 16 environments (Hayes *et al.* 1993). Morex carries the late flowering alleles with additive effects ranging from three to ten days. The peak of the QTL was in either the ABG002-ABG459 interval or in the nearby interval *RbcS*-ABG002 on chromosome 2 (Hayes *et al.* 1993). Both intervals also have been associated with plant height and grain yield. QTL for flowering time has also been reported in comparable map positions with T. Prentice x V. Gold (Kjaer *et al.* 1995) and Dicktoo x Morex crosses (Karsai *et al.* 1997).

Several short daylength photoperiod response genes are known. Three mutations for flowering time under short days have been mapped. These are *Eam*8 (*Ea*$_k$) mapped on chromosome 5L(1H) toward the telomere (Takahashi and Yasuda 1971), *Eam9* (or *Ea9*) mapped on 4L(4H) (Yasuda and Hayashi 1980), and *Eam10* (*Ea*$_{sp}$) mapped on chromosome 3L(3H) (Gallagher *et al.* 1991). Mutant plants reach flowering earlier under short day conditions. A fourth gene, a spontaneous mutant designated *Eam7* (*Ea7*), has been mapped on chromosome 6S (6H) near the centromere (Stracke and Borner 1998). *Eam7* affected flowering time both in short and long days although its effect was three times stronger in short days compared to long days.

Flowering time under short days has also been studied using QTL analysis. Laurie *et al.* (1995) mapped a QTL, *Ppd-H2*, on barley chromosome 5L(1H), approximately 3 cM distal to the PSR162 marker. *Ppd-H2* affects flowering time under controlled short day conditions and in fall planting but not in spring planting (Laurie *et al.* 1995). Pan *et al.* (1994) reported two intervals (*iPgd2*-BCD265C and *HorD*-ABG452) on chromosome 5L that affect flowering time under short days. Dicktoo chromosome regions of these intervals delayed the flowering time by 12-17 days in controlled environments (8 h light), and 8 days in fall-sown field experiments.

The complex nature of flowering time, including minor QTL and epistatic effects, can be analyzed using molecular marker technology. Development of isogenic lines is encouraged for this purpose (Pan *et al.* 1994; Laurie *et al.* 1995). Our objectives were to detect loci affecting flowering time in short and/or long day conditions, and to achieve high resolution mapping of two flowering loci mapped as QTL on chromosomes 2S (Hayes *et al.* 1993) and 5L in the Steptoe x Morex population. Isogenic lines developed for these flowering loci will be very useful in studying the epistatic interactions among them (Karsai *et al.* 1997; Laurie *et al.* 1995; Gallagher *et al.* 1991). High-resolution mapping will also facilitate the alignment of mutation-QTL-candidate gene data as well as map based cloning of the flowering genes.

MATERIAL AND METHODS

The Steptoe x Morex 150 DHL mapping population was used for QTL mapping. Long day flowering time was measured on each field grown DHL as single rows in 1996 and 1997 at Pullman, WA. The 1997 trial had two replications while the 1996 trial was not replicated. Planting date was April 27 in 1996 and May 13 in 1997. Flowering under short days was observed in a controlled growth room environment with 11 h 30 min light/12 h 30 min dark cycle and 18°C /13 °C day/night temperature. One plant from each DHL was evaluated.

Based on the map position and completeness of marker data, 149 markers were selected to obtain a skeletal map consisting of markers spaced approximately 6-8 cM apart. Marker distances were confirmed using Mapmaker EXP (Lander *et al.* 1987). QTL analysis was conducted using MQTL software which can utilize data repeated over environments (Tinker and Mather 1995a; 1995b). The 1996 and 1997 data constituted the two environments for flowering observations at the field condition. Linear regression based simple interval mapping (Haley and Knott 1992) of MQTL was used. Test statistics for QTL main effects and QTL x environment effects were calculated. Threshold values for test statistics to check type 1 error rate were calculated for both QTL main effects and QTL x environment interaction effects using permutations replicated 5,000 times for 5 % and 10,000 times for 1 % significance levels, respectively (Churchill and Doerge 1994). Additive effects calculated by MQTL are for two alleles at each locus and, therefore, are twice those obtained with other QTL software.

Based on the preliminary results with QTL mapping, near isogenic lines (NILs) were developed for two genomic regions on chromosome 2S and chromosome 5L. To produce chromosome 5 NILs, SM23 and SM84 DHLs were backcrossed four times to Morex and selfed. Recombinants from the target region were selected in the BC_4F_2 and homozygous NILs were isolated from the BC_4F_3. Several RFLP markers were used to select the Steptoe alleles of a large target area (about 50 cM) on chromosome 5 in every generation starting with BC_2F_1. For chromosome 2 NILs, SM179 was backcrossed to Steptoe three times with selections using molecular markers. NILs were selected from the BC_3F_3 generation. The genetic backgrounds of the chromosome 5 isogenic lines were checked using markers every 20-25 cM on the SM map in the BC_4F_1 while this procedure was not applied for chromosome 2 NILs. In order to accelerate generation turnover, 20-day old embryos were grown in tissue culture to start a new generation without waiting for seed maturation. The MS basal medium (Murashige and Skoog 1962) supplemented with vitamins and 30 g/L maltose without hormones was used. Seedlings were directly transferred to pots when they reached 5-10 cm height. RFLP markers and two morphological markers, short rachilla hair and pubescent leaf on chromosomes 7 and 3 were used in this study. DNA isolation, Southern transfer and hybridization methods, and morphological marker descriptions were as described by Kleinhofs *et al.* (1993).

Flowering time of all NILs and two parents were detected in field conditions grown as single rows in 1998 at Pullman, WA. Ten plants from segregating lines were also observed in greenhouse conditions. Day/night duration was approximately 16 h/8 h. Temperature varied between 18 and 25 °C. Chromosome 5 NILs along with parents and one chromosome 2 NIL, K5, with a large fragment from the chromosome 2 flowering QTL region were grown in controlled environments of growth room and growth chamber. In the first experiment, 17 NILs and parents were grown in a growth room with 11 h light / 13 h dark period under 23 °C constant temperature. In the second experiment, 10 NILs and parents were grown in growth chambers with 10 h 30 min light/13 h 30 min dark with 20 °C / 13 °C day/night temperature. Most plants grown in short daylength conditions were genotyped using molecular markers around the target regions.

Flowering time was measured as the time from planting to the time when 50 % of the plants had awns emerged. Short daylength observations were subjected to statistical analysis since the NILs showed a continuous distribution. Analysis of variance was performed based on the principles explained by Steel *et al.* 1997. Completely randomized block design with three replications (each plant was a replication) was used. SAS software was used for analysis of variance (SAS Institute 1991). Long day observations, on the other hand, could be grouped in discrete classes, hence, statistical analysis was not performed.

RESULTS

QTL mapping

The SM DHL population grown under long day field conditions showed a clear transgressive segregation for flowering time (Fig. 1). As the average of two years, Steptoe and Morex reached flowering in 50 and 52 d, respectively. Flowering time of the DHLs varied from 46 to 63 d. Simple interval mapping using the 150 DHLs grown under field conditions (long days) yielded one significant QTL in the interval of ABC156A-MWG858 (Table 1). This QTL, named *Phr-2S* (PHotoperiod Response), explained 52.4 % of the variation and the Morex allele from the QTL region delayed the flowering time by about six days. The QTL x environment interaction was also significant (P<0.01).

The SM DHL population showed a non-normal distribution for short daylength flowering time (Fig. 2). The DHLs formed two sub-populations with a break around 145 d. Flowering times of the parents Steptoe and Morex were nearly at the extreme locations of the frequency diagram. QTL mapping with 150 SM DHLs under controlled short daylength conditions detected one significant QTL at the MWG518 marker of chromosome 5 (Table 1, Fig. 3). This QTL, designated *Phr-5L*, explained 16.5 % of the variation and Steptoe alleles of the QTL region delayed the flowering by 28 days.

Near isogenic line (NIL) analysis

Near isogenic lines developed by transferring *Phr-2S* QTL from Morex into Steptoe (Fig. 4) formed two discrete phenotype classes for flowering time (data not shown). The Morex late flowering QTL region introduced into Steptoe background caused a prostrate growth habit and delayed flowering time for about 11 d under both greenhouse and Pullman, WA field conditions. The D7 NIL carried a Morex fragment from *Tef4* [elongation factor 1α (Nielsen *et al.* 1997)] to ABG005 but did not have late flowering, implying that the *Phr-2S* locus was distal to *Tef4* (Fig. 4). The presence of MWG858 and *RbcS* Morex alleles at the distal side in the K1C NIL, but absence of late flowering, placed the *Phr-2S* locus proximal to MWG858, i.e. in the 0.9 cM interval of MWG858-*Tef4*. The presence of the late flowering phenotype in the eight NILs (C1-1, C5, K4, D2, C1-7, L18, K1B and K5) which carry the Morex alleles from this interval confirmed the location. Heterozygous plants, detected by codominant RFLP markers, revealed that early flowering was dominant to late flowering. The NIL, K5, with the late flowering region from Morex had Steptoe like flowering phenotype under controlled short day environment indicating that *Phr-2S* expresses only under long day conditions.

The flowering time of 19 NILs, each containing a fragment from Steptoe chromosome 5 *Phr-5L* region in a Morex background, were observed under short daylength conditions in two experiments. The first experiment was conducted with 16 NILs, and Steptoe and Morex, in a growth room with 10 h 30 min light at 23 °C constant temperature. Development of narrow leaves about 1 cm wide indicated late flowering. As plants proceeded toward flowering, larger leaves were formed. Morex flowered 100 d after planting while Steptoe needed 165 d (Table 2). The flowering times of seven NILs (A2, A11, A15, A35, A52, A54 and B2) were very similar to Morex. Eight NILs (A4, A12, A24, A39, B17, F12, F15 and F16) flowered 112 to 126 d after planting. The NIL, G7, was in the middle with a flowering time of 107 d. One plant from the line F16 (designated F16A) was heterozygous for three markers (Fig. 5). The heterozygous F16A plant had the Morex like flowering phenotype although the other two F16 plants were in the late flowering NIL group, indicating that early flowering is dominant to late flowering (Table 2, Fig. 5). However, this finding needs to be verified by observation of additional NILs heterozygous for the markers in the target region or by observation of the progeny of the heterozygous F16A plant.

The second experiment was conducted in a growth chamber with 10 h 30 min light and 13 h 30 min dark; 20 °C day/ 13 °C night temperature conditions. In this experiment, three NILs that were not observed in the previous experiment were used along with eight NILs that were used previously, and Steptoe and Morex. Morex flowered 89 d after planting, as the average of three plants (Table 2). Steptoe flowered 157 d after planting. The NILs A11, A15 and G7 had similar flowering time to Morex. Seven other lines (A4, A38, A40, B17, F11, F12 and F15) flowered after 101-111 d.

Although the differences between the NILs showed a continuous variation, we grouped them as early and late for the sake of simplicity of representation (Fig. 5). Because of its clear Morex like flowering phenotype in the second experiment, G7 was placed in the early flowering group. All of the early flowering NILs had Morex alleles of PSR162 and BCD592 (cosegregates with PSR162 in SM map and in NILs) except F16A, which was heterozygous for both markers. Presence of the Steptoe alleles of MWG518 and other markers in the proximal side of the chromosome in early flowering NILs A11 and B2 implies that the *Phr-5L* is distal to MWG518. Absence of Steptoe alleles of MWG518 and other markers at the distal side in the F15 NIL also shows that the locus is distal to MWG518. Presence of the Steptoe allele of MWG518 but absence of PSR162 (and BCD592) in the late flowering line A12 means that *Phr-5L* is in MWG518-PSR162 (BCD592) interval. Two other late flowering NILs (F11 and B17), which carry Morex alleles of MWG092 and other markers distal to it, clearly show that *Phr-5L* can not be distal to MWG092. Morex NILs that carry Steptoe alleles of the *Phr-5L* region flowered at a time similar to Morex in field conditions (long days) indicating the photoperiod sensitivity of this locus.

DISCUSSION

Steptoe and Morex, which have a similar long daylength flowering time produced DHL progeny with clear transgressive segregation for flowering time. QTL mapping identified a major QTL, *Phr-2S*, in the interval of ABC156A-MWG858 of chromosome 2 that accounted for more than half of the variation in flowering time. Evaluation of NILs with the Morex fragments from *Phr-2S* region in a Steptoe background allowed us to map this flowering locus to a 0.9 cM interval flanked by the markers MWG858 and *Tef4*. Early flowering was dominant to late flowering. The mapping data suggested that *Phr-2S* may be the same as *Ppd-H1* photoperiod response QTL mapped 1 cM proximal to MWG858 in the Igri x Triumph cross (Laurie *et al.* 1995). A yield QTL and a number of agronomic trait QTL mapped in the vicinity of the marker ABG002 (Hayes *et al.* 1993), which cosegregates with MWG858 in SM map, are likely to be the result of *Phr-2S*.

Under field conditions at Pullman, WA, Morex plants flowered one to two days later than Steptoe plants. Considering the 11 d delay in flowering due to the Morex *Phr-2S* in the Steptoe background, Morex appears to have additional genes that accelerate flowering time by approximately nine or ten days. Neither our QTL mapping, nor that of Hayes *et al.* (1993) conducted in sixteen environments using the same SM DHL population identified such a flowering QTL. Morex flowering might be accelerated by a number of minor QTL, rather than a major one like *Phr-2S*, which are difficult to detect with QTL mapping. Another explanation could be that epistatic interactions rather than additive effects play a significant role for such an effect. Failure to detect additional flowering QTL could also be because these loci are in regions with poor marker coverage. Powell *et al.* (1997) reported the detection of new QTL after introducing new markers to their maps. With extensive multilocational data and a confirmed major locus, detection of additional loci for flowering time in the Steptoe x Morex population offers a model system to test the power of new QTL mapping approaches.

QTL analysis for flowering time under short days detected a QTL, designated *Phr-5L*, at the MWG518 marker or just distal to it, on chromosome 5L(1H). *Phr-5L* affects flowering time only under short daylength conditions, which implies that it has photoperiod sensitivity. NIL analysis showed that *Phr-5L* is in the 6.0 cM interval of MWG518-PSR162, being close to PSR162. The map location of this QTL suggests that *Phr-5L* may be the same as the photoperiod response QTL *Ppd-H2* mapped by Laurie *et al.* (1995) using Igri x Triumph DHL population and as short daylength flowering QTL mapped by Pan *et al.* (1994) using the Dicktoo x Morex DH population. Although Pan et al (1994) reported two intervals for the QTL, it is possible that this was an artifact due to the poor marker coverage of chromosome 5(1H) in their population. Based on QTL mapping, Laurie *et al.* (1995) reported that *Ppd-H2* is 3 cM distal to PSR162 in a 16.9 cM interval. Although only one NIL puts *Phr-5L* proximal to PSR162 in our study, a number of other NILs showed that it was very close to PSR162, if not proximal to it. A different map position in our NIL analysis and QTL mapping by Laurie *et al.* (1995) is possibly because of different methods used (i.e. NIL analysis vs. QTL mapping). Similarly, QTL positions of both *Phr-2S* and *Phr-5L* were shifted in our QTL mapping compared to our NIL analysis. If *Phr-5L* and *Ppd-H2* are the same gene, then the map position detected by our NIL analysis would be expected to be more accurate since NIL analysis reduces the effects of other loci and has been reported to be more reliable (Paterson 1998).

Dominant forms of at least four genes are required for short daylength photoperiod sensitivity of barley and the presence of a recessive form of anyone of these genes confers relative photoperiod insensitivity (Gallagher *et al.* 1991). Steptoe has been reported to have the dominant forms of all four genes (*Eam7*, *Eam8*, *Eam9* and *Eam10* on chromosomes 6, 5, 4 and 3 respectively) (Gallager *et al.* 1991). Comparison of map positions of *Eam8* and *Phr-5L* on chromosome 5L using morphological (Franckowiak 1996) and molecular marker maps (Kleinhofs et al. 1993 and unpublished) aligned by *fch7* locus (Kudrna et al. 1996) suggests that the two loci are different (Fig. 6). The closest marker to the *Phr-5L* (PSR162) is about 36 cM proximal to MWG912 and *fch7*, which, in turn, are about 16 cM proximal to *Eam8*. Thus, *Phr-5L* is probably different from *Eam8* and possibly is the fifth locus affecting barley flowering time under short daylength conditions.

The Steptoe fragments from *Phr-5L* region introgressed into Morex background showed continuous variation in flowering time. This continuous variation probably originated, at least in part, from environmental factors and experimental error, which could not be totally eliminated by replications due to limited space in controlled environments. *Phr-5L* delayed flowering time only by 12 to 26 d in the Morex background. The failure to recover Steptoe-like late flowering in the Morex NILs indicates the presence of additional genes for short daylength flowering time in Steptoe, which may act in additive manner or have epistatic interaction with *Phr-5L*.

One Morex background NIL (F16A) turned out to be heterozygous in the *Phr-5L* region. This plant flowered similar to Morex implying that early flowering is dominant at this locus. Although none of the four known short daylength flowering loci confers early flowering in a dominant form, reports involving short daylength flowering time of Steptoe progeny support these data. Gallagher *et al.* (1991) found that the F_1 of Steptoe x Super Precoz (recessive for *Eam10*) flowered earlier than Steptoe, which would not be possible with the four-locus model since Steptoe carries dominant forms of all four known short daylength flowering genes that confer late flowering (Gallagher *et al.* 1991). In this case, Steptoe must have a recessive and Super Precoz a dominant allele of a gene, which accelerates flowering in the heterozygous state. If this locus is the same as *Phr-5L*, then *Phr-5L* is different from the other four short daylength flowering loci since the dominant allele confers early flowering.

In conclusion, we mapped and verified two photoperiod response genes; *Phr-2S* is expressed in long day conditions and may be identical to *Ppd-H1*, and *Phr-5L* is expressed in short day conditions and may be identical to *Ppd-H2*. The *Phr-2S* locus has a major effect on flowering time and resulted in clear-cut phenotypic separation of the NILs. The *Phr-5L* locus effect was less clear with continuous variation. Our data indicate that both Steptoe and Morex have additional genes for flowering time in long and short days, which were not detected in this study. Near isogenic lines produced in this study will be useful for further studies to identify these genes and investigate their interactions.

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Table 1. Summary of QTL effects for field and controlled short daylength flowering time.

Chr.	QTL	Marker or interval	Interval size cM	QTL location	Additive effect[1]	Main effect TS	% variance	QxE TS
				Awn emergence time in field conditions (long days)				
Threshold values :		QTL main effect			5 %= 21.3	1 %= 27.4		
		QTL x env. interaction			5 %= 3.2	1 %= 4.2		
2	*Phr-2S*	ABC156A-MWG858	5.4	4.4	-5.7	222.7**	52.4	9.6**
				Awn emergence time in controlled short day environment				
Threshold values for QTL main effect					5 %= 12.6		1 %= 15.7	
5	*Phr-5L*	MWG518			28.1	27.0**	16.5	

** P<0.01. [1], negative additive effects indicate that the Morex allele has higher value.

Table 2. Flowering time of NILs under short daylength conditions

NIL	MWG 518	PSR 162	No of plants	Flowering time (day)	NIL	MWG 518	PSR 162	No of plants	Flowering time (day)
Experiment 1 Growth room 11 h light 23/23 °C day/night temperature					Experiment 2 Growth chamber 10 h 30 min light 20/13 °C day/night temperature				
Steptoe	A[1]	A	3	165 A**	Steptoe	A	A	3	157 A**
F12	A	A	3	126 B	A38	A	A	3	111 B
A39	A	A	2	121 BC	A40	A	A	3	111 B
A4	A	A	3	119 CD	A4	A	A	3	107 BC
B17	A	A	3	119 CD	F12	A	A	3	105 BC
F16	A	A	2	118 CDE	B17	A	A	3	103 BC
A12	A	B	3	114 DE	F15	B	A	3	102 C
A24	A	A	3	113 DEF	F11	A	A	3	101 C
F15	B	A	3	112 EF	G7	B	B	3	91 D
G7	B	B	3	107 FG	A15	B	B	3	90 D
A52	B	B	3	102 GH	Morex	B	B	3	89 D
A2	B	B	3	101 H	A11	A	B	3	88 D
A11	A	B	3	101 H					
A35	B	B	3	100 H					
B2	A	B	3	100 H					
A54	B	B	3	100 H					
Morex	B	B	3	100 H					
A15	B	B	3	99 H					
F16A	H	H	1	99					

** means by the same letter are not different at 1 % level of probability.

[1] A Steptoe, B Morex. F16A was not included in statistical analysis.

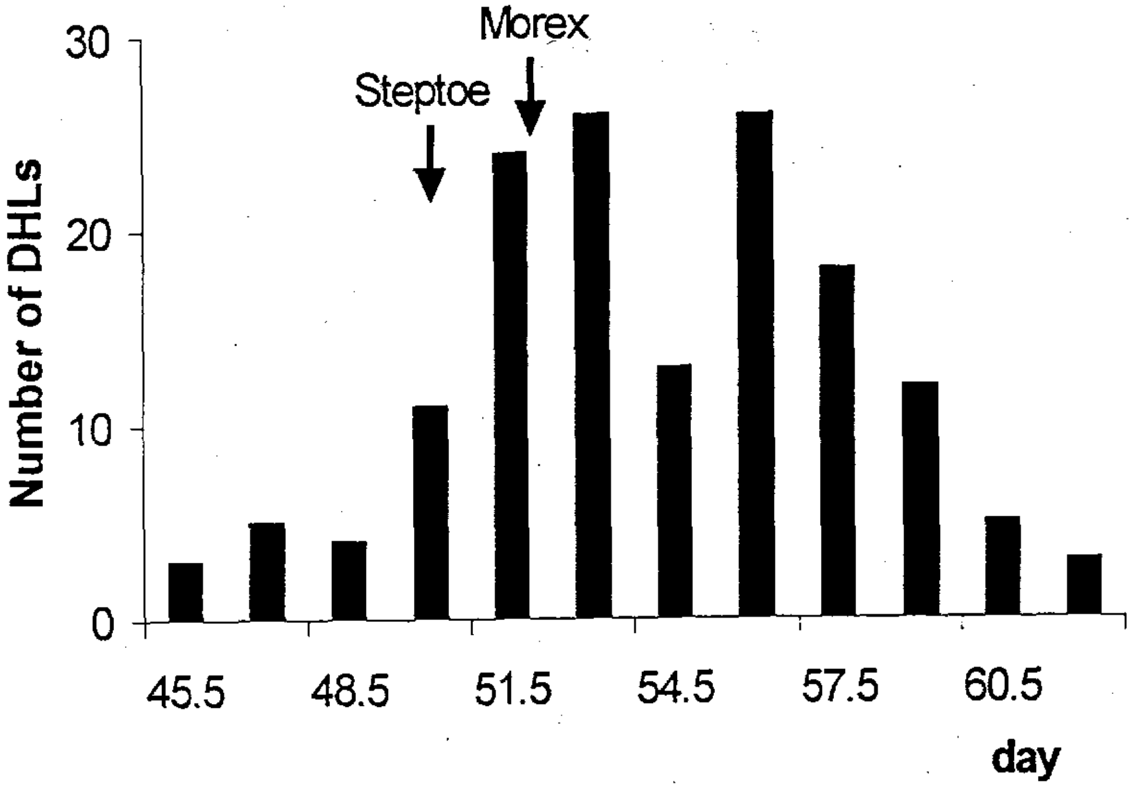


Fig 1. Frequency distribution of SM DHLs for flowering time under long day condition (field planting)

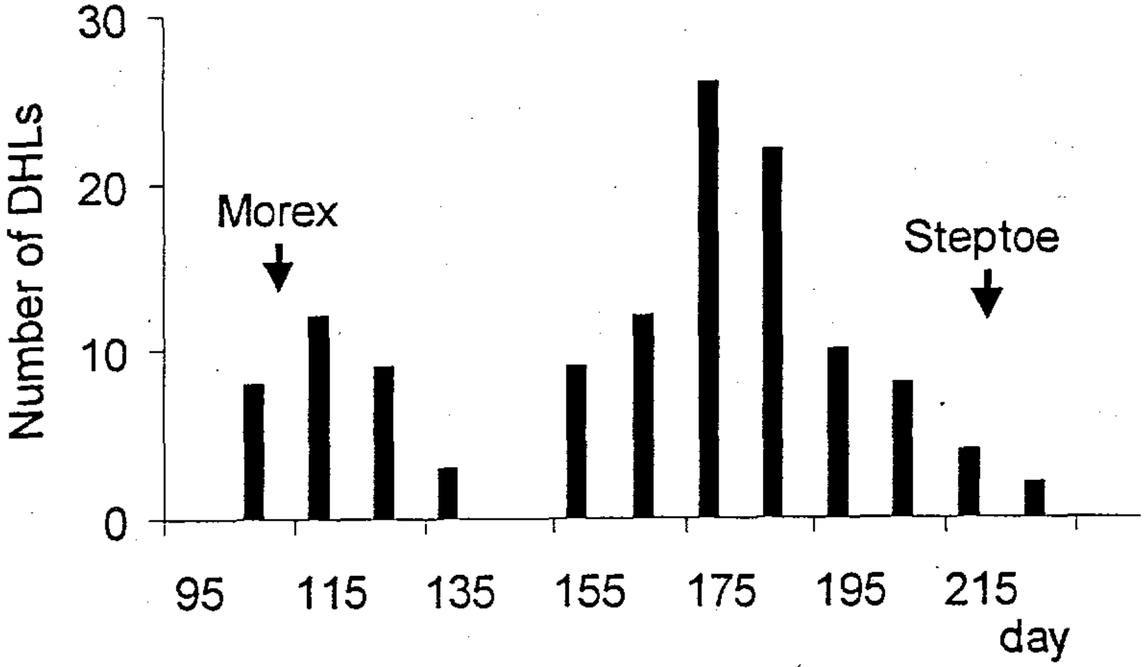


Fig 2. Frequency distribution of SM DHLs for flowering time under controlled short day environments

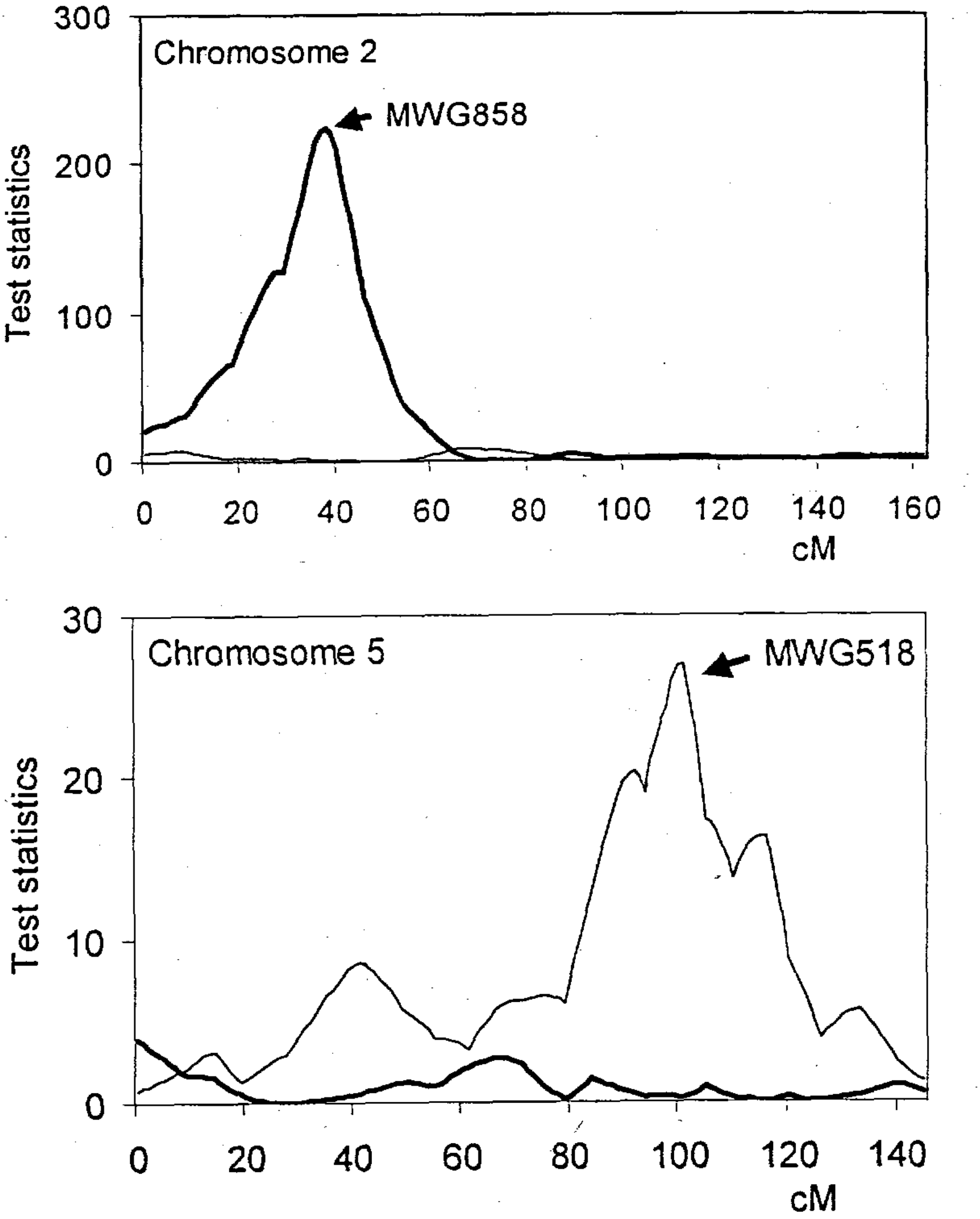


Figure 3. QTL mapping of flowering time under field and controlled short daylength conditions

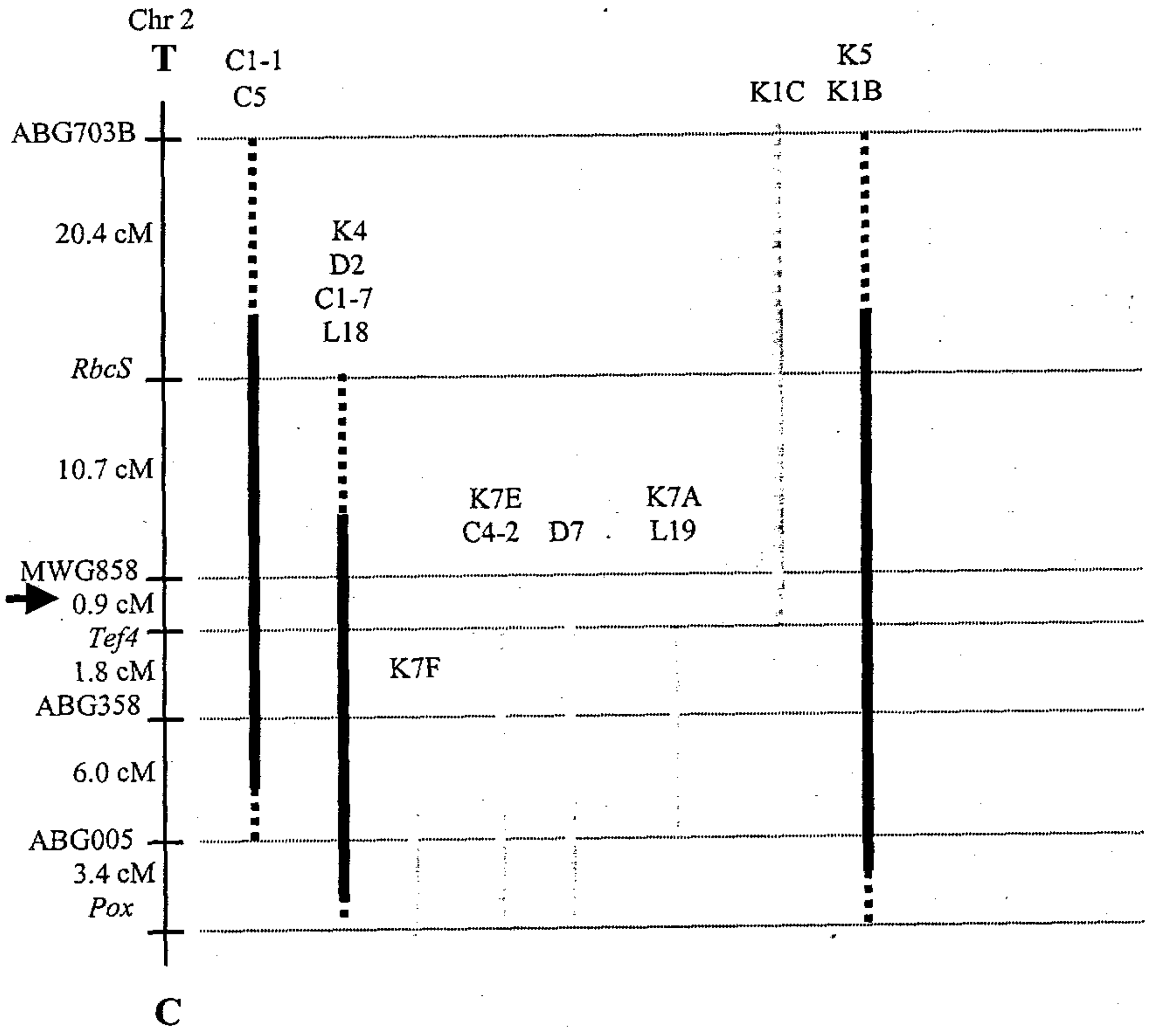


Figure 4. High resolution mapping of long day flowering QTL, *Phr-2S* on chromosome 2. Solid lines show introgressed Morex fragments while dashed lines show cross-over region. NILs represented with dark lines have late flowering phenotype while the ones with gray lines have Steptoe-like early flowering phenotype. Arrow shows the location of *Phr-2S* gene. C and T shows directions of centromere and telomere, respectively.

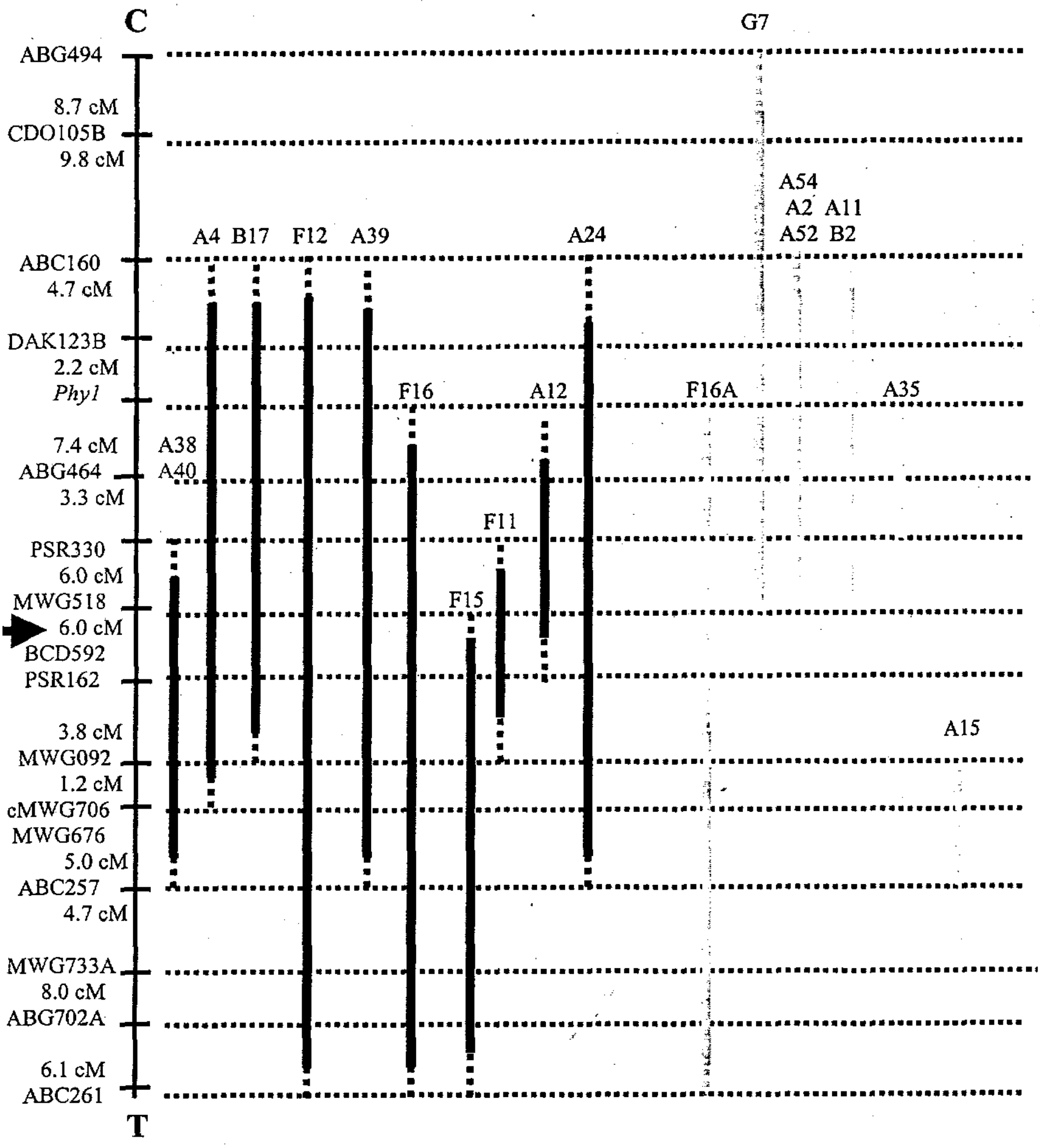


Figure 5. High resolution mapping of short daylength flowering QTL, *Phr-5L*. Solid thick lines show Steptoe fragments while dashed lines show cross-over region. Solid thin line in F16A shows heterozygous region. Dark lines have late flowering phenotype. Arrow shows the location of *Phr-5L* gene. C and T shows the centromere and telomere directions, respectively.

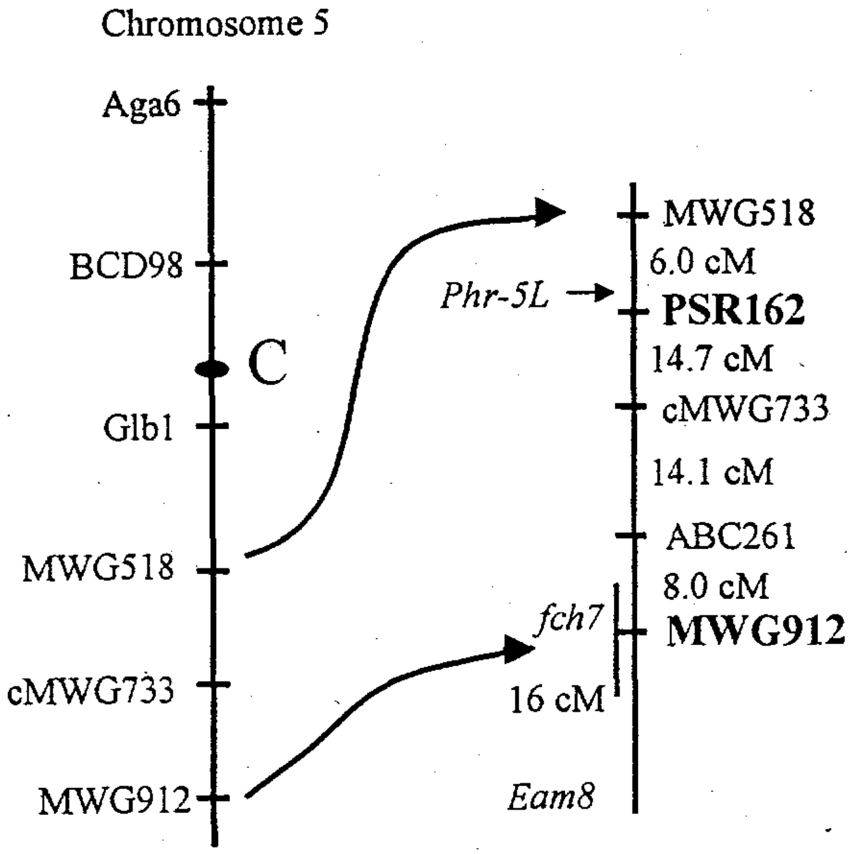


Fig. 6. Comparison of map locations of *Phr-5L* and *Eam8* on chromosome 5L. C shows the location of centromere. Marker distances are from SM map except *fch7-Eam8*, which is from barley morphological marker map (Franckowiak 1996). Alignment of MWG912 and *fch7* is based on bulked segregant analysis (Kudrna *et al.* 1996)

CHAPTER 5

PERSPECTIVES AND PROSPECTS

QTL mapping is used to identify quantitative trait loci in many crop species. Typical size of mapping populations (100-400 lines) can detect only the major QTL (Beavis 1998), which have been, in many cases, already identified in classical breeding programs and manipulated extensively. Minor QTL could be more interesting for plant breeding since they have not been targeted before. A major part of the variation remaining unexplained in QTL mapping populations is probably due to minor QTL or epistatic interactions between different QTL, which can not be detected with a typical mapping population. Failure of recovery of QTL effects, partly or completely, in a new genetic background is possibly due to minor QTL and epistatic interactions. An alternative method to detect minor QTL could be a "step-by-step" approach where one or a few QTL are fixed in a cross, preferably using NILs or advanced backcross lines, and creating a new segregating population. Epistatic interactions involving the fixed QTL would be expected to be simplified in these populations.

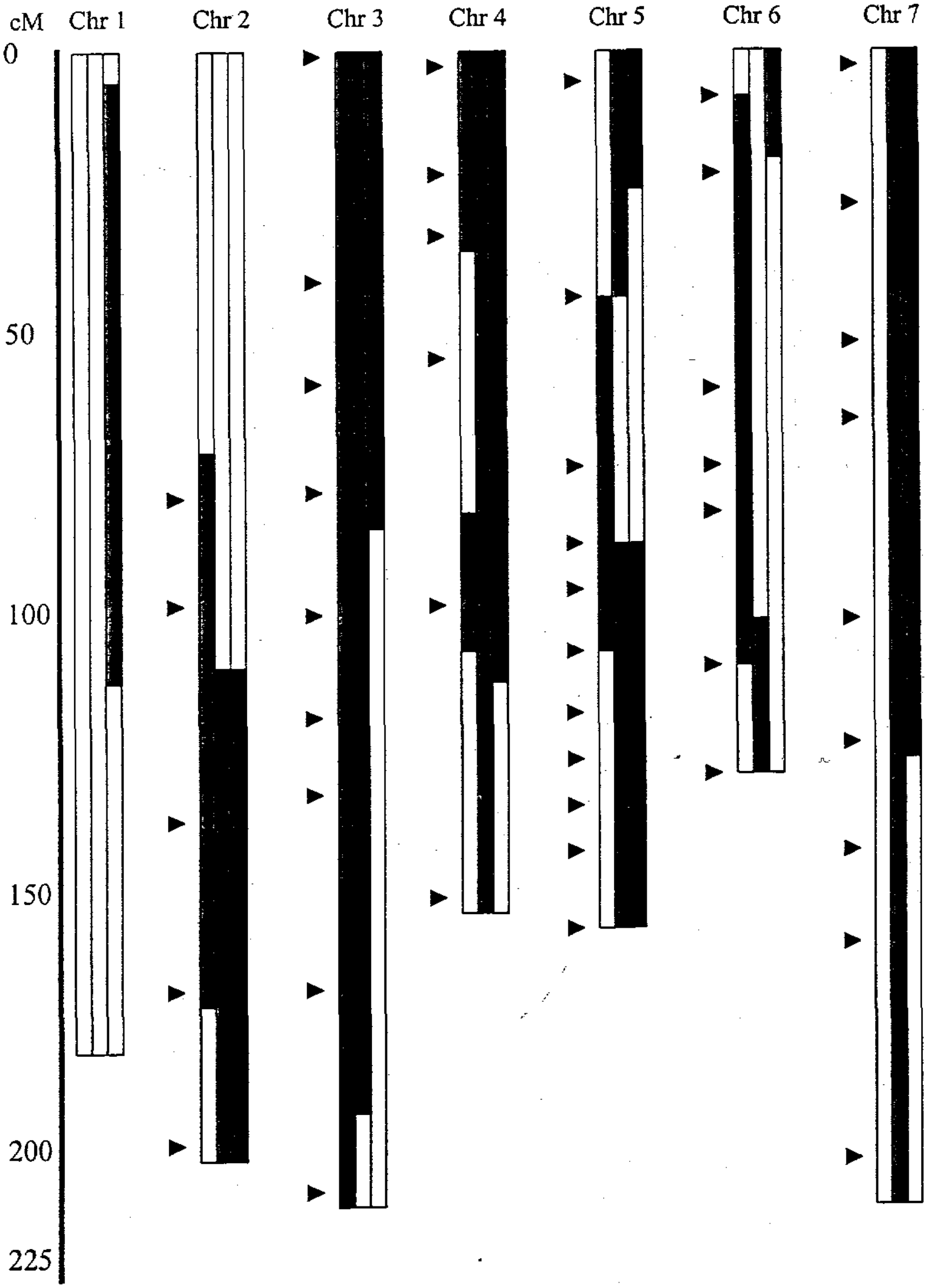
We have verified the presence of yield QTL by mapping yield related traits in two of the three previously reported yield QTL regions in barley. However, these QTL did not affect the yield when transferred from one cultivar to another. The largest-effect yield QTL detected in the cross between high yielding Steptoe and moderate yielding Morex cultivars, designated *QTL-3*, was not a major factor in theoretical yield difference but may be important in harvestable yield difference. Finding the loci responsible for the high Steptoe yields remains a major task. Having fixed *QTL-3* in Morex isogenic lines, the next strategy may be to prepare a new mapping population between Morex NIL with *QTL-3* and Steptoe. The new population will have less variation because a major variation due to *QTL-3* will be fixed. Reduction in the variance will help detect QTL with relatively small effects. Epistatic interactions involving *QTL-3* will also be simplified and loci interacting with *QTL*-3, if present, are likely to be identified.

The continuous distribution in many quantitative traits we studied were converted into discrete phenotype classes in near isogenic lines. Simplification of phenotypic distribution in NILs facilitated high resolution mapping of the quantitative traits. When combined with transferability of RFLP markers, NIL analysis offers an excellent system for map based cloning of genes for quantitative traits. Map based cloning of loci controlling quantitative traits can be further facilitated by using the morphological genes with known map positions found throughout the barley genome. These mutants or morphological markers can be used to pre-screen a segregating population for recombinations. Putative recombinants can be further analyzed by molecular markers and used in map based cloning of the gene of interest.

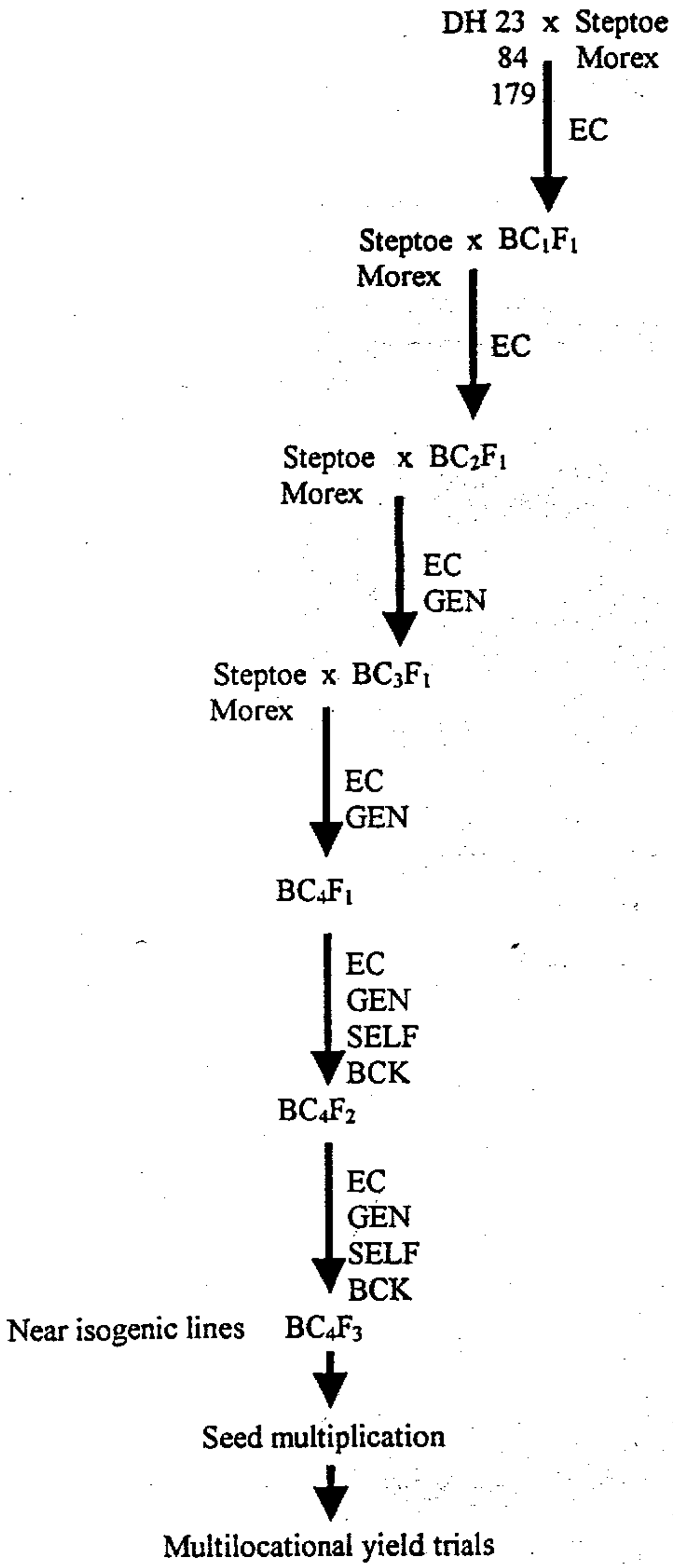
In conclusion, the present study raises questions about the nature of yield QTL that will require further research to answer. The knowledge generated will help us to better manipulate agronomically important traits in crop plants and hopefully to increase crop productivity.

APPENDICES

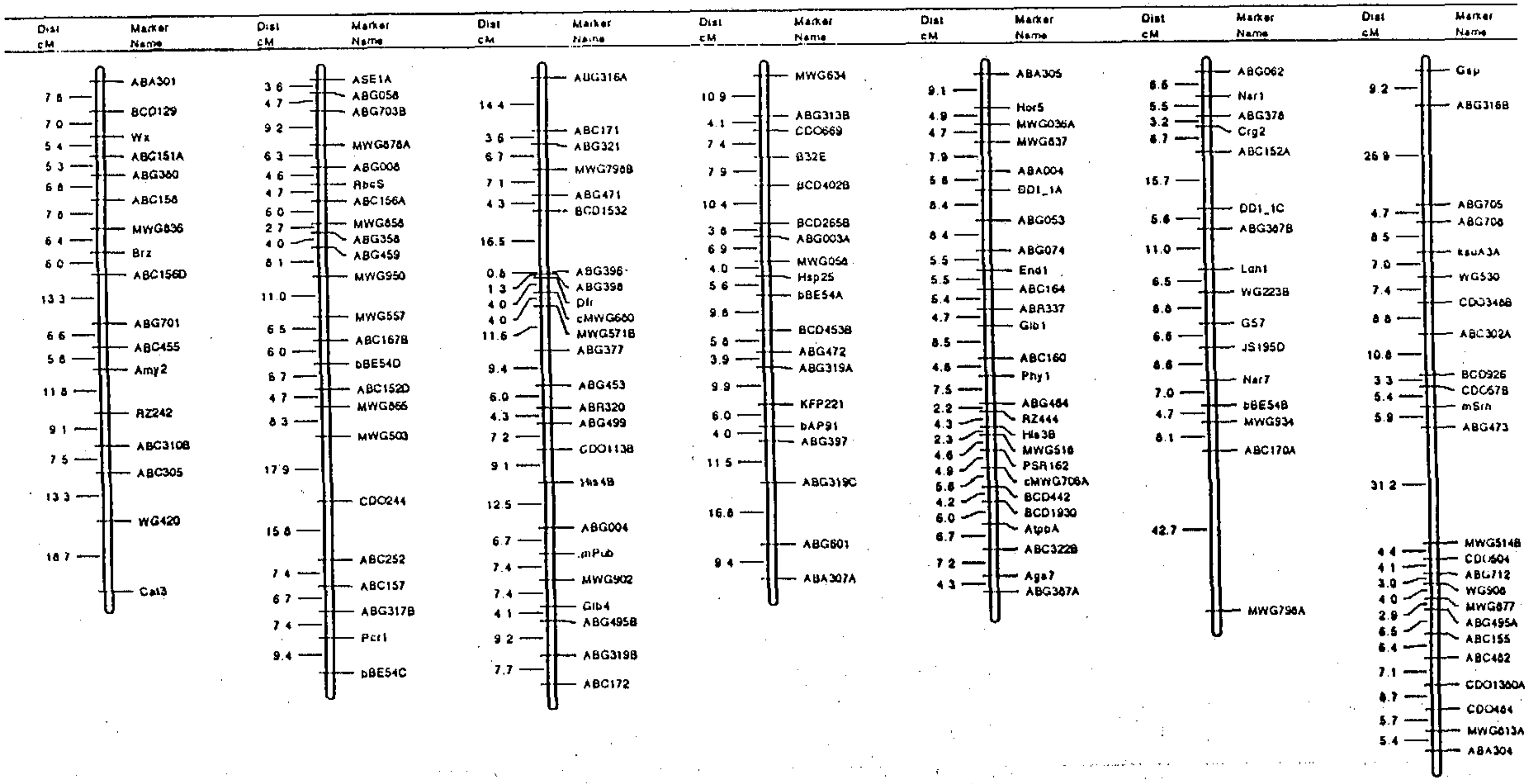
Appendix A. Steptoe and Morex chromosomal fragments of S/M DH lines 23 (left) 84 (middle) and 179 (right). Shaded fragments are from Steptoe. Arrows show the location of markers used to check genomic background in the BC_4F_1 generation.



Appendix B. Molecular marker assisted backcross scheme



EC: Embryo culture
GEN: Genotyping
BCK: Background checking
SELF: Selfing



Appendix C. Steptoe x Morex skeletal map used in QTL mapping