Mapping genes for yield and quality in barley ; a practical test of QTL analysis
by Ji-ung Jeung

A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Plant Sciences
Montana State University

Abstract:
Genes modifying traits affecting agronomic performance and grain quality have been characterized using the techniques of quantitative trait locus (QTL) analysis. These have been most generally attempted in model plant and animal populations which are most often characterized by parents which are genetically quite different. Plant breeders improve crops most efficiently using parents which are more closely related, but which differ for a few important genes. In this project, three populations derived from a single relatively narrow plant breeding cross were developed and evaluated to determine whether QTL analysis could be productively utilized within the context of a practically-oriented plant improvement program.

There are several problems associated with narrow parental divergence and genomewide genetic analysis. Generating a representative linlcage map is challenging and may indeed be impossible. Measuring and interpreting the actions of genes with relatively small effects, especially linked genes, is problematic as well. One of the three populations was used to generate a linkage map comprising 50 anchor markers and 191 AFLP markers. The map covered all seven barley chromosomes with an average distance between markers of 12cM.

QTL analysis was based on results gathered from three years' replicated field experiments and initially the map constructed in the 'Fiber-2' mapping population. A substantial proportion of the variance for grain yield, βglucan content, flowering date and plant height was accounted for by a few, relatively well-marked genes. Composite interval mapping provided a clearer picture of the impacts of these genes on phenotype than did simple interval mapping, and strongly supported the contention that the n and Ik2 genes both impacted grain glucan content.

A search for digenic interactions suggested that the waxy gene acted as a controller of gene expression for flowering date and plant height, a result not previously noted.

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APPROVAL

of a thesis submitted by

Ji-ung Jeung

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

Thomas K. Blake _____ (Signature) 3/24/00 Date

Approved for the Department of Plant Sciences

Norman F. Weeden _____ (Signature) 3/28/00 Date

Approved for the College of Graduate Studies

Bruce R. McLeod _____ (Signature) 3-31-00 Date

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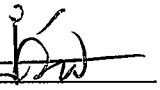
Signature 

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ABSTRACT

Genes modifying traits affecting agronomic performance and grain quality have been characterized using the techniques of quantitative trait locus (QTL) analysis. These have been most generally attempted in model plant and animal populations which are most often characterized by parents which are genetically quite different. Plant breeders improve crops most efficiently using parents which are more closely related, but which differ for a few important genes. In this project, three populations derived from a single relatively narrow plant breeding cross were developed and evaluated to determine whether QTL analysis could be productively utilized within the context of a practically-oriented plant improvement program.

There are several problems associated with narrow parental divergence and genome-wide genetic analysis. Generating a representative linkage map is challenging and may indeed be impossible. Measuring and interpreting the actions of genes with relatively small effects, especially linked genes, is problematic as well. One of the three populations was used to generate a linkage map comprising 50 anchor markers and 191 AFLP markers. The map covered all seven barley chromosomes with an average distance between markers of 12cM.

QTL analysis was based on results gathered from three years' replicated field experiments and initially the map constructed in the 'Fiber-2' mapping population. A substantial proportion of the variance for grain yield, β-glucan content, flowering date and plant height was accounted for by a few, relatively well-marked genes. Composite interval mapping provided a clearer picture of the impacts of these genes on phenotype than did simple interval mapping, and strongly supported the contention that the *n* and lk_2 genes both impacted grain glucan content.

A search for digenic interactions suggested that the *waxy* gene acted as a controller of gene expression for flowering date and plant height, a result not previously noted.

CHAPTER 1

INTRODUCTION

Waxy - Hulless Barley for Human Food

Barley (*Hordeum vulgare* L.) is primarily used in the malting and brewing industry and for animal feed. Only 2% of U.S. barley is used for food (Berglund *et al.*, 1992). Barley is available in both hulled and hulless types. Hulless barleys have some advantages for food uses, because hulless barley does not require pearling prior to consumption. Hulless barley can be milled directly to obtain a meal, or it may be steamed or boiled and directly consumed (Bhatty, 1986). Where barley is a major part of the human diet, naked types are preferred. Hulless barley is now primarily used as food in subsistence farming mountain regions of the world because people generally prefer and purchase rice and wheat as their economic conditions improve (Bhatty, 1986).

Waxy barley has been reported to contain high levels of soluble fiber, especially β-D-glucan (Newman *et al.*, 1990; Bengtsson *et al.*,1990; Oscarsson *et al.*, 1997). Soluble fiber has been shown to reduce serum cholesterol levels in rats (Hecker *et al.*, 1998), chicks (Bengtsson *et al.*, 1990), and humans (Newman,1989). Besides the cholesterol-lowering

effects, possible positive effects on mineral and vitamin bioavailability, blood glucose levels and colon cancer have been suggested (Åman *et al.*, 1989).

Due to the high water-holding capacity of soluble fiber, gumminess occurs when high levels of hulless barley are incorporated in food. There have been various attempts to determine the acceptability of a wide variety of food products in which waxy-hulless barely is incorporated (Berglund *et al.*, 1992), and evaluate the use of a barley β-D-glucan concentrate as a fiber-enriching food ingredient (Hecker *et al.*, 1998).

Gene Sources of Waxy-Hulless Barley

Two recessive genes - hulless(*n*) and waxy(*wx*) - have been utilized in barley to produce hulless, high fiber food barley. A gene reducing awn length(*lk2*) was also incorporated into many early hulless, waxy lines, initially as an easy-to-see morphological marker. Awn length reduction could have a pleiotropic positive effect on grain fiber content by reducing late season photosynthesis and starch deposition.

Hulless barleys are distributed widely in the world, and were independently selected by early agriculturalists in many subsistence farming environments. The relative frequencies of naked forms tended to decrease steadily towards the west from the east. Hulless types are infrequently found in Europe where hulless barley was used very little (Takahashi, 1955)

The physiological importance of the awn in small grains has been pointed out (Grundbacher, 1963; Paluska, 1979). The discomfort of handling rough-awned varieties led breeders to produce high-yielding, smooth-awned varieties of barley (Hayes and Wilcox,

1922). Short awned and smooth awned varieties are common in southern Korea, Japan, and China (Takahashi, 1955).

A series of barley isogenic lines was developed from Compana (CI 5438;2 row, feed barley), Betzes (CI 6398; 2 row, malting barley), and Titan (CI 7055; 6 row, feed barley) cultivars with varying combinations of the non-waxy (*WxWx*), waxy (*wxwx*), hulled (*NN*), hulless (*nn*) and long awn (Lk_2Lk_2) and short awn (lk_2lk_2) genes by Dr. R. F. Eslick and Dr. E. A. Hockett (described in Fox, 1981; Xue *et al.*, 1997). The original genetic sources were Waxy Oderbrucker for *wxwx*, and Sermo for *nn* and lk_2lk_2. Consequently, there are eight (2^3) different genotypes in each isogenic series (Fox, 1981). These three genes are all carried on barley chromosome 1, with lk_2 and *n* in close proximity on the long arm (Kleinhofs, 1996).

Genetic and Physiological Effects of *wx*, *n* and lk_2

Barley Endosperm and the Waxy Gene

Waxy mutant types can be distinguished from normal types by iodine staining. Waxy mutant types stain red while normal types produce a deep blue color due to the interaction between iodine and unbranched amylose.

Although the main carbon source used in grain development is sucrose. There have been multiple reports supporting that sucrose supply during the development of barley endosperm may not be limiting even under environmental conditions in which starch

deposition is reduced (Chevalier and Lingel, 1983; Felker *et al.*, 1984; MacLeod and Duffus, 1988).

Normal barley endosperm contains about 25% amylose and 75% amylopectin. The starch of mature barley endosperm shows various amylose - amylopectin ratios from low (about 2% amylose, waxy) to high (about 40% amylose, high-amylose) depending on genotype (Oscarsson *et al.*, 1997).

In barley endosperm, starch is deposited into membrane-bound granules, and these granules are classified according their size and shape: type A (lenticular, 10 - 48μm) and type B (spherical, 1 -10μm). The large A-granules contain about 90% of the starch by volume (Borém *et al.*, 1999). The large A-granules increase in number and size during the early stages of grain development, then the number is constant and the size is increased in late stages. The small B-granules increase in number throughout the middle and late stages (McDonald *et al.*,1991). In wheat, one model has been developed for the wheat A-type granule in which there is a core of very-low-amylose starch containing little lipid and an outer shell of high-amylose starch with a comparatively high lipid content. This pattern appears to be followed in barley as well (McDonald *et al.*,1991).

There is now good evidence indicating that the waxy gene is the structural gene for the granule-bound, ADP-glucose-dependent, starch synthase I isoform, and that this enzyme is responsible for amylose synthesis (John, 1992). The barley *wx* locus was cloned by Rohde *et al.* (1988). McDonald *et al.* (1991) and Oscarsson *et al.* (1997) observed that the numbers of A-type granules per endosperm was significantly higher in waxy barleys than in wild

types, and suggested that the B-type starch granules of waxy genotypes represented a smaller proportion of the total starch at maturity in waxy lines.

Oliveria *et al.* (1994) found that the surface area and volume of A- and B-type granules was generally greater for malting cultivars than feed cultivars. Borém *et al.* (1999) conducted QTL analysis with the Steptoe (feed cultivar) x Morex (malting cultivar) mapping population on the size, shapes and relative proportions of A- and B-type granules to estimate the relationship between starch granule characters and the quality of barley malting for brewing. Although they detected QTLs affecting the starch granule characters, and the alleles from Morex contributed to larger granules, those QTLs were not associated with malting quality.

In an X-ray diffraction study, the amylose-lipid complex was most obvious in high-amylose starch genotypes. Waxy genotypes did not show an amylose-lipid complex (Czuchajowska *et al.*, 1998). Czuchajowska *et al.*(1998) also observed that waxy barley contained significantly more high-molecular-weight-amylopectin and low-molecular-weight-amylopectin but less intermediate-molecular-weight-amylopectin than did nonwaxy and high-amylose barley.

β-D-Glucans

Over 96% of total grain cellulose is present in the barley hull with very little found in the endosperm cell walls. Branched chain β-(1→3;1→4)-D-glucans (β-D-glucans) are major components of barley and oat endosperm cell walls. Linear polymers with β-(1→3) and β-(1→4) linkages are also found (Izydorczyk *et al.*, 1998). The amount of β-D-glucan

in barley seed is generally 3-7%, which is mostly present in the endosperm cell walls; the starch endosperm cell walls contain around 70% β-D-glucan and 20% arabinoxylan, whereas the aleurone cell walls contain about 26% β-D-glucan and 67% arabinoxylan (Ballance and Manners, 1978; Åman *et al.*, 1989). The β-D-glucan deposition occurs relatively late in grain development (Åman *et al.*, 1989).

There are two methods used to measure β-D-glucan content of barley grain. β-D-glucan may be extracted in either a highly acidic or basic solution and content estimated by measurement of viscosity of the extract. The results vary according to the conditions of extraction (Fleming and Kawakami, 1977). β-D-glucan may be more directly measured with a streamlined enzymatic method (McCleary and Mugford, 1997). Following extraction and hydrolysis, glucose (i.e., degraded β-D-glucan) content is measured optically by addition of a glucose oxidase reagent.

The β-D-glucans of barley have long been a problem for poultry feed manufacturers and the malting and brewing industries. In the former case, barley β-D-glucans contributes to the small intestinal viscosity of chicks and the effect was related to altered lipid metabolism as well as reduced lipid and protein digestibility. In the latter case, the high viscosity of barley β-D-glucan solutions can cause significant brewing problems associated with increased filtration times and reduced brewing yield (Bamforth and Barclay, 1993). Improved malting varieties have lower levels of β-D-glucans and a higher proportion of C-hordein (Ellis *et al.*, 1997). The malting and brewing industries have been interested in β-D-glucan levels in barley varieties and in the potential of grain to produce rapidly β-D-glucan hydrolases during germination. Two barley β-D-glucanase isoenzymes EI and EII have been

reported as single or low copy genes. They have been localized on barley chromosomes, and cloned. EI is on barley chromosome 5 (Slakeski *et al.*, 1990), and EII is on barley chromosome 1 (Loi *et al.*, 1988; Høj *et al.*, 1989).

Awn Length

Awns are apical extensions of the lemma. They contain three vascular bundles and are active photosynthetic organs in small grains (Grundbacher, 1963; Paluska, 1979). Eslick and Hockett (1967) reported that lk_2 (short awn) is located on the long arm of barley chromosome 1. Awnless(*lk*) is on the long arm of chromosome 2, and tightly linked to *v* locus and both lk_2 and *lk* are epistatic to the hooded gene *k* (Nilan, 1964). Another short awn gene(lk_5), lies on the short arm of chromosome 4, and may affect the rachilla, causing it to form additional florets (Tsuchiya and Hall, 1978). Awn barbing is conditioned by *r* , which results in lack of teeth on the awn (Nilan, 1964). It lies on the long arm of chromosome 7.

In long-awned genotypes, the awns account up to 90% of CO_2 exchange rate but only 30% of the spike's dark respiration rate (Kjack and Witters, 1974). Therefore, awns have the additional advantage of being the youngest photosynthetic tissue on the plant and they remain photosynthetically active throughout the grain-filling period (Johnson *et al.* 1975). Weyhrich *et al.* (1994) found no pattern in yield superiority of either awn types (awned or awnletted). However, previous investigations suggest that awns were are favorable under semi-arid environments and stress conditions (Qualset *et al.*, 1965).

Hulless Seed

Kernel characters are widely used for distinguishing one cultivar from another. Cultivated barleys can be classified into those in which the lemma and the palea are tightly fused to the pericarp of the caryopsis - covered or hulled barley-, and those in which the chaff is easily separated from the grain - naked or hulless barley- (Briggs, 1978). Helbaek (1966) estimated that hulless barley also appeared 8,000 years ago when six-rowed landraces (*vv*) were derived from two-rowed races (*V*-) by early agriculturalists.

The caryopsis character is controlled by one gene with two common alleles (*N* and *n*) (Nilan, 1964) and the hulless phenotype can easily be moved into hulled barley strains by repeated backcrossing. The naked caryopsis gene (*n*) was mapped by Heun *et al.* (1991) in Proctor x Nudinka 2.9 cM down from RFLP probe CDO673 and cosegregating with RFLP probe BG143. This places it near the centromere and 3.5 cM from *Amy2* locus in the Steptoe x Morex linkage map (Kleinhofs, 1996).

Gaines *et al.* (1985) showed that the cementing substance is produced by the undifferentiated pericarp only two days after flowering. Since the hulls of naked lines are removed easily during threshing, kernel weight and yield reductions of hulless types are proportional to at least the weight of hulls (8-16% of the kernel weight) (Briggs, 1978), and the proportion of other seed components mainly cellulose, hemicellulose, lignin, and a small quantity of protein also changes accordingly (McGuire and Hockett, 1981; Newman *et al.*, 1990).

Murphy and Witcombe (1981, 1986a) reported that Nepal, with its huge range of hulless land races, is the most likely place for the origin of the hulless form. Recently, Hang

et al. (1996) conducted a 'cluster analysis' on thirty six hulless barely accessions from North America, China, Turkey and Central Asia using RAPD markers to estimate the genetic similarities among accessions. The accessions from China, Turkey and Central Asia belong to one main group that could be divided into three sub-clusters while most of the accessions from North America are closely related and belong to one well-defined cluster.

Genetic Analysis to Investigate the Genetic Effects of *wx*, lk_2, and *n* on Glucan Content and Yield Components

Isogenic Line Approaches

Atkins and Mangelsdorf (1942) proposed to use 'Isogenic lines' in studying of gene effect on traits of interest, because it provided an essential uniform genetic background thereby avoiding subsidiary effects caused by various treatments.

To investigate the gene effects of three genes on traits of interest, a series of six barley isogenic lines was developed from Compana (CI 5438; 2 row, feed barley), Betzes (CI 6398; 2 row, malting barley), and Titan (CI 7055; 6 row, feed barley) cultivars with varying combinations of the non-waxy (*WxWx*), waxy (*wxwx*), hulled (*NN*), hulless (*nn*) and long awn (Lk_2Lk_2) and short awn (lk_2lk_2) genes in a seven backcross breeding program by Eslick and Hockett.

McGuire and Hockett (1981) observed that kernel weights were significantly reduced in the short-awn isotypes in comparison of short-awn (lk_2lk_2) versus long awn(Lk_2Lk_2) isotypes of hulled and hulless barley. Awn length, however, had no obvious effect on grain

yield. McGuire and Hockett (1981) tried to estimate the influence of different awn lengths (Lk_2; long awn versus lk_2; short awn) and caryopsis types (*N*; hulled versus *n*; hulless) on yield with isogenic four Betzes lines. They observed that genotypes with the hulled allele averaged about 10% greater yield than hulless genotypes, and the length of awn showed a linear relationship with heavier kernels, but had little effect on grain yield.

Swanston (1995) used isogenic lines developed in the barley cultivar Compana with varying combinations of the genotypes of three genes (*wx, n,* lk_2). He reported that 1) the waxy gene was associated with changes in endosperm structure that increased milling energy, 2) both *wx,* and lk_2 were associated with a significant reduction in grain size with additive effects, 3) a combination of *wx* and *n* did not show additive effects on β-D-glucan content, and 4) lk_2 showed similar effects to *wx* on grain size, milling energy and β-D-glucan content.

Xue *et al.* (1997) used a series of isogenic lines developed in Compana and Betzes with varying combinations of the genotypes of three genes (*wx, nn,* lk_2). More than 20 endosperm-related characters were measured using a General Linear Model procedure for analysis of variance. The effects of cultivar background, hull type, starch type, hull type and the two-way and three-way interactions were tested. The results demonstrated 1) the *n* gene reduced total dietary fiber by preventing the hulls from adhering to the kernel. 2) waxy barleys contained less starch, but more free sugars. 3) Increased total dietary fiber in waxy barleys was due to an increase in total and soluble β-D-glucans. 4) the short awn gene is associated with the increased viscosity, and with the reduced test weight.

Random Inbred Line Approaches

Powell *et al.* (1985) used random inbred lines produced by doubled haploidy (DH) and single seed descent (SSD) with three crosses (Golden Promise x Mazurka, Golden Promise x Ark Royal, and BH4/143 x Ark Royal). In the experimental analysis, they adopted an assumption that if important genes for any trait were linked, the bias of additive genetic variation of SSD derived families would be less than that of DH derived families, due to multiple round of meiosis reducing linkage disequilibrium (Falconer and Mackay, 1996, p368). They observed that 1) β-D-glucan content was controlled by a simple additive genetic system, 2) linkage disequilibrium did not appear to be an important genetic component of β-D-glucan content in barley, and 3) epistasis on β-D-glucan content was detected in the Golden Promise x Mazurka cross only. They also reported a negative genetic relationship between β-D-glucan content, thousand grain weight and plant height with significant linkage disequilibrium levels.

Swanston (1997) also used random inbred lines (SSD method) produced from a Chalky Glenn (6 row) x Waxy Hector (2 row) cross. In his experiment, he produced waxy lines with acceptable grain size, by avoiding the use of dwarfing (Swanston, 1995). Interestingly, the waxy lines were divided into two groups (high milling energy vs. low milling energy), and one group characterized with low milling energy was shown to have very low β-D-glucan content.

Han *et al.* (1995) mapped QTL for barley β-D-glucan content on barley chromosomes 2 and 5.

Limitations of Previous Studies

Isogenic Line Based Genetical Approaches

Theoretically, isogenic line based genetical approaches (McGuire and Hockett, 1981; Swanston, 1995; Xue *et al.*, 1997; Czuchajowska *et al.*, 1998) demand uniform genetic background except the loci introduced into recurrent parents. Multiple steps of backcrossing were used to generate 8 isogenic lines with 2 different donors (Waxy Oderbrucker for *wxwx,* and Sermo for *nn* and lk_2lk_2, Fox, 1981). The eight resulting lines may show different degree of fitness due to the linkage drag (Young and Tanksley, 1989). Therefore, the pattern of 'background effect' on target QTL by partial donor chromosome retention may differ in each isogenic line. Another disadvantage of this approach is that epistatic interactions between target QTL and other QTLs on other genome regions could not be tested (Han *et al.*, 1997).

Random Inbred Line Approaches

The idea of characterizing the individual QTL responsible for variation of quantitative traits among random inbred lines goes back to the early 20's (Sax, 1923). Application of this idea, however, was limited by a lack of segregating markers in populations of interest. A few morphological, disease resistance, isozyme, and seed storage protein markers could be used (Shahal and Tsuchiay, 1990), and attempts were made to

generate stocks containing multiple morphological markers (Benito *et al.*, 1987). Until the advent of RFLP markers, marker genome coverage remained very low.

Powell *et al.* (1985), using random inbred lines, reported that three to five simple additive genes were enough to explain the variation of β-D-glucan contents among lines, Han *et al.*(1995) mapped the QTLs for barley β-D-glucan content with the aid of well distributed molecular markers. They showed that β-D-glucan content was controlled by additive gene action, but their population did not segregate for *wx*, *n*, and lk_2.

In summary, except for the use of isogenic lines, there has been no report in which all three recessive alleles were considered at the entire genome level with all possible expected genetic mechanisms.

Current Status and Considerations

Rossnagel *et al.* (1981) reported that nonwaxy-hulless barley(*WxWx, nn*) yielded 88% of hulled barley on the average of data from 93 station years. Since the yield reductions of hulless types are proportional to at least the weight of hulls (8-16% of the kernel weight) (Briggs, 1978), the productivity differential between the two classes of barley appeared to be quite small.

Meanwhile, many barley breeding programs have developed and released high β-D-glucan barley varieties (Goering *et al.*, 1973; Eslick *et al.*, 1990; Blake *et al.*, 1990). So far, the developed waxy- hulless varieties commonly showed lower grain yield, performing about 20% lower than non-waxy, hulled varieties (Blake, personal communication).

Gill *et al.* (1966) reported that hulless barley has been historically regarded as an inadequate yielder when compared to hulled barley, even when the weight of hulls was considered. Witcombe and Murphy (1986) tested hypotheses with reciprocal crossings between accessions of two groups and compared the important traits' performances among parental, F_1 and F_2 plants. They could not find any evidence of negative pleiotropic interactions. It therefore seems likely that the observations of Gill *et al.* (1966) may result from poorer germplasm in the hulless pool, rather than representing a direct effect of the hulless phenotype. If this is the case, then understanding the magnitude of the effects of the waxy and hulless characters may lead to the design of more efficient food barleys. If not, then determining the interactions of these genes with performance-related characters will help determine whether barley has potential as a food crop in industrialized nations.

The primary objective of this thesis was to discover in greater detail the genetic basis for poor performance in waxy-hulless barley and try to find ways to remedy it. For this objective, a linkage map was generated in a population of recombinant inbred lines derived from a cross between MTaz90-123 (waxy hull-less, short-awned and high fiber content) and MTH6860756 (a high yielding, stripe rust resistant feed barley line). QTL mapping was applied to evaluate putative QTLs of important traits, and all possible conditional- and combined- dialleleic interactions between major QTLs on each trait were surveyed. Finally, hypotheses regarding the genetic control of grain yield and grain fiber content were tested.

CHAPTER 2

LINKAGE MAP CONSTRUCTION IN A REAL BREEDING POPULATION; MTaz90-123 x MTH6860756

Introduction

The idea of using genetic markers to locate the individual QTL responsible for variation in quantitative traits goes back to the early 20's (Sax, 1923). The practical application of this idea, however, was limited by a lack of segregating markers in the populations of interest. A small number of morphological, disease resistance, isozyme, and seed storage protein markers could be used (Shahal and Tsuchiay, 1990), and lines containing several morphological markers have been constructed. Unfortunately, these morphological recessive genes are generally deleterious, and most of the major QTL identified using these stocks map to these marker genes.

In order to detect QTL using linkage disequilibrium analysis, genetic markers must clearly and inexpensively differentiate between parental alleles (Beckmann and Soller, 1983). Markers should be highly polymorphic, abundant and neutral both with respect to the quantitative trait of interest and to reproductive fitness (Falconer and Mackay, 1996 pp 356-378).

Most of the QTL mapping experiments done thus far utilized populations derived from crosses between parents selected to maximize genetic distance (e.g. Hayes and Jyambo, 1993). This may not always be desirable, and may shed little light on realistic crop improvement projects. If the objective of the experiment is to assist breeding for the improvement of specific traits, elite parents are more desirable. This is especially true for crops like barley which have a long history of breeding (Martin *et al.*, 1991; Hayes *et al.*, 1997).

If a real breeding population is used as a mapping population genetic distance betwen the parents may be low, making it difficult to find enough informative markers to obtain even marker spacing on chromosomes. Entire chromosome arms may share a common origin between parents, making a search for informative markers for these arms futile. QTL analysis in wide crosses plainly has greater potential to uncover more significant gene x phenotype interactions and will better support the detection of hard-to-detect nonadditive sources of genetic variance (Rasmusson and Pillips, 1997).

Low copy restriction fragment length polymorphism (RFLP; Bostein *et al.*, 1980) markers, detected using Southern analysis, have been applied to many crop species to generate linkage maps. However, this technique can be cumbersome when applied to practically-oriented plant breeding programs due to the time consuming and expensive procedures.

The polymerase chain reaction (PCR; Saiki *et al.*, 1988) provides a convenient and cost-effective alternative to RFLP analysis. Olson *et al.* (1989) proposed using sequence tagged sites (STSs) as chromosome landmarks, and several hundred barley RFLP clones

were sequenced and converted to STSs (Shin *et al.*, 1991; Tragoonrung *et al.*, 1992; Blake *et al.*, 1996; Sayed-Tabatabaei *et al.*, 1998). Simple sequence repeat (SSR; Weber and May, 1989) or microsatellite loci contain tandem repeat sequences of DNA and vary in repeat size and number. SSR markers are ideal for genetic mapping because of their high level of polymorphism, and their compatibility with PCR-based detection schemes (Liu *et al.*, 1996). Several primer pairs flanking barley SSRs have been developed and incorporated within the barley linkage map (http:\\wheat.pw.usda.gov).

Amplified Fragment Length Polymorphism (AFLP; Vos *et al.*, 1995) analysis has some advantages over other DNA marker techniques. These include the detection of a larger number of polymorphisms within very short period time, and reduced cost. These AFLPs may be anchored to each barley chromosome with barley STS and SSR markers. Consequently, the most expected practical problem in generating a linkage map of a real breeding population - lack of polymorphisms with even distribution across the entire genome- may be ameliorated with AFLP markers.

In this report, we describe the generation of a linkage map with a real breeding population derived from a cross between waxy-hulless barley line (MTaz90-123; *wxwx, nn, lk_2lk_2*) and high yielding feed barley line (MTH6860756; *WxWx, NN, Lk_2Lk_2*). A combination of morphological, protein, STS, and SSR markers were used as anchor markers. As a major tool for 'gap filling', AFLP markers were applied. This study will describe the practical difficulties underlying linkage map construction with a real breeding populations, and suggest possible solutions.

Materials and Methods

Population Development

Three parallel recombinant inbred line populations were developed from the cross between Mtaz90-123(maternal line) and MTH6860756. MTaz90-123 is the best-performing and well adapted waxy, hulless 2 row barley line with short awn (*wxwx, nn, lk_2lk_2*) in Montana. This line derived from the cross of 'Washonupana' (*wxwx, nn, lk_2lk_2*) x MT83424 (Fox, 1981; Blake, personal communication). MTH6860756 (*WxWx, NN, Lk_2Lk_2*) is high yielding, 2 row feed barley.

Three parallel recombinant inbred line (RIL) populations were utilized for QTL analysis. The were called the 'Fiber-1' (F_4 derived, 61 lines), 'Fiber-2'(F_5-derived, 59 individuals) and 'Fiber-3' (F_5-derived, 96 individuals) populations. Seed was advanced from each individual F_5 line until the F_7 generation to generate sufficient seed for replicated year trials. The 'Fiber-2' population was utilized for map construction.

Morphological and Protein Markers

Two morphological markers, *n* (hull type) and *lk_2* (awn length), were screened during the field trials. Waxy endosperm (*wx*) was identified with iodine staining of cleaved mature seeds (Frykman and Bengtsson, 1992). Variation at the hordein storage protein loci (B and C hordein) were characterized by SDS-PAGE electrophoresis of mature seeds (Blake *et al.*, 1982). These markers are described in Table 1.

Table 1. Morphological and biochemical characters evaluated.

Locus	Phenotype	Genotype		Chromosome[a]	
		MTaz90-123	MTH6860756	S x M	Fib-2
wx	waxy endosperm	*wxwx*	*WxWx*	1	1
n	naked caryopsis	*nn*	*NN*	1	1
lk_2	short lawn	lk_2lk_2	Lk_2Lk_2	1	1
Hor 1	C hordeins			5	5
Hor 2	B hordeins			5	5

[a] Chromosomal locations of morphological and biochemical markers integrated into Steptoe x Morex (S x M; Kleinhofs, 1996) linkage map and evaluated locations with 'Fiber-2'(Fib-2) mapping population.

PCR Amplification and Polymorphism Detection of STS and SSR Markers

Approximately 120 STS primer sets (Blake *et al.*, 1996; Sayed-Tabatabaei *et al.*, 1998) and 44 SSR primer sets (Becker and Heun, 1995; Liu *et al.*, 1996) were evaluated for informativeness in the Fiber-2 population. Out of 15 the Becker and Heun's SSR primer sets, 9 primer sets, with different primer sequences assay the same target SSR loci as do 9 of Liu *et al.*'s primer sets (see Table 3).

All STS- and SSR-PCR amplifications were conducted in 30µl volumes of 1X PCR buffer(Promega, Madison, WC) [50mM KCl, 10mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 1.5 mM $MgCl_2$], 0.1 mM each dNTP, 0.3µM of each primer, 0.5 unit of *Taq* polymerase (Promega), and 50ng of genomic DNA template. Typically, STS-PCR amplification conditions were: 5min at 94°C, followed by 35 cycles of 30 sec DNA denaturation at 94°C, 30 sec annealing at 50°C, and 1 min extension at 72°C, and final 5 min. incubation at 72°C. When a large PCR product (> 1.0 kb) was expected (especially 'MWG' group; Sayed-Tabatabaei *et al.*, 1998), 1min and 2 min were used for annealing and extension respectively. For the SSR-PCR, after passing 5min at 94°C, a 'touchdown' PCR consisted of 18 cycles of 94°C for 1 min denaturing and 72°C for 1 min extension. Annealing (30 sec)

temperatures were progressively decreased by 0.5 °C every second cycle from 64 °C to 55 °C. The PCR reaction continued for 30 additional cycles of 1 min denaturation at 94 °C, 1min annealing at 55 °C, and 1 min extension at 72 °C, and final 5 min incubation at 72 °C. PCR was performed in a GeneAmp™ RCR System 9600 (Perkin Elmer, Norwalk, CT) thermal cycler.

The polymorphisms of STS and SSR markers were detected with 6% polyarcylamide gels in 0.5X TBE buffer with 2.0 hour electrophoresis at 250 V, followed by ethidium bromide staining. In the case of large PCR products (usually MWGs), a 1.8 % agarose gel was also used. Initially monomorphic STS-PCR products between MTaz90-123 and MTH6860756 were subsequently digested with several endonucleases. Two units of each restriction endonucleases were added to 10µl aliquots of PCR products. If the size difference of SSR-PCR products was small, a 4% denaturing polyarcylamide gel (8M urea, 1X TBE buffer) were used at 1,500 V. Those SSR PCR products were visualized using a DNA silver-nitrate staining method (Bassam *et al.*, 1991).

AFLP Reactions and Polymorphism Detection

The protocol adopted for the generation of AFLP markers followed Vos *et al.* (1995) with minor modifications. Genomic DNA (100ng) , in a 20µl reaction volume, was restricted with 4 unit of Pst I and Mse I (New England Biolabs, Beverly, MA) at 37 °C for 2 hours. After digestion, without heat inactivation of the restriction enzymes, 10µl of adaptor ligation cocktail was added and the mixture incubated at 15 °C 1 h, 23 °C 1 h, and 37 °C 30 min with programed thermal cycler. The ligation cocktail contains 1 unit of T4

DNA ligase (New England Biolabs), 4mM ATP, 3 pmol of the Pst I adaptor (5'-CTC GTA GAC TGC GTA CAT GCA; CAT CTG ACG CAT GT-5'), and 30 pmol of Mse I adaptor (5'-GAC GAT GAG TCC TGA G; TAC TCA GGA CTC AT-5').

Pre-amplification was conducted with 30ng each of Pst I and Mse I single-nucleotide selective primers(G and A for Pst I, C for Mse I), 5µl of 1:5 diluted ligated DNA, 1X PCR buffer, 0.2mM dNTPs, 3 unit of *Taq* polymerase in 50µl final reaction volume. Pre-amplification PCR-cycle profiles were performed as described by Vos *et al.* (1995). 1µl of 1:5 diluted pre-amplified DNA was selectively amplified using 30ng of each of the Mse I primers and fluorescent labeled Pst I primers with three selective nucleotides (see Table 5), 1 unit of Taq polymerase, 1X PCR buffer and 0.2mM of dNTPs using the PCR-profile described by Vos *et al.* (1995) with a 10 min final extension at 72°C. All PCR reactions were performed using a GeneAmp™ RCR System 9600 (Perkin Elmer).

Fluorescent AFLPs were detected using an ABI 377™ automated DNA sequencer (Perkin Elmer) with 4% denaturing polyacrylamide gels (8M urea, 1X TBE buffer). For the analysis of complex AFLP fingerprint patterns, the software package Genographer (Benham *et al.*, 1999) was used. This program reads in lanes from an ABI 377 and reconstructs them into a gel image which is straightened and sized. Bins can be defined easily and viewed as thumbnails, so that the presence and absence of a band can be scored quickly and easily. AFLPs were named based on the primer combinations used for specific amplifications, the band size, and the band quality. The last capital letters among AFLP marker names represented the relative band quality; S for very strong and clear, A for clear, B for weak but countable.

Genotyping of Progeny Lines

In constructing the 'Fiber-2 linkage map', the major marker generation tool was AFLP analysis. It is very difficult to score AFLPs co-dominantly without special considerations (Liu, 1998, pp77-78; Castiglioni *et al.*, 1999). Therefore, highly homozygous RI lines in all loci (F_n, $n \rightarrow \infty$) are desirable to permit the simple scoring of presence or absence of bands. To mimic F_7 generation RI lines, young leaves of a single plant were selected for the genomic DNA extraction in each progeny line using a CTAB method (Murray and Thompson, 1980) with minor modifications.

Some heterozygous genotypes were detected using the STS markers at very low frequency. Those allele states were treated as a 'missing data' in that marker class. The morphological markers were scored with all three genotypes in the F_5 individual lines during the field trials. The heterozygous (heterogeneous) genotypic RI line data points were also treated as missing data during linkage map construction.

Linkage Map Construction

Linkage analysis of the 59 RI lines was performed using several software packages with the following purposes. To perform a segregation test, and determine the marker order roughly; MAPL (Ukai, 1999) worked well. We used Map Manager XP 0.9 (Manly, 1999) as our primary linkage analysis tool. For pseudo-linkage group separation, and marker interval estimation with Kosambi mapping function we used Mapmaker 3.0 (Lander *et al.*, 1987).

Some linkage groups were defined as 'orphan' groups if they contained no anchor markers. Those groups were evaluated for potential placement on the map through comparative analysis using the randomly selected 56 'Steptoe x Morex' mapping lines.

The basic idea of comparative mapping, assuming co-linear genetic maps, was adopted into the 'Fiber-2' linkage map construction. First, the comparative chromosomal locations of STS and SSR anchor markers were surveyed very carefully across various barley mapping populations to infer the genome coverage of anchor markers in our 'Fiber-2' mapping population (Web site; hppt://genome.cornell.edu/cgi-bin/WebAce/webace?db=graingenes). The barley 'Consensus 2' linkage map (Qi *et al.*, 1996) was primarily used, because this linkage map were essentially generated using data from 4 different genetic maps (Proctor x Nudinka, Igri x Franka, Steptoe x Morex, and Harrington x TR306) and integrating them into one linkage map containing almost 900 markers. SSR markers were integrated into the Steptoe x Morex linkage map by Saghai-Maroof *et al.*(1996). The expected positions of STS- and SSR-markers spread over the genome and mapping in agreement with barley reference maps were initially identified to build a 'linkage skeleton'.

The markers in each pseudo-linkage group were isolated and used in preparing a 'raw file' to run in Mapmaker. Threshold levels was changed with the'default' command from LOD 10 to LOD 3 step by step to separate markers into two groups without generating conflicts with the anchor markers in the 'linkage skeleton'.

All markers were grouped by two-point linkage analysis using series of minimum LOD thresholds from 6.0 to 2.5 with the 'default' option of Mapmaker 3.0 (the higher

number of linkage groups at high threshold level tended to be reduce with lower threshold). During these grouping procedures, the 'linkage map skeleton' was used to track the anchor markers. If there were no conflicting anchor markers in 'joined' groups, with reduced threshold levels, the joined linkage groups were treated a new linkage group. All markers were sorted by assigned chromosomes, and the marker orders were roughly determined by the automated marker ordering procedure in MAPL, and re-tested and confirmed with Map Manager, which offers very convenient editing tools.

The remaining markers were placed to the most probable map position with the 'Report' command in Map Manager. This approach was only utilized to connect linkage fragments to build specific chromosomes.

Results

STS and SSR Markers

Of the Approximately 120 STS primer sets and 44 SSR primer sets evaluated in this population, 31 STSs and 14 SSRs were informative. In the 'Steptoe x Morex' population, approximately 70% of these markers were informative (Table 2). This difference in genetic distance between the parents (36% vs 70%) demonstrates the limited genetic variance within this population when compared to the model 'Steptoe x Morex' population.

Some primer sets amplified multiple bands. In the cases of ABG20.11 and ABG55, BTA002, and MWG938 primer sets amplified two polymorphic bands, which were located on the 'Fiber-2' to well-distinguished loci by linkage analysis (Table A-2; Figure 5). Single monomorphic fragments generated by 11 primer sets were digested with several endonucleases to reveal polymorphisms. This general type of amplification and segregation pattern were commonly observed across all STS markers (Figure 1).

Out of 27 primer sets, 4 showed length polymorphism (ABC156.1, ABC170, ABG054, and MWG620) and 12 showed a presence or absence polymorphism (ABC155, ABC483, ABG20.11, ABG064, ABG380, ABG452, BCD402, BTA002, MWG058, MWG938, MST109, and WTA1) between the parents. The remaining 11 produced monomorphic fragments which contained internal restriction site polymorphisms. Endonucleases were applied to reveal the polymorphism for ABC253, ABC303, ABG55, ABG058, ABG070, ABG609, ABG618, cMWG694, MWG549, MWG2249, and MWG2261 (Table 2).

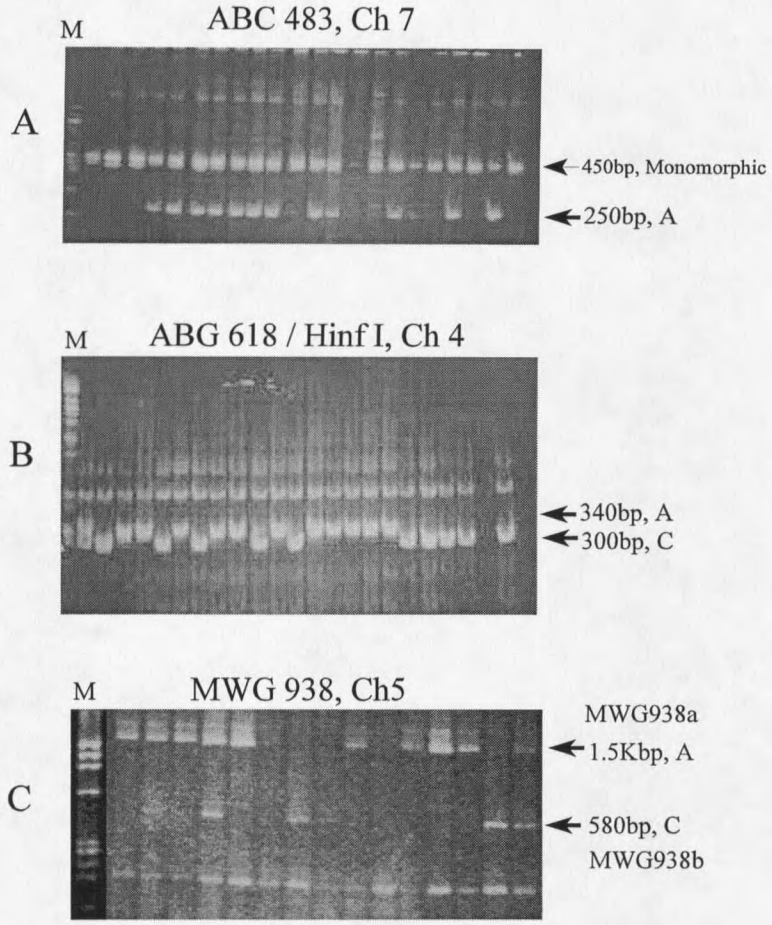


Figure 1. Examples of amplification products and segregation patterns of STS-PCRs in the 'Fiber-2' mapping population. Arrows indicate the segregating loci between MTaz90-123 (A) and MTH6860756(C) with the estimated molecular weights. Letter 'M' indicates DNA size markers, and other lanes represent a set of progeny lines from MTaz90-123 and MTH6860756 cross. Panel A. Primer set ABC483 produced a polymorphic band(250bp) derived from only MTaz90-123 allele. Meanwhile, monomorphic band(450bp) was also produced. Panel B. Primer set ABG618 amplified both parental alleles(550bp), and restriction enzyme (Hinf I) was applied to reveal the polymorphism between two parental alleles (340bp and 300bp were scored as co-dominantly). Panel C. Primer set MWG938 amplified two different loci MWG938a and MWG938b (1.5kbp for MTaz90-123 and 580bp for MTH6860756, respectively). Both two loci were mapped into chromosome 5. These general types of amplification and segregation pattern were commonly observed across all STS markers. The PCR products were analyzed using polyacrylamide gel electrophoresis (PAGF; panel A and panel B) and agarose gel electrophoresis (panel C; mostly for primer sets of 'MWG' group).

Table 2. List of informative STS markers.

Primer name[a]	Band size (bp)[b]		Restriction enzymes[c]	Other bands (bp)[d]	Chromosome[e]		
	A	C			STS	RFLP	Fib-2
ABC155	-	350			7	7	7
ABC156.1	130	110			3	3	5
ABC170	100	120			2	6	2
ABC253	500		Hind III		1	1	1
ABC303	330		Hha I		4	4	4
ABC483	250	-		450	7	7	7
ABG20.11[f]	-	700		1100 + 340 +240	-	-	1
	450	-					7
ABG054	160	180			4	4	4
ABG55[f]	740		Rsa I		5	5	5
	500		Dde I				5
ABG058	1000		Rsa I		2	2	2
ABG064	-	130		500 + 300 + 170	7	7	7
ABG070	450		Bsr I		3	3	3
ABG380	-	320		370 + 350	-	1	1
ABG452	-	330		550	5	5	5
ABG609	500		Rsa I	220 + 120	2	2, 3	2
ABG618	550		Hinf I		4	4	4
BCD402	190	-			-	4	4
BTA002[f]		230		1100	4	4	4
	1300	-					4
cMWG694	1200		Rsa I		2	2	2
MWG058	950	-			4	4	4
MWG938[f]	1500	-			5	5	5
	-	580					5
MWG549	700		Msp I		3, 6,7	3	3
MWG620	850	600			6	6	6
MWG2249	550		Ava II		7	7	7
MWG2261	500		Rsa I		5	5	5
MST109	-	1700		700	6	6	6
WTA1	-	500		460 + 350	-	-	1

Total 31 loci with 27 STS primer sets

[a] Sequences of the STS primers have been published by Blake *et al.* (1996) and Sayed-Tabatabaei *et al.* (1998) except WTA1 and MST109 (Blake, personal communication).

[b] Polymorphic (A=MTaz90-123, C=MTH6860756), and monomorphic.

[c] Endonucleases were used to reveal the polymorphism between two parents.

[d] Non-informative amplified PCR products as common bands between two parents.

[e] Blake *et al.* (1996), Mano *et al.* (1999) and Grain Gene (http://wheat.pw.usda.gov/ ; for ABG380 and BCD402). Column 'Fib-2' indicates the evaluated locations on the 'Fiber-2' linkage map.

[f] Two loci were detected as polymorphic with one primer set, upper rows and lower rows were named 'a' and 'b' in the end of primer name respectively (see Table A-1).

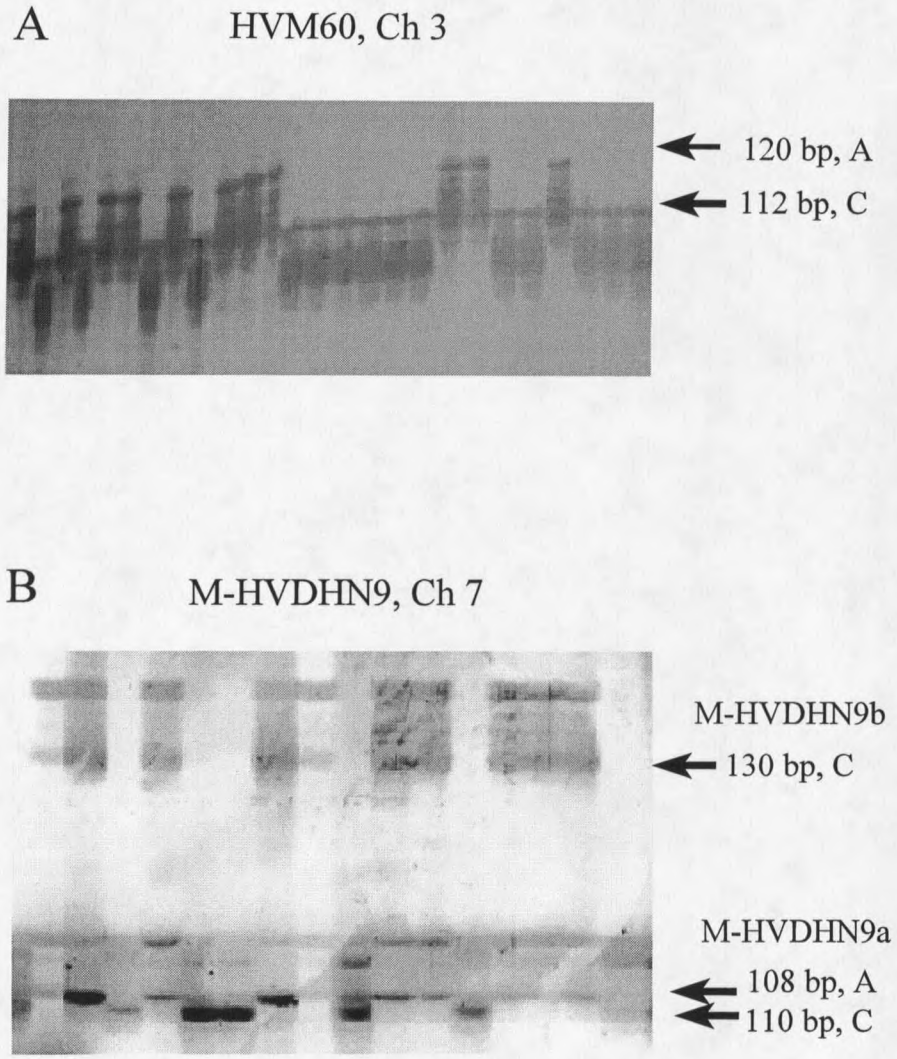


Figure 2. Examples of amplification products and segregation patterns of SSR-PCRs in the 'Fiber-2' mapping population. Arrows indicate the segregating loci between MTaz90-123(A) and MTH6860756(C) with the estimated molecular weights. Lanes represent a set of progeny lines from MTaz90-123 and MTH6860756 cross. Panel A. Primer set HVM60 flanking $(AG)_n$,$(GA)_n$ region, amplified both parental alleles co-dominantly. Panel B. Primer set M-HVDHN9 flanking $(GT)_n$ region, amplified two different loci (130bp for MTH6860756 allele as a dominant marker, and 110bp and 108bp band pairs as co-dominant marker, respectively). Although these two loci, M-HVDHN9a and M-HVDHN9-b, were not separated with the 'Fiber-2' mapping population having 59 lines, it may be possible with increased population size. The PCR products were analyzed using 12% polyarcylamide gels (not in this figure) and 6M urea 4% polyarcrylamide gels (panel A and panel B) following gel staining with ethidium bromide staining and silver-staining, respectively.

Table 3. List of informative SSR markers.

Primer name[a]	Size (bp)[b]		Basic information of SSRs				Chromosome[d]	
	A	C	Source	Repeat	Size(bp)	Reference[c]	S x M	Fib-2
HVM3	210	216	Rubisco activase	$(AT)_{29}$	188	Liu *et al.*	4	4
M-HVWAXYG (HVM4)	290	310	Starch synthase	$(AT)_{9}$	205	Becker and Heun	1	1
M-HVPRP1B (HVM5)	230	210	Pathogenesis-related protein	$(GT)_{6}$, $(AT)_{16}$	167	Becker and Heun	1	1
HVM6	170	168	Old ZZ Library with $(GA)_{10}$ probe	$(GA)_{9}$	175	Liu *et al.*	7	1
HVM36	106	110	Old ZZ Library with $(GA)_{10}$ probe	$(GA)_{13}$	114	Liu *et al.*	2	2
HVM054	160	140	Old ZZ Library with $(GA)_{10}$ probe	$(GA)_{14}$	159	Liu *et al.*	2	2
HVM060	120	112	Old ZZ Library with $(GA)_{10}$ probe	$(AG)_{11}$, $(GA)_{14}$	115	Liu *et al.*	3	3
HVM68	200	206	Old ZZ Library with $(GA)_{10}$ probe	$(GA)_{22}$	204	Liu *et al.*	4	4
M-HVCMA[e] (HVCMA)	120	140	α-amylase inhibitor	$(AT)_{9}$	141	Becker and Heun	1	1
	-	160						1
M-HVDHN9[e] (HVDHN9)	108	110	mRNA for dehydrin-9	$(GT)_{6}$	128	Becker and Heun	7	7
	-	130						7
M-HVCSG (HVCSG)	196	200	Chalcone synthase gene	$(GT)_{4}G_{17}$	196	Becker and Heun	2	2
HVGNIRE	130	128	Nitrate reductase	$(TG)_{6}G_{14}$	136	Becker and Heun		6
Total 14 loci with 12 SSR primer sets								

[a] Sequences of the SSR primers have been published by Becker and Heun (1995) and Liu *et al.* (1996).
The primers names in parentheses indicate the alternative Liu *et al.*'s primer sets flanking the same SSR.
[b] Polymorphic bands (A=MTaz90-123, CA=MTH6860756).
[c] Liu *et al.* (1996) and Becker and Heun (1995).
[d] The determined chromosomal locations of SSRs on Steptoe x Morex (S x M; Liu *et al.*, 1996), and Fiber-2 (Fib-2) mapping population.
[e] Two loci were detected as polymorphic with one SSR primer set. Upper rows and lower rows were named 'a' and 'b' in the end of primer name respectively (see Table A-1).

Forty four SSR primer sets were tested and 12 identified polymorphisms at 14 segregating loci in the 'Fiber-2' population (Table 3). All the primers were initially evaluated using the 'touchdown' PCR methodology. Primer sets which generated a smear of PCR products, produced unexpected fragments with multiple lengths, or amplified no products were not used to test the 'Fiber-2' progeny lines. Twelve out of 14 SSR primer sets produced length polymorphism between two parental alleles due to the different copy number of repetitive sequences (panel A in Figure 2). Minor bands were considered to be artifacts (Liu *et al.*, 1996). M-HVCMA and M-HVDHN9 amplified major products in two distinct size ranges. In both case, one allele was scored as a dominant allele (panel B in Figure 2).

AFLP Markers

Genographer was used to score informative AFLPs and estimate fragment sizes (Figure 3). Some times, the ABI377 automated sequencer failed to detect the DNA size standards utilized by Genographer for lane-to-lane standardization. Naked eye scoring was used to score those AFLP gels. The number of detected informative AFLPs per one primer combination varied from 2 (M-ACG + P-ACG) to 17 (M-CTG + P-GGA), and 10.6 polymorphic AFLPs were detected on average (Table 5). Sometimes, scoring of some AFLPs across lanes, due to background and weak band signal, was not clear.

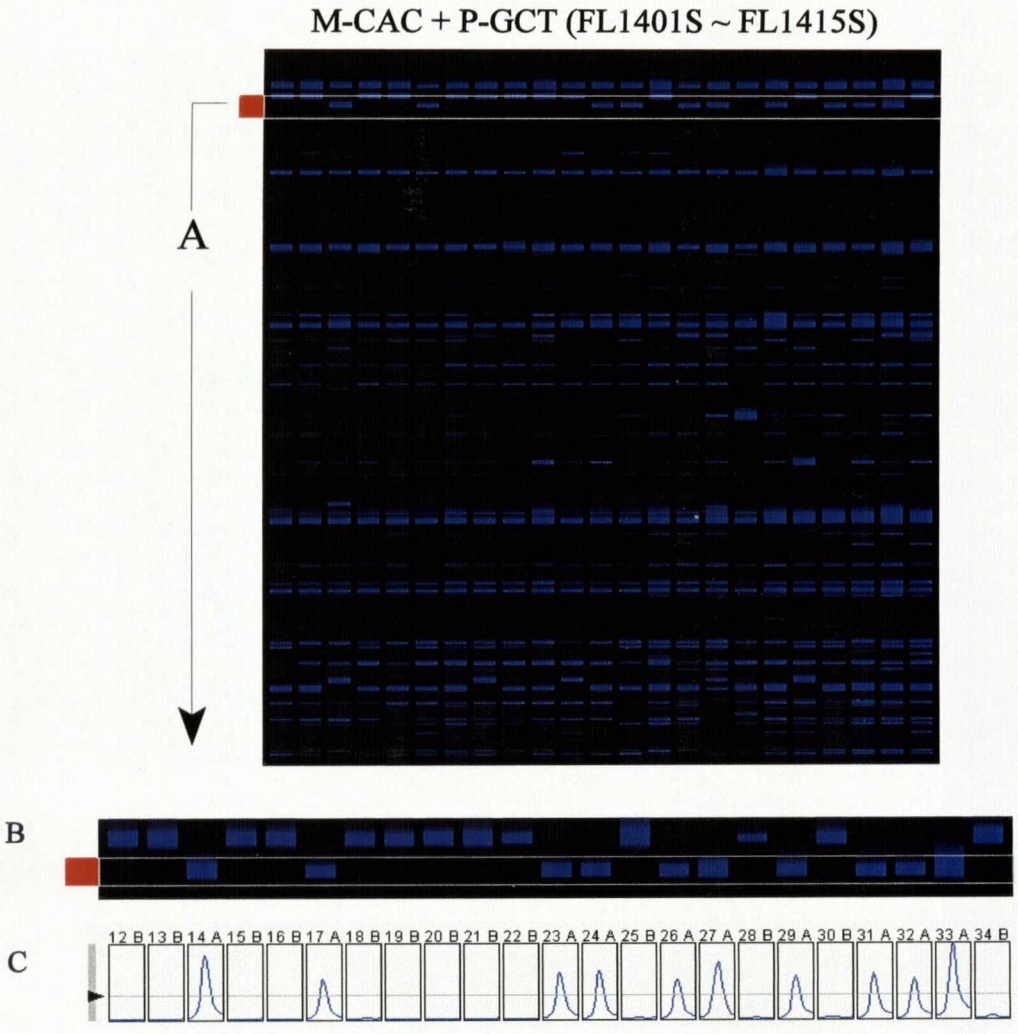


Figure 3. Detection and databasing of informative AFLPs. Amplified AFLPs, with the combination of Mse I and fluorescent labeled Pst I primer set, were detected using ABI377 automated sequencer. Computer software Genographer (Benham *et al.*, 1999) collected lanes from ABI377 and displays a sized and straightened gel image reconstructed from lanes (panel A). Bins were set for the informative AFLPs (two horizontal lanes in panel A and panel B). The bins were automatically scored(panel C). Scoring were exported as a text file to use other mapping programs, such as MapMaker. Panel A. Partial reconstructed gel image of the amplification products of M-CAC + P-GCT primer combination for secondary amplification. One AFLP (FL1412S, 444bp, MTaz90-123 allele) was selected. Panel B. Magnified image. Panel C. The peaks representing amplified band strength were automatically scored after setting the thumbnail (horizontal lane). The progeny lines from #10 to #32 are in this figure.

Table 4. List of informative AFLP markers.

Name[a]	Primer set[b]	Size(bp)[c]	Allele[d]	Chromo-some[e]	Name[a]	Primer set[b]	Size(bp)[c]	Allele[d]	Chromo-some[e]
FL0101A	CAG + ATT	132	A	6	FL1110S	CTG + ATT	366	C	7
FL0102A	CAG + ATT	166	A	6	FL1111S	CTG + ATT	403	A	5
FL0103B	CAG + ATT	199	A		FL1112S	CTG + ATT	650	A	3
FL0104B	CAG + ATT	217	A		FL1201S	CTA + GGA	98	A	
FL0105B	CAG + ATT	244	A		FL1202B	CTA + GGA	280	A	1
FL0106A	CAG + ATT	397	A		FL1203B	CTA + GGA	283	C	
FL0107B	CAG + ATT	456	A		FL1204S	CTA + GGA	298	C	1
FL0201B	CAG + GCA	62	A		FL1205S	CTA + GGA	300	A	1
FL0202S	CAG + GCA	93	A	7	FL1206A	CTA + GGA	322	A	5
FL0203S	CAG + GCA	113	A	1	FL1207B	CTA + GGA	330	A	
FL0204S	CAG + GCA	121	A		FL1208A	CTA + GGA	452	C	5
FL0205B	CAG + GCA	394	A	2	FL1209S	CTA + GGA	490	C	4
FL0206B	CAG + GCA	550	A	5	FL1301S	CTG + GCA	60	A	
FL0207S	CAG + GCA	570	C	1	FL1302A	CTG + GCA	139	A	1
FL0301S	CAC + GCA	55	C	6	FL1303S	CTG + GCA	172	C	6
FL0302B	CAC + GCA	65	C		FL1304S	CTG + GCA	178	A	6
FL0303A	CAC + GCA	75	A	2	FL1305S	CTG + GCA	271	A	1
FL0304S	CAC + GCA	90	C	1	FL1306B	CTG + GCA	276	A	1
FL0305A	CAC + GCA	190	C	3	FL1307B	CTG + GCA	304	A	
FL0306A	CAC + GCA	194	C	3	FL1308S	CTG + GCA	384	C	1
FL0307B	CAC + GCA	270	A	6	FL1309B	CTG + GCA	515	C	1
FL0308S	CAC + GCA	275	C	1	FL1310S	CTG + GCA	580	C	4
FL0309A	CAC + GCA	310	C		FL1311S	CTG + GCA	600	A	4
FL0310A	CAC + GCA	350	A	4	FL1312A	CTG + GCA	680	C	6
FL0311S	CAC + GCA	460	A	3	FL1401S	CAC + GCT	79	A	7
FL0312S	CAC + GCA	650	C	7	FL1402B	CAC + GCT	93	C	
FL0401A	CTA + GCT	60	C	5	FL1403B	CAC + GCT	98	C	
FL0402S	CTA + GCT	158	C	6	FL1404S	CAC + GCT	170	A	2
FL0403S	CTA + GCT	170	A	4	FL1405S	CAC + GCT	175	C	2
FL0404S	CTA + GCT	177	A	2	FL1406A	CAC + GCT	197	A	
FL0405S	CTA + GCT	181	C	7	FL1407B	CAC + GCT	199	C	
FL0406A	CTA + GCT	192	A	7	FL1408B	CAC + GCT	310	A	2
FL0407A	CTA + GCT	194	C	7	FL1409B	CAC + GCT	334	A	6
FL0408B	CTA + GCT	196	C	6	FL1410B	CAC + GCT	337	C	2
FL0409A	CTA + GCT	200	C	4	FL1411B	CAC + GCT	417	C	7
FL0410S	CTA + GCT	234	C	3	FL1412S	CAC + GCT	444	A	3
FL0411S	CTA + GCT	254	A	4	FL1413S	CAC + GCT	449	C	3
FL0412A	CTA + GCT	304	A	6	FL1414S	CAC + GCT	494	A	4
FL0413A	CTA + GCT	368	C	1	FL1415S	CAC + GCT	600	A	7
FL0414S	CTA + GCT	490	C	5	FL1501S	CTC + GGA	145	C	2
FL0415S	CTA + GCT	510	C	5	FL1502B	CTC + GGA	152	A	
FL0416S	CTA + GCT	515	A	5	FL1503A	CTC + GGA	170	C	1
FL0501S	CTT + GGA	76	A	6	FL1504B	CTC + GGA	171	C	
FL0502B	CTT + GGA	84	C	3	FL1505S	CTC + GGA	176	C	1
FL0503A	CTT + GGA	131	A	2	FL1506A	CTC + GGA	205	A	1
FL0504S	CTT + GGA	139	A	1	FL1507A	CTC + GGA	241	C	2
FL0505B	CTT + GGA	160	C		FL1508S	CTC + GGA	343	C	6
FL0506A	CTT + GGA	189	A	7	FL1509S	CTC + GGA	348	A	6
FL0507B	CTT + GGA	419	A		FL1510B	CTC + GGA	362	A	6
FL0508A	CTT + GGA	500	C	1	FL1511B	CTC + GGA	366	C	1
FL0601B	CTC + GCA	58	A	5	FL1512B	CTC + GGA	369	C	5

Table 4. (continued)

Name[a]	Primer set[b]	Size(bp)[c]	Allele[d]	Chromo-some[e]	Name[a]	Primer set[b]	Size(bp)[c]	Allele[d]	Chromo-some[e]
FL0602A	CTC + GCA	83	A	3	FL1513A	CTC + GGA	411	C	1
FL0603S	CTC + GCA	102	C	2	FL1514B	CTC + GGA	433	A	
FL0604B	CTC + GCA	110	A		FL1515A	CTC + GGA	437	C	1
FL0605A	CTC + GCA	147	A	4	FL1601S	CTC + GCT	70	A	4
FL0606A	CTC + GCA	210	C	5	FL1602A	CTC + GCT	172	A	2
FL0607B	CTC + GCA	249	A	2	FL1603A	CTC + GCT	173	C	2
FL0608A	CTC + GCA	270	C	2	FL1604B	CTC + GCT	201	A	3
FL0609S	CTC + GCA	400	C	4	FL1605B	CTC + GCT	282	C	5
FL0701S	CAG + ACG	220	C	4	FL1606B	CTC + GCT	365	A	
FL0702A	CAG + ACG	331	C	2	FL1607B	CTC + GCT	347	A	7
FL0801S	CTT + GCT	161	A	1	FL1608A	CTC + GCT	424	A	7
FL0802S	CTT + GCT	207	C	5	FL1609B	CTC + GCT	460	A	5
FL0803S	CTT + GCT	453	A	7	FL1610B	CTC + GCT	465	C	
FL0804B	CTT + GCT	490	A		FL1611B	CTC + GCT	525	A	
FL0805A	CTT + GCT	495	A	5	FL1612B	CTC + GCT	530	A	3
FL0806S	CTT + GCT	700	C	3	FL1613S	CTC + GCT	570	A	7
FL0901B	CTA + ATC	61	A		FL1614S	CTC + GCT	580	A	3
FL0902B	CTA + ATC	130	C	5	FL1701S	CTG + GGA	67	A	5
FL0903B	CTA + ATC	161	A		FL1702B	CTG + GGA	87	A	7
FL0904S	CTA + ATC	166	A	1	FL1703S	CTG + GGA	96	C	5
FL0905B	CTA + ATC	266	A		FL1704B	CTG + GGA	178	A	
FL0906S	CTA + ATC	327	C	2	FL1705S	CTG + GGA	218	A	4
FL0907B	CTA + ATC	405	C		FL1706S	CTG + GGA	240	C	5
FL0908S	CTA + ATC	434	A	2	FL1707S	CTG + GGA	303	A	7
FL0909A	CTA + ATC	460	C	1	FL1708S	CTG + GGA	304	C	7
FL0910B	CTA + ATC	470	A	1	FL1709A	CTG + GGA	306	C	6
FL1001B	CTG + GCT	73	A		FL1710A	CTG + GGA	383	C	
FL1002B	CTG + GCT	111	A		FL1711A	CTG + GGA	388	C	1
FL1003S	CTG + GCT	123	A	1	FL1712S	CTG + GGA	422	A	7
FL1004S	CTG + GCT	155	C	1	FL1713A	CTG + GGA	435	A	3
FL1005A	CTG + GCT	180	A	7	FL1714B	CTG + GGA	453	A	
FL1006B	CTG + GCT	310	C	7	FL1715B	CTG + GGA	520	A	4
FL1007B	CTG + GCT	315	C		FL1716S	CTG + GGA	570	C	6
FL1008B	CTG + GCT	400	A		FL1717A	CTG + GGA	630	A	3
FL1009B	CTG + GCT	450	A	1	FL1801A	CAC + GAG	56	C	7
FL1010B	CTG + GCT	500	C	3	FL1802A	CAC + GAG	94	A	7
FL1101S	CTG + ATT	74	A	6	FL1803A	CAC + GAG	150	C	3
FL1102B	CTG + ATT	224	A	3	FL1804S	CAC + GAG	252	C	6
FL1103A	CTG + ATT	225	A		FL1805S	CAC + GAG	316	C	4
FL1104S	CTG + ATT	245	C	2	FL1806S	CAC + GAG	324	A	3
FL1105S	CTG + ATT	272	C	1	FL1807S	CAC + GAG	350	A	3
FL1106S	CTG + ATT	308	A	2	FL1808S	CAC + GAG	359	C	3
FL1107S	CTG + ATT	309	C	2	FL1809S	CAC + GAG	364	C	6
FL1108B	CTG + ATT	335	C	2	FL1810A	CAC + GAG	414	C	
FL1109S	CTG + ATT	352	A	1	Total 191 AFLPs				

[a] The last capital letters indicate the relative marker quality; S for very strong, A for clear, and B for weak AFLPs.

[b] Three 3' selective nucleotides. (Mse I + Pst I).

[c] Rough size estimation was applied for the bands over than 500bp.

[d] MTaz90-123 allele (A) and MTH6860756 allele (C).

[e] Chromosomal location of AFLP markers on 'Fiber-2' linkage map (blank=not determined).

Table 5. Distribution of AFLP markers.

All AFLP markers						Markers on the 'Fiber-2' linkage map				
Gel	3' selective nucleotides		Marker quality [a]		Total	Number	Ratio(%)	Marker quality [a]		Covered chromosome (No.) [b]
	Mse I	Pst I	S + A	B				S + A	B	
1	CGA	ATT	3	4	7	2	28.6	2	0	1
2	CAG	GCA	4	3	7	5	71.4	3	2	4
3	CAC	GCA	10	2	12	10	83.3	9	1	6
4	CTA	GCT	15	1	16	16	100.0	15	1	7
5	CTT	GGA	5	3	8	6	75.0	5	1	5
6	CTC	GCA	6	3	9	8	88.9	6	2	4
7	CAG	ACG	2	0	2	2	100.0	2	0	2
8	CTT	GCT	5	1	6	5	83.3	5	0	4
9	CTA	ATC	4	6	10	6	60.0	4	2	3
10	CTG	GCT	3	7	10	6	60.0	3	3	3
11	CTG	ATT	10	2	12	11	91.7	9	2	6
12	CTA	GGA	6	3	9	6	66.7	5	1	3
13	CTG	GCA	9	3	12	10	83.3	8	2	3
14	CAC	GCT	8	7	15	11	73.3	8	3	5
15	CTC	GGA	9	6	15	12	80.0	8	4	4
16	CTC	GCT	6	8	14	11	78.6	7	4	5
17	CTG	GGA	13	4	17	14	82.4	13	1	6
18	CAC	GAG	10	0	10	9	90.0	9	0	4
Total (band number)			128	63	191	Total (%)	150 (78.5%)	121 (80.7 %)	29 (19.3 %)	
Mean (band number)			7.1	3.5	10.6	Marker usage ratio (%)		94.5 %	46.0 %	

[a] All AFLP markers were classified with strong and clear bands (S + A) and weak bands (B) when AFLPs were scored.
[b] The number of chromosomes covered by AFLP markers.

Those were scored as missing data. To control marker informativeness during mapping, all AFLP markers were named dependent on band strength and clarity (Table 4). This type of quality tagging on the AFLP marker names was very helpful in judging and handling problematic markers during segregation analysis and linkage map construction. A survey of different primer combinations, the number of polymorphisms, the band sizes and the distribution of AFLP markers across chromosomes is provided in Table 4.

Segregation Test of All Informative Markers

In total, 241 informative markers (3 morphological, 2 protein, 31 STSs, 14 SSRs, and 191 AFLPs) were used in linkage map construction. Three morphological markers (*lk*$_2$, *n*, and *wx*) were screened during the 1998 field trials and re-confirmed in 1999. The 59 'Fiber-2' mapping lines were scored whether F_5 derived lines contained both phenotypes to infer 3 possible genotypes in each individual lines. The segregation ratio was skewed at the *wx* locus, with 31 homozygous recessives, 20 homozygous dominants and 8 heterozygous (heterogeneous) lines (Table A-1).

All 241 markers, including three morphological markers with considering missing data in those lines having mixed phenotypes, were tested against expected segregation ratio at F_{33} (as ideal recombinant inbred line) generation (Table A-1). The majority of markers showed 1:1 segregation ratio for the two parental alleles (85% and 95% at the significance level $P \leq 0.05$ and $P \leq 0.01$ level, respectively). Only two STS (ABC156.1 and ABG058) and 33 AFLP markers exhibited distorted segregation at the 0.05 level. Twenty-seven of these loci were skewed towards MTaz90-123 (the maternal parent) alleles and 8 towards

MTH6860756 alleles. About 45% of the markers that showed distorted segregation ratio belonged to AFLP marker class 'B (weak band). Table A-1 shows the result of segregation test and genotyping of all 241 loci.

Considering the information collected from 241 loci, the percentage of the MTaz90-123 genome in the 'Fiber-2' progenies was on average 48.7%, ranging from 34.9 (#25) to 65.8% (#46) (Table A-3).

First Attempt to Construct 'Fiber-2' Linkage Map

The first attempt was carried out following 'routine' procedures. Markers were grouped using pairwise linkage analysis to generate linkage groups. Then chromosomes were assigned to linkage groups using anchor probes. Markers were then ordered within each linkage group and multipoint recombination fractions were estimated among adjacent loci.

Mapping was carried out using Mapmaker and STS markers were used anchor probes to assign each barley chromosome. LOD 3.0 and a 50cM maximum distance were used thresholds in grouping procedures. As a result, 29 linkage groups were identified. Among of them, 10 linkage groups with 42 AFLP markers were 'orphan' fragments in which there was no anchor markers.

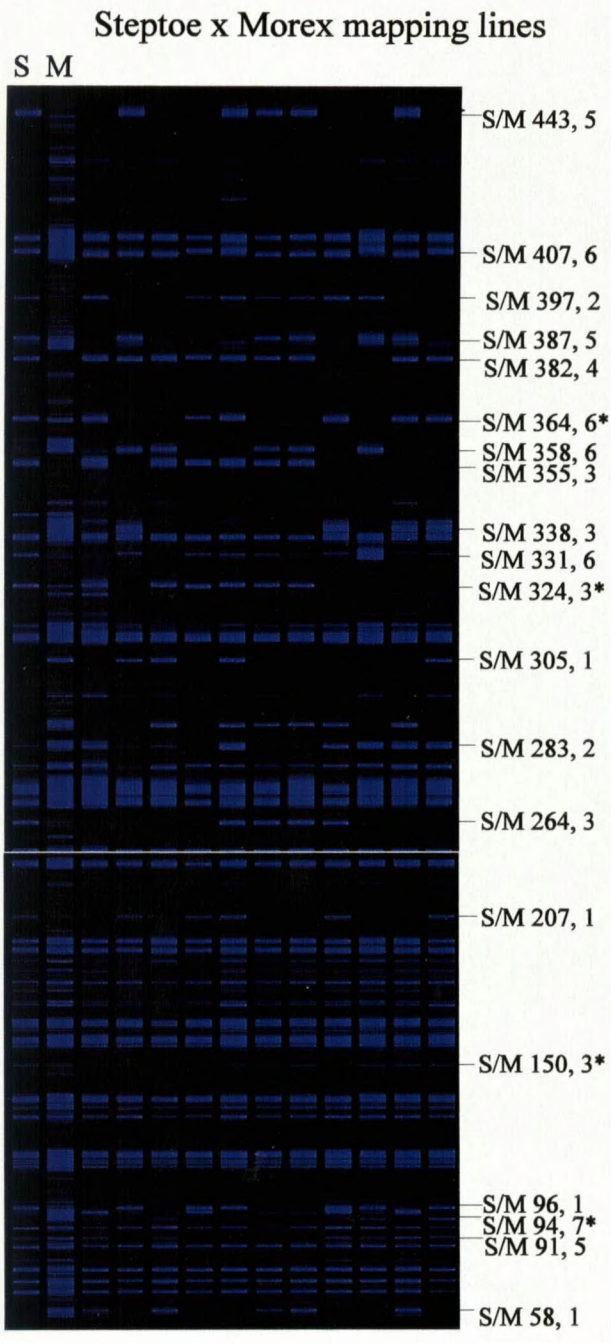
Two psuedo-linkage groups were detected. Chromosomes 1 and 7 were apparently linked (*wx* and M-HVWAXYG as chromosome 1, and ABC483 and HVM6 as chromosome 7 anchor markers, respectively). Also chromosomes 3 and 6 showed significant association

(MWG549 as chromosome 3 and MWG620 as chromosome 6 anchor markers, respectively). Over 45% of the AFLP markers remained outside the boundaries of the anchored map.

Increasing AFLP Marker Utility with Band Size Comparative Analysis

The AFLP technique has been used to estimate genetic diversity in barley (Hayes *et al.*, 1997; Pakniyat *et al.*, 1997). It is possible to consider comparative analysis of AFLPs between two different mapping populations. We have a well-developed map in the Steptoe x Morex population and decided to attempt positional analysis of 'orphan' AFLP fragments in the 'Fiber-2' population by looking for homologous variation in the Steptoe x Morex population. If an AFLP polymorphism could be mapped with certainty in the Steptoe x Morex population, its homologue segregating in Fiber-2 should be at the same position. We looked for 'orphan' fragment AFLP products which segregated in both populations. The fragments had to be identically sized in both populations, and had to segregate in both. Consequently, if any 'Fiber-2' AFLP could be localized, the 'orphan' fragment including the AFLP could be also potentially localized.

Fifty six Steptoe x Morex lines were randomly selected from 150 mapping lines and used as DNA template in AFLP analysis with same primer combinations used in the 'Fiber-2'. The detected Steptoe x Morex AFLPs were placed into the previously constructed linkage map with the 'place' command of Mapmaker program. The band sizes of those assigned AFLPs were compared with AFLPs from the 'Fiber-2'. Using the primer combination (M-CAC + P-GAG), 20 informative AFLPs were detected, and 4 AFLPs had same band size between Steptoe x Morex and the 'Fiber-2' population (Figure 4).



Fiber-2 M-CAC + P-GAG		
Name	bp	Ch.
FL1810A	414	
FL1809S	364 *	6
FL1808S	359	3
FL1807S	350	3
FL1806S	324 *	3
FL1805S	316	4
FL1804S	252	6
FL1803A	150 *	3
FL1802A	94 *	7
FL1801A	56	7

Figure 4. Increasing AFLP marker utility with comparative analysis. AFLP markers which fell into 'orphan' linkage groups were evaluated for potential placement on the 'Fiber-2' linkage map through comparative analysis using the 'Steptoe x Morex'(S/M) population. A primer combination of M-CAC + P-GAG was used to generate AFLPs with 56 S/M lines. Informative S/M AFLPs are reported with the band size and assigned chromosome number. '*' indicate the same band sizes between S/M and 'Fiber-2' AFLPs.

Two orphan groups were assigned to chromosomes using this approach (Figure 5; FL1809S, the end of chromosome 6 long arm and FL1802A, the top of chromosome 7 short arm, respectively). One AFLP (FL1803A) was used to join two linkage fragments into 'one' chromosome 3. The successful outcome of this comparative analysis could be confirmed after building the 'linkage skeleton'(see below). Still we had more linkage groups than chromosomes and we needed better information to line-up those fragments to build more complete maps for each of the seven chromosomes.

Construction of 'Fiber-2' Linkage Map Skeleton

The 'Barley Consensus 2' (Con2) linkage map was primarily used to determine the relative locations of anchor markers among barley chromosomes. If Con2 did not contain the marker, three other linkage maps were screened; Steptoe x Morex (SxM), Igri x Franka (IxF), and Chebec x Herrington (CxH). For the SSR markers, the SSR-integrated SxM linkage map (Saghai-Maroof *et al.*. 1996) was used.

From the first observation in which anchor markers were not scaffolded across the 'Fiber-2' chromosomes, 6 more STS markers were added to anchor the 'anchor empty' regions; ABC253 (Ch 1, 294cM), ABG058 (Ch 2, 16cM), cMWG694 (Ch 2, 140cM), and BCD402 (Ch4, 342cM) (Figure 5 and Table A-2). As we added additional anchor markers, some 'orphan' AFLPs were rescued by additional anchor markers due to their linkage relationships with the anchors. Table 6 shows the distribution of anchor markers expressed with the percentage of chromosome length of each detected population.

Table 6. The construction of linkage map skeleton with previously reported polymorphic markers and their comparative locations on 'Fiber-2' linkage map.

Linkage map skeleton						Locations on 'Fiber-2' linkage map			
CH	Marker	Pop.[a]	Length(cM)	Loc.(cM)	%[b]	CH	Length(cM)	Loc.(cM)	%
1	*wx*	S x M	170	20	11.8	1	325	30	9.2
	M-HVWAXYYG	I x F	200	28	13.7	1	325	29	8.9
	ABG380	Con2	152	32	20.9	1	325	78	24.0
	M-HVCMA	S x M	170	68	40.0	1	325	140	43.1
	n	S x M	170	75	44.2	1	325	156	48.0
	lk_2	S x M	170	85	50.1	1	325	170	52.3
	ABC253	Con2	152	120	78.7	1	325	283	87.1
	M-HVPRP1B	S x M	172	170	99.0	1	325	325	100.0
2	ABG058	Con2	157	12	7.5	2	361	15	4.2
	HVM36	S x M	179	36	20.1	2	361	40	11.1
	HVM054	S x M	179	161	90.1	2	361	268	74.2
	ABG609	Con2	157	143	91.0	2	361	309	85.6
	cMWG694	C x H	157	145	92.1	2	361	131	36.3
	M-HVCSG	S x M	179	170	95.0	2	361	316	87.5
3	ABG070	S x M	185	10	5.4	3	257	0	0.0
	HVM060	S x M	185	93	50.3	3	257	112	43.6
	MWG549	Con2	131	104	79.4	3	257	213	82.9
4	ABC303	Con2	134	49	36.8	4	350	73	20.9
	MWG058	Con2	134	53	39.5	4	350	91	26.0
	HVM3	I x F	137	61	44.7	4	350	87	24.9
	HVM68	S x M	177	90	53.0	4	350	115	32.9
	ABG618	Con2	134	85	63.8	4	350	135	38.6
	ABG054	Con2	134	97	72.1	4	350	148	42.3
	BCD402	Con2	134	124	92.6	4	350	331	94.6
5	MWG938	Con2	150	24	16.3	5	390	43	11.0
	Hor 2	Con2	150	26	17.0	5	390	79	20.3
	Hor 1	Con2	150	35	23.4	5	390	80	20.5
	ABG452	Con2	150	74	49.1	5	390	248	63.6
	ABG55	Con2	150	144	95.9	5	390	387	99.2
6	MWG620	Con2	141	7	4.9	6	313	12	3.8
	ABC170[c]	Con2	141	99	70.5	2	361	56	15.5
7	ABC483	Con2	195	17	8.6	7	335	6	1.8
	M-HVDHN9	S x M	202	112	55.5	7	335	104	31.0

Table 6. (continued)

	Linkage map skeleton					Locations on 'Fiber-2' linkage map			
CH	Marker	Pop.[a]	Length(cM)	Loc.(cM)	%[b]	CH	Length(cM)	Loc.(cM)	%
	ABC155	Con2	195	159	81.5	7	335	169	50.4
7	MWG2249	IxF	239	195	81.6	7	335	319	95.2
	HVM6	S x M	202	188	93.2	1	325	32	9.8
	ABG20.11					1	325	167	51.4
	BTA02	S 4	R4			4	350	305	87.1
	ABC156.1	S 3	R3			5	390	84	21.5
Unreported[d]	MWG2261	S 5	R5			5	390	98	25.1
	MST109	S 6	R6			6	313	5	1.6
	HVGNIRE					6	313	53	16.9
	ABG064	S 6	R7			7	335	0	0.0

[a] The location of anchor markers were surveyed at a web site, http://genome.cornell.edu/cgi-bin/WebAce/webace?db=graingenes with 'Locus' search category. Barley consensus 2(Con2) linkage map was primarily used to determine the relative locations of anchor markers among barley chromosomes. If Con2 did not contain the locus, three other linkage maps were screened; Steptoe x Morex (SxM), Igri x Franka (IxF), and Chebec x Herrington (CxH). SSR markers were integrated into the Sx M linkage map by Saghai-Maroof *et al.*(1996).

[b] The locations of each locus were expressed as the percentage of the linkage map length.

[c] Chromosomal location of the STS-PCR product was previously reported (see Table 2).

[d] The numbers followed by S and R indicate chromosomes (see Table 2), where S and R stand for the wheat-barley additional line test (S) and the original location of RFLP probes (R), respectively.

In total, 36 anchors were incorporated into the 'linkage skeleton' and 7 markers were remained as 'un-localized'. However, 5 of these markers (BTA02, ABC156.1, MWG2261, MST109, and ABG064) still had very useful information- the potential chromosomal locations revealed by 'wheat-barley additional line' test, and the original location of RFLP probes (Table 2; Table 6), so that those markers could be tentatively assigned to chromosomes.

In summary, the 'linkage map skeleton' included a relatively large number of reasonably well-distributed previously-mapped anchor markers. Furthermore, the assigned chromosome numbers and relative positions of anchor markers in each chromosome were also utilized.

Still large gaps remained on every chromosome except chromosome 1. For example, chromosome 2 did not have anchor markers over 70% of its recombinational length (Table 6). The majority of these gaps were filled with AFLP markers.

Pseudo-linkage Separation

The pseudo-linkage of Ch3-Ch6 included 26 markers which separated appropriately into two groups at LOD 5.5. The markers belonging to chromosome 3 were located on chromosome 3's long (m) arm, while those belonging to chromosome 6 marked the short arm. Among of the 14 partial chromosome 6 markers, including MWG620 as an anchor on 'linkage skeleton', MST109 was also included. The potential chromosomal location of MST109 was determined as barley chromosome 6 (Blake *et al.*, 1996; Table 2).

The pseudo-linkage of Ch1-Ch7 also resolved into two groups at LOD 5.0. Among of 17 markers in Ch1-Ch7 pseudo-linkage group, HVM6 (chromosome 7 anchor marker; genotyping data in Table A-1) could not be separated from chromosome 1 anchor markers (*wx* and M-HVWAXYG) even when a very high threshold (LOD 10) was applied, so that map distortion at HVM6 was allowed in the 'Fiber-2' linkage map (Figure 5, top of the chromosome 1).

Linkage Map Construction with All Available Information

Marker grouping was carried out using Mapmaker. All markers were grouped with a high threshold level, LOD 8 and a 50cM maximum distance, and confirmed that there were no conflicting anchor markers. Reduced threshold levels were applied step by step (LOD 0.5

intervals) to increase marker usage. If any group showed an anchor marker conflict, newly incoming markers, due to the reduced threshold, were not used to increase marker number in that group and were treated as 'Still-Orphan'. Threshold level was reduced down to LOD 2.5. During this procedure, 161 markers were assigned to groups having at least one anchor markers on the 'linkage skeleton'. Those markers were imported into both MAPL and MapManager software. The marker orders in each group were rapidly established with the automated ordering procedure in MAPL. The groups assigned to each chromosome were lined up on one chromosome depending on the information of 'linkage map skeleton' with MapManager. The orientation of each group were also determined with MapManager comparing two-point linkage analysis data. The predetermined marker orders with MAPL were re-tested and confirmed.

Using the 'Distribute' and 'Report' commands in MapManager, the remaining 60 markers were placed on the map (mostly those markers treated as 'Still Orphan'). Chromosome 2, 3, and 4 still had disconnected two linkage groups. These groups were connected with most likely AFLP markers (FL1104S for chromosome 2, and FL1808S for chromosome 3). Due to the gap between the short arm region of chromosome 4 and a small group tagged with anchor marker BCD402, three AFLP markers (FL0605A, FL1805A, and FL0411S) were used. During additional marker placement those markers classified as AFLP marker group 'B; weak band but countable (Table 4, Table 5) were not added.

The 199 markers (3 morphological, 2 protein, 30 STSs, 14 SSRs and 150 AFLPs) used for the 'Fiber-2' linkage map covered a distance of 2370 cM, corresponding to approximately 12 cM per marker. The percent of the genome within 20 cM to the nearest marker was 97

%(Figure 5). Chromosome 1 had the largest number of markers. Chromosome 5 displayed the longest genetic length. Forty two markers (ABG20.11b and 41 AFLPs) were not consistently ordered along the respective chromosomes by multi-point analysis and, therefore, were not included in the map. In total, 150 AFLP markers(78%) were used in the 'Fiber-2' linkage map. The best-characterized AFLPs, (class S and A) were almost completely used (95%), while only 46% of class B AFLPs were used (Table 5). The relative location on each chromosome of anchor markers showed good alinement between 'linkage skeleton' and 'Fiber-2' linkage map except cMWG694 tagged region. The map position of this marker was on the bottom of long arm in 'Chebec x Herrington' population, the position in 'Fiber-2' linkage map was on short arm region (Table 6). Seven markers on the 'Fiber-2' linkage map showed segregation distortion ($P \leq 0.001$). Two AFLPs were on chromosome 2, and the other 5 AFLPs were anchored by MWG938 and placed on the short arm of chromosome 5 (Figure 5).

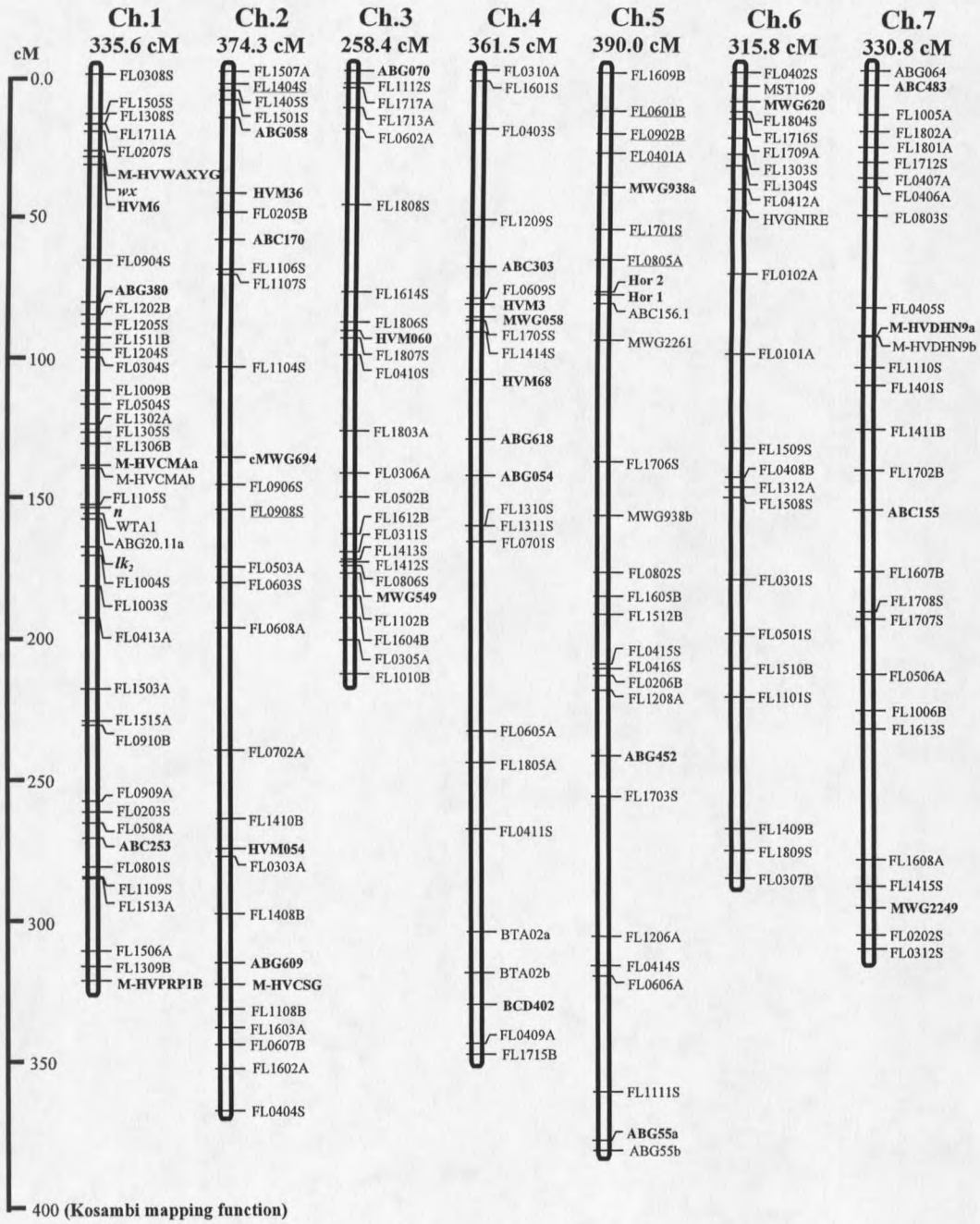


Figure 5. Linkage map of the 'Fiber-2 (MTaz90-123 x MTH6860756)' mapping population. Linkage map containes 199 markers (3 morphological, 2 protein, 30 STS, 14 SSR, and 150 AFLP markers) and covers a distance of 2370 cM. The location of anchor markers used in 'linkage map skeleton' construction (Table 6) are indicated with bold letters. Underlined markers indicate the segregation distortion ($P \leq 0.01$) (Table A-1). Map distance are centimorgans calculated using the Kosambi mapping function. Detail marker intervals are in Table A-2.

Discussion

The morphological markers (*lk*$_2$, *n* and *wx*) were showed 2 times higher heterozygosity levels than expected value (6.25%) in F_5 generation. Three times higher heterozygous types than the expected level were reported by Burr and Burr (1991) in two maize RILs, and Mano *et al.*(1999) reported the level of heterozygosity in barley RILs was 3 times higher than expected level. The result of unintentional selection against plants with low fertility could be one reason of identified high level of heterozygosity (Paran *et al.*, 1995). Another possible reason may be that the relatively high adaptability of heterozygous types to stresses may affect the frequency of genotypes (Mano *et al.*, 1999). During the development of the RIL, there was no fertility barrier between parents, meanwhile, some lines were lost during single seed descent (Blake, personal communication). Also, the population is small (n=59), the morphological markers lie on the same chromosome and this level of deviation from expectation may merely be the result of inadequate sampling.

Segregation ratios of observed genotypic frequencies may depart from the expected frequencies. Altered survival among some classes of gametes or zygotes could be one of the genetical reason (Harushima, *et al.*, 1996). Genotype classification error could be one of the reasons that segregation ratio distortion occurs. Segregation distortion affects linkage tests and the estimation of recombination fractions (Lorieux *et al.*, 1995), and few software can treat the distorted marker classes with very limited range (Manly, 1999).

Segregation ratio was seriously skewed at the Waxy locus (20: 8: 31, for *WxWx: Wxwx: wxwx* at F5 generation) (Table A-1). Bezant *et al.* (1997) reported that segregation distortion was detected on barley chromosome 1, with the *wx* locus in the center of the distorted region, and explained that linkage of a gametophytic factor, *Ga*, to the *wx* locus in barley causing segregation distortion. Frykman and Bengtsson (1992) also reported seriously distorted segregation ratio in waxy-heterozygous barley plants. However, the other markers around *wx* locus on 'Fiber-2' linkage map did not show segregation distortion (Figure 5; Table A-1).

Classification of AFLP markers by their band strength and clarity helped to control problems derived from artifact-based scoring of detected AFLPs (Table 5). Out of 63 class 'B' AFLPs, only 29 (46%) were used, so that the percentage of class 'B' AFLP markers was only 14% in 'Fiber-2' linkage map (Table 6). Five distorted markers AFLP markers ($P \leq 0.01$) were mapped on chromosome 5 (Figure 5).

The level of polymorphism is a reflection of the genetic distance between the two parents. In this population, SSR analysis showed 27% polymorphism (12/44), while 60% polymorphism was reported in the Steptoe x Morex cross (Liu *et al.*, 1996). Comparative AFLP and STS analysis of the Steptoe x Morex vs. the 'Fiber-2' populations showed a similar 2-fold higher polymorphism frequency in Steptoe x Morex. The relative level of polymorphisms in this cross was lower than in of ideal mapping populations, and resulted in gaps in the 'Fiber-2' linkage map.

At first, we expected that the AFLP technique would be a helpful to overcome two potential problems underlying linkage map generation of real breeding populations- 1)

enough number of informative markers and 2) even marker spacing across chromosomes, and it is clear that enough number of markers were successfully developed with the technique (191 AFLP markers). It should be noticed that, under this study, just Pst I and Mse I digested-DNA templates were used to generated AFLP markers from recombinationally active chromosomal regions (Finnegan *et al.*, 1998; Castiglinoi *et al.*, 1999). The 'Fiber-2' linkage map showed that all mapped AFLP markers were randomly distributed across chromosomes and chromosome regions.

The major difficulty in constructing the 'Fiber-2' linkage map was the failure of markers to resolve into seven linkage groups due to the lack of polymorphisms at certain chromosomal areas. These regions are presumably recombinationally active, but monomorphic between the two parents. Consequently, without the aid of anchor markers, the groups containing only AFLPs tended to be 'orphan groups'. Therefore, there should be more effort made in developing and choosing anchor markers to generate linkage maps for real breeding populations, in which genetically close elite lines are the starting point of new breeding programs.

CHAPTER 3

MAPPING QTL CONTROLLING IMPORTANT CHARACTERS IN THE FIBER-2 POPULATION

Introduction

The objective of this chapter is to identify the markers linked to QTLs controlling high fiber content (β-D-glucan) and grain yield, including three other important traits- kernel weight, heading dates and plant height, within 'MTaz90-123 x MTH6860756' population and determine the genetic basis for poor agronomic performance in waxy-hulless barley lines. In this study, the 'Fiber-2' linkage map (chapter 2) was used.

Hulless barley varieties suffer from poor yield (Goering *et al.*, 1973; Eslick *et al.*, 1990; Blake *et al.*, 1990). Three genes, *wx*, lk_2, and *n*, have been implicated as important contributors to hulless waxy barley β-D-glucan content and also have been shown to contribute to grain yield reduction (McGuire and Hockett 1981; Swanston, 1995; Xue *et al.*, 1997; Czuchajowska *et al.*, 1998). These analyses unfortunately confound linkage, linkage drag, background effects and small sample sizes to varying degrees.

The availability of molecular marker-based linkage maps makes it possible to dissect quantitative traits into discrete genetic factors. With a good map and mapping population,

all regions of a genome can be assayed for gene content by interval analysis (Lander and Botstein, 1989; Haley and Knott, 1992). Having the ability to identify specific quantitative trait loci (QTL) may lead to more powerful means of investigating the number of genes impacting a character, their primary effects and their interactions (Edward *et al.*, 1987).

The multilocus approaches that have been developed for marker or interval by phenotype interaction analysis are extensions of standard methods which were adopted to map single genes and their effects on phenotype (Kearsey, 1998). The 'composite interval' concept permits the use of estimation procedures to propose the presence of one or more QTL at different positions throughout the genome, then translate the data into a statistical model which can evaluate the plausibility of the hypothesis.

The most common QTL analysis approaches evaluate intervals between pairs of markers for the presence of QTL (Simple interval mapping; Lander and Botstein, 1989; Haley and Knott, 1992). However, simple interval mapping is an insufficient approach for QTL mapping if multiple QTL of dramatically different effect simultaneously segregate in a population, or if QTL are poorly marked (Liu, 1998; pp 417-444).

Three easily identified genes typically used in construction of high fiber hulless barley varieties (*waxy*, lk_2, and *n*), are generally believed to have major effects on β-D-glucan content and grain yield. All are located on barley chromosome 1 (Kleinhofs, 1996), and lk_2, and *n* are closely linked to each other. Therefore, it is unreasonable to treat these genes as independent factors to estimate those gene effects with 'linear model' or 'simple interval mapping' procedures.

Jansen and Stam (1994) and Zeng (1994) proposed another method called composite interval mapping for dealing with multiple QTLs simultaneously to solve the non-independence problem. This analysis method increases the resolution of QTL location by controlling residual noise in the model using markers other than the markers immediately flanking the segment.

The problems associated with QTL analysis are numerous. If individual QTL effects are small, the number of informative meioses is limiting, or the complexity of the characters of interest is large, marker-assisted selection may not prove helpful. However, marker-assisted selection has proven useful in many instances (Larson *et al.*, 1996; Spaner *et al.*, 1999).

Materials and Methods

Plant Materials

Population development procedures are described in Chapter 2. The 'Fiber-1' population contained 61 individuals, while the 'Fiber-2' and 'Fiber-3' populations contained 59 and 96 lines respectively. The linkage map was generated using the 'Fiber-2' population (Chapter 2).

Field Experiments

Table 7 describes methods of trait measurement, experimental conditions, and agronomic traits measured of the three populations in each field trial. F_5 ('Fiber-2' and 'Fiber-3') and F_4 ('Fiber-1') derived seed was advanced from each individual lines through two years (in 1996, 1997) to generate sufficient seeds for replicated year trials, and β-D-glucan content analyses were performed with the 'Fiber-1' (in 1997) and 'Fiber-2' (in 1996, 1997) populations.

Populations were evaluated over two years in replicated field trials at the Arthur Post Research Farm near Bozeman in irrigated and dryland fields. Lines in each experiment were replicated as two randomized blocks. In 1998, each plot was 4-row (4m per row), seeded at a rate of 40g/plot. In 1999, 2-row plots were utilized. Four agronomic traits, grain yield, β-D-glucan content, heading date and plant height, were measured following the methods described in Table 7.

Table 7. Description of agronomic traits measured and field trial conditions

Agronomic traits		
Trait (Short name)	Units	Method of measurement
Grain yield (YD)	kg/ha	Weight of barley grain harvested per unit area (excluding hull weight in hulless lines).
Kernel weight (KW)	mg/seed	Average weight of an individual barley kernel (excluding hull weight in hulless lines) from a sample 500 grains.
β-D-glucan (GU)	%	β-D-glucan content was determined on duplicates of air-dried ground grain according McCleary and Codd (1991) using a commercial kit (Megazyme International Ireland Ltd., Wicklow, Ireand).
Heading date (HD)	days	Number of days until emergence 50% of heads on main tillers.
Plant height (PH)	cm	Average plant height measured from soil surface to the top of spike (excluding awns).

Field trial conditions [a]									
	Fiber-2			Fiber-3			Fiber-1		
Year	F_5 59 lines			F_5 96 lines			F_4 61 lines		
	Field [b]	Plot [c]	Har. [d]	Field [b]	Plot [c]	Har. [d]	Field [b]	Plot [c]	Har. [d]
96'	Irri.	1	HS	Irri.	1	HS	Irri	1	HS
97'	Irri.	2	HS	Irri.	2	HS	Irri	2	HS
98'	Dry	4	SC	Irri	2	HS	Dry	4	SC
99'	Irri.	2	HS	Irri.	2	HS	Not evaluated		

List of traits measured in each year tested [e]			
96'	GU		
97'	GU	GU	
98'	YD, HD, PH, KW	YD, HD, PH	YD, HD, PH
99'	YD, HD, PH, GU, KW	YD, HD, PH, GU	

[a] Seed was advanced from each individual lines during two years (96' and 97') to generate sufficient seed for replicated year trials in 98' and 99'.
[b] Bozeman dry and Irrigated (Irri.) land fields.
[c] Numbers of row(s) in each plot. One row is approximately 1.5 m^2.
[d] Harvesting methods (Har.). Straw was cut using hand sickles and grain was mechanically threshed (HS) or plots were harvested with a small plot combine (SC).
[e] Grain yield (YD), Heading date (HD), Plant height (PH), β-D-glucan content (GU), and Kernel weight (KW).

Due to seed limitations, one line in the 'Fiber-2' population (line #8) was planted at half the normal seeding rate (0.5g/ft) for each plot in 1998, and this line was treated as missing data for grain yield in 1998.

Straw was cut using hand sickles and grain was mechanically threshed (1 and 2 row plots) or plots were harvested with a small plot combine (4 row plots, in 1998). Harvested grain was cleaned before weighing, and the hulls of hulless lines (*n*) were excluded from the measurement. Ten grams of seeds were randomly chosen and ground and air-dried before assaying the β-D-glucan content using a commercially available enzymatic method (McCleay and Codd, 1991 Megazyme International Ireland Ltd., Wicklow, Ireland).

Kernel weight measurement were conducted separately in 1999 with the seeds produced in 1998 and 1999 for only 'Fiber-2' population without replications to infer the genetic effects of two closely linked genes, *n* and lk_2 (see below).

Analysis of Agronomic Traits

Primary statistical analyses were performed using SAS (SAS Institute, 1988; version 6.10). Analysis of variance was performed to detect differences between years, blocks, and recombinant inbred lines for each trait using the PROC ANOVA and GLM procedures (SAS Institute, 1988).

For the QTL analysis, the 'Fiber-2' field trial data was used. All measured traits were named depend on the trait measured and year tested (YD98, YD99 for grain yield, HD98, HD99 for heading date, PH98, PH99 for plant height, GU96, GU97, GU99 for β-D-glucan contents, and KW98, KW99 for kernel weight).

Single Marker QTL Analysis (SMQ)

Single marker QTL analysis (Falconer and Mackay, 1996; pp361-363) was conducted to screen the association between marker alleles and all scored traits, and establish a reference for simple- and composite- interval mapping. The LRmapqtl procedure in QTL Cartographer (Basten *et al.*,1999) was used for 191 markers in the 'Fiber-2' linkage map. This procedure does not report the R^2 values (the amount of total phenotypic variance that is explained by the specific molecular marker of each markers). Therefore, PROC GLM was used to estimated the R^2 values for each marker for each trait surveyed. We included the 42 remaining 'orphan' markers in the PROC GLM procedure.

QTL Mapping

QTL mapping was conducted using two methods, simple interval mapping (SIM; Lander and Bostein, 1989 for maximum likelihood procedure; Haley and Knott, 1992 for multiple regression approach), and composite interval mapping (CIM; Jansen and Stam, 1994; Zeng, 1994).

Martinez and Curnow (1994) mentioned that one of the advantages of multiple regression based interval mapping is that it is easy to generalize to use the information from more than one pair of markers at the same time, and this advantage (simultaneous use of three markers) reduces the impact of linkage drag-based QTLs in neighboring regions and thus helps permit discrimination between the presence of one or two QTLs. We thought that this might help to distinguish among multiple effects mapping to barley chromosome 1 to

which lk_2 and n are belong. We utilized a multiple regression based QTL mapping program, PLABQTL (Utz and Melchinger, 1999) for this QTL analysis.

Log-transformation of the phenotypic data was used for 3 surveyed traits (HD98, HD99, and GU99) to improve the normality. After conducting analysis, the reported results of three traits were re-transformed for easy comparison with other traits surveyed.

For CIM, the background markers (cofactor) were selected by the 'Forward and Backward stepwise regression' (FB) search option of the SRmapqtl procedure of QTL cartographer. A threshold level($p \leq 0.05$) was used for the partial F-statistics [$p(F\text{-in})$, and $p(F\text{-out})$]. Those pre-selected background markers were used to run composite interval mapping with PLABQTL.

To control type-I error (false positive), empirical critical values were constructed by 1,000 permutation tests (Churchill and Doerge, 1994) in interval mapping for each trait surveyed. The permutation tests were run with PLABQTL on a PC with a 100Mhz CPU. A five percent type-I error level was applied as threshold in each analysis. All detected QTLs were named dependent on the trait surveyed, the interval mapping method used, and the chromosomal position.

Estimation of Additive Effects of Detected QTLs

Each detected QTL was reported by LOD score, percentage of phenotypic variance ($R^2\%$), and additive value under the rejected null hypothesis (no QTL at the tested positon). The phenotypes of individuals in the population are estimated in a combined manner using QTLs and undetectable latent genetic components, such as weak QTLs and interactions.

Simultaneous multiple regression on all detected QTLs was conducted on each trait surveyed to obtain final estimates of the additive value. At this time, those QTLs having non-significant partial R^2 values (the relative importance among detected QTLs) were discarded manually, and we re-ran the multiple regressions on only those QTLs having significant partial R^2 values with the SEQUENCE command in PLABQTL.

Applying permutation test to establish threshold level for CIM analysis has been suggested by some researchers (Tinker and Mather, 1996). Therefore, if any minor QTLs displayed significant *F*-statistics with reasonable R^2 values by SMQ, the closest markers to those minor QTLs were also considered as additional variables for multiple regression models. As a reference to those detected QTLs, the SMQ result (R^2 value and *F*-statistics with the significance level) of the flanking markers closest to each QTL was also reported.

Results

Analysis of Agronomic Traits

Table 8 describes that the ranges of variation among recombinant inbred lines in each trait surveyed. β-D-glucan contents showed unimodal distributions in each population. Population means ranged from 5.56 % to 6.14 %, while the parents showed about 4% and 8% β-D-glucan content except in 1999. In this year, the MTaz90-123 mean was much lower than in 1996 and 1997, and progeny were skewed toward MTaz90-123 (in the 'Fiber-2' population, Skewness was 0.66*).

Heading date analysis showed population means which were skewed toward the maternal the line mean (in the 'Fiber-2' population, Skewness were 1.06* and 0.69*, for HD98 and HD99, respectively). Skewness indicates that the effect of gene substitution might be non-linear for β-D-glucan contents and heading date QTLs in the 'Fiber-2' population. Weller (1992) mentioned the possibility of 'higher-order' QTL effects on traits exhibiting either the skewness or kurtosis.

The 1999 nursery was seeded a month later than would normally be considered optimal. Still, the mean difference between the two parents for heading date was greater in 1999 than in 1998. The mean of kernel weight was also much greater in 1999 than 1998.

None of the measured traits of the 'Fiber-2' mapping population showed bimodal distributions, although heading date data showed some skewness. ANOVA detected significant differences among recombinant inbred lines for each trait in all years tested

(Table 9). Since year x line variation was large for all traits surveyed (Table 10) all trait data over years kept separate and separate QTL analyses were performed for each year.

Single Marker QTL Analysis of 'Fiber-2' Mapping Lines

Table A-2 provides a 'quick view' of putative QTLs for each trait. Chromosomes 1, 2 and 7 carried genes which had a strong effect on phenotype over locations and years.

Grain yield, kernel weight, and β-D-glucan content were affected by genes in the vicinity of *n* and lk_2 on the long arm of chromosome 1. β-D-glucan content was strongly impacted by genes around *wx* on chromosome 1 and by a gene near ABC483 on chromosome 7, and *wx* also affected kernel weight. Plant height and heading date were impacted by genes on chromosomes 1 (ABG380) and 2 (ABC170).

During testing the 42 orphan markers with PROC GLM procedure, FL1810A was identified as another marker heading date and plant height-related genes, and this marker explained over 20% phenotypic variation of heading date and plant height (21%, 28% and 27% for HD98, HD99 and PH99, respectively). The most likely position of FL1810A was estimated around ABG380 on chromosome 1 (30cM apart with LOD 1.2), and displayed a smaller additive effect than ABG380. Meanwhile, regression models did not explain additional variation by adding FL1810A to the effect of ABG380. During interval mapping, therefore, FL1810A was not included in estimating genetic effects with multiple regressions on detected QTLs, since we could not determine a map location for it.

Table 8. Descriptive statistics of agronomic traits measured

Population	Trait [a]	Descriptive statistics [b]							Parental line mean	
		Mean	Std. dev	Coef. var(%)	Skewness	Kurtosis	Min.	Max.	MTaz90-123	MTH6860
Fiber-2	YD98	5156.67	547.00	10.61	-0.03	-0.40	3756.5	6157.5	4251.23	5861.56
	YD99	4159.93	549.63	13.21	0.45	0.06	3073.4	5624.7	4051.33	5182.35
	KW98	42.49	3.961	9.32	-0.02	-0.31	33.0	51.2	37.84	44.42
	KW99	47.24	4.04	8.55	-0.07	-1.26*	40.3	53.9	42.24	48.85
	GU96	5.71	1.10	19.29	0.09	0.33	2.5	8.2	8.1	4.4
	GU97	6.14	1.00	16.33	0.20	-0.64	4.4	8.3	8.0	4.2
	GU99	5.56	1.08	19.36	0.66*	0.17	3.7	8.4	6.2	3.8
	HD98	182.23	2.16	3.56	1.06**	0.40	179.0	188.5	182.0	184.0
	HD99	189.54	2.86	5.78	0.69*	-0.86	186.0	195.5	189.5	193.0
	PH98	84.03	4.05	4.82	0.00	0.06	74	93.5	81.5	85.5
	PH99	83.64	6.95	8.31	-0.23	-0.72	69.2	96.8	83.0	87.3
Fiber-3	YD98	3789.22	921.15	24.31	0.21	-0.55	1560.19	5898.08	3956.11	5397.54
	YD99	4257.41	601.20	14.21	-0.09	-0.09	2641.23	5672.58	3948.63	5131.24

Table 8. (continued)

Population	Trait [a]	Descriptive statistics							Parental line mean	
		Mean	Std. dev	Coef. var(%)	Skewness [b]	Kurtosis [b]	Min.	Max.	MTaz90-123	MTH68607
Fiber-3	GU97	5.40	1.70	31.48	-0.43	1.31*	0.6	10.1	8.1	3.9
	GU99	6.20	1.40	22.58	0.72*	0.53	4.0	10.6	7.5	4.4
	HD98	178.63	2.61	4.45	1.07**	0.24	175.0	186.0	178.5	181.5
	HD99	189.21	2.42	4.92	1.05**	0.45	186.0	196.0	189.0	193.0
	PH98	75.44	4.13	5.47	0.40	0.08	66.5	85.5	71.5	77.0
	PH99	82.17	7.22	8.79	0.40	-0.53	66.5	99.3	81.2	85.2
Fiber-1	YD98	5287.45	559.26	10.58	-0.20	-1.05	4003.53	6309.34	4441.45	5930.27
	HD98	181.70	1.40	2.27	1.04**	2.07	179.0	186.0	182.0	184.0
	PH98	86.3	3.5	4.06	-0.33	-0.59	77.5	92.5	83.0	87.5

[a] Grain yield (YD; kg/ha), Kernel weight (KW; mg/seed), β-D-Glucan content (GU; %), Heading date (HD; days), and Plant height (PH; cm), with the year traits measured.

[b] Standard deviation (Std. Dev), coefficient of variance (C.V. %), minimum (Min.), and maximum (Max.)

Significant deviations from the normal distribution are indicated with * and ** at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 9. ANOVA for agronomic traits of the 'Fiber' populations in each year trials.

Trait(unit)	Year	Source	Fiber-2		Fiber-3		Fiber-1	
		Between	df	MS	df	MS	df	MS
Grain yield	1998[a]	Replicates	1	353.747 **	1	318.141 *	1	0.286
		Lines	57	59.834 **	95	169.699 **	60	63.873 **
		Residual	57	9.524	95	55.687	60	15.262
		Total	115		191		121	
	1999	Replicates	1	20.706	1	525.595 **		
		Lines	58	60.427 **	95	72.288 *		
		Residual	58	19.844	95	51.245		
		Total	117		191			
β-D-Glucan	1996	Replicates	1	0.001				
		Lines	58	2.430 **				
		Residual	58	0.041				
		Total	117					
	1997	Replicates	1	1.096 **	1	0.119		
		Lines	58	2.430 **	94	5.071 **		
		Residual	58	2.017	94	0.068		
		Total	117		189			
	1999	Replicates	1	0.194 **	1	0.418 **		
		Lines	58	2.313 **	95	4.091 **		
		Residual	58	0.022	95	0.027		
		Total	117		191			
Heading date	1998	Replicates	1	0.000	1	318.141 *	1	0.295
		Lines	58	9.814 **	95	169.699 **	60	3.623 **
		Residual	58	0.224	95	55.687	60	0.362
		Total	117		191		121	
	1999	Replicates	1	353.747 **	1	0.034		
		Lines	58	59.834 **	95	16.402 **		
		Residual	58	9.524	95	0.620		
		Total	117		191			
Plant height	1998	Replicates	1	20.347	1	41.255	1	5.541
		Lines	58	32.800 **	95	33.891 **	60	24.376 *
		Residual	58	11.347	95	11.771	60	14.058
		Total	117		191		121	
	1999	Replicates	1	11.204	1	20.672		
		Lines	58	96.564 **	95	102.453 **		
		Residual	58	7.317	95	9.043		
		Total	117		191			
Kernel wt[b] (mg/seed)	1998	Lines	58	15.69				
	1999	Lines	58	16.31				

[a] One line (#8) was missing data.

[b] Kernel weight (Kernel wt) was measured without replications.

*, ** are levels of significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 10. ANOVA for agronomic traits measured in 'Fiber-2' and 'Fiber-3' populations over seasons.

Trait	Source of variance	Fiber-2			Fiber-3		
		59 lines			96 lines		
	Between	df	Mean Square	R^2 (%)[a]	df	Mean Square	R^2 (%)[a]
Grain yield [b]							
	Replicates	1	271.385**	1.9	1	830.786**	2.3
	Lines	58	60.541**	23.7	95	160.324**	39.2
	Years	1	5811.299**	39.3	1	2104.596**	5.8
	Line x Year	57	59.719**	23.0	95	81.6630	24.5
	Residual	116	15.490	12.1	191	53.254	28.2
	Total	233		100.0	383		100.0
Kernel weight [c]	Lines	58	29.086**	26.3			
	Years	1	664.407**	67.7			
	Residual	58	2.908	6.0			
	Totla	117		100.0			
β-D-Glucan							
	Replicates	1	0.701**	0.2	1	0.046	0.0
	Lines	58	4.979**	67.3	95	4.377**	45.3
	Years	2	15.171**	7.1	1	43.560**	4.7
	Line x Year	116	0.891**	24.1	94	4.782**	49.0
	Residual	176	0.031	1.3	190	0.050	1.0
	Total	353		100.0	381		100.0
Heading date							
	Replicates	1	0.017	0.0	1	4.594**	0.0
	Lines	58	24.979**	13.0	95	24.652**	21.6
	Years	1	9584.814**	85.9	1	8381.344**	77.0
	Line x Year	58	1.236**	0.6	95	0.796**	0.7
	Residual	117	0.419	0.4	191	0.395	0.7
	Total	235		100.0	383		100.0
Plant height							
	Replicates	1	30.874	0.4	1	60.1670	0.3
	Lines	58	76.600**	51.5	95	113.333**	56.3
	Years	1	8.695	0.1	1	4143.305**	21.7
	Line x Year	58	52.765**	35.4	95	23.011**	56.3
	Residual	117	9.258	12.6	191	10.362	10.3
	Total	235		100.0	383		100.0

[a] The ratios of the sum of squares of each variance component were expressed with the percent of total sum of square.

[b] One line (#8) was missing data in 1998.

[c] Kernel weights were measured without replications in each year trial.

*, ** are levels of significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

QTL Detection with Interval Mapping; SIM and CIM

To control Type-I error during interval mapping, permutation test were used, and 5% Type-I level was applied to all analysis. Table 11 shows the estimated empirical threshold values for both interval mappings of each trait tested. Figure 7 shows the background markers chosen with stepwise multiple regression for composite interval mapping.

Figure 6-A (SIM) and Figure 8-A (CIM) offer a view of the chromosomal locations of detected QTLs. Scans of test statistics of interval mappings are given in Figure 6-B (SIM) and Figure 8-B (CIM). To compare the chromosomal locations of putative QTLs, map locations of five markers (SIM; Figure 6-B), and 10 chromosome segments (CIM; Figure 8-B) were used as 'reference points' across traits measured and years tested.

The numerical summaries of interval mapping results are organized in Table 12 (SIM) and Table 13 (CIM). No interval mapping methods completely account for all heritable variance ('QTL summary' column), due to extreme individuals (including experimental outliers) and non-perfect algorithms (Kuittinen *et al.*, 1997). Furthermore, some minor CIM QTLs provided uncertain allelic effects, especially where SMQ showed very low significance without clear repulsion linkage as in the cases of the long arm of chromosome 7 in YD98 and HD98 trials. Therefore, to avoid wrong interpretation of interval mapping data, two additional columns are included; 'MR on putative QTLs' and 'SMQ of the closet marker' column in Table 12 and Table 13.

Table 11. Empirical threshold values estimated for simple- and composite- interval mapping [a]

Genome-wise type-I error values for LOD scores									
Simple interval mapping (SIM)									
Type-I error level	YD98	KW98	KW99	GU96	GU97	GU99	HD98	HD99	PH99
30%	2.15	2.21	3.10	2.15	2.23	2.21	2.18	2.21	2.26
25%	2.29	2.29	3.27	2.26	2.32	2.32	2.29	2.33	2.38
10%	2.79	2.78	3.91	2.68	2.89	2.80	2.75	2.83	2.85
5%	3.15	3.06	4.50	2.99	3.28	3.24	3.06	3.19	3.22
1%	3.75	3.97	5.47	3.62	3.88	3.77	3.73	3.95	4.07
Composite interval mapping (CIM) [b]									
Type-I error level	YD98	KW98	KW99	GU96	GU97	GU99	HD98	HD99	PH99
30%	2.76	3.10	4.50	2.96	3.02	2.87	2.76	2.43	2.62
25%	2.92	3.27	4.72	3.08	3.13	3.05	2.88	2.52	2.75
10%	3.46	3.91	5.71	3.74	3.76	3.6	3.50	3.02	3.32
5%	3.84	4.50	6.24	4.25	4.11	4.12	3.85	3.38	3.82
1%	4.96	5.47	7.72	5.14	5.32	5.14	4.64	4.21	4.72

[a] The statistical genome-wise type-I error rate was controlled by means of 1000 permutation tests (Churchill and Doerge, 1994) for each trait surveyed.
[b] Background markers were selected by 'Forward and Backward stepwise regression'(FB) search option of SRmapqtl procedure in QTL Cartographer (version 1.13; Basten *et al.*, 1999). Threshold level 0.05 was used for the partial *F* statistics [$p(F\text{-in})$, and $p(F\text{-out})$].

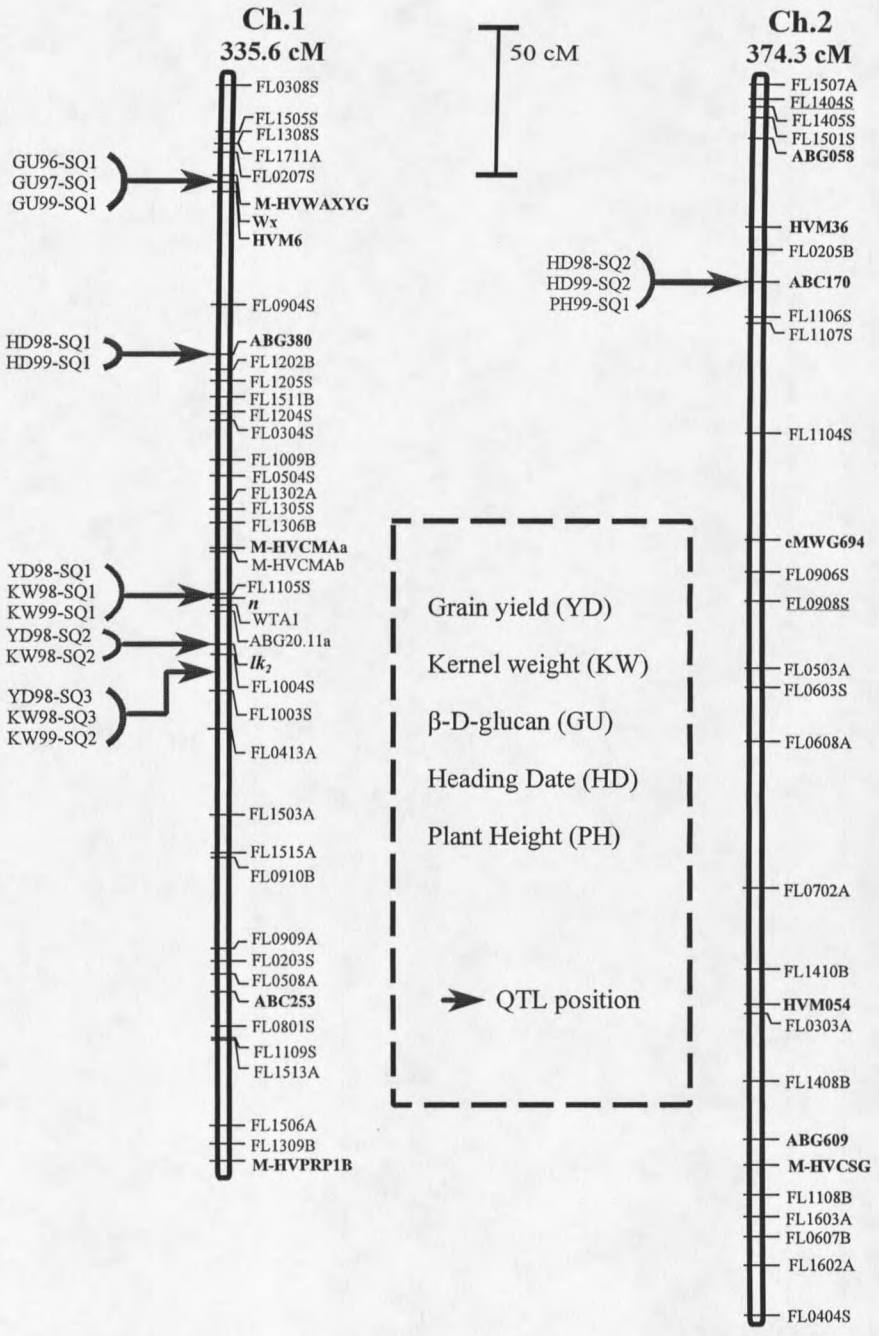


Figure 6-A. The chromosomal locations of putative QTLs for simple interval mapping. Arrows on chromosome 1 and 2 show the putative locations of QTLs associated with each trait surveyed. The detected QTLs are reported with the QTL name used in Table 12. The location of anchor markers used in 'linkage map skeleton' construction (Table 6) are indicated with bold letters, and underlined markers indicate the segregation distortion (P<0.01) (Table A-1). Detail marker intervals are in Table A-2.

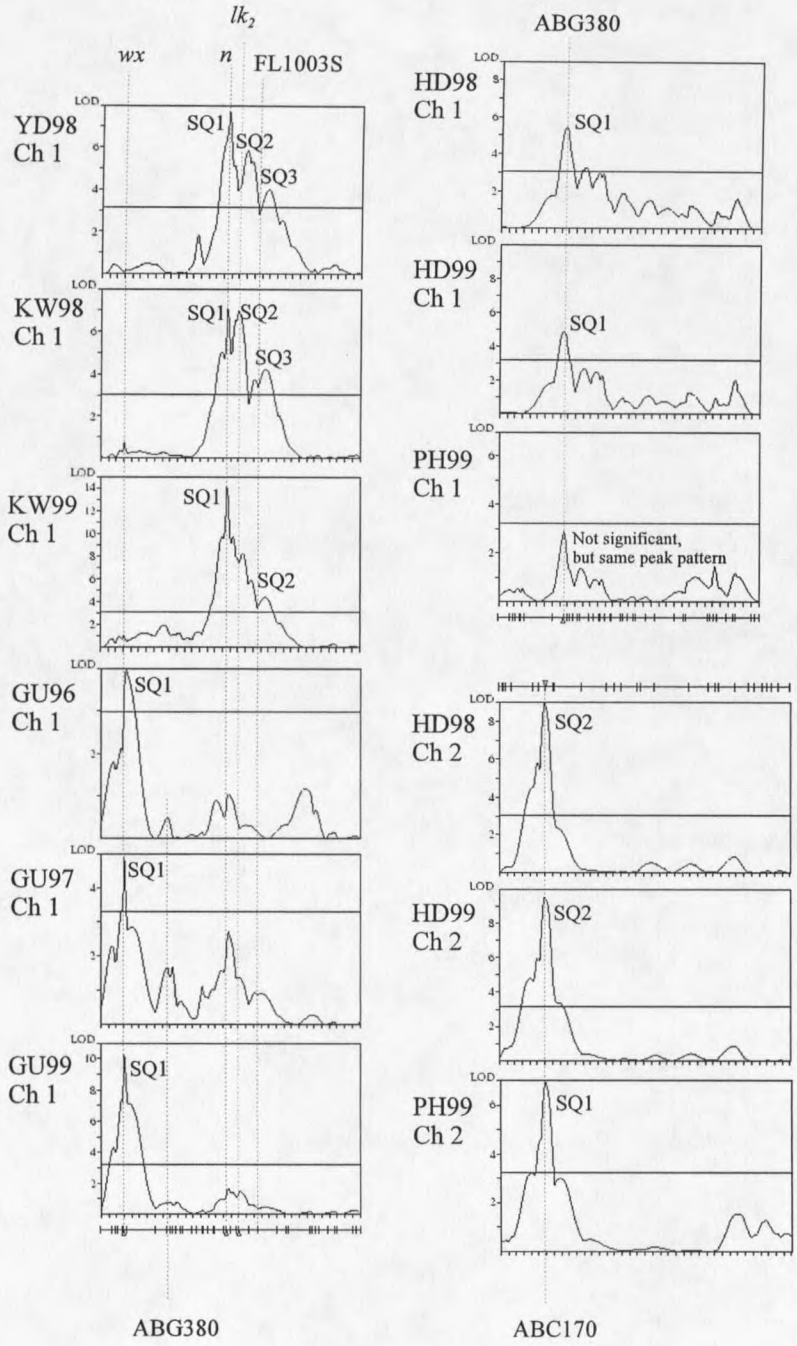


Figure 6-B. Scans of a test statistic for simple interval mapping. Scans are showed for nine traits as indicated. The grids in the small bars locating top and bottom of chromosomes show the marker locations (see Figure 6-A), and dotted lines across traits indicate the marker positions linked to detected QTLs, which are reported in Table 12. Horizontal dashed lines, in each panel, show thresholds for testing SIM at 5% genome-wise-type I error (Table 11).

QTLs for Grain Yield SIM on grain yield exhibited broad, trimodal peaks, with subpeaks near markers *n* (YD98SQ1), 6 cM distal from lk_2 (YD98SQ2), and near FL0413A (YD98SQ3) (Figure 6-B; YD98 Ch1). From the scan alone, it was not clear whether there were one, two, or three QTLs affected grain yield. The position near *n* explained more of the observed variation (R^2, 43.5% at *n*) than the other two positions, and regression models including two other possible QTL positions showed that inclusion of additional loci in the model did not improve the model (Table 12; R^2 % and Part. R^2 % of YD98). This suggested the presence of a single QTL near *n* with 387kg/ha as its estimated additive value attributed by MTH6860756 allele. It was possible, however, that there was more than one locus affecting grain yield near lk_2 and FL0413A.

CIM with 11 background markers (Figure 7), produced surprising results. First, one QTL peak distal to the *n* locus was identified (Figure 8-B; segment C). YD98Q1 was flanked by FL1105S and *n* with 3cM interval with 376kg/ha as the estimated additive value (Table 13). The difference for the peak near the *n* locus between SIM and CIM could be explained by increased QTL resolution with CIM method. Meanwhile, lk_2 still displayed a significant impact on grain yield (LOD 2.6) (Figure 8-B; segment C).

Second, three QTLs were identified on chromosome 7 with very high estimated additive values. Two QTLs, YD98Q3 and YD98Q4 (Figure 8-B; segment I), were tightly linked to each other(14cM) in repulsion phase on chromosome 7 (Table 13; QTL summary). Each QTL displayed 350kg/ha and 560kg/ha additive values by MTaz90-123 allele and MTH6860756 allele, respectively. Liu (1998, pp417-458) mentioned that when QTLs are linked in the repulsion phase, scans of test statistics underestimate their true effect. Only

conditional analysis can resolve the problem of repulsion linkage phase for linked QTLs. SMQ results of these distal markers FL1802A and FL0407A (Table 13; R^2% in SMQ), support Liu's contention (0.1% and 0.7% R^2 values on yield, respectively). Furthermore, YD98Q4 showed a much higher estimated additive value (560kg/ha) than YD98Q1 near the *n* locus (376kg/ha). In both cases, favorable alleles were from MTH6860756. Another QTL on chromosome 7, YD98Q5 (Figure 8-B; segment J), showed 313kg/ha as its estimated additive value attributed by MTaz90-123 allele. This QTL was also identified with SQM (Table A-2).

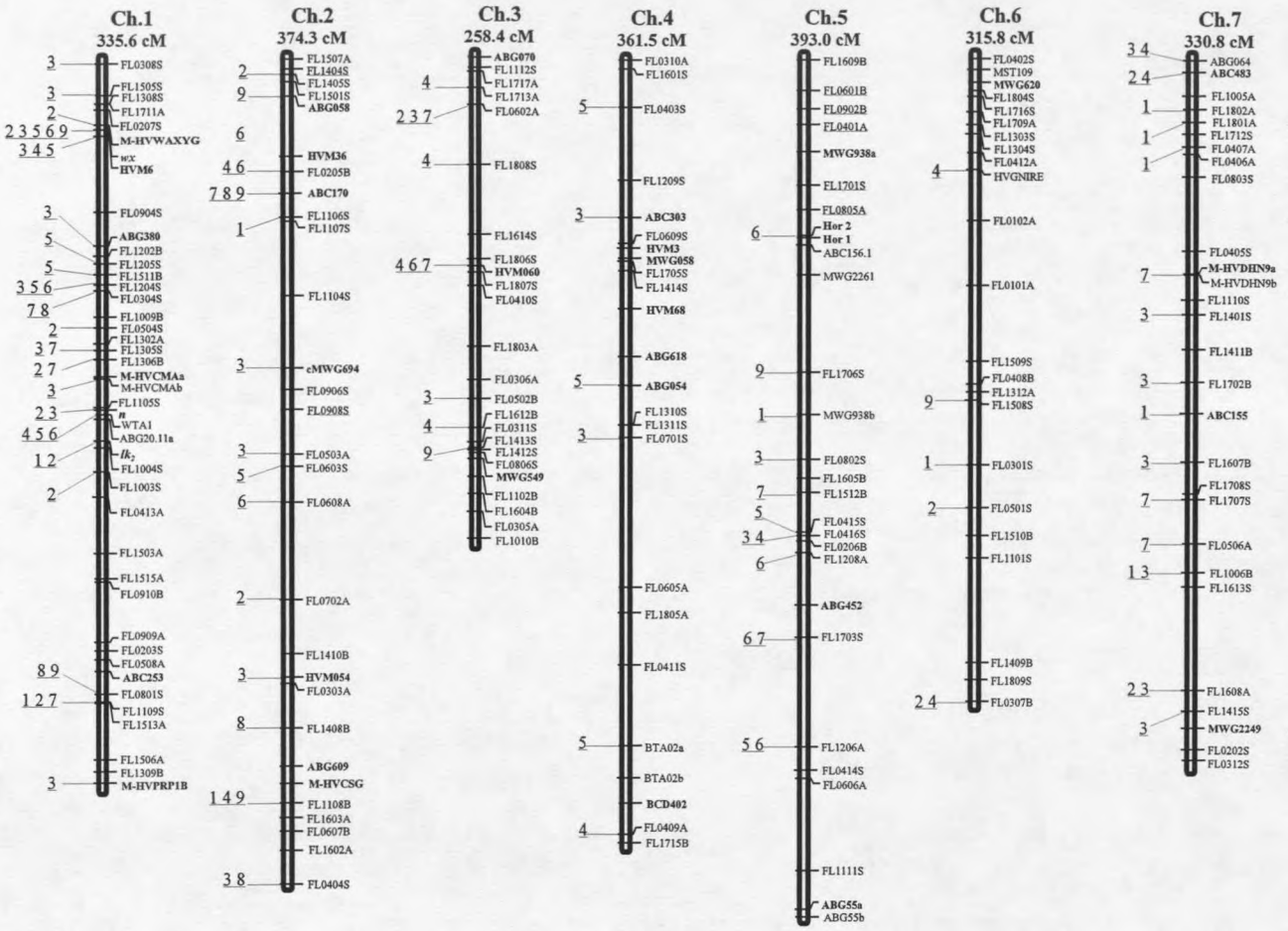
One more QTL, flanked by ABC170 and FL1106S, was detected on chromosome 2. This QTL, YD98Q2 (Figure 8-B;segment E), showed 193kg/ha as its additive value (Table 13; QTL summary).

Simultaneous multiple regression was applied to all 5 putative QTLs to estimate their additive values in combined manner under the assumption of second parent (MTH6860756) always carries the favorable alleles;

$$\underline{\text{YD98(kg/ha)} =}$$

$$\underline{5115 + 446(\text{YD98Q1}) + 173(\text{YD98Q2}) - 278(\text{YD98Q3}) + 385(\text{YD98Q4}) - 184(\text{YD98Q5})}.$$

This model explained 74.4% of phenotypic variation (Table 13; MR on putative QTLs). YD98Q1 near *n* locus showed the biggest additive value, otherwise, the other QTLs were greatly reduced except YD98Q2. Perhaps the repulsion phase linkage between YD98Q3 and YD98Q4 interfered with the liner estimation.



Selected Background markers (B.C)		
ID	Trait	No. of B.C.
1	YD98	11
2	KW98	15
3	KW99	28
4	GU96	13
5	GU97	13
6	GU99	13
7	HD98	12
8	HD99	4
9	PH99	8

Figure 7. Distribution of background markers (cofactor) used in composite interval mapping. Background markers were selected with 'Forward and Backward stepwise regression (FB)' option of Srmapqtl procedure in QTL cartographer (version 1.13; Basten *et al.*, 1999). Threshold level 0.05 was used for the partial *F* statistics [*p*(*F*-in), and *p*(*F*-out)]. Loci marked with various trait IDs indicate selected background markers for each trait measured.

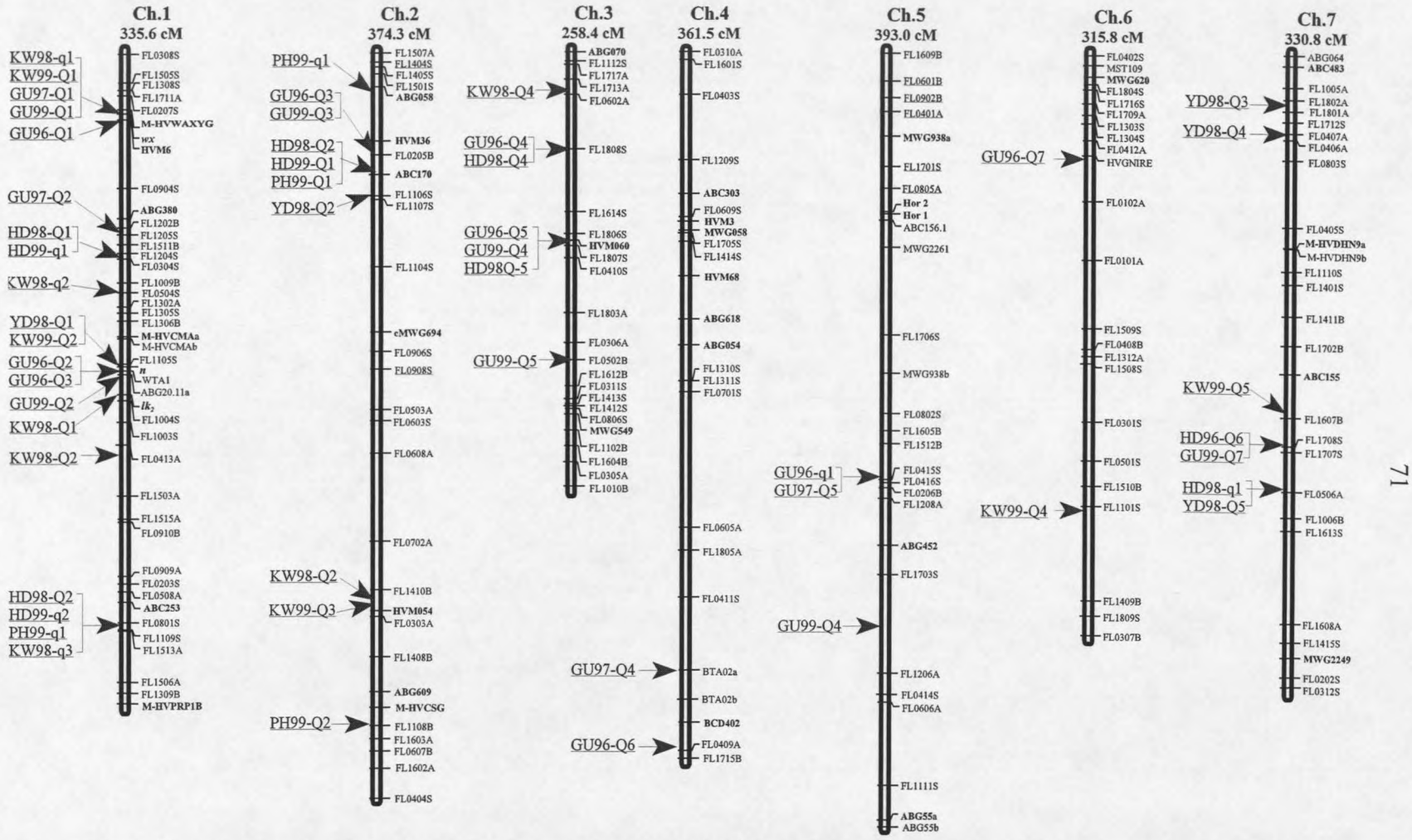


Figure 8-A. The chromosomal locations of detected putative QTLs for composite interval mapping. Arrows on chromosomes show the putative locations of QTLs associated with each trait surveyed. The detected QTLs are reported with the QTL name used in Table 13. Detail marker intervals are in Table A-2.

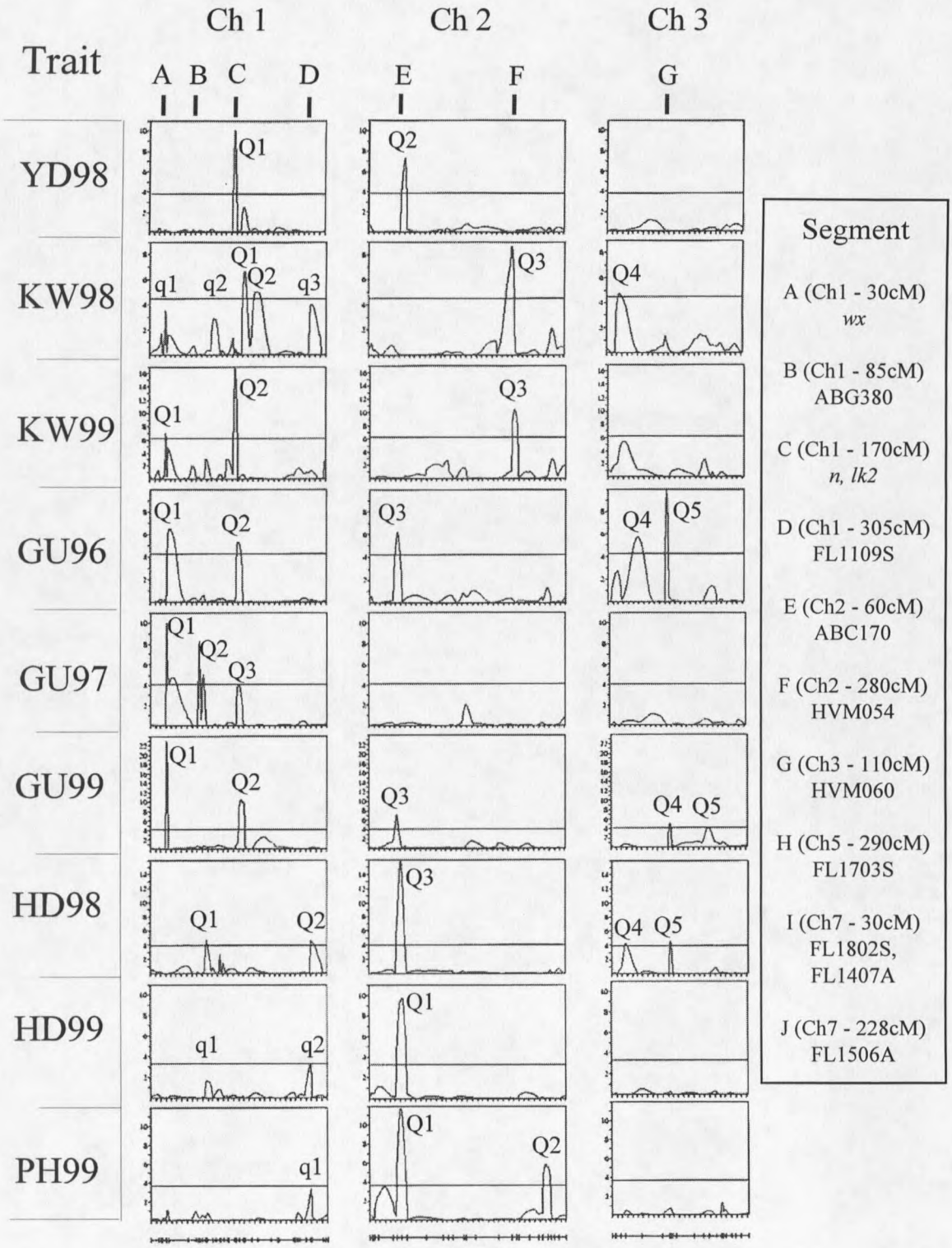


Figure 8-B. Scans of a test statistic for composite interval mapping (continued).

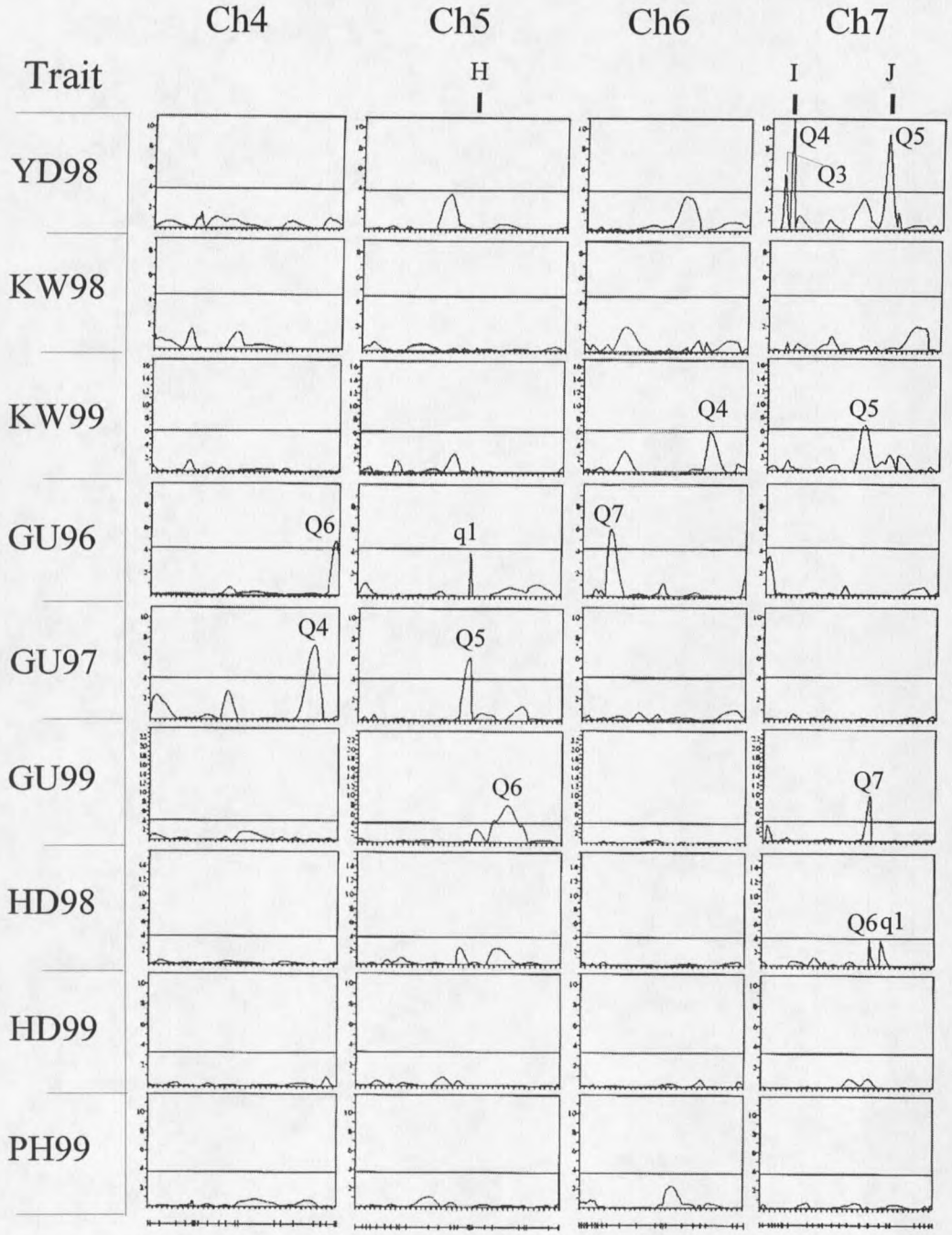


Figure 8-B. Scans of a test statistic for composite interval mapping. Scans are shown for nine traits as indicated, and horizontal dashed lines show the LOD thresholds for testing CIM at 5% genome-wise-type I error (Table 11). The detected QTLs are reported with the QTL names described in Table 13. Regional segments are expressed with their proximal chromosomal locations and anchor markers to compare peak patterns across traits and years.

QTLs for Kernel Weight SIM on kernel weight exhibited a peak pattern with similar to that provided by the analysis of YD98. The position of two subpeaks near *n* locus (KW98SQ1, KW99SQ1) and FL0413A (KW98SQ3, KW99SQ2) appeared to be coincident with YD98Q1 and YD98Q3 (Figure 6-B, Table 12). The position near *n* explained 47% and 75% of observed variation (Table 12; SMQ), for 1998 and 1999 respectively. Therefore this result suggests that the *n* locus affected kernel weight differently in each year (estimated additive values were 2.71mg/seed for KW98, and 3.45mg/seed in 99' by MTH6860756 allele), while FL0413A did not show significant difference between two years (2.83mg/seed and 2.75mg/seed) (Table 12). The position of another subpeak for 98' trial (KWSQ2) was interesting. This subpeak was near the lk_2 region (Table 12) between two yield subpeaks, YD98SQ1 and YD98SQ2 (Figure 6-B). The lk_2 region did not display high significance in 1999.

Multiple regression on detected QTL subpeaks suggested that the loci lk_2 and *n* affected kernel weight more strongly than the other genes (Table 12; Rart. R^2 % in QTL summary column). It was not possible, however, to estimate the 'real' effects of each gene on kernel weight with a linear model due to the linkage between these loci.

CIM with background markers (Figure 7) narrowed the confidence intervals of putative QTLs detected with SIM (Figure 8-B; segment C). As expected by SIM, the lk_2 gene (KW98Q1) displayed the highest additive value (2.24mg/seed) on kernel weight (the Lk_2 allele was positive in this case) and the *n* gene (KW99Q1) showed 2.59mg/seed as its additive value (the *N* allele positive) in 1999 (Table 13, Figure 8-B; segment C). Other positive alleles detected by CIM were contributed by MTH6860756 (Table 13).

Table 12. Summary of detected QTLs with simple interval mapping

Trait	List of detected QTLs												SMQ of the closest marker [d]		
	Position [a]			QTL summary [b]				MR on putative QTLs [c]			Flanking markers				
	Name	Ch	cM	LOD	R^2(%)	Add	Part. R^2(%)	QTL	Int	R^2(Adj)%	Left	Right	R^2(%)	AZ	CA
YD98	SQ1	1	162	7.7	45.8	386.9	12.1 **	386.90	5083	45.8 (43.8)	FL1105S	***n***	43.8	4719	5453
(kg/ha)	SQ2	1	186	5.9	37.3	376.3	0.7				lk_2	**FL1004S**	33.9	4806	5441
	SQ3	1	214	4.0	27.1	391.5	2.0				FL1003S	**FL0413A**	19.8	4907	5389
KW98	SQ1	1	162	7.1	42.5	2.71	5.6		42.2	41.9 (39.8)	FL1105S	***n***	47.2	39.2	44.9
(mg/seed)	SQ2	1	176	7.0	41.9	2.67	10.0 *	2.67			ABG20.11a	lk_2	48.2	39.2	45.0
	SQ3	1	212	4.3	28.2	2.83	2.8				FL1003S	**FL0413A**	14.0	41.3	44.2
KW99	SQ1	1	162	14.1	66.6	3.45	47.5 **	3.45	46.5	66.6 (65.4)	FL1105S	***n***	74.9	43.0	50.4
(mg/seed)	SQ2	1	194	5.9	37.0	2.75	0.9				**FL1004S**	FL1003S	34.7	44.7	49.4
												Lk2	*46.3*	*44.1*	*49.7*
GU96(%)	SQ1	1	32	4.0	26.8	-0.60	26.8 **	-0.60	5.6	26.8 (24.2)	*wx*	**HVM6**	27.1	6.2	5.0
GU97(%)	SQ1	1	30	4.9	31.8	-0.58	31.8 **	-0.58	6.0	31.8 (29.4)	M-HVWAXYG	***wx***	39.7	6.7	5.4
GU99(%)	SQ1	1	30	10.3	55.1	-1.16	55.1 **	-1.16	5.3	55.1 (53.5)	M-HVWAXYG	***wx***	53.5	6.3	4.6
HD98	SQ1	1	84	5.4	34.6	-1.31	27.3 **	-1.01	182.5	63.8 (61.2)	FL0904S	**ABG380**	34.2	183.6	181.0
(days)	SQ2	2	58	9.0	50.3	1.72	44.7 **	1.02			FL0205B	**ABC170**	53.7	181.0	184.

Table 12. (continued)

Trait	List of detected QTLs												SMQ of the closest marker [d]		
	Position [a]			QTL summary [b]				MR on putative QTLs [c]			Flanking markers				
	Name	Ch	cM	LOD	R^2(%)	Add	Part. R^2(%)	QTL	Int	R^2(Adj)%	Left	Right	R2(%)	AZ	CA
HD99	SQ1	1	82	4.9	31.5	-1.74	23.3 **	-1.02	189.8	63.7 (61.0)	FL0904S	**ABG380**	31.1	191.1	187.9
(days)	SQ2	2	58	9.6	52.7	2.29	47.0 **	1.04			FL0205B	**ABC170**	57.5	187.8	192.3
PH99	SQ1	2	58	7.0	41.9	4.95	41.9 **	4.28	84.5	47.9 (40.0)	FL1205B	**ABC170**	44.4	79.2	88.8
(cm)		*1*	*84*	*2.9*	*20.2*	*-1.82*	*10.2 **	*-1.82*				*ABG380*	*20.5*	*86.7*	*80.5*

[a] The position on the chromosome of the detected QTL, in cumulative cM.

[b] LOD scores were calculated from the *F*-value in the multiple regression according to Haley and Knott (1992), and 5% genome level of empirical critical values were applied to control type I error (Table 11). Under the rejected null hypothesis, percentage of the phenotypic variance, R^2(%) and the additive effect(Add) were estimated. It was assumed that the second parent (MTH6860756; paternal line) carries the favorable alleles for the trait under study. The coefficient of determination between the respective QTL and the phenotypic observations, keeping all other QTL effects fixed. Partial R^2 values (Part. R^2) could be used to determine the relative importance of detected QTLs (Utz and Melchinger, 1999), * and ** represent the significant level at 0.05 and 0.001, respectively.

[c] Multiple regressions were conducted to obtain the estimated values of parameters (QTL; detected QTLs with significant Partial R^2 values) and the phenotypic variance (R^2 and adj R^2 %) explained with the QTLs. To compare the deviation of Intercept (Int) from population mean, see Table 8.

[d] The closest marker to the detected QTL (bold lettered flanking marker) was used for the single marker QTL analysis (SMQ), and R^2 value and each genotype means (MTaz90-123; AZ, and MT6860756; CA) are also reported. Additive value of the closet marker allele could be estimated with the formula, (CA - AZ)/2 and be compared with the additive effect(Add) which was estimated under the rejected null hypothesis (see QTL summary column). *F*-statistics of each SMQ are in Table A-2.

Two chromosomal regions were identified by CIM in both years. The Waxy gene (Figure 8-B; segment A) displayed less effect on kernel weight in 1998 (R^2, 15%; KW98q1) than in 1999 (R^2, 19%; KW99Q1), and its estimated additive value in the1999 trial (KW99Q1; 2.5mg/seed) was greater than the SMQ estimation from 1998 (1.8mg/seed). A QTL near HVM054 (Figure8-B; segment F, KW98Q3, KW99Q3) exhibited a small effect on kernel weight (R^2, 7.5% and 4.7%). One QTL (KW98Q2) was detected near FL1003S, to which one subpeak of SIM was located (Figure 6-B; KW98SQ3, KW99SQ2).

Under the assumption that MTH6860756 contributed all of the favorable alleles for kernel weight, the reduced best models are;

KW98(mg/seed) =

42.4 + 1.04(*wx*) + 2.29(KW98Q1) + 1.08(KW98Q2) + 1.50(KW98Q3) ; R^2, 62.5%

KW99(mg/seed) =

46.6 + 0.74(KW99Q1) + 3.25(KW99Q2) + 1.11(KW99Q3) + 0.69(KW99Q5) ; R^2, 77.3%.

KW98Q1 distal to lk_2 , and KW99Q2 near *n* showed the biggest additive values in each year trail. Although, *wx* was included as a QTL for KW98 simultaneous multiple regression on all putative QTLs CIM indicated that there may be undetected QTL for these traits, perhaps with small (such as those peaks on chromosome 1) and/or non-additive effects.

QTLs for β-D-glucan contents Simple interval mapping identified one QTL (waxy gene), while composite interval mapping identified 5-7 QTLs controlling grain β-D-glucan contents. The *wx* locus played the major role controlling β-D-glucan content in the 'Fiber-2' population. Although the R^2(%) value changed over years, putative QTLs in each year distal to *wx* locus displayed additive values from 0.58% to 1.16%, with the *wx* allele contributing positively to this character (Figure 6-B and Table 12).

Composite interval mappins, using 13 background marker sets (Figure 7), detected from 4 to 6 additional QTLs from 1996 to 1999 (Table 13, Figure 8-A, B).

Among the additional QTLs detected by CIM, those QTLs distal to WTA1 (4.5cM right from *n* locus; GU96Q2, GU97Q3, GU99Q2) were detected over years as well as the QTL distal to the *wx* locus (Figure 8-B; segment A and C). The *n* gene which exhibited the largest QTL for grain yield, was also found to have an important genetic role in β-D-glucan content (Table 13; SMQ).

A QTL region distal to ABC170 (GU96Q3, GU99Q3) was also related to plant height and heading date (Figure 8-B; segment E). Another QTL distal to HVM6 on chromosome 3 was also detected over 2 years (Figure 8-B; segment G). MTaz90-123 contributed all of the favorable alleles found for grain B-glucan content.

Regression models including two QTL regions indicated by CIM in every year (*wx* and *n*, Figure 8-B; segment A and C), explained 30.4%, 44.5% and 60.2% of phenotypic variance for GU96, GU97, and GU99 respectively. This suggested that these regions may have higher priority of being fixed for 'high-fiber barley' than the other regions. Regression models explained 67% and 61% of phenotypic variations for GU96 and GU97.

A higher proportion of the phenotypic variance was explained by the regression model for GU99 (84%).

The estimated additive values of GU97Q1 (distal to *wx* locus) and GU97Q2 (near ABG380; Figure 8-B; segment B) showed very different values than those values estimated under the null hypothesis (Table 13; compare the additive values in QTL summary with the Coefficients in MR on putative QTL). Therefore, it was assumed there were still undetected small QTLs and some level of non-additive genetic factors might be involved in β-D-glucan contents, especially in the 1997 trial.

Interestingly, all detected positive β-D-glucan QTL alleles near the 'heading date and plant height' QTL regions (ABG380, ABC170, HVM060) were contributed by MTaz90-123. Among these three regions, ABC170 and HVM060 regions were identified by CIM in GU96 and GU99 trials (GU96Q3, GU99Q3 for ABC170 and GU96Q5, GU99Q4 for HVM060; Figure 8-B; segment E and G). Meanwhile, GU97Q2 was detected near ABG380 in which one major QTL of plant height and heading date was detected by SIM (Figure 6-B; note ABG380 region is responding with GU97 trial).

QTLs for heading date and plant height SIM identified two large QTL affecting both heading date and plant height in the 'Fiber-2' population. This pleiotropic effect was observed in all trials in the regions around ABG380 on chromosome 1 and ABC170 on chromosome 2 (Figure 6-B).

The QTL distal to ABC170 (HD98SQ2, HD99SQ2, and PH99SQ1) displayed the biggest effects on both traits (R^2; 54%, 58%, and 44%, respectively). The allele contributed

by MTH6860756 resulted in a 2-day delay in flowering and about 5cm greater height than that contributed by MTaz90-123 (Table 12; SMQ).

The QTL allele distal to ABG380 (HD98SQ1, HD99SQ1, and marginal QTL for PH99 trial) contributed by MTaz90-123 had a smaller impact on both traits (R2; 34%, 31%, and 21%, respectively). The estimated additive effect of this QTL were 1.5-day lateness and 2 cm tallness on heading date and plant height by MTaz90-123 allele (Table 12; SMQ).

Composite interval mapping failed to detect the ABG380 region for either plant height or heading date (Figure 8-B; segment B). Both the 'Forward' and 'Forward and Backward' stepwise multiple regression approaches (in QTL cartographer, Basten *et al.*, 1999) were applied to select background markers, but both methods failed to indicate the ABG380 marker as a significant marker for plant height and heading date.

Tinker *et al.* (1995) suggested that when a certain region contained two separate small QTLs, simple interval mapping could be influenced by those two small QTLs as a combined effect that could not be strong enough to manifest themselves as separate genes.

Consequently, composite interval mapping might not provide evidence for a QTL at positions where SIM was marginally significant. The ABG380 region effect was marginal using SIM for plant height in 1999, likely due to our late seeding resulting from a wet spring (Figure 6-B; PH99 Ch1).

Table 13. Summary of detected QTLs with composite interval mapping

Trait	List of detected QTLs											SMQ of the closest marker [d]		
	Position [a]			QTL summary [b]				MR on putative QTLs [c]			Flanking markers				
	Name	Ch	cM	LOD	R^2(%)	Add	Part R^2(%)	QTL	Int	R^2(Adj)%	Left	Right	R^2(%)	AZ	CA
YD98	Q1	1	162	10.1	55.0	376	69.1 **	446	5115	74.4(69.0)	FL1105S	***n***	43.8	4719	5453
(kg/ha)	Q2	2	70	7.3	44.2	193	26.2 **	173			ABC170	**FL1106S**	*3.0*	*5078*	*5268*
	Q3	7	26	5.5	35.1	-350	16.8 **	-278			**FL1802A**	FL1801A	*0.1*	*5175*	*5137*
	Q4	7	42	11.0	58.1	560	30.1 **	385			**FL0407A**	FL0406A	*0.7*	*5121*	*5217*
	Q5	7	226	9.5	53.1	-313	26.8 **	-184			FL1707S	**FL0506A**	7.9	5304	4999
KW98	Q1	1	180	6.7	40.6	2.2	56.8 **	2.71	42.4	80.8(74.7)	lk_2	FL1004S	48.2	39.2	45.0
(mg/seed)	Q2	1	206	5.1	32.6	1.7	36.2 **	2.05			**FL1003S**	FL0413A	23.2	40.5	44.3
	Q3	2	272	8.7	49.3	1.7	37.7 **	1.45			**FL1410B**	HVM054	7.5	41.5	43.6
	Q4	3	24	4.8	31.2	1.1	28.2 **	1.25			FL1713A	**FL0602A**	*0.6*	*42.2*	*42.8*
	q1	1	30	3.5	24.1	0.9	30.3 **	1.20			M-HVWAXYG	***wx***	16.5	41.3	44.6
	q2	1	122	2.9	20.2	-1.2	24.2 **	-1.26			FL1009B	**FL0504S**	*1.9*	*42.0*	*43.1*
	q3	1	306	4.0	27.0	-1.0	25.3 **	-1.14			**FL1109S**	FL1506A	*0.2*	*42.7*	*42.3*
KW99	Q1	1	30	7.1	42.6	2.6	13.5 **	0.74	46.6	77.3(73.6)	M-HVWAXYG	***wx***	19.4	45.9	49.5
(mg/seed)	Q2	1	162	16.8	73.0	2.4	71.7 **	3.25			FL1105S	***n***	74.9	43.0	50.4
	Q3	2	276	10.6	56.2	1.4	25.0 **	1.11			FL1410B	**HVM054**	*4.7*	*46.3*	*48.1*
	Q4	6	246	6.3	38.8	1.2	4.6				**FL1101S**	FL1409B	*0.2*	*47.1*	*47.4*
	Q5	7	186	6.9	41.6	1.3	8.4 *	0.69			ABC155	**FL1607B**	6.3	46.1	48.1
PH99	Q1	2	60	11.8	60.3	4.3	45.1 **	3.85	85.4	65.2(59.7)	FL0205B	**ABC170**	44.4	79.2	88.8
(cm)	Q2	2	336	5.9	36.8	-2.8	26.2 **	-3.14			**M-HVCSG**	FL1108B	5.6	85.6	82.2
	q1	1	304	3.4	23.3	-1.9	13.4 **	-1.72			**FL0801S**	FL1109S	8.6	85.8	81.8
	q2	2	28	3.5	23.9	3.1	15.3 **	2.97			**ABG058**	HVM36	*4.6*	*82.3*	*85.3*

Table 13. (continued)

Trait	List of detected QTLs											SMQ of the closet marker [d]		
	Position [a]			QTL summary [b]				MR on putative QTLs [c]			Flanking markers				
	Name	Ch	cM	LOD	R^2(%)	Add	Part R^2(%)	QTL	Int	R^2(Adj)%	Left	Right	R^2(%)	AZ	CA
GU96(%)	Q1	1	36	6.5	39.6	-0.5	42.0 **	-0.59	5.4	66.9(54.2)	**HVM6**	FL0904S	27.1	6.2	5.0
	Q2	1	166	5.3	32.5	-0.4	20.8 **	-0.35			*n*	**WTA1**	7.3	6.1	5.5
	Q3	2	52	6.3	29.0	-0.4	16.7 **	-0.31			**FL0205B**	ABC170	7.1	6.0	5.4
	Q4	3	58	5.7	15.7	0.5	13.9 **	0.30			**FL1808S**	FL1614S	*1.7*	*5.6*	*5.9*
	Q5	3	112	9.9	37.6	-0.5	30.0 **	-0.43			FL1806S	**HVM060**	9.3	6.0	5.3
	Q6	4	354	4.9	27.0	0.4	20.4 **	0.46			BCD402	**FL0409A**	*2.9*	*5.5*	*5.9*
	Q7	6	54	6.1	23.0	0.5	22.8 **	0.48			**HVGNIRE**	FL0102A	*3.0*	*5.6*	*5.9*
	q1	5	218	3.9	19.5	0.3	10.4 *	0.24			**FL0415S**	FL0406S	*0.1*	*5.8*	*5.7*
GU97(%)	Q1	1	30	10.1	54.4	-1.1	40.6 **	-0.55	6.0	60.8(52.6)	M-HVWAXYG*wx*		39.7	6.7	5.4
	Q2	1	92	8.3	47.6	-1.0	19.0 **	-0.34			FL0205B	**FL1205S**	12.4	6.5	5.7
	Q3	1	166	4.1	27.5	-0.3	10.6 *	-0.24			*n*	**WTA1**	17.2	6.6	5.8
	Q4	4	316	7.3	43.4	-0.4	15.2 **	-0.28			FL0411S	**BTA02a**	9.5	6.5	5.8
	Q5	5	216	6.1	37.9	0.4	14.3 **	0.30			**FL1512B**	FL0415S	*2.8*	*5.9*	*6.3*
GU99(%)	Q1	1	30	23.0	83.4	-0.8	84.4 **	-0.90	5.2	83.6(79.3)	M-HVWAXYG*wx*		53.5	6.3	4.6
	Q2	1	172	10.6	56.4	-0.4	41.8 **	-0.39			**WTA1**	ABG20.11a	11.1	6.0	5.3
	Q3	2	52	7.3	43.3	-0.5	30.3 **	-0.35			**FL0205B**	ABC170	*0.9*	*5.7*	*5.5*
	Q4	3	112	5.0	32.5	-0.2	31.3 **	-0.29			FL1806S	**HVM060**	*0.1*	*5.6*	*5.5*
	Q5	3	184	4.3	28.4	0.2	11.8				**FL0502B**	FL1612B	*1.3*	*5.4*	*5.7*
	Q6	5	290	8.0	46.4	0.7	41.8 **	0.77			**FL1703S**	FL1206A	*1.2*	*5.4*	*5.6*
	Q7	7	204	9.8	53.6	0.4	40.9 **	0.49			FL1607B	**FL1708S**	*3.4*	*5.3*	*5.7*

Table 13. (continued)

Trait	List of detected QTLs												SMQ of the closet marker [d]		
	Position [a]			QTL summary [b]				MR on putative QTLs [c]			Flanking markers				
	Name	Ch	cM	LOD	R^2(%)	Add	Part R^2(%)	QTL	Int	R^2(Adj)%	Left	Right	R^2(%)	AZ	CA
HD98	Q1	1	106	4.7	30.9	-0.7	35.2 **	-0.80	182.7	76.8(70.7)	**FL0304S**	FL1009B	21.2	183.4	181.4
(days)	Q2	1	304	4.6	30.2	-0.6	24.3 **	-0.58			**FL0801S**	FL1109S	11.9	183.1	181.6
	Q3	2	60	16.0	71.3	1.6	60.8 **	1.48			FL0205B	**ABC170**	53.8	181.0	184.3
	Q4	3	26	4.4	29.0	0.7	4.8				FL0602A	**FL1808S**	*1.0*	*182.5*	*182.1*
	Q5	3	112	4.7	30.6	0.6	14.2 **	0.42			FL1806S	**HVM060**	*3.7*	*181.9*	*182.7*
	Q6	7	206	4.1	27.2	-0.6	18.5 **	-0.57			**FL1708S**	FL1707S	*0.0*	*182.3*	*182.3*
	q1	7	228	3.7	24.9	0.6	10.3 **	0.40			**FL0506A**	FL1005A	*0.5*	*181.5*	*182.2*
HD99	Q1	2	62	11.9	60.5	2.0	57.4 **	1.99	189.9	69.8(66.3)	**ABC170**	FL1106S	31.1	191.1	187.9
(days)	q1	1	106	2.4	16.8	-0.7	20.3 **	-0.81			**FL0304S**	FL1009B	17.9	190.9	188.4
	q2	1	302	3.3	22.7	-0.9	22.2 **	-0.87			ABC253	**FL0801S**	*15.5*	*190.8*	*188.5*

[a] The position on the chromosome of the detected QTL, in cumulative cM.

[b] LOD scores were calculated from the *F*-value in the multiple regression according to Haley and Knott (1992), and 5% genome level of empirical critical values were applied to control type I error (Table 11). Some minor QTLs (started with q-), among those QTLs under the pre-selected threshold level, were also added to the multiple regression model, depend on the SMQ results. Under the rejected null hypothesis, percentage of the phenotypic variance, R^2(%) and the additive effect(Add) were estimated. It was assumed that the second parent (MTH6860756; paternal line;CA) carries the favorable alleles for the trait under study. The coefficient of determination between the respective QTL and the phenotypic observations, keeping all other QTL effects fixed. Partial R^2 values (Part. R^2) could be used to determine the relative importance of detected QTLs (Utz and Melchinger, 1999), * and ** represent the significant level at 0.05 and 0.001, respectively.

[c] Multiple regressions were conducted to obtain the estimated values of parameters (QTL; detected QTLs with significant Partial R^2 values) and the phenotypic variance (R^2 and adj R^2 %) explained with the QTLs. To compare the deviation of Intercept (Int) from population mean, see Table 8.

[d] The closest marker to the detected QTL (bold lettered flanking marker) was used for the single marker QTL analysis (SMQ), and R^2 value and each genotype means (MTaz90-123; AZ, and MT6860756; CA) are also reported . Additive value of the closet marker allele could be estimated with the formula, (CA -.AZ)/2 and be compared with the additive effect(Add) which was estimated under the rejected null hypothesis (see QTL summary column). *F*-statistics of each SMQ are in Table A-2. The markers indicated with Italic letters were non-significant in SMQ.

However, another region on chromosome 1 (Figure 8-B; segment D) was detected in both years as a QTL for heading date and plant height (HD98Q2, HD99q2, PH99q1).This positive QTL allele was contributed by the MTaz90-123 (Table 13). Still, the ABC170 region showed very significant effects on both traits tested using either CIM or SIM (Table 13 and Figure 8-B; segment E).

In the 1998 trial chromosome 7 displayed another possibility of repulsion linkage. Two QTLs, HD98Q6 and HD98q1 (Figure 8-B; segment J), were linked to each other(20cM) in repulsion phase on chromosome 7. Each QTL displayed less than one day (0.6 day) additive values by MTaz90-123 allele and MTH6860756 allele, respectively (Table 13; QTL summary).

Among additional HD98 QTLs, HD98Q5 (near HVM060, Figure 8-B; segment G) was also detected by CIM as a significant β-D-glucan factor (GU96Q5, GU99Q4). Another QTL, HD98Q4, was also detected by CIM for its impact on β-D-glucan content (GU96Q3).

Another plant height QTL was detected on chromosome 2 (PH99Q2, Figure 8-B). The positive allele for this QTL was contributed by MTaz90-123 allele with 2.85 cm as its estimated additive value (Table 13).

Simultaneous multiple regression on all putative QTLs detected by CIM explained 77%, 70% , and 65% phenotypic variations for each HD98, HD99 and PH99 (Table 13; MR on putative QTLs. However, the regression model containing only ABC170 and ABG380 (SIM result) explained 63% 64% and 48% R^2 value for HD98, HD99 and PH99 (Table 12).

The reason that few QTLs were identified for heading date and plant height in 1999 might be because due to late seeding the effective vegetative period for the crop was

shortened. The late seeding date of this experiment could be the cause of the poor resolution we observed for plant height, flowering date and grain yield in 1999. Otherwise, the limited population size of the 'Fiber-2' population implies that only QTLs with large effects could reach statistical significance (Kanpp and Bridges, 1990). Indeed, a few hundred individuals would be required to detect segregating QTL responsible for very small amount of the phenotypic variance (1-5%; Weller, 1992).

Discussion

Figure 6-A (SIM) show the chromosome regions that contain the most significant QTLs in 'Fiber-2' population and Figure 8-A (CIM) includes additional significant QTLs in the 'Fiber-2' population.

Chromosome 1 produced the most significant effects on grain yield and kernel weight (distal to *n* locus) and β-glucan contents (distal to *wx* locus). The mutant alleles resulting in high glucan content and naked caryopsis were introduced from Waxy Oderbrucker and Sermo (Chapter 1). The short arm of chromosome 2 (distal to ABC170) carried genes affecting all traits tested.

Hayes *et al.* (1993) detected a QTL affecting flowering time and plant height on the short arm of chromosome 2 distal to ABG002. Bezant et al. (1996) also reported a similar result. Liu *et al.*(1996) integrated SSR markers into Stepto x Morex linkage map, among of them HVM036 was near ABG002. In the 'Fiber-2' linkage map, HVM36 is also located on the short arm of chromosome 2.

Han *et al.* (1995) reported a β-glucan QTL which mapped between Chs1B and ABC162 interval in the Steptoe x Morex linkage map, and in the middle of the interval, ABG002 is located. Borěm *et al.*(1999) also found a QTL affecting the overall mean starch granule volume, the proportion of A granules, the mean volume of A granules, the mean maximum diameter of A granules and the mean F-shape of B granules, which mapped to the same region.

Coincidence between heading date and β-D-glucan content was also seen in the HVM60 region on chromosome 3. This region contained genes with significant impact on β-glucan content (GU96Q5 and GU99Q4) and heading date (HD98Q5). HVM60 was mapped near ABG453 in the Steptoe x Morex linkage map by Liu *et al.*(1996), where QTLs for heading date and grain protein content reside. As it was mentioned before, all detected β-D-glucan QTLs near the 'heading date and plant height' QTL regions (ABG380, ABC170, HVM060) were contributed by MTaz90-123 alleles which also contributed to reduced plant height and earlier heading date.

Chromosome 5 contained 5 distorted markers and displayed the biggest apparent map length (390cM). Two QTLs were detected by CIM (GU97Q5 and GU99Q6) in the middle of the long arm,. Han *et al.* (1995) also reported a QTL for β-glucan content on Steptoe x Morex chromosome five's long arm.

Two grain yield QTLs were mapped in repulsion phase in on the satellite of barley chromosome 7 in the 'Fiber-2' linkage map. Spaner *et al.*(1999) reported that the ABG483 region contained QTLs for grain yield, plant height, maturity and lodging severity in the 'Harrington' x 'TR306' population.

Despite the small size of 'Fiber 2' population evaluated here, QTL with major effects were detected and provided new information for further studies. Composite interval mapping showed its strength in our analyses. It proved to have better precision in detecting QTLs over simple interval mapping due to its ability to simultaneously take into account effects of other QTLs lying outside the interval region being queried, providing much stronger QTL detection power than SIM.

It was problematic to estimate each of the *n* and lk_2 effects on 3 traits (YD, KW, and GU) due to the linkage between two loci. The comparison of peak patterns around these two loci suggested that they responded to each trial condition with different effects across traits tested (Figure 6-B, Figure 8-B).

Hockett (1981) reported that if the hull weight (10% of grain weight) was allowed, yield was equal between hulless and hulled isogenic lines. Most previous experiments (with isogenic lines) suggested that most of the effects of *n* locus were accounted for by the removal of the fibrous hulls (Bhatty, 1986; Swanston, 1995; Xue *et al.*, 1997), not by possible negative pleiotropic effect of the *n* locus region.

We expected strong interactions between the *n*, lk_2 and *wx* genes because of previous isogenic analyses (Chapter 1). While the final estimation of genetic effects of each detected QTLs suggested that non-additive genetic factors maybe involved in each or the traits tested interactions between detected QTLs were not identified during interval mapping analysis. These questions will be further addressed in Chapter 4.

CHAPTER 4

EVALUATION OF GENETIC EFFECTS UNDERLYING MAJOR QTL IN THE FIBER-2 POPULATION

Introduction

Naked versus covered caryopsis is a simple genetic character (Nilan, 1964), and the genetic removal of the hull improves the value of barley for human food. Although many barley breeding programs have sought to increase the grain yield of hulless barleys, their performance remains lower than hulled barleys (chapter 1).

Interval mapping showed that *n* gene region had a greater impact on grain yield and kernel weight than other detected QTLs, and played an important genetic role in the determination of β-D-glucan content in the 'Fiber-2' population. These pleiotrophic effects were identified over seasons (chapter 3).

It was not clear whether the *n* locus could still maintain negative effects on naked barley lines' grain yield and kernel weight even after the hull weight was compensated for in hulless lines. Murphy and Witcombe (1981, 1986b) reported that they could not find strong evidence of large pleiotropic effect on major agronomic traits by the caryopsis covering gene or closely linked genes. Studies with isogenic lines (chapter 1) and released

non-waxy hulless barleys (Hockett, 1981; Helm *et al.*, 1992, 1996a and 1996b) do not offer enough information about possible diluted effects of *n* locus on agronomic traits by excluding the hull weight and high fibrous hull components.

There have been many reports about the potential of lk_2 locus on grain yield as secondary photosynthesis organ, especially during grain development (Grundbacher, 1963; Schaller *et al.*, 1972; Kjack and Witters, 1974; Paluska, 1979 ; Qualset *et al.*, 1965; McGuire and Hockett, 1981). Isogenic line studies reported additional additive effects of lk_2 on β-D-glucan content in barley (Fox, 1981; Xue *et al.*, 1997).

Both *n* and lk_2 were detected by interval mapping for grain yield, kernel weight and β-D-glucan content (chapter 3), but their linkage caused problems in dissecting their individual effects. A comparisons of mean values of recombinant genotypes would resolve this uncertainty. Fifty nine lines (the population size of the 'Fiber-2' population) did not provide a sufficient number of recombinant individuals. We therefore merged results from the 'Fiber-2' and 'Fiber-3' populations to provide more recombinant individuals.

Oscarsson *et al.* (1997) reported that waxy and high amylose barleys (which have been reported as another class of high fiber barleys) have thicker endosperm cell walls than other barleys. Similar observations were made by Swanston (1997).

Borém *et al.* (1999) detected QTLs related to the size and shape of barley starch granules (A granules), and this region also displayed large QTL for heading date and plant height. They suggested that QTL for late heading and for increased plant height coincided with QTL for large starch granules. Interval mapping of the 'Fiber-2' population showed that there might be some coincidence between β-D-glucan content and heading date . Some

chromosomal regions to which putative β-D-glucan QTLs were located also responded to CIM with heading date. All those overlapped QTLs suggested earlier heading and shorter plant height may be favorable for increasing β-D-glucan content, and this tendency may be also reflected in small kernel weight. Powell *et al.* (1985) also detected large negative correlations for β-D-glucan content with thousand grain weight and with plant height with random inbred lines prepared by crossings between normal endosperm barleys (chapter 1).

Lark *et al.* (1995) proposed that if a chromosomal region contains two genes which modify a trait of interest, they might interact with other genes differently, and this difference may provide evidence for the presence of linked genes, rather than one gene with multiple interactions. Differentiation of the genetic effect of two linked genes, *n* and lk_2, may be possible with their analytical approaches. Furthermore, interval mapping of the 'Fiber-2' also display the possibility interactions between major QTLs for β-D-glucan contents and heading date and/or plant height.

The major goals of this chapter are to evaluate the effect of *n* locus on agronomic traits, find approaches to differentiate of two linked QTLs (*n* - lk_2), and determine which of two linked genes is more likely to be responsible for an effect of interest with limited population size. For this aim, the contribution of hulls to kernel weight was estimated and evaluated against the genetics underlying yield and kernel weight. All possible digenic interactions between major QTLs, tagged by four markers (*wx*, *n*, lk_2, and ABC170), were also surveyed.

Materials and Methods

Population Merging and Data Preparation

The plant materials and measured traits are described in Chapter 3. Fifty nine lines of the 'Fiber-2' population was not enough to generate reasonable subgroup sizes (recombinant lines between two loci of interested). Therefore, another population, 'Fiber-3' with 96 individuals, was merged with the 'Fiber-2' population to generate 'Fiber (merged)' population (166 lines). Both the 'Fiber-2' and the 'Fiber-3' populations are F_5-derived, and evaluated in the same trial years. Digenic interactions between major QTLs were surveyed with the'Fiber (merged)' population.

Four markers (*wx*, lk_2, *n*, and ABC170) were considered as flanking markers of major QTLs detected in the 'Fiber-2' population (Chapter 3). The genotypes of each lines were assayed using the of ABC170 primer set (co-dominant marker; Table 2) with the DNA templates prepared from multiple plants from each line. The genotypes for lk_2 and *n* were inferred during field trials, and cleaved multiple grains were stained with an iodine solution to determine the waxy genotypes (Frykman and Bengtsson, 1992). In all cases, mixed lines were excluded from the analysis.

To estimate the proportion of hulls in kernel weight, nine mixed (heterogeneous) lines for *n* locus were selected from the 'Fiber-2' population (see Table 16), and 1,000 kernels were counted for each inferred genotype (*NN* and *nn*) and reported with mg/seed.

Under the assumption that most genes have been fixed in each line, the weight difference between two inferred genotypes was considered as the 'estimated hull weight'.

Statistical Analysis

The phenotypic correlation coefficients among traits measured based on means of the 'Fiber-2' population were estimated. To compare the genotypic effect of each gene on traits measured, the 'Fiber-2' population lines were divided into two sub-populations depending on the genotypes for the *n* and *wx* locus, and the phenotypic correlation coefficients among traits measured were re-estimated within each sub-population.

The mean value of hull weight (percent to kernel weight of hulled genotype) was added to all hulless lines in the 'Fiber-2' population (weighted). The contribution of hulls to kernel weight was estimated with the 'PROC ANOVA' or 'PROC GLM' procedures in SAS. The weighted grain yield and kernel weight data were also used for SIM analysis with a software package, 'PlabQTL'. In surveying possible digenic interactions between major QTLs, a software package, 'EPISTAT' (Chase *et al.*, 1997) and the 'PROC SORT' and 'PROC GLM' in SAS were used for the main effect estimation of a locus in a specific genetic background (MLG).

Results

Correlation among traits

Genetic correlation among traits is caused by linkage, pleiotropy, pedigree relationships and environmental interactions (Falconer and Mackay, 1996, pp 312-334). The correlation coefficients observed among the 'Fiber-2' field trials (Table 14) suggested that chromosome segments associated with reduced kernel weight were strongly associated with increased values of β-D-glucan contents across seasons. Grain yield showed negative correlations with β-D-glucan contents and heading date. Interestingly, plant height showed significant negative correlation with β-D-glucan contents in 1996 and 1999 while heading date was significant positively correlated with glucan content in 1997. CIM also detected similar results (Table 13, Figure 8-B).

Table 14. Phenotypic correlation coefficients among traits measured based on means of 'Fiber-2' mapping population.

KW98	0.378**							
KW99	0.537**	0.818**						
GU96	-0.207	-0.404**	-0.391**					
GU97	-0.232	-0.531**	-0.517**	0.555**				
GU99	-0.262*	-0.497**	-0.477**	0.610**	0.649**			
HD98	-0.091	0.080	-0.292*	-0.060	0.257*	-0.008		
HD99	-0.038	0.146	-0.216	-0.052	0.197	-0.050	0.936**	
PH99	0.085	0.271*	-0.015	-0.292*	0.009	-0.284*	0.722**	0.760**
	YD98	KW98	KW99	GU96	GU97	GU99	HD98	HD99

Grain yield (YD), Kernel weight (KW), β-D-glucan content (GU), Heading date (HD), and Plant heihgt (PH) with the trail years.

*, ** are levels of significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 15 offers comparisons of the *n* and *wx* gene effects on barley grain traits (grain yield, kernel weight, and β-D-glucan content). The positive correlation between grain yield and kernel weight identified in Table 14 was mainly derived from the *wx* gene effect. In both genotypes of *n* locus, correlations were not significant, although the signs were opposite in each genotype. Interestingly the correlation between grain yield and β-D-glucan content showed negative numbers in both hulless and waxy lines, but the correlation exhibited positive values in both hulled and nonwaxy lines.

In all four genotypes, kernel weight showed a negative correlation with β-D-glucan content. Waxy lines displayed a significant negative correlation with fiber content. The negative correlation was greater in hulless lines than hulled lines in the 1998 trial, but the pattern was altered in 1999.

The mean differences between hulless and hulled lines (Table 15; 5.7 and 7.4 mg/seed for KW98 and KW99 trial and 733.7 kg/ha for YD98 trial) may be considered as the negative pleiotrophic effect of the *n* gene. Since the hulls are not positive contributors to food barley, we needed to determine how much of the negative yield impact of the chromosome 1 genes near *n* were the result of hull loss and how much represented a real reduction in productivity for developing 'high fiber hulless barley' as human food.

Correlation coefficient analysis suggested that the *wx* gene impacted kernel weight very strongly (Table 15; Part B), although SIM did not detect the *wx* locus as significant marker for kernel weight (Table A-2, Figure 6-B). We thought this odd situation may be derived from unconsidered hull weight of hulless lines in the 'Fiber-2' population (see below).

Table 15. Phenotypic correlation coefficients among 'grain traits' based on the means of 4 inferred genotypes in the 'Fiber-2' population.

Part A. Hull type

Hulless (*nn*) - 22 lines							B.V [b]	hulled (*NN*) - 28 lines						Mean [a]
Mean [a]	Trials							Trials						
4719.4	YD98						733.7	YD98						5453.1
39.2	KW9	0.078					5.7	KW98	-0.187					44.9
43.0	KW9	0.002	0.796**				7.4	KW99	-0.127	0.561**				50.4
6.1	GU96	-0.226	-0.433*	-0.183			-0.7	GU96	0.021	-0.354	-0.562**			5.5
6.7	GU97	-0.393	-0.385	-0.335	0.561**		-0.9	GU97	0.431*	-0.332	-0.304	0.475*		5.8
6.1	GU99	-0.326	-0.427*	-0.243	0.594**	0.581**	-0.8	GU99	0.218	-0.297	-0.552**	0.721**	0.546**	5.3
		YD98	KW98	KW99	GU96	GU97			YD98	KW98	KW99	GU96	GU97	

Part B. Waxy type

Waxy (*wxwx*) - 30 lines							B.V [b]	Nonwaxy (*WxWx*) - 21 lines						Mean [a]
Mean [a]								Trials						
5053.8	YD98						252.2	YD98						5306.1
41.4	KW9	0.555**					3.2	KW98	-0.313					44.6
45.9	KW9	0.704**	0.817**				3.6	KW99	-0.024	0.648**				49.5
6.3	GU96	-0.243	-0.350	-0.264			-1.3	GU96	0.372	-0.230	-0.212			5.1
6.7	GU97	-0.466*	-0.599**	-0.533**	0.345		-1.3	GU97	0.380	-0.176	-0.101	0.369		5.4
6.3	GU99	-0.340	-0.573**	-0.385*	0.305	0.318	-1.7	GU99	0.276	-0.065	-0.191	0.641**	0.499*	4.6
		YD98	KW98	KW99	GU96	GU97			YD98	KW98	KW99	GU96	GU97	

[a] Grain yield (YD, kg/ha), Kernel weight (KW, mg/seed), and β-D-glucan content (GU, %) with the trail years.
[b] Breeding value (B.V) = (Mean of normal genotype lines - Mean of mutant genotype lines)
*, ** are levels of significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Estimation of Hull Weights and Evaluation of Their Attributions to Grain Traits

If a portion of the mean differences between two genotypes (*nn* and *NN*) in the 'Fiber-2' population were simply factorable by adding the hull weights to hulless lines, and the hull weight is relatively stable to environmental changes, it may be possible to treat the hull weight as a statistical constant. We could then reduce the artificial effects of hulls on both grain yield and kernel size during interval mapping.

Under the assumption mentioned above, we tested kernel weight in nine mixed (heterogeneous) lines in the 'Fiber-2' population (Table 16). The proportion of hulls in each line ranged widely, from 9.3% (#24 in 98) up to 16.9% (#59 in 99') of the kernel weight. The mean percent of hull weight was estimated as 13.8% and 14.5% for each trial year (Table 16, Part A). ANOVA detected significant variation among lines and over seasons (49.8% and 46.0% partial R^2 for hulled and hulless lines, respectively; Table 16, Part B).

To test whether hull weights are controlled by simple genetics, the estimated hull weight was considered for all hulless lines in the 'Fiber-2' population (for mixed lines, half the amount of mean hull weight was considered). ANOVA on both 'before (raw data)' and 'after (weighted)' kernel weights display very different partial R2 (%) values for variance sources (Table 16, Part C). This result suggested that the *n* gene alone is not sufficient to explain the variation in hull weights in this population of lines. Estimation of phenotypic effects (R^2; with SMQ analysis) of the lk_2 and *n* genes in both 'before' and 'after' weighting the data provided surprising results (Table 16, Part D). The R^2 value of *n* was reduced in 1998 (45.1% in KW98 and 6% in WKW98), while the lk_2 gene retained a high R^2 value (20.4%).

Table 16. Hull weight estimation and its contributions to kernel weight in the 'Fiber-2' population.

Part A. Estimation of hull weight with comparing two phenotypes in each F_5-derived lines [a]

Mixed lines (*NN* + *nn*)		1998				1999			
line ID (#)	Awn type	*NN*		*nn*	Hull wt %	*NN*		*nn*	Hull wt %
31	lk_2 lk_2	43.26		38.54	10.91	49.58		43.60	12.06
9		45.42		38.00	16.34	49.60		42.32	14.68
29	Lk_2 lk_2	44.54	47.2	37.42	39.6 15.99	49.06	51.5	41.62	43.3 15.17
50		46.28		38.48	16.85	51.62		42.56	17.55
52		52.54		44.52	15.26	55.66		46.70	16.10
24		46.10		41.80	9.33	51.54		45.44	11.84
49	Lk_2 Lk_2	45.88	46.3	39.22	40.7 14.52	48.74	51.0	41.00	44.1 15.88
56		47.46		41.98	11.55	49.62		44.90	9.51
59		45.56		39.64	12.99	54.20		45.04	16.90
Mean (mg/seed)		46.34		39.96	13.77 %	51.07		43.69	14.46 %

Part B. ANOVA for kernel weight of two phenotypes tested in part A [b]

		Hulled type (*NN*)			Hulless type (*nn*)		
Source	d.f.	Part R^2(%)	MS	*F*-value	Part R^2(%)	MS	*F*-value
Year	1	49.8	100.73	50.78**	46.00	62.65	82.71**
Line	8	42.3	10.70	5.40**	49.50	8.43	11.13**
Error	8	7.9	1.98		4.50	0.7575	
Total	17	100.0			100.00		

Part C. ANOVA for kernel weight of 59 'Fiber-2' mapping lines over seasons, before (KW) and after (WKW) considering hull weight of hulless lines [c]

		Kernel weight (KW)			Weighted kernel weight (WKW)		
Source	d.f.	Part R^2(%)	MS	*F*-value	Part R^2(%)	MS	*F*-value
Year	1	26.3	664.41	228.45**	41.00	685.21	226.00**
Line	58	67.7	1687.01	10.00**	48.50	808.37	4.60**
Error	58	6.0	168.69		10.50	175.85	
Total	117	100.0			100.00		

Part D. Comparison the phenotypic effects for Lk_2 and *N* on kernel weight, before (KW) and after (WKW) considering hull weight of hulless lines in 'Fiber-2' mapping population

	Loci	KW98	WKW98	KW99	WKW99
R^2 (%)	lk_2	46.4	20.4	43.1	24.7
	n	45.1	6.0	71.2	24.5

[a] In each 'mixed' line, 1000 kernel were counted for each inferred genotype (*NN* and *nn*) separately, weighted and reported with mg/seed. The proportion of hull weight (Hull wt %) was estimated from the difference of kernel weight between two phenotypes.

[b] ANOVA was conducted for each phenotypes of 9 'mixed' lines separately.

[c] The weight loss of hulless types, due to the discarded hulls during threshing, was weighted by adding estimated hull weight of hulless lines with the mean of 'Hull wt %' in part A for each year.

In 1999, the *n* gene effect on kernel weight was not diminished using the weighted kernel weight (WKW99; R^2, 24.5%). These result strongly suggested that the KW98 dataset was mainly influenced by lk_2, while in 1999, *n* impacted kernel weight very strongly.

We used weighted kernel weight (KWK) and compared the results of SIM and CIM over the YD98, KW98 and KW99 datasets (Figure 9). SIM with KWK confirmed the result of SMQ analysis on KWK (Table 16, Part D). Scans of test statistics clearly displayed the lk_2 effect on KW98 trial (Figure 9; column B), and *n* locus effect on KW99 trial. CIM results of YD98 (lk_2 region responded to CIM) and KW99 (lk_2 region is non-significant) suggested that the R^2 value of lk_2 in KW99 (43%) was detected due to linkage between *n* and lk_2 loci. For the same reason, the lk_2 effect on YD98 (Table 12; R^2, 43.8% and 33.9% for *n* and lk_2 respectively, and Figure 9; column C) appears real.

SIM with WYD (weighted YD98 data with the estimated hull weight) suggested that the genetic effects of both *n* and *lk2* on YD98 were smaller than on KW98 (Figure 9; note that the horizontal dashed lines in the column B indicate LOD score 2.5). As a conclusion, SMQ or SIM analysis, which have been used most commonly, overestimated the *n* locus effect on grain yield and kernel weight and therefore could seriously bias the interpretation of gene actions of lk_2 locus and *wx* locus (see below) on both traits in the 'Fiber-2' population. Figure 9 demonstrates the power of CIM. Highly significant peaks (contributed by MTaz90-123 alleles) were around the *wx* gene in SIM with WKW, and this region was detected by CIM using either weighted or unweighted data.

Weyhrich *et al.* (1994) reported that they found no pattern in yield superiority of either awn types (awned or awnletted) in wheat. Qualset *et al.*(1965) suggested that awns

were favorable under semi-arid environments and stress conditions. Altered genetic roles between lk_2 and *n* locus in KW98 and KW99 may fit into Oualset's interpretation when we think of water supply condition in both years (Table 7).

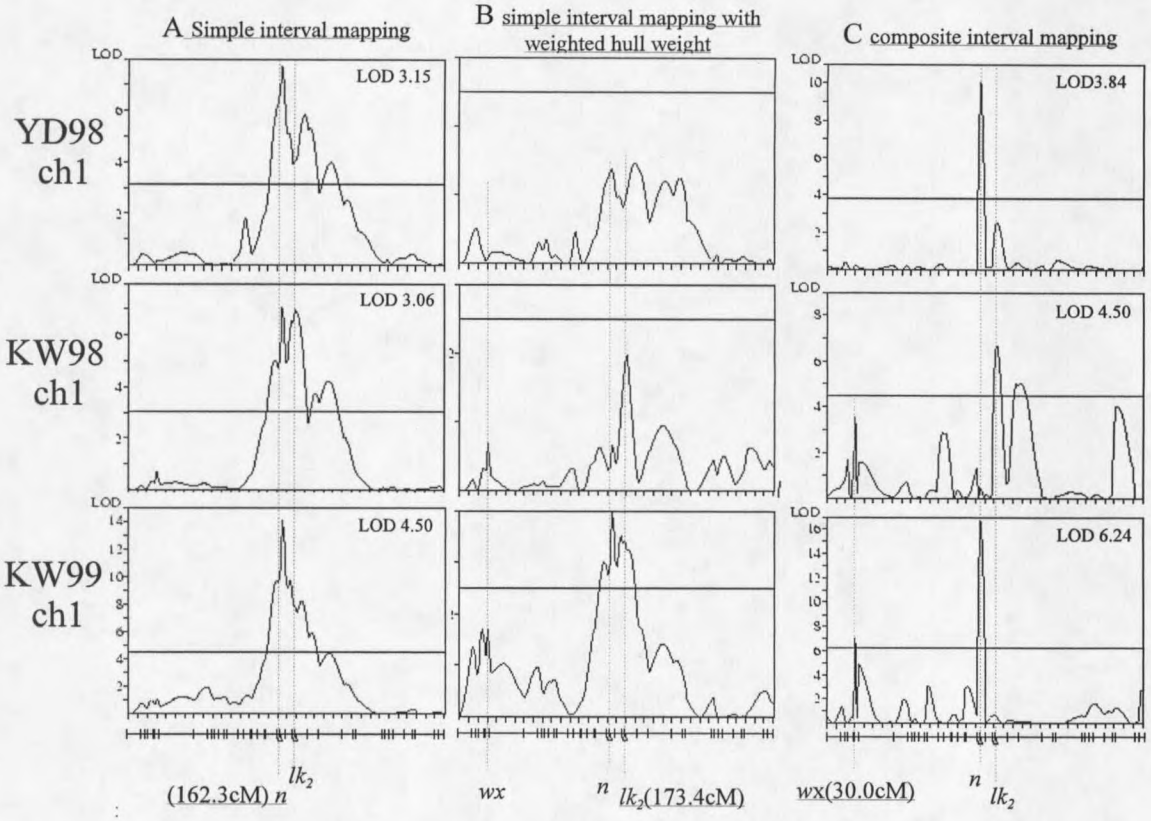


Figure 9. Graphical approaches to evaluate the genetic effects of the *n* locus region and differentiate two closely linked genes, *n* and lk_2. Column A. Scans of test statistics of SIM without considering the weight of hulls in hulless lines. Column B. Scans of test statistics of SIM with 'weighted' YD98, KW98 and KW99 datasets with the estimated hull weight (Table 16). Column C. Scans of test statistics of CIM without considering the weight of hulls in hulless lines. Small grid lines on bars under each column indicate marker positions on chromosome 1, and dotted lines across each column indicate chromosomal region tagged by three markers, *n*, lk_2 and *wx*. Horizontal dashed lines in each panel show the LOD threshold for testing interval mapping at 5% genome-wise-type I error, and those scores are shown in each panel (note the lines in column B indicate LOD score 2.5).

Main Effect Estimation of a Locus within a Specific Genetic Background (MLG)

We searched for possible interactions between major QTLs with 'EPISTAT'. We detected just one significant digenic interaction between lk_2 and *wx* locus for heading date in 1999 (Figure 10).

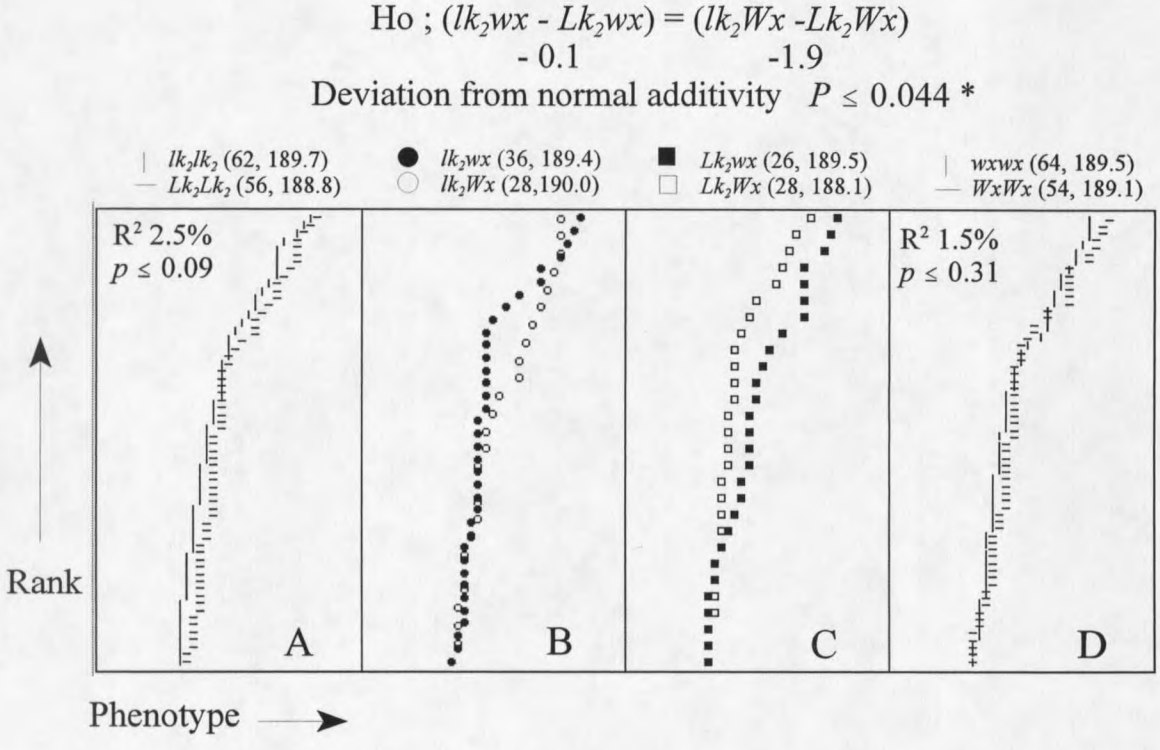


Figure 10. Cumulative distributions of heading date (HD99 trial) for the 'Fiber (merged)' individuals with particular genotypes at the lk_2 and *wx* loci. Total 118 lines from the 'Fiber-2' and 'Fiber-3' were used. In each genotype class, the number of individuals and the mean are parenthesized. Panel A and Panel D are exhibiting that neither locus has a significant independent effect on HD99 trial. In contrast, the recombinant classes (Panel B and Panel C), display difference in heading date. Under the null hypothesis (Ho), the difference (1.8 day) represent the 'deviation from normal additivity' and the probability was established with Monte Carlo simulations ($P \leq 0.044$ *). This unique interaction pattern was defined as 'co-adapted interaction' by Chase *et al.* (1997). Computer software 'Epistat' (Chase *et al.*, 1997) was used for this analysis.

Neither locus was detected as a 'major QTL' for heading date with either SIM or CIM (Figure 8-B; segment A, C). Panel B and C in Figure 10 show that the *wx* locus interacts with both allele (lk_2, Lk_2) and the degree of interaction is stronger between *wx* locus with Lk_2 allele (panel C) than the other combination. The results from 'EPISTAT' suggested that there were no significant interactions between major QTLs in the 'Fiber-2' population.

Although the mean differences among recombinant sub-groups were clear, it did not give us any further information for judging whether linked two QTLs are influencing a certain trait independently, or whether one apparent QTL is merely the result of linkage to another. Wade (1992) pointed out that attempting to detect epistasis by means of the interaction variance could not give us as much as power of detecting main effects in the ANOVA models, and it could be the main reason that most quantitative genetic studies have considered epistasis as statistical noise.

Therefore, we decided to include another numerical reference for each particular genotypic combination by the alleles of two loci. Instead of detecting an interaction term, we focused on detecting the main effect of a locus in specified genetic backgrounds (MLG). When digenic interaction was estimated between two we divided the population into two sub-groups depend on the locus 1 genotypes (homogeneous for 'A' sub-group, and homogeneous for 'a' sub-group). In each sub-group, the relative amount of explainable phenotypic variation (R^2 %) and the *p* value of *F*-statistics of locus 2 was estimated. The same procedure was applied in estimating R^2 value and the significance of locus 1 within each locus 2 sub-group (within B and b).

The MLG approach gave us a powerful tool for judging two linked QTLs, *n* and lk_2. The combination of *wx* and lk_2 genes in the GU99 dataset (Table 17; GU99 waxy + awn) displayed very clear additive gene action for β-D-glucan content. In the GU99 (waxy + hull) subgroup the additive effect of the *n* genotype is reduced to non-significance within the *wx* genotype sub-population (R^2, 1.7% ns). In the *Wx* genotype subpopulation, *n* was highly significant, controlling more than 28% of the phenotypic variation ($p \leq 0.001$). When *wx* genotype was considered in each *n* locus genotype sub-population (*nn* and *NN*), the liner model of waxy locus was interfered within *n* sub-population (R^2 39% **), but the model still showed significance for the GU99 dataset.

The estimated breeding values (B.V.) also show the level of interference by specific genetic backgrounds through analysis of sub-populations. For example, when the *n* locus is considered within *Wx* (normal barley) sub-population, the mean difference between each *n* locus genotype (*nn* and *NN*) was 0.79 %, meanwhile, within the *wx* (waxy) sub-population, *nn vs. NN* mean difference was 0.31%. This difference was from the *nn-wxwx* (homogenous for both mutant genes) combination (7.04%). If absolute additive action was responsible, the mean of *nn-wxwx* should be 7.52% .

When two homogeneous mutant genes are in one line of the 'Fiber-2' population, the line would display much lower β-D-glucan content (7.04%) than the expected value (7.52%). The mean (7.04%) is lower than *wxwx-*lk_2 lk_2 genetic combination (7.22%), although *nn* genotypes show a higher R^2 value and β-D-glucan content (28.75%, 5.36%) in the *Wx* sub-population than among *wxwx* genotypes (11.6%, 5.23 %).

Table 17. Surveying interactions between major QTLs by means of 'main effect estimation of a locus within a specific genetic background'.

GU99						**GU99**					
waxy* + *awn		6.39	5.53	-0.86	9.3**	***waxy* + *hull***		6.36	5.67	-0.69	6.1**
	5.98 %	lk_2	Lk_2	B.V.	R^2		**6.04 %**	*n*	*N*	B.V.	R^2
6.87	*wx*	7.22	6.41	-0.81	11.4**	6.91	*wx*	7.04	6.73	-0.31	1.7ns
4.93	*Wx*	5.23	4.65	-0.58	11.6**	4.96	*Wx*	5.36	4.57	-0.79	28.75**
-1.94	B.V.	-1.99	-1.76			-1.95	B.V.	-1.68	-2.16		
47.7**	R^2	48.9**	50.1**			49.3**	R^2	39.1**	62.9**		
HD99						**HD99**					
170* + *hull		190.12	188.84	-1.28	5.4*	**170 + *awn***		189.83	188.85	-0.98	3.2+
	189.52 d	*n*	*N*	B.V.	R^2		**189.35 d**	lk_2	Lk_2	B.V.	R^2
187.94	170az	188.29	187.61	-0.68	6.7*	187.84	170az	188.12	187.59	-0.53	4.1+
192.49	170ca	192.80	192.01	-0.79	2.9ns	192.26	170ca	192.55	191.87	-0.68	2.0ns
4.55	B.V.	4.51	4.40			4.42	B.V.	4.43	4.28		
62.2**	R^2	65.7**	57.1**			59.0**	R^2	63.2**	54.2**		
PH99						**PH99**					
170 + *hull*		83.58	81.21	-2.37	2.5+	**170 + *awn***		83.46	81.27	-2.19	2.1+
	82.47 cm	*n*	*N*	B.V.	R^2		**82.37 cm**	lk_2	Lk_2	B.V.	R^2
78.43	170az	78.83	78.07	-0.76	0.6ns	78.35	170az	78.68	78.07	-0.61	0.4ns
90.05	170ca	90.52	89.28	-1.24	1.46ns	90.12	170ca	91.02	88.92	-2.10	4.4ns
11.62	B.V.	11.69	11.21			11.77	B.V.	12.34	10.85		
55.4**	R^2	61.8**	46.8**			55.6**	R^2	63.7**	46.9**		

Table 17. (continued)

HD99						**PH99**					
awn* + *waxy		189.45	189.07	-0.38	0.5ns	***awn* + *waxy***		81.92	83.54	1.62	1.2ns
	189.28 d	*wx*	*Wx*	B.V.	R^2		**82.66 cm**	*wx*	*Wx*	B.V.	R^2
189.67	*lk2*	189.40	190.04	0.64	1.5ns	83.85	*lk2*	82.23	86.10	3.87	7.34*
188.84	*Lk2*	189.52	188.16	-1.36	6.7ns	81.35	*Lk2*	81.54	81.16	-0.38	0.1*
-0.83	B.V.	0.12	-1.88			-2.50	B.V.	-0.69	-4.94		
2.5ns	R^2	0.10	13.5**			3.0+	R^2	0.2ns	11.1**		
GU99						**HD99**					
***waxy* + 170**		5.86	6.07	0.21	0.5ns	**170 + *waxy***		189.78	189.10	-0.68	1.5ns
	5.93 %	170az	170ca	B.V.	R^2		**189.47 d**	*wx*	*Wx*	B.V.	R^2
6.85	*wx*	6.85	6.85	0.00	0.1ns	187.98	170az	188.12	187.83	-0.29	1.2ns
4.89	*Wx*	4.77	5.13	0.36	5.5+	192.36	170ca	192.90	191.72	-1.18	5.9ns
-1.96	B.V.	-2.08	-1.72			4.38	B.V.	4.78	3.89		
49.6**	R^2	56.6**	37.6**			58.3**	R^2	62.8**	54.2**		

1. Two populations ('Fiber-2' and 'Fiber-3') were merged and generated total 165 individuals. Depend on the inferred genotypes of each line, just homogeneous lines were used to estimate digenic interactions for each particular marker combination.
2. Waxy type (*wx, Wx*), Awn length (awn; lk_2, Lk_2), hullness (hull; *n, N*), and ABC170 (170; ca for MTH6860756 allele, az for MTaz90-123 allele).
3. (The first panel as an example from now) The marker combination of particular trait measured, and the mean value of total individuals tested are indicated at the top-left of each panel (GU99 *waxy* + *awn*, 189.28). Reft-side column (for *waxy*) and upper-row (for *awn*) are showed the each genotype mean and breeding values (B.V. ; under the assumption that second parent, MTH6860756, always contains the favorable alleles - *Wx, Lk2, N*, and ABC170ca) with R^2 value and *F*-test result (**, *, +, and ns for $p \leq$ 0.01, 0.05, 0.1, and non-significant, respectively). The means of four subgroups (recombinant types of two marker loci) are given at the bottom-right region (7.22, 6.41, 5.23 and 4.65). To estimate the phenotypic effect of one marker (*awn*) within each genotype (*wx* and *Wx* subgroup) of the other marker (*waxy*), individuals were divided into two subgroup depend on the genotype, and SMQ was conducted in each subgroup for awn (R^2 %, 11.4** and 11.6** within *wx* and *Wx* subgroup, respectively). Same procedures for *waxy* in each *awn* subgroup (R^2 %, 48.9** and 50.1** for lk_2 and Lk_2, respectively).

Therefore it may be concluded that the lk_2 locus and the *n* locus are both real QTLs for β-D-glucan content in the 'Fiber (merged)' population.

MLG showed that the *n* locus is likely a real QTL for heading date (R^2, 6.7%). The lk_2 locus showed significance in the HD99(R2 3.2% +) dataset due to the linkage with *n* (Table 17; HD99 170 + hull and HD99 170 + awn).

MLG on HD99 and PH99 of the combination of *lk2* locus and *wx* locus (Table 17; HD99 awn + waxy, PH99 awn + waxy) provided strange results. The lk_2 locus was a significant genetic source influencing the both heading date (R^2 13.5%**) and plant height (R^2, 11.1%**) within *Wx* sub-population, but not within the *wx* population (in this case, *n* locus was likely to be linked to lk_2 locus; data not shown). It appears as though the homogeneous waxy mutant (*wxwx*) genotype tends to suppress the effect of lk_2lk_2 genotye on plant height and heading date. Panel B and C of Figure 11 ('EPISTAT' result of HD99 awn + waxy), however, suggest that another factor may be involved. The cumulative distributions of heading date display that each one sub-population could be divided into two groups (lower part with no clear gap and big gap at the upper part between each genotype combination).

This situation was also detected using the MLG approach on HD99 of the combination of ABC170 and *wx* locus (Table 17; HD99 170 + waxy). The linear model failed to predict the results within the *WxWx* genotype sub-population. In other cases, the *wxwx* genotype sub-populations failed to fit the linear model (Table 17).

Discussion

Poor performance of hulless barley lines has been reported by many barley breeders (chapter 1). Generally, many reports did not consider the yield reductions contributed by the weight of hulls (9 - 17 % in the 'Fiber-2' population) of hulless lines. The estimation of hull weight in the 'Fiber-2' population, and further analysis with 'weighted' data of kernel weight and grain yield suggested that grain traits of hulless barley lines have been underestimated, simply due to the excluded hulls. We could not find any evidence supporting the hypothesis that the two mutant genes (lk_2 and n) interfere meaningfully with quantitative traits in the 'Fiber-2' population (Murphy and Witcombe ,1981, 1986b). Furthermore, it may be also possible to infer that 'poor-performance' derived from the breakdown of favorable gene interactions of Western elite lines during crossing, and it could be remedied with routine breeding procedures. In real breeding programs, only QTLs with new, desirable alleles from the low yield parent would be useful for further yield improvement (Larson *et al.*, 1996). The finding of grain yield and heading date QTLs located on chromosome 7 with repulsion phase supports this idea.

Some attempts to increase QTL detection power have been reported (Goffinet and Mangin, 1998; Mangin *et al.*, 1999), but there are still limitations our ability to detect two closely linked QTLs. Lark *et al.* (1995) proposed that surveying interactions across entire genomes with molecular markers could be an approach to differentiate two closely linked QTLs. The 'Fiber-2' population has 241 markers, but the small population size (59) was not

big enough to generate reasonable sizes of recombinant types between two loci, and 'Fiber (merged)' population was not evaluated with all markers used in developing the 'Fiber-2' linkage map. By comparing the main effect of one locus under each genotype specific sub-population gave us the power to differentiate two linked QTL, *n* and lk_2 with 3 markers. This approach did not demand the generation of huge interaction variance to detect interactions, and gave us a clearer interpretation in surveying possible interactions between major QTLs of the 'Fiber-2' population.

We tried to find some evidence of heading date and plant height may be also involved in determining the β-D-Glucan content as an additional genetic sources (short plant height and early heading date for the normal endosperm lines), Table 18 showed a totally unexpected result (tall plant height and late heading date are more favorable).

Table 18. Phenotypic correlation coefficients among three traits in the 'Fiber (merged)' population based on means of two inferred waxy genotypes.

Waxy (*wxwx*) - 75 lines					Nonwaxy (*WxWx*) - 61 lines			
Mean [a]	Trials			B.V [b]	Trials			Mean [a]
6.8	GU99			-1:9	GU99			4.9
189.7	HD99	-0.083		-0.7	HD99	0.251		189.0
82.3	PH99	-0.074	0.770**	0.9	PH99	0.304*	0.753**	83.2
		GU99	HD99			GU99	HD99	

[a] β-D-glucan content (GU, %), Heading date (HD), and Plant height (PH) with the trail years.
[b] Breeding value (B.V) = (Mean of normal genotype lines - Mean of mutant genotype lines)
*, ** are levels of significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Borém *et al.* (1999) reported that the alleles from Morex resulted in larger starch granules and the huge heading date and plant height QTL on chromosome 2 was also

identified as a major starch granule QTL. Han *et al.* (1995) reported one β-D-Glucan QTL locating on the same region (chapter 3). Ironically, the high β-D-Glucan QTL was contributed by Morex, a malting barley.

Correlation coefficient data from the 'Fiber (merged)' population may suggest that β-D-Glucan, in the normal endosperm barleys, should be considered as another storage component during grain filling period, in agreement with Tinker *et al.* (1996).

Waxy lines did not display any significant correlation between β-D-Glucan content and heading and plant height in 99' trial (Table 18). This result indicate that there might be some genetic factors modifying heading and plant height QTLs within waxy mutant background. During MLG analysis we found some evidence suggesting that the *waxy* mutant gene may influence both heading date and plant height in an epistatic. Significant negative correlations between β-D-Glucan content and plant height in the 1996 and 1999 trials, and positive correlation of high β-D-Glucan content and late heading date suggest that there might be complex interactions among *wx* and other genetic factors depending on the environment.

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APPENDIX

Table A-1. Segregation, χ^2 goodness-of-fit analysis and genotypes of 241 loci of 59 mapping lines in the 'Fiber-2' population.

Marker name	Ch[a]	Segregation test [b]				Lines of the 'Fiber-2' population (A; MTaz90-123, C; MTH6860756, -; Missing data)					
		A	C	To	χ^2	1 - 10	11 - 20	21 - 30	31 - 40	41 - 50	51 - 59
lk2	1	23	28	51	0.49	CCCAACAC-A	CAAACCAAAC	AACC-CAA-C	ACAC--ACCA	AAACCCACC-	C-ACCC-CC
n	1	22	28	50	0.72	CCCAACAC-A	CAACCCAAAC	AAC-ACAC-C	-CACCACCCA	ACACACAC--	C-AAC-CC-
wx	1	31	20	51	2.37	A--ACA-A-A	AAAAAAAAAA	ACCCACCAAA	-CACCACACC	AC-AAC-CCA	-CAAAACCC
Hor1	5	36	23	59	2.86	CAAACACCCA	ACAAACAACA	AAAAACCAAC	AAACCCAAAA	CAAACCAAAC	ACCCACAAC
Hor2	5	37	22	59	3.81	CAAACACCCA	ACAAACAACA	AAAAACCAAC	AAACCCAAAA	CAAACCAAAC	ACCCAAAAC
ABC155	7	28	31	59	0.15	ACCCAACCCC	CCACCAACAC	AAAAACCCCC	CAACCACCCC	AAAAACAACA	ACCACCAAA
ABC156.1	5	38	20	58	5.59 *	-AAACACACA	ACAAACAACA	AAAAACAAAC	AAACCCAAAA	CAAACCAACC	ACCCACAAA
ABC170	2	30	23	53	0.92	CAACAACC-C	CA-AAAAACA	CCACAAC-AA	A--ACCC-CC	AAAACAAACC	CACAACACA
ABC253	1	32	27	59	0.42	CCCAAACCCA	CACCCCAAAA	AACCAAAACA	CCAAAACCAA	AACCCCACCA	CACCAAAAA
ABC303	4	31	26	57	0.44	-CACCAAC-C	ACCAAACACC	CCAAAAAAAC	ACCAAAAAAA	CCCCCAACCA	CAACCAACA
ABC483	7	29	25	54	0.30	A-C-CACA-A	-CCAACCAAA	ACCC-CCCAA	CAACCAAACC	ACCAAACAAA	ACAAAACCC
ABG20.11a	1	26	33	59	0.83	CCCAACACCA	CAAACCAAAC	AACCCCAACC	CCACCAACCA	AAACCCACAC	CAAACCCCC
ABG20.11b		31	28	59	0.15	ACCACACAAC	AAAACACCAA	ACCCACCCAA	AAACCACACC	ACCACAACAA	CCAAAACCC
ABG054	4	28	29	57	0.02	ACAA-CCC-C	ACCCACCACC	AAAAACCACA	AACACAAAAA	CCCCACCACA	ACCACACAC
ABG55a	5	33	26	59	0.83	ACACCACACC	CCAAAAAAAC	AACCAAACAA	ACCCCCAAAA	ACACAAACCA	AAAACCCCC
ABG55b	5	34	25	59	1.37	ACACAACACC	CCAAAAAAAA	AACCAAACAA	ACCCCCAAAA	ACACAAACCC	AAAACCCCC
ABG058	2	34	17	51	5.67 *	---A-CAC-A	ACAAAACAAC	AAAAAAACAA	ACAAC-AACC	ACCC-ACAAC	CAAAACA-A
ABG064	7	27	30	57	0.16	--CCCCCAAC	ACCACCCAAA	ACCCACCCAA	CAACCAAAAC	ACCAAACCAA	ACCAAACCC
ABG070	3	33	22	55	2.20	-ACC-CAAAC	AAACCCACCA	AACACAAACA	CCCAAAAA-A	CAAA-CCCAC	ACAAAAAAC
ABG380	1	30	29	59	0.02	ACCAAAAACA	ACCCCCAAAA	AACCACAACC	CCCCCCACCA	ACCAACCCAA	CAAACACAA
ABG452	5	23	33	56	1.79	CAAA-ACC-A	CCACCAAACA	CCAAACCCCC	CCACCAACCA	CACA-CCCCC	CACACACCA
ABG609	2	23	31	54	1.19	-AAA-AAC-A	ACCCACCCCC	AACCAACCCC	CACACCAACC	CCAA-CCCA-	CACACAACC
ABG618	4	27	29	56	0.07	ACAACCCCCC	ACCCAACACC	CAA-CAACAC	AACACAAAAA	CCCC-CAACA	A-CAAACCC
BCD402	4	25	32	57	0.86	-CACCCCCAA	CCCACCCAA-	CAACACCCCC	CCACAACAAA	AAAAAACCCC	CCAACCACA
BTA02a	4	29	30	59	0.02	CACACCCACA	CCAACCAACA	CCCCCCAACC	CAAAAACACC	AAACAAACAC	CCAAACACA
BTA02b	4	32	27	59	0.42	CCACCCACAA	CCAACCAACA	CACCACAACC	CAAAAACAAA	AAAAAACCAC	CCAACCACA

Table A-1. (continued)

Marker name	Ch[a]	Segregation test [b]				Lines of the 'Fiber-2' population (A; MTaz90-123, C; MTH6860756, -; Missing data)					
		A	C	To	χ^2	1 - 10	11 - 20	21 - 30	31 - 40	41 - 50	51 - 59
cMWG694	2	28	25	53	0.17	--CCCAC--C	CAAACACCAA	ACAAACCAAA	CAAA-CACCA	CCAAA-CACA	CCAACACCA
MWG058	4	30	28	58	0.07	ACAACAA-AC	AACAACCACC	CCAACACACC	ACCAAAAAAA	CCCCCCACCA	CACACAAAC
MWG938a	5	34	25	59	1.37	CCAAAACCCA	AAAACCCACA	AACAAACAAC	CAACACAACA	CCCACAAAAC	CAACAACAC
MWG938b	5	24	34	58	1.72	CCCCCCCACC	ACACCACCCC	CCAAAACACC	CACCACAACC	AACCCCCAA-	CCAAAAAAA
MWG549	3	27	32	59	0.42	ACAACACACC	CACCACCCCC	AACAACAAAA	ACCAACCCCA	CCCCACCCCC	AAAAAAACC
MWG620	6	29	29	58	0.00	CACCAACACC	CACAAAACAA	CAAAAAAAAC	AACCCCCCAC	CCCCCCAAAA	CCCCAC-AA
MWG2249	7	30	26	56	0.29	--ACAACAAC	CAAAACAAAC	ACCCAACCCA	CAACCCCCC-	AAACACAACA	CCCCAAAAA
MWG2261	5	34	23	57	2.12	CAAACACC-C	CCAAACAACA	AAAAACCAAC	AAAAACAACA	CCCA-AAACC	AACCCCAAA
MST109	6	30	29	59	0.02	CACCAACACC	CACAAAACAA	ACAAAAAAAC	ACCCCCCAAC	CCACCAAAAA	CCCCACCCA
WTA1	1	24	35	59	2.05	CCCAACACCA	CAAACCAAAA	CACCACACCC	CCACCACCCA	ACACACACCC	CAAACCCCC
HVM3	4	29	28	57	0.02	ACAACAACAC	AACAACCACC	CCAACACACC	ACAAA-AACA	CC-CCAACCA	CCCACAAAC
M-HVWAXY	1	35	23	58	2.48	AAAACACAAA	AAAAAAAAAA	ACCCACCAAA	ACACCACACC	ACCAACCCCA	ACCAAACC-
M-HVPRP1B	1	29	29	58	0.00	CCCCAACCCA	CAACAAACAA	ACACACCCCC	CAAAAACCAA	ACCAAAACCC	CCC-CAAAA
HVM6	1	34	22	56	2.57	ACAACACACA	AAAAAAAAAA	ACCCACCAAA	ACACCACACC	AC-AACCCCA	ACCAAAA--
HVM36	2	36	23	59	2.86	AAACAACCAA	CCAAAAAACC	CACAAAACAA	AAAACCAACC	AAAACCAACC	CACACCACA
HVM054	2	27	31	58	0.28	-AACACACAC	CACACCCCCA	AACCCAACCA	CCCAACACCC	CCAAACACAA	CCAAAAACC
HVM060	3	30	28	58	0.07	-CCCAACACA	ACACAAACCC	CCCAACCACC	CAACACCAAC	CAACCCAAAA	CCAAAAAAA
HVM68	4	31	21	52	1.92	ACCA-CACAC	AACAAACA--	CCAACACACA	A-CAAAAAAA	CCCC-CAC-A	AAACAACA-
M-HVCMAa	1	27	32	59	0.42	CCCAAAACCA	ACAACCAAAC	CAACCCAAAC	ACACCCCCCA	ACACACACAC	CCCACACCA
M-HVCMAb	1	28	31	59	0.15	CCCAAAACCA	AAAACCAAAC	CAACCCAAAC	ACACCCCCCA	ACACACACAC	CCCACACCA
M-HVDHN9a	7	25	32	57	0.86	CCCCCCCC-C	ACCACACCAC	AACCACCACC	CAAAACCCCC	AA-ACAACAC	AAAAACACA
M-HVDHN9b	7	31	28	59	0.15	AAAACCAACA	CAACACAACA	CCAACAACAA	ACCCCAAAAA	CCACACCACA	CCCCCACAC
M-HVCSG	2	23	35	58	2.48	-AAAACACAA	CCCCCCCCCA	CACCAACCCC	CACACCCACC	CCAAACCCAC	CACAAAACC
HVGNIRE	6	26	32	58	0.62	CACCAACA-C	CACAAAACAC	ACCACAAAAC	AACACCCCCC	CCACCCAACA	ACCCACCAC
FL0101A	6	40	19	59	7.47 *	AAAACAAAAC	AAAACAAAAC	CAAAAAAAAA	CAAAAAAACC	CAACCCAACA	AACCCCCCA
FL0102A	6	21	38	59	4.90 *	CAACCACCAC	CACACCACCC	ACCAACACAC	CCCCCCCACC	CAACCCAACA	AACCCCCCA

Table A-1. (continued)

Marker name	Ch[a]	Segregation test [b]				Lines of the 'Fiber-2' population (A; MTaz90-123, C; MTH6860756, -; Missing data)					
		A	C	To	χ^2	1 - 10	11 - 20	21 - 30	31 - 40	41 - 50	51 - 59
FL0103B		31	28	59	0.15	ACAACAACCC	AAACCCAAAC	AAAAAAAAAA	CCACAACCCC	CAACCCCACC	AACCACACC
FL0104B		40	16	56	10.29 **	CAAA-AAAAA	AAACCAAAA-	AAAAAAAAAA	ACCAAAAACC	CAAACCAACA	AA-CCCCCA
FL0105B		21	35	56	3.50	ACCC-CAACC	AACACCCCA-	AAACACCACC	CCAAACCAAC	CAACCCCACC	CA-CCCCCC
FL0106A		36	22	58	3.38	ACCA-AAAAA	AAACCCCAAC	CAAAACAAAA	ACCAAACAAC	AAACCCAACA	ACACCACCA
FL0107B		27	32	59	0.42	AAAACCACCA	AAAAACACAC	AACACCCACC	CACCACCCAC	ACACACCACC	ACCCCCAAC
FL0201B		40	19	59	7.47 *	AAACAACCAC	CAACAACCCA	ACAAAAACCA	CACAAAAAAC	CACAAAAAAA	AAACAAAAC
FL0202S	7	24	35	59	2.05	ACACAACCCC	CAAAACACAC	ACCCCACCCA	CCACCCCCAC	ACACACCCCA	CCCCAAAAA
FL0203S	1	34	25	59	1.37	ACCACAAAAC	CACCCAAAAA	AACCAAAACC	CACACAACAA	AAACCCCCAA	CACCACAAA
FL0204S		37	22	59	3.81	ACAACAACAA	CACAAACAAA	AACCCAAAAA	AACAAACAAA	AAAACCCCAC	ACACCCCCA
FL0205B	2	37	21	58	4.41 *	AAACAACCCA	CAAAAAAACC	CAAAA-CAAA	AACACCAACC	AAAACCAACA	CACAACACA
FL0206B	5	23	33	56	1.79	CACAAACCCC	ACCCAAAACA	ACCAA-ACCC	CCCCC-AACC	CCCAAC-ACC	CACAAACCC
FL0207S	1	34	23	57	2.12	AAAACACAAA	AACAAAAAAA	ACCCA-CCAA	ACACC-CACC	ACCAAAACAA	CCCAAACCC
FL0301S	6	20	39	59	6.12 *	CCAACACACC	CCCCCCCCCC	CCCCCCAAAA	ACACAACCAC	CCACCCAACC	ACACCCCAA
FL0302B		30	29	59	0.02	AAACACCACA	ACAAACCCCC	CCAAAACCAC	AACCAAAACC	CCAAAAAACC	ACAACCCCC
FL0303A	2	26	33	59	0.83	AAACACAAAC	CACCCCCCCA	AACCCAACCA	CCCAACCCCC	CCAAACACAA	CCCAAAACC
FL0304S	1	27	32	59	0.42	ACCAAAAACA	ACCCCCAAAA	CCCCAAAAAC	CCCCCCACCA	ACCAACCCAA	CCCACCCAA
FL0305A	3	29	30	59	0.02	ACCAACCACC	AACCACCCCC	CCCAACAAAA	ACCAAACACA	CCACCCCACC	AAAAAAACA
FL0306A	3	28	31	59	0.15	CCAAAACACC	CACAAACCAC	ACCACCAACA	ACCAACCCCA	CCCCCCCACC	AAAAAAACA
FL0307B	6	31	28	59	0.15	CAACCCCACA	AAACAACCAC	CACAACACCA	CAAAAACACC	CAACAACAAC	ACACACCCA
FL0308S	1	23	36	59	2.86	AAACCCCAAC	CCCCACCCCA	CCCCACCCAA	ACACCACACC	ACCAAACCAA	CCCAAACCC
FL0309A		28	28	56	0.00	AAAACCC-AA	CCAAACAACA	CCACC-CCAA	ACCACCAAAA	CCCCAA-ACC	CACCAACAC
FL0310A	4	29	28	57	0.02	CAAACCCCAA	AACCAAACAC	ACCAC-AACC	ACCACACCAC	CAAAAC-CCA	AAACCAACC
FL0311S	3	31	28	59	0.15	ACAAAACACC	CACCACCCAC	AACAAAACAA	ACCAACCCCA	CCCCACAACC	AAAAAAACC
FL0312S	7	24	34	58	1.72	ACACAACACC	CAAAACACAC	CCCCCCCCCA	CAACCCCCCC	ACACAC-ACA	CCCCAAAAA
FL0401A	5	41	18	59	8.97 **	ACAAAACACA	AAAAACCAAA	AACAAACAAC	CAAACAAAAA	CCAACCAAAC	AAACAACAC
FL0402S	6	25	34	59	1.37	CACCAACACC	CACAAAACAC	ACAAAACAAC	ACCCCCCCAC	CCACCACCAA	CCCCACCCA

Table A-1. (continued)

Marker name	Ch[a]	Segregation test [b]				Lines of the 'Fiber-2' population (A; MTaz90-123, C; MTH6860756, -; Missing data)					
		A	C	To	χ^2	1 - 10	11 - 20	21 - 30	31 - 40	41 - 50	51 - 59
FL0403S	4	33	26	59	0.83	AAAACCCAAA	ACCCAACCCC	CCCACAAAAC	CAAAAAAAAA	CAACCCCCCA	AAAACAACC
FL0404S	2	39	20	59	6.12 *	CAACACAAAA	AAACAACCAA	AACAAACCAC	CACAAACAAC	CAAAAAACAA	CACAACAAC
FL0405S	7	27	32	59	0.42	CCACCCACAC	ACCACACAAC	AACCACCAAC	AAAAACCCCC	ACAACACCAC	CACAACCCA
FL0406A	7	33	25	58	1.10	ACCACACAAA	ACAAAACAAA	ACCCCACCAA	CAACCCCAAC	AACAAA-AAA	CCCAAACCC
FL0407A	7	32	26	58	0.62	ACAACACAAA	ACCAAACAAA	ACCCCACCAA	CAACCCCAAC	ACCAAA-AAA	CCCAAACCC
FL0408B	6	30	27	57	0.16	CAAAAACACC	AAAAAAACAC	ACCACAACAA	AACCC-ACCC	CCCCCC-ACA	CAACAACAC
FL0409A	4	27	30	57	0.16	ACACCCACAA	CCCCCCAACA	CACCAACACC	CAACA-CAAA	AACAAA-CAC	ACCCCCACA
FL0410S	3	29	30	59	0.02	ACACAACACA	ACACACACCC	CCCAAAAACC	CACCACCAAC	CACCCCAACA	CCCAAAAAA
FL0411S	4	34	25	59	1.37	CCCCCCCAAA	CAAACAACCA	ACACAACAAC	CAAACCAACA	AAAACACAAA	ACAAACACC
FL0412A	6	27	31	58	0.28	CACCAAACCC	CACAAAACAC	ACAAACAAAC	AACCCCACCC	CCACCC-ACA	ACACACCAC
FL0413A	1	32	26	58	0.62	CCCAACACAA	CACACCAAAC	AAACCAAAAC	CCACCCACAA	ACAAAC-AAA	CCACCCAAA
FL0414S	5	28	31	59	0.15	CCCACCCCCA	CCACACAACC	CAAAACCAAC	ACCCACCACC	AACACCAAAA	ACAAACAAC
FL0415S	5	24	34	58	1.72	CACAAACACC	ACCCACAACA	CCCAACAACC	CCCCCAAACC	CCCAAC-ACC	CACAAACCC
FL0416S	5	25	33	58	1.10	CACAAACACC	ACCCAAAACA	CCCAACAACC	CCCCCAAACC	CCCAAC-ACC	CACAAACCC
FL0501S	6	31	28	59	0.15	AAACCACACC	CCCAACAAAC	CCACCAAAAA	AAACAACCAA	AAACCCAACC	ACCCCACCA
FL0502B	3	32	27	59	0.42	ACAAAACACC	CAACAAACAA	ACCACAAAAA	ACCAACCCCA	CCACCCCACC	AAAAAACCC
FL0503A	2	30	29	59	0.02	ACCCAACAAC	CACACCCCAA	ACAACAACCA	CACCAAACCA	ACAACCAACA	CACACCACA
FL0504S	1	32	27	59	0.42	CCCAAAACCA	ACCCCCAAAA	CACCAAAAAC	ACACCAACAA	ACACACACCA	CACACACAA
FL0505B		29	30	59	0.02	CAAAACCACA	CAAAAAAACC	CCACACCCAC	ACCCCCAAAA	CCCACCAACC	CACAAAACC
FL0506A	7	30	29	59	0.02	ACCCAACCCA	CCACCACCAA	ACCAAAAAAC	AAAAACACCC	AACACCAACA	CCCAACCCA
FL0507B		35	24	59	2.05	CACAACCCAA	ACCACAAAAA	CACAACCAAA	AAAACCAACA	CACAAACCCC	AACAAAACC
FL0508A	1	30	27	57	0.16	CCCACAAAAC	CACCCCAAAA	AACCA-AACA	CACACACCAA	AACCCC-CAA	CACCACAAA
FL0601B	5	43	16	59	12.36 **	ACCAAACACC	AACAACCAAA	AACAAAAACC	CAAAAAAAAA	CAAACAAAAC	AAAAAACAA
FL0602A	3	31	28	59	0.15	CACCAACCAC	ACCCCCACCA	AACAAACACA	CCCACAACCA	AAAAAACACC	CAAAAACAC
FL0603S	2	26	33	59	0.83	ACCCAACAAC	CACACCCCAA	ACCAAACCCA	CACCACACCC	CCAACCAACA	CACACCACA
FL0604B		17	42	59	10.59 **	CCACCCACCC	CCCCCACAAA	CCCCCCACCC	CCCCCCAAAC	CCCCACCAAC	CAACAACCC

Table A-1. (continued)

Marker name	Ch[a]	Segregation test [b]					Lines of the 'Fiber-2' population (A; MTaz90-123, C; MTH6860756, -; Missing data)					
		A	C	To	χ^2		1 - 10	11 - 20	21 - 30	31 - 40	41 - 50	51 - 59
FL0605A	4	34	24	58	1.72		CCCACCCCAA	CAAACAACAC	ACACC-AAAA	CAAAACCACA	ACAAACAAAC	ACACAAACA
FL0606A	5	32	26	58	0.62		CCCACCACCA	CCACACAAA-	CAAAAACAAC	ACCCACCACC	AAAACCAAAA	ACAAACAAC
FL0607B	2	26	32	58	0.62		CCCCACACAA	AAACCACCC-	ACCAACCCCC	CCCACCAACC	CAAAAACCAA	CACAAAACC
FL0608A	2	24	33	57	1.42		ACACAAAAAC	CAAAACACA-	ACCCC-CCCC	CACACCACCC	CCAACCACCC	CACAACACC
FL0609S	4	30	28	58	0.07		ACAACAACAC	AACAACCAC-	CCAACACAAC	ACCAAAAACA	CCCCCAACCA	CCCACAAAC
FL0701S	4	29	29	58	0.00		ACCACCCACC	CCCAACCACC	CAAAACCCAA	AAAACCACCA	CCAAACAC-A	ACACACAAA
FL0702A	2	28	30	58	0.07		ACAACCAAAC	CACAACCACC	CACCCAAAAC	CCAAAACCAC	CCAAACAC-A	CACCACCAC
FL0801S	1	27	32	59	0.42		ACCAAACCCC	CACCCAACAA	AACCACAACA	CCACCACCAA	AACCCCACCA	CACCCAAAC
FL0802S	5	25	34	59	1.37		CCCCACCACC	ACAAACACCC	CCAAAACCCC	CCCCCAAACC	CACCAAAAAA	CCCAAAACC
FL0803S	7	39	18	57	7.74	*	ACAAAACAAA	ACAAAACAAA	ACCCAACCAA	AAAACACACC	ACCAAA-AAA	CACAAA-CA
FL0804B		30	27	57	0.16		CCAACACACC	CACCAACCAC	ACCACAACAA	AAACCAAACC	CCCAAC-ACC	CAAAAA-AA
FL0805A	5	43	14	57	14.75	**	AAAACAACAA	ACAAACAACA	AAAAACCAAC	AAACCAAAAA	AAAACA-AAC	AAACAC-AA
FL0806S	3	26	31	57	0.44		ACAAAACACC	CACCACCCCC	AACAACACAA	ACCAACCCCA	CCCCAC-ACC	AAAACA-CC
FL0901B		41	17	58	9.93	**	CAAAAAAAAC	CAAACCAAAA	AAAAAAAACA	AAACCAACCA	-AACCAAAAA	CACACCACA
FL0902B	5	41	17	58	9.93	**	ACAAAAAACC	AACAACCAAA	AACCAAAACC	AAAAAAAAAA	-CAACCAAAC	AAACAACAC
FL0903B		37	21	58	4.41	*	AAAAAAACCC	CCAACCCCAA	ACCAAAAACA	AAAAAAAACA	-AAACAAACC	CCAAAACCC
FL0904S	1	39	20	59	6.12	*	ACAAAAAAAA	ACCCACAAAA	ACCCAAAAAC	CCACCAACCA	ACAAACAAAA	AACACACAA
FL0905B		35	23	58	2.48		ACCAAACCAA	AAAACAACAC	CAACCCCAAA	CCCACAAAAA	-ACACCCCAA	CCAAAAAAA
FL0906S	2	31	27	58	0.28		ACCAACCACC	CAAACACCAA	CCAAAACAAA	CAAACCACCA	-CAACAAACA	CCCACACCA
FL0907B		30	28	58	0.07		CAACAAAACC	AAAACCCACA	ACCAAAACAA	ACACCACCCA	-AACCCAAAA	CCCCCCACC
FL0908S	2	39	19	58	6.90	**	ACAAAAAACC	CAAACAACAA	ACAAAAAAAA	CAAAAAACCA	-CAACAAACA	CCCACACCA
FL0909A	1	37	21	58	4.41	*	ACAACAAAAC	CACCCAAAAA	AACCAAAACC	CAAACAACAA	-AACCCAAAA	CACCACAAA
FL0910B	1	33	25	58	1.10		CCAACAACAC	CCCCCAAAAA	AACCAAAACC	CAAACAACAA	-ACCCCAAAA	CACCACAAA
FL1001B		33	26	59	0.83		CAACACCAAA	AACACCACAA	CCACCCAACC	CACAAAAAAC	CAAACAACCA	CCCAACAAA
FL1002B		30	29	59	0.02		CACCAAAACC	CCCAAACCCA	ACCCAAAAAA	AACCCAAAAC	CCAAACAACA	AACCCCCCC
FL1003S	1	28	31	59	0.15		CCCAACACCA	CAACCCAAAC	CCCCACACAC	ACACAACCCA	AAACACAACA	CCCACCAAA

Table A-1. (continued)

Marker name	Ch[a]	Segregation test [b]					Lines of the 'Fiber-2' population (A; MTaz90-123, C; MTH6860756, -; Missing data)					
		A	C	To	χ^2		1 - 10	11 - 20	21 - 30	31 - 40	41 - 50	51 - 59
FL1004S	1	27	32	59	0.42		CCCAACAACA	CAACCCAAAC	AACCACACAC	ACACCACCAA	AAACCAACCA	CCCACCCCC
FL1005A	7	35	24	59	2.05		ACAACACAAA	ACAAAACAAA	ACCCACCCAA	AAACCCCACC	ACCAAAAAAA	CCCAAACCA
FL1006B	7	33	26	59	0.83		AACCCACCAA	CCACCCACAA	CAAAACAAAA	AAAAACACCC	AAAACAAACA	CCCCACCCA
FL1007B		39	20	59	6.12	*	CAACCAAACC	AAACAACCCA	ACAAAAACCA	CACCAACAAA	AACAAACAAA	AAAAAACCA
FL1008B		38	21	59	4.90	*	AACAAAAACC	ACCAACACCA	ACACCAACCA	AAAAAAAAAC	AACAAACCAC	CCAAACAAA
FL1009B	1	32	27	59	0.42		CCCAAAAACA	ACCCCCAAAA	CACCAAAAAC	ACAACCACCA	ACCCACACCA	CACACAAAA
FL1010B	3	32	27	59	0.42		ACCAACCACA	AACCCCCCCC	CCCAAAAAAA	ACCCACCCAA	AAAAACAACC	AAAAAAACC
FL1101S	6	36	23	59	2.86		ACCCCACACC	CAAAAAAAAC	ACAACCAAAA	AAACAAACAA	AAACCAAACC	ACACCCCAA
FL1102B	3	23	36	59	2.86		CCCAACCACC	AACCCCCCCC	CCCAACAACA	ACCAACCCCA	CCCCACACCC	AAAAAAACC
FL1103A		27	32	59	0.42		ACAAACAACC	AACAAACACC	ACCCCACCCC	CCCCCCCAAA	CCACACACAC	AAAACAACC
FL1104S	2	28	31	59	0.15		CACAAACCAA	CAAACACCCA	CCCCAAACAA	CAACACCCCA	CCACCAAACA	CACACCCCA
FL1105S	1	22	37	59	3.81		CCCAACACCA	CAACCCAAAC	AACCACACAC	ACACCACCCA	ACACACCCCC	CCCACCCCC
FL1106S	2	34	25	59	1.37		CAAAACCCCA	CAAAAAACCC	CCACAACAAA	AACACCACAA	AACACAAACC	CACAACACA
FL1107S	2	32	27	59	0.42		CAAAACCCCA	CAAAAAACCC	CCACAACCAA	ACCACCACAA	AACACAAACC	CACAACACA
FL1108B	2	28	31	59	0.15		CCAAACACAA	ACACCACCCA	CACAAACCCC	CCAACCAACC	CCAAAACCAC	CACAAAACC
FL1109S	1	26	33	59	0.83		CCCAAACCCC	CACCCAACAA	AACCACAACA	CCAACACCAA	AACCCCCCCA	CACCCAAAC
FL1110S	7	20	39	59	6.12	*	CCCCCACCAC	ACCCCACCAC	CACCACAACC	CAACCCCCCC	ACAACCCCAC	ACAACCACA
FL1111S	5	26	33	59	0.83		ACCCCCACCA	CCACAAAACC	AACCAAACAA	ACCACCCACA	ACCCACACCC	AAACCCCAC
FL1112S	3	34	25	59	1.37		CACCAAAAAC	ACACCCACCA	AACAAAAACA	CCCAAAACAA	CCAAACCCAC	CCAAAAAAC
FL1201S		22	35	57	2.96		ACACAACACC	CACCC-ACCA	CCCCACCACC	C-CCACCCAA	CCAACAACCA	ACACCACAC
FL1202B	1	31	26	57	0.44		ACCAAAAACA	ACCCA-AAAA	ACCCACAAAC	C-ACCCACCA	ACCAACCCAA	CACACACAA
FL1203B		28	29	57	0.02		CAACCCCCAC	CAAAC-CCCC	CAAACACCCA	A-AAAACAAC	CAACCAAACC	ACACAAACC
FL1204S	1	29	28	57	0.02		ACCAAAAAAA	ACCCC-AAAA	CCCCACAAAC	C-CCCCACCA	ACCAACACAA	CACACCCAA
FL1205S	1	33	24	57	1.42		ACCAAAAAAA	ACCCA-AAAA	ACCCACAAAC	C-CCACACCA	ACCAACACAA	CACACACAA
FL1206A	5	34	23	57	2.12		CAAACCCCCA	CCACA-ACCC	CAAAAACAAC	A-ACACAACC	AAAAACCAAA	ACAAAAAAC
FL1207B		33	23	56	1.79		CAACAACAAC	ACCAA-ACCA	CCACAACACA	C-CCACAAAC	CCAACA-AAA	CAACAAAAA

Table A-1. (continued)

Marker name	Ch[a]	Segregation test [b]					Lines of the 'Fiber-2' population (A; MTaz90-123, C; MTH6860756, -; Missing data)					
		A	C	To	χ^2		1 - 10	11 - 20	21 - 30	31 - 40	41 - 50	51 - 59
FL1208A	5	25	32	57	0.86		CACAAACCCC	ACACA-ACCA	CCCAAAACCC	C-CACAAACC	CCAAACCACC	CACAAACCC
FL1209S	4	28	29	57	0.02		ACAACAACAC	ACCAA-CCCA	CCACCCAAAC	C-CACAAACA	ACAACAACCA	CACCCAACC
FL1301S		31	28	59	0.15		CCCAAAACAC	ACACACCACC	ACCAAAACAA	AACCCCAACA	AACCACCAAA	CACACACCA
FL1302A	1	27	32	59	0.42		CCCACAACCA	ACCCCCAAAA	CACCCAAACC	ACACCCACCA	ACAAACACCA	CCCACACAA
FL1303S	6	26	33	59	0.83		CACCAACACC	CACAAAACAC	ACAACAAAAC	AACCCCACCC	CCAACCCACC	CCACACCAC
FL1304S	6	29	30	59	0.02		CACCAACACC	CACAAAACAC	ACAACACAAC	AACCCCACCC	CCAAAAAACA	CCACACCAC
FL1305S	1	29	28	57	0.02		CCCAAAACCA	ACCCCCAAAA	AACCCAAACC	ACACCCACCA	ACAAAC-CCA	CACA-ACAA
FL1306B	1	31	27	58	0.28		CCCAAAACCA	ACCACCAAAC	AAACCAAACC	ACACCCACCA	ACAAACACAA	CACA-ACCA
FL1307B		23	34	57	2.12		CCACACACCC	CCCACCCAAC	CCCCCAAACC	ACACCCACCA	ACAAAC-CAA	CACA-ACCA
FL1308S	1	32	26	58	0.62		AAAACACAAA	AACAAAAAAA	CCCCACCCAA	AAACCACACC	ACCCAACCAA	CCCA-ACCC
FL1309B	1	26	30	56	0.29		CCCCAACCCA	CAACAAACAA	CCAC-ACCCC	CAAAAACCAA	AACCAA-CCC	CCCC-AAAC
FL1310S	4	29	28	57	0.02		ACAACCCACC	CCCCACCACC	CAAAACCAAA	AACACAACCA	CCAAAC-ACA	ACAC-CAAA
FL1311S	4	29	28	57	0.02		ACAACCCACC	CCCCACCACC	CAAAACCAAA	AACACAACCA	CCAAAC-ACA	ACAC-CAAA
FL1312A	6	28	29	57	0.02		CACAAACACC	AACCAAACAC	ACCACAACAA	AACCCCACCC	CCAACC-ACA	CAAC-ACAC
FL1401S	7	25	33	58	1.10		ACCCCACCCC	ACCCCACCAC	AACAACAACC	CAACACCCCC	AAAACC-CAA	ACAACCACA
FL1402B		32	26	58	0.62		CCACCCAAAA	ACAAACCACA	ACAACACCAC	AAAAAAAACC	CCCACC-CAC	AAAACCACA
FL1403B		25	33	58	1.10		CCCAACCCCA	ACCCACCCCA	CCAACACACC	AAACCCAAAC	CCCCAA-CAC	CAAACCAAA
FL1404S	2	39	19	58	6.90	**	CACACCACAA	ACAAAACAAC	AAAAAAACAA	AAAACAAACC	AACCAC-AAC	CACAAAACA
FL1405S	2	37	21	58	4.41	*	CACACCACAA	ACAAAACAAC	AAAACAACAA	AAAACAAACC	AACCAC-AAC	CACAACACA
FL1406A		37	21	58	4.41	*	CAAACAAAAA	ACCAACCCAA	ACAACCACCA	CAACCAACAA	AACACA-AAC	CAAAAAACA
FL1407B		21	37	58	4.41	*	CCCAAAAAAC	CACACACCAA	CCCCCACCCA	CCACCCCCAC	CCCAAC-AAC	CCCCACACC
FL1408B	2	23	35	58	2.48		CAAAACACCC	ACCCCCCCCA	CCCCAAACCC	CCCACCCAAA	CCCAAC-CAA	CACAAAACC
FL1409B	6	30	28	58	0.07		CAACCCCACA	AAAAAACAAC	CACCCCAACA	CAAAAAAACC	CCCCAA-ACC	AAACCACCC
FL1410B	2	28	30	58	0.07		ACCCAAACAC	CACACCCACA	AACCCAAAAA	CCCAACCCCC	CCAAAC-CAA	CCCAAAACA
FL1411B	7	33	25	58	1.10		ACACCACCCC	CCACCAACAC	ACCAAAAACA	CAACAACCAC	AAAAAC-AAC	AAAACCAAA
FL1412S	3	28	30	58	0.07		ACAAAACACC	CACCACCCCC	AACAACACAA	ACCAACCCCA	CCCCAC-ACC	AAAAAAACC

Table A-1. (continued)

Marker name	Ch[a]	Segregation test [b]				Lines of the 'Fiber-2' population (A; MTaz90-123, C; MTH6860756, -; Missing data)					
		A	C	To	χ^2	1 - 10	11 - 20	21 - 30	31 - 40	41 - 50	51 - 59
FL1413S	3	27	31	58	0.28	CCAAAACACC	CACCACCCCC	AACAACACAA	ACCAACCCCA	CCCCAC-ACC	AAAAAAACC
FL1414S	4	25	33	58	1.10	CCAACAACCC	AACCACCACC	CCAACCCACC	ACCAAAAAAA	CCCCCC-CCA	ACCACAAAC
FL1415S	7	30	28	58	0.07	ACACAAAAAC	CAAAACACAC	ACCCCACACA	CAACCCCCAC	AAAAAC-ACA	CCCCCCAAA
FL1501S	2	36	23	59	2.86	CCAACCACAA	ACAAAACAAC	AAAACAACAA	AAAACAAACC	AACCACCAAC	CCCAACACA
FL1502B		36	23	59	2.86	CAAACAACCC	CCACAACAAA	ACACCCACAC	AAAACCAACA	ACCAAACACA	AAAACAAAA
FL1503A	1	28	31	59	0.15	ACCAACACAA	CCCACCAAAC	AACCCAAAAC	ACAACCACAA	ACAACCACCC	CCCCCCAAC
FL1504B		38	21	59	4.90 *	CAAACAAACC	AACACCAAAC	AACAAAACAC	AAACAACACA	ACAACAAACC	CAACAAACA
FL1505S	1	32	27	59	0.42	AAAACACAAA	ACCAAAAAAA	ACCCACCCAA	ACACCACACC	ACCCAACCAC	CCAAAACCC
FL1506A	1	30	29	59	0.02	CCCCAACCCA	CAACCAACAA	ACACAACCCC	CAAAAACCAA	AACCAACACA	CCCCCAAAA
FL1507A	2	40	19	59	7.47 *	CACACCACAA	ACAAAACAAC	AAAAAAACAA	AAAAAACACC	ACCCACAAAA	CACAAAACA
FL1508S	6	27	32	59	0.42	CCAAAACACC	CACCAAACCC	ACCACAACAA	AACCCCACCC	CCAACCAACA	CCACAACAC
FL1509S	6	39	20	59	6.12 *	AAAAAACAAC	CAAAAAACAC	ACCACAAAAA	AACACCACCA	CCAACCAACA	AAAAAACAC
FL1510B	6	27	32	59	0.42	ACCCCACACC	CACACAAAAC	CCACCCAAAA	AACCAACCAA	AACCCCCACA	ACCCCCCAA
FL1511B	1	32	27	59	0.42	AAAAAAAAAA	ACCCACAAAA	ACCCACAAAC	CCCCCCACCA	ACCCACACAA	CACACCCAA
FL1512B	5	24	35	59	2.05	CACCACCACC	ACACCCACAC	CCAAAACCCC	CCCCCAACCC	CCAAAACACA	CACAAACAC
FL1513A	1	26	33	59	0.83	CCCAAACCCC	CACCCAACAA	AACCACAACA	CCAACACCAA	AACCCCCCCA	CACCCAAAC
FL1514B		30	29	59	0.02	CAACACACAC	ACAACACCCA	AAACACCCAC	CAAACAAAAC	CCCCCACCAA	CCAAAAACC
FL1515A	1	28	31	59	0.15	ACCAACACAA	CCCCCCAAAC	AACCCAAAAC	ACAACCACAA	ACAACCACCA	CCCCCCAAC
FL1601S	4	30	29	59	0.02	AAAACCCCAA	AAACAAACAC	ACCACAAACC	CCCACACCAC	CAAACCCCCA	AAACCAACC
FL1602A	2	32	27	59	0.42	CCCAACACCA	AAACAACCAC	AACAAACCCC	CACACCAACC	CAAAAAACAA	CACAAAACC
FL1603A	2	29	30	59	0.02	CCCAACACAC	AAACCACCCA	AACAAACCCC	CCCACCAACC	CCAAAAACAA	CACAAAACC
FL1604B	3	29	30	59	0.02	AACAACCACC	AACACCAACC	CCCAACAAAA	ACCAACCCCA	CCCCACCACC	AAAAAAACC
FL1605B	5	24	35	59	2.05	CCCCACCACC	ACACACACCC	CCACAACCCC	CCCCCAACCC	CAAAAAAACA	CACAAACAC
FL1606B		33	26	59	0.83	CCCAAAAAAA	CCACACCCAA	AAACACACCA	CAAACAACCA	AAAAACCCAA	ACACCACCC
FL1607B	7	26	33	59	0.83	ACCCAACACA	CCACCCACAC	CCCAACACAC	AAAACCCCCC	CAAAACAACA	CCCCAAACC
FL1608A	7	36	22	58	3.38	AAACAACAAA	CAAAACACAA	AACCCCCACA	CAACCCCCAC	AAAAAC-AAA	ACAACCAAA

Table A-1. (continued)

Marker name	Ch[a]	Segregation test [b]					Lines of the 'Fiber-2' population (A; MTaz90-123, C; MTH6860756, -; Missing data)					
		A	C	To	χ^2		1 - 10	11 - 20	21 - 30	31 - 40	41 - 50	51 - 59
FL1609B	5	33	25	58	1.10		ACAAAACCCA	ACCAACCAAA	AACAACCACC	CAAACAAAAA	CCCACA-AAC	AAACCACCC
FL1610B		29	29	58	0.00		ACACCCAAAC	AACCCCCAAA	AACACCAAAC	ACACCAAACC	CAAACA-ACA	ACCCCCACC
FL1611B		39	18	57	7.74	*	CACACAAACA	ACC-AAAAAA	ACACACCACA	AAACCAAAAA	ACCAAA-AAC	CAAACAAAA
FL1612B	3	30	27	57	0.16		ACAAAACACC	CAA-CCCCCC	AACACCACAA	ACCAACCCCA	CAACAC-ACA	AAAAAAACC
FL1613S	7	38	21	59	4.90	*	CACCCAACAA	CAACCCACAA	AAAAACAAAA	AAAAAAACCC	AAAACAAACA	CCCCACAAA
FL1614S	3	30	28	58	0.07		ACAAAACCCA	ACCAACACCC	CCCAACCAAC	AACACCCAAC	CCAACC-AAA	CCCAAAAAA
FL1701S	5	39	19	58	6.90	**	CAAAAACCCA	ACAAACCACA	AAAAACCAAC	AAAAACAAAA	-CAACAAAAC	ACACACCAA
FL1702B	7	30	28	58	0.07		ACCCAACCAC	CAACAAACAC	CCCCAACACA	CACCCACCAC	-AAAACAACA	ACAACCAAA
FL1703S	5	24	34	58	1.72		CAACACACCA	CCAAAAACCA	CCAAAACACC	CCACCCCCCA	-ACCCCCCCC	CACAAACCA
FL1704B		19	39	58	6.90	**	AAACCCAACC	CCCCCCCCAA	AAACCAACCC	CCAACACCAC	-CAACCCACC	CCCCCCCCC
FL1705S	4	28	30	58	0.07		ACAACAACAC	AACAACCACC	CCAACACACC	ACCAAAAAAA	-CCCCCCCCA	CCCACAAAC
FL1706S	5	28	30	58	0.07		CCCCACCACC	ACACCCACCA	ACACAAAAAC	CAACACAACC	-CCAACAACC	CCCAAAAAA
FL1707S	7	27	31	58	0.28		ACCCAACACC	CCACCACCAC	CCCCCCAAAA	AACAACCACC	-AAAACAACA	CACAAACCC
FL1708S	7	25	33	58	1.10		ACCCAACACC	CCACCACCAC	CCCCCCAAAA	AACACCCACC	-AAAACCACA	CACAAACCC
FL1709A	6	28	30	58	0.07		CACCAACACC	CCCAAAACAC	ACCACAAAAC	AACCCCACAA	-AAACCCACA	CCACACCAC
FL1710A		17	41	58	9.93	**	CAACCCACAC	CCCCACAAAC	CCCACACCCC	CCCCACCCCC	-CAACCCACA	CACCCCACC
FL1711A	1	32	26	58	0.62		AAAACACAAA	AACAAAAAAA	CCCCACCCAA	AAACCACACC	-CCCAACCAA	CCCAAACCC
FL1712S	7	31	27	58	0.28		ACAACACAAA	ACAAAACAAA	ACCCCACCCA	AAACCCCACC	-CCCAACAAA	CCCAAACCA
FL1713A	3	28	29	57	0.02		CACCAAACAC	ACCCCCACCA	AACAACAACA	CACACAACCA	-CAAAA-CCC	CCAAAACCC
FL1714B		31	26	57	0.44		ACCACCACCA	ACACACCAAA	ACACACACCC	AAACCAACCC	-CCACC-AAC	AAAAAAAAA
FL1715B	4	25	32	57	0.86		CCCCCCACAA	CCCACCAACA	CACCAACACC	CAACAACAAA	-ACCAA-CAC	ACCCCCACA
FL1716S	6	28	29	57	0.02		CACCAACACC	CACAAAACAC	ACAAAAAAAC	AACCCCCCAC	-CAACC-AAA	CCCCACCAC
FL1717A	3	32	24	56	1.14		CACCAA-AAC	ACACCCACCA	AACAAAAACA	CCCACAACAA	-CAAAC-CAC	CCAAAAAAC
FL1801A	7	31	28	59	0.15		ACAACACAAA	ACCAAACCAA	ACCCCCCCAA	CAACCCCACC	ACCAAAAAAA	CCCAAACCA
FL1802A	7	31	28	59	0.15		ACAACACAAA	ACAAAACAAA	ACCCCCCCAA	CAACCCCACC	AACAAACCAA	CCCAAACCC
FL1803A	3	27	32	59	0.42		ACACACCACA	CACCAACCAC	AACACAAAAA	CCCAACACCA	CCCCCCAACC	CCCAAAACC

Table A-1. (continued)

Marker name	Ch[a]	Segregation test [b]				Lines of the 'Fiber-2' population (A; MTaz90-123, C; MTH6860756, -; Missing data)					
		A	C	To	χ^2	1 - 10	11 - 20	21 - 30	31 - 40	41 - 50	51 - 59
FL1804S	6	29	30	59	0.02	CACCAACACC	CACAAAACAC	ACAAAAAAAC	AACCCCCCAC	CCACCCAAAA	CCCCACCAA
FL1805S	4	31	27	58	0.28	CCCCCCAAAA	CAAACACCAC	ACACCAA-AC	CAACACAAAC	CCAAACAAAC	ACACAAACC
FL1806S	3	27	30	57	0.16	ACCCAACACA	ACACACACCC	CCCAACC-CC	CAACCCCAAC	CAACAC-AAA	CCCAAAAAA
FL1807S	3	29	29	58	0.00	ACCCAACACA	ACACAAACCC	CCCAACC-CC	CACCACAAAC	CAACCCAAAA	CCCAAAAAA
FL1808S	3	29	29	58	0.00	ACCAAAACAC	CCACCCACCA	ACACACC-AC	ACCCCCACAA	AAAACCCACA	CACAAAAAC
FL1809S	6	27	30	57	0.16	CAACCCCACA	AACAAACAAC	CACACAA-CA	CAAAAACACC	CCCCAC-AAC	ACACCCCCC
FL1810A		27	30	57	0.16	ACCCCAAAAC	ACCCCCACAA	AACACCA-CC	AAACACCAAA	CCCCAC-CAA	ACACCACAC

Inferred genotypes of 3 markers at F_5 generation

Marker name	Ch[a]	Segregation test [b]					H; Heterogeneous (inferred by phenotypes)					
		A	H	C	χ^2		1 - 10	11 - 20	21 - 30	31 - 40	41 - 50	51 - 59
lk$_2$	1	23	8	28	5.83		CCCAACACHA	CAAACCAAAC	AACCHCAAHC	ACACHHACCA	AAACCCACCH	CHACCCHCC
n	1	24	9	26	8.24	*	CCCAACACHA	CAACCCAAAC	AACHACACHC	HCACCACCCA	ACACACACHH	CHAACHAAH
wx	1	31	8	20	7.57	*	AHHACAHAHA	AAAAAAAAAA	ACCCACCAAA	HCACCACACC	ACHAACHCCA	HCAAAACCC

[a] Chromosomal locations of each locus on the 'Fiber-2' linkage map.

[b] * and ** are levels of significance at $p \leq 0.05$ and $p \leq 0.01$, respectively.

Table A-2. Marker intervals of the 'Fiber-2' linkage map with the results of single marker QTL analysis on traits measured.

'Fiber-2' linkage map					Single Marker QTL analysis on quantitative traits measured [a]								
Order		Interval [b] cM(Kosambi)		Locus	YD98 *F*-value	KW98 *F*-value	KW99 *F*-value	GU96 *F*-value	GU97 *F*-value	GU99 *F*-value	HD98 *F*-value	HD99 *F*-value	PH99 *F*-value
Ch1	1	0.0	0.0	FL0308S	0.215	0.382	0.023	1.534	2.049	0.881	0.307	0.306	0.818
	2	14.5	14.5	FL1505S	2.026	1.034	3.781	8.371 **	11.286 **	18.959 ****	0.020	0.333	2.063
	3	19.1	4.6	FL1308S	1.548	0.014	1.218	7.029 *	7.080 *	15.668 ***	0.004	0.339	1.422
	4	22.8	3.7	FL0207S	1.523	1.093	3.265	6.189 *	9.079 **	19.895 ****	0.027	0.366	2.918
	5	28.5	5.7	M-HVWAXYG	0.000	1.249	1.835	10.087 **	14.842 ***	32.878 ****	0.307	0.000	2.124
	6	30.0	1.5	*wx*	1.873	10.031 **	12.601 ***	16.859 ***	32.343 ****	54.924 ****	0.423	0.050	2.734
	7	33.3	3.3	HVM6	0.524	0.724	1.811	19.029 ****	9.318 **	27.344 ****	0.589	0.552	1.608
	8	69.9	36.6	FL0904S	1.845	0.646	4.453 *	0.001	1.546	1.202	8.208 **	8.694 **	1.674
	9	84.4	14.5	ABG380	0.127	1.013	8.861 **	2.261	7.766 **	2.916	29.558 ****	25.768 ****	14.666 ***
	10	89.1	4.7	FL1202B	0.028	0.000	2.307	1.528	5.106 *	1.887	15.165 ***	12.354 ***	6.340 *
	11	92.8	3.7	FL1205S	0.149	0.025	2.602	0.039	7.449 **	0.986	12.409 ***	10.547 **	5.880 *
	12	97.5	4.7	FL1511B	0.283	0.452	4.933 *	0.136	2.257	2.702	9.391 **	5.445 *	2.540
	13	102.2	4.7	FL1204S	0.037	0.026	3.165	0.096	2.651	1.034	10.065 **	7.596 **	5.685 *
	14	105.9	3.7	FL0304S	0.099	0.224	3.079	0.035	2.475	1.224	15.329 ***	12.467 ***	6.365 *
	15	117.6	11.7	FL1009B	2.107	0.218	2.830	0.442	0.638	0.065	9.849 **	7.916 **	2.391
	16	123.3	5.7	FL0504S	9.171 **	1.131	5.932 *	0.129	0.676	0.261	12.390 ***	11.458 **	4.159 *
	17	130.1	6.8	FL1302A	1.829	2.937	5.132 *	0.641	5.323 *	0.889	13.465 ***	9.721 **	3.079
	18	132.8	2.7	FL1305S	1.987	3.030	6.269 *	0.100	2.753	0.224	15.056 ***	11.097 **	4.880 *
	19	137.5	4.7	FL1306B	4.533 *	8.006 **	11.278 **	1.022	3.130	0.577	6.620 *	3.684	1.542
	20	145.4	7.9	M-HVCMAa	9.276 **	12.306 ***	20.477 ****	3.731	4.532 *	1.202	4.366 *	1.616	0.249
	21	146.3	0.9	M-HVCMAb	10.587 **	16.173 ***	25.231 ****	4.114 *	4.556 *	2.673	3.679	0.969	0.086
	22	159.4	13.1	FL1105S	28.803 ****	23.670 ****	59.221 ****	1.942	6.731 *	4.057 *	9.019 **	4.349 *	0.494
	23	162.3	2.9	*n*	27.330 ****	44.916 ****	137.100 ****	4.656 *	11.639 **	7.107 **	8.740 **	3.893	0.673
	24	166.8	4.5	WTA1	22.295 ****	22.515 ****	52.445 ****	4.498 *	11.825 **	7.109 **	5.960 *	3.204	0.473
	25	174.7	7.9	ABG20.11a	16.689 ****	36.448 ****	44.459 ****	2.288	5.774 *	5.429 *	3.054	1.191	0.975

Table A-2. (continued)

'Fiber-2' linkage map					Single Marker QTL analysis on quantitative traits measured [a]								
Order		Interval [b] cM(Kosambi)		Locus	YD98 *F*-value	KW98 *F*-value	KW99 *F*-value	GU96 *F*-value	GU97 *F*-value	GU99 *F*-value	HD98 *F*-value	HD99 *F*-value	PH99 *F*-value
Ch1	26	178.4	3.7	lk_2	15.542 ***	47.857 ****	42.307 ****	0.529	9.099 **	7.627 **	2.910	1.424	0.382
	27	190.2	11.8	FL1004S	24.092 ****	12.227 ***	30.285 ****	1.268	2.616	2.776	6.905 *	4.843 *	0.882
	28	201.9	11.7	FL1003S	12.807 ***	17.199 ***	17.717 ****	0.678	3.976	1.689	3.679	2.708	0.044
	29	228.0	26.1	FL0413A	10.041 **	8.667 **	9.037 **	1.122	0.952	1.244	3.630	2.045	2.242
	30	239.8	11.8	FL1503A	7.634 **	3.708	5.486 *	1.327	0.209	0.090	2.567	3.037	1.457
	31	241.6	1.8	FL1515A	6.214 *	2.781	4.547 *	1.598	0.226	0.066	5.650 *	5.999 *	3.558
	32	270.1	28.5	FL0910B	0.171	0.751	0.392	5.737 *	1.885	0.801	0.880	0.869	2.833
	33	273.8	3.7	FL0909A	1.184	0.097	0.082	2.484	1.076	0.041	1.239	1.762	2.898
	34	277.5	3.7	FL0203S	0.148	0.463	0.084	3.119	1.116	0.130	4.434 *	4.795 *	7.306 **
	35	282.5	5.0	FL0508A	0.879	0.168	0.486	1.096	0.237	0.077	3.156	2.449	2.670
	36	293.8	11.3	ABC253	1.294	0.358	0.597	0.442	0.128	0.686	2.325	2.216	1.016
	37	302.9	9.1	FL0801S	1.928	0.021	1.698	0.494	0.240	0.554	7.660 **	10.454 **	5.388 *
	38	305.6	2.7	FL1109S	1.048	0.132	0.258	0.014	0.051	0.001	6.448 *	6.659 *	4.939 *
	39	325.1	19.5	FL1506A	0.030	0.330	0.457	0.180	0.022	0.741	0.010	0.146	0.943
	40	330.3	5.2	FL1309B	0.156	0.865	0.576	0.451	0.244	0.843	0.252	0.188	0.152
	41	335.6	5.3	M-HVPRP1B	0.007	0.085	0.002	0.206	0.874	0.039	0.031	0.002	0.074
Ch2	1	0.0	0.0	FL1507A	1.090	0.501	0.520	2.509	0.854	0.048	0.036	0.556	1.791
	2	4.1	4.1	FL1404S	1.737	1.040	0.734	1.015	0.207	0.158	0.894	2.717	2.310
	3	6.1	2.0	FL1405S	1.435	1.375	0.476	0.014	0.607	0.111	1.354	3.824	3.100
	4	9.1	3.0	FL1501S	1.165	0.696	0.168	0.064	0.664	0.029	1.018	2.749	1.616
	5	16.2	7.1	ABG058	1.371	0.149	0.089	0.748	0.318	0.188	0.704	2.527	2.280
	6	44.9	28.7	HVM36	0.029	2.443	0.003	0.743	1.923	0.254	20.277 ****	20.507 ****	14.489 ***
	7	51.7	6.8	FL0205B	0.067	1.378	0.911	3.508	1.761	0.266	28.808 ****	31.010 ****	23.422 ***
	8	60.4	8.7	ABC170	0.029	0.692	3.030	0.007	1.073	0.048	66.486 ****	80.571 ****	31.887 ***

Table A-2. (continued)

'Fiber-2' linkage map					Single Marker QTL analysis on quantitative traits measured [a]								
Order		Interval [b] cM(Kosambi)		Locus	YD98 *F*-value	KW98 *F*-value	KW99 *F*-value	GU96 *F*-value	GU97 *F*-value	GU99 *F*-value	HD98 *F*-value	HD99 *F*-value	PH99 *F*-value
Ch2	9	70.4	10.0	FL1106S	1.742	0.160	4.253 *	0.471	3.460	1.047	15.091 ***	20.984 ****	12.422 ***
	10	72.2	1.8	FL1107S	1.759	0.018	2.149	0.399	3.918	0.755	11.260 **	16.840 ***	14.631 ***
	11	106.4	34.2	FL1104S	0.248	0.397	0.002	0.003	0.048	0.259	0.593	1.688	1.952
	12	140.1	33.7	cMWG694	2.402	0.589	0.035	3.474	3.548	0.003	0.116	0.443	0.625
	13	150.0	9.9	FL0906S	0.070	0.101	0.554	2.761	0.222	0.885	0.060	0.092	0.331
	14	158.2	8.2	FL0908S	0.000	0.192	0.036	1.130	0.739	0.563	0.267	0.377	0.076
	15	178.7	20.5	FL0503A	0.199	0.301	0.041	0.182	0.188	0.869	0.234	0.085	0.262
	16	184.4	5.7	FL0603S	1.190	0.254	0.014	0.158	0.000	0.308	1.409	0.139	0.296
	17	201.3	16.9	FL0608A	0.032	1.145	1.176	0.022	0.214	0.016	1.782	1.336	0.792
	18	245.6	44.3	FL0702A	1.064	0.045	0.319	0.769	0.002	0.214	2.260	2.071	0.181
	19	270.2	24.6	FL1410B	2.572	4.440 *	1.621	1.078	0.641	0.017	0.072	0.136	0.004
	20	279.4	9.2	HVM054	1.774	4.793 *	2.758	0.144	0.676	0.422	0.051	0.119	0.398
	21	283.1	3.7	FL0303A	1.726	0.925	1.544	0.003	0.012	2.111	0.787	1.091	1.408
	22	303.6	20.5	FL1408B	0.167	1.920	0.387	0.917	1.424	4.456 *	3.158	3.385	5.680 *
	23	321.4	17.8	ABG609	0.128	0.169	0.002	0.076	0.394	0.898	0.569	0.715	4.014 *
	24	329.1	7.7	M-HVCSG	0.014	0.545	0.021	0.049	0.785	3.848	0.035	0.102	3.780
	25	338.3	9.2	FL1108B	0.302	0.842	0.202	0.301	1.859	5.263 *	0.051	0.011	5.602 *
	26	345.1	6.8	FL1603A	0.423	0.002	0.318	0.175	0.417	3.142	0.606	0.227	4.759 *
	27	351.2	6.1	FL0607B	2.858	0.475	0.359	0.000	0.827	1.330	0.000	0.058	3.073
	28	359.8	8.6	FL1602A	0.000	0.242	0.611	0.596	0.560	1.534	0.544	0.081	2.645
	29	374.3	14.5	FL0404S	1.005	1.193	0.083	2.757	4.199 *	2.817	0.023	0.001	2.687
Ch3	1	0.0	0.0	ABG070	3.729	0.094	0.183	1.470	0.683	3.519	1.300	2.045	6.313 *
	2	6.2	6.2	FL1112S	1.064	0.018	0.749	1.384	0.461	4.862 *	1.800	2.376	6.008 *
	3	7.1	0.9	FL1717A	0.040	0.243	2.174	0.460	0.894	2.819	0.642	0.813	2.835

Table A-2. (continued)

'Fiber-2' linkage map					Single Marker QTL analysis on quantitative traits measured [a]								
Order		Interval [b] cM(Kosambi)		Locus	YD98 *F*-value	KW98 *F*-value	KW99 *F*-value	GU96 *F*-value	GU97 *F*-value	GU99 *F*-value	HD98 *F*-value	HD99 *F*-value	PH99 *F*-value
Ch3	4	16.9	9.8	FL1713A	0.281	2.385	2.850	0.011	0.205	1.230	0.305	0.136	0.743
	5	25.4	8.5	FL0602A	0.034	0.363	0.057	0.658	0.642	3.503	0.333	0.327	0.003
	6	57.3	31.9	FL1808S	0.718	0.008	0.125	0.998	0.177	2.390	0.590	1.667	1.009
	7	94.8	37.5	FL1614S	0.032	0.472	0.930	2.947	1.316	0.014	2.984	0.880	1.751
	8	108.3	13.5	FL1806S	0.069	0.033	0.016	4.877 *	0.480	0.073	0.296	0.001	0.356
	9	112.1	3.8	HVM060	0.913	0.023	0.195	5.607 *	0.004	0.080	2.167	0.575	0.004
	10	114.8	2.7	FL1807S	2.778	0.756	1.132	2.071	0.862	0.502	1.141	0.101	0.266
	11	121.6	6.8	FL0410S	2.240	1.722	2.408	0.826	2.211	0.834	0.851	0.012	0.025
	12	155.8	34.2	FL1803A	0.635	0.009	0.136	0.455	1.300	0.840	0.008	0.011	0.609
	13	173.6	17.8	FL0306A	1.635	0.075	0.993	0.345	0.240	0.050	0.033	0.143	4.479 *
	14	183.9	10.3	FL0502B	0.044	0.000	0.028	0.087	0.040	0.755	0.008	0.015	2.650
	15	197.9	14.0	FL1612B	0.042	2.780	3.416	3.096	0.004	0.546	0.314	0.421	2.358
	16	206.3	8.4	FL0311S	0.219	0.017	0.554	0.825	0.195	0.703	0.352	0.313	2.452
	17	209.3	3.0	FL1413S	0.134	0.107	0.650	0.959	0.170	0.494	0.003	0.023	4.481 *
	18	210.3	1.0	FL1412S	0.019	0.047	0.661	1.842	0.051	0.265	0.063	0.087	3.484
	19	211.3	1.0	FL0806S	0.162	0.059	0.791	1.812	0.003	0.308	0.211	0.381	2.318
	20	215.4	4.1	MWG549	0.251	0.152	0.030	2.147	0.041	0.002	0.827	0.510	0.954
	21	225.7	10.3	FL1102B	0.031	0.055	0.453	0.778	0.102	0.347	1.018	0.785	0.033
	22	233.6	7.9	FL1604B	0.431	0.563	0.548	1.568	0.607	0.092	0.234	0.186	1.948
	23	243.9	10.3	FL0305A	0.018	0.132	0.627	1.686	0.748	0.140	0.047	0.087	2.057
	24	258.4	14.5	FL1010B	2.425	1.124	0.893	0.622	0.008	0.453	0.071	0.618	0.191
Ch4	1	0.0	0.0	FL0310A	0.399	0.776	0.398	2.045	0.955	0.066	0.352	0.265	0.006
	2	3.8	3.8	FL1601S	0.028	0.046	0.002	0.634	1.495	0.087	0.646	0.780	0.848
	3	21.6	17.8	FL0403S	0.593	3.305	4.743 *	0.332	2.477	1.505	0.220	0.942	1.535

Table A-2. (continued)

'Fiber-2' linkage map					Single Marker QTL analysis on quantitative traits measured [a]								
Order		Interval [b] cM(Kosambi)		Locus	YD98 *F*-value	KW98 *F*-value	KW99 *F*-value	GU96 *F*-value	GU97 *F*-value	GU99 *F*-value	HD98 *F*-value	HD99 *F*-value	PH99 *F*-value
Ch4	4	55.2	33.6	FL1209S	0.924	2.945	3.215	0.572	0.455	0.302	0.025	0.370	0.142
	5	72.4	17.2	ABC303	2.474	2.977	7.234 **	0.515	2.369	0.062	1.098	0.268	0.813
	6	84.3	11.9	FL0609S	1.009	2.414	3.638	0.178	0.152	0.077	0.051	0.059	0.294
	7	86.1	1.8	HVM3	0.000	0.369	1.488	0.029	0.020	0.139	0.051	0.023	0.185
	8	89.8	3.7	MWG058	0.037	5.553 *	3.746	0.270	1.078	0.013	0.128	0.946	0.030
	9	91.6	1.8	FL1705S	0.015	2.397	3.230	0.098	0.094	0.212	0.063	0.561	0.066
	10	96.5	4.9	FL1414S	0.381	3.400	3.801	0.006	0.391	0.128	0.502	1.091	0.053
	11	114.7	18.2	HVM68	1.285	4.088 *	2.981	1.137	0.004	0.003	0.857	1.070	0.941
	12	136.8	22.1	ABG618	0.231	7.654 **	3.449	0.105	4.265 *	2.571	0.392	0.556	0.170
	13	149.7	12.9	ABG054	0.486	5.526 *	1.856	0.002	0.305	0.456	1.214	2.084	1.192
	14	166.8	17.1	FL1311S	0.174	0.810	0.765	0.515	0.236	0.149	0.117	0.005	3.903
	15	173.2	6.4	FL0701S	0.053	0.045	0.076	2.853	0.208	0.438	0.000	0.001	2.112
	16	242.6	69.4	FL0605A	1.138	4.084 *	1.088	0.447	3.465	0.207	0.526	2.092	1.238
	17	254.7	12.1	FL1805S	0.011	0.895	0.628	0.209	0.325	0.030	0.060	0.079	0.434
	18	278.5	23.8	FL0411S	0.028	0.825	0.040	0.118	0.328	0.117	6.024 *	6.207 *	0.733
	19	316.3	37.8	BTA02a	0.862	5.962 *	2.286	3.203	5.996 *	1.662	0.964	1.694	0.481
	20	330.8	14.5	BTA02b	0.465	3.109	1.178	0.036	2.573	0.037	0.190	0.092	2.040
	21	341.8	11.0	BCD402	1.426	1.632	0.594	0.637	2.638	1.292	0.022	0.049	0.846
	22	357.7	15.9	FL0409A	0.069	0.136	0.570	1.656	0.861	0.011	0.024	0.157	6.120 *
	23	361.5	3.8	FL1715B	0.117	0.002	0.070	1.282	0.768	0.008	0.032	0.060	3.691
Ch5	1	0.0	0.0	FL1609B	0.004	1.331	0.618	1.110	0.067	1.257	0.001	0.418	1.065
	2	14.9	14.9	FL0601B	0.898	0.820	0.758	0.268	0.362	0.526	0.225	1.437	1.182
	3	22.8	7.9	FL0902B	0.542	0.264	0.029	0.003	0.529	0.461	0.920	3.289	1.152
	4	30.7	7.9	FL0401A	0.630	0.757	0.013	1.642	0.401	1.685	0.028	0.102	0.060

Table A-2. (continued)

'Fiber-2' linkage map					Single Marker QTL analysis on quantitative traits measured [a]								
Order		Interval [b] cM(Kosambi)		Locus	YD98 *F*-value	KW98 *F*-value	KW99 *F*-value	GU96 *F*-value	GU97 *F*-value	GU99 *F*-value	HD98 *F*-value	HD99 *F*-value	PH99 *F*-value
Ch5	5	42.4	11.7	MWG938a	0.002	0.016	0.023	0.473	0.228	0.571	0.858	0.921	0.041
	6	57.8	15.4	FL1701S	0.343	0.196	1.819	0.000	0.044	0.145	4.894 *	4.984 *	1.407
	7	69.5	11.7	FL0805A	0.202	0.046	0.277	0.859	0.267	1.068	2.922	1.768	0.262
	8	80.5	11.0	Hor 2	0.070	0.353	0.564	0.045	0.699	0.018	1.868	1.187	0.192
	9	81.4	0.9	Hor 1	0.117	0.193	0.552	0.100	0.922	0.120	2.672	1.995	0.410
	10	85.1	3.7	ABC156.1	0.106	0.646	0.821	0.055	1.659	0.076	0.671	0.241	0.499
	11	98.8	13.7	MWG2261	1.359	2.802	6.552 *	2.018	0.035	0.641	2.934	3.516	2.267
	12	143.4	44.6	FL1706S	0.063	0.001	0.020	0.120	0.575	1.620	0.259	0.012	0.351
	13	162.6	19.2	MWG938b	2.915	1.532	4.310 *	0.728	0.208	1.850	2.434	1.315	0.151
	14	183.1	20.5	FL0802S	0.018	0.005	0.002	0.597	1.073	0.977	1.485	0.854	0.343
	15	192.2	9.1	FL1605B	0.833	0.138	0.020	0.001	1.195	2.555	0.028	0.000	0.165
	16	197.9	5.7	FL1512B	0.274	0.764	0.003	0.333	1.661	3.976	0.821	0.612	0.421
	17	217.6	19.7	FL0415S	0.042	0.018	0.029	0.157	0.048	0.097	0.201	0.099	0.124
	18	218.5	0.9	FL0416S	0.318	0.177	0.024	0.093	0.022	0.094	0.170	0.047	0.150
	19	221.6	3.1	FL0206B	0.029	0.009	0.037	0.000	0.034	0.012	0.004	0.010	0.483
	20	227.1	5.5	FL1208A	0.354	0.782	0.840	0.134	2.041	4.911 *	0.196	0.421	0.129
	21	250.5	23.4	ABG452	0.028	0.068	0.125	0.111	0.028	0.027	0.049	0.146	0.132
	22	264.8	14.3	FL1703S	0.348	0.056	0.276	0.198	0.158	0.410	1.485	2.528	0.611
	23	315.8	51.0	FL1206A	0.110	0.220	0.179	0.575	0.439	0.301	0.909	1.570	0.315
	24	326.6	10.8	FL0414S	0.316	1.456	0.269	2.598	2.575	2.233	1.274	1.452	2.568
	25	330.3	3.7	FL0606A	1.167	2.770	0.859	0.993	2.250	0.674	0.136	0.160	0.665
	26	372.5	42.2	FL1111S	1.925	0.713	3.346	1.287	1.895	1.858	0.421	0.030	1.600
	27	390.3	17.8	ABG55a	3.690	0.426	2.485	2.314	2.158	1.818	0.418	0.056	1.304
	28	393.0	2.7	ABG55b	3.028	0.331	2.323	0.701	1.002	0.403	0.020	0.002	0.712

Table A-2. (continued)

'Fiber-2' linkage map					Single Marker QTL analysis on quantitative traits measured [a]								
Order		Interval [b] cM(Kosambi)		Locus	YD98 *F*-value	KW98 *F*-value	KW99 *F*-value	GU96 *F*-value	GU97 *F*-value	GU99 *F*-value	HD98 *F*-value	HD99 *F*-value	PH99 *F*-value
Ch6	1	0.0	0.0	FL0402S	0.106	0.010	0.168	0.661	2.218	0.003	1.638	2.686	1.894
	2	4.6	4.6	MST109	0.566	0.037	0.467	1.140	2.353	0.077	2.447	2.849	2.488
	3	11.4	6.8	MWG620	0.823	1.791	1.328	1.829	6.248 *	0.737	1.433	0.959	1.042
	4	15.1	3.7	FL1804S	0.004	0.010	0.004	0.972	3.652	0.210	1.647	1.819	3.210
	5	17.0	1.9	FL1716S	0.305	0.106	0.195	0.800	1.097	0.021	1.738	1.705	3.207
	6	25.9	8.9	FL1709A	0.018	0.464	0.002	2.702	0.967	0.455	0.153	0.085	1.367
	7	31.6	5.7	FL1303S	0.099	0.452	0.003	1.696	1.834	0.055	2.423	3.002	5.593 *
	8	36.2	4.6	FL1304S	0.001	0.705	0.063	0.784	0.089	0.071	2.152	3.172	6.368 *
	9	45.3	9.1	FL0412A	0.152	2.268	1.067	0.166	0.043	0.510	0.425	0.775	4.105 *
	10	53.5	8.2	HVGNIRE	0.569	0.164	0.023	2.700	2.298	0.203	1.339	1.941	6.805 *
	11	78.7	25.2	FL0102A	0.615	2.287	0.583	0.341	1.491	0.045	2.235	2.812	4.225 *
	12	109.8	31.1	FL0101A	0.092	0.007	0.313	0.014	0.320	0.001	0.004	0.127	0.114
	13	147.6	37.8	FL1509S	0.033	0.051	0.058	0.051	0.389	0.207	0.076	0.536	5.989 *
	14	158.0	10.4	FL0408B	0.869	0.004	0.565	0.034	0.905	0.569	0.068	0.333	5.656 *
	15	162.7	4.7	FL1312A	0.334	0.107	0.369	0.041	0.847	0.010	0.010	0.042	2.926
	16	167.4	4.7	FL1508S	0.402	0.208	0.280	0.024	0.798	0.000	0.734	1.035	6.503 *
	17	198.5	31.1	FL0301S	1.083	0.054	0.193	0.002	0.019	0.170	0.024	0.042	2.333
	18	220.0	21.5	FL0501S	1.504	0.045	0.429	0.092	0.005	0.000	1.521	0.569	6.087 *
	19	233.1	13.1	FL1510B	0.006	0.401	0.495	0.012	0.110	1.085	0.190	0.649	1.151
	20	244.8	11.7	FL1101S	0.618	0.898	0.087	0.162	0.481	1.314	1.282	0.962	5.697 *
	21	295.8	51.0	FL1409B	0.053	0.187	0.492	1.948	1.350	2.182	0.023	0.540	0.428
	22	304.4	8.6	FL1809S	0.056	0.005	0.030	1.037	0.228	2.279	0.524	1.075	1.255
	23	315.8	11.4	FL0307B	1.475	0.000	0.135	0.607	0.298	0.001	0.662	1.976	0.373

Table A-2. (continued)

'Fiber-2' linkage map					Single Marker QTL analysis on quantitative traits measured [a]								
Order		Interval [b] cM(Kosambi)		Locus	YD98 *F*-value	KW98 *F*-value	KW99 *F*-value	GU96 *F*-value	GU97 *F*-value	GU99 *F*-value	HD98 *F*-value	HD99 *F*-value	PH99 *F*-value
Ch7	1	0.0	0.0	ABG064	0.007	0.289	0.221	2.269	4.971 *	2.224	0.636	0.867	1.367
	2	6.4	6.4	ABC483	0.002	0.370	0.760	12.970 ***	12.711 ***	13.062 ***	0.003	0.002	1.224
	3	17.9	11.5	FL1005A	0.069	0.086	0.489	11.048 **	10.285 **	8.568 **	0.297	0.767	2.763
	4	23.6	5.7	FL1802A	0.069	0.021	0.507	7.947 **	7.340 **	5.768 *	0.174	0.005	0.003
	5	29.3	5.7	FL1801A	0.301	0.106	0.002	6.278 *	5.113 *	3.748	0.012	0.090	0.777
	6	36.1	6.8	FL1712S	0.001	0.395	0.147	4.776 *	3.646	3.220	0.060	0.391	0.808
	7	41.9	5.8	FL0407A	0.429	0.906	0.164	3.583	3.290	3.326	0.010	0.038	0.188
	8	44.6	2.7	FL0406A	0.252	0.033	0.010	4.478 *	4.010	2.157	0.000	0.040	0.070
	9	55.3	10.7	FL0803S	0.262	0.241	0.027	5.145 *	1.220	2.488	1.506	1.477	3.421
	10	90.5	35.2	FL0405S	0.002	0.723	0.073	0.022	1.305	0.000	0.840	1.233	0.020
	11	101.0	10.5	M-HVDHN9a	1.310	1.248	0.001	1.025	1.504	0.005	1.695	1.072	0.021
	12	112.8	11.8	FL1110S	0.369	1.479	0.623	0.517	0.522	0.102	0.001	0.004	1.234
	13	119.8	7.0	FL1401S	3.441	3.254	0.993	3.813	2.246	0.144	0.376	1.618	2.360
	14	136.6	16.8	FL1411B	0.075	0.408	0.022	0.137	0.001	0.803	0.630	0.060	0.000
	15	151.9	15.3	FL1702B	0.399	1.583	0.171	0.173	0.039	0.384	1.218	0.443	0.977
	16	166.5	14.6	ABC155	0.078	2.190	2.758	1.899	0.140	0.086	0.028	0.569	0.028
	17	190.1	23.6	FL1607B	0.016	3.131	3.860	2.031	0.083	0.411	0.031	0.016	0.708
	18	205.3	15.2	FL1708S	0.555	0.037	0.011	1.842	0.033	1.904	0.006	0.000	0.565
	19	207.1	1.8	FL1707S	0.943	0.300	0.000	1.954	0.361	1.912	0.012	0.004	0.840
	20	227.7	20.6	FL0506A	4.788 *	1.895	0.013	0.509	0.325	0.343	1.433	0.870	0.835
	21	242.2	14.5	FL1006B	2.497	1.108	0.112	0.015	0.142	0.824	2.430	3.461	1.274
	22	249.0	6.8	FL1613S	0.896	2.603	0.186	0.690	0.047	0.169	1.504	1.956	0.960

Table A-2. (continued)

'Fiber-2' linkage map					Single Marker QTL analysis on quantitative traits measured [a]								
Order		Interval [b] cM(Kosambi)		Locus	YD98 *F*-value	KW98 *F*-value	KW99 *F*-value	GU96 *F*-value	GU97 *F*-value	GU99 *F*-value	HD98 *F*-value	HD99 *F*-value	PH99 *F*-value
Ch7	23	297.2	48.2	FL1608A	0.033	3.752	2.446	1.685	2.042	2.827	0.449	0.202	0.002
	24	307.7	10.5	FL1415S	1.149	2.463	0.956	0.005	0.225	0.327	0.405	0.090	0.159
	25	315.8	8.1	MWG2249	2.829	4.577 *	3.528	2.095	0.842	1.708	1.349	1.890	4.206 *
	26	325.0	9.2	FL0202S	1.190	1.054	1.133	2.641	0.183	1.207	0.000	0.002	0.348
	27	330.8	5.8	FL0312S	0.948	0.910	1.199	5.958 *	0.825	1.568	0.297	0.287	2.742
		195 loci											

Pairs having identical genotypes in 59 mapping lines

Map order			
Ch1	3	with	FL1711A
Ch1	38	with	FL1109S
Ch4	14	with	FL1310S
Ch7	11	with	M-HVHDN9b

Total 199 loci on the 'Fiber-2' linkage map

[a] Grain yield (YD), Kernel weight (KW), β-D-glucan contents (GU), Heading date (HD) and Plant height (PH) with the year tested. *, **, *** and **** are levels of significance at $P \leq 0.05$, 0.01, 0.001 and 0.0001, respectively.

[b] Kosambi mapping function was used. Accumulative map size and marker intervals with cM.

Table A-3. The amount of maternal genome (MTaz90-123) in 59 lines of the 'Fiber-2' population.

Line	Total 241 loci		'Fiber-2' linkage map 199 loci		Chromosome 1 43 loci	
	M	MTaz90-123 (%)	M	MTaz90-123 (%)	M	MTaz90-123 (%)
1	12	52.0	12	50.3	0	44.2
2	6	61.3	6	64.6	1	16.3
3	2	49.4	2	52.8	1	25.6
4	1	40.4	1	40.2	0	90.7
5	9	42.2	6	39.7	0	69.8
6	0	36.5	0	34.4	0	74.4
7	2	57.3	2	62.2	1	65.1
8	3	45.4	2	46.6	0	46.5
9	13	57.0	14	58.0	3	39.5
10	0	52.3	0	49.7	0	81.4
11	1	49.6	1	50.5	0	48.8
12	0	49.8	0	49.7	0	58.1
13	1	50.8	1	50.0	0	34.9
14	2	47.3	1	47.4	0	34.9
15	0	43.6	0	42.1	0	32.6
16	9	53.0	6	51.3	3	37.2
17	0	43.2	0	39.0	0	97.7
18	0	50.2	0	49.7	0	83.7
19	1	40.0	1	39.7	0	97.7
20	8	48.5	6	51.9	0	72.1
21	0	39.4	0	39	0	69.8
22	0	55.2	0	53.3	0	58.1
23	0	61.4	0	64.6	0	16.3
24	2	48.5	2	47.7	1	0.0
25	3	34.9	3	31.8	2	74.4
26	8	45.1	7	44.1	2	37.2
27	0	42.3	0	41.5	0	74.4
28	7	40.6	6	36.5	0	69.8
29	2	43.9	2	41.5	2	55.8
30	0	52.7	0	54.9	0	30.2
31	2	48.5	2	50.3	2	46.5
32	11	44.8	8	45.5	3	20.9
33	1	45.8	1	45.9	0	83.7
34	0	51.9	0	49.7	0	30.2
35	2	61.1	2	61.1	1	14.0
36	7	52.6	7	56.4	2	60.5
37	0	46.1	0	47.2	0	46.5
38	1	50.0	1	54.1	0	18.6

Table A-3. (continued)

Line	Total 241 loci		'Fiber-2' linkage map 199 loci		Chromosome 1 43 loci	
	M	MTaz90-123 (%)	M	MTaz90-123 (%)	M	MTaz90-123 (%)
39	1	59.2	1	62.4	0	37.2
40	1	49.2	1	47.9	0	81.4
41	26	50.7	18	47.5	3	93.0
42	0	59.8	0	60.0	0	32.6
43	4	41.8	4	39.8	2	44.2
44	0	41.9	0	42.6	0	41.9
45	7	45.7	7	43.6	0	69.8
46	1	65.8	1	68.0	0	20.9
47	54	39.0	41	35.1	5	55.8
48	0	41.9	0	43.1	0	14.0
49	4	54.4	4	55.0	1	48.8
50	4	41.4	4	39.3	2	74.4
51	1	64.2	1	65.5	1	7.0
52	3	51.3	3	52.1	2	46.5
53	2	57.7	0	61.0	0	18.6
54	1	37.5	1	33.5	1	65.1
55	8	40.8	6	37.6	4	27.9
56	1	39.6	1	39.7	1	60.5
57	6	45.5	5	45.8	1	39.5
58	2	56.5	2	54.4	1	60.5
59	4	51.9	4	50.3	3	53.5
Mean (%)		48.7		48.9		50.0

Amount of the maternal alleles (MTaz90-123 %) in each individual line was expressed with the percentage of total number of loci in total informative markers, markers on Fiber-2 linkage map, and the markers on chromosome 1. M indicates the number of missing markers.