Genetic Mapping of Beta-glucan Content and Beta-glucanase Activity Loci in Malting Barley (*Hordeum vulgare* L.)

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ABSTRACT

Low concentrations of (1,3;1,4)-β-D-glucan are desired in malt/wort created from barley (*Hordeum vulgare* L.) to ensure efficiency during brewing. This goal can be achieved through low grain β-glucan content, high malt β-glucanase activity, or a combination thereof. Evaluation of these traits is time-consuming and expensive since malting must occur before analysis, thus they are ideal for the identification of associated markers to permit marker-assisted selection.

Two genetic mapping approaches were taken to evaluate these traits. First, a bi-parental QTL mapping population was developed from the Western Canadian two-row, spring, hulled varieties CDC Meredith and CDC Kindersley. CDC Meredith has normal to higher grain β-glucan content and higher wort β-glucan content, while CDC Kindersley has normal to lower grain β-glucan content and very low wort β-glucan content. Secondly, a 92 member association mapping (AM) population was assembled consisting of two-row, spring, hulled elite malting breeding lines and varieties derived from eight different barley breeding programs focused on Western Canada. Both populations were grown over a two year period and assessed for kilned malt β-glucanase activity, grain, malt, and wort β-glucan content, plus green malt β-glucanase activity in the AM population. Both populations were genotyped using the Barley 9K iSELECT Genotyping BeadChip and the QTL population was also genotyped with DArTseq markers.

As expected, green malt β-glucanase activity typically had a stronger correlation to malt/wort β-glucan content than did either kilned malt β-glucanase activity or grain β-glucan content. Ten unique QTLs (four for grain β-glucan, two for malt β-glucan, and three for kilned malt β-glucanase) were identified in the QTL population and eight marker-trait associations were identified in the AM population for grain β-glucan, green/kilned malt β-glucanase, or a combination thereof. One common genomic region on chromosome 2H was identified in both studies which was associated with grain β-glucan content. In the QTL and AM populations, six and five QTLs and marker-trait associations identified were novel, respectively, while the others overlay previously identified regions. In some cases, underlying genes were identified which may explain the observed associations. Lines with high β-glucanase activity and heat stability were identified in the AM population, thus through crossing, lines possessing both of these beneficial traits could be generated. Further trials to collect data on these traits would be desirable to validate the identified markers and confirm the β-glucanase heat stability data.

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LIST OF ABBREVIATIONS

AAFC	Agriculture and Agri-Food Canada
AC	Agriculture Canada
AFLPs	Amplified Fragment Length Polymorphisms
AM	Association Mapping
ANOVA	Analysis of Variance
AU	Agricore United
BARI	Busch Agricultural Resources Inc.
BARLEX	Barley Genome Explorer
BG	β-glucan
BGase	β-glucanase
BP	Base Pair(s)
CDC	Crop Development Centre
DArTseq	Diversity Arrays Technology Sequencing
EI	β-glucanase isoenzyme I, coded by *HvGlb1*
EII	β-glucanase isoenzyme II, coded by *HvGlb2*
FAOSTAT	Food and Agriculture Organization of the United Nations Statistics Division
FCDC	Field Crop Development Centre
FDR	False Discovery Rate
G x E	Genotype by Environment Interaction
HSD	Honest Significant Difference
IBD	Identical by Descent
IBSC	International Barley Genome Sequencing Consortium
IBSC_2012	2012 map generated by the International Barley Genome Sequencing Consortium
Indel	Insertion/Deletion
K	Kinship (Familial Relatedness)
KCRF	Kernen Crop Research Farm
LD	Linkage Disequilibrium
LOD	Logarithm of Odds
LRR	Leucine-Rich Repeat
LS	Least Square

MCMC	Markov Chain Monte Carlo
MDS	Multidimensional Scaling
MK	CDC Meredith x CDC Kindersley
MMAS	Molecular Marker-Assisted Selection
NGS	Next Generation Sequencing
NTSYS	Numerical Taxonomy System
OWB	Oregon Wolfe Barley
P	Genome-wide Relatedness
PCA	Principal Components Analysis
PGSB	Plant Genome and Systems Biology
Q	Population Structure
QTL	Quantitative Trait Loci/Locus
RAPDs	Randomly Amplified Polymorphic DNA
RCBD	Randomized Complete Block Design
RFLPs	Restriction Fragment Length Polymorphisms
RIL	Recombinant Inbred Line
SF	Crop Science Seed Farm
SNP	Single Nucleotide Polymorphism
SSRs	Simple Sequence Repeats
UDP	Uridine Diphosphate
U of S	University of Saskatchewan
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
USDA-ARS	United States Department of Agriculture Agricultural Research Service

1.0 INTRODUCTION

Barley (*Hordeum vulgare* L.) is the fourth most widely-produced cereal in the world after maize, rice, and wheat (FAOSTAT 2013). The high malt extract yield obtained from barley makes it the most popular grain for malting and subsequently brewing (Fischbeck 2002). One of the most important malting quality characteristics of barley is malt/wort β-glucan concentration because high amounts of this hemicellulose will result in viscous wort (Briggs 1978), which will in turn slow lautering and filtration times during brewing and increase haze and precipitates in the resulting beer (Izydorcyzk and MacGregor 2000; Jin *et al.* 2004). β-glucan may also reduce the rapidity of endosperm modification (Martin and Bamforth 1980), in part due to its prevalence in the endosperm cell walls (Fincher 1975), by obstructing the action of enzymes responsible for hydrolysing starches and modifying proteins during malting and mashing (Swanston and Ellis 2002). Malt/wort β-glucan content is influenced by both grain β-glucan content and malt β-glucanase activity, the primary enzyme which degrades the polysaccharide during the germination stage of the malting process.

Low concentrations of grain β-glucan content, high β-glucanase activity, or a combination thereof are desired in malting barley varieties. However, the exact mechanisms behind β-glucan synthesis and degradation are not fully understood. β-glucanase is known to exist as two isoenzymes, encoded by *HvGlb1*and *HvGlb2* (Loi *et al.* 1988; Slakeski *et al.* 1990), but it is likely that factors beyond these two enzymes are responsible for influencing β-glucanase activity. Of the two known isoenzymes, EII (encoded by *HvGlb2*) is more thermostable than EI (encoded by *HvGlb1*) (Barber *et al.* 1994; Loi *et al.* 1987). Barley varieties containing more heat stable β-glucanase would result in greater enzyme survival after kilning, allowing for further degradation of β-glucan during the mashing step of brewing. Varieties containing both high β-glucanase activity and high enzyme stability would be the most desirable.

Genomic regions associated with traits such as β-glucan and β-glucanase can be identified through quantitative trait loci (QTL) mapping or association mapping (AM). These two methods are complementary to one another in that markers identified via QTL mapping can

be assessed for broader utility by determining if the same marker is also identified in an AM population; conversely, any markers identified in AM populations can be validated in bi-parental QTL mapping populations. By identifying genomic regions which play a role in controlling these traits, one may begin to elucidate the mechanisms behind β-glucan synthesis and degradation, in addition to aiding in screening barley populations for these traits.

This study examined two different elite, two-row, hulled malting barley populations important to Western Canada: 1) a 100 member recombinant inbred line (RIL) population derived from a cross between varieties CDC Meredith and CDC Kindersley (MK population) that was used for QTL mapping, and 2) a 92 member association mapping population (AM population) containing breeding lines and varieties derived from eight malting barley breeding programs which focus on production in Western Canada. These populations were grown for three and two site-years, respectively, in and around Saskatoon, Saskatchewan, as a randomized complete block design (RCBD). β-glucan content was analyzed in the grain, malt, and wort for both populations, while β-glucanase activity was measured in kilned (finished) malt for both populations and in green (unfinished) malt for the AM population. Both populations were genotyped with single nucleotide polymorphism (SNP) markers via the Barley 9K Infinium Assay using the iSELECT Genotyping BeadChip (Illumina, Inc., San Diego, CA), and the MK population was additionally genotyped by the DArTseq method (Diversity Arrays Technology Pty. Ltd., Canberra, Australia).

1.1 Objectives and Hypotheses

This study had two objectives and related hypotheses concerning barley β-glucan content and high β-glucanase activity. The objectives were:

1) Assess the malt (green and kilned) β-glucanase activity and grain, malt, and wort β-glucan content in a QTL and an AM population to understand the range in variability and correlations of these traits.

2) Generate a linkage map and identify QTL linked to β-glucanase activity in the CDC Meredith x CDC Kindersley population. Use an association mapping population to identify loci influencing β-glucanase activity.

The hypotheses for these objectives were:

1) Green malt β-glucanase activity is more correlated to both malt and wort β-glucan content than is kilned malt β-glucanase activity. Differences in β-glucanase heat stability will be identified between barley genotypes.

2) Novel and previously identified genomic regions, including the *HvGlb1* and *HvGlb2* loci, controlling grain, malt, and wort β-glucan and green and kilned malt β-glucanase will be identified in the MK and AM populations.

2.0 LITERATURE REVIEW

2.1 Barley

2.1.1 Production

Barley (*Hordeum vulgare* L.) is believed to have multiple centres of diversity (Zohary and Hopf 2000) and is thought to have been domesticated in the southern part of the Fertile Crescent (Badr *et al.* 2000). Ancestors of *H. spontaneum* are believed to be the progenitors of modern two-row and possibly six-row barley (Briggs 1978). This notion of monophyletic origin has been supported through research utilizing amplified fragment length polymorphisms (Castiglioni *et al.* 1998).

Today, two-row malting barley dominates barley production on the Canadian Prairies as six-row malting barley varieties continue to lose market share (Canadian Grain Commission 2015). According to the Canadian Grain Commission (2015), the dominant barley variety in Western Canada in 2015 was AC Metcalfe, at the time occupying 38.5 % of the acreage, followed by CDC Copeland (35.4 %) and CDC Meredith (5.2 %). The three Prairie Provinces saw 2,513 thousand hectares seeded in 2015, roughly 900 thousand hectares fewer than were seeded a decade earlier. This downward trend is unsurprising and projected to continue because barley is competing for acreage with other crops of higher market value. Despite barley's water use efficiency and tolerance of short growing seasons, making it a practical choice in temperate environments with limited rainfall, feed quality barley tends to have lower market prices than a crop such as wheat (Blake *et al.* 2011).

According to the Food and Agriculture Organization of the United Nations Statistics Division (FAOSTAT), barley ranked fourth amongst cereals (behind maize, rice, and wheat) for tonnes produced in 2013 (FAOSTAT 2013). In Canada, barley was the fourth most produced crop overall in 2015 at over 8.2 million tonnes, behind wheat, canola, and (grain) corn (Statistics Canada 2015); Western Canada specifically produced approximately 7.8 million tonnes of barley that year (Canadian Grain Commission 2015).

2.1.2 Uses

Given the high quality standards that malting barley must meet, only approximately 25 % of the barley produced on the Canadian Prairies is selected for malting purposes each year (Canadian Grain Commission 2015). Despite the majority of the barley acreage being dedicated to malting varieties, most barley ends up being used as either feed or forage (Canadian Grain Commission 2015). Minor uses of barley grain include the production of fuel ethanol due to interest in and subsequent research into renewable energy (Blake *et al.* 2011), the production of high-maltose syrup for use in the food and pharmaceutical industries, and the extraction of β-glucan for use as a thickening agent in pharmaceuticals (Zhou 2009).

As a feed grain, barley competes primarily with maize and sorghum (Blake *et al.* 2011). Barley can be incorporated into the diets of livestock as grain or, for ruminants, as hay, forage, or silage (Briggs 1978; Fischbeck 2002). Barley straw is of limited nutritional value but can be chemically treated to improve its crude protein and digestibility (Petersen and Munck 1993). Spent grains and dried distillers solubles are also turned into animal feed (Briggs 1978).

With the bulk of barley used for feed, comparatively little is used for food. Human consumption of barley can come in the form of barley flour (used to make heavy breads), or the whole grain can be roasted, parched, popped, or pearled (Zhou 2009). Barley not used in baking often makes its way into soups, stews, sauces, porridge, and cereal (Zhou 2009). Given the health benefits associated with β-glucan and other soluble dietary fibres, and the fact that barley is relatively low in fat and rich in antioxidants, it is slowly being incorporated into more diets (Baik *et al.* 2011). While barley can be used to make a few non-alcoholic drinks such as barley water, barley tea, or barley coffee, it is more often associated with alcoholic beverages (Von Bothmer *et al.* 2003; Zhou 2009). Barley malt is often used to make malt or spirit vinegars, or distilled to produce potable spirits like malt whisky, although it is most widely known for its use in making beer (Briggs 1978).

2.2 The Malting Process

Though many cereals can be used for malt, barley is often the cereal of choice worldwide because it yields a high malt extract (Fischbeck 2002). Malt extract refers to the solid compounds brought into solution during the mashing process of brewing; the solution is then called wort (Briggs 1978). With higher levels of malt extract, less initial malt is needed to create

a comparable amount of wort. Low levels of seed protein are desirable because protein and starch content are inversely correlated and it is the carbohydrates of the starchy endosperm within the seed that contribute the most to malt extract. Malting barley typically contains 60 to 65 % starch and 10.5 to 13.5 % protein (Schwarz and Li 2011).

The malting process occurs in three basic steps: steeping, germination, and kilning. Malting is the controlled germination of the seed followed by controlled drying to cease the germination process. The aim is to produce malt with a characteristic flavour and colour, a desired endosperm modification, and a high enzyme activity without sacrificing too much dry weight, though on average, 10 % dry weight is lost (Delcour and Hoseney 2010). The internal changes in a barley kernel brought on by the malting process, specifically during germination, allow for a series of hydrolytic events to occur, broadly encompassing the degradation of cell walls, starch, and protein, which result in good quality malt (Hayes *et al.* 2003).

Good malt is a result of good grain quality. Fully mature barley of an acceptable malting variety is cleaned to remove chaff, broken kernels, weed seeds, insects, and other contaminants. To be accepted as malt, the barley grain must be free from disease, frost damage, heat damage, weathering and staining, DON (Fusarium head blight toxin), and chemical residues (Brewing and Malting Barley Research Institute 2010). According to the Brewing and Malting Barley Research Institute (2010), high quality malting barley should have a germination rate of at least 95 %, moisture content of no more than 13.5 %, protein content between 11 and 12.5 % (on a dry weight basis), and have fewer than 5 % peeled or broken kernels. The grain should be plump and of a uniform size and show no signs of pre-harvest germination. Accepted grain is typically sized by its plumpness, as a uniform germination rate requires a uniform rate of water infiltration during steeping (Delcour and Hoseney 2010). Quality characteristics such as protein content and thousand seed weight indicate the barley's potential yield of malt extract and enzymes (Pollock 1962a).

In the steeping process, the grain is periodically immersed in water until it reaches a moisture content of 41 to 49 %, depending on the end use of the malt, as moisture contents of 41 to 45 % are preferred for brewing (Briggs 1978). This step takes at least 24 h, typically being declared sufficient within 72 h. Prolonged steeping can have adverse effects on the grain including poor germination and irregular growth, so aeration periods are necessary (Pollock 1962b). Steeping is generally carried out at temperatures of 12° to 16°C to slow the growth rate

of microorganisms, minimize water loss, and reduce the risk of subsequent germination problems (Briggs 1978; Pollock 1962b). Aeration, kernel agitation, and steep water exchange are all necessary during the process to reduce microbial contamination and maintain a source of oxygen for the seed (Delcour and Hoseney 2010). At the completion of the steeping process, the water is drained and the grain is placed in germination beds (Delcour and Hoseney 2010).

The germination process typically takes four to six days, but may be as many as twelve, depending on the desired amount of endosperm modification (Delcour and Hoseney 2010; Pollock 1962b). Although the germination beds are sprinkled with water during the germination period to maintain the relative humidity in the beds close to 100 %, constant air movement and relatively low temperature (12 to 18°C) decrease the likelihood of fungal or bacterial development (Delcour and Hoseney 2010; Schuster 1962). The grain may be mixed and turned periodically to prevent matting of roots and to provide more uniform growth opportunities (Briggs 1978). The temperature, moisture content of the grain, and amount of air forced through the grain beds all impact the germination rate, with the goal of maximizing endosperm modification (Delcour and Hoseney 2010).

Endosperm modification increases the kernel's friability and causes the synthesis and release of bound amylolytic, proteolytic, and cytolytic enzymes (Pollock 1962b). Starch molecules are primarily hydrolysed into fermentable sugars and non-fermentable dextrins (Schwarz and Li 2011). Along with starch-degrading enzymes, the enzymes synthesized during germination hydrolyse proteins, cell wall constituents, and nucleic acids into smaller molecules which are necessary later in the brewing process, particularly during fermentation (Hayes *et al.* 2003). Fermentable carbohydrates comprise the majority of the malt extract which are converted into alcohol during brewing (Schwarz and Li 2011). Temperature, pH, and yeast species, along with duration of the process and the relative amounts of other additives in the initial malt blend, influence the type of beer produced (Briggs 1978).

Germination is considered complete when endosperm modification is deemed sufficient, generally once the acrospire averages between one-third and three-fourths the length of the kernel or accounts for about 5 % of the kernel weight (Delcour and Hoseney 2010; Pollock 1962b). At this point, kernel moisture is roughly 43 to 45 % (Schuster 1962). The germinated grain, known as green malt, is then dried with heat during kilning to produce kilned or finished malt.

Kilning serves to cease the germination process, drying the malt to moisture contents of ≤7 % so that it may be stored easily, the rootlets readily removed, and the kernels themselves crushed without difficulty during milling (Briggs 1978; Hayes *et al.* 2003). Kilning can be used to develop a distinctive flavour, aroma, and colour in the malt (Pollock 1962a). Temperatures vary depending on the desired qualities of the malt, though the heating process is almost always gradual and completed in stages. Kilning time typically takes anywhere from 16 h to 40 h (Schwarz and Li 2011). Kilning at lower temperatures will ensure less enzyme degradation, however kilning can be done in such a way that enzymes are totally degraded or slightly enhanced from the levels found in green malt (Briggs 1978). In general, high enzyme activity malts are produced at the expense of flavour, so little enzyme activity remains in malts with high flavour and dark colour (Delcour and Hoseney 2010). After kilning, the kernels are immediately scoured to remove the rootlets and the finished malt can be stored as is or ground into grist (Schuster 1962).

2.2.1 β-glucan and β-glucanase in the Malting Process

(1,3;1,4)-β-D-glucan (β-glucan) is a non-starch, soluble dietary fibre of cereals that is hydrolysed by the enzyme β-glucanase. β-glucans and arabinoxylans, a different type of hemicellulose, collectively comprise over 90 % of the cell wall within the starchy endosperm of barley (Hrmova *et al.* 1997). During germination, starchy endosperm cell walls hydrolyse more rapidly than those of the aleurone (Morrall and Briggs 1978). If degradation is insufficient, the resulting high levels of β-glucans make the malt undesirable because it will produce more viscous wort and create difficulties in the brewing process (Briggs 1978; Vis and Lorenz 1997). As with other cell wall polysaccharides that are hydrolysed during malting, the presence of wort β-glucan leads to slower lautering and filtration rates, increased cloudiness in the beer (Swanston and Ellis 2002; Izydorcyzk and MacGregor 2000; Jin *et al.* 2004), and possibly even increased beer viscosity (Li *et al.* 2005).

If β-glucan is not broken down, diffusion of germination enzymes and the subsequent mobilization of kernel reserves is hampered (Wang *et al.* 2004). However, germinated barley contains enzymes which catalyze the degradation of β-glucan and due to the rapid decrease in viscosity seen in the malt, it can be concluded that these enzymes are indeed internal rather than a product of microorganisms on the grain (Briggs 1978). (1,3;1,4)-β-glucan endohydrolase (β-

glucanase) is the enzyme believed to be primarily responsible for this degradation. Roughly half of the grain β-glucan present is broken down during the malting process (Ellis *et al.* 1997). As such, the barley cultivars that make the best malt have low levels of β-glucan, high activity levels of β-glucanase, or a combination thereof.

The best indicators of the final β-glucan content in beer are the levels of β-glucan in either the malt or the malt Congress extracts (wort obtained under a particular mashing schedule), both of which have a greater correlation to beer β-glucan content than does grain β-glucan content (Edney *et al.* 1998). However, malt quality is determined by numerous traits, including diastatic power (starch-degrading capacity of the malt), Kolbach index (soluble to total protein ratio), malt extract, and viscosity (Jin *et al.* 2011). These factors are largely controlled by genetics, although Jin *et al.* (2011) found that three of the four are not independent of each other. The Kolbach index was negatively correlated with viscosity and positively correlated with malt extract while malt extract was negatively correlated with viscosity (Jin *et al.* 2011). Evans *et al.* (1999) reported similar findings, with the Kolbach index being negatively correlated to β-glucan and viscosity in both wort and beer.

Wang *et al.* (2004) examined the levels of β-glucan and β-glucanase before and after the malting process using eight barley cultivars grown at seven different locations. Grain β-glucan content was, on average, 3.4 % while malt β-glucan content was substantially less, averaging 0.7 %. This mean reduction of nearly 80 % was a direct result of enzyme activity during the malting process. β-glucanase activity in the grain was low while malt β-glucanase levels were on average eight times greater. The study noted that malt β-glucan content appeared to be more dependent on the malt β-glucanase activity level than on the original grain β-glucan content; in addition, grain β-glucanase activity was found to be a good indicator of malt β-glucanase activity. However, the β-glucanase activity in the mature seed was small.

β-glucanase activity in the malt depends upon a variety of factors, including barley variety, growing environment, and malting conditions (Jin *et al.* 2011). β-glucanase levels are very low in mature barley grain and remain unchanged throughout the steeping process, increasing greatly only during the germination process before being rapidly destroyed during even mild kilning (Bamforth and Martin 1983). Georg-Kraemer *et al.* (2004) found that there was an average loss of 60 % of the β-glucanase activity after kilning. A similar study by Barber *et al.* (1994) found that β-glucanase activity was reduced by 16 to 26 % from green to kilned

malt, depending on the variety. When the green malt was heated at 48°C for ten minutes, losses of β-glucanase activity ranged from 39 to 63 %.

β-glucan degradation during germination can be promoted through increased moisture, modestly increased temperature, or both (Kuusela *et al.* 2004). In general, β-glucanase activity throughout the entire germination period can be increased with higher moisture levels, and while a higher germination temperature enhances β-glucanase synthesis at the start of germination, it results in lower β-glucanase activity at later stages (Kuusela *et al.* 2004). For improved β-glucanase survival, lower kilning temperatures would be desired (Bamforth and Martin 1983), unless the heat lability of the existing β-glucanase enzymes can be improved.

2.3 (1,3;1,4)-β-D-glucans

(1,3:1,4)-β-D-glucans (β-glucan), sometimes referred to as mixed-linkage glucans, are not widely distributed outside the Poaceae family of plants (Fincher 2009b). Within some members of this family, specifically barley, oat, and rye, β-glucans are the major constituent of the cell walls (Fincher 2009b; Fincher and Stone 2004). In the cell walls of barley, β-glucan comprises 70 to 75 % of the starchy endosperm (Ballance and Manners 1978; Fincher 1975), 26 % of the aleurone (Bacic and Stone 1981), 10 to 19 % of the four-day-old coleoptile (Fincher 2009b; Sakurai and Masuda 1978), 16 % of the young leaf (Fincher 2009b), and 5 % of the stem (Kokubo *et al.* 1989; Fincher 2009b). Despite its abundance in endosperm cell walls, β-glucan here represents a minimal portion of total grain β-glucan, as the majority exists in the unmodified region of the endosperm which is not immediately accessible to β-glucanase (Gianinetti 2009). In germinating grain, variation in total β-glucan content ranged from 4.0 to 7.4 % (Anker-Nilssen *et al.* 2008), but in a study which included four waxy mutant (low amylose) varieties, β-glucan content varied from 2.5 to 8.3 % (Holtejølen *et al.* 2006).

In general, β-glucan concentrations are higher in hulless, waxy mutant, low amylose starch varieties of barley than in varieties with normal starch (Holtekjølen *et al.* 2006). Typically, barley cultivars high in β-glucan tend to have thicker husk and bran layers, high kernel elastic moduli, and high compressional viscosities (López-Perea *et al.* 2012). In hulless varieties, more β-glucan is stored in the aleurone layer and at the border between the aleurone and subaleurone layers, whereas in hulled varieties, there is a more uniform distribution of β-glucan in the kernel's endosperm (Wirkijowska *et al.* 2012).

While some hemicelluloses, such as hexoses, are composed of linked polymers, β-glucans are formed by chains of monomers (Delcour and Hoseney 2010). β-glucans can be subdivided by their solubility: soluble in hot water, soluble in alkali solution but insoluble in hot water, or insoluble in both hot water and alkali solution (Forrest and Wainwright 1977). The water solubility of β-glucan is influenced by its molecular structure via its ratio of (1,3)-β- to (1,4)-β-linkages (Fincher and Stone 2004). The content of water-soluble β-glucans is significantly influenced by the growth temperature of barley during grain filling, with solubility and molecular weight tending to increase with temperature (Anker-Nilssen *et al.* 2008).

Because grasses are part of a more recently evolved group of plants, it is likely that β-glucan evolution also occurred relatively recently (Fincher 2009a; Kellogg 2000). However, the precise evolution of β-glucan is unknown. β-glucan may have evolved independently in a number of diverse species or evolved early on as a cell wall component of fungi and primitive plants, but was selected against in all but those species from which grasses descended (Burton and Fincher 2009). Outside of the grasses, β-glucan or β-glucan-related components have been found in species such as some lichens (Olafsdottir and Ingólfsdottir 2001), bryophytes (Popper and Fry 2003), and horsetail (Sørensen *et al.* 2008). Comparative genomics techniques and genome sequencing have aided in tracing the evolution of β-glucan through the identification of β-glucan synthesis genes (Fincher 2009a). Evidence suggests that a single, new biosynthetic gene would have been sufficient for the first synthesis of the mixed-linkage glucans, but their degradation was likely the result of the adaptation of multiple existing hydrolytic enzymes (Fincher 2009a; Fincher and Stone 2004).

2.3.1 Functional Roles

β-glucans are an important component of primary and secondary cell walls in the grasses (Fincher 2009a). β-glucan is pliable and less inert than cellulose, yet still strong enough to provide a degree of support to the cell wall (Delcour and Hoseney 2010; Fincher 2009b). It is readily found in secondary cell walls, emphasizing the importance of its structural role in certain regions, such as expanding cell organs and vascular tissue (Gibeaut and Carpita 1993). Its asymmetrical structure means it and other non-cellulosic polysaccharides form a gel-like matrix within the cell wall, giving it a certain level of flexibility, porosity, extensibility, and compressibility which thereby allows multiple essential cell functions to take place (Fincher

2009a; Woodward *et al.* 1983). For instance, its porosity is conducive for the transfer of water, nutrients, and other small molecules across the cell wall and into, or from, the starchy endosperm (Fincher 2009b). In addition, soluble β-glucan can comprise up to 18 % of the total stored glucose in the walls of the starchy endosperm of cereal grains and thus functions as a storage polysaccharide (Morrall and Briggs 1978). As such, it would be mobilized during germination, and it has been proposed that it can also provide an energy source for other growing tissues, such as in leaves during periods of darkness when β-glucanase levels rise (Roulin *et al.* 2002).

High levels of β-glucans are undesirable in the malting and brewing industries, as well as in animal stock feed. For the same reason it can form a gel-like matrix in the cell wall, the presence of β-glucan increases the viscosity of liquids, leading to poor wort separation and the need for increased beer filtration time (Fincher and Stone 2004). In monogastric animals, such as pigs and poultry, β-glucan's high viscosity has an anti-nutritional effect as it results in slower diffusion of digestion enzymes and absorption of nutrients, ultimately causing slower growth rates (Fincher and Stone 2004). In poultry specifically, the poor digestibility of high β-glucan feeds usually results in the condition of sticky feces, increasing hygiene and handling problems faced by producers (Classen 1996; Fincher and Stone 2004).

In humans, β-glucan is an important soluble dietary fibre. Oat and barley β-glucan is associated with lower plasma cholesterol levels and a lower risk of heart disease development (Delcour and Hoseney 2010; Othman *et al.* 2011). The former effect is likely due to β-glucan's formation of a viscous layer in the small intestine which then inhibits the uptake of dietary cholesterol, slows the absorption of nutrients, and reduces the reabsorption of bile acids leading to the increased formation of bile acids from LDL cholesterol (Lia *et al.* 1995; Lund *et al.* 1989; Shelat *et al.* 2011). β-glucan is also believed to boost the immune system, thereby helping to prevent or combat infections, and it is thought to have anti-coagulant, anti-cytotoxic, anti-mutagenic, and anti-tumorigenic properties (Mantovani *et al.* 2008; Volman *et al.* 2008).

β-glucan has a variety of industrial uses. It can be cast as biodegradable films, used to increase the foam and emulsion stabilizing ability of a substance, and used to increase water absorption in dough if used as a flour supplement (Fincher and Stone 2004). Its ability to form a gel has potential use as a partial salt replacement, such as in cases where chicken breast meat is processed under high pressure (Omana *et al.* 2011). Other forms of β-glucan, such as fungal (1,3)-β-D-glucan, are being used to create gels in the pharmaceutical industry for applications

such as drug delivery (Lehtovaara and Gu 2011).

2.3.2 Structure and Molecular Arrangement

β-glucan molecules are named on the basis of their linkages and configuration. It is synthesized by the polymerization of β-D-glucopyranosyl monomers via the first carbon of one monomer with the third or fourth carbon of another (Burton and Fincher 2009; Fincher and Stone 2004). The most common form of β-glucans in cereals, (1,3;1,4)-β-D-glucan, results when (1,3)-β- and (1,4)-β-linkages join together β-D-glucopyranosyl residues to create unbranched, linear chains of polysaccharides (Fincher and Stone 2004). In cereals, β-glucans tend to have a degree of polymerization between 300 and 1,850 residues, depending on the species, extraction procedure, and molecular mass determination method (Fincher and Stone 2004). The solubility of these molecules decreases as the degree of polymerization rises, generally becoming completely insoluble in water when the degree of polymerization exceeds 100 residues (Mantovani *et al.* 2008).

According to Fincher and Stone (2004), the ratio of (1,4)-β- to (1,3)-β-linkages generally falls in the range of 2.2 to 2.6:1. While this ratio is relatively constant, the order of linkages is not set and instead appears in irregular patterns. While regions of two or three adjacent (1,4)-β-linkages are the most frequent, stretches of 5-20 adjacent (1,4)-β-linkages are not uncommon and can comprise up to 10 % of the polysaccharide chain. Conversely, (1,3)-β-linkages are rarely found together and are instead separated by at least two (1,4)-β-linkages. Therefore, (1,4)-β-linked oligoglucosides (cellodextrins) form cellotriosyl units, cellotetraosyl units, or longer units, with each set of units in the final polymer separated by a single (1,3)-β-linked monomer (Staudte *et al.* 1983). Upwards of 90 % of the total water-soluble β-glucan derived from barley flour at 40ºC is in the form of cellotriosyl and cellotetraosyl units linked by individual (1,3)-β-linkages (Staudte *et al.* 1983). Conversely, alkali-extractable β-glucans have large amounts of adjoining (1,4)-β-linkages, high ratios of cellotriosyl to cellotetraosyl units, and high ratios of (1,4)-β- to (1,3)-β-linkages (Izydorcyzk *et al.* 1998).

Fincher and Stone (2004) found that the ratio of trisaccharide to tetrasaccharide units in barley falls between 2.9 and 3.4:1. The size of the overall polysaccharide is variable, resulting in differing molecular masses, but the average molecular mass of β-glucans ranges from 48,000 to 3,000,000, with the larger molecules having a greater degree of polymerization. While the

average molecular mass ratio for barley β-glucan is 1.18 (weight average molecular weight over number average molecular weight), certain β-glucan molecules associated with small amounts of protein may have molecular masses of up to 40,000,000.

Although linear, a chain of β-glucan has many molecular kinks (Woodward *et al.* 1983). These kinks are a direct result of the (1,3)-β-linked glucosyl residues (Woodward *et al.* 1983) and as a result the molecules cannot align to themselves over extended regions (Fincher and Stone 2004). Similarly, the formation prevents comprehensive intermolecular alignment, meaning the microfibrils formed are not well-structured (Woodward *et al.* 1983). Given the kinks in its structure, barley β-glucan is generally considered to be a relatively flexible molecule (Woodward *et al.* 1983). In solution, water-soluble β-glucans are able to adopt a somewhat inflexible, partially extended, snake-like chain formation which results in their gelling properties, but in a solid state, the chain appears slightly more helical in nature (Fincher and Stone 2004).

Johansson *et al.* (2004) found that roughly 25 % of the total β-glucan content in barley is insoluble. This insoluble β-glucan is bonded non-covalently with other components of the cell wall (Woodward *et al.* 1983). β-glucans in barley are not covalently bonded with pentosans, but appear to be part of a matrix in the cell wall with water- and alkali-soluble arabinoxylans, and ultimately support cellulose microfibrils that are associated with arabinoxylans and mannose-rich polysaccharides (Fincher 1975; Forrest and Wainwright 1977). β-glucan and arabinoxylan compose roughly 75 % and 25 % of this matrix, respectively (Fincher 1975). Because some β-glucans in the cell walls are entangled with a portion of arabinoxylans, the latter can only be released with the solubilisation of the former (Izycorcyzk *et al.* 1998). As such, insoluble β-glucans improve the structural integrity of endosperm cell walls (Forrest and Wainwright 1977).

2.3.3 Synthesis

The mechanisms of β-glucan biosynthesis are still not entirely understood, but it is suspected that a variety of genes encode enzymes which play a role in synthesizing β-glucans. The discovery of cellulose synthase genes (*CesA*) led to the subsequent discovery of cellulose synthase-like genes (*Csl*), with the latter gene family thought to encompass the genes for β-glucan synthesis (Burton *et al.* 2006). Quantitative trait loci (QTL) mapping has been useful for identifying particular genes linked with β-glucan synthesis, thereby providing more order to the genes and gene families responsible.

Both the *CslF* and *CslH* gene families contribute to grass β-glucan synthesis to some degree, but likely act independently of one other (Doblin *et al.* 2009). β-glucan synthesis is almost certainly the result of either multiple enzymes or a multi-enzyme complex, possibly in combination with proteins and other cofactors, the components of which have not yet been identified (Buckeridge *et al.* 1999; Burton *et al.* 2006; Fincher 2009b). Barley is believed to have only one *CslH* gene (Doblin *et al.* 2009), while multiple *CslF* genes exist (Burton *et al.* 2008).

The *CslF* group of genes are likely responsible for β-glucan synthesis in cereals, given that the genes are thought to be cereal-specific (Hazen *et al.* 2002). The first seven *HvCslF* genes, as documented by Burton *et al.* (2008), were divided into two classes on the basis of their intron-exon arrangements. Four genes (*HvCslF3*, *HvCslF4*, *HvCslF8*, and *HvCslF10*) were mapped to a single locus on barley chromosome 2H where a known QTL for β-glucan content exists, while the remaining three were mapped to chromosomes 1H (*HvCslF9*), 5H (*HvCslF7*), and 7H (*HvCslF6*). The genes on chromosomes 1H and 7H fell near known QTL regions responsible for grain β-glucan content. Schreiber *et al.* (2014) identified three additional genes, mapping them to 2H (*HvCslF12* and *HvCslF13*) and 7H (*HvCslF11*). They noted *HvCslF13* as a suspected pseudogene as it gives rise to a truncated protein, but observed restricted expression of *HvCslF11* and *HvCslF12* in the root and leaf, respectively. These genes may be involved in β-glucan synthesis in those regions, but biosynthesis could not be authenticated.

In barley, *HvCslF6* appears to be the key factor in determining the synthesis of β-glucans, as a mutation in this gene can result in plants lacking this hemicellulose (Taketa *et al.* 2012) or showing a significant decrease in its content in grain (Hu *et al.* 2014). Increased expression of either *HvCslF4* or *HvCslF6* results in higher levels of seed β-glucan (Burton *et al.* 2011), and differential expression of *HvCslF6* late in grain development (>30 days after pollination) through *cis*-regulation may explain quantitative differences observed between barley lines (Wong *et al.* 2015). When rice *CslF* genes were isolated and inserted into *Arabidopsis*, a plant which contains neither *CslF* genes nor β-glucans (Hazen *et al.* 2002; Zablackis *et al.* 1995), the presence of β-glucans was detected in the cell walls of the transgenic plants (Burton *et al.* 2006). The gain-of-function finding indicated that the products of the inserted *CslF* genes are, therefore, required for β-glucan synthesis, but as it depended on various innate factors such as the appropriate donor and acceptor substrates, it is likely that it was through an interaction with other genes that the β-

glucan was synthesized (Burton *et al.* 2006).

A similar experiment using the cellulose synthase-like *HvCslH1* gene on chromosome 2H of barley also produced β-glucan synthesis in *Arabidopsis* plants (Doblin *et al.* 2009). Low levels of the *CslH* gene transcripts, designated *HvCslH1*, can be found in a variety of barley tissues, including floral, vegetative, and developing grain tissue (Doblin *et al.* 2009). Slightly higher levels are present in leaf tips where the cells are no longer actively growing (Doblin *et al.* 2009). However, the greatest presence of *HvCslH1* is in cells undergoing secondary cell wall thickening, notably lignified cells such as sclerenchyma fibre or xylem (Doblin *et al.* 2009).

Shu and Rasmussen (2014) suggested the glucan synthase-like 3 gene (*HvGSL3*) played a minor role in β-glucan synthesis in barley, acting either through biosynthesis or regulation, although the major β-glucan-associated gene identified in their study was *HvCslF6*. The *Lys3* locus is believed to be involved in the regulation of *HvCslF6*, being functional and active during the majority of seed development prior to desiccation (Christensen and Scheller 2012). However, β-glucan in the primary and secondary cell walls of vegetative issues is likely under the control of a different regulatory mechanism (Christensen and Scheller 2012). *Lys5* mutants show increased incorporation of β-glucan into their cell walls, which is proposed to be part of a feedback mechanism where an increase in β-glucan is related to an increased cytosolic concentration of ADP-glucose and, by consequence, a high concentration of UDP-glucose (Christensen and Scheller 2012). UDP-glucose acts as a substrate for β-glucan synthesis and requires a cofactor of either magnesium or manganese (Gibeaut and Carpita 1993). If the *CslH* and *CslF* genes only synthesize one type of linkage, a currently unidentified enzyme such as a glucosyltransferase may be responsible for the other type (Doblin *et al.* 2009).

β-glucan is not synthesized in dividing cells, but rather accumulates in the starchy endosperm cell walls of developing grains during cell enlargement (Buckeridge *et al.* 1999; Carpita and Gibeaut 1993). A similar pattern is found in barley coleoptiles, where β-glucan increases during cell elongation and decreases during maturation (Gibeaut *et al.* 2005). According to Becker *et al.* (1995), (1,3)-β-glucan (callose) synthesis depends on the presence of micromolar levels of calcium ions, whereas (1,3;1,4)-β-D-glucan synthesis does not. Both types of β-glucan are actively produced in membranes obtained from barley leaves and coleoptiles, but not those obtained from roots. Optimal synthesis for β-glucan was at pH 8.5 between 20 and 25°C and β-glucan synthesis peaked approximately 85 to 90 h after planting, declining after 90 h

(Becker *et al.* 1995). Wilson *et al.* (2006) found that β-glucan appeared four to five days after pollination within already formed and still developing anticlinal and periclinal cell walls.

At the subcellular level, it was believed that β-glucan synthesis took place primarily in the Golgi apparatus, where other hemicelluloses are synthesized (Gibeaut and Carpita 1993; Hazen *et al.* 2002). It was thought that cellodextrin synthesis occurs in the endoplasmic reticulum or the Golgi, with polysaccharide assembly either occurring there (but remaining in a largely inaccessible form to labelling antibodies used to identify its presence until modification at the plasma membrane) or at the plasma membrane (Doblin *et al.* 2009; Wilson *et al.* 2006). From the Golgi, the polysaccharide or its components would be transported via vesicles to the plasma membrane and then across the extracellular space to be subsequently incorporated into the cell wall (Gibeaut and Carpita 1993). Kim *et al.* (2015) found that in *Brachypodium*, *CslF6* appears capable of producing both β-glucan linkage types and that the fluorescent protein-tagged version of this gene protein was located in the Golgi, meaning that β-glucan was channelled through the Golgi to the cell wall. Other evidence supports the hypothesis that β-glucan synthesis in various grasses (including barley) occurs primarily at the plasma membrane, as antibodies to *CslF6* congregated in the plasma membrane and their presence in this area was verified through membrane fractionation experiments (Wilson *et al.* 2015). The same study also found that antibodies to *CslH1* were chiefly observed in the endoplasmic reticulum and the Golgi rather than the plasma membrane (Wilson *et al.* 2015).

2.3.4 Degradation

β-glucan is hydrolysed during cell wall breakdown, and its degradation is widely believed to occur in two steps. While this biphasic model is popular, some degradation models suggest the process is monophasic and solely attribute β-glucan degradation to the enzyme β-glucanase (EC 3.2.1.73) (Gianinetti *et al.* 2007). However, β-glucanase cannot break down at least seven to eight percent of the β-glucan present in cell walls, so it is probable that numerous enzymes are responsible for the degradation of β-glucan and that they degrade the polysaccharide in at least two steps (Gianinetti *et al.* 2007). While many such enzymes are proposed to be responsible, the exact enzymes involved and their degree of involvement is currently unknown.

In the biphasic model, the first step is solubilisation and the second is polymer digestion (Bamforth 2010), although it was initially thought that proteolysis was the first step in β-glucan

degradation (Forrest and Wainwright 1977). The enzymes responsible for the first step are sometimes simply referred to as solubilases (Bamforth 2010). β-glucanase itself is not known to act as a solubilase (Bamforth *et al.* 1997). Given the unorganized structure of hemicellulose in cell walls, some β-glucan may be inaccessible to the enzymes which hydrolyse it, so enzymes responsible for hydrolysing other hemicelluloses, such as pentosan or arabinose, must be present for more complete degradation of β-glucan to occur (Bamforth 2010; Delcour and Hoseney 2010).

β-glucan from isolated starchy endosperm cell walls can be solubilised by both endo-β-glucanase and endo-xylanase (Kanauchi and Bamforth 2002). (1,4)-endo-β-glucanase (cellulase) is proposed to be a solubilase (Yin *et al.* 1989) and is thought to hydrolyse long blocks of (1,4)-β-linked glucosyl residues present between two single (1,3)-β-linkages (Yin and MacGregor 1989). Wilhelmi and Morgan (2001) observed that cellulase acts as a solubilase under dilute conditions, but when more concentrated, it will cleave small cello-oligomer residues of β-glucan. Another proposed solubilase enzyme is carboxypeptidase, which was found to be most active during the earliest stages of barley germination, with activity peaking after approximately three days (Bamforth *et al.* 1979). β-glucan solubilase has been consistently shown to be resistant to heating at 65°C, with a residual activity of roughly 60 % (Bamforth *et al.* 1997), and speculation of the presence of a thermostable β-glucan solubilase enzyme persists (Bamforth 2010). It is likely that the enzymes involved in the solubilisation process have different thermosensitivities, so an increase in temperature would result in a decrease of certain enzyme activities (Gianinetti *et al.* 2007; Yin and MacGregor 1989).

β-glucanase is thought to be largely responsible for the second step of β-glucan degradation, which involves the splitting of β-glucan into smaller molecules (Gianinetti *et al.* 2007). While β-glucanase can readily hydrolyse soluble β-glucan, other enzymes are necessary to break down insoluble β-glucan (Gianinetti *et al.* 2007). Manners and Marshall (1969) identified five enzymes in malted barley extract which hydrolyse β-glucosidic linkages, three of which were β-glucanases (endo-(1,4)-β-glucanase, endo-(1,3)-β-glucanase, and endo-(1,3;1,4)-β-glucanase) and two of which were β-glucosidases (true glucosidases, not exo-β-glucanases). The hydrolysing enzymes present in this depolymerisation step, such as the 60-kDa β-glucosidase BGQ60, remove glucose from the non-reducing end of β-glucan and reduce the polysaccharide to glucose units, particularly 3(O)-β-cellobiosyl D-glucose and 3(O)-β-cellotriosyl D-glucose

(Hrmova *et al.* 1997; Leah *et al.* 1995). Endo-(1,3)-β-glucosidase, encoded by *Eng1* on chromosome 4H, hydrolyses β-glucan and releases UDP-glucose in the Golgi which is required for β-glucan production (Islamovic *et al.* 2013).

The different bonds within β-glucan are targeted by different enzymes (Delcour and Hoseney 2010). (1,3)-β-linkages appear to be targeted preferentially over (1,4)-β-linkages (Leah *et al.* 1995). However, it is unclear if the rate at which the bonds are cleaved is equal for both linkages (Delcour and Hoseney 2010). The actual rate of degradation likely depends on the level of enzyme activity in the malt, the amount of grain β-glucan present, and the accessibility of the β-glucan molecule (Gianinetti *et al.* 2007).

Gajdošová *et al.* (2007) found that temperature influences degradation rate, noting that the decline in soluble β-glucan content in barley bran and flour during storage can be slowed slightly, though not stopped, when samples are stored at cooler temperatures (8°C) as opposed to room temperature (25°C). Moisture content of the grain is another factor impacting β-glucan content. Soluble β-glucan content drops when high moisture content barley is stored for 35 to 90 days (Svihus *et al.* 1997). Conversely, insoluble β-glucan content under these conditions is higher unless additional degrading enzymes like β-glucanase are added (Svihus *et al.* 1997). In both cases, enzyme addition results in reduced acid extract viscosity and improved fibre degradation (Svihus *et al.* 1997).

2.4 β-glucanase

There are three general classes of β-glucanase found in barley: (1,4)-β-glucan 4-glucanohydrolase, (1,3;1,4)-β-glucan 4-glucanohydrolase (EC 3.2.1.73; also called (1,3;1,4)-β-D-glucan endohydrolase or (1,3;1,4)-β-glucanase), and (1,3)-β-glucan glucanohydrolase. The second of these enzymes is considered the most important because of its greater abundance in the endosperm of germinating barley grain (Woodward and Fincher 1982). As previously mentioned, the purpose of β-glucanase is to hydrolyse β-glucan. β-glucanase activity is low in mature barley grain, but it is rapidly synthesized during the germination process (Xue *et al.* 2008). β-glucanase activity appears to be positively correlated to total protein content, but high levels of salt-soluble protein have a significant negative correlation with β-glucanase activity (Xue *et al.* 2008). As high protein levels are considered unfavourable for malting barley, resulting in decreased malt yield due to less starch content, the optimal balance between protein

content and β-glucanase activity can be difficult to achieve (Briggs 1978).

Ballance *et al.* (1976) studied the distribution and development of endo-β-glucanase activity in barley. They found that endo-(1,4)-β-glucanase activity occurred primarily in the hull and pericarp tissues, but was also detectable in the endosperm late in the germination process. Endo-(1,3)-β-glucanase activity was concentrated in the embryo and scutellum prior to steeping, but increased considerably during germination in the endosperm and aleurone. (1,3;1,4)-β-glucanase activity, although concentrated in the aleurone and endosperm, was also found in small amounts in the hull, embryo, and scutellum.

Two isoenzymes of barley (1,3;1,4)-β-glucanase are known. The first isoenzyme, hereafter referred to as EI, is encoded by the *HvGlb1* gene on chromosome 1H of barley, whereas the second isoenzyme, EII, is encoded by the *HvGlb2* gene on chromosome 7H (Loi *et al.* 1988; Slakeski *et al.* 1990). In general, the EI and EII isoenzymes are expressed in different tissues and at different times (Hrmova *et al.* 1997; Stuart *et al.* 1986). EI synthesis occurs predominantly in the scutellum, but is expressed in other tissues during seedling development, while EII synthesis is restricted to the aleurone (Slakeski and Fincher 1992; Stuart *et al.* 1986). Neither enzyme requires calcium for activity, but the amount of enzyme secretion is increased in the presence of both calcium and gibberellic acid (Stuart *et al.* 1986). However, this stimulus provided by gibberellic acid is of limited practical importance within the malting and brewing industry given the larger impacts of germination time and mashing temperature on activity levels (Bourne and Pierce 1970).

Synthesis of mRNA encoding β-glucanase in the aleurone layer appears to be hormonally regulated (Mundy and Fincher 1986). mRNA synthesis is increased by gibberellic acid and decreased by abscisic acid, with mRNA preferentially increased for EII over EI (Mundy and Fincher 1986; Litts *et al.* 1990). In the scutellum, EI mRNA predominates during the germination process (Slakeski and Fincher 1992). Slakeski and Fincher (1992) found that while mRNA for EI was present in leaf tissue about eight days after germination and continued to rise in that tissue for approximately twelve more days, no mRNA for EII was detected because of its tissue-specific synthesis. Similarly, mRNA for EI was found at high levels in young roots around three to six days after germination, while EII was either not detected or found at very low levels. As such, transcription of EI occurs in young leaves, the scutellum, and aleurone, while EII is transcribed only in the aleurone layer of germinated grain (Slakeski *et al.* 1990).

While quite similar in terms of their kinetic properties and substrate specificities, the isoenzymes differ considerably in terms of their chemical and enzymatic properties (Woodward and Fincher 1982). EI and EII differ in molecular weight, isoelectric point, and percentage of associated carbohydrate, being 28 kDa, pH 8.5, and 0.7 %, and 33 kDa, > pH 10, and 3.6 %, respectively (Woodward and Fincher 1982). β-glucanase is heat labile, with EII being more stable than EI, meaning EII is stable over a greater range of temperatures although it shows slightly lower enzyme activity than EI (Barber *et al.* 1994; Loi *et al.* 1987; Woodward and Fincher 1982). This thermostability is perhaps due to a preference for guanine (G) and cytosine (C) nucleotides in the wobble position of its codons, as these base pairs form stronger bonds than adenine (A) and thymine (T) (Fincher *et al.* 1986). It may also be a result of the greater carbohydrate content of EII, as it appears to be a glycoprotein (Woodward and Fincher 1982). The greater molecular weight and isoelectric point of EII may be explained by the fact that it contains about 40 more amino acid residues than EI, with the majority of these amino acids being basic or hydrophobic (Woodward and Fincher 1982).

2.4.1 Genetic Inheritance

As previously mentioned, the isoenzymes of β-glucanase are synthesized from the *HvGlb1* and *HvGlb2* genes on barley chromosomes 1H and 7H, respectively (Litts *et al.* 1990; Wolf 1991). Both genes are characterized by a single large intron within the coding region. The *HvGlb1* gene contains one intron of 2,490 bp and two exons of 73 and 929 bp (Litts *et al.* 1990). The 4,849 bp gene region, including the 5' leader and 3' flanking regions, codes for a 334 amino acid polypeptide that is the active enzyme (Jin *et al.* 2011). Typically, there is less variation in this gene in cultivated barley compared to wild barley (Jin *et al.* 2011).

The coding region for the *HvGlb2* gene is shorter than the *HvGlb1* gene, being only 1,002 bp, but it is interrupted by a 2,952 bp intron (Wolf 1991). The gene encodes a 306 amino acid protein (Fincher *et al.* 1986). Upstream of the coding region, the known sequence includes 1,035 bp, with 171 bp being found downstream of this region (Wolf 1991). Given the sequence homology of the amino acids produced in the NH_2-terminal, with only three of forty differing between EI and EII, and the 92 % overall homology between the coding regions, it is likely that the two genes were derived from a single ancestral gene (Fincher *et al.* 1986; Wolf 1991; Woodward *et al.* 1982).

2.5 Quantitative Trait Loci (QTL) Mapping

Quantitative trait loci (QTL) mapping is the identification and subsequent chromosomal mapping of genes associated with particular traits. Populations derived from bi-parental crosses which are segregating for the traits of interest are saturated with molecular markers and phenotyped. Initially, QTL mapping in barley relied on genetic maps created using random amplified polymorphic DNA (RAPDs) (Thomas *et al.* 1995), restriction fragment length polymorphisms (RFLPs) (Backes *et al.* 1995), and amplified fragment length polymorphisms (AFLPs) (Yin *et al.* 1999), with subsequent genetic maps created from simple sequence repeats (SSRs) (Xue *et al.* 2009) and diversity arrays technology (DArT) markers (Wenzl *et al.* 2004). However, with the exception of the last marker type, these genetic maps typically had poor genome coverage and thus QTLs were missed or suffered from poor resolution. DArT markers have been used to create medium density genetic maps (Wenzl *et al.* 2004) and have been combined with other marker types to create consensus maps with greater genome coverage (Wenzl *et al.* 2006). More recently, single nucleotide polymorphisms (SNPs) assayed on high throughput genotyping platforms, such as the Illumina GoldenGate assay or Infinium assay (Schmalenbach *et al.* 2011), have alleviated the issue of poor genome coverage, and there has been some interest in the use of insertion and deletion (indel) markers for genetic map production (Zhou *et al.* 2015). Nevertheless, resolution of QTL is still limited by the available number of recombinations present in a segregating population.

QTL analysis can allow for more efficient selection of malting quality during the breeding process (Han *et al.* 1995). For example, QTL pyramiding was used to shift non-malting germplasm into a suitable malting profile without the loss of other desirable traits (Emebiri *et al.* 2009). As a result of QTL analysis in barley that has helped to pinpoint genomic regions in the barley genome associated with grain, malt, and wort β-glucan, and β-glucanase activity in green and kilned malt, marker-assisted selection for these traits has become possible. However, the need to identify new alleles of these genes in order to make further improvements to already elite malting germplasm remains.

As of April 2016, GrainGenes 2.0 (Carollo *et al.* 2005; http://147.49.50.65/GG3/) listed 14 unique QTLs for β-glucan content (three for grain, ten for malt, and one for wort) and 15 QTLs for β-glucanase (three for green malt, six for kilned malt, and six which were undefined in

terms of their association with green or kilned malt), of which nine were unique. Han *et al.* (1995) identified grain β-glucan QTLs on chromosomes 1H (Glb1-ABC160) and 2H (ABG703-Chs1b; Adh8-ABG019), and malt β-glucan QTLs on chromosomes 1H (His3B-iPgd2; ABG702-ABA002), 3H (ABG471-Dor4A), 4H (WG622-ABG313B), and 7H (Brz-ABC156D; ABG011-ABC455). Mather *et al.* (1997) and Szűcs *et al.* (2009) mapped additional malt β-glucan QTLs on chromosomes 3H (Upg2, near ABG471; ABG607-MWG847) and 5H (CDO504, corresponding to POPA1_1472; MWG632, near MWG602A), while Oziel *et al.* (1996) found one wort β-glucan QTL on chromosome 5H (apADH-apt59A). Han *et al.* (1995) also discovered green malt β-glucanase QTLs on chromosomes 1H (Glb1-ABC160; ABC160-ABG464; His3B-iPgd2), 4H (ABG484-WG464), and 5H (Ale-ABC302), and kilned malt β-glucanase QTLs on chromosomes 1H (ABC160-ABG464; ABG464-His3B), 5H (Ale-ABC302; ABG707-ABG463), and 7H (Brz-ABC156D; ABG011-ABC455). Zwickert-Menteur *et al.* (1996) identified a green malt β-glucanase QTL on 1H (ABG464-His3B) which overlapped with a kilned malt β-glucanase QTL identified by Han *et al.* (1995).

2.6 Association Mapping

Association mapping (AM), also known as linkage disequilibrium (LD) mapping, detects QTLs based on the strength of the correlation between mapped genetic markers and specific traits (Mackay and Powell 2007). While QTL mapping is conducted on a population derived from a bi-parental cross, AM is carried out on a population of genetically dissimilar lines. As a result, AM tends to have a greater mapping resolution than is possible with QTL mapping because a larger number of recombination events are present in these more diverse populations (Rostoks *et al.* 2006). The increased resolution offered by AM increases the likelihood of identifying markers closer to the gene of interest within the genome, which in turn makes it more useful to plant breeders. The ability to use a diversity of lines also means that special populations do not need to be created for mapping, and thus AM fits well within the normal activities of breeding programs. One of the greatest dangers involved with AM is the possibility of generating false positives due to structure (close genetic relationships) present in the AM population, and thus measures of genetic variability are commonly included as a covariate during the AM procedure (Zhao *et al.* 2007).

Association mapping involves the examination of the non-random association of alleles

(linkage disequilibrium) at distinct loci on the same chromosome using estimates of their positions based on previously generated genetic maps (Mackay and Powell 2007; Rostoks *et al.* 2006). LD is caused by mutation, selection, migration, population admixture, population bottlenecks, founder effects, and genetic drift (Mackay and Powell 2007). While there is a clear relationship between genetic distance and LD, there is no obvious relationship between LD and physical distance (Rostoks *et al.* 2006).

Linkage disequilibrium is an important parameter in AM because mapping resolution is dependent on how rapidly LD decays with genetic distance (Mackay and Powell 2007). In barley, it has been shown that LD decays at different rates depending on the germplasm being examined. LD is typically higher in populations that have greater population structure (Malysheva-Otto *et al.* 2006). For example, LD analyzed across a 215 kb region surrounding the barley *Ha* (hardness) locus extended to at least 212 kb in cultivated germplasm, but only to 83 kb in landraces (with complete decay at 98 kb) (Caldwell *et al.* 2006). Wild barley often has lower levels of LD (Morrell *et al.* 2005). This relationship is key as the LD value determines the density of markers required for AM. Comadran *et al.* (2011) examined LD in cultivated barley and determined that, with genome-wide association scans, 87.3 % of the SNPs mapped within 5 cM of their position on the consensus map generated by Close *et al.* (2009), and 50 % of the SNPs mapped within 1 cM. In addition, Comadran *et al.* (2011) found that outside of centromeric regions, mapping resolution was reasonably high so SNPs associated with simple traits or QTLs with strong additive effects may be close enough to the gene in question to be used with reasonable confidence in marker-assisted selection.

Association mapping can take two approaches: whole genome scans or use of candidate genes. Low resolution, whole genome scans examine the entire genome (typically in populations with high LD) to identify candidate regions, followed by fine scale, high resolution scans to identify candidate genes using populations with low LD (Caldwell *et al.* 2006). The candidate gene AM approach targets specific genes that are suspected of being involved with the trait of interest. This requires prior evidence or well-known biochemical pathways which implicate specific genes.

Recognizing the need for a reference genome sequence in barley, the International Barley Genome Sequencing Consortium (2012) developed a physical map of 4.98 Gb, with more than 3.90 Gb anchored to a high-resolution genetic map. Continued work on mapping barley's 5.1 Gb

genome through next generation sequencing (Mascher *et al.* 2013a) and comparative genomic hybridization to identify copy number variation (Muñoz-Amatriaín *et al.* 2013) has aided in the development of databases such as Barleymap (Cantalapiedra *et al.* 2015; http://floresta.eead.csic.es/barleymap/) and Ensembl Plants (Kersey *et al.* 2014; http://plants.ensembl.org/index.html), which provide a framework of known genetic/physical map information. Individual population information can be compared to this reference genome, which may aid in using information derived from AM and QTL mapping in barley breeding.

2.7 Molecular Marker-Assisted Selection in Barley

Identifying QTLs for important traits has allowed for molecular marker-assisted selection (MMAS). QTL regions with large, consistent effects over different environments are best suited to MMAS (Han *et al.* 1997). MMAS permits selection of traits based on genetic markers linked to the genotype rather than the phenotype, increasing accuracy by eliminating environmental effects (Emebiri *et al.* 2009). When MMAS is used in combination with QTL pyramiding, selection for malt quality can become substantially more effective and more efficient, and it can be useful to select within populations for low-heritability traits like malt β-glucan content or for traits which are difficult and/or costly to assay (Emebiri *et al.* 2009; Han *et al.* 1997). Both recessive and dominant genes can be identified using MMAS, and tightly-linked markers decrease or eliminate the need for phenotypic screening (Thomas 2002).

Candidate markers for MMAS are identified through high-resolution mapping (Collard *et al.* 2005). The best markers are those closely linked to a particular trait (< 1 cM from the gene), which minimizes the likelihood of recombination occurring between the marker and trait locus during gamete formation. An ideal marker is located within the gene controlling the trait. Markers used for MMAS are typically validated by assessing how reliably they can predict the phenotype outside of the population in which they were identified (Collard *et al.* 2005).

While there are numerous marker types that can be used for MMAS, SNPs are an extremely useful marker type that have been extensively used in barley. These bi-allelic markers provide a comprehensive picture of the barley genome, in part because they are highly abundant and less prone to mutations than other markers, such as microsatellites (Kota *et al.* 2001). Partial genome sequencing in barley has identified tens of thousands of SNPs and led to the development of high density, high quality SNP maps (Comadran *et al.* 2012; Close *et al.* 2009).

High throughput genotyping platforms associated with these SNPs (e.g. Illumina GoldenGate and Infinium Genotyping Assays) have been particularly beneficial for identifying QTL, and alternate genotyping platforms (e.g. TaqMan and KASP) have been valuable for conducting MMAS.

MMAS can be effective for traits, such as disease resistance, for which phenotyping is time-consuming, labour intensive, or prone to false positives (i.e. escapes). Resistance to Barley Yellow Mosaic Virus complex using the resistance genes *rym4* and *rym5* (Rae *et al.* 2007), resistance to true loose smut using the *Un8* gene (Zang *et al.* 2015), resistance to scald using the *Rrs2* gene (Hanemann *et al*. 2009), and resistance to covered smut using the *Ruhq* gene (Grewal *et al.* 2008) have been successful examples of how this technology can improve the selection process in breeding programs. However, MMAS for malting quality QTLs has been limited because many QTLs are not validated and many of the QTL target alleles are already present within elite malting germplasm (Meyer *et al.* 2001). Coupled with the fact that malting quality is determined by multiple genes, it has been difficult to use MMAS for malting quality traits in barley breeding. However, QTLs observed in association studies are likely to be more relevant, if conducted within a population of elite malting germplasm, and more robust than those discovered through bi-parental QTL studies using wide crosses (Rae *et al.* 2007). In addition, ongoing research to fine map and ultimately clone the genes underlying QTLs will permit subsequent sequencing of these genes across a wide range of germplasm to identify novel and/or the most useful alleles.

3.0 PROJECT I: QUANTITATIVE TRAIT LOCI (QTL) MAPPING FOR β-GLUCAN AND β-GLUCANASE IN AN ELITE MALTING BARLEY POPULATION

3.1 Introduction

High levels of (1,3;1,4)-β-glucan (β-glucan) in malt are undesirable because the resulting high wort β-glucan creates viscosity problems during the brewing process (Briggs 1978). As such, malting barley varieties must produce low levels of malt β-glucan. These low levels can be achieved through low initial β-glucan content in the grain or high malt (1,3;1,4)-β-glucanase (β-glucanase) activity, the primary enzyme which hydrolyses β-glucan. This degradation occurs chiefly during the germination stage of the malting process, once β-glucanase has been synthesized, as much of the enzyme is denatured during kilning.

Biosynthesis of β-glucan is still not fully understood, but multiple genes are believed to be involved, so it is likely the result of multiple enzymes or a multi-enzyme complex (Buckeridge 1999; Fincher 2009b). Cellulose synthase-like (*Csl*) genes, the most probable candidates, are divided into families (e.g. *CslF* and *CslH*) and have been mapped to several barley chromosomes. For example, *HvCslF* genes have been located to chromosomes 1H, 2H, 5H, and 7H (Burton *et al.* 2008; Schreiber *et al.* 2014), while *HvCslH1* is on chromosome 2H (Doblin *et al.* 2009). The entire set of enzymes involved in the degradation of β-glucan is also not entirely understood, but β-glucan is known to be hydrolysed by β-glucanase. This enzyme exists as two isoenzymes in barley, encoded by *HvGlb1* and *HvGlb2* which are found on chromosomes 1H and 7H, respectively (Loi *et al.* 1988; Slakeski *et al.* 1990).

Quantitative trait loci (QTL) mapping has been a critical tool used over the past 20 years to identify regions of the barley genome associated with numerous traits, including those associated with malting quality. Szűcs *et al.* (2009) identified malting quality-related QTL in the Oregon Wolfe Barley (OWB) population, finding three QTLs for grain β-glucan (chromosomes 1H and 2H), 10 QTLs for malt β-glucan (chromosomes 1H, 3H, 4H, 5H, and 7H), one QTL for wort β-glucan (chromosome 5H), five QTLs for β-glucanase activity (chromosomes 1H, 4H, and 5H), one QTL for green malt β-glucanase activity (chromosome 1H), and three QTLs for kilned malt β-glucanase activity (chromosomes 1H and 7H). Some of the QTLs in this population, as with other populations, are located at known positions of β-glucan synthesis genes, such as the cluster of *HvCslF* genes on chromosome 2H and the *HvCslF6* gene on chromosome 7H (Burton *et al.* 2008; Pauli *et al.* 2015; Shu and Rasmussen 2014; Szűcs *et al.* 2009). *HvGlb1* on

chromosome 1H is within another region commonly identified in such QTL studies (Han *et al.* 1995; Szűcs *et al.* 2009; Zwickert-Menteur *et al.* 1996). Not all QTLs identified overlap with genes that may readily account for the observations made, and in narrower crosses common quality-related QTL regions may be monomorphic, emphasizing the need for continued studies to learn more about the genes which appear to affect various traits.

Comparison of QTLs linked to traits, such as β-glucan and β-glucanase, identified in different mapping populations has been made simpler by the development of consensus maps. Consensus maps combine different marker types (spanning the evolution of markers from RFLPs to SNPs) and integrate map order variations to provide a single, high marker density linkage map with increased genome coverage (Muñoz-Amatriaín *et al.* 2011). With such maps, new QTLs linked to a trait can be compared against previously known QTLs to determine if novel genes have been identified (i.e. if the QTL is located within a previously unmapped location in the genome) or if potentially new alleles have been identified (i.e. should the new QTL overlap with prior ones). The barley consensus map created by Muñoz-Amatriaín *et al.* (2011) contains 2,994 SNP loci, an improvement on those built in the past (Close *et al.* 2009; Wenzl *et al.* 2006), and work such as theirs aided in the development of the genome-wide physical map by the International Barley Genome Sequencing Consortium (2012) which covers 4.98 Gb. Resolution of such maps is being improved through various methods such as population sequencing (e.g. Mascher *et al.* (2013a)).

Markers underlying QTLs associated with traits of interest can be used for molecular marker-assisted selection (MMAS). Selecting for such markers in place of the trait itself can be extremely valuable, especially in cases where the trait is expensive, time-consuming, and has a low throughput. These three criteria would certainly apply to malting quality traits, including β-glucan and β-glucanase, where malting must be performed to produce the malt which will be analyzed (a process that takes 7 to 10 days), the cost per sample is approximately $50, and only a few thousand samples per year can be evaluated. As such, germplasm screening can be streamlined, allowing germplasm with the marker for the trait of interest to be identified earlier and advanced for further evaluation.

Although previous QTLs linked to β-glucan and β-glucanase have been mapped, there is a constant requirement to identify new QTLs and/or alleles for these traits in order to further improve elite malting barley varieties. As such, the goals of this study were to identify genomic

regions associated with four malting quality traits (grain β-glucan content, malt β-glucan content, wort β-glucan content, and kilned malt β-glucanase activity) in a bi-parental mapping population created from two malting barley varieties grown in Western Canada to determine if any unique loci or alleles controlling these traits could be found. One of the parents is known to produce malt with very low wort β-glucan content while the second parent produces moderate to higher wort β-glucan content. These traits were assessed over three site-years in Saskatchewan, Canada, and the correlations between them were calculated. A linkage map comprised of SNP and DArTseq markers was constructed to allow for the identification of significant β-glucan- and β-glucanase-related QTLs in this population.

3.2 Materials and Methods

3.2.1 Plant Material

A two-row, spring malting barley population was used in this study and consisted of 196 $F_{4:8}$ recombinant inbred lines (RIL) generated from a cross between CDC Meredith and CDC Kindersley (hereafter called the MK population). CDC Meredith has normal to slightly higher grain β-glucan content and normal to higher wort β-glucan content, while CDC Kindersley has normal to slightly lower grain β-glucan content and very low wort β-glucan content. This population was used for QTL mapping.

A subset of the MK population, 100 lines including five check varieties, was grown as a two replication randomized complete block design (RCBD) trial at the Crop Science Seed Farm (SF) in 2012. In 2013, this population was grown as a two replication RCBD at two locations (Kernen Crop Research Farm (KCRF) and the SF). The KCRF, located at NW 5-36-4 W3, has a Sutherland clay-clay loam texture, while the SF, located at 35-36-5 W3, has an Elstow clay loam soil texture. Plots were grown as microplots (1.2 m x 1.2 m) at the SF and as yield plots (1.2 m x 3.6 m) at the KCRF. Plots were sprayed to control weeds. Grain was cleaned using a Rationel Kornservice seed cleaner (model SLN; A/S Rationel Kornservice, Esbjerg, Denmark) and sized over a 5.5/64” slotted screen.

3.2.2 Micromalting

Malt was prepared using a Joe White Micromalting System (Joe White Maltings Ltd., Adelaide, Australia) designed to handle 120 samples of barley grain (50 g) in each batch. Each

replication was loaded into the machine as a separate batch. The unused baskets in each batch were filled with an alternative variety of barley grain to maintain balance within the micromalter. Internal checks between malting batches were CDC Copeland and CDC Mayfair.

Samples were steeped at 13°C using the following regime: 10 h wet steep, 18 h air rest, 8 h wet steep, and 12 h air rest. Samples were germinated for 96 h at 15°C with 100 % relative humidity. Kilning was carried out over 48 h as follows: 6 h increase from 30° to 48°C, 16 h at 48°C, 8 h increase from 48° to 66°C, 10 h at 66°C, 2 h increase from 66° to 85°C, and 6 h at 85°C.

3.2.3 Sample Preparation

All malt samples were scoured to remove the rootlets and, as with the grain, ground to pass through a 0.5 mm screen using a Retsch® Ultra Centrifugal Mill ZM 200 (Retsch, Verder Scientific Inc., Haan, Germany). The mill was cleaned after each sample. β-glucan content was determined in the grain, kilned malt, and wort. β-glucanase activity was quantified in kilned malt.

3.2.4 β-glucan Assay

β-glucan content was analyzed using the mixed-linkage β-glucan kit offered by Megazyme (Megazyme International Ireland Ltd., Wicklow, Ireland), which follows the McCleary method (McCleary and Glennie-Holmes 1985). This method was used to determine β-glucan content in both the grain (at 0.5 g) and the malt (at 1.0 g), with a larger sample size being used in the latter analysis to compensate for the small amounts of β-glucan likely to be detectable.

For grain β-glucan content determination, 0.5 g of ground sample was weighed into centrifuge tubes. To each tube, 1.0 mL of aqueous ethanol (50 % v/v) and 5.0 mL of sodium phosphate buffer (20 mM, pH 6.5) was added prior to mixing the tubes on a vortex mixer. For malt β-glucan content determination, 1.0 g of ground sample was weighed into centrifuge tubes. To each sample, 5.0 mL of aqueous ethanol (50 % v/v) was added. The tubes were incubated for 5 min in a boiling water bath, the contents mixed on a vortex mixer, another 5.0 mL of ethanol added, and the contents again mixed. The tubes were then centrifuged for 10 min at 3,000 rpm (1,000 g), the supernatant discarded, and the pellet resuspended in 10.0 mL of ethanol. The

tubes were centrifuged for a further 10 min at 3,000 rpm (1,000 g), the supernatant discarded, and the pellet once again resuspended in 5.0 mL of sodium phosphate buffer.

Tubes from either procedure were then incubated in a boiling water bath (approximately 97°C) for 2 min, stirred on a vortex mixer, heated for another 2 min, vortexed, and heated for 1 min in a water bath before being vortexed again. The tubes were cooled to 40°C, and 5 mL of double distilled water and 0.2 mL of lichenase enzyme (endo-(1,3;1,4)-β-D-glucan-4-glucanohydrolase; 50 U/mL in 20 mM sodium phosphate buffer, pH 6.5) was added to each tube. Once capped, the tubes were vortexed and incubated at 40°C in a shaking water bath for 1 h. After this time, 19 mL of double distilled water was added to the tubes, the contents mixed, and the tubes centrifuged for 10 min at 3,000 rpm (1,000 g). To three separate test tubes per sample, 0.1 mL of supernatant was added; the reaction blank tube received 0.1 mL of sodium acetate buffer (50 mM, pH 4.0) while the other two test tubes, the reaction (as two technical replications), received 0.1 mL of β-glucosidase enzyme (2 U/mL in 50 mM sodium acetate buffer, pH 4.0). All tubes were incubated at 40°C for 15 min. After incubation, 3.0 mL of GOPOD reagent (glucose oxidase/peroxidase and 4-amnioantipyrine in 0.4 % w/v ρ-hydroxybenzoic acid and sodium azide, pH 7.4) was added to each tube, which were incubated for 20 min at 40°C before the absorbance at 510 nm was read against a blanked spectrometer.

A control sample, containing the barley flour standard supplied with the kit, was evaluated with each set of test samples. Change in absorbance was determined by subtracting the reaction blank (0.1 mL distilled water, 0.1 mL sodium acetate buffer, 3.0 mL of GOPOD reagent) for each sample from each of the observed absorbance of each technical replication for each sample. Similarly, the absorbance of the reaction blank was subtracted from the absorbance of the standard to determine the absorbance of 100 µg of the D-glucose standard (0.1 mL of 1.0 mg/mL in 0.2 % w/v benzoic acid, 0.1 mL sodium acetate buffer, 3.0 mL of GOPOD reagent). The conversion factor (*F*) between absorbance values and µg glucose was determined in the following way:

$$Conversion\ Factor\ (F) = \frac{100\ (\mu g\ of\ D\text{-}glucose)}{Absorbance\ of\ 100\ \mu g\ of\ D\text{-}glucose}$$

The moisture of each of the ground samples was also determined. Metal weighing dishes were weighed empty, their weights recorded and tared, and 1.0 g of the ground samples was added. The lids of the dishes were immediately closed. Once all samples were weighed out and

the oven pre-heated to 130°C, the lids on the dishes were cracked to allow moisture to escape and the dishes were placed in the oven for 1 h. After that time, the lids were again closed on the dishes and they were placed in a desiccator until cool, upon which time the final weights were recorded to determine moisture lost. Percent moisture was then determined according to the following formula:

$$Moisture\ Content\ (\%) = \frac{Water\ Weight\ (g)}{Dry\ Weight\ (g)}\ x\ 100\%$$

The fresh sample weight used in the β-glucan assays was corrected to account for moisture using the following formula, with the corrected dry weight (*W*) determined in milligrams for use in a later equation:

$$Corrected\ Dry\ Weight\ (mg) = Fresh\ Weight\ (g)\ x\ \frac{100 - Moisture\ Content\ (\%)}{100}\ x\ 1000$$

β-glucan content was calculated for both technical replications of each sample in the following manner:

$$\beta\text{-}glucan\ Content\ (\%\ w/w) = \Delta A\ x\ \frac{F}{W}\ x\ 27$$

where ΔA is the absorbance of the sample subtracted by the reaction blank, *F* is the quotient of the absorbance conversion factor, and *W* is the corrected dry weight. The final β-glucan content was reported as an average of the two technical replications for each sample in each biological replication at each location of each year. If large discrepancies were found between samples (≥0.5 difference in absorbance reading), then the assay was repeated for that particular sample.

Wort β-glucan content was assessed using a FlowAccess® Skalar, utilizing the San^{++} Continuous Flow Analyzer (Skalar Analytical B.V., Breda, Netherlands). Control malt samples run with each batch of samples (CDC Copeland and AC Metcalfe) were also analyzed. Wort was made by adding 7.5 g of malt from each sample into a 500 mL Erlenmeyer flask and adding 60 mL of degassed 70°C water. The flasks were stoppered, lightly swirled to ensure suspension of the malt, and set in a shaking water bath for 1 h at 70°C. After this time, the stoppers were removed and the flasks were submerged in cool water for approximately 15 min. The contents were filtered through P8 coarse filter paper set in funnels to drain the liquid (sweet wort) into

clean flasks. Once filtered, the sweet wort was vortexed before being transferred into test tubes and placed in the Skalar alongside test tubes filled with distilled, degassed water (to serve as blanks). Five standards of increasing β-glucan concentration (50, 100, 200, 300, and 400 parts per million (ppm)) and the two control malt samples were measured at the beginning of each run and again halfway through each run to account for potential baseline drift. A calcofluor solution (5 mg/L Fluorescent Brightener 28 in 104 mM glycine buffer, pH 10.0) was used to induce fluorescence in the sample for reading by the fluorimeter. The final β-glucan content was reported in ppm.

3.2.5 β-glucanase Activity Assay

β-glucanase activity in the kilned malt was determined using the assay of *endo*-β-glucanases using beta-glucazyme tablets from Megazyme. The kit supplied 60 mg β-glucazyme tablets (T-BGZ1000), which were used in conjunction with a Trizma® Base solution (2 % w/v), a sodium acetate stock buffer (1 M, pH 4.2), and an extraction/dilution buffer (25 mM sodium acetate, pH 4.2). The Trizma® Base solution was prepared by dissolving 20 g of Trizma® Base in 1 L of water. The stock buffer was prepared by adding 60.0 mL of glacial acetic acid to 800 mL of double distilled water. The pH of the solution was adjusted to 4.2 through the addition of 4 M sodium hydroxide solution, and the final volume was adjusted to 1 L without compromising pH. The extraction/dilution buffer was made up by adding 25 mL of stock buffer solution to 950 mL of double distilled water, adjusting the pH to 4.2 through the addition of 1 M hydrochloric acid, and bringing the final volume up to 1 L without compromising pH.

The CDC Copeland control sample of kilned malt was assayed with each batch of test samples. For each sample, 0.5 g of kilned malt was weighed into centrifuge tubes. To these tubes, 5.0 mL of extraction/dilution buffer (25 mM sodium acetate, pH 4.2) was added and the tubes were stirred thoroughly on a vortex mixture. The mixture was allowed to incubate at room temperature for 15 min, with mixing every five minutes. After centrifugation at 3,000 rpm (1,000 g) for 10 min, 0.5 mL of the supernatant was transferred into test tubes and incubated at 30°C for 5 min. Each sample and the reaction blank (0.5 mL of extraction/dilution buffer) received one β-glucazyme tablet at 20 s intervals. After precisely 10 min, 10.0 mL of Trizma® Base solution (2 % w/v) was added to terminate the reaction. The tubes were allowed to stand for 5 min at room temperature before being centrifuged. The absorbance of the supernatant was

read at 590 nm against the substrate blank on a blanked spectrometer. When the absorbance of the reaction solution was above 1.2, the enzyme extract was diluted with the extraction/dilution buffer and the assay repeated. β-glucanase activity, measured in U/kg malt and considered as the amount of enzyme required to release one micromole of D-glucose reducing sugar equivalents per minute, was calculated using the following formula:

$$\beta\text{-glucanase Activity (U/kg Malt)} = 94 \times Abs^2 + 381 \times Abs + 28$$

where the corrected absorbance value (*Abs*) accounted for both the dilution factor and the absorbance of the substrate blank. The three constants given in the formula are specific to this particular assay.

3.2.6 Statistical Analysis

Statistical analysis was primarily conducted using the statistical software SAS v. 9.4 (SAS Institute Inc., Cary, NC). Proc Mixed in SAS was used for the analysis of variance (ANOVA), where replications (blocks) were treated as random factors and entry (breeding line), year, and location were treated as fixed factors. Least square (LS) means values were generated using Proc Mixed and adjusted using the Tukey test. Proc Corr was used to generate correlations between traits based on LS means. Normality according to the Shapiro-Wilk test was assessed using Proc Univariate on LS means. Basic statistics were generated using the phenotype summary option in JMP Genomics v. 7 (SAS Institute Inc., Cary, NC).

Variance components used to determine heritability were generated using the statistical software R v. 3.1.2 (R Core Team 2014) and the linear mixed effects model package lme4 (Bates *et al.* 2014). Variance components were produced using the lmer function, where entry, replication, site-year, and all combinations of interactions (using two and three factors) were treated as random factors. With the exception of the combination of the environmental and error variance terms, broad sense heritability (h^2) was calculated according to equation 8 in Vargas *et al.* (2013), where genotype variance (σ^2_g) is divided by the sum of three terms: genotype variance, G x E (genotype by environment interaction) variance (σ^2_{ge}) divided by the number of locations (test environments, t), and error variance (σ^2_e) divided by the number of locations (t) multiplied by the number of replications (r). This equation is identical to the one proposed by

Fehr (1987) to calculate heritability on an entry-mean basis using variance components, given below:

$$h^2 = \frac{\sigma^2_g}{\sigma^2_e/rt + \sigma^2_{ge}/t + \sigma^2_g}$$

3.2.7 Genotyping

The MK population was genotyped with single nucleotide polymorphism (SNP) markers using the Barley 9K Infinium Assay at the Biosciences Research Laboratory, USDA-ARS (Fargo, ND) on the iSELECT Genotyping BeadChip (Illumina, Inc., San Diego, CA), and by the DArTseq method (Diversity Arrays Technology Pty. Ltd., Canberra, Australia) which utilizes representative sequencing on Next Generation Sequencing (NGS) platforms. SNP genotyping data from the iSELECT platform was visually screened using Illumina GenomeStudio software (Illumina, Inc., San Diego, CA) to ensure correctness of the automated calls. Markers that were monomorphic, had greater than 10 % failed or null reactions, or showed highly skewed segregation ($p<0.01$) were removed to create a high quality set of genotyping data. DArTseq data were similarly screened and both genotyping data sets were combined. Identical markers and markers mapping to more than one locus as determined by the International Barley Sequencing Consortium (IBSC) map on Barleymap (Cantalapiedra *et al.* 2015; http://floresta.eead.csic.es/barleymap/) were removed from the data set.

3.2.8 Linkage Map Creation and QTL Mapping

The genetic map was created using 159 RILs from the MK population. Linkage groups were determined using JoinMap 4.0 (Kyazma B.V., Wageningen, Netherlands). Default calculation settings were used when building the groupings tree (initial groups created with independent LOD scores from 2.0 to 10.0 and loci were removed if there was a goodness-of-fit jump greater than 5.00). Marker order within the initial linkage groups was established using the regression mapping algorithm as described in Stam (1993) under its default settings, with the exception that no third round to fit loci was performed and the Kosambi mapping method (Kosambi 1944) was used instead of the Haldane mapping method (Haldane 1919). General map groupings were verified by checking genome positions with bi-parental and consensus

genetic maps (the Hordeum-OWB-OPA2008 map available on GrainGenes 2.0 (Carollo *et al.* 2005; http://147.49.50.65/GG3/) and the HarvEST Barley v. 1.83 (University of California, Riverside, Riverside, CA) consensus map created by Muñoz-Amatriaín *et al.* (2011)), as well as the IBSC map on Barleymap.

Linkage groups and marker order data were input into MapQTL 5.0 (Kyazma B.V., Wageningen, Netherlands). QTL mapping parameters were maintained at their default values and phenotypic data were analyzed separately by site-year due to significant G x E. A permutation test was run to determine the significant LOD threshold for each trait at each site-year. The LOD values were taken at approximately the 95 % confidence level for genome-wide significance, as suggested by van Ooijen (1999); when the exact relative cumulative count 0.9500 at the genome-wide level was not present for a particular trait in a particular site-year, the next highest value was used. Interval mapping was conducted to identify QTLs linked to kilned malt β-glucanase activity and grain, malt, and wort β-glucan content. The Kruskal-Wallis test was run on all unmapped markers in this population using MapQTL 5.0 to determine potential associations between these unmapped markers and the four studied traits; only those found to be significant at $\alpha = 0.0001$ were reported.

The BARLEX: The Barley Genome Explorer (Colmsee *et al.* 2015; http://apex.ipk-gatersleben.de/apex/f?p=284:10:9124939587496), Ensembl Plants (Kersey *et al.* 2014; http://plants.ensembl.org/index.html), PGSB: Plant Genome and Systems Biology (International Barley Genome Sequencing Consortium 2012; http://pgsb.helmholtz-muenchen.de/plant/index.jsp), and Barleymap databases were used to identify putative explanatory genes underlying any QTL detected. Known QTLs linked to β-glucan and β-glucanase were identified using the GrainGenes database. HarvEST: Barley, Ensembl Plants, Barleymap, and the Barley_PhysMap (http://pgsb.helmholtz-muenchen.de/cgi-bin/gb2/gbrowse/Barley_PhysMap/) database from Gbrowse (Generic Genome Browser v. 2.55) were used in conjunction with maps on GrainGenes (Hordeum-OWB-OPA2008, Hordeum-OPA-2009, Barley-Consensus2, and Hordeum-Consensus2005-SNP) to determine relative positions of genes and proximity of the observed QTLs to known QTLs. Enzyme and gene function were identified using the InterPro: protein sequence analysis and classification (Mitchell *et al.* 2015; http://www.ebi.ac.uk/interpro/) and CAZy: carbohydrate-active enzymes (Lombard *et al.* 2014; http://www.cazy.org/) databases.

3.3 Results

3.3.1 Analysis of Phenotypic Data

Summary statistics for the four traits analyzed are shown in Table 3.1. In the MK population, grain β-glucan content and β-glucanase activity were higher in 2012 than in 2013 (Table 3.1) while malt and wort β-glucan content were higher in 2013 than in 2012 (Table 3.1). The MK population showed transgressive segregation for all traits in all site-years (Table 3.1). As expected, CDC Kindersley showed higher β-glucanase activity and lower malt/wort β-glucan content than CDC Meredith over all three site-years (Table 3.1). Grain β-glucan content was similar between both parents, as expected, except at the 2013 KCRF site where CDC Kindersley had noticeably lower β-glucan content (Table 3.1). Normality of the traits was assessed using the LS mean values for each site-year since blocks were not a significant source of variation (data not shown). All traits in all site-years were normally distributed, except malt β-glucan content at SF in 2012 which displayed a slight negative skew (Table 3.2). Attempts to improve the normality of the data using a log transformation did not improve the distribution. However, visual inspection of the untransformed data indicated that it met the assumption of normality and so was analyzed for QTLs.

Entries (breeding lines) were a significant source of variation for all traits examined (Table 3.3). Site and year were significant factors influencing variability in grain β-glucan content, while year, the interaction between entry and year, and the interaction between entry and site were significant factors affecting variability in both kilned malt β-glucan and wort β-glucan content (Table 3.3). In addition, site was a significant source of variability for malt β-glucan content (Table 3.3). Consequently, marker-trait associations from the different site-years were analyzed for QTLs separately for all traits except kilned malt β-glucanase activity.

No significant correlations were seen between grain β-glucan content and kilned malt β-glucanase activity (Table 3.4). Grain β-glucan content was observed to be significantly correlated with both malt and wort β-glucan content in both 2013 site-years (Table 3.4) while kilned malt β-glucanase activity was significantly correlated to both malt and wort β-glucan content in the 2012 site-year (Table 3.4). Malt and wort β-glucan content were highly correlated in all site-years (Table 3.4).

The heritability of all four traits was moderate to high (ranging from 0.54 to 0.73) (Table 3.5).

Table 3.1. Summary statistics for the four traits evaluated in the MK population grown in 2012 and 2013.

			Parent Means		MK Population			
Year	Site[1]	Trait[2]	CDC Meredith	CDC Kindersley	Mean	Minimum	Maximum	Standard Deviation
2012	SF	Grain BG	3.9	4.1	4.2	3.7	4.8	0.25
		Malt BGase	1,180	1,368	1,336	1,069	1,608	112
		Malt BG	0.8	0.7	0.6	0.3	1.1	0.18
		Wort BG	614	474	477	125	984	193
2013	KCRF	Grain BG	4.0	3.4	3.6	3.1	4.1	0.21
		Malt BGase	1,224	1,355	1,295	1,030	1,519	101
		Malt BG	1.2	0.8	1.0	0.5	1.5	0.21
		Wort BG	1,038	656	815	331	1,204	173
2013	SF	Grain BG	3.6	3.4	3.6	3.0	4.0	0.22
		Malt BGase	1,257	1,451	1,281	1,016	1,510	109
		Malt BG	1.0	0.8	0.8	0.4	1.2	0.19
		Wort BG	848	754	654	278	1,030	175

[1] KCRF: Kernen Crop Research Farm; SF: Seed Farm.
[2] BG: β-glucan (% w/w for grain and malt BG, ppm for wort BG); BGase: β-glucanase (U/kg malt).

Table 3.2. P-values obtained from the Shapiro-Wilk normality test for four traits measured on the MK population in 2012 and 2013.

Year	Location[1]	Trait	P-value
2012	SF	Grain β-glucan	0.35
		Kilned Malt β-glucanase	0.82
		Malt β-glucan	0.03
		Wort β-glucan	0.14
2013	KCRF	Grain β-glucan	0.74
		Kilned Malt β-glucanase	0.74
		Malt β-glucan	0.99
		Wort β-glucan	0.35
2013	SF	Grain β-glucan	0.85
		Kilned Malt β-glucanase	0.33
		Malt β-glucan	0.23
		Wort β-glucan	0.48

[1] SF: Seed Farm; KCRF: Kernen Crop Research Farm.

Table 3.3. P-values obtained for the effect of entry, year and interactions for four traits measured on the MK population in 2012 and 2013.

Trait	Factor	P-value
Grain β-glucan	Entry	<0.01
	Site	<0.01
	Year	0.03
	Entry* Site	0.93
	Entry*Year	0.07
Kilned Malt β-glucanase	Entry	<0.01
	Site	0.80
	Year	0.44
	Entry*Site	0.93
	Entry*Year	0.10
Malt β-glucan	Entry	<0.01
	Site	0.05
	Year	0.03
	Entry*Site	<0.01
	Entry*Year	<0.01
Wort β-glucan	Entry	<0.01
	Site	0.09
	Year	0.03
	Entry*Site	0.03
	Entry*Year	0.01

Table 3.4. Pearson correlation coefficients among four traits measured in the MK population in 2012 and 2013.

Trait[1]	Site-Year[2]	Grain β-glucan	Kilned Malt β-glucanase	Malt β-glucan	Wort β-glucan
Grain BG	SF 2012	1			
	KCRF 2013	1			
	SF 2013	1			
Kilned Malt BGase	SF 2012	0.16	1		
	KCRF 2013	-0.04	1		
	SF 2013	0.09	1		
Malt BG	SF 2012	0.09	0.24*	1	
	KCRF 2013	0.43***	-0.17	1	
	SF 2013	0.35*	0.06	1	

Table 3.4. Continued

Trait[1]	Site-Year[2]	Grain β-glucan	Kilned Malt β-glucanase	Malt β-glucan	Wort β-glucan
Wort BG	SF 2012	0.13	0.28*	0.92***	1
	KCRF 2013	0.38**	-0.13	0.91***	1
	SF 2013	0.36**	0.03	0.96***	1

[1] BG: β-glucan; BGase: β-glucanase.
[2] KCRF: Kernen Crop Research Farm; SF: Seed Farm.
* Significant at $\alpha = 0.05$.
** Significant at $\alpha = 0.001$.
*** Significant at $\alpha = 0.0001$.

Table 3.5. Broad sense heritability estimates for the four traits evaluated in the MK population grown in 2012 and 2013.

Trait	Phenotypic Variance	Genetic Variance	Environmental Variance	G x E Variance	Heritability
Grain β-glucan	0.16	0.03	0.13	0.01	0.54
Kilned Malt β-glucanase	17,989	6,403	2,073	1,670	0.73
Malt β-glucan	0.30	0.02	0.05	0.01	0.65
Wort β-glucan	32,634	22,958	17,695	2,379	0.72

3.3.2 Analysis of Genotypic Data

After removing markers that were monomorphic, had a high percentage of failed calls, showed skewed segregation ($p<0.01$), or were duplicated between platforms, a total of 4,241 segregating markers (801 SNP markers and 3,439 DArTseq markers) remained for mapping in the MK population. A total of 1,515 SNP and DArTseq markers were mapped to unique loci (340 SNP markers and 1,175 DArTseq markers), leaving 2,725 markers unmapped (461 SNP markers and 2,264 DArTseq markers). Mapped markers are listed by linkage group in Tables A1 and A2 in Appendix A.

3.3.3 Genetic Linkage Map

The 1,515 markers mapped in the MK population were divided into nine linkage groups (Fig. 3.1) representing the seven barley chromosomes. Chromosomes 2H and 7H were each split into two linkage groups based on marker positions within the consensus map, while every other chromosome was represented by a single linkage group (Fig. 3.1). Chromosome 1H was the longest linkage group at 129.8 cM while the shortest linkage group at 16.9 cM belonged to the short arm of chromosome 7H (Table 3.6). The largest gap between adjacent loci was found in

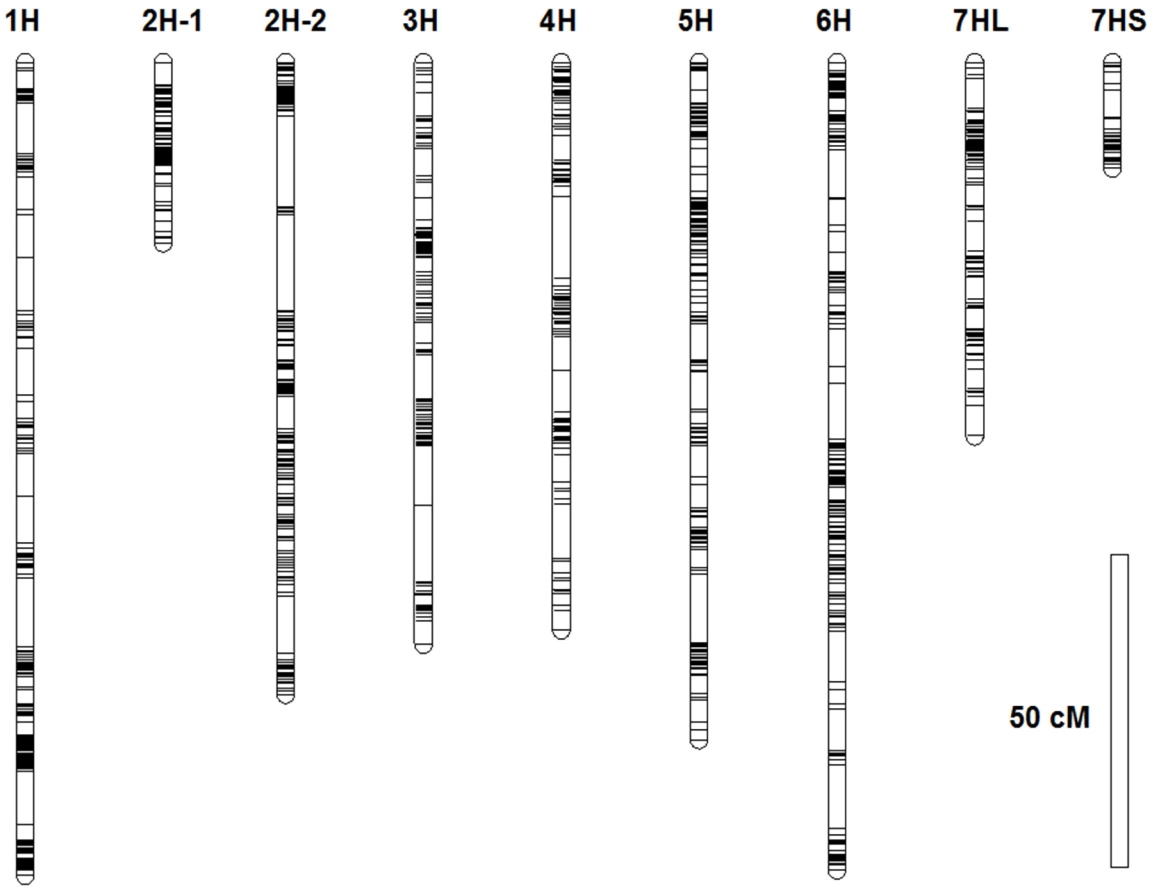

Figure 3.1. A genetic linkage map created using 159 $F_{4:8}$ recombinant inbred lines derived from the cross CDC Meredith x CDC Kindersley. The map includes 1,515 markers distributed over nine linkage groups with a total length of 756.5 cM. The barley chromosome number (based on marker positions within the consensus map) is indicated at the top of each linkage group. Where multiple markers mapped to the same locus, only one marker has been used to represent that locus. Black bars within each linkage group indicate the position of markers, and a scale indicating genetic distance is provided at the bottom of the figure.

Table 3.6. Summary information for the genetic map created in the MK population using a combination of DArTseq and SNP markers.

Chromosome	Markers in Linkage Group	Linkage Group Length (cM)	Average Distance[1] (cM)	Largest Gap[1] (cM)
1H	211	129.8	0.6	11.2
2H-1	115	28.8	0.3	3.5
2H-2	215	100.9	0.5	15.2
3H	168	92.9	0.6	12.3
4H	156	90.6	0.6	13.1
5H	223	108.1	0.5	11.1
6H	214	128.9	0.6	10.3
7HS	53	16.9	0.3	4.3
7HL	160	59.6	0.4	4.9
Summary:	1,515	756.5	0.5	9.5

[1] Between loci.

linkage group 2H-2 while the average distance between loci across all linkage groups was 0.5 cM, with average distances ranging from 0.3 cM to 0.6 cM (Table 3.6). Chromosome 5H contained the most markers of any linkage group (Table 3.6).

3.3.4 QTL Analysis

A total of 10 unique QTLs were identified for the four traits evaluated in the MK population across the three site-years (Table 3.7). However, it is possible that the two grain β-glucan 2H-2 QTLs, albeit approximately 8.4 Mb apart, are part of a single QTL given their proximity to the previously identified QTL QBgn.StMo-2H.1 (Han *et al.* 1995). Four unique QTLs were found on chromosomes 1H, 2H, and 7H for grain β-glucan content (Table 3.7, Fig. 3.2). The chromosome 1H grain β-glucan content QTL peaked at 100.1 cM (Table 3.7, Fig. 3.2), which falls at position 107 cM on the IBSC_2012 map, or at approximately position 439.7 Mb on the barley physical map. This QTL spans the iPgd2 to ABG702 marker interval (based on the Barley-Consensus2-1H, Hordeum-Consensus2005-SNP-1H, and Hordeum-OWB-OPA2008-1H maps) and locates between two known malt β-glucan QTLs (QBgnm.StMo-1H.1, peak resides between markers His3B and iPgd2, and QBgnm.StMo-1H.2, peak resides between markers ABG702 and ABA002) identified by Han *et al.* (1995). The QTL observed in this study does not fall within the support interval of either of the mentioned Han *et al.* (1995) QTLs but this could be due to the poorer mapping resolution of the earlier study. This QTL overlaps two UDP-glycosyltransferase superfamily protein genes, MLOC_17987.1 (at 435.6 Mb) and MLOC_70129.1 (at 435.5 Mb) (Table 3.8).

The peak of the 2H-2 grain β-glucan content QTL identified at the 2013 KCRF site fell at 4.5 cM (Table 3.7, Fig. 3.2). The peak marker, 4184974, is located near marker 3269257, which is associated with bowman_contig_1983701. This contig overlaps with morex_contig_52735 and is located at 7.7 Mb on chromosome 2H. MLOC_64239.1, believed to code for a UDP-glycosyltransferase superfamily protein, is found at 8.2 Mb (Table 3.8). On the Hordeum-OWB-OPA2008-2H map, a known grain β-glucan content QTL, QBgn.StMo-2H.1 (Han *et al.* 1995), falls less than 2 cM from marker POPA3_1446 (12_31446) which is associated with morex_contig_38698. This contig is located at 2.2 Mb on chromosome 2H, so the observed QTL is likely the same as that reported by Han *et al.* (1995).

Table 3.7. Quantitative trait loci (QTL) associated with grain, malt, and wort β-glucan content and kilned malt β-glucanase activity that were identified in the MK population grown in 2012 and 2013.

Site-Year[1]	LOD Threshold	Chr[2]	Position (cM)	Peak Marker[3]	LOD Value	% Variance Explained	Approximate QTL Range (cM)
Grain β-glucan							
2013 SF	3.1	1H	100.1	3257635	3.34	17.5	96 - 100
2013 KCRF	3.0	2H-2	4.5	4184974	3.04	16.1	4 - 5
2013 SF	3.1	2H-2	23.7	3267214	3.21	16.9	23 - 24
		7HL	47.5	11_20247	5.83	23.5	43 - 48
2012 SF	3.1	7HL	34.2	3986721	5.83	31.9	25 - 59
Malt β-glucan							
2013 KCRF	3.0	1H	127.4	12_30231	6.02	29.3	121 - 129
		4H	39.3	3662708	3.96	20.6	36 - 49
Wort β-glucan							
2013 KCRF	3.0	1H	127.4	12_30231	4.4	22.4	124 - 130
Kilned Malt β-glucanase							
All	3.0	2H-1	26.9	3257572	3.69	19.2	25 – 27
		6H	62.0	5339062	4.78	24.0	48 – 67
		7HL	22.8	4184560	3.24	24.6	22 – 24

[1] KCRF: Kernen Crop Research Farm; SF: Seed Farm.
[2] Chromosome.
[3] Marker within the QTL with the highest LOD value.

The 2H-2 grain β-glucan content QTL associated with the 2013 SF site had a peak LOD score at 23.7 cM (Table 3.7, Fig. 3.2), which correlates to approximately 12.6 cM on the IBSC_2012 map based on contig alignments in Barley_PhysMap which contained DArTseq marker 3924813 and SNP marker SCRI_RS_128162 located within the QTL. This position correlates to approximately 16.1 Mb on the physical map. On the IBSC_2012 map, SNP marker SCRI_RS_128162 mapped to the same position as POPA2_0724 (11_20724) which is approximately 15 cM away from the grain β-glucan QTL QBgn.StMo-2H.1, identified by Han *et al.* (1995), based on comparison to markers AGB703b and Chs1b found using the Hordeum-OWB-OPA2008-2H, Hordeum-OPA-2009-2H, and Barley-Consensus2-2H maps. In addition, POPA2_0724 and therefore the QTL observed in this study appears to fall between ABG8 (ABG008) and Chs1b, which is within the support interval for the Han *et al.* (1995) QTL. MLOC_81137.1, a putative UDP-glucuronosyl/UDP-glucosyltransferase, falls at position 15.7 Mb (Table 3.8).

The chromosome 7HL grain β-glucan content QTL peak position was at 34.2 cM at the 2012 SF site and at 47.5 cM at the 2013 SF site (Table 3.7, Fig. 3.2), which respectively fall at approximately 97.1 cM and 102.9 cM on the IBSC_2012 map or 539.1 Mb and 552.7 Mb on the physical map. This QTL displayed a wide interval in 2012, ranging from 25 to 59 cM, or approximately 92 to 116 cM on the IBSC_2012 map, with a narrower range in 2013 (43 to 48 cM or 100 to 102 cM on the IBSC_2012 map). These intervals spanned two genes (MLOC_52796.1 at position 537.1 Mb and MLOC_70708.1 at position 558.3 Mb) which code for glycosyltransferase enzymes (Table 3.8).

Table 3.8. Putative genes underlying quantitative trait loci (QTL) associated with grain, malt, and wort β-glucan content and kilned malt β-glucanase activity identified in the MK population grown in 2012 and 2013.

Site-Year[1]	Chr[2]	Position (cM)	Peak Marker[3]	Underlying Gene	Physical Map Position (Mb)	Predicted Function	Prior QTL
Grain β-glucan							
2013 SF	1H	100.1	3257635	MLOC_17987	435.6	UDP-glycosyltransferase superfamily protein	QBgnm.StMo-1H.1 (Han *et al.* 1995; Szűcs *et al.* 2009), QBgnm.StMo-1H.2 (Han *et al.* 1995; Szűcs *et al.* 2009)
				MLOC_70129	435.5	UDP-glycosyltransferase superfamily protein	
2013 KCRF	2H-2	4.6	4184974	MLOC_64239	8.2	UDP-glycosyltransferase superfamily protein	QBgn.StMo-2H.1 (Han *et al.* 1995; Szűcs *et al.* 2009)
2013 SF	2H-2	23.7	3267214	MLOC_81137	15.7	UDP-glucuronosyl/UDP-glucosyltransferase	QBgn.StMo-2H.1 (Han *et al.* 1995; Szűcs *et al.* 2009)
2013 SF	7HL	47.5	11_20247	MLOC_52796	537.1	glycosyltransferases (family 14)	-
2012 SF	7HL	34.2	3986721	MLOC_70708	558.3	glycosyltransferases (family 31)	-
Malt β-glucan							
2013 KCRF	1H	127.4	12_30231	-	-	-	QMe.StMo-1H.5 (Hayes *et al.* 1993; Szűcs *et al.* 2009)

Table 3.8. Continued

Site-Year[1]	Chr[2]	Position (cM)	Peak Marker[3]	Underlying Gene	Physical Map Position (Mb)	Predicted Function	Prior QTL
Malt β-glucan							
2013 KCRF	4H	39.3	3662708	MLOC_34770	510.3	UDP-glycosyltransferase superfamily protein	-
Wort β-glucan							
2013 KCRF	1H	127.4	12_30231	-	-	-	QMe.StMo-1H.5 (Hayes *et al.* 1993; Szűcs *et al.* 2009)
Kilned Malt β-glucanase							
All	2H-1	26.9	3257572	MLOC_52642	614.3	concanavalin A-like lectin/glucanase	-
	6H	62.0	5339062	MLOC_62475	480.2	glycoside hydrolase	-
				MLOC_67192	471.2	endoglucanase 6	-
	7HL	22.8	4184560	-	-	-	-

[1] KCRF: Kernen Crop Research Farm; SF: Seed Farm.
[2] Chromosome.
[3] Marker within the QTL with the highest LOD value.

Two unique QTLs were identified for malt β-glucan content on chromosomes 1H and 4H (Table 3.7, Fig. 3.3). The chromosome 1H QTL was also associated with wort β-glucan content at the same site-year (Table 3.7, Fig. 3.4). The 1H malt/wort β-glucan content QTL had a peak at 127.4 cM, which is equivalent to 132.5 cM on the IBSC_2012 map and 461.3 Mb on the physical map. This QTL maps within 2 cM of the malt extract QTL QMe.StMo-1H.5 (Hayes *et al.* 2003). This QTL maps to position 153.0 cM on the Hordeum-OWB-OPA2008-1H map, and overlies the POPA1_0041 (11_10041) marker which maps to position 130.7 cM on the IBSC_2012 map. POPA1_0041 co-segregates with POPA2_0603 (ABG373) (HarvEST: Barley), one of the significant markers linked to the malt extract QTL QMe.StMo-1H.5 (Hayes *et al.* 1993). The 4H malt β-glucan content QTL had a peak LOD score at 39.3 cM (97 cM on the IBSC_2012 map). The QTL peak, at 513.6 Mb, lays approximately 3.4 Mb from MLOC_34770.1 (at 510.3 Mb), predicted to be a UDP-glycosyltransferase superfamily protein (Table 3.8).

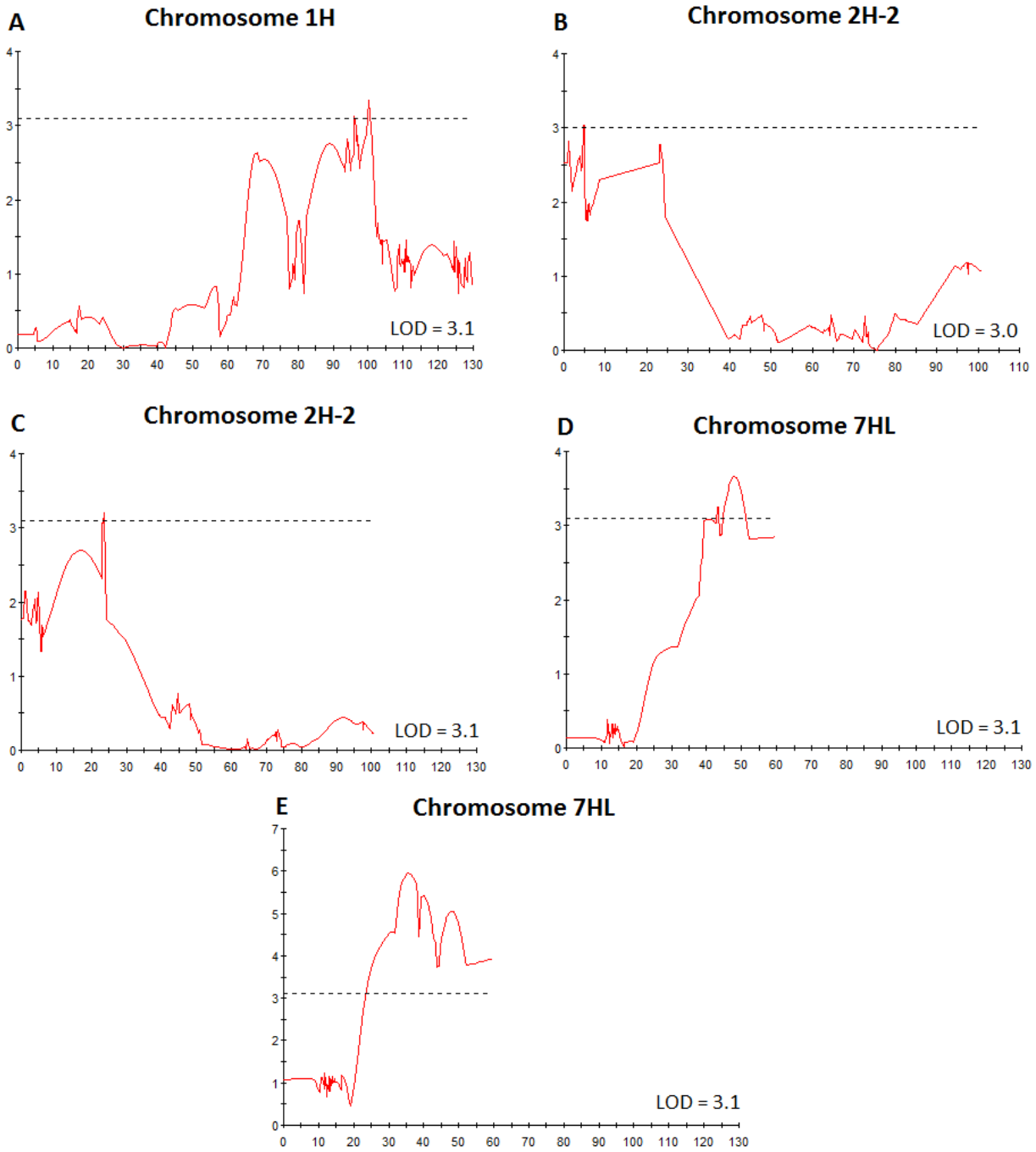

Figure 3.2. QTLs identified in the MK population for grain β-glucan content. (A) chromosome 1H at Seed Farm in 2013, (B) chromosome 2H-2 at Kernen Crop Research Farm in 2013, (C) chromosome 2H-2 at Seed Farm in 2013, (D) chromosome 7HL at Seed Farm in 2013, and (E) chromosome 7HL at Seed Farm in 2012. The dotted line represents the significant LOD threshold for the trait in that particular site-year and is also noted in the bottom corner of each graph.

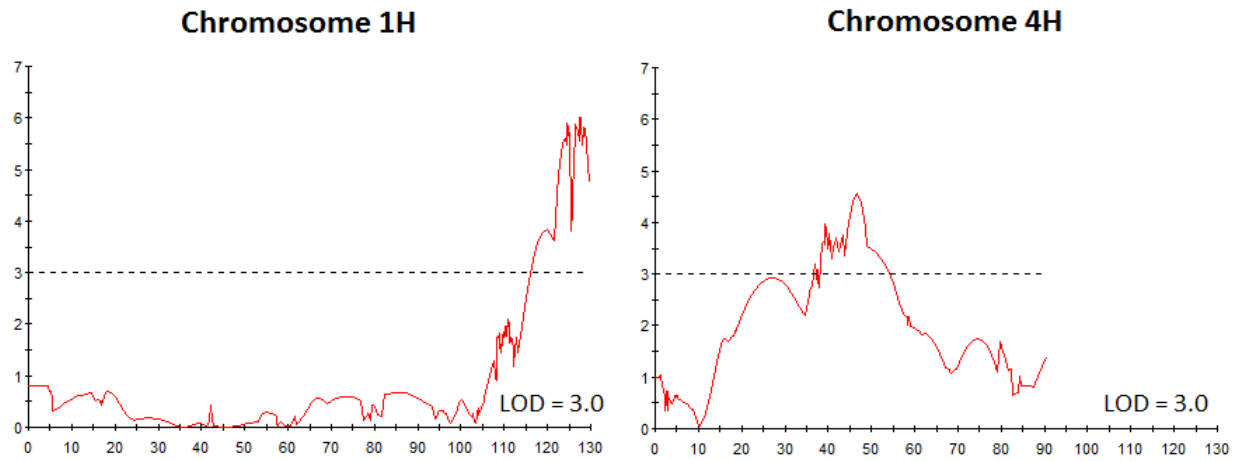

Figure 3.3. QTLs identified in the MK population for malt β-glucan content on chromosomes 1H and 4H at Kernen Crop Research Farm in 2013. The dotted line represents the significant LOD threshold for the trait in that particular site-year and is also noted in the bottom corner of each graph.

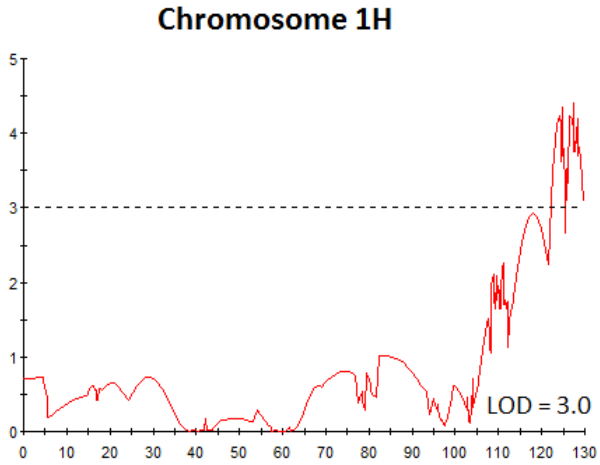

Figure 3.4. QTL identified in the MK population for wort β-glucan content on chromosome 1H at Kernen Crop Research Farm in 2013. The dotted line represents the significant LOD threshold for the trait in that particular site-year and is also noted in the bottom corner of the graph.

Three unique QTLs associated with kilned malt β-glucanase activity were found on chromosomes 2H, 6H, and 7H (Table 3.7, Fig. 3.5). The 2H-1 kilned malt β-glucanase activity QTL peaked at 26.9 cM, or 141.9 cM on the IBSC_2012 map. At 616.1 Mb, the QTL peak falls approximately 2 Mb from MLOC_52642.1 (at 614.3 Mb), which likely encodes a concanavalin A-like lectin/glucanase (Table 3.8). The 6H kilned malt β-glucanase activity QTL peaked at 62.0 cM, or approximately 68.5 cM on the IBSC_2012 map or 471.1 Mb on the physical map. This QTL overlaps both a probable glycoside hydrolase and a probable endoglucanase 6 gene (Table 3.8). The glycoside hydrolase gene, MLOC_62475.1 at position 480.2 Mb, falls

approximately 9.1 Mb from the QTL peak, while the endoglucanase 6 gene, MLOC_67192.1 at position 471.2, falls approximately 0.1 Mb from the QTL peak.

The DArTseq marker 4184560 located near the peak of the 7H kilned malt β-glucanase activity QTL at 22.8 cM is located within barke_contig_279132. This contig overlaps with morex_contig_52928 and is located at 520.4 Mb on 7H.

Table B1 in Appendix B details the methods used to identify the underlying genes of the observed QTLs. Table C1 in Appendix C lists unmapped markers with potential associations with the studied traits identified in the Kruskal-Wallis test using a significance level of $\alpha = 0.0001$.

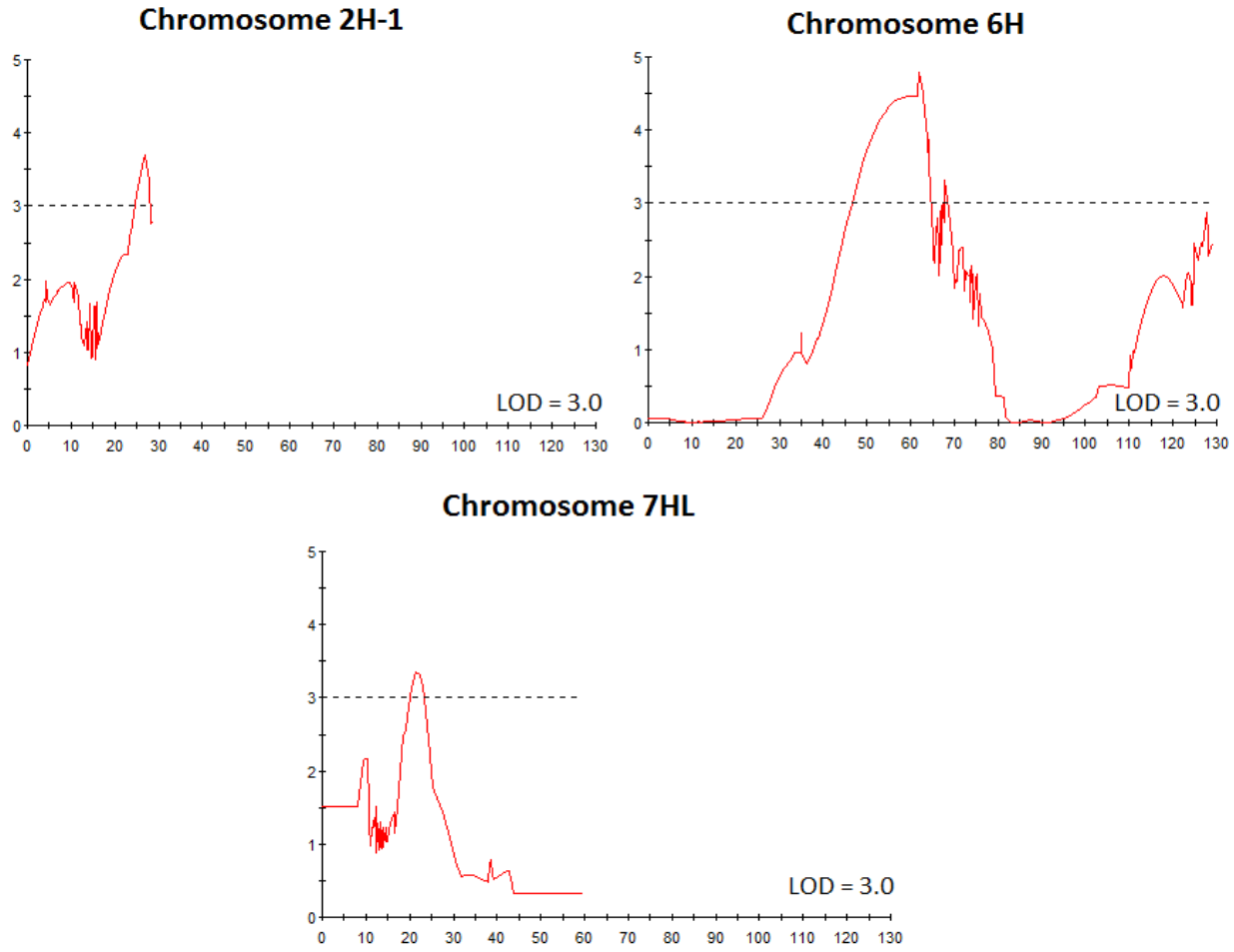

Figure 3.5. QTLs identified in the MK population for kilned malt β-glucanase activity on chromosomes 2H-1, 6H, and 7HL at all site-years (2012 Seed Farm, 2013 Kernen Crop Research Farm, and 2013 Seed Farm). The dotted line represents the significant LOD threshold for the trait in that particular site-year and is also noted in the bottom corner of each graph.

3.4 Discussion

The observed values for each trait fell within expected ranges. In this study, grain, malt, and wort β-glucan ranged from 3.0 to 4.8 %, 0.03 to 1.5 %, and 125 to 1,204 ppm, respectively (Table 3.1). Holtekjølen *et al.* (2006) analyzed barley varieties of diverse genetic origin from barley-growing areas in the northern hemisphere, chiefly those from Norway, Denmark, Finland, and Sweden but also including hulless varieties and those with atypical amylose/amylopectin ratios which mainly originated from Canada, and observed grain β-glucan ranging from 2.4 to 8.3 %. Anderson *et al.* (1978) recorded grain β-glucan ranging from 3.6 to 6.4 % in a set of Canadian and Australian varieties (the Canadian varieties were chosen on the basis of low extractable non-starch polysaccharides content), and Wei *et al.* (2009) reported values from 2.6 to 6.2 % observed in a segregating doubled haploid population derived from a cross between the two-row Australian malting variety Gairdner and a six-row salt-tolerant variety CM72. Evans *et al.* (1999) observed malt β-glucan values between 0.005 to 0.053 % in a population of Australian, German, Finnish, and Canadian malting varieties, while Pauli *et al.* (2015) reported a wider range (284 to 1,247.8 ppm) among lines included in the Barley Coordinated Agricultural Project, which consisted of feed, food, and malt lines from ten different USA breeding programs. Wang *et al.* (2004) reported the highest values for malt β-glucan, ranging from 0.39 to 1.44 %, within eight Asian cultivars of two-row, winter barleys (both feed and malt) popular in southern China. Beattie *et al.* (2010) studied a population of elite North American malting germplasm and found wort β-glucan content ranging from nine to 1,307 ppm, and Emebiri *et al.* (2009) observed values ranging from 52.8 to 1,200.5 ppm within doubled haploids derived from introgressing Harrington and Morex alleles into Australian malt quality breeding lines. The wider range in values for these three traits reported in other studies is not surprising given that they typically evaluated a set of less/unrelated germplasm, as compared to this study, which recorded values on lines derived from a bi-parental cross.

Kilned malt β-glucanase activity in this study ranged from 1,016 to 1,608 U/kg malt (Table 3.1). Zwickert-Menteur *et al.* (1996) observed markedly lower values, ranging from 177 to 679 U/kg malt when they analyzed 126 doubled haploids derived from a cross between North American barley varieties Steptoe (six-row feed) and Morex (six-row malt), which were both released in 1978. The wider range in reported values from this study can again be attributed to the greater genetic diversity present in the varieties used because the malt and feed varieties

would display greatly differing malting profiles, which would be reflected in their progeny. The high values reported in this study can be attributed to the CDC Kindersley parent which averaged 1,391 U/kg overall with the highest level observed in this study being 1,571 U/kg malt. This variety was released in 2013, approximately 35 years after the varieties used in the cross by Zwickert-Menteur *et al.* (1996), and highlights the improvements that can be achieved through plant breeding. Additionally, the transgressive segregation observed in the population, with several progeny showing higher β-glucanase activity than CDC Kindersley, indicates that further improvements can be made. This statement would also apply to the three other traits for which transgressive segregation was observed within the population.

Given that both β-glucanase activity and grain β-glucan content are the two major factors which impact malt and wort β-glucan content, the significant correlations observed between these traits in this study are to be expected. The content of β-glucan in the grain at the beginning of the malting process should impact its content at the end of malting and after mashing, and the amount of enzyme activity present will determine the degree of β-glucan hydrolysis. Here, grain β-glucan content was significantly correlated to final malt and wort β-glucan content in two of the three site-years, with malt β-glucanase activity correlated in the remaining site-year, indicating, as with other studies, the importance of both traits.

In their study of Chinese cultivars, Wang *et al.* (2004) noted that malt β-glucan content was positively and significantly ($p<0.05$) correlated with grain β-glucan content, but negatively and significantly ($p<0.05$) correlated with malt β-glucanase activity, with correlation coefficients of 0.2883 and -0.2801, respectively. Edney *et al.* (1998) observed a significant ($p<0.001$) correlation of 0.23 between grain and malt β-glucan content in Canadian cultivars and breeding lines, but Jin *et al.* (2011) looked at wild and cultivated Tibetan barley and found that malt β-glucan content was mainly controlled by kilned malt β-glucanase activity, with a significant ($p<0.01$) correlation of -0.442. However, the same study also found a slight negative correlation between grain and malt β-glucan of -0.174. Henry (1989) observed a significant ($p<0.05$) correlation of 0.36 between β-glucanase activity and the amount of β-glucan degraded during malting in Australian varieties, along with a significant ($p<0.05$) correlation of 0.57 between grain β-glucan and malt β-glucan content. Similarly, de Sá and Palmer (2004) observed a significant ($p<0.00$) correlation of -0.90 between green malt β-glucanase activity and total malt β-glucan content in the Scottish varieties Chariot and Decanter. However, they found that the

correlation between β-glucan degradation and endo-β-glucanase activity in individual grains was not always significant, nor was β-glucan degradation always correlated to the physical modification of the endosperm in this case, indicating that β-glucan content and malt β-glucanase activity are not necessarily reliable indicators of the degree of endosperm modification during malting.

Most of the grain β-glucan is hydrolysed during the malting process, primarily in the germination stage, and little additional hydrolysis occurs during mashing, making the high correlation observed between malt and wort β-glucan logical. This also explains why the correlations between wort β-glucan and both grain β-glucan and kilned malt β-glucanase closely mirror those of malt β-glucan. Ingversen *et al.* (1989) noted a correlation of 0.71 (significant at $p<0.01$) between these traits in Danish barley breeding lines, leading them to conclude that malt β-glucan content analysis is a good indicator of malt quality as it relates to wort β-glucan content. Edney *et al.* (1998) also saw a significant ($p<0.001$) correlation of 0.86 between malt and wort β-glucan.

Interestingly, in the two site-years that β-glucanase activity was not correlated with malt or wort β-glucan content in this study, grain β-glucan content was (Table 3.5). It appears that, at least in the MK population, the influence of grain β-glucan content and β-glucanase activity on malt or wort β-glucan contents are linked. The lack of a significant correlation between grain β-glucan content and β-glucanase activity, however, is not unexpected for two reasons: 1) most β-glucanase is synthesized during germination and 2) there is typically no relationship between grain β-glucan content and protein content, so β-glucanase present in mature grain would have little effect on β-glucan. Previous studies (de Sá and Palmer 2004; Ellis *et al.* 1997; Wang *et al.* 2004) have noted this same lack of significant relationship.

The heritability values associated with grain, malt, and wort β-glucan were higher than other reports. The complex control of β-glucan synthesis, as indicated by the numerous *Csl* genes that may play roles in its formation (Burton and Fincher 2014; Burton *et al.* 2006, 2008; Doblin *et al.* 2009), would be indicative of lower heritability and may explain the lower heritability values observed in other studies. Hang *et al.* (2007) studied hulless and hulled USA breeding lines and varieties and concluded that grain β-glucan is influenced by the environment to the point that nearly half of the variability observed in their studied population was influenced by site-year; Islamovic *et al.* (2013) had a similar observation in a study of lines derived from a

cross between six-row feed varieties Falcon (non-waxy) and Azhul (waxy), with genotypic variance accounting for 42.9 % of grain β-glucan. In hulless accessions, Mežaka *et al.* (2011) saw markedly higher broad-sense heritability values for grain β-glucan (0.97), but this value dropped to 0.37 in hulled accessions, which is more akin to what Han *et al.* (1997) observed for the heritability of malt β-glucan (38.8 %) in a subset of the original Steptoe x Morex mapping population.

Like β-glucan biosynthesis, the hydrolysis of this carbohydrate is not fully understood, with a range of possible enzymes, such as solubilases and β-glucosidase (Bamforth 2010; Manners and Marshall 1969), playing roles. The heritability value for β-glucanase activity is easier to explain given that only two genes related to its expression (*HvGlb1* and *HvGlb2*) have been identified, and while it is probable that there are other underlying genes playing a role in its expression, these unknown factors are unlikely to have as great an influence on β-glucanase activity as the previously identified genes. Jin *et al.* (2011) found that β-glucan content and β-glucanase activity were both primarily controlled by genetic factors, but also impacted by the environment, with grain and malt β-glucan content from the previous year being able to explain 38.75 % and 41.22 %, respectively, of the variation for the current year and the previous year's β-glucanase activity explaining 21.32 % of the observed variation for the current year. Zwickert-Menteur *et al.* (1996) saw narrow-sense heritability values ranging from 0.81 to 0.94 and 0.71 to 0.91 for green and kilned malt β-glucanase activity, respectively.

The gaps observed in the constructed genetic map for the MK population primarily resulted from the fact that the population was derived from a cross between two elite malting barley varieties, CDC Meredith and CDC Kindersley, which were developed by the same breeding program. As such, it is unsurprising that there would be some common parentage between the two varieties, and thus genetically fixed regions of the genome. 'Manley' is common to both pedigrees, and a significant number of other breeding materials derived from the AAFC-Brandon and AAFC-Winnipeg programs is found in both pedigrees, potentially indicating further shared germplasm (Figs. 3.6 and 3.7). In addition, selection for good malting quality would result in certain relevant regions of the genome being conserved. Consequently, both β-glucanase genes, *HvGlb1* on 1H and *HvGlb2* on 7H, fall within regions of the genome which are genetically fixed in the studied population.

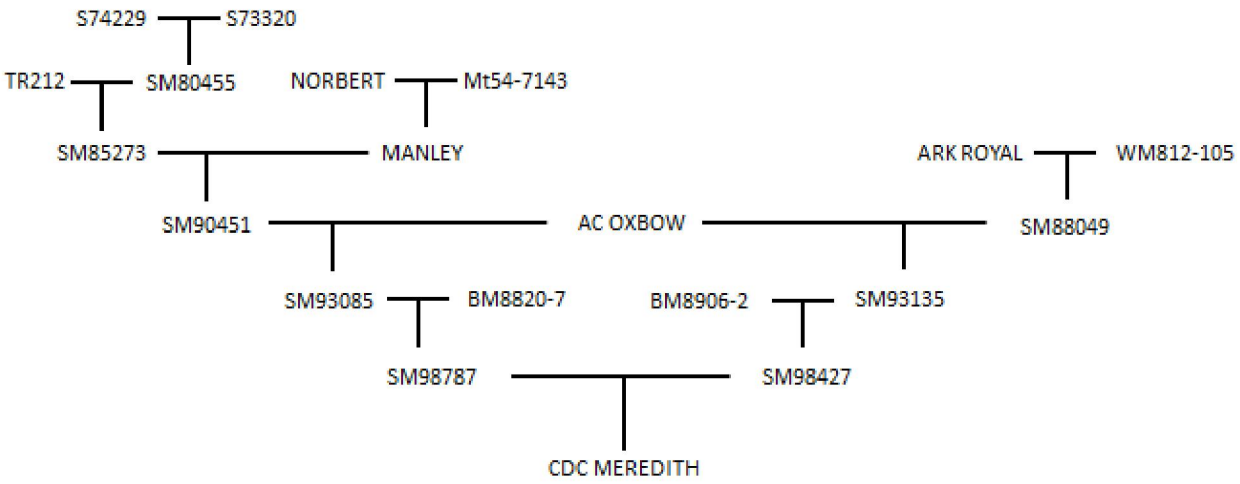

Figure 3.6. Partial pedigree for CDC Meredith. Breeding lines from the Crop Development Centre are designated with S or SM numbers, lines from the AAFC-Brandon breeding program are denoted with BM numbers, and lines from the AAFC-Winnipeg breeding program are denoted with WM numbers. AC Oxbow, Norbert, and TR212 are from the AAFC-Winnipeg program, Manley is from the CDC program, and Ark Royal is a UK variety.

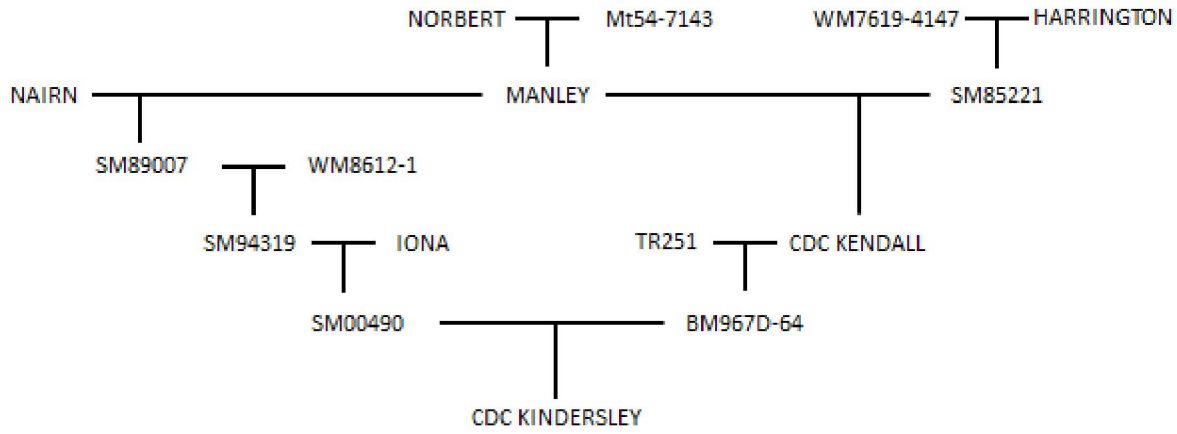

Figure 3.7. Partial pedigree for CDC Kindersley. Breeding lines from the Crop Development Centre are designated as with S or SM numbers, lines from the AAFC-Brandon breeding program are denoted with BM numbers and lines from the AAFC-Winnipeg breeding program are denoted with WM numbers. Norbert is from the AAFC-Winnipeg program, TR251 is from the AAFC-Brandon program, Manley, Harrington, and CDC Kendall are from the CDC program, and Iona is from the AAFC-Charlottetown program.

For some of the observed QTLs, we were able to identify underlying candidate genes that have a high probability of explaining the relationship to our traits of interest. The chromosome 1H grain β-glucan content QTL observed in this study mapped between two previously identified malt β-glucan content QTLs observed in previous studies (Han *et al.* 1995; Zwickert-Menteur *et al.* 1996). Its proximity to the previously identified QTLs suggests either a cluster of genes important to β-glucan reside in this region, or perhaps a lack of precision in the QTL mapping

indicating that the current QTL is identical to one of the previously mapped QTLs. Genes MLOC_17987.1 (at 435.6 Mb) and MLOC_70129.1 (at 435.5 Mb) on chromosome 1H, predicted to code for UDP-glycosyltransferase superfamily proteins, may explain the presence of this QTL as they reside within 0.2 Mb of the peak QTL marker (Table 3.8).

Both grain β-glucan content QTLs on 2H-2, although observed in different site-years, may be part of the known grain β-glucan QTL QBgn.StMo-2H.1 (Han *et al.* 1995). MLOC_81137.1 (at 15.7 Mb), predicted to be a UDP-glucuronosyl/UDP-glucosyltransferase, resides within 2 cM of the 2013 SF 2H-2 QTL peak (Table 3.8). A second gene (MLOC_64239.1 at 8.2 Mb) coding for an enzyme in the same superfamily is found underlying the other 2H-2 QTL identified in the 2013 KCRF site (Table 3.8). UDP-glucosyltransferases catalyze the formation of glycophospholipids rather than β-glucans from UDP-glucose, but the reactions would draw upon the same substrate pool and can occur in tandem (Becker *et al.* 1995).

The QTL interval for grain β-glucan content on chromosome 7H encompasses the genes MLOC_52796.1 (at 537.1 Mb) and MLOC_70708.1 (at 558.3 Mb), which are predicted glycosyltransferases (families 14 and 31) (Table 3.8). These genes, which respectively fall approximately 2.6 Mb and 18.5 Mb from the peak, are involved in cell wall metabolism and are a probable reason for the presence of a QTL linked to grain β-glucan. Among other enzymes, glycosyltransferase family 14 includes type II arabinogalactan β-glucuronosyltransferase, which has been found to be involved in the biosynthesis of another cell wall polysaccharide, arabinogalactan, in *Arabidopsis* (Knoch *et al.* 2013). Glycosyltransferase family 31 includes β-1,3-galactosyltransferases, which are involved in cell wall biosynthesis (Edwards *et al.* 1999). This enzyme specifically relates to galactomannans, which only make up approximately 2 % of the endosperm cell walls in barley, but are considered a major polysaccharide component of the cell wall nonetheless (Ballance and Manners 1978; Edwards *et al.* 1999).

The strong positive correlation between malt and wort β-glucan content was reflected in the identification of a common QTL on chromosome 1H in the same site-year (2013 KCRF). This QTL also coincides with the malt extract QTL QMe.StMo-1H.5 (Hayes *et al.* 1993), which is unsurprising given that the quantity of malt extract is related to how many solids are solubilized in the mashing process to produce wort (Briggs 1978). A greater extract concentration could result from higher enzymatic activity (both protein and carbohydrate related) which would result in more soluble compounds in the wort and lower concentrations of β-glucan

(Briggs 1978). No highly probable candidate genes for the 4H malt β-glucan content QTL were identified.

The 2H-1 kilned malt β-glucanase QTL encompasses a predicted concanavalin A-like lectin/glucanase gene, MLOC_52642.1 (at position 614.3 Mb) (Table 3.8). The concanavalin A-like domain is found in proteins belonging in glycosyl hydrolase family 16, including (1,3;1,4)-β-glucanase. The kilned malt β-glucanase QTL on 6H identified in this population contains a gene (MLOC_62475.1 at 480.2 Mb) believed to encode glycoside hydrolase (family 3), which includes β-glucosidases and β-D-glucan exohydrolase, among other cell wall degrading enzymes (Table 3.8). The endoglucanase 6 gene (MLOC_67192.1 at 471.2 Mb) is a putative endo-1,4-β-glucanase, part of the glycoside hydrolase family 9 (Table 3.8). The chromosome 7HL QTL did not fall near candidate genes with a high probability of explaining the observed QTL.

Glycosyl hydrolases break the glycosidic bond between two or more carbohydrates, as in the case of β-glucan. β-glucosidase and endoglucanase are components of cellulase, the multi-enzyme complex which hydrolyses cellulose (Singhania *et al.* 2012). The barley β-D-glucan exohydrolase studied by Varghese *et al.* (1999) is possibly involved in the partial hydrolysis of matrix-phase polysaccharides, including (1,3;1,4)-β-glucans. Given that all these enzymes play some role in the loosening or degradation of the cell wall, it is feasible that the QTLs for β-glucanase activity in these regions are due to the breakdown of β-glucan itself or of surrounding cell wall components that would result in the increased accessibility of β-glucan upon which β-glucanase would be free to act.

This study identified a number of QTLs linked to four important malting barley traits, namely grain, malt, and wort β-glucan content, and kilned malt β-glucanase activity. Only one QTL (grain β-glucan on chromosome 7H) was identified in multiple environments. While some of the QTLs appear to be in regions of the genome previously associated with these traits (grain β-glucan content on chromosomes 1H and 2H), a number of novel loci were identified for all four traits. Many of the QTLs had underlying genes with predicted functions that provide plausible explanations for the presence of the QTL. Additional phenotyping in the MK population to confirm these QTLs would add strength to the current findings and support future work to validate the QTLs in other populations and further examine the underlying genes.

4.0 PROJECT II: ASSOCIATION MAPPING (AM) FOR β-GLUCAN AND β-GLUCANASE IN AN ELITE MALTING BARLEY POPULATION

4.1 Introduction

(1,3;1,4)-β-glucan, hereafter referred to as β-glucan, is a hemicellulose found most commonly in the Poaceae family (Fincher 2009b). High concentrations of this polysaccharide are undesirable in the malt produced from malting barley varieties because it reduces the efficiency of the lautering and filtration processes during brewing (Briggs 1978). The enzyme (1,3;1,4)-β-glucanase, hereafter simply called β-glucanase, exists as two isoenzymes in barley, encoded by the *HvGlb1* and *HvGlb2* genes (Loi *et al.* 1988; Slakeski *et al.* 1990), and is largely responsible for the breakdown of β-glucan. As such, low levels of malt/wort β-glucan can be achieved through a combination of low grain β-glucan content and high malt β-glucanase activity.

β-glucanase is synthesized during the germination process and acts to degrade β-glucan at that time. Green (unkilned) malt has a higher level of β-glucanase activity than does kilned malt due to heat denaturation of the enzyme experienced during kilning. The EI β-glucanase isoenzyme (encoded by *HvGlb1*) is more heat labile than the EII isoenzyme (encoded by *HvGlb2*) (Loi *et al.* 1987, 1988; Slakeski *et al.* 1990). The heat stability of EII is one reason this isoenzyme is considered more important to reducing β-glucan content in the germinating grain, despite its synthesis being restricted to the aleurone, while EI is produced in multiple seed tissues (Stuart *et al.* 1986). Some β-glucan degradation occurs after the kilning process, at least in part due to the residual heat stable β-glucanase, which results in different levels of malt and wort β-glucan contents (Loi *et al.* 1987). Knowledge of the relative heat stability of β-glucanase in barley lines may allow a better estimate to be made of final wort β-glucan content. In addition, identifying lines with improved heat stable β-glucanase would further reduce wort β-glucan content and improve brewery performance.

QTL mapping and association mapping (AM) are two methods that can identify associations between regions of the genome and traits of interest. However, association mapping can evaluate a greater amount of phenotypic variability than what would be possible with QTL mapping. In addition, the more diverse genetic background of an AM panel means its findings are likely relevant to a wider diversity of germplasm because unlike bi-parental crosses of inbred lines which can only study two alleles at any given locus at once, the AM panel may contain

multi-allele loci (Flint-Garcia *et al.* 2003), and novel genes identified in these panels can be validated in bi-parental crossing populations (Kraakman *et al.* 2006).

The basis of identifying marker-trait associations with AM is linkage disequilibrium (LD), the non-random association of alleles between loci, that can be created from processes such as mutation, selection, migration, population admixture, population bottlenecks, founder effects, and genetic drift (Mackay and Powell 2007). As such, AM is highly dependent on pre-existing population structure, and both familial and genome-wide relatedness between lines. A diverse population with less structure yields fewer false marker-trait associations than one with high levels of structure. With this in mind, if an AM panel can be created to minimize structure, or at least one where structure is dealt with using various estimates of relatedness, the greater number of recombination events sampled in such populations would provide an opportunity to obtain a more precise genomic region related to the trait of interest.

Barley has a large genome size (5.1 Gb) and numerous repetitive elements (estimated to comprise 80 % of the genome), which slowed the production of a reference sequence for the barley genome (Mascher *et al.* 2013b). However, the International Barley Genome Sequencing Consortium (2012) developed a physical and genetic framework within which genomic regions relating to high- and low-confidence genes were identified. Mascher *et al.* (2013b) built upon the barley genetic/physical framework to develop an exome capture assay and then anchored and ordered next-generation sequencing contig assemblies via population sequencing (Mascher *et al.* 2013a). These resources have been extremely useful for identifying candidate genes residing near markers linked to important traits using strategies such as bulked segregant analysis, QTL mapping, and AM. For example, Zang *et al.* (2015) used fine mapping to assign true loose smut resistance gene *Un8* to fingerprinted contig 546 on the barley physical map. Similarly, Qin *et al.* (2015) characterized and fine mapped *HvSGRA*, a novel barley stage green-revertible albino gene, to within two adjacent fingerprinted contigs (contig_34437 and contig_46434) on the physical map. This map was also used to fine map *Rps6*, an intermediate host resistance gene to wheat stripe rust, to fingerprinted contig 320 (Dawson *et al.* 2016). Other work has demonstrated the effectiveness of mapping-by-sequencing when used in combination with a reference genome (Mascher *et al.* 2014).

Continued improvement of barley malt quality, in part through reduction of malt β-glucan content, may be achieved by identifying favourable combinations of elite malting barley parents

with complementary allele combinations. A first step towards this goal is to genetically and phenotypically characterize suitable elite germplasm which is adapted to the target production region. The goal of this study was to identify genomic regions associated with five malting quality traits (grain, malt, and wort β-glucan content, and green and kilned malt β-glucanase activity) using an AM panel composed of 92 elite breeding lines and varieties targeted for production in Western Canada which display a wide range in values for the traits. These traits were assessed over two site-years in Saskatchewan, Canada, and correlations and comparisons between them were made. Particular interest was given to green and kilned malt β-glucanase activity which may highlight lines containing more heat stable isoforms of β-glucanase.

4.2 Materials and Methods

4.2.1 Plant Material

The association mapping population (hereafter called the AM population) used in this study consisted of 92 two-row, spring elite malting breeding lines and varieties important to Western Canadian barley production. The lines and varieties were derived from eight different barley breeding programs: Agricore United (AU), Coors, Cargill, Busch Agricultural Resources Inc. (BARI) Canada, University of Saskatchewan Crop Development Centre (U of S CDC), Agriculture and Agri-Food Canada (AAFC) Brandon, Lacombe Field Crop Development Centre (FCDC), and AAFC-Lethbridge. This population, which included currently popular malting varieties like AC Metcalfe and CDC Copeland, as well as older varieties like Harrington and newer varieties like Merit 57, CDC Meredith, and Bentley, contained lines with a wide range of grain β-glucan content and β-glucanase activity.

The AM population was grown in 2011 with one replication at the Kernen Crop Research Farm (KCRF) and in 2013 as a two replication randomized complete block design (RCBD) at the KCRF. The KCRF, located at NW 5-36-4 W3, has a Sutherland clay-clay loam texture. Plots were grown as yield plots (1.2 m x 3.6 m) and were sprayed to control weeds. Grain was cleaned using a Rationel Kornservice seed cleaner (model SLN; A/S Rationel Kornservice, Esbjerg, Denmark) and sized over a 5.5/64” slotted screen.

4.2.2 Micromalting and Freeze Drying

The malting regime was carried out as described in Project I. For green malt analysis, samples were removed from the micromalter prior to the kilning stage. The germinated grain from each basket was immediately transferred to an aluminum weighing dish and covered with perforated aluminum foil. The samples were then frozen at -30°C and kept under low (less than 15 %) relative humidity until freeze dried. Freeze drying was carried out by POS Bio-Sciences (Saskatoon, SK) according to their standard freeze drying regime. One complete replication was freeze dried per batch.

4.2.3 Phenotyping

Grain, malt, and wort β-glucan content, and kilned malt β-glucanase activity were assessed as described in Project I. Green malt β-glucanase activity was assessed using the same method as kilned malt β-glucanase activity.

4.2.4 Genotyping

The AM population was genotyped with single nucleotide polymorphism (SNP) markers via the Infinium Assay at the Biosciences Research Laboratory, USDA-ARS (Fargo, ND) using the Barley 9K iSELECT Genotyping BeadChip (Illumina, Inc., San Diego, CA). A set of high quality markers were generated using the same filtering process as described in Project I, with the exception that a note was made of markers showing a minor allele frequency of less than 5 % within the population. Using the International Barley Genome Sequencing Consortium (IBSC) map as a reference map (International Barley Genome Sequencing Consortium 2012), hereafter referred to as the IBSC_2012 map, approximate positions of mapped SNP markers were identified using the Find Markers option on Barleymap.

4.2.5 Statistical Analysis

The data were analyzed as described in Project I. Heritability values were calculated as described in Project I with the exception that the least square (LS) means of the traits in 2013 were input into the equation along with the trait values from the single replication in 2011. This same strategy was used to calculate Tukey's honest significant difference (HSD) values at

$p \leq 0.05$. The HSD values were generated with the one-way ANOVA t-test function using SAS Enterprise Guide v. 7.1 (SAS Institute Inc., Cary, NC).

4.2.6 Association Mapping, LD Estimation, and Candidate Gene Identification

Population structure (Q) was determined using the model-based Bayesian clustering method implemented in STRUCTURE v. 2.3.4 (Pritchard *et al.* 2000; Falush *et al.* 2003, 2007) and using the modelling method described by Yu *et al.* (2006). Subpopulations were evaluated using a subset of the genotyping data. As such, 42 SNP markers were evaluated, three from each chromosome arm. These markers had little to no missing data and were spaced approximately 20 to 30 cM apart. The linkage ancestry model and correlated allele frequencies were used to test groups from one to 10 with 10 runs per group. A burn-in length of 100,000 iterations was used. These were followed by 100,000 Markov chain Monte Carlo (MCMC) iterations. Subpopulations were identified using Structure Harvester (Earl and vonHoldt 2012; http://taylor0.biology.ucla.edu/structureHarvester/), which utilizes the method described by Evanno *et al.* (2005).

Genome-wide relatedness (P) was used to control population structure and was obtained in two different ways: multidimensional scaling (MDS; P_M) and principal components analysis (PCA; P_P). For the MDS method, the Relationship Matrix function in JMP Genomics v. 7.1 (SAS Institute Inc., Cary, NC) was used to obtain identical by descent (IBD) values (K). The generated data were then input into the Multidimensional Scaling feature in JMP Genomics. Four dimensions or eigenvectors, as defined by Bowley 1999, were retained (P_M) and used in the AM analysis based on a visual inspection of the plot comparing distance correlation against the number of dimensions to determine where the slope of the graph changed.

Genome-wide relatedness values generated based on PCA were used to control population structure as in the model suggested by Price *et al.* (2006). Marker data (scored as AA = 00, AB = 01, BB = 11, and missing = -999) was used to produce the SimQual module association value matrix based on the DICE coefficient (Dice 1945) using NTSYSpc (v. 2.20N, Setauket, NY). The association values were input into the Dcenter (double centre) module to convert the original symmetrical similarity matrix to scalar product form. These transformed data were then used to compute eigenvectors and eigenvalues (which define the regression coefficients, as noted in Bowley 1999) using the Eigen module. As with the MDS method, the

distance correlation versus dimensions plot was assessed to determine the number of significant principal components. The first two eigenvectors (principal components) were used for visualization of the relationships.

NTSYSpc was also used to produce a dendrogram illustrating the relationships among the 92 lines. The association values generated from the SimQual module were used to cluster lines in the SAHN module using the unweighted pair group method with arithmetic mean (UPGMA) method outlined by Sneath and Sokal (1973), which was used to produce the dendrogram (Tree plot module). The dendrogram's validity was assessed by producing cophenetic values in an ultrametric association value matrix (Coph module) that was used in a goodness-of-fit test of the cluster analysis to the original similarity matrix produced using the MxComp (matrix comparison) module. The correlation between these two matrices, r, was completed using the 2-way Mantel test method (Mantel 1967).

Using these population structure evaluation methods, several different linear mixed models were evaluated using JMP Genomics with phenotypic data analyzed separately by site-year. These were: 1) P_P + K (using PCA), 2) P_M + K (using MDS), 3) Q + K, and 4) naïve (which included no population control methods). The naïve model was included to evaluate the effectiveness of the other three models with respect to controlling population structure and was generated using the SNP-Trait Association function in JMP Genomics. Significant markers were identified in all models using a false discovery rate (FDR) of 0.05 as calculated by the method of Benjamini and Hochberg (1995) which controls for multiple testing. A marker was also considered linked to a trait if its p-value was among the top three p-values reported in more than one model and in both site-years. All model analyses were run twice, once with all 6,261 polymorphic markers and once with only the polymorphic markers mapped to unique loci (1,697 in total, including three unmapped markers from the first analysis with very small p-values).

The square of the correlation coefficient, r^2, was the linkage disequilibrium (LD) measure examined using the Linkage Disequilibrium method in JMP Genomics. The NONEHWD haplotype estimation method was used for this analysis and LD calculations were performed for all pairs of markers within each chromosome. Only markers mapped to unique loci (via the IBSC_2012 map) were included in this analysis. A fitted curve for a nonlinear regression model, used to estimate LD decay, was generated using the linkage disequilibrium equation provided by Hill and Weir (1988):

$$r^2 = \frac{10 + d}{(2 + d)(11 + d)} \; x \; [1 + \frac{(3 + d)(12 + 12d + d^2)}{n(2 + d)(11 + d)}]$$

where *d* is the distance (in cM) between the pairwise markers and *n* is the population size.

Genomic positions for polymorphic SNP markers were determined using the position of barley genetic markers on the IBSC_2012 map on Barleymap, which is a genome-wide physical map derived from the six-row spring cultivar Morex. Loci with multiple map positions were excluded from the analysis. In this way, the genotypic data was partitioned into eight groups, one group for each of the seven barley chromosomes and one representing unmapped markers. Where possible, markers were additionally placed on the barley physical map available on Ensembl Plants.

As described in Project I, Ensembl Plants, PGSB: Plant Genome and Systems Biology, and Barleymap databases were used to identify putative explanatory genes underlying any marker-trait associations detected. In addition, the BARLEX: The Barley Genome Explorer and the Triticeae Toolbox (https://triticeaetoolbox.org/barley/) databases were used to place unmapped markers within the barley genome. Enzyme families and gene function were identified using the InterPro: protein sequence analysis and classification and CAZy: carbohydrate-active enzymes databases.

4.3 Results

4.3.1 Analysis of Phenotypic Data

Summary statistics for the five traits analyzed are shown in Table 4.1. In the AM population, average grain β-glucan content was higher in 2013 than 2011, while both green and kilned malt β-glucanase activity levels were lower in 2013 than 2011 (Table 4.1). As a result of these two factors, both malt and wort β-glucan contents were higher in 2013 than in 2011 (Table 4.1). Normality of the traits was assessed using the single replication value from 2011 and the LS mean values from 2013 (blocks were not a significant source of variation, data not shown). Most traits in both site-years were normally distributed. The exceptions were malt β-glucan content in 2011, which showed a slight negative skew, and wort β-glucan content in 2013, which showed a slight positive skew (Table 4.2). Attempts to improve the normality of the data using a

square or cube transformation (positive skew) or a log transformation (negative skew) did not improve the distribution. As with the MK population, a visual inspection of the untransformed data indicated that it met the assumption of normality and so were analyzed for associations.

Entry, year, and the interaction between entry and year were a significant source of variability for all traits examined with the exception of year for green malt β-glucanase activity (Table 4.3). As a result, marker-trait associations were analyzed separately by site-year.

Tukey's HSD values (Table 4.4) indicate that significant differences existed between some lines for all traits except wort β-glucan. Comparisons of lines based on the HSD value are provided for each trait in Appendix D (Tables D1 to D5).

No significant correlations were recorded between grain β-glucan content and green or kilned malt β-glucanase activity in the AM population (Table 4.5). Grain β-glucan content was observed to be significantly correlated with malt and wort β-glucan content in both site-years and in the KCRF 2013 site-year, respectively (Table 4.5). Both green and kilned malt β-glucanase activities were significantly correlated to both malt and wort β-glucan content in both site-years (Table 4.5). Malt and wort β-glucan were highly correlated in both site-years (Table 4.5). Finally, there was a strong correlation between green and kilned malt β-glucanase activity (Table 4.5).

The heritability of β-glucan content in grain, malt, and wort was moderate (ranging from 0.47 to 0.69), while the heritability of β-glucanase activity was higher (ranging from 0.80 to 0.88) (Table 4.6).

Table 4.1. Summary statistics for the five traits evaluated in the AM population grown in 2011 and 2013.

Year	Trait[1]	Mean	Minimum	Maximum	Standard Deviation
2011	Grain BG	4.0	2.9	5.0	0.45
	Green Malt BGase	1,981	1,242	2,529	241
	Kilned Malt BGase	1,335	633	1,797	224
	Malt BG	0.9	0.3	2.3	0.38
	Wort BG	740	137	1,302	287
2013	Grain BG	4.2	2.8	5.1	0.38
	Green Malt BGase	1,977	1,424	2,493	212
	Kilned Malt BGase	1,205	640	1,847	188
	Malt BG	1.4	0.7	2.4	0.32
	Wort BG	1,057	606	1,428	162

[1] BG: β-glucan (% w/w for grain and malt BG, ppm for wort BG); BGase: β-glucanase (U/kg malt).

Table 4.2. P-values obtained from the Shapiro-Wilk normality test for five traits measured in the AM population in 2011 and 2013.

Year	Location[1]	Trait	P-value
2011	KCRF	Grain β-glucan	0.10
		Green Malt β-glucanase	0.52
		Kilned Malt β-glucanase	0.47
		Malt β-glucan	0.01
		Wort β-glucan	0.09
2013	KCRF	Grain β-glucan	0.08
		Green Malt β-glucanase	0.64
		Kilned Malt β-glucanase	0.20
		Malt β-glucan	0.46
		Wort β-glucan	0.01

[1] KCRF: Kernen Crop Research Farm.

Table 4.3. P-values obtained for the effects of entry, year and their interaction for five traits measured on the AM population grown in 2011 and 2013.

Trait	Factor	P-value
Grain β-glucan	Entry	<0.01
	Year	<0.01
	Entry*Year	<0.01
Green Malt β-glucanase	Entry	<0.01
	Year	0.61
	Entry*Year	0.01
Kilned Malt β-glucanase	Entry	<0.01
	Year	<0.01
	Entry*Year	<0.01
Malt β-glucan	Entry	<0.01
	Year	<0.01
	Entry*Year	0.01
Wort β-glucan	Entry	<0.01
	Year	<0.01
	Entry*Year	<0.01

Table 4.4. Tukey's honest significant difference test values ($p \leq 0.05$) for the AM population grown in 2011 and 2013.

Trait	HSD Value
Grain β-glucan	1.3
Green Malt β-glucanase	464
Kilned Malt β-glucanase	584
Malt β-glucan	1.5
Wort β-glucan	1,200

Table 4.5. Pearson correlation coefficients among five traits measured in the AM population in 2011 and 2013.

Trait[1]	Site-Year[2]	Grain β-glucan	Green Malt β-glucanase	Kilned Malt β-glucanase	Malt β-glucan	Wort β-glucan
Grain BG	KCRF 2011	1				
	KCRF 2013	1				
Green Malt BGase	KCRF 2011	0.08	1			
	KCRF 2013	0.03	1			
Kilned Malt BGase	KCRF 2011	0.11	0.75***	1		
	KCRF 2013	0.18	0.82***	1		
Malt BG	KCRF 2011	0.27*	-0.51***	-0.31*	1	
	KCRF 2013	0.44***	-0.52***	-0.43***	1	
Wort BG	KCRF 2011	0.2	-0.50***	-0.32*	0.90***	1
	KCRF 2013	0.43***	-0.38**	-0.28*	0.88***	1

[1] BG: β-glucan; BGase: β-glucanase.
[2] KCRF: Kernen Crop Research Farm.
* Significant at $\alpha = 0.05$.
** Significant at $\alpha = 0.001$.
*** Significant at $\alpha = 0.0001$.

Table 4.6. Broad sense heritability estimates for the AM population grown in 2011 and 2013.

Trait	Phenotypic Variance	Genetic Variance	Environmental Variance	G x E Variance	Heritability
Grain β-glucan	0.18	0.10	0.01	0.04	0.69
Green Malt β-glucanase	51,360	40,450	0	4.37×10^{-10}	0.88
Kilned Malt β-glucanase	51,151	33,842	8,388	0	0.80
Malt β-glucan	0.22	0.10	0.10	6.6×10^{-15}	0.63
Wort β-glucan	54,094	31,805	49,844	2,430	0.47

4.3.2 Analysis of Green and Kilned Malt β-glucanase Activity

A comparison of green and kilned malt β-glucanase activity indicated a substantial range in variability for both traits. On a dry weight basis, green malt β-glucanase activity was

measured between 1,000 and 2,750 U/kg malt in 2011 and between 1,250 and 2,500 U/kg malt in 2013 (Figs. 4.1A and 4.1B). As shown in Figs. 4.1C and 4.1D, kilned malt β-glucanase activity (also on a dry weight basis) overlapped with the green malt β-glucanase activity and resided between 500 and 2,000 U/kg malt in both 2011 and 2013. The loss of β-glucanase activity after kilning (i.e. difference between green and kilned values) within an individual barley line ranged from 300 to 1,200 U/kg in 2011 and 500 to 1,200 U/kg in 2013 (Fig. 4.2). Green, kilned, and loss of malt β-glucanase activity across all 92 breeding lines in the AM population (averaged across 2011 and 2013) are presented in Appendix E (Fig. E1)

In each year, there was almost a two-fold difference in activity recorded for green malt β-glucanase among lines within the population (Table 4.7), while an approximate three-fold difference was observed for kilned malt β-glucanase over this period. Similarly, there was about a two-fold difference in enzyme heat lability observed each year (Table 4.7). Consistently across site-years, TR585 and TR250 were the lines with the highest β-glucanase activity in both green and kilned malt, while TR05286, TR03273, and TR05288 showed high kilned malt β-glucanase activity (Table 4.7). TR03191 appeared to have consistently high green malt β-glucanase activity across site-years (Table 4.7). TR635 had consistently low green and kilned malt β-glucanase activity across site-years (Table 4.7), while TR986, TR984, TR982, and TR633 also showed both low green and kilned malt β-glucanase activity, but not to the same consistency as TR635 (Table 4.7).

The mean loss of β-glucanase activity after kilning varied from 24.8 to 51.9 % (Table 4.7). The most heat stable lines included TR03273, TR704, and TR250, which showed the lowest loss in enzyme activity at one of the site-years and in the mean values across both site-years (Table 4.7). The most heat labile line was TR635, which demonstrated the largest percent loss in enzyme activity in one site-year and in the mean values across both site-years, although other heat labile lines included TR992, TR03191, TR990, and TR988 (Table 4.7). It was interesting to note that although TR03191 had high levels of green malt β-glucanase activity in both years, it also showed the third largest loss of activity after kilning in 2011 and the second largest loss of activity in the mean values across both site-years (Table 4.7), falling within the top 15 lines for largest loss of activity in 2013 (data not shown). TR03189, with the fifth highest green malt β-glucanase activity levels across both site-years (Table 4.7), was similar to TR03191 and also showed a consistently large loss of activity during kilning, averaging within the top 15

lines across both site-years (data not shown). This loss, which averages 42 % across both site-years, is greater than that of TR05671, which although having high green malt β-glucanase activity loses approximately 37 % of β-glucanase activity after kilning (data not shown). In contrast, TR03273 had consistently high β-glucanase activity in the kilned malt, despite not having high green malt β-glucanase activity, because of the heat stability of its enzyme activity (averaging just 26.8 % lost across both site-years (Table 4.7). The same is true of both TR05286 and TR05288, whose loss of β-glucanase activity fell just outside the lowest five and lowest 15, respectively (data not shown). The kilned malt β-glucanase activity reported for TR250 and TR585 was high (Table 4.7), but in both cases this was in large part due to their high green malt β-glucanase activities (Table 4.7). TR585 saw a consistently larger loss of activity than TR250, losing approximately 28 to 37 % compared to 23 to 26 % (data not shown).

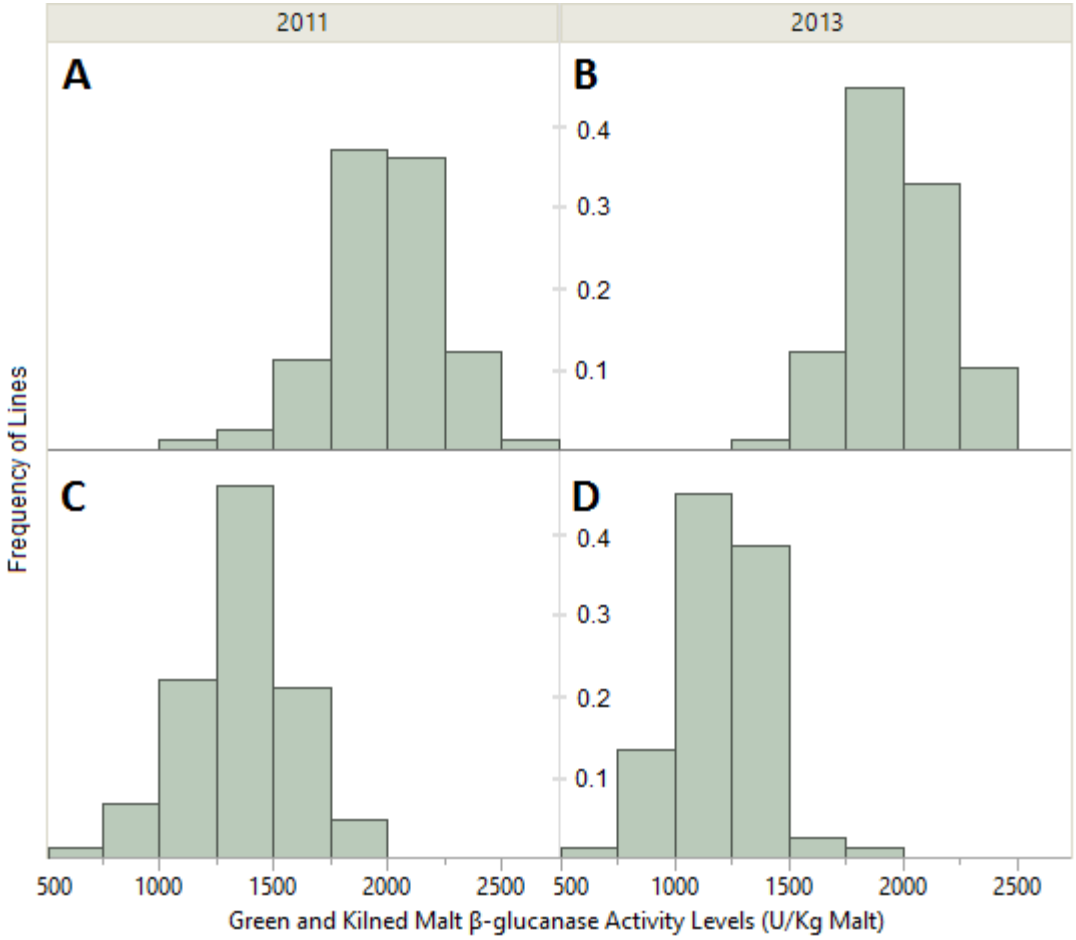

Figure 4.1. Distributions of green and kilned malt β-glucanase activity on a dry weight basis in the AM population in 2011 and 2013. (A) and (B) show green malt β-glucanase activity in 2011 and 2013, respectively, while (C) and (D) show kilned malt β-glucanase activity in 2011 and 2013, respectively.

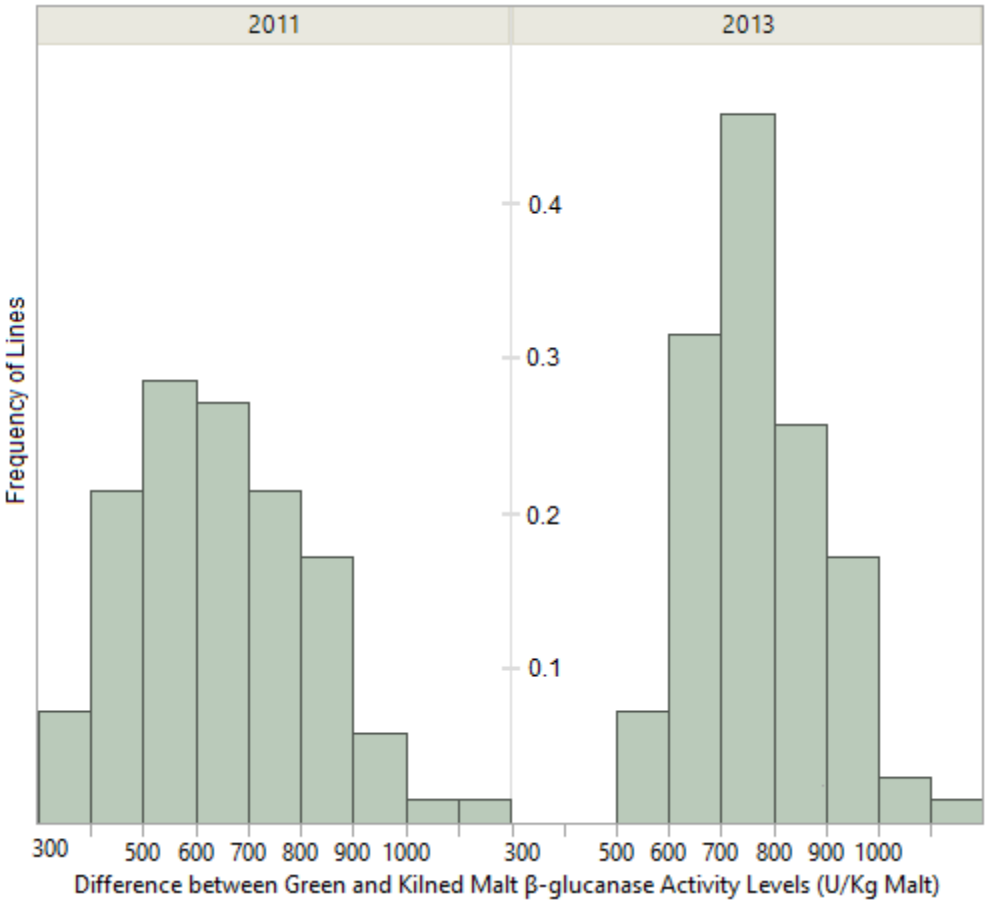

Figure 4.2. Distribution of malt β-glucanase activity lost after kilning (i.e. the difference between green and kilned malt β-glucanase activity on a dry weight basis) within individual barley lines from the AM population grown in 2011 and 2013.

Table 4.7. Green, kilned, and loss (after kilning) of malt β-glucanase activity in a subset of barley lines (indicated in parentheses) from the AM population representing the extreme values for these traits. Data is presented for the KCRF 2011 and KCRF 2013 site-years, and the mean across site-years.

Site-Year	Statistic	Green Malt BGase[1]	Kilned Malt BGase	Percent Loss
KCRF 2011	Minimum	1,242 (TR635)	633 (TR635)	20.1 (TR490)
		1,385 (TR633)	835 (TR441)	20.5 (TR584)
		1,486 (TR982)	870 (TR984)	20.5 (TR133)
		1,510 (TR986)	896 (TR986)	21.2 (TR965)
		1,551 (TR441)	956 (TR990)	21.3 (TR03273)
	Maximum	2,529 (TR585)	1,797 (TR585)	49.4 (TR992)
		2,496 (TR05671)	1,788 (TR250)	49.1 (TR635)
		2,341 (TR04196)	1,772 (TR03273)	48.8 (TR03191)
		2,341 (TR250)	1,751 (TR05286)	47.1 (TR990)
		2,337 (TR03191)	1,715 (TR05288)	46.2 (TR441)

Table 4.7. Continued

Site-Year	Statistic	Green Malt BGase[1]	Kilned Malt BGase	Percent Loss
KCRF 2013	Minimum	1,424 (TR635)	640 (TR635)	25.9 (TR250)
		1,576 (TR574)	769 (TR982)	27.1 (TR704)
		1,593 (TR982)	791 (TR988)	31.0 (TR964)
		1,600 (TR984)	841 (TR633)	31.5 (TR01715)
		1,614 (TR441)	884 (TR984)	31.7 (TR643)
	Maximum	2,493 (TR250)	1,847 (TR250)	55.0 (TR635)
		2,469 (TR03191)	1,623 (TR05286)	53.2 (TR988)
		2,417 (TR05286)	1,534 (TR05288)	51.7 (TR982)
		2,390 (TR01655)	1,499 (TR03273)	50.8 (TR985)
		2,357 (TR585)	1,466 (TR585)	49.5 (TR993)
Mean	Minimum	1,333 (TR635)	636 (TR635)	24.8 (TR250)
		1,509 (TR633)	876 (TR984)	26.8 (TR03273)
		1,540 (TR982)	899 (TR986)	27.0 (TR704)
		1,570 (TR574)	901 (TR988)	27.5 (TR05287)
		1,573 (TR986)	907 (TR633)	27.6 (TR05286)
	Maximum	2,443 (TR585)	1,817 (TR250)	51.9 (TR635)
		2,417 (TR250)	1,687 (TR05286)	50.3 (TR03191)
		2,403 (TR03191)	1,636 (TR03273)	48.1 (TR990)
		2,355 (TR05671)	1,632 (TR585)	47.9 (TR988)
		2,338 (TR03189)	1,625 (TR05288)	47.0 (TR992)

[1] BGase: β-glucanase.

4.3.3 Analysis of Genotypic Data

After removing markers that were monomorphic and had a high percentage of failed calls, a total of 6,261 polymorphic SNP markers were identified in the AM population. Approximate positions of these markers were determined using the International Barley Genome Sequencing Consortium 2012 (IBSC_2012) reference map on Barleymap. A total of 1,313 markers displayed a minor allele frequency of less than 5 %, but were included in the analysis to account for the possibility of rare alleles. Among the polymorphic markers, 1,103 were not present on the IBSC_2012 reference map, leaving a subset of 5,158 markers which were placed

at 1,694 unique loci (Table 4.8, Fig. 4.3). Appendix F (Tables F1 and F2) lists the chromosome and position of the 1,694 markers polymorphic in the AM population which were present on the IBSC_2012 consensus map.

While marker positions are only approximate because the mapping was not completed in this particular population, the noted positions demonstrate the coverage of the genome. The average distance between loci was 0.6 cM, with average distances by chromosome ranging from 0.5 to 0.8 cM (Table 4.8). The largest linkage group was created for chromosome 5H and was 169.4 cM in length, while the shortest linkage group was associated with chromosome 4H and was 115.2 cM long (Table 4.8). Across the entire map, the largest distance between adjacent loci was 11.0 cM on chromosome 4H (Table 4.8).

Table 4.8. Summary information for the genetic map created from 5,158 SNP markers polymorphic in the AM population. Data based on marker map positions in the IBSC_2012 reference map.

Chromosome	Markers in Linkage Group	Unique Loci	Linkage Group Length (cM)	Average Distance[1] (cM)	Largest Gap[1] (cM)
1H	518	179	132.8	0.8	4.5
2H	864	303	149.4	0.5	4.1
3H	786	272	155.0	0.6	7.2
4H	543	167	115.2	0.7	11.0
5H	1,051	318	169.4	0.5	8.7
6H	683	223	126.6	0.6	1.4
7H	713	232	140.9	0.6	4.5
Summary:	5,158	1,694	989.3	0.6	5.9

[1] Between markers.

4.3.4 Population Characteristics

The population chosen for association mapping was composed of breeding lines and varieties from eight different breeding programs. As shown in Fig. 4.4, the most unrelated lines have a distance coefficient of 0.62, as calculated by the unweighted pair group method with arithmetic mean (UPGMA), while the most closely related lines have a distance coefficient of nearly 1.00. The correlation between the original association value matrix and the cophenetic/ultrametric association value matrix was 0.88, indicating that the dendrogram has a high level of validity.

Some general patterns appeared in the data which corresponded to activities within the various breeding programs. For example, the majority of lines from each program were clustered together, but there were outliers that clustered with other programs, reflecting the

exchange of germplasm between the programs. This pattern was easier to observe in Fig. 4.5 when the population was evaluated using principal components analysis (PCA).

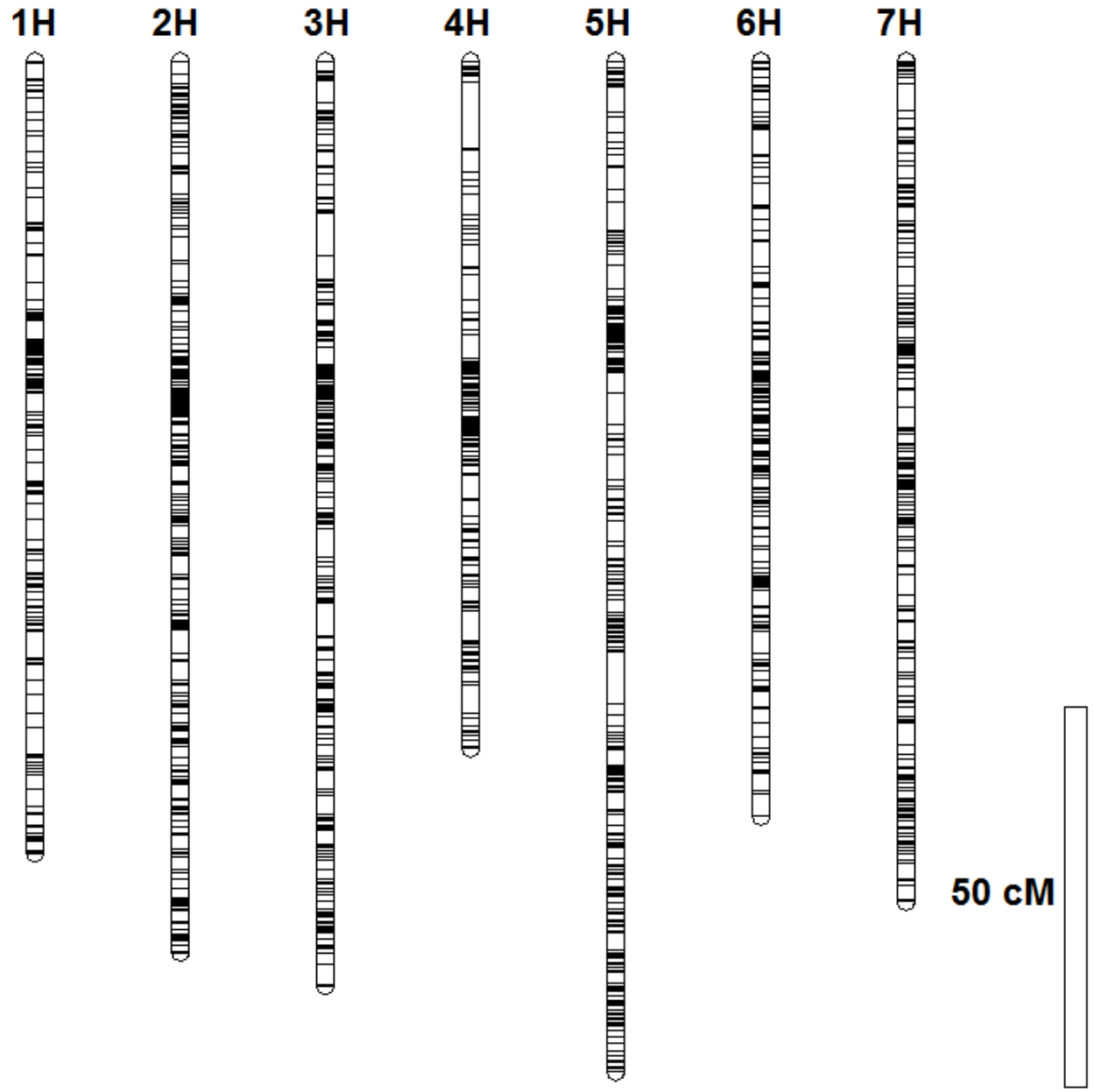

Figure 4.3. A genetic map representing the approximate positions of 5,158 polymorphic markers present in the AM population created using map data from the International Barley Genome Sequencing Consortium 2012 (IBSC_2012) reference map. This map includes seven linkage groups with 1,694 unique loci and spans 989.3 cM. The barley chromosome number is indicated at the top of each linkage group.

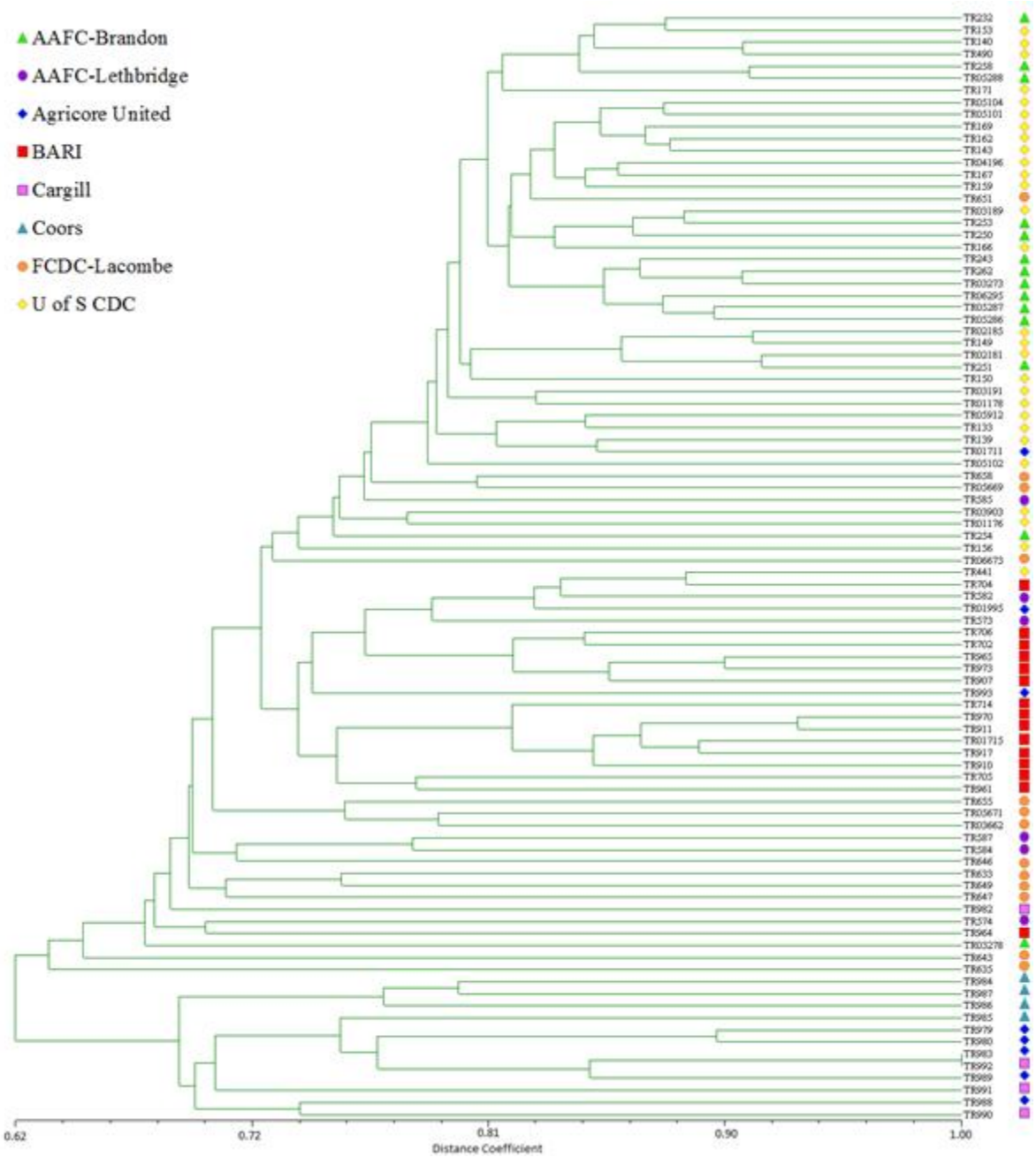

Figure 4.4. Unweighted pair group method with arithmetic mean (UPGMA) dendrogram showing the relationship between the 92 lines of the AM population. Line identification numbers are listed on the right, along with symbols corresponding to the breeding program of origin.

Of the two methods most commonly used to detect population structure, the Evanno method (Evanno *et al.* 2005) was chosen over the Pritchard method (Pritchard *et al.* 2000). Based on this analysis, three subpopulations were identified within the studied AM population (Fig. 4.6).

LD was calculated using the 1,694 unique loci, which resulted in 214,353 pairwise comparisons across the genome (Table 4.9). A total of 35,729 loci pairs (16.7 %) were in significant ($p<0.01$) LD, of which 1,137 loci pairs (0.5 %) were in complete LD (Table 4.9). The horizontal line in Fig. 4.7 denotes the point above which loci pairs were in LD at the 99 % confidence level, while the curved line represented the trend of expected LD decay. Genome-wide LD was 27.8 cM (Table 4.9). The longest LD occurred on chromosome 5H at 42.3 cM and the shortest LD was seen on chromosome 6H at 20.0 cM (Table 4.9).

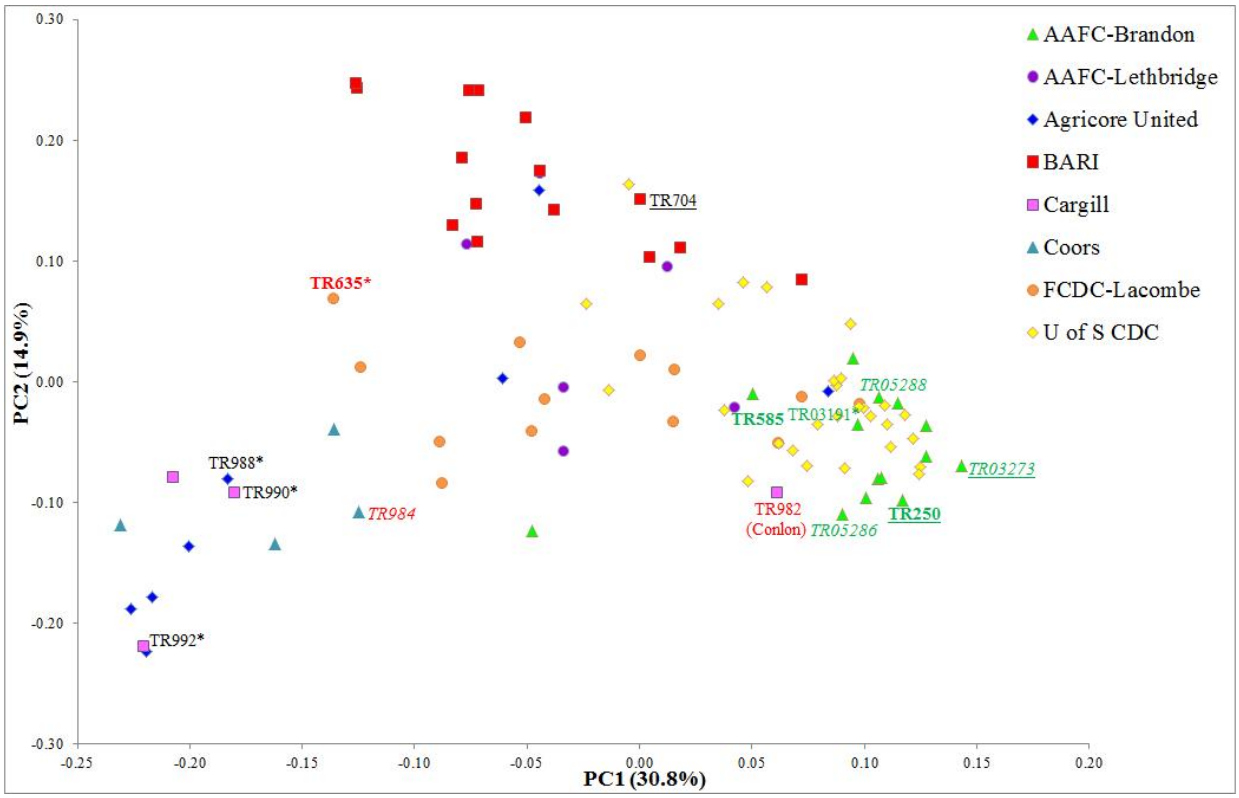

Figure 4.5. Scatter plot of the 92 lines within the AM population created using the first two principal components analysis (PCA) axes. The percentage of variance explained by each component is indicated on the axes. Lines within the AM population are divided by breeding program, as indicated by the different symbols. Lines displaying consistently high (TR250, TR585) and low (TR635) green and kilned malt β-glucanase activity are noted in bold green and bold red, respectively. Lines displaying consistently high green malt β-glucanase activity (TR03191) are noted in green, while those which are consistently low (TR982) are noted in red. Lines with consistently high kilned malt β-glucanase activity (TR03273, TR05286, TR05288) are noted in italicized green whereas those which are consistently low (TR984) are noted in italicized red. Lines with a consistently low percent loss of activity (TR704, TR250, TR03273) are underlined, while lines with a consistently high percent loss of activity (TR992, TR990, TR988, TR635, TR03191) are marked with an asterisk.

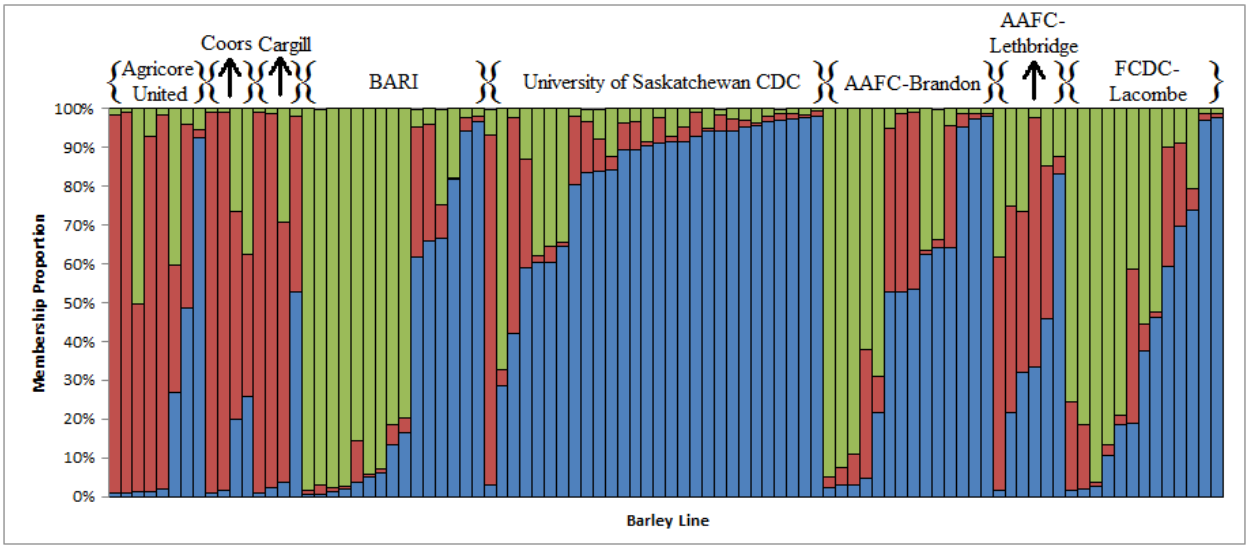

Figure 4.6. Distribution of genomic membership into three subpopulations identified using the linkage ancestry model in Structure. Each vertical bar represents one of the 92 lines in the AM population, with a colour representing the proportion of the genome assigned to one of three subpopulations. Breeding institutions from which lines were derived are indicated across the top.

Table 4.9. Total loci pairs and significant ($p<0.01$) loci pairs in LD for each chromosome and across the entire genome within the AM population. Average LD was determined using the genetic distance present between all significant loci pairs.

Chromosome	Total Loci Pairs	Significant Loci Pairs ($p<0.01$)	Loci Pairs in Complete Disequilibrium	Average LD (cM)
1H	15,931	2,171 (13.6 %)	106 (.05 %)	24.8
2H	45,753	7,265 (15.9 %)	708 (0.33 %)	34.1
3H	36,856	4,224 (11.5 %)	73 (0.03 %)	22.4
4H	13,861	2,385 (17.2 %)	63 (0.03 %)	21.8
5H	50,403	9,915 (19.7 %)	75 (0.03 %)	42.3
6H	24,753	4,189 (16.9 %)	66 (0.03 %)	20
7H	26,796	5,580 (20.8 %)	46 (0.02 %)	28.9
Genome-wide	214,353	35,729 (16.7 %)	1,137 (0.53 %)	27.8

4.3.5 Association Mapping Analysis

Association mapping was carried out using four different models: $P_P + K$, $P_M + K$, $Q + K$, and naïve. The results were not impacted by markers with a minor allele frequency (that is, removal did not noticeably reduce the false discovery rate), so those markers were retained. Model comparisons by trait for both site-years indicated that no single model performed best for every trait, and in most cases, there was very little difference between the models, including the naïve model with no correction for population structure (Figs. 4.8 and 4.9). With respect to the naïve model, this was somewhat unexpected as it is often the case that a large number of highly

significant marker-trait associations are observed. The expected behaviour of this model was seen most clearly for kilned malt β-glucanase activity in 2013 (Fig. 4.8B) and for both malt and wort β-glucan content in 2011 (Figs. 4.9B and 4.9C) in which there was a bowing of the curve away from the expectation (dashed) line.

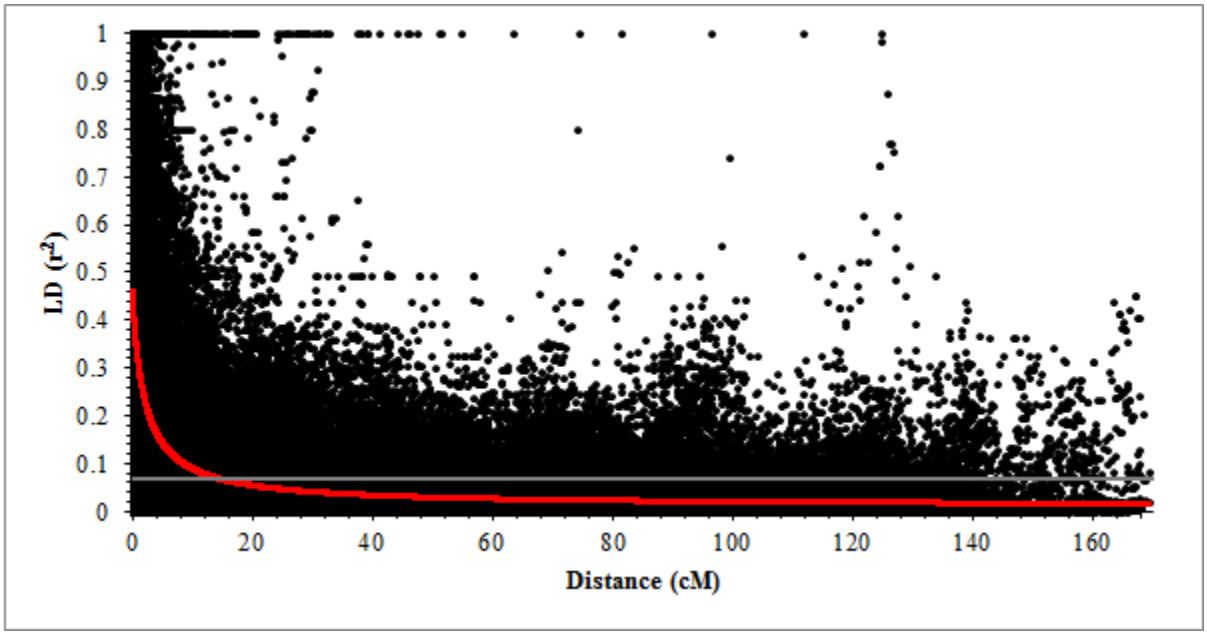

Figure 4.7. Genome-wide linkage disequilibrium (LD) decay plot for pairwise intrachromosomal marker comparisons. LD was represented by the square of the correlation coefficient, r^2. The horizontal line indicates pairs of markers in significant LD (above the line) as determined using the NONEHWD haplotype estimation and a p-value <0.01. The fitted curve represents the expected LD decay between adjacent loci based on the nonlinear regression equation given by Hill and Weir (1988).

No markers were considered significant after FDR correction when all 6,162 markers were included in the AM analysis. When only markers mapped to unique loci (1,697 markers, including three unmapped markers with very low p-values from the first analysis) were considered in the analysis, eight markers were found to be significantly associated with at least one of grain β-glucan content, green malt β-glucanase activity, or kilned malt β-glucanase activity (Table 4.10). Four of these markers (12_30968, 11_10895, 12_10704, and SCRI_RS_184274) had minor allele frequencies of less than 5 %. Six of the markers (12_30968, 11_10895, SCRI_RS_146936, SCRI_RS_202800, 11_20897, and SCRI_RS_11640) were significantly associated to traits after FDR correction (Table 4.10). No markers linked to either malt or wort β-glucan content were identified. In some cases, candidate genes which have a high probability of explaining the observed association with the traits of interest could be identified. Appendix G (Table G1) provides a description of the process to determine the

location of marker-trait associations observed for grain β-glucan content and green and kilned malt β-glucanase in the current study on other barley genetic and physical maps, along with the presence of underlying genes and QTL previously identified for these traits.

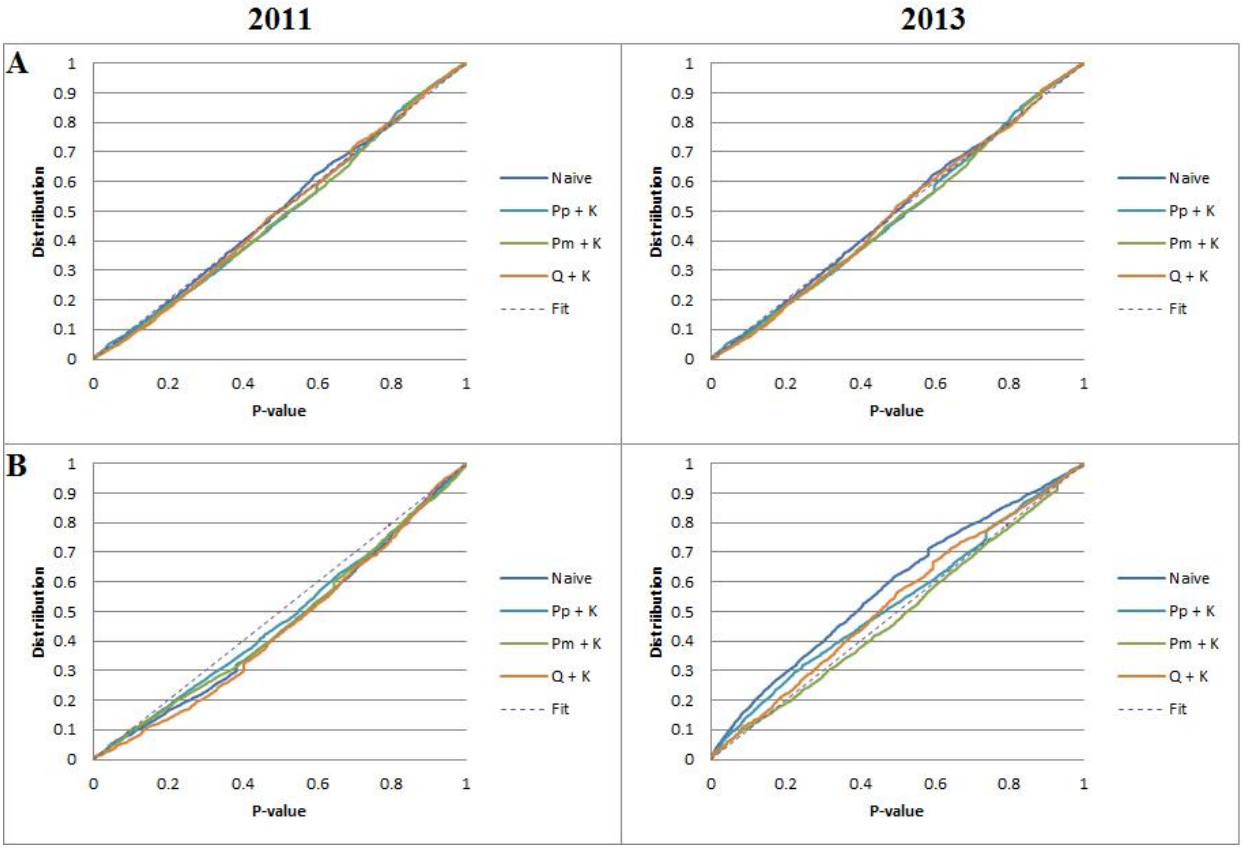

Figure 4.8. P-value distribution plots for (A) green malt β-glucanase activity and (B) kilned malt β-glucanase activity at KCRF in 2011 (left plots) and 2013 (right plots). The naïve model did not correct for kinship or population structure. The P_P + K model corrected for kinship and population structure using principal components analysis values for structure, the P_M + K model corrected for kinship and population structure using multidimensional scaling values for structure, and the Q + K model corrected for kinship and population structure using Structure-derived Q values for structure.

SNP marker 12_30968, located on chromosome 1H, was associated with both grain β-glucan content and green malt β-glucanase activity. The marker resides within morex_contig_49842 and falls at approximately 523.9 kb on the barley physical map. SNP marker 11_10895, located at approximately 637.0 kb on chromosome 1H (Table 4.11), is positioned less than 150 kb from SNP marker 12_30968 and was also associated with grain β-glucan content (Table 4.10).

The SCRI_RS_184274 marker was associated with both green and kilned malt β-glucanase activity (Table 4.10). This marker has been mapped to the same position on chromosome 1H as SCRI_RS_224686 (using the Morex x Barke map derived from Close *et al.*

2009), which is associated with morex_contig_42904 at 6.6 Mb on the physical map. Explanatory candidate genes related to the marker-trait association observed between this marker and green/kilned malt β-glucanase activity could not be identified.

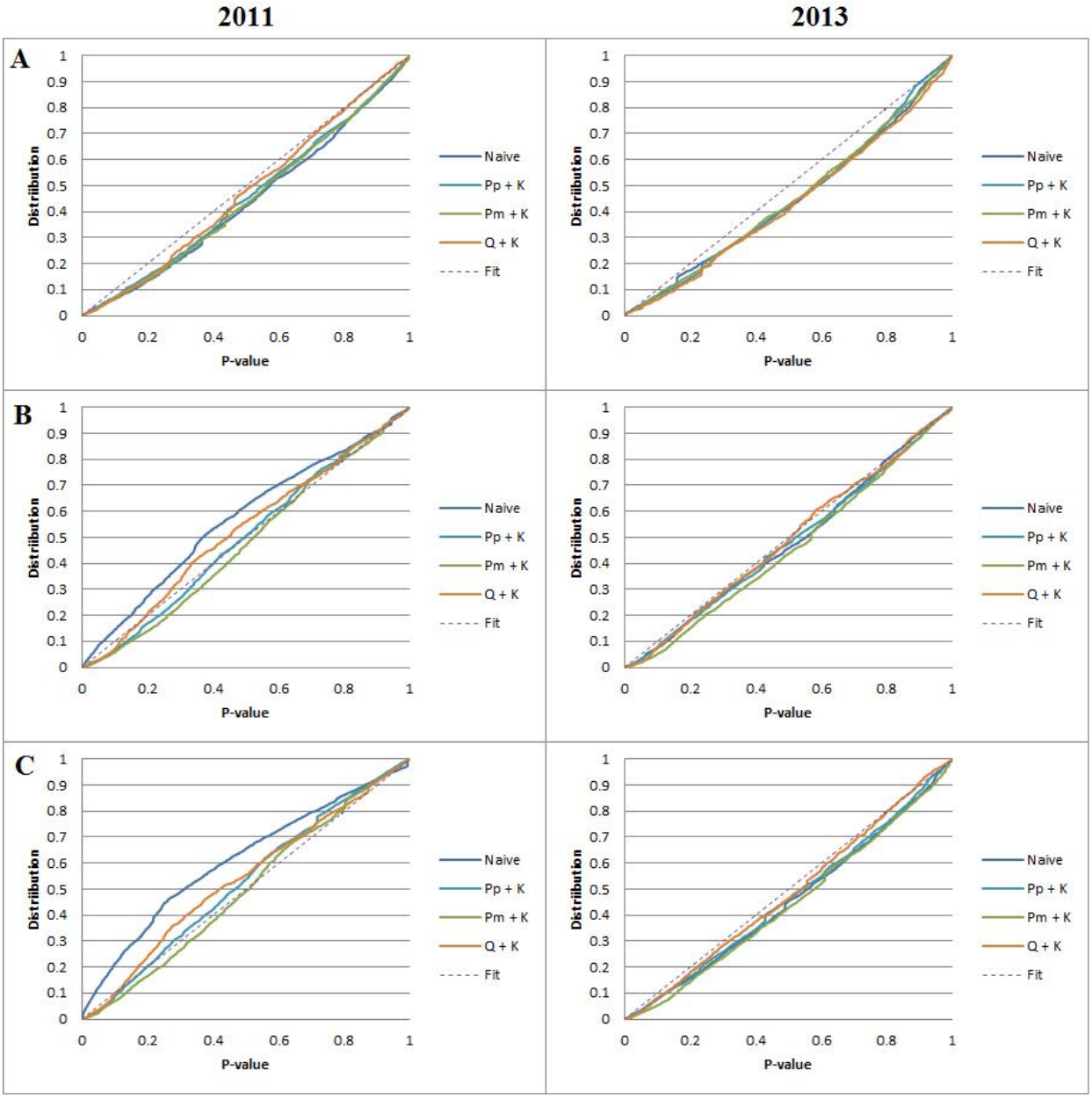

Figure 4.9. P-value distribution plots for (A) grain β-glucan content, (B) malt β-glucan content, and (C) wort β-glucan content at KCRF in 2011 (left plots) and 2013 (right plots). The naïve model did not correct for kinship or population structure. The P_P + K model corrected for kinship and population structure using principal components analysis values for structure, the P_M + K model corrected for kinship and population structure using multidimensional scaling values for structure, and the Q + K model corrected for kinship and population structure using Structure-derived Q values for structure.

Table 4.10. Marker-trait associations identified in the AM population for five traits measured at KCRF in 2011 and 2013.

Marker	Chr[1]	Position[2]	Year	Trait(s)[3]	Model		P-value Range
					FDR	No FDR[4]	
12_30968	1H	0.0	2011	Grain BG, Green BGase		Naïve, P_M + K	0.0002-0.0015
			2013	Grain BG, Green BGase	Naïve	P_P + K, P_M + K, Q + K	0.0001-0.0005
11_10895	1H	0.2	2011	Grain BG		Naïve	0.0001-0.0014
			2013	Grain BG		Naïve, P_P + K, P_M + K	<0.0001-0.0008
SCRI_RS_184274	1H	9.9	2011	Green/Kilned BGase		Naïve, P_M + K, Q + K	0.0001-0.0005
			2013	Green/Kilned BGase		Naïve, P_M + K, Q + K	<0.0001-0.0005
SCRI_RS_146936	2H	4.3	2011	Green BGase		P_P + K	0.0016
			2013	Grain BG	Naïve	Q + K	<0.0001-0.0004
SCRI_RS_202800	5H	164.7	2011	Kilned BGase	Naïve, Q + K		<0.0001
11_20897	U (5H)	0	2011	Kilned BGase	Naïve, Q + K		<0.0001
12_10704	6H	100.9	2011	Green BGase		Naïve, P_M + K	0.0002-0.0004
			2013	Green/Kilned BGase		Naïve, P_M + K	0.0002-0.0014
SCRI_RS_11640	U (6HS)	0	2013	Green BGase	Naïve	P_M + K, Q + K	<0.0001

[1] Chromosome.
[2] Approximate position on IBSC_2012 (Cantalapiedra *et al.* 2015), in cM.
[3] BG: β-glucan; BGase: β-glucanase.
[4] To be included, markers must have a low p-value range (only the lowest three for each trait in each model in each year were considered) and this low range must be consistent across years (2011 and 2013).

Marker SCRI_RS_146936, associated with green malt β-glucanase activity and grain β-glucan content, is present at 7.5 Mb on the physical map of chromosome 2H and resides within morex_contig_2556038 (Table 4.11). This gene is 700 kb from MLOC_64239.1 (located at 8.2 Mb), which encodes a UDP-glycosyltransferase (Table 4.11). MLOC_13397.1 (at 7.7 Mb) falls in this region and is associated with gibberellin receptor GID1L2 (Table 4.11). The observed QTL here is likely the same as that of known QTL QBgn.StMo-2H.1 (Han *et al.* 1995; Szűcs *et al.* 2009), which falls at approximately 2.2 Mb on the barley physical map.

Table 4.11. Putative genes underlying the marker-trait associations with grain β-glucan content and green and kilned malt β-glucanase activity as identified in the AM population grown in 2011 and 2013

Marker	Chr[1]	Position in Physical Map (Mb)	Candidate Gene(s)	Position in Physical Map (Mb)	Predicted Function	Prior QTL
12_30968	1H	523.9 kb	MLOC_13737	918.7 kb	concanavalin A-like lectin/glucanase subgroup	-
			MLOC_60692	1.4	concanavalin A-like lectin/glucanase, subgroup	-
11_10895	1H	637.0 kb	-	-	-	-
SCRI_RS_184274	1H	6.6	-	-	-	-
SCRI_RS_146936	2H	7.5	MLOC_64239	8.2	UDP-glycosyl-transferase	QBgn.StMo-2H.1 (Han *et al.* 1995; Szűcs *et al.* 2009)
			MLOC_13397	7.7	gibberellin receptor	QBgn.StMo-2H.1 (Han *et al.* 1995; Szűcs *et al.* 2009)
SCRI_RS_202800	5H	546.0	-	-	-	QBgnm.HaTR-5H.2 (Mather *et al.* 2003; Szűcs *et al.* 2009)
11_20897	U (5H)	541.7	-	-	-	QBgnm.HaTR-5H.2 (Mather *et al.* 2003; Szűcs *et al.* 2009)
12_10704	6H	518.2	MLOC_59341	516.0	concanavalin A-like lectin/glucanase	-
SCRI_RS_11640	U (6H)	410.8 kb	-	-	-	-

[1] Chromosome.

Marker SCRI_RS_202800, associated with kilned malt β-glucanase activity (Table 4.10), resides with morex_contig_2548020 and is located at 546.0 Mb on chromosome 5H of the barley physical map (Table 4.11). SNP marker 11_20897, also associated with kilned malt β-glucanase activity (Table 4.10), was not mapped on the physical reference map, but is associated with morex_contig_127315 and has been mapped to position 177.9 cM on chromosome 5H (Muñoz-Amatriaín *et al.* 2011) which is located at approximately 541.7 Mb on the physical map of chromosome 5H, near SNP marker SCRI_RS_202800 (Table 4.11). Both markers were

associated with kilned malt β-glucanase activity and were likely part of the same QTL. This QTL may be QBgnm.HaTR-5H.2, identified by Mather *et al.* (2003) and again by Szűcs *et al.* (2009), which falls at approximately 552.0 Mb on the physical map.

The SNP 12_10704 marker, which was associated with both green and kilned malt β-glucanase activity, is present within morex_contig_1593246 at position 518.2 Mb in the physical map. Located nearby at 516.0 Mb is MLOC_59341.1, which is predicted to encode a concanavalin A-like lectin/glucanase (Table 4.11).

SNP marker SCRI_RS_11640, associated with green malt β-glucanase activity, is present on the physical reference map at approximately 0.4 Mb on chromosome 6HS but no explanatory candidate genes could be found.

4.4 Discussion

Phenotypic values for the five traits measured in the AM population displayed a greater range than recorded in the MK population. This finding is not unexpected given the broader genetic diversity within the AM population. Means of the five traits tended to be similar to or slightly higher than those seen in the MK population, and consequently the standard deviations observed were typically greater in the AM population. Tukey's HSD values indicated that there were significant differences between the AM population lines for each of the traits, except wort β-glucan content. Grain, malt, and wort β-glucan content in this study across both site-years ranged from 2.8 to 5.1 %, 0.3 to 2.4 %, and 137 to 1,428 ppm respectively. Holtekjølen *et al.* (2006) observed grain β-glucan ranging from 2.4 to 8.3 % in a study of barley varieties (hulled and hulless) from Norway, Denmark, Finland, Sweden, and Canada. Higher β-glucan values are associated with hulless genotypes (Mežaka *et al.* 2011), which were not represented in this study. Pauli *et al.* (2015) examined feed, food, and malt barley lines from ten different breeding programs in the USA and reported a range in malt β-glucan values from 284 to 1,247.8 ppm. The higher malt β-glucan values observed in this study is likely due to a combination of environmental factors and differing genetics within the studied barley lines. Beattie *et al.* (2010) reported wort β-glucan values ranging from nine to 1,307 ppm in a population of elite North American malting germplasm, a range which encompasses the values observed in this study. The current study observed a range in green and kilned malt β-glucanase activity across both site-years from 1,242 to 2,529 U/kg malt and 633 to 1,847 U/kg malt. Zwickert-Menteur *et al.*

(1996) studied doubled haploids derived from a cross between feed barley (Steptoe) and malting barley (Morex) and reported green and kilned malt β-glucanase activity levels range from 481 to 1,243 U/kg malt and 177 to 414 U/kg malt, respectively. The higher values observed in this study are to be expected, given the overall improvement of β-glucanase activity in malt varieties achieved through breeding efforts and the fact that the lines in this study were all bred for malt quality as opposed to feed, which would have a poorer malting quality profile.

The lack of significant correlation between grain β-glucan content and malt β-glucanase activity was expected, given that most β-glucanase is synthesized after germination and that there is typically no relationship between β-glucan and protein content, so β-glucanase present in the mature grain would not affect β-glucan. The positive correlation seen between grain β-glucan and malt and wort β-glucan was reasonable because initial grain β-glucan content should impact the content of β-glucan remaining after the malting and mashing processes. Since β-glucanase activity is another major factor in determining malt and wort β-glucan content, the correlation between these traits was logical. The strong correlation observed between malt and wort β-glucan is due to most of the β-glucan being hydrolysed during the malting process with little additional hydrolysis occurring during mashing. Finally, the strong correlation between green and kilned malt β-glucanase activity indicated that heat lability of the β-glucanase enzyme did not differ much across genotypes, although the lack of complete correlation indicates that some differences do exist.

The heritability of grain β-glucan content and kilned malt β-glucanase activity was slightly greater than those recorded in the MK population, with malt β-glucan content being about the same and wort β-glucan content being less. The difference in heritability between the two populations was not unexpected because the populations have an entirely different structure and formation, with the most relevant factor being that the AM population had more genetic diversity than the MK population (which would impact the calculation of heritability).

With respect to β-glucanase activity, a combination of high activity and greater heat stability would be the most effective manner to reduce wort β-glucan content. Consistently high green malt β-glucanase activity was recorded for TR250, TR585, and TR03191, with TR250 and TR585 also having high kilned malt β-glucanase activity, along with TR05286, TR03273, and TR05288 (Table 4.7). The high levels of kilned malt β-glucanase activity in these lines resulted from different mechanisms. For lines such as TR250 and TR585, high kilned malt β-glucanase

activity was due more to their high green malt β-glucanase activity, since each line lost a relatively large proportion of activity after kilning (28 to 37 % for TR585 and 23 to 26 % for TR250). TR03191 was an even more extreme case of heat lability in the β-glucanase activity as this line had consistently high green malt β-glucanase activity, but also one of the highest proportions of heat labile enzymatic activity, losing 50.3 % of its β-glucanase activity. In contrast, TR03273 did not have extremely high green malt β-glucanase activity, but because it had very good heat stability (losing just 26 % of its activity) this line had amongst the highest kilned malt β-glucanase activity.

Since the goal of a malting barley program is to release varieties that produce low malt/wort β-glucan, lines such as TR250 and TR585 would be the most desirable as breeding parents. Other lines showing good heat stability, such as TR03273 and others without high levels of kilned malt β-glucanase activity (e.g. TR704), would also provide value as parents to impart a greater proportion of heat stability if combined with a second parent containing high levels of green malt β-glucanase activity.

It has been established that the isoenzyme encoded by *HvGlb2* is more heat stable than that of the one encoded by *HvGlb1* (Brunswick *et al.* 1987; Loi *et al.* 1987), though the exact reasons behind this thermostability are unclear. Using the more thermostable bacterial β-glucanases as a basis, Jensen *et al.* (1996) adapted the codon usage of a thermostable β-glucanase gene in bacteria to mimic the structure of the *HvGlb2* gene and was able to create transgenic plants which synthesized the thermostable version of the enzyme. In this case, given the molecular structure of the enzyme (Keitel *et al.* 1993), the thermostability of the bacterial β-glucanase is suggested to be due to the formation of hydrogen bonds between N- and C-terminal β-sheets (Jensen *et al.* 1996). Niu *et al.* (2010) noted the importance of particular N- and C-terminal residues on the thermostability of lichenase from *Clostridium thermocellum*, finding that residues at positions 10 to 25 of the N-terminal and 1 to 9 of the C-terminal are vital for high thermostability. Again, the hydrogen bonds between these terminal residues were implicated in the thermostability of the enzyme, while the Thr-Pro box between the catalytic and dockerin domains of the enzyme was believed to positively affect its catalytic efficiency (Niu *et al.* 2010). In addition, Keitel *et al.* (1994) observed that calcium ions stabilize *Bacillus* β-glucanase, allowing for enhanced activity of the enzyme at higher temperatures. This stabilizing effect was also observed in an examination of α-amylase from barley malt (Buckow *et al.* 2007). The

thermostability of α-glucosidase is dependent upon particular N-glycosylation recognition sites, as determined by Clark *et al.* (2004) upon point mutagenesis of barley α-glucosidase in genomic regions identified through a comparison with the more heat stable sugar beet α-glucosidase. In barley limit dextrinase, certain amino acid substitutions in the gene confer greater heat stability to the enzyme (Yang *et al.* 2009). It is possible that minor genetic differences such as these play a role in determining the thermostability of barley β-glucanase.

The first component axis of the PCA plot provided some discrimination between lines based on β-glucanase activity (Fig. 4.5). Lines with high green and/or kilned malt β-glucanase activity tended to cluster towards the upper end of the x axis while low β-glucanase activity lines clustered (with more dispersion) towards the opposite end of the axis. The exception was TR982 (Conlon) from the Cargill program, which had low β-glucanase activity (data not shown) but was more closely clustered with lines displaying high β-glucanase activity. Inherent in this differentiation based on β-glucanase activity was the link to breeding program. The CDC and AAFC-Brandon programs tended to represent the majority of lines with high activity, with TR585 from the AAFC-Lethbridge program being an exception. The AAFC-Brandon and CDC programs routinely share germplasm, thus it is not unexpected to observe common malt quality performance, specifically β-glucanase activity, in lines derived from these programs. The reason behind the clustering of TR585 with the AAFC-Brandon and CDC lines is less clear, other than perhaps unknown, but common parentage with CDC or AAFC lines. Conversely, lines from FCDC-Lacombe and Coors represented nearly all the lines with the lowest activity, with TR441 (Harrington) from the CDC program being the exception (data not shown). This is an interesting observation for Harrington as it was known to produce higher wort β-glucan levels.

Studies in barley have reported a wide range of genome-wide LD, with the extent of the LD strongly influenced by population structure and whether or not the population was composed of elite or wild germplasm. Rostoks *et al.* (2006) found highly significant intrachromosomal LD (r^2>0.5, p>0.001) in their combined European elite spring/winter barley breeding population extending up to 60 cM, with an average of 3.9 cM. When only the spring two-row subset was studied LD extended up to 15 cM with a mean of 1.53 cM. Similarly, Beattie *et al.* (2010) reported a genome-wide LD of 1.9 cM (r^2>0.05, p<0.001) and 13 cM (99^{th} percentile of unlinked markers) using the same elite AM population as this study. Comadran *et al.* (2011) observed that blocks of LD (r^2=0.5) over the entire genome decayed quickly in elite accessions of cultivated

barley, the vast majority (>95 %) after 3 cM. Hamblin *et al.* (2010) observed LD decaying at distances of 20 to 30 cM when studying a subset of lines from the Barley Coordinated Agricultural Project, which consisted of malt, feed, food, and fuel targeted barley germplasm from 10 different breeding programs in the USA. Caldwell *et al.* (2006) observed that LD obtained from sequence data surrounding the *Ha* locus decayed on average at 212 kb in elite germplasm while it extended to less than 100 kb in landraces and wild germplasm.

Comadran *et al.* (2009) examined LD in a highly structured association mapping population representing landraces, as well as old and new cultivars grown around the Mediterranean basin. They found a large range in LD values, varying from marker pairs 50 cM apart and showing significant LD, to those closely linked and in complete equilibrium. Overall, mean LD in their population was determined to be 3.2 cM (r^2=0.15). Wild barley populations (*H. spontaneum*) generally have low levels of LD with loci linked at a distance of ≥7 cM and have an LD level similar to that of unlinked loci (Morrell *et al.* 2005). Genome-wide LD of 27.8 cM observed in this study was greater than other reports using elite germplasm populations. This greater value seems to be indicative of population structure (Gupta *et al.* 2005), but analysis of the population structure indicates that it does not have a significant influence on this population. Increased LD can be attributed to a small population size (Gupta *et al.* 2005), yet the population analyzed here, while smaller than in some studies (Caldwell *et al.* 2006; Comadran *et al.* 2009), was approximately as large as those used in previous studies (Beattie *et al.* 2010; Rostoks *et al.* 2006). Numerous other factors influence LD, yet it is difficult to pinpoint which ones may have resulted in the large LD values observed in this study given the lower LD values reported by Beattie *et al.* (2010) in a study using the same population.

Evaluation of population structure in AM population using the method of Evanno *et al.* (2005) indicated the presence of three subpopulations (Fig. 4.6). This finding was in contrast to the four subpopulations identified by Beattie *et al.* (2010) in the same population using the Pritchard method (Pritchard *et al.* 2000). The CDC lines tended to show a greater proportion of the blue subpopulation than other programs (Fig. 4.6), with a few lines from other programs (excepting the Coors and Cargill programs) displaying a similar distribution which is reflected in the clustering of the PCA plot (Fig. 4.5). Likewise, the red subpopulation was prominent among the Agricore United, Coors, and Cargill lines (Fig. 4.6) while the green subpopulation was prominent among some of the lines from the BARI, AAFC-Brandon, and FCDC-Lacombe

programs. Overall, this analysis indicated some degree of population structure within the AM population. The dendrogram (Fig. 4.4) and PCA plot (Fig. 4.5) related similar information and separated the AM population to a certain extent based on breeding program. There was a high degree of similarity in lines derived from the CDC and AAFC-Brandon programs which, as previously mentioned, have a long history of germplasm exchange. Most of the Coors, Cargill, and Agricore United lines were clustered apart from the other programs, reflecting the lack of exchange between these three programs and the others. The BARI lines also tended to cluster as a distinct group (Figs. 4.4 and 4.5), but with more overlap to older CDC lines which is an accurate reflection of germplasm exchange between the programs. Similarly, the FCDC-Lacombe lines clustered as a more distinct set, but again with some overlap to more recent CDC and AAFC-Brandon lines. The FCDC program has always incorporated a greater degree of CYMMYT germplasm which may explain the clustering pattern of breeding lines from this program (Figs. 4.4 and 4.5).

The p-value plots (Figs. 4.8 and 4.9) did not display a large degree of population structure. The naïve model featured no correction for either kinship or population structure, yet it was not markedly distinguishable in either year from the other three models which corrected for these factors for most traits, exceptions being kilned malt β-glucanase activity in 2013 (Fig. 4.8B) and both malt and wort β-glucan content in 2011 (Figs. 4.9B and 4.9C). As such, any significant markers detected using the naïve model for the traits where correction for kinship or population structure had no noticeable impact were considered equally valid to markers identified using models which attempted to correct for population structure. In contrast, any marker-trait associations observed in the naïve model when it did show obvious deviation from the fitted line (e.g. kilned malt β-glucanase activity in 2013 and malt/wort β-glucan content in 2011) were unlikely to be real associations (and were ignored). In general, there were only minor differences between these models which were reflected in both the p-value plots (Figs. 4.8 and 4.9) and marker-trait associations observed (Table 4.10).

The reference genome and associated positions of SNPs present on the Barley 9K iSELECT BeadChip used to assay the AM panel in this study allowed the genomic location and potential candidate genes to be determined for SNPs linked to the traits studied. Markers 11_10895 and 12_30968 are found in close proximity to one another on chromosome 1H at positions 0.0 cM and 0.2 cM on the IBSC_2012 physical reference map, respectively, or at 523.9

kb and 637.0 kb on the barley physical map. It is therefore likely that they are both associated with the same genes linked to grain β-glucan content. Marker 12_30968 was also associated with green malt β-glucanase activity. MLOC_60692.1 and MLOC_13737.2, predicted to contain concanavalin A-like lectin/glucanase domains (Table 4.11) which are found in enzymes like glycosyl hydrolases and xylanases/endoglucanases (Dong *et al.* 2011), are both highly probable candidate genes which may explain the association seen with green malt β-glucanase. SNP marker SCRI_RS_184274, located at position 9.9 cM (6.6 Mb) on chromosome 1H, does not have a candidate gene to potentially explain its relationship with green/kilned malt β-glucanase activity.

SNP marker SCRI_RS_146936, located at approximately 7.5 Mb on chromosome 2H, resides within 5.4 Mb of a previously identified marker associated with grain β-glucan (Mohammadi *et al.* 2014) and near QBgn.StMo-2H.1 (Han *et al.* 1995; Szűcs *et al.* 2009), a known grain β-glucan QTL. Close to these markers can be found a UDP-glycosyltransferase-associated predicted gene (MLOC_64239.1 at 8.2 Mb). UDP-glycosyltransferases catalyze the addition of glycosyl groups from a UTP-sugar to another molecule, forming glycophospholipids and ultimately drawing upon the same substrate pool as the enzymes which catalyze the formation of β-glucans (Becker *et al.* 1995), which makes this gene a highly possible reason for the observed association with grain β-glucan content. MLOC_13397.1 (at 7.7 Mb), considered a highly probable candidate gene and associated with gibberellin receptor GID1L2, is involved in gibberellin signalling (Ueguchi-Tanaka *et al.* 2007) and also falls in this region. Stuart *et al.* (1986) found that gibberellic acid enhances the secretion of β-glucanase in barley aleurone and scutella layers which may explain this locus's association with green malt β-glucanase activity.

SCRI_RS_202800, located at 164.7 cM (546.0 Mb) on chromosome 5H, resides near marker 11_20897. This marker has been mapped to the same position as marker 12_21393 on Hordeum-OPAConsensus-2011-5H, residing at 177.9 cM (Muñoz-Amatriaín *et al.* 2011). This region (approximately at position 541.7 Mb) is located within 10 Mb of SCRI_RS_202800 and is likely associated with the same kilned malt β-glucanase activity QTL, which also falls near QBgnm.HaTR-5H.2, a malt β-glucan QTL (Mather *et al.* 2003; Szűcs *et al.* 2009).

The MLOC_59341.1 gene found on chromosome 6H at position 516.0 Mb contains a concanavalin A-like lectin/glucanase domain. Some enzymes with this domain act upon cell wall components (Dong *et al.* 2011), cleaving both β-glucans and components such as xylans.

This gene would explain the association between green/kilned malt β-glucanase activity, making it a highly probable candidate gene, and SNP marker 12_10704 located on the same chromosome at 100.8 cM (518.2 Mb).

SCRI_RS_11640 resides 0.4 Mb from the terminal end of the short arm of chromosome 6H but does not fall near any candidate genes which may explain its association with grain β-glucan.

This study identified a number of barley lines containing high levels of β-glucanase activity, higher proportions of heat stable β-glucanase, or a combination of both. These lines will be valuable parents in efforts to breed varieties that produce lower wort β-glucan. Loci associated with three important malting barley traits, namely grain β-glucan content and green and kilned malt β-glucanase activity, were identified. Most of the markers linked to these traits were identified in both environments used to evaluate these traits, providing more confidence in their relevance. Some of the markers were located near genes with predicted functions that provide possible explanations for the association and some fell in QTL regions identified in previous studies. Additional trials to collect data on these traits would be desirable to confirm the β-glucanase data and the identified markers.

5.0 GENERAL DISCUSSION

5.1 Conclusions and Future Research

β-glucan content and β-glucanase activity continue to be of interest for barley breeders intent on producing malting varieties. While QTLs concerning both traits have been documented in various populations in previous studies (Han *et al.* 1995; Pauli *et al.* 2015; Szűcs *et al.* 2009; Zwickert-Menteur *et al.* 1996), markers associated with these traits have not yet been developed for use across multiple barley populations. In addition, it is not always clear which underlying genes are responsible for the associations observed or what role these genes may play in influencing the traits in question. This study strove to understand the range of variability and the correlations between malt (green and kilned) β-glucanase activity and grain, malt, and wort β-glucan content that were present in elite malting barley germplasm relevant to Western Canada. The information related to green malt β-glucanase activity was of particular interest because little information exists on this and it would also allow an assessment of heat stability in conjunction with kilned malt β-glucanase activity. This study was also interested in identifying genomic regions associated with these traits. Two different genetic populations were used to accomplish these goals. Firstly, a QTL mapping population was created from a bi-parental cross between two varieties grown in Western Canada (CDC Meredith and CDC Kindersley) and secondly, an association mapping population was assembled using elite breeding lines and varieties grown in Western Canada.

Observations made in the MK population indicated that both grain β-glucan content and kilned malt β-glucanase activity were correlated predictors of final malt/wort β-glucan content. However, the two traits were never found to both be correlated in the same site-year; rather, it was observed that one or the other was correlated to final malt/wort β-glucan content. In contrast, both grain β-glucan content and kilned malt β-glucanase activity were found to be correlated to final malt/wort β-glucan content in the same site-year in the AM population. Malt β-glucanase activity (green and kilned) was consistently correlated with malt/wort β-glucan content, while a significant correlation with those traits was seen in only one site-year with grain β-glucan content. When comparing green and kilned malt β-glucanase activity, green malt β-glucanase activity showed consistently higher correlations to malt/wort β-glucan content, indicating it was a better predictor than kilned malt β-glucanase activity and supporting our

hypothesis. Malt β-glucanase activity was observed to be lost in all lines after kilning, and because most β-glucan degradation occurs during germination (and prior to kilning), it is logical that measurement of activity prior to kilning should correlate better to final β-glucan in the malt/wort.

Comparisons of β-glucanase activity in green and kilned malt in the AM population permitted an examination of heat lability of the β-glucanase enzyme across the population. A wide range in heat stability was observed in the population, ranging from 24.8 to 51.9 % loss of activity. This wide range in values also supported the hypothesis that variability would be observed for this trait. A number of lines displayed consistently high green malt β-glucanase activity (TR03191), high kilned malt β-glucanase activity (TR05286, TR03273, and TR05288), or both (TR250 and TR585). It was interesting to note that high levels of kilned malt β-glucanase activity could result from different mechanisms. For lines such as TR250 and TR585, high kilned malt β-glucanase activity was due more to their high green malt β-glucanase activity, since each line lost a relatively large proportion of activity after kilning (28 to 37 % for TR585 and 23 to 26 % for TR250). In contrast, TR03273 did not have extremely high green malt β-glucanase activity, but because it had very good heat stability (losing just 26 % of its activity) this line had amongst the highest kilned malt β-glucanase activity. With respect to breeding for improved malting quality, a combination of high activity and greater heat stability would be the most effective manner to reduce wort β-glucan content. Future work should focus on collecting additional green and kilned malt β-glucanase activity data on the AM population (or perhaps just those showing high activity or good heat stability) to confirm the findings from this study. In addition, based on the data already collected there are a number of interesting parents which should be crossed to combine high activity and heat stability (e.g. TR250 x TR03273).

A total of 11 loci, 10 unique, were linked to the four traits studied in the MK population, of which five loci appeared to be in regions of previously identified QTLs. Three of these QTLs were associated with grain β-glucan content (only two of which were unique QTLs) while the other two were linked to malt and wort β-glucan content and were the same malt extract QTL rather than β-glucan QTLs. It was interesting that no QTL associated with malt β-glucanase activity in the present study was previously identified, or that they coincided with the *HvGlb1* and *HvGlb2* loci. This finding refutes our hypothesis that these two genes would be identified as being associated with β-glucanase activity. Sequencing of *HvGlb1* and *HvGlb2* in these two

varieties has been initiated, but no differences have been found in the coding region of *HvGlb2* (data not shown) and although not complete, sequencing of the *HvGlb1* coding region has also revealed no polymorphisms. Given the supposed importance of these genes and the lack of variation in the coding region, there are perhaps expression differences which differentiate these two varieties.

It is known that the β-glucanase genes *HvGlb1* and *HvGlb2* are differentially expressed in different tissues and at different times (Hrmova *et al.* 1997; Slakeski and Fincher 1992; Stuart *et al.* 1986). In future work, it would be interesting to evaluate the expression of the β-glucanase genes in CDC Meredith and CDC Kindersley at different times over seed development (when only *HvGlb1* is expressed (Slakeski *et al.* 1990)) and germination (when both genes are expressed). Woodward and Fincher (1982) observed differences between the activity levels of the two isoenzymes during the fourth day of germination, but examining the expression of these genes over time within the parents of this bi-parental cross may give a clue as to what factors may be underlying the different β-glucanase activity levels seen.

The three loci linked to β-glucanase activity in the MK population provide an alternate explanation for differences in β-glucanase activity observed between these two varieties. Each of these loci encompasses genes with putative functions, or containing domains, which are related to enzymatic degradation of cell wall components. Future work should focus on examining sequence and expression differences in these genes between CDC Meredith and CDC Kindersley, as well as validating their importance in other populations segregating for β-glucanase activity.

Eight markers were associated with three traits (grain β-glucan content, green and kilned malt β-glucanase activity) in the AM population. Once again, the *HvGlb1*and *HvGlb2* loci were not associated with β-glucanase activity (again in opposition to our hypothesis). This finding suggests that, at least in the elite malting germplasm used in these studies, these two loci are fixed which would not be unreasonable given the selection pressure to reduce malt/wort β-glucan in elite malting barley germplasm. It was also interesting to note that only one trait-associated locus was identified in both the MK and AM populations, that being the chromosome 2H (position 4.3 to 4.5 cM) grain β-glucan content QTL. Identifying this QTL in both populations may not be surprising given that it is likely the previously identified QTL QBgn.StMo-2H.1 (Han *et al.* 1995; Szűcs *et al.* 2009), indicating this QTL has significance across a wider range of

germplasm. Counting this grain β-glucan QTL, two previously identified QTLs were identified in this study (the other being QBgnm.HaTR-5H.2 (Mather *et al.* 2003; Szűcs *et al.* 2009)) that were associated with three different markers. As with the QTL identified in the MK population, several potentially new loci were identified which will warrant further validation in bi-parental populations or in breeding germplasm. Many of these have putative genes residing nearby which strengthens the confidence in the associations observed. The identification of novel and previously identified loci confirms our hypothesis that both would be found for the traits evaluated in these studies.

This study provided several pieces of novel information that should assist efforts to improve malting barley quality through reduction in malt and wort β-glucan content. Firstly, a number of loci impacting grain, malt, and wort β-glucan, as well as malt β-glucanase activity, were identified. Many of these loci had genes residing in close proximity with putative functions that plausibly explained the association and would warrant further study. The eventual development of markers for these traits will increase the efficiency of selection in breeding programs. Secondly, information pertaining to lines with high levels of green malt β-glucanase activity and lines with more heat-stable β-glucanase activity were identified. Combining parents that show high activity with those that have heat stability should allow further reduction in malt and wort β-glucan in future varieties.

6.0 LITERATURE CITED

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7.0 APPENDICES

Appendix A

Linkage Groups Constructed the CDC Meredith x CDC Kindersley Population

Table A1. Chromosome assignment and map position for the SNP and DArTseq markers mapped on chromosomes 1H to 4H in the MK population.

Chromosome 1H		Chromosome 2H-1		Chromosome 2H-2		Chromosome 3H		Chromosome 4H	
Marker	**Pos[1]**	**Marker**	**Pos**	**Marker**	**Pos**	**Marker**	**Pos**	**Marker**	**Pos**
4170074	0.0	4169624	0.0	3257075	0.0	3396484	0.0	4014268	0.0
3266635	0.8	3398463	3.5	3270262	0.2	3665185	0.9	3257648	0.6
3986115	1.3	3986514	3.6	3261392	0.6	4791016	1.2	5259083	1.0
3260608	4.2	3273347	3.8	SCRI_RS_174552	0.8	3398462	2.0	3662953	1.2
4194529	4.4	4016246	4.1	SCRI_RS_122	1.1	3985620	3.2	3273360	1.4
4190414	4.5	3987280	4.3	SCRI_RS_10642	1.2	SCRI_RS_220221	4.8	5245937	2.3
4016076	4.7	3257692	4.3	5249876	2.0	3258925	8.5	5250613	2.4
3810297	4.7	3255324	4.5	12_30132	2.2	3272604	9.0	3662427	2.4
4184570	4.9	3270634	4.6	4184803	3.0	5249706	9.1	3395767	2.5
4006349	5.2	3263075	4.6	3264340	3.4	SCRI_RS_146798	9.3	3267914	2.7
5246292	5.2	3260781	4.8	3271986	3.8	3271271	10.4	3264462	2.7
SCRI_RS_66630	5.3	3261857	5.1	3987206	3.9	3398152	11.3	3264814	2.8
3272213	5.4	SCRI_RS_144102	5.6	4000278	4.2	5336955	11.6	3267588	2.9
3262934	5.6	SCRI_RS_6458	5.9	3254927	4.4	3663613	11.6	3274241	3.0
5249599	5.7	SCRI_RS_238605	6.2	4184974	4.6	3269519	11.7	4189638	3.0
3434198	5.8	12_30351	6.2	3397386	4.8	3662528	11.8	4188383	3.2
SCRI_RS_194326	6.1	5249603	6.5	5246768	4.8	3270001	11.9	3255472	3.7
3255648	6.4	11_10376	6.7	5246432	4.8	5250140	12.0	5246875	3.8
5250790	14.6	SCRI_RS_182627	6.8	3264126	4.8	4173171	12.9	3266338	3.8
5245738	14.9	SCRI_RS_181112	6.9	3433331	4.8	3265858	13.2	SCRI_RS_189457	4.5
4007492	15.3	3258185	7.1	3269345	4.8	3910385	13.7	12_31151	4.5
3431112	15.6	3271081	7.6	3270427	4.9	SCRI_RS_126369	18.0	SCRI_RS_13454	4.5

Chromosome 1H		Chromosome 2H-1		Chromosome 2H-2		Chromosome 3H		Chromosome 4H	
Marker	**Pos[1]**	**Marker**	**Pos**	**Marker**	**Pos**	**Marker**	**Pos**	**Marker**	**Pos**
3262073	16.1	12_10579	7.8	3662379	4.9	SCRI_RS_216174	18.8	12_31219	4.5
3926181	16.5	SCRI_RS_134241	8.0	3268119	4.9	11_10702	19.0	SCRI_RS_157974	4.6
5257050	16.6	3268810	8.5	3433243	4.9	SCRI_RS_224468	21.6	SCRI_RS_222098	4.6
3911032	16.6	3255317	8.6	3662230	4.9	5259069	25.1	SCRI_RS_66181	4.6
5253289	16.7	3255790	9.5	3432969	4.9	3270973	26.4	11_20272	4.6
12_30948	16.7	SCRI_RS_193100	9.7	3269257	4.9	3261711	26.6	SCRI_RS_214071	4.6
SCRI_RS_169668	16.8	3259685	9.8	3266434	4.9	3262195	26.9	SCRI_RS_142792	4.7
4193211	16.8	3261705	10.4	3663464	4.9	5257556	27.1	SCRI_RS_148782	4.7
3265900	16.8	3666153	10.5	5247264	5.0	5253904	27.2	SCRI_RS_2513	4.7
4793318	16.9	11_20715	10.7	3264303	5.0	3267247	27.4	11_11186	4.8
3999830	16.9	SCRI_RS_138045	10.8	3985415	5.0	3270087	27.4	SCRI_RS_238901	4.8
SCRI_RS_189465	16.9	SCRI_RS_201191	11.1	3275020	5.0	3271254	27.4	SCRI_RS_216897	4.8
3666587	17.1	SCRI_RS_194195	11.6	3271610	5.0	3272256	27.5	12_30873	4.8
5242190	17.5	12_10623	12.0	3255333	5.0	4793310	27.5	SCRI_RS_171481	4.8
5251061	18.2	SCRI_RS_230951	12.0	4789473	5.1	3256524	27.6	11_10610	5.1
4330638	23.5	12_30914	12.0	3268440	5.3	3661935	27.6	3432529	5.3
3274386	24.2	11_10551	12.0	3270465	5.3	3268259	27.6	3260267	5.3
4015913	31.2	SCRI_RS_168471	12.1	3270936	5.5	3663647	27.7	3255760	5.6
3396127	39.7	11_10826	12.2	3267895	5.5	3396509	27.7	3258813	5.6
3810410	40.2	11_10566	12.2	4188317	5.6	3255588	27.8	3264842	5.7
7575861	41.3	12_30352	12.2	3986147	5.6	4330908	27.8	6277138	6.1
3429866	41.7	12_31209	12.2	3397066	5.7	3269873	27.8	3257381	6.4
3661958	42.1	11_20494	12.2	3270149	5.7	4190354	27.9	SCRI_RS_140963	7.4
3430788	42.3	SCRI_RS_13322	12.3	3927574	5.8	4195615	27.9	3270769	8.3
7585018	42.4	3432469	12.9	3925663	5.9	3274014	27.9	4170253	8.6
5255584	42.4	3256557	13.1	3433598	6.0	3272068	28.0	3396336	8.9
3396078	42.7	3274124	13.6	6277610	6.0	3266779	28.1	11_10387	9.7
4006759	43.8	4006323	13.6	5250023	6.2	4170317	28.6	12_31422	10.2
5245903	43.9	5249557	13.7	3398676	6.4	3256147	28.7	3264338	10.3

Chromosome 1H		Chromosome 2H-1		Chromosome 2H-2		Chromosome 3H		Chromosome 4H	
Marker	**Pos[1]**	**Marker**	**Pos**	**Marker**	**Pos**	**Marker**	**Pos**	**Marker**	**Pos**
3429543	43.9	3274226	13.7	4191221	6.7	5250507	28.9	3263267	10.7
3267864	44.0	3268787	13.7	3271427	7.1	5259113	29.0	3430919	11.6
3664413	45.6	4174123	13.7	3261670	7.4	5250431	29.2	SCRI_RS_201871	15.5
3987469	53.0	3258616	13.8	3263719	7.7	5239555	29.3	SCRI_RS_192689	16.0
5249668	54.1	3254894	13.8	4195327	7.8	6428837	29.4	SCRI_RS_179398	16.3
3272590	56.9	4793231	13.8	3262234	8.6	3257262	29.4	3276423	17.0
4330924	57.4	3256862	13.9	3924813	23.1	3262365	29.4	3264936	17.2
5250978	57.5	3661850	13.9	4192502	23.1	5257482	29.4	3258056	17.9
4197053	57.8	3398703	14.1	5253975	23.3	3398858	29.7	4169582	18.1
3274404	58.0	3395918	14.3	3267214	23.7	3398852	29.9	3430967	18.5
3398685	58.3	3272858	14.3	4505558	23.8	3255995	30.0	3999808	18.5
12_30147	59.6	5251389	14.5	3269818	24.4	11_21272	30.4	3259416	18.5
SCRI_RS_182406	59.6	3266838	14.6	5245489	39.6	11_20952	30.6	5253863	18.6
SCRI_RS_196439	60.0	3263253	14.6	3260737	39.9	SCRI_RS_14107	31.0	3273958	18.6
SCRI_RS_169123	60.1	5250215	14.6	3259614	40.5	SCRI_RS_144559	31.2	3430774	18.6
SCRI_RS_1473	60.2	5250092	14.6	3271721	40.8	SCRI_RS_132388	33.3	3987582	18.8
SCRI_RS_823	60.7	3431344	14.7	3255315	41.1	SCRI_RS_174582	34.1	3431341	18.9
3255612	61.7	5250025	14.7	3398230	41.3	3259817	34.6	3271194	19.1
SCRI_RS_188360	62.1	3264637	14.7	6437274	41.7	3275999	35.1	3268359	19.8
3261781	62.4	3663096	14.7	SCRI_RS_174063	42.1	3987142	35.5	11_20044	21.4
3986734	69.2	4013696	14.8	3812343	42.4	3254649	36.6	3433484	34.5
3255958	76.8	4790752	14.8	5247137	42.7	SCRI_RS_193258	37.0	3266924	35.7
3257203	77.5	3433329	14.8	11_10216	43.0	3257629	37.5	3272193	36.4
3268199	78.4	4198415	14.9	11_21366	44.1	3273319	38.5	3262541	37.0
3269227	78.6	3985508	14.9	SCRI_RS_7026	44.3	5242775	38.5	3274974	37.3
3269606	78.7	3255874	15.0	11_21261	44.4	3986355	38.5	3256633	37.4
4015605	79.0	3985819	15.1	3985320	44.9	3917326	38.6	3273244	37.6
3257475	79.5	3258781	15.1	3262951	45.2	4197038	38.6	3270049	37.7
SCRI_RS_171501	80.1	3269831	15.3	3263393	45.3	3263765	38.7	3276623	37.9

Chromosome 1H		Chromosome 2H-1		Chromosome 2H-2		Chromosome 3H		Chromosome 4H	
Marker	**Pos[1]**	**Marker**	**Pos**	**Marker**	**Pos**	**Marker**	**Pos**	**Marker**	**Pos**
SCRI_RS_154646	80.3	5243910	15.3	5258089	47.6	3262039	38.8	4790153	37.9
SCRI_RS_236160	80.4	4193811	15.3	11_10891	47.8	3398103	39.1	4506017	38.3
3255326	80.6	5249830	15.3	3259145	48.2	3258330	40.1	3262804	38.7
3433744	81.6	3261759	15.3	3258433	48.3	5258125	40.6	7575908	39.1
3397559	81.6	3260658	15.4	3259795	48.4	3269731	40.7	3662015	39.2
SCRI_RS_78578	82.2	5249827	15.4	3268521	48.5	3397526	40.7	3263662	39.3
11_21392	93.4	4172786	15.4	3274890	48.6	SCRI_RS_108971	41.0	5253864	39.3
3254983	94.0	5250670	15.4	4791234	48.7	3666552	41.5	5255547	39.3
3262888	94.2	3275644	15.5	3395761	48.8	3664905	44.8	3263696	39.3
SCRI_RS_91596	94.5	3269655	15.6	3263518	49.0	3254786	45.9	3662708	39.3
SCRI_RS_218473	94.9	3270346	15.7	12_30748	50.5	3269778	46.0	5255888	39.4
3260679	95.0	3925168	15.8	SCRI_RS_5552	50.6	3273155	46.1	3661809	39.4
3261997	95.4	3266740	15.8	3255981	50.9	3264087	46.3	5253238	39.4
3258919	95.9	5250512	15.8	3434268	51.2	5250640	46.7	3255726	39.5
4791459	96.0	4194991	16.0	3276242	51.3	3255266	53.8	3264385	39.5
3274121	96.1	3987455	16.1	3256024	51.4	3263834	53.9	5255021	39.8
4013553	96.1	3262907	16.3	3272092	51.5	5247581	54.2	3986793	39.9
3264357	96.3	5251026	16.5	3256569	51.6	3269679	54.5	3986139	40.0
3265059	96.5	3270270	16.5	3273364	51.7	3271889	54.6	3986825	40.0
3263243	96.6	3434226	17.7	5256130	51.8	3664761	54.9	3266308	40.0
3985893	96.7	4792060	17.9	3255920	51.8	3987537	54.9	3275686	40.0
3265183	96.9	5246348	19.2	3271627	51.9	3265551	54.9	3275353	40.0
3275341	96.9	3812644	19.8	3272645	52.0	3258606	55.3	5256714	40.1
3271596	97.0	3396048	22.3	3431635	52.3	3270985	55.4	4194910	40.3
3985307	97.5	5250205	22.9	4191432	52.4	3274510	55.4	3266161	40.9
3264618	97.6	3264235	23.5	3265948	52.4	3276516	55.6	3398742	41.2
3258267	98.1	3266028	23.5	6267848	52.5	3267583	55.6	3266555	41.4
3262692	99.7	5250423	23.6	3271666	52.5	5249551	56.3	4188689	41.8
3257635	100.1	SCRI_RS_134343	25.3	5242927	52.6	SCRI_RS_160707	56.7	SCRI_RS_141214	42.5

Chromosome 1H		Chromosome 2H-1		Chromosome 2H-2		Chromosome 3H		Chromosome 4H	
Marker	**Pos[1]**	**Marker**	**Pos**	**Marker**	**Pos**	**Marker**	**Pos**	**Marker**	**Pos**
3262736	102.3	3257572	26.9	3433663	52.7	3433883	56.7	3254773	42.9
3257661	102.6	12_31527	27.8	3809851	52.9	5251266	57.0	SCRI_RS_210971	43.3
6437527	102.9	SCRI_RS_122297	27.8	6283623	53.2	SCRI_RS_193132	57.5	SCRI_RS_168399	43.7
3665744	102.9	11_10072	27.9	7582813	58.5	SCRI_RS_151711	57.6	5240324	49.1
5250257	103.2	SCRI_RS_149233	28.1	3398833	59.2	SCRI_RS_164290	57.6	3271042	55.9
4006891	103.3	3271599	28.8	3260977	59.6	4169900	57.8	SCRI_RS_237913	56.9
3431942	103.6			3663460	59.7	3273453	57.8	11_20740	57.0
3264838	103.8			3266079	59.7	3263016	58.3	SCRI_RS_160624	57.0
4330209	103.9			3263085	59.9	3920522	58.3	11_10467	57.0
3268095	103.9			3665632	60.4	5255362	58.5	SCRI_RS_218491	57.3
11_10338	104.1			3432062	60.4	3265767	59.0	SCRI_RS_4583	57.4
6436032	104.2			3257890	60.6	3395831	59.0	3985762	58.0
3258374	105.4			3270560	60.7	5255977	59.5	3263527	58.1
3665883	107.5			SCRI_RS_174444	60.8	5249951	59.6	3663398	58.2
3914865	107.7			5251162	61.9	5250619	59.6	3272704	58.2
3912181	107.8			3396491	62.1	5254009	59.7	3397326	58.2
3666525	107.9			5245552	62.2	4195312	59.9	3985498	58.2
3663415	108.0			3255158	62.6	5250484	59.9	3271588	58.5
3913063	108.1			3254659	62.7	3267521	60.0	3255392	58.5
3264674	108.2			5242592	63.2	3268539	60.1	3662462	58.6
5249738	108.3			5253909	63.4	3259859	60.1	5250955	58.9
3257059	108.4			3258846	63.7	4169489	60.2	3925223	59.8
3265592	108.6			3275583	64.0	SCRI_RS_13434	60.5	3811027	59.9
3256574	108.7			3256231	64.2	12_31329	60.7	4016300	60.2
4785822	108.9			SCRI_RS_206245	64.5	3271173	61.0	3263178	60.3
3256816	109.0			SCRI_RS_171029	64.5	SCRI_RS_231027	61.1	SCRI_RS_20701	60.7
3270356	109.0			6430342	65.0	11_20523	61.1	SCRI_RS_157650	60.7
5245913	109.1			3256490	65.6	3921704	70.7	SCRI_RS_148392	60.8
7582820	109.2			3262358	65.9	4790478	83.0	3432531	61.6

Chromosome 1H		Chromosome 2H-1		Chromosome 2H-2		Chromosome 3H		Chromosome 4H	
Marker	Pos[1]	Marker	Pos	Marker	Pos	Marker	Pos	Marker	Pos
12_21172	109.3			3257527	66.3	3266068	83.2	3430690	62.7
3274720	109.4			3256386	66.6	3263550	83.5	4184700	67.0
11_10911	109.5			3398515	67.5	SCRI_RS_164675	84.4	3263645	68.1
3257989	109.6			3259995	68.8	SCRI_RS_160216	84.4	7575938	68.5
11_20908	109.9			3432495	69.4	SCRI_RS_191130	84.7	4007030	69.6
SCRI_RS_137116	109.9			5249856	69.6	SCRI_RS_219322	84.7	3918240	70.4
11_11528	109.9			SCRI_RS_126877	70.1	SCRI_RS_235869	84.7	3262463	79.2
12_21522	110.3			11_10498	70.2	11_20222	84.8	3985520	79.6
SCRI_RS_162765	110.4			11_10297	70.2	11_11276	84.8	4330538	81.5
11_10722	110.4			12_30691	70.4	SCRI_RS_108543	84.8	11_21397	82.2
SCRI_RS_4522	110.4			3432565	70.8	SCRI_RS_149160	84.8	SCRI_RS_157832	82.7
SCRI_RS_170110	110.4			SCRI_RS_155546	72.2	SCRI_RS_156056	84.8	12_31164	82.8
11_10586	110.5			SCRI_RS_144776	72.6	SCRI_RS_190529	84.8	3662898	84.1
SCRI_RS_147611	110.5			11_10909	72.6	SCRI_RS_161186	84.8	4790590	84.3
SCRI_RS_159031	110.6			12_30042	73.0	SCRI_RS_117006	84.9	3434182	84.8
7231240	110.9			12_21288	73.1	SCRI_RS_211003	84.9	5244025	86.6
3254849	111.0			SCRI_RS_131325	73.1	SCRI_RS_224377	84.9	4169373	87.4
3665864	111.0			12_30251	73.1	SCRI_RS_122796	85.0	3395914	90.6
3913890	111.2			11_20891	73.2	SCRI_RS_200011	85.1		
3260549	111.3			SCRI_RS_30973	73.2	5243083	86.6		
5249866	111.3			SCRI_RS_106444	73.2	3254977	86.9		
7576319	111.4			SCRI_RS_187110	73.2	3262230	87.0		
7576331	111.5			SCRI_RS_186443	73.2	4790141	87.3		
3271509	111.5			SCRI_RS_6099	73.2	3271279	87.4		
4197452	111.6			11_20602	73.3	3272478	87.6		
3268065	111.7			12_31288	73.3	3433905	87.6		
4000050	111.7			SCRI_RS_206569	73.3	5255382	88.0		
3271436	111.8			SCRI_RS_41905	73.3	5262321	88.6		
4196015	111.9			SCRI_RS_194216	73.4	4789227	89.2		

Chromosome 1H		Chromosome 2H-1		Chromosome 2H-2		Chromosome 3H		Chromosome 4H	
Marker	Pos[1]	Marker	Pos	Marker	Pos	Marker	Pos	Marker	Pos
4191229	112.0			SCRI_RS_195938	73.5	6436034	92.9		
3276064	112.1			11_10996	73.5				
3268888	112.3			11_20387	73.6				
3266596	112.6			SCRI_RS_169913	73.6				
3663852	112.8			SCRI_RS_125325	73.7				
3432535	113.1			3999987	74.1				
3396129	121.7			4330430	74.4				
6422819	124.2			3262943	75.6				
3398019	124.3			4188266	75.9				
3927555	124.4			5249525	76.3				
3429901	124.4			4782868	78.0				
3811281	124.4			3663791	78.3				
3397686	124.5			3258146	78.9				
6422820	124.5			3255448	79.4				
6282440	124.6			3433189	79.8				
3662219	124.7			3395919	80.3				
6428451	124.7			3262207	80.6				
3263296	124.7			4790678	81.2				
3929874	124.9			3269076	82.1				
3270053	125.0			3270612	82.4				
3664616	125.1			3987252	82.7				
3266141	125.5			3261182	83.3				
3268290	125.6			12_30696	84.5				
3266405	125.7			11_10196	84.5				
3270187	125.8			3262333	85.3				
3257379	126.3			12_30480	95.8				
11_20840	127.0			4010377	96.1				
SCRI_RS_153896	127.3			4193047	96.3				
SCRI_RS_158298	127.3			3266841	96.5				

Chromosome 1H		Chromosome 2H-1		Chromosome 2H-2		Chromosome 3H		Chromosome 4H	
Marker	**Pos[1]**	**Marker**	**Pos**	**Marker**	**Pos**	**Marker**	**Pos**	**Marker**	**Pos**
SCRI_RS_150935	127.4			5255819	96.8				
12_30231	127.4			3256362	97.5				
12_30934	127.5			3276571	97.7				
12_31081	127.6			3259580	97.7				
SCRI_RS_195067	127.7			3257840	97.7				
SCRI_RS_132472	127.7			3257089	97.7				
11_10443	127.8			3275736	97.9				
SCRI_RS_167999	128.0			3262144	97.9				
3256243	128.3			3258359	97.9				
3432637	128.5			3257039	98.1				
3265674	128.7			3255412	98.5				
3665831	128.9			5250946	98.8				
3257198	129.8			4192630	99.0				
				3258441	99.2				
				11_20498	100.0				
				SCRI_RS_135355	100.3				
				4193513	100.9				

[1]Position in cM.

Table A2. Chromosome assignment and map position for the SNP and DArTseq markers mapped on chromosomes 5H to 7H in the MK population.

Chromosome 5H		Chromosome 6H		Chromosome 7HS		Chromosome 7HL	
Marker	**Pos[1]**	**Marker**	**Pos**	**Marker**	**Pos**	**Marker**	**Pos**
7576313	0.0	3985333	0.0	3270084	0.0	12_31357	0.0
4193147	0.3	3262304	1.3	5250323	0.4	3256429	0.9
3264847	0.7	12_31233	1.7	3274193	0.4	3274037	1.8
3257767	0.9	SCRI_RS_153023	2.0	5244519	0.7	7576358	2.5
3264342	1.0	11_10120	2.2	12_30219	1.5	SCRI_RS_104566	7.2
5255941	1.0	SCRI_RS_194023	2.3	3273803	3.4	3664593	7.6

Chromosome 5H		Chromosome 6H		Chromosome 7HS		Chromosome 7HL	
Marker	**Pos[1]**	**Marker**	**Pos**	**Marker**	**Pos**	**Marker**	**Pos**
3430513	1.2	11_21521	2.4	5254130	4.4	3255279	7.7
5240419	4.4	3397466	2.9	4011123	4.5	3270091	8.0
3271412	6.4	3272099	3.2	5250262	8.8	4170237	9.1
3919717	6.7	3396287	3.3	5251569	8.9	3662073	9.3
3273889	7.0	3265734	3.5	5250112	9.0	3663939	9.5
3434152	7.3	3271445	3.6	3263854	10.7	3663702	9.8
3272516	7.6	3262629	3.7	4016623	11.2	3255268	10.2
3397253	7.8	3397854	3.8	3986742	11.6	3255998	10.3
3918968	8.0	3431236	3.9	4192108	11.6	6281994	10.6
3925631	8.2	3430735	4.0	3257722	11.8	3260730	10.8
4194475	8.5	7584089	4.1	3986411	11.9	3915617	10.9
3271897	8.5	3256331	4.3	4191667	12.3	5249369	11.1
3913781	8.6	3256442	4.8	3662570	12.5	3255420	11.2
3258637	8.8	3254803	5.0	5243047	12.5	3432078	11.6
3272738	8.9	3255658	5.2	3267309	12.7	3262895	11.6
3432914	8.9	6437575	5.3	3986397	12.7	4007217	11.6
4184877	9.0	6429855	5.5	3264486	12.7	3265423	11.7
3432871	9.4	3432628	5.7	3270415	12.7	3256549	11.7
3811936	9.5	3264109	7.7	3273754	12.7	3260628	11.9
3433858	9.5	6248534	7.8	3396243	12.7	3433813	11.9
3263122	9.8	SCRI_RS_89111	8.3	12_30530	13.0	3269463	12.2
4173716	10.1	SCRI_RS_138977	8.6	SCRI_RS_166520	13.0	3255202	12.3
SCRI_RS_160975	11.0	3665413	8.8	SCRI_RS_127224	13.0	3255948	12.3
SCRI_RS_220005	11.1	3917281	9.0	SCRI_RS_145415	13.0	3255714	12.4
SCRI_RS_155101	11.3	7576300	9.2	11_20495	13.0	7582827	12.4
11_20686	11.4	3985342	9.4	SCRI_RS_179528	13.1	3432448	12.4
3257703	11.5	3922369	9.8	4330399	13.2	3256325	12.4
3273919	11.7	3264806	10.7	3275136	13.3	4792770	12.5
3269146	11.7	3268586	11.0	3271747	13.4	6281626	12.6

Chromosome 5H		Chromosome 6H		Chromosome 7HS		Chromosome 7HL	
Marker	**Pos[1]**	**Marker**	**Pos**	**Marker**	**Pos**	**Marker**	**Pos**
3272491	11.8	3256543	11.6	3270046	13.4	3255331	12.6
3397263	11.8	3275877	11.8	3986376	13.4	3929146	12.7
3258245	12.2	3433418	12.1	3271430	13.5	3265821	12.8
SCRI_RS_122949	13.8	3258338	12.4	3263757	13.5	3255690	12.8
3270201	16.6	3666048	12.8	3263443	13.5	3256857	12.8
12_30642	17.9	5257604	13.2	3275116	13.6	3256154	12.9
7243954	20.6	3397412	14.0	5247518	13.6	5249615	12.9
3812168	21.6	3260541	14.0	3397508	13.8	5256403	12.9
SCRI_RS_181376	21.8	3664377	21.5	3665122	13.9	3254896	12.9
3257497	22.2	5241039	21.8	3430630	14.0	3262794	13.0
3254667	22.4	SCRI_RS_186897	25.9	3916112	14.4	3256559	13.0
3272420	22.7	11_10023	26.0	5249564	15.1	4172313	13.0
3260567	22.7	3259310	26.9	SCRI_RS_162631	15.2	3255045	13.0
SCRI_RS_138608	22.8	3261043	30.3	SCRI_RS_223021	15.4	3258312	13.1
11_20545	23.0	3267959	33.3	SCRI_RS_204453	15.5	3276655	13.2
3262374	23.1	3265835	33.5	3262511	15.8	4187056	13.2
SCRI_RS_107772	23.1	3666849	33.5	SCRI_RS_47235	16.2	5250471	13.2
SCRI_RS_238426	23.1	3911378	33.6	6282000	16.8	7575929	13.2
SCRI_RS_222314	23.2	3395785	33.6			4781099	13.2
SCRI_RS_181379	23.2	3985920	33.8			3270276	13.2
SCRI_RS_173583	23.3	3258149	33.8			4175301	13.2
SCRI_RS_239368	23.3	3274282	33.9			3257780	13.2
SCRI_RS_230034	23.4	3663778	34.3			5259877	13.2
11_10901	23.4	3912737	34.3			3919842	13.2
SCRI_RS_14455	23.4	7582774	34.5			3257582	13.2
11_21041	23.5	7584822	34.5			4014313	13.3
11_10820	23.5	3396506	34.9			3810962	13.3
3256846	23.8	3999831	34.9			3260878	13.3
3262902	24.1	5251055	34.9			3911777	13.3

Chromosome 5H		Chromosome 6H		Chromosome 7HS		Chromosome 7HL	
Marker	**Pos[1]**	**Marker**	**Pos**	**Marker**	**Pos**	**Marker**	**Pos**
3254674	24.2	6281957	34.9			3913926	13.3
SCRI_RS_189369	24.4	3663731	34.9			3920568	13.3
11_11497	24.9	5245000	35.1			3432092	13.3
SCRI_RS_7678	24.9	3927788	35.9			3915635	13.3
SCRI_RS_3429	25.1	3259122	36.3			4186929	13.3
3272502	25.2	SCRI_RS_138556	36.7			6427345	13.3
3431042	25.3	5247271	38.8			3270110	13.3
3266982	25.4	3931064	39.9			3265834	13.4
3274978	25.4	7583926	40.0			3661733	13.4
3275399	25.5	7211808	40.2			7575892	13.5
3811169	25.6	7234719	40.8			3257390	13.5
3259618	25.9	12_31485	41.8			3262197	13.5
3264151	25.9	11_10994	42.5			3259976	13.5
3265758	26.1	SCRI_RS_209824	48.6			3921835	13.5
4187827	26.1	3664337	51.2			3432552	13.6
3262631	26.2	11_21069	60.1			3666172	13.6
4016929	26.4	SCRI_RS_5787	60.8			3271140	13.7
3268435	26.7	SCRI_RS_231205	61.0			3918949	13.8
SCRI_RS_222839	27.2	SCRI_RS_236779	61.2			3274782	13.9
11_11490	27.3	3257517	61.4			3924761	14.0
SCRI_RS_204809	27.5	SCRI_RS_165594	61.7			3256130	14.0
3268025	27.9	11_11349	61.7			11_10700	14.1
11_21355	28.5	5339062	62.0			6435204	14.1
12_31221	28.6	4186706	62.7			4793426	14.5
3264786	28.6	3276009	63.3			12_30492	14.7
3271270	28.6	3263620	63.4			11_10083	14.7
3397489	29.0	3666337	64.0			11_10073	14.8
3433653	29.8	3272972	64.2			12_30004	14.8
3255776	29.8	4790790	64.3			12_10459	14.9

Chromosome 5H		Chromosome 6H		Chromosome 7HS		Chromosome 7HL	
Marker	**Pos[1]**	**Marker**	**Pos**	**Marker**	**Pos**	**Marker**	**Pos**
3268884	30.0	3272457	65.2			11_10055	14.9
3266104	30.0	3270863	65.2			SCRI_RS_149647	14.9
3257360	30.2	3397450	65.3			12_10125	15.3
3257369	30.2	5255925	65.3			11_21079	15.6
3430536	30.6	3263295	65.3			12_30997	15.9
3256074	31.1	3268204	65.4			SCRI_RS_207354	15.9
3986854	32.1	3665137	65.4			12_30199	16.6
SCRI_RS_152484	32.4	4006643	65.5			12_30998	16.6
3261975	33.7	5255942	65.5			11_10299	17.0
3260427	33.9	3259308	65.8			11_10431	18.6
5249702	34.0	3432440	66.2			11_10153	19.1
3255904	34.9	12_30573	66.4			SCRI_RS_139962	19.5
3270557	36.4	11_20746	66.5			4184560	22.8
5251608	37.3	SCRI_RS_221951	66.5			3273200	23.1
3430424	38.3	SCRI_RS_143317	66.5			5249674	23.4
5241992	38.3	SCRI_RS_81903	66.6			3275087	23.4
3263455	38.4	SCRI_RS_172605	66.6			5249654	25.4
3263811	38.5	SCRI_RS_150951	66.7			3267599	30.2
4007087	39.9	SCRI_RS_173878	66.7			4006615	30.9
3986537	40.4	SCRI_RS_187506	66.7			3272158	31.1
3266000	40.5	SCRI_RS_171096	66.7			SCRI_RS_174699	31.3
3273008	40.6	3257450	66.7			4010667	31.7
4006504	41.0	3665192	66.9			12_30630	31.7
3266953	41.2	3256687	67.0			SCRI_RS_229415	31.7
3258342	41.8	3256727	67.1			SCRI_RS_195937	31.7
5337201	47.5	3262199	67.2			SCRI_RS_153505	31.7
5333733	47.5	4016295	67.3			SCRI_RS_202464	31.9
5259179	47.6	3256259	67.3			SCRI_RS_14491	32.0
4185573	47.6	3665572	67.4			4171796	32.8

Chromosome 5H		Chromosome 6H		Chromosome 7HS		Chromosome 7HL	
Marker	**Pos[1]**	**Marker**	**Pos**	**Marker**	**Pos**	**Marker**	**Pos**
3913088	47.8	3274728	67.8			3262690	33.0
3663228	47.9	4793312	67.9			3267784	33.5
3272672	47.9	3267432	69.8			3258052	34.1
3255521	48.0	3273936	69.8			3986721	34.2
3260924	48.4	3264461	69.9			3255524	37.8
3923945	49.1	SCRI_RS_144337	69.9			6282658	38.4
3254781	49.4	3266299	70.2			3276353	38.7
5259661	55.3	3265308	70.3			3275935	38.9
5339050	55.7	4192583	70.6			3255665	39.2
3256758	57.7	3276863	70.6			3257479	42.5
4187660	58.3	3274374	70.7			6284399	42.7
3263087	58.5	3930200	70.9			3270777	43.2
5248320	58.9	3274791	71.0			3265619	43.3
5255615	58.9	SCRI_RS_166239	71.3			5251680	43.3
3271957	58.9	5258767	71.6			3270900	43.4
3269842	58.9	4169476	71.9			3254668	43.5
3266240	58.9	SCRI_RS_181031	72.4			SCRI_RS_231275	43.8
4011721	59.0	3257992	72.6			11_20092	43.8
4015721	59.1	SCRI_RS_139297	73.4			11_20623	44.1
3666073	59.7	3270051	73.5			SCRI_RS_109484	44.1
3930776	59.8	3259196	73.5			11_10853	44.3
SCRI_RS_189923	60.0	4017325	74.0			3254848	44.3
SCRI_RS_135254	60.5	3264135	74.1			3270896	44.4
SCRI_RS_8256	60.8	3267644	74.1			3271167	44.4
12_11298	60.8	5336934	74.3			5241101	44.9
SCRI_RS_199964	60.8	3396205	74.8			3270758	45.2
SCRI_RS_212515	61.1	4790394	74.9			11_10563	46.5
5245538	66.1	7206932	75.1			SCRI_RS_7217	46.6
SCRI_RS_178985	67.5	3265724	75.5			11_20247	47.5

Chromosome 5H		Chromosome 6H		Chromosome 7HS		Chromosome 7HL	
Marker	**Pos[1]**	**Marker**	**Pos**	**Marker**	**Pos**	**Marker**	**Pos**
5241688	71.2	3264464	75.6			3986865	48.9
3267950	71.2	4792004	75.8			3271333	52.1
3928467	71.6	3258445	75.9			3269912	52.5
4006868	71.8	4015356	76.1			4189463	52.5
SCRI_RS_119308	72.3	4188770	76.9			3268963	52.5
SCRI_RS_120829	72.5	3264992	77.9			4187508	52.6
3985206	72.5	7585070	78.6			SCRI_RS_175568	53.4
3276465	72.6	3397436	78.7			3987051	54.8
3665706	74.2	7585071	78.9			5250694	59.6
3661914	74.6	4011743	79.4				
4186261	74.8	11_20783	80.2				
3261378	75.0	3255979	80.6				
3255907	75.0	3432742	80.8				
3264997	75.3	3665270	81.1				
3265426	75.3	3257200	81.4				
SCRI_RS_147618	75.7	3276155	81.8				
SCRI_RS_172024	75.7	5251488	82.5				
SCRI_RS_138221	75.8	5259098	83.2				
SCRI_RS_214241	75.9	4191800	84.6				
SCRI_RS_157897	75.9	3256061	85.0				
SCRI_RS_225268	76.0	3256854	85.3				
11_21390	76.2	3255554	85.7				
3268279	76.5	3255572	86.4				
5256380	76.8	3272844	87.5				
4010218	77.3	4187883	87.9				
3275707	77.8	3429680	88.3				
11_21061	80.6	4175549	88.5				
3256595	81.0	3269951	89.5				
3261683	81.1	3255294	89.7				

Chromosome 5H		Chromosome 6H		Chromosome 7HS		Chromosome 7HL	
Marker	**Pos[1]**	**Marker**	**Pos**	**Marker**	**Pos**	**Marker**	**Pos**
4186679	81.6	3272153	90.2				
3257142	92.7	3662935	90.9				
6280268	92.8	5256978	98.8				
3257187	93.1	3262839	100.1				
3398037	93.4	3259125	102.3				
5257201	93.4	3258625	103.3				
3262462	93.7	3271560	109.8				
3273637	93.8	11_21467	110.2				
3395834	93.9	3272889	110.4				
6277067	94.0	4790293	110.8				
3273198	94.1	3256459	111.4				
3261393	94.2	3430826	112.1				
SCRI_RS_206867	94.9	3664332	124.2				
SCRI_RS_206848	95.0	3915911	124.3				
SCRI_RS_179268	95.0	4793171	124.4				
4006641	95.1	5255614	124.5				
SCRI_RS_153937	95.1	5253465	124.6				
3262406	95.1	4012548	124.8				
3260185	95.1	6256971	124.8				
3913339	95.2	3267233	124.8				
3661950	95.2	3662666	124.9				
11_20097	95.5	3395765	125.8				
12_30533	95.5	4014685	126.5				
3255640	95.6	3271832	126.6				
3261740	95.7	3433346	126.9				
3256890	95.8	7583504	127.0				
4016843	95.9	7582796	127.1				
3664633	96.0	3396453	127.3				
3269362	96.2	3255751	127.6				

Chromosome 5H		Chromosome 6H		Chromosome 7HS		Chromosome 7HL	
Marker	**Pos[1]**	**Marker**	**Pos**	**Marker**	**Pos**	**Marker**	**Pos**
4790393	96.5	3254806	127.9				
3275509	96.6	12_30956	128.1				
3263386	96.6	11_20537	128.9				
3917423	96.8						
3262380	97.6						
4016722	97.8						
3987524	100.8						
3272347	101.3						
3259242	101.8						
SCRI_RS_170734	105.3						
3432380	106.5						
3254874	108.1						

[1]Position in cM.

Appendix B

Cross Referencing of Currently Identified β-glucan and β-glucanase QTLs in the CDC Meredith x CDC Kindersley Population

Table B1. A description of the process to determine the location of QTL observed for four different traits (kilned malt β-glucanase and grain, malt, and wort β-glucan) in the current study on other barley genetic and physical maps, the presence of underlying genes and QTL previously identified for these traits.

Site-Year	Chromosome	Bridging Marker	Position in MK Map (cM)	Position in IBSC_2012 Map (cM)	Position in Physical Map (Mb)	Comments
Grain β-glucan						
2013 SF	1H	3257635	100.1	107.0	439.7	GrainGenes and the Barley-Consensus2-1H, Hordeum-Consensus2005-SNP-1H, and Hordeum-OWB-OPA2008-1H maps to identify prior QTL
2013 KCRF	2H-2	3269257	4.9	4.4	7.65	Barley_PhysMap to place markers near QTL peak via associated contigs (bowman_contig_1983701 (3269257) and morex_contig_52735); used GrainGenes, the Hordeum-OWB-OPA2008-2H map, and morex_contig_38698 (overlapping 12_31446) to place QTL
2013 SF	2H-2	SCRI_RS_128162	-	12.6	16.1	Haldane mapping method to place SCRI_RS_128162 near peak marker; Barley_PhysMap to place markers near QTL peak via associated contigs (barke_contig_395419 (3924813) and morex_contig_42767 (SCRI_RS_128162)); used GrainGenes and the Hordeum-OWB-OPA2008-2H, Hordeum-OPA-2009-2H, and Barley-Consensus2-2H maps to place QTL (11_20724 maps to same position as SCRI_RS_128162 on IBSC_2012)
	7HL	SCRI_RS_7217	46.6	102.9	552.7	Flanking marker within genetic linkage map which could be placed on IBSC_2012
2012 SF	7HL	3258052	34.1	97.1	539.1	Flanking marker within genetic linkage map which could be placed on IBSC_2012
Malt β-glucan						
2013 KCRF	1H	12_30231	127.4	132.5	461.3	QMe.StMo-1H.5 maps to 153.01 cM on Hordeum-OWB-OPA2008-1H and overlaps 11_10041, which maps to 130.74 cM on IBSC_2012
	4H	3263662	39.3	97.2	513.6	Flanking markers within genetic linkage map which could be placed on IBSC_2012

Site-Year	Chromosome	Bridging Marker	Position in MK Map (cM)	Position in IBSC_2012 Map (cM)	Position in Physical Map (Mb)	Comments
Wort β-glucan						
2013 KCRF	1H	12_30231	127.4	132.5	461.3	QMe.StMo-1H.5 maps to 153.01 cM on Hordeum-OWB-OPA2008-1H and overlaps 11_10041, which maps to 130.74 cM on IBSC_2012
Kilned Malt β-glucanase						
All	2H-1	3257572	26.9	141.9	616.1	-
	6H	11_11349	61.7	68.5	471.1	Flanking marker within genetic linkage map which could be placed on IBSC_2012
	7HL	4184560	22.8	86.0	520.4	Barley_PhysMap to place markers near QTL peak via associated contigs (barke_contig_279132 (4184560) and morex_contig_52928)

Appendix C

Unmapped Markers in the CDC Meredith x CDC Kindersley Population Associated with β-glucan and β-glucanase

Table C1. Kruskal-Wallis test results on unmapped markers which were polymorphic in the MK population grown in 2012 and 2013. Markers significant at $\alpha = 0.0001$ are listed, along with chromosome and position based on other maps (if present).

Site-Year	Marker	K*	Chromosome	Position (cM)	Source Map
Grain β-glucan					
2012 SF	3257057	18.70	7H	92.1	Cantalapiedra *et al.* (2015)
	SCRI_RS_150049	17.68	7H	97.1	Cantalapiedra *et al.* (2015)
	3257520	16.11	7H	97.1	Cantalapiedra *et al.* (2015)
	12_31261	15.99	7H	97.1	Cantalapiedra *et al.* (2015)
	5250728	19.71	7H	97.2	The International Barley Genome Sequencing Consortium (2012)
	SCRI_RS_152402	18.85	7H	97.2	Cantalapiedra *et al.* (2015)
	6277356	18.33	7H	97.2	The International Barley Genome Sequencing Consortium (2012)
	SCRI_RS_175893	18.20	7H	97.2	Cantalapiedra *et al.* (2015)
	5337149	17.86	7H	97.2	The International Barley Genome Sequencing Consortium (2012)
	3256783	17.62	7H	97.2	Cantalapiedra *et al.* (2015)
	SCRI_RS_141732	17.49	7H	97.2	Cantalapiedra *et al.* (2015)
	3256993	17.28	7H	97.2	Cantalapiedra *et al.* (2015)
	3254837	15.54	7H	97.2	Mascher *et al.* (2013)
	3661812	21.07	7H	97.3	The International Barley Genome Sequencing Consortium (2012)
	6283021	20.08	7H	97.3	The International Barley Genome Sequencing Consortium (2012)
	12_20684	18.92	7H	97.3	Mascher *et al.* (2013)
	3263780	19.21	7H	98.3	Cantalapiedra *et al.* (2015)
	SCRI_RS_174327	18.92	7H	98.3	Cantalapiedra *et al.* (2015)
	11_20824	18.81	7H	98.3	Cantalapiedra *et al.* (2015)
	3256780	17.56	7H	98.3	The International Barley Genome Sequencing Consortium (2012)
	3910706	19.96	7H	98.6	The International Barley Genome Sequencing Consortium (2012)
	3432725	16.91	7H	98.6	The International Barley Genome Sequencing Consortium (2012)

Site-Year	Marker	K*	Chromosome	Position (cM)	Source Map
Grain β-glucan					
2012 SF	3255194	18.59	7H	99.2	Cantalapiedra *et al.* (2015)
	3665583	22.17	7H	U	Unmapped JoinMap Groupings
	5262434	16.72	7H	U	Unmapped JoinMap Groupings
Malt β-glucan					
2013 KCRF	3257249	17.29	4H	83.2	Mascher *et al.* (2013)
	11_20358	21.04	4H	83.5	Cantalapiedra *et al.* (2015)
	SCRI_RS_230980	15.45	4H	U	Unmapped JoinMap Groupings
Kilned Malt β-glucanase					
All	3270583	17.18	2H	140.8	Cantalapiedra *et al.* (2015)
	3397358	16.14	2H	140.8	The International Barley Genome Sequencing Consortium (2012)
	SCRI_RS_171198	15.63	2H	140.8	Cantalapiedra *et al.* (2015)
	3663908	16.47	2H	141.0	The International Barley Genome Sequencing Consortium (2012)
	SCRI_RS_188722	15.63	2H	141.0	Cantalapiedra *et al.* (2015)
	6428402	16.98	2H	141.9	The International Barley Genome Sequencing Consortium (2012)
	3255810	16.62	2H	142.1	Cantalapiedra *et al.* (2015)
	3269850	19.34	2H	U	Unmapped JoinMap Groupings
	3265700	17.87	2H	U	Unmapped JoinMap Groupings
	5249633	16.65	2H	U	Unmapped JoinMap Groupings
	SCRI_RS_115440	16.37	6H	64.1	Cantalapiedra *et al.* (2015)
	3267937	16.11	6H	66.1	Cantalapiedra *et al.* (2015)
	3265871	17.52	6H	72.2	Cantalapiedra *et al.* (2015)
	3269865	17.89	6H	U	Unmapped JoinMap Groupings

Appendix D

Tukey's HSD Values for the Five Traits Evaluated in the Association Mapping Population

Table D1. Tukey's HSD values for grain β-glucan content in the AM population grown in 2011 and 2013.

Tukey Grouping						Mean	Line
			A			4.9	TR643
	B		A			4.7	TR02185
	B		A			4.7	TR01178
	B		A	C		4.7	TR582
	B	D	A	C		4.7	TR03278
E	B	D	A	C		4.6	TR649
E	B	D	A	C		4.6	TR05101
E	B	D	A	C		4.6	TR973
E	B	D	A	C		4.6	TR243
E	B	D	A	C		4.6	TR140
E	B	D	A	C		4.5	TR647
E	B	D	A	C		4.5	TR646
E	B	D	A	C		4.5	TR03903
E	B	D	A	C		4.5	TR05102
E	B	D	A	C		4.5	TR702
E	B	D	A	C	F	4.4	TR651
E	B	D	A	C	F	4.4	TR917
E	B	D	A	C	F	4.4	TR162
E	B	D	A	C	F	4.4	TR585
E	B	D	A	C	F	4.4	TR05671
E	B	D	A	C	F	4.4	TR01715
E	B	D	A	C	F	4.4	TR05288
E	B	D	A	C	F	4.3	TR705
E	B	D	A	C	F	4.3	TR441
E	B	D	A	C	F	4.3	TR714
E	B	D	A	C	F	4.3	TR250
E	B	D	A	C	F	4.3	TR658
E	B	D	A	C	F	4.3	TR910
E	B	D	A	C	F	4.3	TR159
E	B	D	A	C	F	4.3	TR01176
E	B	D	A	C	F	4.3	TR01995
E	B	D	A	C	F	4.3	TR706
E	B	D	A	C	F	4.2	TR990
E	B	D	A	C	F	4.2	TR02181
E	B	D	A	C	F	4.2	TR907
E	B	D	A	C	F	4.2	TR05287

Tukey Grouping							Mean	Line
E	B	D	A	G	C	F	4.2	TR251
E	B	D	A	G	C	F	4.2	TR03191
E	B	D	A	G	C	F	4.2	TR584
E	B	D	A	G	C	F	4.2	TR970
E	B	D	A	G	C	F	4.2	TR633
E	B	D	A	G	C	F	4.2	TR169
E	B	D	A	G	C	F	4.1	TR253
E	B	D	A	G	C	F	4.1	TR171
E	B	D	A	G	C	F	4.1	TR03189
E	B	D	A	G	C	F	4.1	TR965
E	B	D	A	G	C	F	4.1	TR984
E	B	D	A	G	C	F	4.1	TR149
E	B	D	A	G	C	F	4.1	TR143
E	B	D	A	G	C	F	4.1	TR05912
E	B	D	A	G	C	F	4.1	TR258
E	B	D	A	G	C	F	4.1	TR232
E	B	D	A	G	C	F	4.1	TR167
E	B	D	A	G	C	F	4.1	TR254
E	B	D	A	G	C	F	4.1	TR490
E	B	D	A	G	C	F	4.1	TR987
E	B	D	A	G	C	F	4.1	TR133
E	B	D	A	G	C	F	4.1	TR262
E	B	D	A	G	C	F	4.1	TR911
E	B	D	A	G	C	F	4.1	TR992
E	B	D	A	G	C	F	4.0	TR989
E	B	D	A	G	C	F	4.0	TR05669
E	B	D	A	G	C	F	4.0	TR06673
E	B	D	A	G	C	F	4.0	TR655
E	B	D	A	G	C	F	4.0	TR988
E	B	D	A	G	C	F	3.9	TR03273
E	B	D	A	G	C	F	3.9	TR166
E	B	D	A	G	C	F	3.9	TR982
E	B	D	A	G	C	F	3.9	TR05104
E	B	D	A	G	C	F	3.9	TR139
E	B	D	A	G	C	F	3.9	TR964
E	B	D	A	G	C	F	3.9	TR06295
E	B	D	A	G	C	F	3.8	TR153
E	B	D	A	G	C	F	3.8	TR980
E	B	D	A	G	C	F	3.8	TR985
E	B	D	A	G	C	F	3.8	TR03662
E	B	D	A	G	C	F	3.8	TR704
E	B	D	A	G	C	F	3.8	TR04196

Tukey Grouping							Mean	Line
E	B	D	A	G	C	F	3.8	TR993
E	B	D	A	G	C	F	3.8	TR961
E	B	D	A	G	C	F	3.7	TR986
E	B	D	A	G	C	F	3.6	TR587
E	B	D		G	C	F	3.6	TR156
E	B	D		G	C	F	3.5	TR991
E	B	D		G	C	F	3.5	TR05286
E	B	D		G	C	F	3.5	TR983
E	B	D		G	C	F	3.4	TR979
E		D		G	C	F	3.4	TR573
E		D		G		F	3.4	TR150
E				G		F	3.4	TR01711
				G		F	3.1	TR574
				G			2.9	TR635

Table D2. Tukey's HSD values for malt β-glucan content in the AM population grown in 2011 and 2013.

Tukey Grouping		Mean	Line
	A	2.3	TR643
B	A	1.9	TR582
B	A	1.8	TR633
B	A	1.7	TR649
B	A	1.7	TR714
B	A	1.7	TR140
B	A	1.6	TR982
B	A	1.6	TR984
B	A	1.6	TR05101
B	A	1.6	TR647
B	A	1.6	TR986
B	A	1.6	TR973
B	A	1.5	TR587
B	A	1.5	TR988
B	A	1.5	TR702
B	A	1.5	TR06673
B	A	1.5	TR490
B	A	1.5	TR03278
B	A	1.4	TR02181
B	A	1.4	TR01995
B	A	1.4	TR651
B	A	1.4	TR01178

Tukey Grouping		Mean	Line
B	A	1.4	TR917
B	A	1.3	TR964
B	A	1.3	TR989
B	A	1.3	TR987
B	A	1.3	TR635
B	A	1.3	TR05287
B	A	1.3	TR162
B	A	1.3	TR143
B	A	1.3	TR441
B	A	1.2	TR706
B	A	1.2	TR06295
B	A	1.2	TR243
B	A	1.2	TR01715
B	A	1.2	TR03903
B	A	1.2	TR910
B	A	1.2	TR991
B	A	1.2	TR646
B	A	1.2	TR574
B	A	1.2	TR262
B	A	1.1	TR979
B	A	1.1	TR658
B	A	1.1	TR965
B	A	1.1	TR584
B	A	1.1	TR05671
B	A	1.1	TR961
B	A	1.1	TR232
B	A	1.1	TR05288
B	A	1.1	TR133
B	A	1.1	TR985
B	A	1.1	TR573
B	A	1.0	TR05102
B	A	1.0	TR970
B	A	1.0	TR911
B	A	1.0	TR258
B	A	1.0	TR159
B	A	1.0	TR704
B	A	1.0	TR149
B	A	1.0	TR05104
B	A	1.0	TR169
B	A	1.0	TR166
B	A	1.0	TR655
B	A	0.9	TR990

Tukey Grouping		Mean	Line
B	A	0.9	TR993
B	A	0.9	TR03662
B	A	0.9	TR03273
B	A	0.9	TR01176
B	A	0.9	TR250
B	A	0.9	TR705
B	A	0.9	TR251
B	A	0.9	TR980
B	A	0.9	TR585
B	A	0.9	TR153
B	A	0.8	TR05669
B	A	0.8	TR05912
B	A	0.8	TR03189
B		0.8	TR253
B		0.7	TR992
B		0.7	TR907
B		0.7	TR139
B		0.7	TR254
B		0.7	TR983
B		0.7	TR01711
B		0.7	TR05286
B		0.7	TR04196
B		0.7	TR02185
B		0.7	TR171
B		0.7	TR167
B		0.7	TR150
B		0.6	TR156
B		0.5	TR03191

Table D3. Tukey's HSD values for wort β-glucan content in the AM population grown in 2011 and 2013.

Tukey Grouping	Mean	Line
A	1057	TR979
A	861.6	TR980
A	655.9	TR983
A	1199	TR988
A	1150.7	TR989
A	879.7	TR993
A	1140.3	TR01995
A	666.8	TR01711

Tukey Grouping	Mean	Line
A	1213.4	TR984
A	966.2	TR985
A	1146.4	TR986
A	983.9	TR987
A	1175.8	TR982
A	976.5	TR991
A	676.1	TR992
A	647.5	TR990
A	1043.1	TR964
A	943.9	TR965
A	914.1	TR961
A	783.9	TR970
A	1090	TR973
A	1086.2	TR702
A	804.8	TR704
A	639.5	TR705
A	938.6	TR706
A	1097.4	TR714
A	885.9	TR01715
A	586.3	TR907
A	874.3	TR910
A	861	TR911
A	988.8	TR917
A	1000.9	TR441
A	1124.8	TR490
A	922.7	TR133
A	587.9	TR139
A	1149.6	TR140
A	1090	TR143
A	1007.2	TR06295
A	822.9	TR149
A	589.6	TR150
A	713.1	TR153
A	492	TR156
A	798.9	TR159
A	1061.7	TR162
A	912	TR166
A	574.7	TR167
A	760.2	TR169
A	620.6	TR171
A	771.2	TR01176
A	1161.2	TR01178

Tukey Grouping	Mean	Line
A	1136.7	TR02181
A	592.5	TR02185
A	720.7	TR03189
A	371.3	TR03191
A	1046.1	TR03903
A	540.5	TR04196
A	1270	TR05101
A	960.2	TR05102
A	762	TR05104
A	700.8	TR05912
A	852.3	TR232
A	953.3	TR243
A	739.9	TR250
A	727.5	TR251
A	631.7	TR253
A	548.1	TR254
A	789.3	TR258
A	949.5	TR262
A	800.7	TR03273
A	1111.8	TR03278
A	623.5	TR05286
A	964.7	TR05287
A	903.9	TR05288
A	850.2	TR573
A	919	TR574
A	1145.6	TR582
A	870.8	TR584
A	705.6	TR585
A	1037	TR587
A	1073.2	TR633
A	994.2	TR635
A	1289	TR643
A	997.9	TR646
A	1241.4	TR647
A	1271.1	TR649
A	1119.9	TR651
A	717.4	TR655
A	1012	TR658
A	858.6	TR03662
A	709.4	TR05669
A	895.3	TR05671
A	1155.9	TR06673

Table D4. Tukey's HSD values for green malt β-glucanase activity in the AM population grown in 2011 and 2013.

Tukey Grouping																	Mean	Line
									A								2,443	TR585
			B						A								2,417	TR250
			B						A								2,403	TR03191
			B						A			C					2,355	TR05671
			B		D				A			C					2,338	TR03189
	E		B		D				A			C					2,329	TR05286
	E		B		D				A			C		F			2,321	TR05288
	E		B		D				A		G	C		F			2,315	TR06295
	E		B		D		H		A		G	C		F			2,285	TR262
	E		B	I	D		H		A		G	C		F			2,245	TR655
	E		B	I	D		H		A		G	C		F			2,244	TR04196
	E	J	B	I	D		H		A		G	C		F			2,233	TR03273
	E	J	B	I	D		H		A		G	C		F			2,232	TR232
K	E	J	B	I	D		H		A		G	C		F			2,202	TR253
K	E	J	B	I	D		H		A		G	C		F			2,196	TR907
K	E	J	B	I	D		H		A		G	C		F	L		2,186	TR153
K	E	J	B	I	D		H		A		G	C	M	F	L		2,178	TR139
K	E	J	B	I	D		H		A		G	C	M	F	L		2,150	TR167
K	E	J	B	I	D		H		A		G	N	C	M	F	L	2,136	TR01711
K	E	J	B	I	D		H		A		G	N	C	M	F	L	2,136	TR251
K	E	J	B	I	D		H		A		G	N	C	M	F	L	2,122	TR01178
K	E	J	B	I	D		H		A		G	N	C	M	F	L	2,112	TR05287
K	E	J	B	I	D		H		A		G	N	C	M	F	L	2,104	TR149
K	E	J	B	I	D		H		A		G	N	C	M	F	L	2,090	TR992
K	E	J	B	I	D		H		A		G	N	C	M	F	L	2,082	TR973
K	E	J	B	I	D		H		A		G	N	C	M	F	L	2,075	TR02185
K	E	J	B	I	D		H		A		G	N	C	M	F	L	2,075	TR171
K	E	J	B	I	D		H		A		G	N	C	M	F	L	2,068	TR169
K	E	J	B	I	D		H		A		G	N	C	M	F	L	2,067	TR258
K	E	J	B	I	D		H		A		G	N	C	M	F	L	2,065	TR05912
K	E	J	B	I	D		H		A		G	N	C	M	F	L	2,061	TR159
K	E	J	B	I	D		H		A		G	N	C	M	F	L	2,059	TR01176
K	E	J	B	I	D		H		A	O	G	N	C	M	F	L	2,052	TR980
K	E	J	B	I	D		H		A	O	G	N	C	M	F	L	2,048	TR584
K	E	J	B	I	D		H	P	A	O	G	N	C	M	F	L	2,041	TR02181
K	E	J	B	I	D		H	P	A	O	G	N	C	M	F	L	2,039	TR573
K	E	J	B	I	D	Q	H	P	A	O	G	N	C	M	F	L	2,035	TR911
K	E	J	B	I	D	Q	H	P	A	O	G	N	C	M	F	L	2,035	TR05104
K	E	J	B	I	D	Q	H	P	A	O	G	N	C	M	F	L	2,035	TR987

Tukey Grouping																			Mean	Line
K		E	J	B		I	R	D	Q	H	P	A	O	G	N	C	M	F	L	2,029	TR587
K		E	J	B		I	R	D	Q	H	P	A	O	G	N	C	M	F	L	2,027	TR910
K		E	J	B		I	R	D	Q	H	P	A	O	G	N	C	M	F	L	2,020	TR254
K		E	J	B		I	R	D	Q	H	P	A	O	G	N	C	M	F	L	2,011	TR983
K		E	J	B		I	R	D	Q	H	P	A	O	G	N	C	M	F	L	2,009	TR03662
K		E	J	B		I	R	D	Q	H	P	A	O	G	N	C	M	F	L	2,008	TR05102
K		E	J	B	S	I	R	D	Q	H	P	A	O	G	N	C	M	F	L	1,997	TR243
K		E	J	B	S	I	R	D	Q	H	P	A	O	G	N	C	M	F	L	1,993	TR646
K		E	J	B	S	I	R	D	Q	H	P	A	O	G	N	C	M	F	L	1,984	TR03278
K		E	J	B	S	I	R	D	Q	H	P	A	O	G	N	C	M	F	L	1,981	TR704
K		E	J	B	S	I	R	D	Q	H	P		O	G	N	C	M	F	L	1,973	TR150
K		E	J		S	I	R	D	Q	H	P	T	O	G	N	C	M	F	L	1,935	TR985
K		E	J		S	I	R	D	Q	H	P	T	O	G	N	C	M	F	L	1,926	TR651
K		E	J		S	I	R	D	Q	H	P	T	O	G	N	C	M	F	L	1,923	TR647
K		E	J		S	I	R	D	Q	H	P	T	O	G	N	C	M	F	L	1,919	TR133
K		E	J		S	I	R	D	Q	H	P	T	O	G	N	C	M	F	L	1,909	TR162
K		E	J		S	I	R	D	Q	H	P	T	O	G	N	C	M	F	L	1,906	TR01995
K		E	J		S	I	R	D	Q	H	P	T	O	G	N	C	M	F	L	1,899	TR05669
K		E	J		S	I	R	D	Q	H	P	T	O	G	N	C	M	F	L	1,897	TR706
K		E	J		S	I	R	D	Q	H	P	T	O	G	N	C	M	F	L	1,896	TR991
K		E	J		S	I	R	D	Q	H	P	T	O	G	N		M	F	L	1,887	TR658
K		E	J		S	I	R	D	Q	H	P	T	O	G	N		M	F	L	1,883	TR06673
K		E	J		S	I	R	D	Q	H	P	T	O	G	N		M	F	L	1,879	TR917
K		E	J		S	I	R	D	Q	H	P	T	O	G	N		M	F	L	1,878	TR156
K		E	J		S	I	R	D	Q	H	P	T	O	G	N		M	F	L	1,877	TR990
K		E	J		S	I	R	D	Q	H	P	T	O	G	N		M	F	L	1,877	TR649
K		E	J		S	I	R	D	Q	H	P	T	O	G	N		M	F	L	1,876	TR166
K		E	J		S	I	R		Q	H	P	T	O	G	N		M	F	L	1,871	TR140
K			J		S	I	R		Q	H	P	T	O	G	N		M	F	L	1,861	TR705
K			J		S	I	R		Q	H	P	T	O	G	N		M		L	1,852	TR01715
K			J		S	I	R		Q	H	P	T	O		N		M		L	1,850	TR964
K			J		S	I	R		Q	H	P	T	O		N		M		L	1,850	TR989
K			J		S	I	R		Q	H	P	T	O		N		M		L	1,849	TR143
K			J		S	I	R		Q	H	P	T	O		N		M		L	1,844	TR965
K			J		S	I	R		Q	H	P	T	O		N		M		L	1,842	TR979
K			J		S	I	R		Q	H	P	T	O		N		M		L	1,840	TR490
K			J		S	I	R		Q	H	P	T	O		N		M		L	1,823	TR03903
K			J		S	I	R		Q		P	T	O		N		M		L	1,821	TR970
K			J		S	I	R		Q		P	T	O		N		M		L	1,810	TR05101
K			J		S	I	R		Q		P	T	O		N		M		L	1,798	TR961
K			J		S	I	R		Q		P	T	O		N		M		L	1,797	TR993
K			J	U	S		R		Q		P	T	O		N		M		L	1,778	TR582

Tukey Grouping											Mean	Line
K	U	S	R	Q	P	T	O	N	M	L	1,765	TR714
	U	S	R	Q	P	T	O	N	M	L	1,729	TR988
	U	S	R	Q	P	T	O	N	M		1,716	TR702
	U	S	R	Q	P	T	O	N			1,680	TR643
	U	S	R	Q	P	T	O				1,593	TR984
	U	S	R	Q	P	T					1,583	TR441
	U	S	R	Q		T					1,574	TR986
	U	S	R			T					1,570	TR574
	U	S				T					1,540	TR982
	U					T					1,509	TR633
	U										1,333	TR635

Table D5. Tukey's HSD values for kilned malt β-glucanase activity in the AM population grown in 2011 and 2013.

Tukey Grouping											Mean	Line
						A					1,817	TR250
			B			A					1,687	TR05286
			B			A		C			1,636	TR03273
			B		D	A		C			1,632	TR585
			B		D	A		C			1,625	TR05288
	E		B		D	A		C			1,549	TR232
	E		B		D	A		C	F		1,531	TR05287
	E		B		D	A	G	C	F		1,501	TR06295
	E		B		D	H	A	G	C	F	1,491	TR05671
	E		B		D	H	A	G	C	F	1,489	TR258
	E		B	I	D	H	A	G	C	F	1,483	TR584
	E	J	B	I	D	H	A	G	C	F	1,481	TR907
	E	J	B	I	D	H	A	G	C	F	1,478	TR05912
	E	J	B	I	D	H	A	G	C	F	1,461	TR167
K	E	J	B	I	D	H	A	G	C	F	1,459	TR153
K	E	J	B	I	D	H	A	G	C	F	1,446	TR704
K	E	J	B	I	D	H	A	G	C	F	1,443	TR253
K	E	J	B	I	D	H	A	G	C	F	1,434	TR254
K	E	J	B	I	D	H	A	G	C	F	1,425	TR139
K	E	J	B	I	D	H	A	G	C	F	1,420	TR243
K	E	J	B	I	D	H	A	G	C	F	1,417	TR05104
K	E	J	B	I	D	H	A	G	C	F	1,397	TR05102
K	E	J	B	I	D	H	A	G	C	F	1,383	TR02185
K	E	J	B	I	D	H	A	G	C	F	1,379	TR133
K	E	J	B	I	D	H	A	G	C	F	1,378	TR04196
K	E	J	B	I	D	H	A	G	C	F	1,376	TR262
K	E	J	B	I	D	H	A	G	C	F	1,360	TR911

Tukey Grouping											Mean	Line
K	E	J	B	I	D	H	A	G	C	F	1,352	TR03189
K	E	J	B	I	D	H	A	G	C	F	1,352	TR01178
K	E	J	B	I	D	H	A	G	C	F	1,347	TR251
K	E	J	B	I	D	H	A	G	C	F	1,346	TR573
K	E	J	B	I	D	H	A	G	C	F	1,342	TR171
K	E	J	B	I	D	H	A	G	C	F	1,337	TR910
K	E	J	B	I	D	H	A	G	C	F	1,319	TR651
K	E	J	B	I	D	H	A	G	C	F	1,317	TR169
K	E	J	B	I	D	H	A	G	C	F	1,314	TR587
K	E	J	B	I	D	H	A	G	C	F	1,310	TR647
K	E	J	B	I	D	H	A	G	C	F	1,300	TR01711
K	E	J	B	I	D	H	A	G	C	F	1,297	TR150
K	E	J	B	I	D	H	A	G	C	F	1,296	TR965
K	E	J	B	I	D	H	A	G	C	F	1,291	TR01995
K	E	J	B	I	D	H	A	G	C	F	1,290	TR973
K	E	J	B	I	D	H	A	G	C	F	1,283	TR991
K	E	J	B	I	D	H	A	G	C	F	1,281	TR961
K	E	J	B	I	D	H	A	G	C	F	1,279	TR649
K	E	J	B	I	D	H	A	G	C	F	1,277	TR149
K	E	J	B	I	D	H	A	G	C	F	1,273	TR03662
K	E	J	B	I	D	H	A	G	C	F	1,268	TR03191
K	E	J	B	I	D	H	A	G	C	F	1,264	TR03903
K	E	J	B	I	D	H	A	G	C	F	1,260	TR980
K	E	J	B	I	D	H	A	G	C	F	1,259	TR490
K	E	J	B	I	D	H	A	G	C	F	1,256	TR05101
K	E	J	B	I	D	H	A	G	C	F	1,254	TR01715
K	E	J	B	I	D	H	A	G	C	F	1,253	TR166
K	E	J	B	I	D	H	A	G	C	F	1,252	TR140
K	E	J	B	I	D	H	A	G	C	F	1,237	TR01176
K	E	J	B	I	D	H	A	G	C	F	1,237	TR964
K	E	J	B	I	D	H	A	G	C	F	1,236	TR970
K	E	J	B	I	D	H		G	C	F	1,230	TR655
K	E	J	B	I	D	H		G	C	F	1,223	TR706
K	E	J	B	I	D	H	L	G	C	F	1,219	TR143
K	E	J	B	I	D	H	L	G	C	F	1,205	TR02181
K	E	J	B	I	D	H	L	G	C	F	1,203	TR03278
K	E	J	B	I	D	H	L	G	C	F	1,198	TR714
K	E	J	B	I	D	H	L	G	C	F	1,192	TR05669
K	E	J	B	I	D	H	L	G	C	F	1,188	TR159
K	E	J	B	I	D	H	L	G	C	F	1,186	TR646
K	E	J	B	I	D	H	L	G	C	F	1,176	TR979
K	E	J	B	I	D	H	L	G	C	F	1,170	TR917

Tukey Grouping											Mean	Line
K	E	J	B	I	D	H	L	G	C	F	1,164	TR705
K	E	J	B	I	D	H	L	G	C	F	1,156	TR582
K	E	J	B	I	D	H	L	G	C	F	1,153	TR162
K	E	J	B	I	D	H	L	G	C	F	1,147	TR989
K	E	J	B	I	D	H	L	G	C	F	1,137	TR987
K	E	J	B	I	D	H	L	G	C	F	1,133	TR702
K	E	J	B	I	D	H	L	G	C	F	1,129	TR983
K	E	J	B	I	D	H	L	G	C	F	1,117	TR156
K	E	J	B	I	D	H	L	G	C	F	1,115	TR643
K	E	J	B	I	D	H	L	G	C	F	1,109	TR992
K	E	J		I	D	H	L	G	C	F	1,071	TR06673
K	E	J		I	D	H	L	G	C	F	1,069	TR985
K	E	J		I	D	H	L	G		F	1,048	TR658
K	E	J		I		H	L	G		F	989	TR574
K	E	J		I		H	L	G		F	974	TR990
K		J		I		H	L	G		F	961	TR993
K		J		I		H	L	G			922	TR982
K		J		I		H	L				915	TR441
K		J		I		H	L				907	TR633
K		J		I			L				901	TR988
K		J					L				899	TR986
K							L				877	TR984
							L				636	TR635

Appendix E

Green, Kilned, and Difference in β-glucanase Activity within the Association Mapping Population

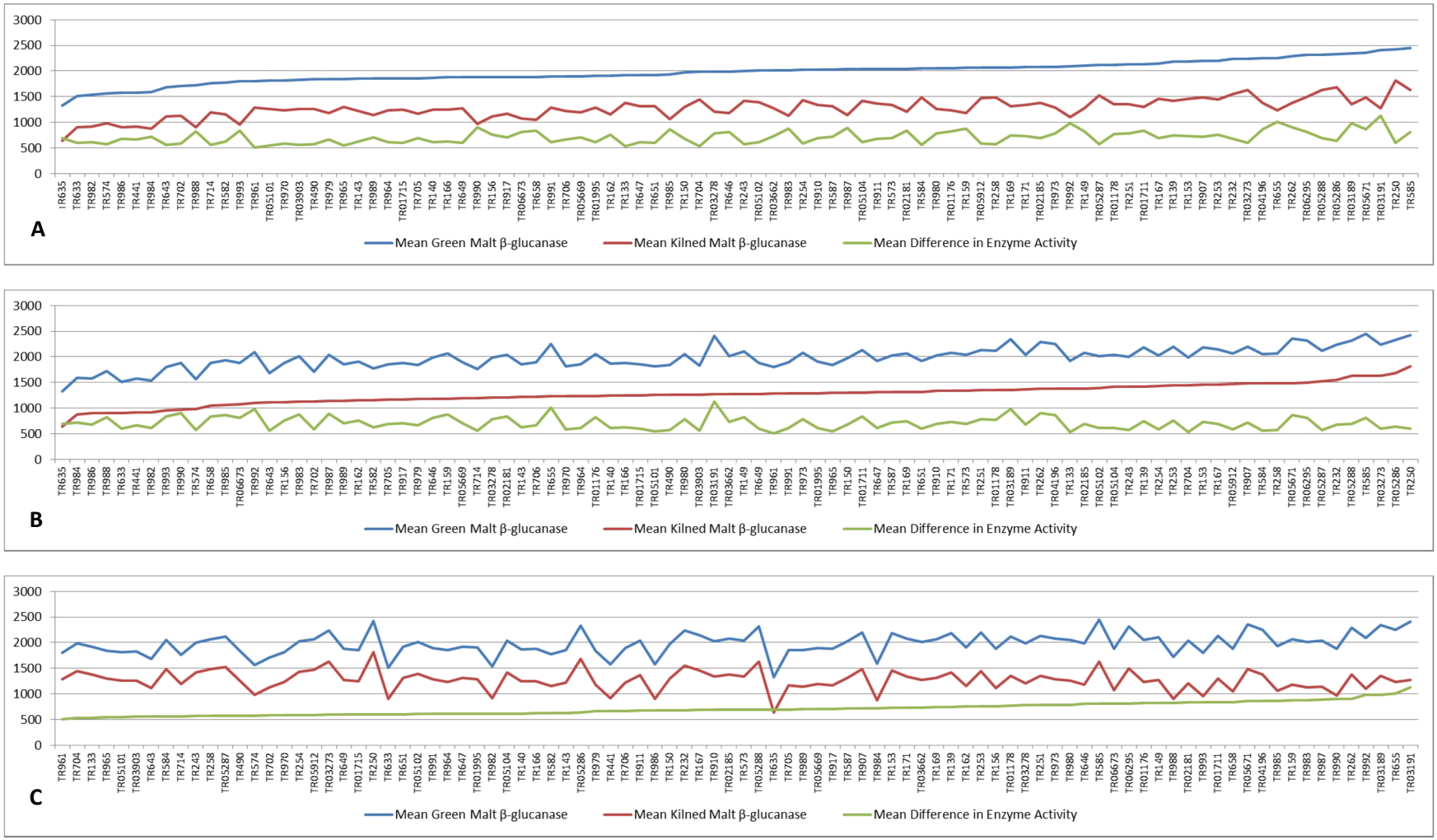

Figure E1. Comparison of average β-glucanase activity among the 92 lines in the AM population grown in 2011 and 2013. (A) lines ordered by increasing green malt β-glucanase activity, (B) lines ordered by increasing kilned malt β-glucanase activity, and (C) lines ordered by increasing difference in β-glucanase activity.

Appendix F

Chromosome and Map Positions of Polymorphic Markers in the Association Mapping Population

Table F1. Chromosome and approximate position of polymorphic markers on chromosomes 1H to 4H in the AM population based on the IBSC_2012 reference map.

Chromosome 1H		Chromosome 2H		Chromosome 3H		Chromosome 4H	
Marker	**Pos[1]**	**Marker**	**Pos**	**Marker**	**Pos**	**Marker**	**Pos**
11_21354	0.0	SCRI_RS_142851	0.0	12_31428	0.0	SCRI_RS_164381	0.8
SCRI_RS_142714	0.2	SCRI_RS_135585	2.1	12_10103	1.6	11_21228	1.1
SCRI_RS_82277	3.0	SCRI_RS_173486	2.3	SCRI_RS_173491	1.9	12_21117	1.1
12_30715	3.2	SCRI_RS_174552	3.8	11_11453	2.4	12_31511	1.3
11_20502	4.1	SCRI_RS_146936	4.3	SCRI_RS_168529	2.7	SCRI_RS_154517	1.9
12_11311	4.7	SCRI_RS_198370	4.4	SCRI_RS_119379	3.1	SCRI_RS_110334	2.3
SCRI_RS_165910	5.0	SCRI_RS_184395	4.5	12_31409	3.1	12_31486	2.4
12_11011	6.0	SCRI_RS_227507	5.4	11_20529	3.3	11_10208	3.5
11_21226	8.5	SCRI_RS_168604	5.5	SCRI_RS_132388	7.0	12_30540	14.5
SCRI_RS_162524	8.6	SCRI_RS_146997	5.8	11_10886	8.3	SCRI_RS_180891	14.9
SCRI_RS_184274	9.9	SCRI_RS_133377	6.3	SCRI_RS_130264	8.6	SCRI_RS_105960	15.0
SCRI_RS_200512	11.8	SCRI_RS_169758	6.4	11_20595	8.9	11_10113	18.5
SCRI_RS_148560	12.6	11_21377	7.3	12_30915	9.2	11_11136	19.9
12_30588	15.1	SCRI_RS_177330	7.5	SCRI_RS_129198	9.4	SCRI_RS_162743	20.9
SCRI_RS_130592	16.9	12_10592	7.8	SCRI_RS_193258	9.5	SCRI_RS_98443	22.2
SCRI_RS_205669	17.9	SCRI_RS_221776	8.3	SCRI_RS_239142	9.6	SCRI_RS_190426	25.6
SCRI_RS_13972	18.5	SCRI_RS_149407	8.3	11_10112	9.8	11_20411	26.4
SCRI_RS_128285	21.3	12_31497	8.4	SCRI_RS_142420	10.5	SCRI_RS_7704	26.5
11_10030	22.8	11_20563	8.6	SCRI_RS_108971	11.5	SCRI_RS_225074	26.6
12_30876	27.1	SCRI_RS_173604	8.9	11_20172	12.1	11_20001	27.5
SCRI_RS_151047	27.3	12_30155	9.2	SCRI_RS_65533	14.1	11_21374	28.1
12_30241	27.4	SCRI_RS_605	9.6	11_20742	15.0	SCRI_RS_228232	29.0
SCRI_RS_123187	27.9	SCRI_RS_209516	10.4	11_10565	15.1	SCRI_RS_157832	30.0
11_10757	28.1	SCRI_RS_144545	10.5	SCRI_RS_214280	15.2	12_31164	30.7
11_10744	28.4	SCRI_RS_136287	11.6	SCRI_RS_153718	17.4	12_30864	34.4
SCRI_RS_239784	30.5	SCRI_RS_141564	12.1	SCRI_RS_151808	17.5	12_11300	34.6
12_31276	32.2	SCRI_RS_226348	12.5	SCRI_RS_192352	17.9	11_21122	35.9
SCRI_RS_230133	32.6	SCRI_RS_152744	12.6	11_20982	18.7	SCRI_RS_145412	40.0
SCRI_RS_161702	37.0	SCRI_RS_155957	12.8	11_10559	20.8	SCRI_RS_183399	42.1
12_30336	40.1	SCRI_RS_231889	13.4	SCRI_RS_103614	22.8	12_30992	43.3
SCRI_RS_153785	41.6	SCRI_RS_194812	13.5	11_20794	23.1	SCRI_RS_161528	43.3
SCRI_RS_140837	42.1	SCRI_RS_154683	14.4	SCRI_RS_189757	23.9	11_20012	43.3
11_10764	42.4	SCRI_RS_198643	15.4	11_20968	24.9	12_10371	43.5
SCRI_RS_160545	42.7	SCRI_RS_159228	17.5	SCRI_RS_121895	25.3	11_20114	44.9
11_10275	42.8	SCRI_RS_150590	17.9	12_30192	25.5	11_11180	44.9
11_21095	43.0	SCRI_RS_153672	18.1	11_20410	32.7	11_10048	45.7
11_10597	43.1	SCRI_RS_233272	18.6	SCRI_RS_230486	36.5	SCRI_RS_140586	49.7
11_21134	43.2	11_10180	18.9	11_10672	36.8	11_10668	49.9
11_20617	43.3	12_30871	22.2	SCRI_RS_153732	36.8	11_10667	50.4
SCRI_RS_132461	43.4	11_20394	23.2	12_10968	37.4	SCRI_RS_119778	50.6
11_10526	46.5	SCRI_RS_12516	23.6	11_20607	37.4	11_10793	50.6

Chromosome 1H		Chromosome 2H		Chromosome 3H		Chromosome 4H	
Marker	**Pos[1]**	**Marker**	**Pos**	**Marker**	**Pos**	**Marker**	**Pos**
12_30683	46.6	11_10216	23.8	SCRI_RS_173721	37.5	SCRI_RS_217322	50.7
12_31467	46.7	SCRI_RS_115905	24.5	SCRI_RS_136959	37.6	11_21073	50.9
SCRI_RS_124727	46.8	SCRI_RS_153693	24.8	SCRI_RS_9439	37.8	SCRI_RS_224697	50.9
11_11293	47.1	SCRI_RS_115892	25.4	SCRI_RS_102837	38.0	12_31310	51.0
12_10235	47.1	11_21187	26.2	SCRI_RS_115045	38.6	SCRI_RS_121003	51.2
11_21000	47.3	11_10891	27.7	11_20193	38.7	SCRI_RS_74014	51.3
11_11064	47.5	SCRI_RS_231897	28.2	11_10863	40.0	12_31414	51.4
SCRI_RS_149971	47.7	11_20864	29.4	11_20647	40.5	12_31360	51.5
11_10985	47.8	11_21304	33.5	11_21145	40.7	SCRI_RS_104697	51.6
12_30762	48.0	12_30748	33.9	11_11002	43.4	SCRI_RS_229704	51.9
11_10438	48.0	12_10847	34.0	SCRI_RS_146425	43.7	11_10028	51.9
11_20757	48.0	SCRI_RS_5552	36.8	SCRI_RS_229693	43.9	11_10379	52.2
SCRI_RS_193640	48.1	SCRI_RS_174935	37.8	12_30785	44.3	SCRI_RS_194649	52.3
SCRI_RS_138691	48.2	11_10525	38.8	SCRI_RS_151254	45.2	SCRI_RS_153459	52.3
12_31272	48.4	SCRI_RS_120182	39.0	12_30064	45.5	11_10411	52.4
SCRI_RS_130590	48.4	SCRI_RS_78277	39.4	11_10601	45.7	12_30427	53.0
SCRI_RS_116685	48.5	SCRI_RS_89851	39.5	11_21093	46.2	11_11042	53.0
11_20810	48.6	11_10399	39.7	SCRI_RS_143810	46.5	11_20610	53.3
12_11169	48.7	11_10837	39.9	SCRI_RS_154769	46.9	11_10568	54.0
11_11326	48.7	SCRI_RS_134823	40.1	SCRI_RS_115769	47.0	11_10046	54.2
11_10933	48.8	11_21057	40.1	SCRI_RS_189409	48.0	11_10527	54.3
12_30110	48.9	SCRI_RS_135619	40.2	SCRI_RS_222735	50.9	SCRI_RS_14612	54.6
11_10520	48.9	SCRI_RS_174444	40.5	11_10137	51.2	11_20482	54.8
11_11359	49.1	12_30432	40.8	11_10328	51.3	SCRI_RS_150615	55.4
11_21217	49.2	SCRI_RS_141655	41.9	12_30039	51.4	SCRI_RS_182937	55.7
SCRI_RS_109060	49.3	SCRI_RS_171029	43.8	12_30622	51.4	11_10914	55.8
12_30478	49.8	SCRI_RS_194318	44.4	11_10008	51.5	SCRI_RS_168603	56.2
11_21361	50.1	12_10715	45.0	12_31012	51.5	SCRI_RS_13552	56.2
12_30786	50.4	SCRI_RS_152206	46.4	11_11099	51.6	11_10509	56.6
SCRI_RS_198546	50.6	11_10084	47.3	11_10224	51.7	SCRI_RS_170560	57.3
11_11256	50.9	SCRI_RS_201800	48.4	11_10349	51.8	12_31186	57.5
12_10198	51.5	12_10219	48.5	11_11401	52.0	SCRI_RS_189180	58.1
SCRI_RS_56976	52.5	SCRI_RS_237094	48.6	12_31011	52.1	11_20723	58.4
SCRI_RS_1445	52.7	SCRI_RS_221843	49.4	SCRI_RS_109716	52.4	SCRI_RS_13430	59.6
SCRI_RS_100503	53.3	11_11505	49.5	11_11016	52.6	11_11431	59.8
11_20095	53.5	12_31127	49.7	11_20276	52.9	SCRI_RS_165912	60.1
SCRI_RS_145305	53.7	12_31169	50.1	11_20325	53.1	12_30620	60.3
12_10201	53.8	11_10679	50.3	11_10225	53.3	SCRI_RS_110333	60.6
SCRI_RS_130666	54.1	SCRI_RS_198460	50.5	11_20801	53.7	12_30755	60.6
SCRI_RS_121978	54.2	SCRI_RS_173531	50.7	11_10728	54.2	SCRI_RS_147712	60.7
11_10552	54.2	11_10297	50.9	SCRI_RS_1799	54.5	12_31385	60.8
12_30821	54.5	12_30691	51.5	SCRI_RS_152172	54.5	SCRI_RS_146174	61.1
SCRI_RS_152795	54.9	11_20674	51.6	SCRI_RS_222102	54.8	11_11224	61.5
11_10176	55.3	11_10733	51.8	SCRI_RS_173186	55.1	SCRI_RS_182555	61.7
SCRI_RS_192779	55.4	SCRI_RS_182050	52.2	SCRI_RS_174324	55.2	SCRI_RS_108509	62.0
SCRI_RS_21483	55.5	11_20929	52.4	SCRI_RS_168665	55.4	SCRI_RS_154704	62.3
SCRI_RS_182431	55.7	SCRI_RS_151535	52.8	SCRI_RS_179275	55.7	SCRI_RS_140349	62.5
SCRI_RS_145336	58.9	SCRI_RS_175065	52.9	SCRI_RS_141109	55.8	11_20072	62.7

Chromosome 1H		Chromosome 2H		Chromosome 3H		Chromosome 4H	
Marker	**Pos[1]**	**Marker**	**Pos**	**Marker**	**Pos**	**Marker**	**Pos**
11_21431	59.4	SCRI_RS_187110	53.3	SCRI_RS_182328	56.1	11_10846	63.2
11_20432	60.1	SCRI_RS_155546	53.7	11_10158	56.4	SCRI_RS_139806	63.4
SCRI_RS_116661	60.8	11_20602	54.2	SCRI_RS_124607	56.4	11_10627	64.0
11_10043	61.0	11_11015	54.3	SCRI_RS_197825	56.7	SCRI_RS_195017	64.3
SCRI_RS_206795	61.2	SCRI_RS_125325	54.3	11_21502	57.1	SCRI_RS_197405	64.6
12_30295	61.5	12_31288	54.4	SCRI_RS_158616	57.2	SCRI_RS_154658	64.6
SCRI_RS_133886	62.3	11_11302	54.4	11_20659	57.2	SCRI_RS_146941	65.4
SCRI_RS_157039	62.8	SCRI_RS_185513	54.7	SCRI_RS_200042	57.4	SCRI_RS_179444	66.1
12_10166	65.2	11_10422	55.1	SCRI_RS_135581	57.5	11_21504	66.6
12_30438	67.3	SCRI_RS_222933	55.4	11_20704	57.5	11_10610	66.7
11_20229	67.3	SCRI_RS_131325	55.5	SCRI_RS_231801	58.0	11_11513	67.0
SCRI_RS_160234	70.3	SCRI_RS_204254	55.5	SCRI_RS_137934	58.1	SCRI_RS_143825	67.0
SCRI_RS_153727	70.7	SCRI_RS_186664	55.5	11_10335	58.6	SCRI_RS_148392	67.5
11_21126	70.9	11_10996	55.6	SCRI_RS_230975	59.0	SCRI_RS_160624	67.6
11_10006	71.0	SCRI_RS_171237	55.6	SCRI_RS_107261	59.1	SCRI_RS_89959	67.6
SCRI_RS_1473	71.2	SCRI_RS_144891	55.6	SCRI_RS_164731	59.4	SCRI_RS_159331	67.7
12_30498	71.9	SCRI_RS_122871	55.6	SCRI_RS_211224	59.5	11_10829	69.0
SCRI_RS_156208	72.4	12_30853	55.6	12_30616	59.6	12_30994	69.3
11_20121	72.6	12_30251	55.7	11_11394	59.7	SCRI_RS_165833	73.4
SCRI_RS_222357	74.0	SCRI_RS_198865	55.8	SCRI_RS_6793	59.9	11_10247	73.5
SCRI_RS_181353	76.8	11_20039	55.8	SCRI_RS_171837	60.7	SCRI_RS_164399	73.7
12_31229	80.2	SCRI_RS_13952	56.0	11_20351	60.8	SCRI_RS_200957	76.3
11_10471	81.7	SCRI_RS_236995	56.0	SCRI_RS_76971	61.7	SCRI_RS_214059	77.6
11_20434	82.1	11_20458	56.0	SCRI_RS_10288	61.8	SCRI_RS_171874	78.5
11_20550	82.5	11_10012	56.2	12_30754	61.9	11_20670	78.6
SCRI_RS_181300	83.7	SCRI_RS_11204	56.2	SCRI_RS_224360	62.0	11_21353	78.7
SCRI_RS_139690	85.8	12_10474	56.2	SCRI_RS_138291	62.5	11_10724	78.7
SCRI_RS_236623	86.0	SCRI_RS_55840	56.3	11_10350	62.7	SCRI_RS_13460	78.8
SCRI_RS_144315	86.0	12_30042	56.4	11_10276	63.0	SCRI_RS_125524	80.2
SCRI_RS_135092	86.5	SCRI_RS_117951	56.5	SCRI_RS_175710	63.2	SCRI_RS_169134	80.5
SCRI_RS_121048	86.5	SCRI_RS_161169	56.7	11_20521	63.7	11_21151	81.4
SCRI_RS_177788	86.6	SCRI_RS_4969	57.0	SCRI_RS_13778	63.7	SCRI_RS_162410	83.0
SCRI_RS_166168	86.7	SCRI_RS_116920	57.1	12_30743	64.0	11_20358	83.5
11_20475	86.8	SCRI_RS_191136	57.1	11_10683	64.2	11_20178	83.6
SCRI_RS_189168	87.5	SCRI_RS_1502	57.2	SCRI_RS_104564	64.6	11_20689	84.5
SCRI_RS_114308	88.0	11_20585	57.2	12_31346	64.7	SCRI_RS_188944	86.0
11_21373	88.2	SCRI_RS_208320	57.3	SCRI_RS_149160	64.9	SCRI_RS_138835	86.4
SCRI_RS_197263	88.9	11_10685	57.4	SCRI_RS_117006	66.2	SCRI_RS_168074	87.0
SCRI_RS_213675	90.2	SCRI_RS_134925	57.4	SCRI_RS_189039	67.5	11_11398	87.7
11_10396	90.3	11_21399	57.6	SCRI_RS_207408	67.6	SCRI_RS_141214	88.1
11_11277	91.2	SCRI_RS_168400	58.1	SCRI_RS_137852	67.9	SCRI_RS_233242	90.6
11_20971	91.5	11_10436	58.3	11_21163	68.0	SCRI_RS_157760	90.9
SCRI_RS_199689	92.5	SCRI_RS_200879	58.5	11_20093	68.2	11_20732	91.2
SCRI_RS_142282	93.1	11_10632	58.6	12_30677	68.2	SCRI_RS_168399	91.3
11_10522	93.7	SCRI_RS_125516	58.6	SCRI_RS_223611	68.3	SCRI_RS_173947	91.3
11_20754	94.2	11_10909	58.8	SCRI_RS_219894	68.6	SCRI_RS_129218	91.5
11_20169	94.6	SCRI_RS_83731	58.9	SCRI_RS_162539	69.0	11_20384	92.1
SCRI_RS_235968	95.2	SCRI_RS_134606	58.9	12_31262	69.9	12_30718	97.2

Chromosome 1H		Chromosome 2H		Chromosome 3H		Chromosome 4H	
Marker	Pos[1]	Marker	Pos	Marker	Pos	Marker	Pos
11_20125	95.6	SCRI_RS_188255	59.1	SCRI_RS_13780	70.3	12_10271	97.3
11_20780	100.1	12_30108	59.4	SCRI_RS_233246	72.3	11_20838	97.6
12_31181	100.1	12_11278	59.5	11_20063	73.0	12_30554	97.7
11_20625	100.3	11_20690	60.4	SCRI_RS_167410	73.2	11_10334	98.2
11_20220	100.8	SCRI_RS_206529	60.5	SCRI_RS_155763	74.8	11_20701	98.3
SCRI_RS_147329	100.9	11_21166	60.7	11_21348	75.7	11_11292	99.1
12_30532	100.9	11_21110	60.8	11_20136	76.0	SCRI_RS_188827	99.2
12_10149	101.1	11_21144	62.5	12_11468	76.1	SCRI_RS_207853	99.6
11_21392	103.8	11_10265	62.7	SCRI_RS_231362	76.1	12_30988	100.2
SCRI_RS_157953	106.2	11_10651	62.8	SCRI_RS_177399	76.2	11_20044	100.6
11_20383	109.4	SCRI_RS_155808	63.5	12_10134	76.4	12_30987	100.6
11_20959	111.8	11_10017	63.6	12_30325	77.1	SCRI_RS_197256	101.3
SCRI_RS_127646	116.3	SCRI_RS_173757	64.2	SCRI_RS_240090	77.3	11_10614	101.3
11_20908	116.5	SCRI_RS_155470	64.2	SCRI_RS_826	77.4	11_10510	101.6
12_21172	116.5	12_20985	64.2	12_10583	77.5	SCRI_RS_160461	102.0
11_11528	116.6	SCRI_RS_163975	64.5	11_10747	77.7	SCRI_RS_9549	102.4
11_10586	117.5	SCRI_RS_198848	64.6	12_31367	78.3	SCRI_RS_148330	104.0
SCRI_RS_48925	118.0	SCRI_RS_145381	64.8	SCRI_RS_198609	78.4	11_20974	104.6
11_10722	118.5	11_21251	64.9	SCRI_RS_1509	83.1	12_11233	109.2
SCRI_RS_162765	119.0	11_21205	65.4	SCRI_RS_192761	83.8	SCRI_RS_148782	109.2
11_21140	119.1	12_31021	66.1	12_11322	84.0	12_10032	110.2
SCRI_RS_160475	119.7	11_20667	66.4	12_30250	84.6	11_20668	111.3
SCRI_RS_169881	122.0	SCRI_RS_150266	66.9	SCRI_RS_159340	86.2	12_30873	112.1
SCRI_RS_130577	122.1	SCRI_RS_16024	67.3	11_21438	86.3	SCRI_RS_113536	112.3
SCRI_RS_165338	124.9	11_20528	67.4	11_20999	86.4	SCRI_RS_7809	112.5
SCRI_RS_130891	126.0	11_21078	67.9	11_20130	86.4	SCRI_RS_222936	113.0
SCRI_RS_196025	126.1	SCRI_RS_159024	70.5	SCRI_RS_120503	86.7	11_21130	113.1
SCRI_RS_13869	128.1	12_30696	70.8	11_20628	87.4	11_10269	113.7
SCRI_RS_10956	128.3	SCRI_RS_207237	70.9	SCRI_RS_138193	88.1	SCRI_RS_151357	114.9
11_21384	129.4	SCRI_RS_172667	71.0	11_20626	88.2	SCRI_RS_188829	115.2
SCRI_RS_214888	130.0	SCRI_RS_7517	72.6	SCRI_RS_165444	88.5		
11_11105	130.2	SCRI_RS_2273	73.0	12_30640	88.8		
12_30517	130.4	SCRI_RS_17898	73.7	SCRI_RS_3125	89.9		
11_10041	130.7	SCRI_RS_133539	74.3	11_21083	90.0		
11_20594	130.8	SCRI_RS_174318	74.4	11_21381	90.1		
SCRI_RS_132472	132.2	SCRI_RS_156323	74.4	SCRI_RS_238430	90.4		
11_20509	132.4	11_21242	75.2	SCRI_RS_161385	90.6		
12_30934	132.5	SCRI_RS_139193	75.6	SCRI_RS_170206	90.6		
SCRI_RS_167999	132.5	11_10823	76.1	11_21493	90.7		
12_20429	132.7	SCRI_RS_154398	76.2	SCRI_RS_140043	96.3		
11_20772	132.7	SCRI_RS_235860	76.5	SCRI_RS_187928	96.4		
11_10443	132.8	11_20781	76.7	11_20023	96.5		
		11_11214	76.8	SCRI_RS_8667	96.5		
		SCRI_RS_137263	77.1	SCRI_RS_172266	96.6		
		11_11100	77.3	11_10753	98.2		
		SCRI_RS_224512	77.9	SCRI_RS_116542	98.5		
		11_10287	79.9	SCRI_RS_230069	98.7		
		12_30897	80.0	11_10312	100.3		

Chromosome 1H		Chromosome 2H		Chromosome 3H		Chromosome 4H	
Marker	Pos[1]	Marker	Pos	Marker	Pos	Marker	Pos
		SCRI_RS_196853	80.6	SCRI_RS_131897	100.4		
		12_31205	80.6	11_10584	102.5		
		SCRI_RS_180028	80.9	11_11503	102.7		
		11_11533	81.0	SCRI_RS_141195	103.0		
		SCRI_RS_3376	81.1	12_30927	103.1		
		SCRI_RS_10670	81.5	SCRI_RS_136593	103.7		
		12_10936	81.8	SCRI_RS_198594	103.8		
		SCRI_RS_219074	82.3	12_31220	104.3		
		11_21351	82.5	SCRI_RS_164290	104.4		
		11_21037	82.8	12_30926	104.5		
		SCRI_RS_116694	86.0	SCRI_RS_236528	104.8		
		11_10214	86.6	11_11330	105.0		
		SCRI_RS_165795	86.8	SCRI_RS_150944	107.0		
		SCRI_RS_160616	86.9	11_10918	107.2		
		SCRI_RS_222876	88.5	12_30274	107.8		
		11_10138	90.2	SCRI_RS_203905	108.0		
		11_21175	90.3	12_30924	108.3		
		11_11307	91.0	11_11172	108.4		
		11_10900	92.2	11_10044	108.6		
		12_10649	92.7	11_20650	108.9		
		SCRI_RS_160958	92.8	11_10821	109.0		
		SCRI_RS_209969	93.6	12_10205	109.8		
		11_11250	94.0	12_30081	111.3		
		12_31333	94.3	12_10188	111.8		
		SCRI_RS_115690	94.4	11_10280	112.7		
		SCRI_RS_157097	94.6	SCRI_RS_194623	113.4		
		11_10398	94.6	11_11196	114.7		
		SCRI_RS_150494	94.7	SCRI_RS_146012	116.4		
		SCRI_RS_147985	94.8	11_20612	117.0		
		SCRI_RS_142188	94.9	12_30973	117.6		
		11_20923	94.9	SCRI_RS_167755	118.2		
		11_10916	95.2	12_30092	118.6		
		11_11323	99.3	11_20527	118.9		
		11_11480	100.4	11_10381	122.0		
		SCRI_RS_128484	100.5	11_20085	122.6		
		11_20064	103.8	11_11328	123.2		
		SCRI_RS_154703	104.2	12_11047	126.1		
		SCRI_RS_8671	104.6	SCRI_RS_213950	126.7		
		11_10352	105.9	SCRI_RS_175038	127.0		
		SCRI_RS_223885	106.4	SCRI_RS_10016	127.3		
		11_10429	107.2	12_30137	128.0		
		SCRI_RS_204145	107.3	SCRI_RS_204422	128.1		
		SCRI_RS_114164	107.6	11_10570	128.5		
		11_10707	107.7	SCRI_RS_204462	128.6		
		11_11236	108.0	SCRI_RS_206881	128.7		
		SCRI_RS_151617	108.1	SCRI_RS_231382	128.8		
		SCRI_RS_156220	108.2	SCRI_RS_174582	131.3		
		SCRI_RS_183974	109.4	12_11208	131.6		

Chromosome 1H		Chromosome 2H		Chromosome 3H		Chromosome 4H	
Marker	Pos[1]	Marker	Pos	Marker	Pos	Marker	Pos
		11_10404	110.9	12_21386	131.7		
		SCRI_RS_12444	111.3	SCRI_RS_167698	132.2		
		SCRI_RS_153811	111.7	SCRI_RS_144559	132.9		
		SCRI_RS_149429	112.0	SCRI_RS_14107	133.3		
		11_21220	112.3	11_20952	133.4		
		SCRI_RS_235261	113.5	SCRI_RS_173459	133.8		
		11_20511	113.9	11_21272	135.5		
		11_10092	114.0	12_31161	135.6		
		SCRI_RS_130279	114.1	12_21376	137.1		
		SCRI_RS_209218	114.2	SCRI_RS_205957	137.5		
		SCRI_RS_119513	114.4	SCRI_RS_110995	137.6		
		SCRI_RS_179555	114.9	12_31500	137.9		
		SCRI_RS_708	116.8	12_20421	138.7		
		SCRI_RS_211291	118.0	12_20505	139.3		
		11_21370	118.7	SCRI_RS_154449	139.6		
		11_10383	119.0	SCRI_RS_65010	139.7		
		11_21088	119.8	12_30921	140.9		
		SCRI_RS_119426	120.0	SCRI_RS_173623	142.2		
		SCRI_RS_149273	120.5	12_20198	142.6		
		11_20215	120.7	12_30767	142.6		
		12_31268	120.8	SCRI_RS_126351	143.0		
		11_10065	121.0	11_21008	143.1		
		12_30351	121.2	11_21523	143.5		
		SCRI_RS_201191	123.7	SCRI_RS_229623	144.1		
		11_10376	123.9	11_20605	144.6		
		SCRI_RS_144102	124.8	SCRI_RS_194256	144.9		
		11_20895	124.9	SCRI_RS_180847	145.3		
		12_10579	125.2	11_11411	145.5		
		SCRI_RS_134241	125.4	SCRI_RS_156315	145.6		
		SCRI_RS_204022	125.9	12_10014	145.8		
		SCRI_RS_193100	126.2	11_10694	146.1		
		SCRI_RS_11645	127.2	SCRI_RS_142818	147.2		
		SCRI_RS_133224	128.3	12_30736	148.2		
		11_21274	129.3	11_20057	148.4		
		11_20590	129.8	SCRI_RS_146798	148.6		
		11_21181	129.8	12_20176	149.6		
		12_31461	132.0	12_30229	151.3		
		11_10826	132.6	SCRI_RS_236603	154.8		
		11_10551	132.9	SCRI_RS_139876	155.0		
		11_10566	133.3				
		11_20994	135.6				
		11_11380	136.1				
		11_20293	137.0				
		SCRI_RS_139073	138.6				
		SCRI_RS_173017	140.3				
		SCRI_RS_134343	140.5				
		11_10791	140.8				
		SCRI_RS_141500	140.9				

Chromosome 1H		Chromosome 2H		Chromosome 3H		Chromosome 4H	
Marker	Pos[1]	Marker	Pos	Marker	Pos	Marker	Pos
		SCRI_RS_200267	140.9				
		SCRI_RS_13450	141.0				
		SCRI_RS_153293	141.1				
		SCRI_RS_152274	141.2				
		SCRI_RS_147278	141.5				
		11_10072	141.6				
		SCRI_RS_225633	142.2				
		SCRI_RS_73620	142.3				
		11_20561	142.4				
		SCRI_RS_172136	142.4				
		SCRI_RS_149233	144.1				
		12_31180	144.3				
		SCRI_RS_170209	144.4				
		SCRI_RS_161281	145.6				
		SCRI_RS_161224	146.3				
		SCRI_RS_195051	146.5				
		11_20681	146.9				
		SCRI_RS_204579	147.1				
		SCRI_RS_158043	147.3				
		SCRI_RS_139737	148.2				
		SCRI_RS_123364	149.3				
		SCRI_RS_165239	149.4				

[1] Position in cM.

Table F2. Chromosome and approximate position of polymorphic markers on chromosomes 5H to 7H in the AM population based on the IBSC_2012 reference map.

Chromosome 5H		Chromosome 6H		Chromosome 7H	
Marker	Pos	Marker	Pos	Marker	Pos
12_30543	0.0	11_20232	0.3	12_10036	0.2
11_20894	0.1	SCRI_RS_168487	0.3	11_10682	0.4
SCRI_RS_109375	1.1	11_20212	1.0	SCRI_RS_200389	0.6
12_30984	1.1	SCRI_RS_194023	1.4	12_30472	0.7
12_30975	1.6	11_21521	2.6	11_21443	0.9
SCRI_RS_31797	1.9	11_10120	4.0	12_31411	1.3
SCRI_RS_90293	2.2	12_31233	4.4	11_10121	1.5
SCRI_RS_236068	3.0	11_10669	4.8	SCRI_RS_229445	1.6
12_31022	3.2	SCRI_RS_56	4.9	12_20839	2.1
SCRI_RS_168359	3.7	SCRI_RS_153928	5.0	11_20534	2.6
12_30591	4.0	11_11406	6.4	11_11031	3.8
12_31326	4.2	12_30651	8.6	SCRI_RS_129323	8.3
SCRI_RS_155555	8.6	11_20294	9.4	SCRI_RS_171301	9.7
11_21365	9.3	SCRI_RS_141842	10.1	SCRI_RS_166323	11.1
SCRI_RS_108541	12.0	11_20262	10.6	SCRI_RS_152931	11.5
SCRI_RS_98293	13.4	11_21032	10.8	SCRI_RS_140481	12.8
SCRI_RS_149877	14.5	SCRI_RS_231003	11.1	11_21437	13.2
SCRI_RS_184564	15.6	SCRI_RS_203036	11.3	11_10851	13.3
12_30167	17.6	SCRI_RS_139713	15.7	SCRI_RS_147695	13.6
11_21065	17.6	11_20415	15.7	SCRI_RS_142893	13.9

Chromosome 5H		Chromosome 6H		Chromosome 7H	
Marker	**Pos**	**Marker**	**Pos**	**Marker**	**Pos**
11_20873	17.9	11_10023	16.0	SCRI_RS_126944	15.4
12_30531	21.6	11_10165	17.0	11_20722	16.8
11_11048	23.6	12_30842	17.7	11_10025	17.6
SCRI_RS_189936	28.3	SCRI_RS_189122	19.4	SCRI_RS_162631	19.7
11_10688	28.7	SCRI_RS_4551	20.3	11_20495	20.8
12_10499	29.1	SCRI_RS_154574	24.0	12_30530	21.0
SCRI_RS_104699	29.8	11_10136	24.5	SCRI_RS_148318	21.2
SCRI_RS_27067	30.1	SCRI_RS_139117	24.5	SCRI_RS_78255	21.8
SCRI_RS_187327	30.4	SCRI_RS_231372	24.6	SCRI_RS_223021	21.9
SCRI_RS_144042	30.4	12_30603	26.5	SCRI_RS_204453	22.7
SCRI_RS_205508	31.1	SCRI_RS_189878	28.5	SCRI_RS_145156	23.0
11_10621	31.7	11_10676	30.0	12_31351	23.8
SCRI_RS_156295	31.8	12_30673	30.1	12_30780	23.8
12_30410	31.9	12_31308	30.2	11_10965	23.9
SCRI_RS_3114	32.4	12_31485	34.4	11_20162	24.1
11_21207	34.1	11_10799	35.6	SCRI_RS_140096	24.2
11_20845	34.2	12_30122	37.0	SCRI_RS_143967	24.5
11_20730	34.3	11_10427	37.4	SCRI_RS_160723	26.8
12_11318	38.1	12_30358	37.5	SCRI_RS_150053	27.3
SCRI_RS_220645	39.5	SCRI_RS_154121	37.8	11_10451	27.6
12_10864	39.6	SCRI_RS_235711	38.0	SCRI_RS_15864	28.5
12_30707	40.0	12_30521	39.7	11_20192	28.7
SCRI_RS_133674	41.2	12_30361	41.0	SCRI_RS_163976	29.8
SCRI_RS_207769	41.2	SCRI_RS_205857	43.6	SCRI_RS_138457	30.6
SCRI_RS_157728	41.3	11_10129	43.8	12_30083	32.1
SCRI_RS_185617	41.3	11_10494	43.9	11_20126	32.1
11_10260	41.5	SCRI_RS_141429	44.9	SCRI_RS_140831	32.8
SCRI_RS_187598	41.6	SCRI_RS_115036	45.2	12_30893	34.4
12_30105	41.7	12_30665	45.2	11_10838	37.6
SCRI_RS_204593	41.8	SCRI_RS_170814	46.0	12_31305	38.8
SCRI_RS_159069	41.8	SCRI_RS_7397	46.2	12_10218	39.7
SCRI_RS_179958	41.9	SCRI_RS_139451	46.3	12_30065	40.5
12_31312	42.0	11_21030	46.5	SCRI_RS_91662	40.7
SCRI_RS_222345	42.2	12_30516	46.7	12_10979	41.4
SCRI_RS_189103	42.4	11_10462	48.6	SCRI_RS_8200	42.0
12_31257	42.9	11_10355	48.8	12_10368	42.3
SCRI_RS_68142	43.0	SCRI_RS_147342	49.0	11_21528	42.5
11_11432	43.3	11_20743	49.0	SCRI_RS_155795	43.3
11_21447	44.0	SCRI_RS_143259	49.1	SCRI_RS_187827	43.8
SCRI_RS_142259	44.1	12_30316	49.2	SCRI_RS_186130	44.0
SCRI_RS_181514	44.2	SCRI_RS_168455	49.2	SCRI_RS_208770	44.4
12_31423	44.2	SCRI_RS_209824	49.3	SCRI_RS_185707	46.4
SCRI_RS_41519	44.4	SCRI_RS_140158	49.7	12_31490	47.0
SCRI_RS_234579	44.4	SCRI_RS_133544	50.2	SCRI_RS_150016	47.5
SCRI_RS_160321	44.4	11_20291	50.4	SCRI_RS_125000	47.6
SCRI_RS_195073	44.5	12_30510	50.5	SCRI_RS_157816	47.7
11_11506	44.8	SCRI_RS_161288	50.8	SCRI_RS_162708	47.9
11_20396	45.1	11_20675	50.9	SCRI_RS_164730	48.0

Chromosome 5H		Chromosome 6H		Chromosome 7H	
Marker	**Pos**	**Marker**	**Pos**	**Marker**	**Pos**
11_11128	45.2	SCRI_RS_232893	51.9	12_30545	48.3
SCRI_RS_135425	45.2	12_30569	52.1	12_30752	48.4
SCRI_RS_159056	45.4	12_10910	52.2	SCRI_RS_172335	48.4
SCRI_RS_209398	45.4	SCRI_RS_187832	52.5	SCRI_RS_164437	48.7
SCRI_RS_171243	45.6	SCRI_RS_153797	52.6	12_30371	48.9
SCRI_RS_236595	45.7	SCRI_RS_131341	52.7	SCRI_RS_202653	49.2
12_10508	45.8	SCRI_RS_136897	52.8	SCRI_RS_168494	49.7
11_20332	46.2	SCRI_RS_148432	52.9	SCRI_RS_212956	49.8
SCRI_RS_147462	46.3	SCRI_RS_166120	53.1	SCRI_RS_171008	49.9
SCRI_RS_164250	46.5	12_30473	53.3	11_20790	50.7
SCRI_RS_160506	46.5	SCRI_RS_164602	53.3	SCRI_RS_136556	51.0
SCRI_RS_134798	46.7	11_20656	53.5	SCRI_RS_178619	51.3
SCRI_RS_180342	46.9	11_21014	53.6	SCRI_RS_137626	52.3
SCRI_RS_153476	47.0	SCRI_RS_142727	53.7	12_30576	53.2
12_30454	47.1	SCRI_RS_205256	53.8	11_10346	54.8
SCRI_RS_13964	47.2	12_30311	54.3	SCRI_RS_229041	55.0
SCRI_RS_133042	47.2	SCRI_RS_186147	54.4	SCRI_RS_200890	55.1
12_31183	47.6	SCRI_RS_207083	54.9	11_10721	57.9
SCRI_RS_196041	47.6	11_20600	55.0	11_20750	61.5
11_20961	47.6	12_31479	55.0	11_20975	61.8
12_10034	47.7	SCRI_RS_144162	55.2	11_21270	62.0
12_31280	47.7	SCRI_RS_162961	55.3	12_30149	62.4
11_10671	47.7	SCRI_RS_126229	55.4	SCRI_RS_182	64.0
11_11240	47.8	11_20835	55.5	11_11098	64.2
12_30111	47.8	SCRI_RS_187725	55.5	SCRI_RS_139962	64.8
SCRI_RS_165313	47.9	12_30395	55.6	11_11028	65.4
11_20501	47.9	11_10377	55.7	11_20195	66.4
SCRI_RS_114549	48.0	SCRI_RS_206213	56.0	12_30125	66.6
11_11221	48.1	12_30144	56.2	SCRI_RS_195940	67.3
SCRI_RS_215787	48.1	SCRI_RS_153707	56.4	12_30496	67.4
11_21200	48.2	SCRI_RS_10810	56.4	SCRI_RS_140553	67.6
11_21508	48.3	11_20184	56.8	11_10299	67.8
12_30515	48.8	SCRI_RS_192599	56.8	12_30997	67.8
SCRI_RS_137893	49.7	SCRI_RS_114351	57.2	11_20885	68.1
11_20265	49.7	SCRI_RS_147666	57.2	SCRI_RS_219081	68.1
11_21414	49.9	SCRI_RS_187343	57.3	SCRI_RS_132722	68.2
SCRI_RS_169088	50.0	SCRI_RS_222315	58.3	SCRI_RS_132879	68.4
SCRI_RS_157941	50.1	SCRI_RS_206986	58.6	12_31452	68.4
12_30745	50.1	11_10189	59.3	SCRI_RS_169639	69.3
SCRI_RS_162773	50.4	SCRI_RS_206704	59.5	SCRI_RS_214425	69.4
SCRI_RS_171407	50.6	11_11067	59.8	12_30344	69.5
SCRI_RS_215710	50.8	SCRI_RS_129321	59.8	12_30481	70.2
12_30007	51.3	SCRI_RS_211289	59.9	12_10698	70.3
11_11249	51.5	SCRI_RS_4697	60.0	SCRI_RS_10407	70.4
SCRI_RS_223699	51.6	SCRI_RS_226342	60.1	11_20828	70.4
11_10641	51.9	SCRI_RS_232544	60.2	SCRI_RS_160279	70.5
SCRI_RS_174091	51.9	SCRI_RS_206731	60.2	12_30563	70.6
SCRI_RS_205803	52.3	SCRI_RS_217259	60.3	SCRI_RS_167383	70.6

Chromosome 5H		Chromosome 6H		Chromosome 7H	
Marker	**Pos**	**Marker**	**Pos**	**Marker**	**Pos**
11_21121	52.3	SCRI_RS_143492	60.3	12_10459	70.7
SCRI_RS_221999	55.6	12_10758	60.4	11_10773	70.8
SCRI_RS_205235	55.6	SCRI_RS_226330	60.5	11_10209	70.8
11_20367	61.0	11_21339	60.5	SCRI_RS_112718	70.9
11_10127	62.5	11_21310	60.6	11_21302	70.9
SCRI_RS_227918	63.2	12_30765	60.7	12_11477	71.0
11_21133	63.5	SCRI_RS_158011	61.7	SCRI_RS_162419	71.0
11_20096	64.6	SCRI_RS_133948	61.9	11_21178	71.1
SCRI_RS_166296	66.0	11_11483	62.8	SCRI_RS_151367	71.3
SCRI_RS_160332	70.1	SCRI_RS_205595	63.3	11_10534	71.6
SCRI_RS_159611	70.2	11_21225	63.5	11_10700	71.7
SCRI_RS_161222	71.2	12_10348	63.7	11_10442	72.5
12_31427	71.7	SCRI_RS_170672	64.1	SCRI_RS_175164	73.1
SCRI_RS_11206	73.3	SCRI_RS_115440	64.1	11_20880	73.9
SCRI_RS_140487	73.5	11_10455	65.3	12_30565	74.3
SCRI_RS_203938	74.7	SCRI_RS_139937	65.7	11_11445	75.2
11_10524	74.9	SCRI_RS_236457	65.8	11_11239	76.0
SCRI_RS_212784	75.6	SCRI_RS_231205	65.9	SCRI_RS_230083	76.4
11_21150	75.9	11_10040	66.1	11_20896	76.6
12_20359	77.1	11_20714	66.6	12_30199	76.7
11_10518	80.4	11_20468	66.8	SCRI_RS_229585	76.9
11_11473	81.3	11_11329	67.9	12_31199	77.0
11_10414	83.5	12_31289	68.1	SCRI_RS_206747	77.3
11_20850	83.6	SCRI_RS_225072	68.2	11_20083	77.4
12_10408	83.6	11_11349	68.5	SCRI_RS_194291	77.7
SCRI_RS_206061	84.5	12_31250	68.8	SCRI_RS_225636	78.0
SCRI_RS_4923	84.6	11_20784	68.8	12_30419	78.1
11_10622	85.6	11_11459	68.9	12_30996	79.8
11_20018	86.1	SCRI_RS_124856	68.9	SCRI_RS_104566	80.1
SCRI_RS_137196	86.9	11_20744	69.3	SCRI_RS_127791	81.5
12_31279	87.4	11_20053	70.0	11_21103	82.0
11_11350	87.6	11_20336	71.4	SCRI_RS_4562	84.4
SCRI_RS_230566	88.7	SCRI_RS_137215	71.7	SCRI_RS_134640	84.6
11_20392	89.4	SCRI_RS_167655	72.2	SCRI_RS_148722	86.0
11_20549	90.2	SCRI_RS_221951	73.0	SCRI_RS_168994	89.5
11_20795	92.4	SCRI_RS_153435	73.6	11_10301	91.2
SCRI_RS_156633	93.0	11_21224	73.8	12_30630	91.4
SCRI_RS_169223	93.4	SCRI_RS_164341	74.0	SCRI_RS_193197	91.9
SCRI_RS_219728	93.6	11_10608	74.2	SCRI_RS_195937	92.0
11_10024	93.7	SCRI_RS_206976	74.6	11_20808	92.1
11_21314	93.9	SCRI_RS_154582	75.5	12_31294	92.2
SCRI_RS_138826	93.9	SCRI_RS_184811	76.1	11_10169	93.8
11_21168	94.6	SCRI_RS_238393	78.1	SCRI_RS_136590	93.9
11_10834	94.7	SCRI_RS_1937	78.4	12_31261	97.1
11_11361	94.9	11_10331	79.6	SCRI_RS_152402	97.2
12_30848	95.1	SCRI_RS_153166	81.3	SCRI_RS_197282	97.3
11_21061	95.1	SCRI_RS_173232	81.7	SCRI_RS_139808	97.3
11_10094	95.5	11_20465	81.9	SCRI_RS_149645	98.2

Chromosome 5H		Chromosome 6H		Chromosome 7H	
Marker	**Pos**	**Marker**	**Pos**	**Marker**	**Pos**
SCRI_RS_13960	95.9	11_10815	84.0	11_20824	98.3
SCRI_RS_13549	96.3	11_11153	85.1	SCRI_RS_112204	98.4
11_20805	96.6	SCRI_RS_159441	85.7	12_10897	99.1
SCRI_RS_174177	96.6	SCRI_RS_8702	86.3	11_20385	100.0
11_11200	96.7	SCRI_RS_187841	86.5	12_10652	102.3
SCRI_RS_129893	97.1	SCRI_RS_164156	86.6	SCRI_RS_7217	102.9
11_11273	97.3	11_20783	86.8	SCRI_RS_172243	103.1
SCRI_RS_157897	98.1	12_30626	86.9	SCRI_RS_124251	103.5
11_10477	98.6	12_30698	87.2	SCRI_RS_153261	104.8
SCRI_RS_120829	98.9	SCRI_RS_183055	87.3	SCRI_RS_152752	106.5
SCRI_RS_9991	107.6	SCRI_RS_138716	87.5	SCRI_RS_161285	107.1
SCRI_RS_134711	109.7	SCRI_RS_147599	87.8	SCRI_RS_172331	107.4
11_20883	111.3	SCRI_RS_7640	87.9	12_30797	108.1
SCRI_RS_199964	112.6	SCRI_RS_139987	88.2	SCRI_RS_204424	109.9
SCRI_RS_212515	112.9	SCRI_RS_222802	88.2	SCRI_RS_152504	110.3
SCRI_RS_195217	113.1	11_10978	88.7	12_31254	110.6
12_30524	113.5	SCRI_RS_194345	91.2	SCRI_RS_204483	110.8
SCRI_RS_198525	114.0	11_10595	91.7	SCRI_RS_128439	114.5
12_31361	114.0	SCRI_RS_152675	93.0	12_31374	116.1
SCRI_RS_126419	114.9	SCRI_RS_188103	93.1	SCRI_RS_233901	116.9
SCRI_RS_161728	115.1	SCRI_RS_124224	94.0	11_20354	118.3
SCRI_RS_179388	115.2	11_10734	94.6	11_11243	118.5
11_11507	115.3	SCRI_RS_151574	94.8	11_21229	119.6
12_30377	118.0	12_31353	94.8	12_20832	119.8
12_31041	118.1	11_20531	94.9	11_10182	120.0
SCRI_RS_148876	118.1	11_10015	95.0	SCRI_RS_1383	120.2
SCRI_RS_237805	118.4	11_20342	95.6	SCRI_RS_146324	120.4
SCRI_RS_51000	118.6	SCRI_RS_234362	99.2	11_21104	120.8
11_20347	118.8	SCRI_RS_8034	100.4	SCRI_RS_154003	121.8
12_30169	119.0	11_20036	100.9	SCRI_RS_235853	122.3
SCRI_RS_167563	119.1	11_20467	101.0	SCRI_RS_151387	123.7
SCRI_RS_24549	119.4	SCRI_RS_128218	101.3	11_21160	123.9
11_21422	119.6	SCRI_RS_189619	102.1	11_10797	124.1
SCRI_RS_150691	119.7	12_30734	103.8	SCRI_RS_193330	124.2
SCRI_RS_170159	120.1	11_20355	104.8	SCRI_RS_162972	124.6
12_31286	120.2	SCRI_RS_151263	105.2	SCRI_RS_220680	125.1
11_21325	120.4	SCRI_RS_147455	105.3	SCRI_RS_198785	125.3
11_11375	120.7	SCRI_RS_162836	105.6	SCRI_RS_200937	125.5
12_30067	121.5	12_31495	105.7	SCRI_RS_155078	125.7
SCRI_RS_138146	121.7	SCRI_RS_131119	108.3	12_31241	126.1
SCRI_RS_168141	122.4	11_21467	108.6	SCRI_RS_178933	126.3
SCRI_RS_122458	122.4	12_21477	111.0	11_10885	126.6
11_10146	122.4	SCRI_RS_161343	113.2	SCRI_RS_134057	126.7
12_30129	122.5	11_11187	115.0	11_10454	127.2
11_10705	122.5	SCRI_RS_203457	115.9	SCRI_RS_16316	128.3
12_30861	122.6	SCRI_RS_171887	116.0	11_10550	128.5
SCRI_RS_133602	125.5	11_10748	116.0	SCRI_RS_141470	128.7
11_10783	125.6	SCRI_RS_205578	116.2	12_31325	129.4

Chromosome 5H		Chromosome 6H		Chromosome 7H	
Marker	**Pos**	**Marker**	**Pos**	**Marker**	**Pos**
11_11080	125.8	SCRI_RS_206827	116.8	SCRI_RS_197886	129.5
SCRI_RS_186901	125.8	SCRI_RS_159529	117.5	SCRI_RS_206322	130.0
11_10095	126.1	SCRI_RS_235672	118.8	12_20640	130.8
11_10521	128.1	12_31283	119.0	12_20241	131.0
SCRI_RS_166857	128.1	11_10390	119.1	11_11012	131.3
SCRI_RS_228463	128.2	11_21112	119.3	11_10130	131.7
11_11532	129.4	SCRI_RS_152414	122.2	11_11275	132.2
SCRI_RS_140356	129.7	SCRI_RS_10811	122.9	SCRI_RS_86194	132.7
SCRI_RS_78626	129.7	SCRI_RS_223104	126.5	SCRI_RS_184902	133.9
SCRI_RS_157238	129.8	12_30956	126.6	11_20962	134.0
SCRI_RS_188141	130.6	SCRI_RS_144034	126.6	11_20117	134.4
11_10819	130.9			11_20586	137.0
11_10658	131.2			SCRI_RS_14119	137.3
SCRI_RS_171042	131.6			11_10547	138.2
12_31217	131.7			SCRI_RS_157208	140.4
SCRI_RS_4658	133.5			SCRI_RS_168479	140.6
SCRI_RS_123457	134.7			12_30166	140.6
SCRI_RS_223157	135.0			SCRI_RS_167617	140.7
SCRI_RS_235550	135.1			11_10174	140.9
11_21077	135.4			SCRI_RS_123211	140.9
11_20568	136.1				
SCRI_RS_6203	136.1				
SCRI_RS_157026	136.3				
SCRI_RS_225632	136.4				
12_31165	137.1				
11_20388	137.2				
SCRI_RS_125258	138.4				
11_10363	138.5				
SCRI_RS_196061	138.5				
SCRI_RS_172379	138.7				
SCRI_RS_193063	138.8				
12_10904	139.0				
SCRI_RS_152365	139.4				
12_30795	139.8				
11_10217	139.9				
12_31170	140.0				
11_11490	140.1				
SCRI_RS_143179	140.1				
SCRI_RS_222839	141.0				
SCRI_RS_3429	142.0				
SCRI_RS_138735	142.7				
SCRI_RS_180920	143.0				
SCRI_RS_14455	144.0				
SCRI_RS_153575	144.1				
SCRI_RS_155688	144.3				
11_10582	144.7				
11_20078	144.8				
11_21024	144.9				

Chromosome 5H		Chromosome 6H		Chromosome 7H	
Marker	**Pos**	**Marker**	**Pos**	**Marker**	**Pos**
SCRI_RS_19741	145.7				
11_20560	146.1				
11_20646	149.1				
11_10336	149.1				
SCRI_RS_130982	149.4				
11_10901	149.4				
12_30162	149.6				
SCRI_RS_192640	149.7				
SCRI_RS_155322	150.1				
11_20334	150.3				
11_20829	151.1				
SCRI_RS_159482	151.4				
12_30003	151.9				
SCRI_RS_145388	152.0				
11_10251	152.4				
11_20686	152.5				
SCRI_RS_224671	152.6				
11_10869	154.5				
11_20644	155.0				
SCRI_RS_184029	155.3				
SCRI_RS_13318	155.4				
SCRI_RS_152162	155.5				
11_10778	155.6				
11_10600	155.6				
11_20546	155.8				
SCRI_RS_230046	156.6				
11_20536	157.4				
12_11450	157.7				
12_31239	157.9				
SCRI_RS_203575	158.1				
12_20816	158.9				
11_10254	159.5				
SCRI_RS_141634	159.7				
12_30656	159.8				
SCRI_RS_160831	160.5				
SCRI_RS_240083	160.7				
SCRI_RS_13882	161.1				
11_10405	161.1				
SCRI_RS_179582	161.1				
11_10736	161.4				
SCRI_RS_13914	161.4				
12_30494	161.5				
11_10236	161.7				
12_11018	161.8				
12_30769	162.5				
SCRI_RS_145394	163.6				
12_30504	164.7				
11_21155	165.8				

Chromosome 5H		Chromosome 6H		Chromosome 7H	
Marker	**Pos**	**Marker**	**Pos**	**Marker**	**Pos**
SCRI_RS_178615	166.0				
11_21052	166.7				
12_31292	166.8				
SCRI_RS_237948	167.6				
SCRI_RS_174123	167.7				
SCRI_RS_136415	167.9				
11_10401	168.5				
12_30360	168.8				
SCRI_RS_1928	168.9				
11_20132	169.4				

[1] Position in cM.

Appendix G

Cross Referencing of Currently Identified β-glucan and β-glucanase QTLs in the Association Mapping Population

Table G1. A description of the process to determine the location of marker-trait associations observed for five different traits (green and kilned malt β-glucanase and grain, malt, and wort β-glucan) in the current study on other barley genetic and physical maps, the presence of underlying genes and QTL previously identified for these traits.

Marker	Trait(s)[1]	Chr[2]	Position in IBSC_2012 Map (cM)	Position in Physical Map (Mb)	Nearby MLOC	Year(s)	Prior QTL	Comments
12_30968	Grain BG, Green BGase	1H	0.0	0.5	MLOC_65169	Both	-	BARLEX to place marker within morex_contig_49842; Ensembl Plants to identify candidate genes
11_10895	Grain BG	1H	0.2	0.6	MLOC_57430	Both	-	Barleymap to place marker; Ensembl Plants to identify candidate genes
SCRI_RS_184274	Green/Kilned BGase	1H	9.9	6.6	MLOC_54234	Both	-	Morex x Barke map (Triticeae Toolbox)derived from Close *et al.* (2009) to place SCRI_RS_224686 (associated with morex_contig_42904, determined using BARLEX) at same position as marker; Barleymap to place marker on IBSC_2012 map; Ensemble Plants to identify candidate genes using morex_contig_42904 position
SCRI_RS_146936	Grain BG, Green BGase	2H	4.3	7.5	MLOC_40063	Both	QBgn.StMo-2H.1 (Han *et al.* 1995; Szűcs *et al.* 2009)	BARLEX to place marker within morex_contig_2556038; Ensembl Plants to identify candidate genes
SCRI_RS_202800	Green/Kilned BGase	5H	164.7	546.0	MLOC_59786	2011	QBgnm.HaTR-5H.2 (Mather *et al.* 2003; Szűcs *et al.* 2009)	BARLEX to place marker within morex_contig_2548020; Ensembl Plants to identify candidate genes

Marker	Trait(s)[1]	Chr[2]	Position in IBSC_2012 Map (cM)	Position in Physical Map (Mb)	Nearby MLOC	Year(s)	Prior QTL	Comments
12_10704	Green/Kilned BGase	6H	100.9	518.2	MLOC_48791	Both	-	BARLEX to place marker within morex_contig_1593246; Ensembl Plants to identify candidate genes
11_20897	Kilned BGase	U (5H)	-	541.7	MLOC_12759	2011	QBgnm.HaTR-5H.2 (Mather *et al.* 2003; Szűcs *et al.* 2009)	Mapped to 177.9 cM on chromosome 5H by Muñoz-Amatriaín *et al.* (2011); BARLEX to assign to morex_contig_127315; Ensembl Plants to identify candidate genes
SCRI_RS_11640	Green BGase	U (6HS)	-	0.4	MLOC_42874	2013	-	Mapped using the physical map generated with BLAST and the Ensembl Plants genome assembly (082214v1) via the Triticeae Toolbox: Barley

[1] BG is β-glucan; BGase is β-glucanase.
[2] Chromosome.