IDENTIFYING SIX-ROW BARLEY GENOTYPES FOR SPECIFIC BREWER NEEDS USING A DNA FINGERPRINT

A Thesis
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

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In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Department:
Plant Sciences

May 2016

Fargo, North Dakota

North Dakota State University
Graduate School

Title
Identifying six-row barley genotypes for specific brewer needs using a DNA fingerprint

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The Supervisory Committee certifies that this *disquisition* complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Six-rowed barley (*Hordeum vulgare* L.) malt is an important raw material of beer. Each barley cultivar has its own unique malt profile for specific traits. The two biggest brewers in the United States, Anheuser-Busch InBev (ABI) and MillerCoors Brewing Company (MillerCoors), have different malt profiles for their ideal malt. MillerCoors wants moderate levels of protein and enzymatic activity while ABI wants higher levels of protein and enzymatic activity. Two cultivars that have the ideal malt profile for each company are Robust for MillerCoors and Stander for ABI. The pedigree of these two cultivars is very narrow; thus, understanding the genetic basis for the differences observed between Robust and Stander may help us in developing new cultivars that meet specific brewers' needs. The objectives of this investigation is to use the Robust x Stander doubled-haploid population to develop a genetic haplotype that helps distinguish six-rowed barley lines for ABI and MillerCoors.

ACKNOWLEDGEMENTS

This work would have not been possible without the support of several people I would like to acknowledge here.

My advisor Dr Richard Horsley for his insightful comments and suggestions and for always believing in me.

Our barley project technicians, Martin Hochhalter and Jason Faller, for the help in seed preparations and all planting related activities.

The laboratory technicians John Barr, James Gillespie, and Theja Wijetunga who helped immensely in all malting quality evaluations and analyses.

Dr Jawahar Jyoti for statistical analyses guidance.

My fellow graduate students for general understanding of thesis formatting requirements and data analyses.

Undergraduate students working in the barley breeding program for their technical support.

DEDICATION

This thesis is dedicated to my parents whom always believed in me and always offered unconditional love and support. I would also like to dedicate this work to my fiancée for always offering continuous support and help in this time as a graduate student.

PREFACE

This thesis includes the totality of my work as a Master's student from 20012 to 2016 at North Dakota State University. During this research I was able to begin to understand the genetic diversity and relationship contained in modern day barley cultivars and attempt to construct a genetic blueprint that would help distinguish cultivars used by the two largest brewing companies in the United States.

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INTRODUCTION

Barley (*Hordeum vulgare* L.) malt used for brewing is often a blend of several malting barley cultivars present in proportions specified by the brewer. Malt made from each cultivar has a unique profile of enzymes, proteins, and carbohydrates that are desired by the maltster and brewer. To ensure new cultivars meet the requirements of the end user, breeders are provided the ideal ranges for each of the traits that must be met before the cultivar will be recognized as a malting barley cultivar. The two biggest brewers in the United States, Anheuser-Busch InBev (ABI) and MillerCoors Brewing Company (MillerCoors), have different specifications for the malt they use. MillerCoors prefers cultivars with moderate levels of protein modification and enzymatic activity while ABI prefers cultivars with higher levels of protein modification and enzymatic activity.

Cultivars that represent the ideal malt profile for ABI and MillerCoors are Stander (Rasmusson et al., 1993) and Robust (Rasmusson and Wilcoxson, 1983), respectively. Both cultivars were developed at the University of Minnesota. Stander has the pedigree Robust*2/3/'Cree'/'Bonanza'//'Manker'/4/Robust/'Bumper' and 'Robust has the pedigree 'Morex'/Manker (Rasmusson and Wilcoxson, 1983). The cultivar Robust or a sib thereof appears in the pedigree of Stander at least four times. The kinship of the cultivars is very similar; yet, the two differ phenotypically for agronomic performance and malt quality. An F_1-derived doubled-haploid population consisting of 193 lines from the cross Robust x Stander was developed and evaluated for agronomic performance in field trials in North Dakota. Grain from these trials was malted and phenotyped for many malt quality traits. The close relatedness but large phenotypic differences between Robust and Stander should allow us to more precisely

identify chromosome regions with the QTL responsible for the differences between the two cultivars.

LITERATURE REVIEW

Barley

Barley is in the genus *Hordeum*, which consists of 32 species and 45 taxa including diploid (2n = 2x = 14), tetraploid (2n = 4x = 28), and hexaploid (2n = 6x = 42) cytotypes (Bothmer et al., 2003). All species in the genus *Hordeum* have three one-flowered spikelets at a rachis node. The two lateral spikelets are sterile in two-rowed types and fertile in six-rowed types. According to Bothmer et al. (2003), there is biological diversity among the species in the genus *Hordeum* in terms of growth habit (annual or perennials) and mode of reproduction (inbreeding or self-incompatible). Based on the genepool concept, cultivated barley and *Hordeum spontaneum* are in the primary genepool while *Hordeum bulbosum* is in the secondary genepool. All the other species in the genus *Hordeum* are in the tertiary genepool (Bothmer et al., 2003).

Barley has been an important crop since the beginning of agriculture development. It is believed to have played an important role in human transition from hunting and gathering to an agrarian lifestyle. Its genus name, *Hordeum*, comes from the word by which Roman gladiators were known, "hordearii," or "barley men" due to the crop's nutritional value that provided these warriors the strength and endurance they needed (Ullrich, S.E., 2011). To this day, barley is used as animal and human feed, and for malt production.

Domestication and U.S. Commercialization

The first theory of the origin of barley was that the two-rowed type was directly derived from *Hordeum vulgare ssp. spontaneum* in the Fertile Crescent in southwest Asia (Bothmer et al., 2003). However, this theory did not explain the occurrence of cultivated six-rowed barley types. With the discovery of six-rowed types with brittle rachis in western China in the 1930's, it

was firmly believed that the six-rowed phenotype evolved from *Hordeum acriocrithon*. Some phylogenetic studies using markers closely linked to *btr1/btr2* genes (genes controlling rachis brittleness) determined that cultivated barley consists of two geographic types, western and eastern (Pourkheirandish and Komatsuda, 2007; and references cited therein), supporting the two independent domestication hypotheses of barley proposed by Takahashi in 1955 (Pourkheirandish and Komatsuda, 2007). According to the review by Burger et al. (2008), consensus about domestication of barley has not been obtained. Archaeological remains of two-rowed barley from Ali Kosh were dated back to 9000 BP with sporadic six-rowed elements among the two-rowed materials, supporting the hypothesis that six-rowed types were derived from two-rowed barley (Pourkheirandish and Komatsuda, 2007). The other evidence that supports the hypothesis that six-rowed barley was derived from two-rowed barley is the existence of more than 90 mutant lines from two-rowed barley for one of the genes controlling row type (*vrs1*) in chromosome 2H. In addition to the two common theories, there are some discussions about domestication of barley in some other countries like Ethiopia (Orabi et al., 2007 and references therein), Morocco (Molina-Cano et al., 1999 and references cited therein), and Tibet (reference cited in Molina-Cano et al., 1999). However, the most favored theory among the barley scientists at present is a single evolutionary line from *Hordeum vulgare* ssp. *spontaneum* to cultivated two-rowed barley, which was then changed to a six-rowed spike in cultivated barley by mutation during the domestication process (Bothmer et al., 2003; Pourkheirandish and Komatsuda, 2007).

The domestication process of barley was associated with gradual accumulation of traits that facilitated agricultural production (Bothmer et al., 2003; Pourkheirandish and Komatsuda, 2007). According to Pourkheirandish and Komatsuda (2007) and references cited therein, there

were three key traits important in the domestication of barley. The first trait was a non-brittle rachis, which resulted in efficient harvest without loss of grains in the cultivated barley. However, the traits of brittle rachis along with rough awn were crucial for natural dispersal in the wild types. The most important non-brittle rachis genes for barley domestication are *btr1* and *btr2*, which are mapped to chromosome 3H, but remain to be cloned (Bothmer et al., 2003; Pourkheirandish and Komatsuda, 2007). The homozygous recessive of these genes independently make barley non-brittle, indicating that the mutation is a loss of function. *Btr1Btr2* (double dominant genotypes) strongly constrict the rachis node, whereas one recessive allele *btr1Btr2* or *Btr1btr2* does not result in constriction of the rachis node and avoids brittleness (Pourkheirandish and Komatsuda, 2007).

The second important trait in the domestication process of barley was the selection for six-rowed barley (Pourkheirandish and Komatsuda, 2007), which resulted in three times the number of seeds compared to two-rowed barley and possibly increased yield (Bothmer et al., 2003; Pourkheirandish and Komatsuda, 2007). According to Pourkheirandish and Komatsuda (2007), there are at least five independent loci controlling the six-rowed spike phenotype in barley (*vrs3, vrs1, vrs4, vrs5 or int-c,* and *vrs2*) mapped in chromosomes 1H to 5H, respectively. Of these loci, *vrs1* located in the long arm of chromosome 2H in the homozygous recessive form was found in all six-row genotypes while the dominant form was found in wild and two-rowed barley genotypes. The mutations at the *vrs1, vrs3, and vrs4* loci were only detected in induced mutant lines. The last locus (*vrs5 or int-c*), which was observed in genotypes, modifies the degree of fertility in lateral spikelets and produces an intermediate spike type (Bothmer et al., 2003; Pourkheirandish and Komatsuda, 2007). Hence, the two loci (*vsr1* and vrs5 or int-c) seem important in the conversion from two-rowed to six-rowed phenotypes during the domestication

process of barley. A true two-rowed spike has the genotype *Vrs1Vrs1int-cint-c*. A true six-rowed spike has the genotype *vrs1vrs1Int-cInt-c*.

The third trait of domestication importance in barley was the naked or hulless caryopsis (Bothmer et al., 2003; Pourkheirandish and Komatsuda, 2007), which is controlled by a single recessive gene (*nud*) located in the long arm of chromosome 7H (Pourkheirandish and Komatsuda, 2007). Remains of hulless kernels have been found in Ali Kosh about 8000 BP and the change to a non-brittle rachis preceded it. In addition to the above traits, reduced dormancy is important in cultivation of barley and required in cultivars (Bothmer et al., 2003). Pourkheirandish and Komatsuda (2007) reported that seed dormancy is a quantitative trait that is affected by several alleles at multiple loci, with two of the more common QTL being *SD1* and *SD2*, both of them located in chromosome 5H.

Important Traits Considered by the Barley Breeder

There are multiple traits involved in determining the utility of barley cultivars for production by growers and use for malting and brewing. These traits include those associated with agronomic performance, disease resistance, and barley and malt quality. Important agronomic traits include heading date; resistances to lodging, stem breakage, and disease resistance; and plant height. For barley quality purposes, important traits that are measured on clean grain include kernel plumpness, 1000-kernel weight, test weight, protein, and moisture content. Malt quality traits include malt extract, wort protein, wort color, Kolbach Index, α-amylase activity, diastatic power (DP), wort ß-glucan concentration, and free amino nitrogen (FAN) concentration. For a cultivar to maintain its competitiveness with other crops and to be thought of favorably by producers, it must be financially viable compared to other crops they produce and have the barley and malt quality desired by end users.

Agronomic Traits

Agronomic traits that are priorities during the evaluation of new breeding lines for release include heading date, plant height, lodging, and stem breakage.

Heading date, or spike emergence, is important for cereal cultivars to adapt to their respective environments and in maximizing yield potential (Bezant *et al.,* 1996). Barley grown in the northern Great Plains must not be too early as that could impact yield potential. Additionally, if the heading date is too late, this could result in grain fill during the hotter and dryer periods of summer or delay harvest of the crop to a time when the farmer wants to be harvesting another crop, such as canola (*Brassica napus* L.) or wheat (*Triticum aestivum* L.).

Lodging is when the plant is no longer in an upright position during the growing season after it is exposed to climate changes in its environment (Pinthus, 1973). Factors that impact lodging include plant height, straw strength, and soil fertility. Lodged plants often have decreased translocation of carbohydrates to the developing seed, which can result in low-weight seed with higher protein contents. Lodging close to maturity may not impact seed weight, but it does interfere with ease of harvest. If stems break when the crop has reached harvest maturity but harvest is delayed, yield losses may occur due to spikes that that either break and fall to the ground or are unable to be collected by the combine for threshing.

Deoxynivalenol (DON) Accumulation in Barley

Deoxynivalenol is a mycotoxin produced by the pathogen *Fusarium graminearum* Schwabe [telomorph *Gibberella zea* (Schwein)] that causes Fusarium head blight (FHB). High levels of FHB can negatively impact grain yield and/or grain quality. The products of the fungus can harm animals and humans alike (Paulitz, and Steffenson. 2011). Humans who ingest DON can experience acute toxicosis symptoms and animals such as swine may have vomiting and

hyperestrogenism. Since these mycotoxins are perceived as problematic by end users and consumers, assays to determine the level of mycotoxin present are now standard tests in barley at the point of purchase.

Barley Quality

Barley quality traits include moisture content, test weight, thousand-kernel weight (TKW), kernel plumpness, great protein, and barley color. Deficiencies in one or more of these traits may be indicative of grain that may not be suitable for malting. End use quality problems can arise from different causes, such as poor seed handling practices, unfavorable environmental conditions, disease, and improper grain storage (Schwarz and Li, 2011). Poor seed quality can result in low germination, which directly affects malt extract, flavor, and reduces processing performance and ease. The American Malting Barley Association (AMBA) has produced guidelines that detail the ideal values for six-rowed and two-rowed barley and malt used for brewing adjunct and all-malt beers (AMBA, 2014).

Moisture content, an important criterion for determining the safety of storing barley, is the percent of water found in the kernels in barley dry matter (Burger and La Berge, 1985). Moisture content helps calculate other quality factors on a dry basis (db) and ensures safe storage.

The test weight, or hectoliter weight, of barley is dependent on factors like cultivar, environmental conditions during production, sample cleanliness, presence of awns after threshing, and grain drying (Schwarz and Li, 2011). Test weight is a density measure expressed in kg hL^{-1} (most common measurement today) and is the specific amount of barley required to fill a Standard Winchester bushel (Bu) measure of 2150.42 in^3. The commercial weight of

barley is 62 kg hL^{-1}. The buyer may discount the purchase price if the test weight is below 59.3 kg hL-1.

Thousand-kernel weight is literally the weight of exactly 1000 kernels after the removal of broken grains and foreign material (Schwarz and Li, 2011). Values for thousand-kernel weight are expressed to the nearest one-gram for 1000 kernels. Also, TKW should be conducted on a moisture free basis because higher moisture will increase the TKW. Thousand-kernel weight is highly correlated with seed plumpness. Higher TKW is desired because the heavier grain generally has higher starch content. In North Dakota, six-rowed barley TKW generally ranges from 30 to 36 g with an average of about 34 g.

Kernel or seed plumpness is determined by mechanically sorting 100 g of seed using different sized sieves with rectangular openings. Sieve sizes include 19.0 x 2.8-mm, 19.0 x 2.4-mm, and 19.0 x 2.0-mm rectangular openings (Schwarz and Li, 2011). Plump seeds are those that remain on top of the 2.8-mm and 2.4-mm sieves while those that pass through both sieves are considered thin. A desirable ratio of plumps to thin kernels is 9:1. Grain with less than 85% plump kernels may be discounted by the buyer or refused for use for malting.

Determing grain protein concentration is an easy and quick method of determing the sutablitity of a sample for malting . Methods used to determine grain protein concentration include determination of nitrogen by combustion analysis or the determination of protein by near-infrared (NIR) spectroscopy (Schwarz and Li, 2011). Important factors impacting protein content include soil type, crop rotation, fertilizer application, and yield potential (Garstang and Spink, 2011). Higher grain protein levels will yield lower levels of fermentable extract (DeClerck, 1958; Schwarz and Li, 2011). In the US the ideal six-rowed malting barley protein content is ≤13.0% (AMBA, 2014).

Acceptable kernel color or brightness is generally light yellow-straw color with a bright appearance (Schwarz and Li, 2011). Grain buyers commonly use visual appearance as a way to select against barley that may be contaminated with disease or weathered (dark kernels), or harvested prematurely (green kernels). Barley color is a measure of brightness and is usually expressed as an L-value of the tristimulus color scale (Shellhammer, 2009; Schwarz and Li, 2011).

Malt Quality

Genetic and environmental factors can influence barley malt quality. Characteristics viewed as desirable include plump and uniform kernel size, moderate levels of protein, and high enzymatic activity. Plump kernels are related to higher malt extract and the uniform seed size ensures more consistent water update and germination during malting. High enzymatic activity may result in better carbohydrate degradation during malting and brewing. Moderate levels of soluble protein are needed for yeast nutrition during fermentation and foam stability in the final beer, but a high level of protein results in lower malt extract, which is a major economic factor in breweries.

Modification is the term utilized to describe the physical and biochemical changes that occur in the endosperm during the malting process (Lewis and Young, 1995). During the whole malting procedure, maltsters and brewers monitor the level of modification occurring because the more cell wall and protein that is degraded the easier it will be for the enzymes to access the starch granules that lay beneath. The degree of modification determines what mashing schedule is the most appropriate to obtain the most extract (Ullrich, S.E., 2011). Well-modified malt may require only a single temperature rest for saccharification.

Friability is a direct measure of malt modification (Schwarz and Li, 2011). The friability method is based on the observation that the endosperm of well-modified malt should be easily crushed while the endosperm of poorly modified malt is hard and steely. The instrument used for this procedure is called a Friabilimeter (Pfeuffer, Germany) and the value for friability is determined as 100 percent minus the percent of barley endosperm that is not crushed by a rubber roller set against rotating screen. Modified endosperm will be crushed and pass through the rotating screen into a removable pan.

Growth count is the measure of the acrospires of 100 selected kernels during germination relative to total kernel length (Schwarz and Li, 2011). Maltsters view this trait as a simple test for modification and uniformity of germination. Lengths are classified from 0-0.25, 0.25-0.5, 0.5-0.75, and to over 1/1 (overgrown). The desired average length of the acrospires is traditionally 0.75 although this might be a low estimate for some brewers across the United States (Kunze, 1999; Schwarz and Li, 2011).

Another measure of modification is the fine-coarse extract difference. This is the difference in malt extract of malt milled at two different gap settings on a malt mill. The malt milled with a wider gap is referred to as the coarse-extract and that with the closer gap settings is the fine-grind extract. The measure of modification predicts how malt will perform related to extract yield and rate of wort separation (Bamforth, and Barclay, 1993). The maximum fine-coarse difference currently desired by the AMBA is 1.2% (AMBA, 2014).

Measures of malt modification done on the wort include wort viscosity, wort ß-glucan concentration, and the ratio of wort protein to malt protein. This ratio is referred to as the S/T value or the Kolbach Index. Wort viscosity is measured in centipoises (cP) and values $\leq$ 1.50 cP

are desired (AMBA, 2014). Values > 1.50 cP may indicate potential problems with longer than desired lautering times during brewing (Burger and LaBerge, 1985).

Measurement of wort ß-glucan determines the total amount of ß-glucan in the wort, but provides no information on the different sizes of molecules that may be present. Depending on their molecular weight or size and conformation, ß-glucans have been found to have an impact on the wort and beer viscosity, lautering time, and membrane plugging for brewers using mash filters instead of a lautering tun (Schwarz and Li, 2011). The AMBA desires wort ß-glucan levels <120 mg L^{-1} (AMBA, 2014).

The Kolbach index is a direct measurement of protein modification (Schwarz and Li, 2011) and is expressed as a percentage (S/T x 100). The desired range in S/T by the AMBA is between 42%-47% (AMBA, 2014). Malt with values < 42% is considered under-modified and malt with values > 47% is considered over-modified. Under-modified malt may have lower than desired malt extract values while over-modified malt may have higher than desired malt loss and wort color.

Components of protein modification are wort protein, wort color, and FAN. Wort protein is determined using a spectrophotometer at a wavelength of 430 nm and desired values are 5.20-5.70% (AMBA, 2014). Values > 5.70% may result in higher than desired wort color. Wort color is measured with a spectrophotometer at a 430 nm wavelength in °SRM, which is very similar to the °Lovibond scale. The AMBA desired wort color for six-rowed barley ranges from 1.8-2.5 °SRM (AMBA, 2014). The method to determine FAN is extremely sensitive due to the fact that both peptide and amino acids contain only a single free amino group. The amount of FAN desired in wort made from six-rowed malt is 210 mg L^{-1} (AMBA, 20014.

Extract is expressed as a percentage of malt on a dry basis and as mentioned before is one of the most economically valued parameters in malt. The method to determine extract, which uses a Congress Mash, dates back to the beginning of the nineteenth century (Schwarz and Li, 2011). The content of the Congress Mash is essentially composed of carbohydrates that can be broken down into disaccharide maltose and branched dextrins (Burger and La Berge, 1985). Malt extract > 79% is desired for six-rowed malt.

Collectively, the enzymes α-amylase, ß-amylase, limit dextrinase, and α-glucosidase are responsible for hydrolyzing starch to fermentable sugars. Diastatic power is a measure of the capacity of the malt to convert starch into fermentable sugars (Schwarz and Li, 2011). Some scientists consider DP to be a measure of β-amylase since this enzyme has a much greater activity than any other starch-degrading enzyme in this assay. Diastatic power is reported in °ASBC in accordance with the American Society of Brewing Chemists (ASBC) and the current desired value is > 150 °ASBC (AMBA, 2014).

The measurement of α-amylase activity determines the dextrinizing capacity of malt and is reported in dextrinizing units (DU). Like ß-amylase, this enzyme also degrades starch and larger dextrins, which helps to reduce wort viscosity and provide more substrate for ß-amylase (Schwarz and Li, 2011). The AMBA desires values for α-amylase > 50 DU (AMBA, 2014).

The starch debranching enzyme in malt, commonly referred to as limit dextrinase, is responsible for degrading or hydrolyzing the alpha 1,6 glucosidic bonds in amylopectin and branched dextrins (McCafferty, 2004). Limit dextrinase has become an important enzyme to study because of its capacity to degrade non-fermentable dextrins to fermentable sugars; however, the AMBA has not specified any desired value or range of values for this enzyme.

ß-amylase is a heat sensitive exoenzyme that releases maltose by hydrolyzing bonds from the non-reducing ends of the dextrins generated by α-amylase or the α-(1-6) side chains of amylopectin (Bamforth, and Barclay, 1993). As stated earlier, ß-amylase is the main enzyme measured in the DP assay since the of number ß-amylase molecules is larger than other starch degrading enzymes. Additionally, the importance of ß-amylase is supported by the fact that maltose is the primary sugar present in wort. The AMBA has not specified a desired value or range for ß-amylase.

α-glucosidase is yet another important enzyme responsible for producing fermentable sugars during brewing. This enzyme is responsible for hydrolyzing maltose and other small maltodextrins to glucose (Fincher, 2011). All of the aforementioned enzymes work together to degrade starch. The AMBA has no desired value or range specified for α-glucosidase.

The carbohydrates that come from the degradation of starch compose approximately 92% of the solids found in wort. The most important sugars and dextrins in solution are those comprised of glucose units. Of these sugars, maltose is the most abundant at 50-60% of the total, followed by maltotriose. The fermentable sugars are the energy source for the yeast's major metabolic products, which are alcohol and carbon dioxide (Briggs et al., 2004). The methods utilized to measure fermentable sugars rely on high performance liquid chromatography (HPLC).

Molecular Marker Research on Malt Quality

Molecular studies throughout the years to determine the genetic basis of economically important traits in barley quality have considered malt quality traits as a high priority. However, due to limited funding and the complexity of malt quality, there is still a lack of genetic information needed to develop an effective marker-assisted selection (MAS) strategy. In previous studies using microarrays and expressed sequence tags (ESTs), the numbers of genes

estimated to control six malt quality traits ranged from 11-102 (Lapitan et al. 2009). However, the function of many of these genes remains unknown. Emebiri et al. (2009) reported on an MAS strategy for pyramiding QTL controlling malt quality, kernel plumpness, and disease resistance.

In a QTL analysis conducted by Marquez-Cedillo et al. (2009) using three mapping populations ('Harrington' x Morex, Harrington x TR306, and 'Steptoe' x Morex), they found phases of desired alleles for several traits. In the Harrington x Morex population the aforementioned authors discovered malt quality QTL associated with the *Amy2* locus in chromosome 7H and *hordein* loci in chromosome 1H.

More recent molecular studies include one using six-rowed malting barley lines from the University of Minnesota (UM) that were genotyped with 1,524 single nucleotide polymorphisms (SNPs) (Munoz-Amatriain et al., 2010). Using micro-malting and the Barley1 GeneCHip SNP array, the authors associated the genotype and phenotype data. They identified 49 genes that were associated with different malt quality traits. In 2011, Zhou et al. constructed a 550 marker high-density map using 95 doubled-haploid (DH) lines from the cross 'Mikamo Golden' x Harrington. They identified QTL in chromosomes 2H and 5H associated with malt quality. The QTL in chromosome 2H was associated with malt extract and the QTLs in chromosome 5H were associated with malt extract, soluble nitrogen, and Kolbach index. Using the information from the study, they created cleaved amplified polymorphic sequence (CAPS) markers that could be utilized for MAS of malt extract (Zhou et al., 2011).

Previous NDSU Research

As part of their PhD research, Pedraza-Garcia (2011) and Lewis (2012) used the Robust x Stander DH population for mapping agronomic and malt quality traits. Pedraza-Garcia (2011)

used 73 doubled-haploid lines to identify QTL for FAN in chromosomes 5H and 6H, wort color in chromosomes 2H and 5H, wort protein and S/T in chromosome 6H, and fermentable sugars in chromosomes 4H, 5H, and 6H. Interestingly, in chromosomes 5H and 6H, Pedraza-Garcia (2011) found QTL that regulate levels of α-amylase activity, DP, S/T, wort protein, FAN, and wort color, which are the majority of malt quality traits that differentiate the malt quality needs of ABI and MillerCoors.

Lewis (2012) continued the work mapping agronomic and malt quality traits using the Robust x Stander population. New to her research was the addition of 138 lines, which brought the population size to 193 lines. Agronomic data were collected from all locations but only six locations where micro-malted. The revised map was constructed using 102 SNP, SSR, and DArT markers. QTL controlling malt quality and carbohydrates traits were mapped to chromosomes 4H, 5H, and 6H. QTL controlling agronomic traits were mapped to chromosomes 4H and 6H. Missing from the work of Lewis (2012) was phenotyping the full population for malt quality at a sufficient number of environments to draw conclusions for mapping malt quality traits.

MATERIALS AND METHODS

Plant Material and Field Evaluation

The Robust x Stander F_1-dervided DH population used by Lewis (2012) is the same population used for the present research. The population consists of 193 DH lines. Checks used in the experiments included the parents (Robust and Stander), and 'Tradition'. During the 2011-2014 growing seasons, the population and checks were grown in replicated yield trial experiments sown near Fargo, McVille, Ray (Nesson Valley), and Osnabrock, ND. Entries in the yield trial experiments were assigned to experimental units using a simple square lattice design. The experiments at Nesson Valley were irrigated. Experimental units included seven rows spaced 19 cm apart. Plot length was 244 cm and there was 46 cm between plots. The seeding rate for all locations was 247 seeds m^{-2} at non-irrigated sites and 341 seeds m^{-2} at Nesson Valley.

Agronomic data collected from each field experiment included heading date, plant height, and scores for lodging and stem breakage. Heading date was recorded as the number of days after 31 May when at least 50% of the spikes were emerged from at least 50% of the plants in an experimental unit (each individual plot). Plant height was measured in cm from the ground up, excluding the awns. Scores for lodging resistance and stem breakage were assigned using scales. A 1-10 scale (1 = no lodging and 10 = severe lodging) was used for rating lodging severity and a 1-5 scale (1 = no breakage and 5 = severe breakage) was used for rating stem breakage severity at harvest maturity.

At maturity, grain was harvested using a plot combine, dried in a forced-air dryer to approximately 100 g kg^{-1} if needed, threshed, and cleaned. Data collected on the clean included yield (Mg ha^{-1}), test weight (kg hL^{-1}), kernel plumpness (percent of 100 g of kernels remaining on top of 19.0 x 2.4-mm and 19.0 x 2.8-mm rectangular-slotted sieves), and grain protein (g kg^{-}

^{1}). Grain protein, kernel moisture, and kernel color were determined using the Foss Infratec 1241-grain analyzer, which uses near-infrared spectroscopy (NIR).

Malt Quality Evaluation

Grain for malting consisted of entries combined across replicates within an environment and sized using a 19.0 x 2.4-mm sieve. Grains remaining on top of the sieve were used for malting. Eighty grams of grain on a dry basis from the selected yield trial experiments was micro-malted in Dr. Paul Schwarz's barley and malt quality laboratory at NDSU using the methods described by Karababa et al. (1993). This protocol included steeping the grain to 437 g kg^{-1} moisture at 16°C, 4 d of germination at 95% relative humidity and 16°C, and kilning using a ramped schedule. Malted samples were analyzed for malt loss (%), malt moisture (%), fine grind extract (%DB), wort protein (%), S/T (%), α-amylase activity (20^{o} dextrinizing units; DU), DP (^{o}ASBC), wort viscosity (cP), wort color (°L), wort FAN (mg L^{-1}), wort β-glucan content (mg L^{-1}), limit dextrinase activity (Units kg^{-1}), ß-amylase activity (Units g^{-1}), and α-glucosidase (Units mL^{-1}). The concentrations of fructose, glucose, maltose, and maltotriose (g of sugar/100mL of wort) were determined using ASBC method Wort-14B (ASBC, 2004). This method utilizes an HPLC outfitted with a Hi-Plex Na 300 x 7.7-mm column (Agilent, Santa Clara, CA).

For this project, most methods used beginning with Malt- or Wort-followed by a number are described in the American Society of Brewing Chemists (ASBC) Methods of Analysis (ASBC, 2004). Malt loss was measured as a percentage of any barley dry matter lost during the malt process. Moisture was analyzed as a percentage of total weight and was determined with the incorporation of a modified ASBC standard Oven Drying method, Malt-3. Fine grind extract also was measured as a percentage and was determined using ASBC method Malt-4. Soluble protein measurement was determined using ASBC method Malt-5, which consists of determining the

total nitrogen in the laboratory wort calculated back to its dry basis. α-amylase and DP were determined using a modification of ASBC method Malt-6 that is described in Karababa et al. (1993). Wort color was determined with a spectrophotometer at 430 nm following ASBC procedure Wort-9. Wort ß-glucan was determined using flow injection analysis as described in Wort-18 ASBC. α-glucosidase measurement was based on the method of Sissons and MacGregor (1994). Limit dextrinase activity was determined using Megazyme (Ireland) assay of limit-dextrinase in cereal flours (McCleary, 1992). ß-amylase was determined as described in the Megazyme ß-amylase assay kit.

Statistical Analysis

Agronomic Data

Agronomic data from an individual location were analyzed as a simple square lattice using Agrobase Generation II (Agronomix Software; Winnipeg, Manitoba, Canada). For data collected from only one location, *F*-tests were considered significant at $P \leq 0.05$ and mean separation was done using an *F*-protected least significant difference (LSD) at $P=0.05$. When data for a trait were collected from more than one environment, adjusted entry means from a single environment were used for the combined analysis across environments as a randomized complete block design using the PROC GLM and the LSMEANS functions of SAS/STAT (SAS Institute, 2011). In the combined analyses, environments were considered a random effect and entries were considered a fixed effect. Mean separation was done using the PDIFF option of the LSMEANS statement. *F*-tests and mean separation tests were considered significant at $P \leq 0.05$. The denominator of the *F*-test for the environment x entry source of variation was the pooled error mean square (MS) and the denominator of the *F*-test for the entries source of variation was

the environment x entry MS. The PDIFF command in SAS/STAT (SAS Institute, 2011) was used to conduct mean separation at P=0.05.

Malt Quality Data

Malt data were collected from environments that contained an acceptable range of protein in the grain. Due to the high cost and time consuming process of laboratory malting and quality analyses, entries from three environments were malted and evaluated for quality. In the statistical analyses, each location was considered a replicate. The PROC GLM function of SAS/STAT (SAS Institute, 20011) was used to obtain entry means. *F*-tests and mean separation tests were considered significant at $P \leq 0.05$. Because we are bulking entries across replicates from a location, there is no environment x entry term for testing; thus, this source of variation is the error term.

QTL Mapping

The molecular map used for QTL mapping was developed by Correa-Heilman (2013). QTL in the present study were identified using QTL Cartographer V2.5 (Wang et al., 2007). Composite interval mapping (CIM) was conducted using the default parameters for cofactor selection suggested in QTL Cartographer V2.5 (Wang et al., 2007) along with the Forward and Backward Regression method. Threshold values for each environment were determined by a permutation test with 1000 iterations at $P = 0.05$.

RESULTS AND DISCUSSION

Linkage Map

The linkage map of the Robust x Stander DH population utilized in this study was constructed by Heilman-Correa (2013). The population was genotyped using the Barley iSelect SNP chip (http://bioinf.hutton.ac.uk/iselect/app/), which is based on the illumina (San Diego, CA) Infinium genotyping assay. Less than 6% of the 7,842 SNP assays on the array identified polymorphisms. The constructed map contains 88 non-cosegregating SNP markers that were arranged into eight linkage groups (Figure 1). The map includes segments of all chromosomes except 7H. Chromosome 5H is represented by three linkage groups. Compared to the reference map of Close et al. (2009), the Robust and Stander map represents around 18% of the barley genome. The map represents 13% of chromosome 1H, 2% of chromosome 2H, 6% of chromosome 3H, 17% of chromosome 4H, 47% of chromosome 5H and 42% of chromosome 6H. Large gaps in the map are probably a result of fixed loci controlling economically important traits such as malt quality and agronomic performance.

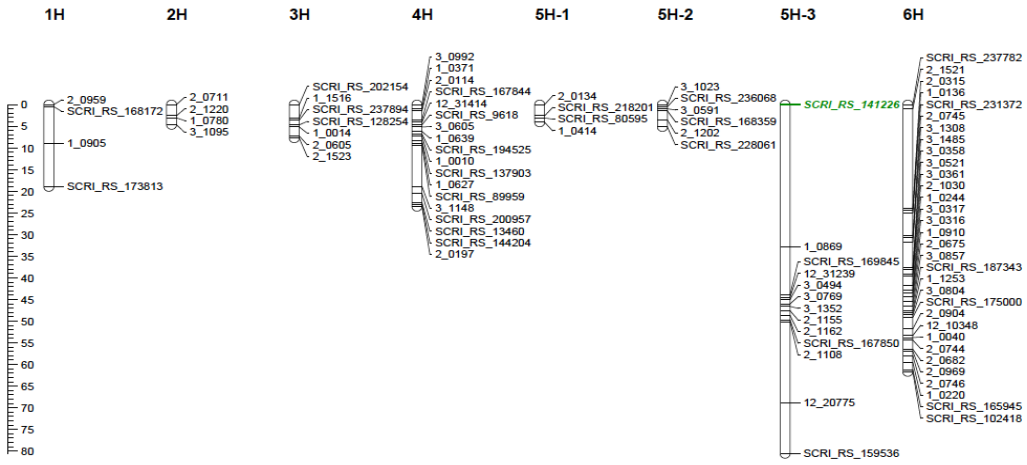

Figure 1. Linkage map for the Stander/Robust population built with 88 SNP non-cosegregating markers from the Barley Infinium iSelect SNP Chip (http://bioinf.hutton.ac.uk/iselect/app/).

Analyses of Agronomic Traits

Analyses of Phenotype

Phenotypic data were collected on days to heading, plant height, lodging, stem breakage, deciduous awns, and yield. In the combined ANOVA across environments, the *F*-tests for environment x entry and entry were significant ($P \leq 0.05$). Table 1 illustrates mean values based on individual and combined analyses of data from up to seven environments in North Dakota from 2011 to 2014.

Significant differences in days to heading between Robust and Stander were observed in three of the seven environments; in addition, there were significances between the progeny in all environments. These results suggest that days to heading in Robust and Stander are controlled by different genes. Combined across environments, mean values for heading date were 31.7 d for Robust, 32.1 d for Stander, and 31.8 d for the DH population. Days to heading and maturity have been well studied in barley and multiple authors have described different QTL controlling this trait. Cuesta-Marcos et al. (2008) described major QTL affecting heading date found near the centromere of chromosome 2H in a 120 DH population derived form a spring by winter cross while Yu et al. (2009) found QTL related to heading date in chromosomes 1H, 2H and 5H. These findings back the previous statement where different genes can be controlling this trait.

Plant height varied greatly across the different lines and environments. Values ranged from 53 to 110 cm in length (Table 1). Across environments, mean plant heights were 88.0 cm for Robust, 75.2 cm for Stander, and 81.8 cm for the progeny. Like days to heading, different genes control plant height. Data for lodging were recorded only at Nesson Valley in 2013. When Stander was released, it represented a big improvement in lodging resistance or standability of

malting barley cultivars available for growers in the upper Midwest US; hence, the name Stander.

Table 1. Mean heading date; plant height; yield; and scores for, lodging, stem breakage, and deciduous awns (DA) of Robust, Stander, and the Stander x Robust doubled haploid progeny based on the individual and combined analyses of data from up to seven environments in North Dakota (2011-2014).

	Env[†]	Robust	Stander	Population			
		----------Mean----------		Mean	LSD[‡]	SD[§]	Range
Heading date (days after 31 May)	11 NV	32.9a[¶]	32.9a	33.4	3.2	1.6	30-35
	11 MC	42.0a	42.7a	42.6	1.7	0.8	40-44
	12 FA	19.5a	20.5b	19.8	2.5	1.3	17-22
	12 OS	32.5a	33.5b	32.2	1.6	0.8	30-37
	13 NV	28.4a	27.9a	28.1	1.8	0.9	26-29
	13 FA	28.5a	28.2a	28.1	1.3	0.7	26-31
	14 OS	38.0a	39.0b	38.6	1.5	0.8	37-40
	Combined	31.7a	32.1a	31.8	1.9	1.0	17-44
Plant height (cm)	11 NV	79.2b	73.0a	75.5	7.1	3.6	65-83
	11 MC	85.7b	76.2a	82.2	5.1	2.6	76-95
	12 FA	74.4b	67.2a	68.7	7.7	3.9	53-77
	12 OS	102.7b	83.3a	90.1	13.7	6.9	70-102
	13 NV	107.3b	82.1a	97.1	10.2	5.2	82-110
	13 FA	74.8b	60.0a	69.9	6.6	3.4	59-77
	14 OS	91.7b	84.4a	88.8	5.2	2.6	81-94
	Combined	88.0b	75.2a	81.8	7.9	4.0	53-110
Lodging (1-9)[††]	13 NV	4.5b	1.0a	1.8	2.8	1.4	1-6
Stem breakage (1-5)[‡‡]	12 OS	4.0b	1.5a	2.4	2.0	1.0	1-5
DA (1-5)[§§]	12 OS	3.6a	3.6a	3.0	1.7	0.9	1-4
	13 NV	3.0b	1.0a	1.9	1.5	0.7	1-3
	Combined	3.2a	2.2a	2.5	1.6	0.8	1-4
Yield (T/ha)	11 NV	3.1b	2.4a	2.6	0.6	0.3	1-3
	11 MC	2.3a	3.0b	3.1	0.7	0.4	2-4
	12 FA	3.1a	3.3a	3.4	0.7	0.3	2-4
	12 OS	4.8b	4.1a	4.3	0.9	0.4	3-4
	13 NV	6.8a	6.7a	7.0	1.7	0.9	5-8
	13 FA	3.7a	3.4a	3.5	0.5	0.3	2-4
	Combined	4.0a	3.8a	4.0	0.8	0.4	1-8

[†]Env = Environments in North Dakota include 11NV=2011 Nesson Valley, 11MC=2011 McVille, 12FA=2012 Fargo, 12OS=2012 Osnabrock, 13NV=2013 Nesson Valley, 13FA=2013 Fargo, and 14OS=2014 Osnabrock.

[‡]LSD=Least significant difference at P=0.05.

[§]=Standard deviation

[¶]Means for parents between columns followed by the same letter are not significantly different ($P \leq 0.05$) as determined by an F-test.

[††]Score of 1=no lodging and 9=severe lodging.

[‡‡]Score of 1=no stem breakage and 5=severe stem breakage.

[§§]Score of 1=no deciduous awns and 5=severe deciduous awns.

Mean lodging across environments was 2.6 for Robust, 1.0 for Stander, and 1.8 for the progeny (Table 1). Lodging can affect yield, grain quality, and even malt quality (Day and Dickson, 1958).

Stem breakage data were collected at one location, 2012 Osnabrock. Stem breakage data are collected just prior to harvest to identify genotypes with strong straw so they can be harvested using straight combining. The NDSU barley breeding program considers a stem breakage score > 3.0 unacceptable. Mean stem breakage scores in my study were 4.0 for Robust, 1.5 for Stander, and 2.4 for the population. The range in stem breakage scores for the progeny ranged from 1-4.

Data on deciduous awns were collected at two locations. Deciduous awn is defined as the breaking off of the lemma awn prior to harvest and is considered an undesirable trait. When the awn breaks off, it often tears off a portion of the lemma, which results in the "skinning" of the kernel. Barley with skinned kernels can be discounted or even rejected when it is sold. An intact husk on the kernel is important for malting because the husk protects the elongating coleoptile. If the kernel is skinned, the coleoptile may be susceptible to damage or even breaking during germination. If the coleoptile breaks off, the kernel is killed and the malting process for the kernel ceases. Little to no research has been done on mapping of deciduous awns possibly since it's not consistently expressed in every environment. Combined across locations, the mean score for deciduous awns was 3.2 for Robust, 2.2 for Stander and 2.5 for the population. A score > 3.0 for deciduous awn is considered unacceptable by the NDSU barley-breeding program.

Yield data were collected from all environments. However, yield per se can be a very hard trait to study because so many traits impact it, including abiotic and biotic stresses. Across

environments, mean yield was 4.0 Mg ha^{-1} for Robust, 3.8 Mg ha^{-1} for Stander, and 4.0 Mg ha^{-1} for the population.

QTL Analyses

The QTL mapping software QTL Cartographer v. 2.5 (Basten et al. 1994, 2000; Wang et al. 2001) was used to analyze data from each environment separately. Significant QTL have been identified in previous studies for all of the aforementioned agronomic traits; however, for a QTL to be a candidate for marker-assisted selection (MAS) it must be detected in most of the environments where the trait is measured. For this study, QTL were considered meaningful if they were detected in > 50% of the environments.

Quantitative trait loci for heading date (Appendix Table A1) were identified in chromosomes 4H, 5H-3 and 6H; however, none met the threshold of being detected in > 50% of the environments. Lewis (2013) detected the same QTL in chromosome 4H in her evaluation of the population.

Two QTL for plant height were identified, but only one met the criteria of being identified in > 50% of the environments (Table 2 and Appendix Table A2). A QTL in chromosome 6H from 44.41 to 47.61 cM was detected in four of the seven environments (Table 2). On average, the QTL explained 12.3% of the phenotypic variation for plant height in the population. Pedraza-Garcia (2011) and Lewis (2012) also identified QTL for plant height in the region, but using a smaller Robust x Stander DH population. Another study conducted using the Oregon Wolfe Barley DH population also identified a QTL for plant height in the same region of chromosome 6H (Cistue et al. 2011).

Table 2. Quantitative trait loci for plant height detected in >50% of environments using composite interval mapping analysis of the Robust x Stander doubled-haploid population.

Location[†]	Chromosome	Position (cM)[‡]	Left Marker	Right Marker	LOD[§]	Additive effect	R^2
12OS	6H	44.41	1_0910	2_0675	2.34	-1.21	0.05
11NV	6H	45.51	1_0910	2_0675	8.12	-1.30	0.16
12FA	6H	46.51	3_0857	SCRI_RS_187343	5.26	-1.08	0.10
13FA	6H	47.61	SCRI_RS_187343	1_1253	11.58	-1.43	0.18

[†]Locations in North Dakota include 11NV=2011 Nesson Valley, 11MC=2011 McVille, 12FA=2012 Fargo, 12OS=2012 Osnabrock, 13NV=2013 Nesson Valley, 13FA=2013 Fargo, and 14OS=2014 Osnabrock.
[‡]Position is that for the peak value in the QTL.
[§]Threshold values for each environment were determined by a permutation test with 1000 iterations at $P = 0.05$.

Data for lodging and stem breakage were collected at one environment and data for deciduous awn were collected at two environments; thus, the threshold of the QTL being detected in > 50% of the environments is not really meaningful for these traits. I would feel more comfortable with the results from these traits if there were data from a minimum of three environments. Two QTL for lodging resistance were found, one in chromosome 3H and the other in chromosome 6H (Table 3). Both QTL explained very little of the phenotypic variation ($R^2 \leq 0.05$). Plant height and lodging are both traits that have previously been linked to a QTL found in the long arm of chromosome 3H (Hayes et al. 1993). The QTL in the region they mention may be the *sdw1* locus, which is a semi-dwarfing gene in barley; however, neither Stander or Robust likely have the *sdw1* allele associated with reduced plant height. Thus, the QTL I identified is a unique QTL responsible for reduced plant height. However, it is hard to determine if the QTL I identified is associated with *sdw1* because the papers discussing the mapping of this locus are quite old and don't use markers that appear in current consensus maps. The QTL detected in chromosome 6H was about 15 cM proximal to the one identified for plant height in my study (Table 2). However, even though it makes sense that shorter plants tend to

lodge less, the distance between the plant height and lodging QTL seems to be great enough that I can't conclude that the two traits are being controlled by the same QTL.

Quantitative trait loci for stem breakage were identified in chromosomes 2H and 4H. The one in chromosome 4H explained 14% of the phenotypic variation for stem breakage in the population. Stem breakage is not a trait that is typically mapped, so there are no papers with results that can be used for comparisons to the results I obtained.

Deciduous awn is another trait that is not regularly phenotyped by barley-breeding programs. Three QTL were detected, one each in chromosomes 2H, 3H and 6H. The QTL in chromosome 6H was detected in both environments and on average explained about 9% of the phenotypic variation. Lewis (2012) identified a QTL in the same region using a subset of the population I used. Over 100 additional DH lines were generated in 2011-2012 to increase the population size so the results would be more robust. Lewis (2012) had around 70 plants in her population.

Identifying QTL for yield is nebulous because it can be impacted by so many abiotic and biotic stresses that occur throughout the growth of the plant. Even if data are collected on the yield components of plants m^{-2}, number of $spikes^{-1}$, number of kernels $spike^{-1}$, and grain weight, it is difficult to separate the genetic from the environment effects. In the present study, QTL for yield were identified in chromosomes 1H, 2H, 3H, 4H, 5H-3 and 6H; however, none of these QTL were identified in > 50% of the environments (Appendix Table A3). In fact, only the QTL in chromosome 6H was detected in more than one environment. Therefore, it's incorrect to claim any marker-trait associations for yield based on the results of my study.

Table 3. Quantitative trait loci for lodging, stem breakage, and deciduous awn detected in >50% of environments using composite interval mapping analysis of the Robust x Stander doubled-haploid population.

Location[†]	Chromosome	Position (cM)[‡]	Left marker	Right marker	LOD[§]	Additive effect	R^2
			----------Lodging----------				
13NV	3H	0.01	SCRI_RS_202154	1_1516	2.32	0.22	0.04
13NV	6H	30.81	3_1308	3_1485	2.59	-0.26	0.05
			----------Stem breakage----------				
12OS	2H	0.01	2_0711	2_1220	2.72	-0.19	0.05
12OS	4H	5.21	3_0605	1_0639	6.99	0.31	0.14
			----------Deciduous awn----------				
13NV	2H	2.61	2_1220	1_0780	3.81	-0.17	0.07
13NV	3H	2.01	SCRI_RS_202154	1_1516	2.43	0.14	0.04
13NV	6H	41.71	2_1030	1_0244	4.71	-0.20	0.10
12OS	6H	44.41	1_0910	2_0675	4.36	-0.30	0.08

[†]Locations in North Dakota include 11NV=2011 Nesson Valley, 11MC=2011 McVille, 12FA=2012 Fargo, 12OS=2012 Osnabrock, 13NV=2013 Nesson Valley, 13FA=2013 Fargo, and 14OS=2014 Osnabrock.
[‡]Position is that for the peak value in the QTL.
[§]Threshold values for each environment were determined by a permutation test with 1000 iterations at $P = 0.05$.

Malt Quality

Phenotypic Analyses

When discussing phenotypic results for malt quality, it is not only important to describe the progeny values in relation to the parents, but also in relation to the Ideal Commercial Malt Criteria provided by the American Malting Barley Association (AMBA, 2008) (http://ambainc.org/media/AMBA_PDFs/Pubs/Guidelines_for_Breeders.pdf; verified 2 April 2016.

Mean values of the parents and the population for traits typically measured by maltsters are presented in Table 4. Barley protein and color are traits that can be measured when the grain is sold. The AMBA guidelines indicate that grain must be below 13.5% protein but they don't

provide a desired value for kernel color. Grain color is measured in ^{o}L and higher values are indicative of kernels with brighter color. Kernel discoloration can be caused by diseases or weathering. Mean protein for Robust was significantly greater than that of Stander's and above the level desired by the AMBA. Mean percent protein of the population was intermediate to the two parents and greater than the desired level of 13.5%. The ^{o}L-value for Stander was significantly greater than that of Robust and the mean value of the population was similar to that of Robust.

For traits measured on malt, significant differences between the parents were observed for extract, α-amylase, wort protein, S/T, wort color, and FAN (Table 4). Malt extract of Stander was two percentage points greater than that of Robust; yet, the values for both cultivars were less than the AMBA desired level of > 79%. The two percentage-point difference observed between Robust and Stander is considered substantial by maltsters and brewers. The mean extract of the progeny was intermediate to that of the parents. α-amylase of Stander was nearly 20 DU greater than that of Robust. Even though both values for α-amylase are well above the ideal commercial malt criteria (>50 DU) in this study, a concern of many brewers using Robust malt was its lower α-amylase. Mean α-amylase of the progeny was intermediate to that of the two parents.

Wort protein, S/T, wort color, and FAN are traits related to protein modification during malting. The higher values of Stander vs. Robust for each of these traits was expected as Stander is known for its high levels of protein modification. Anheuser-Busch InBev prefers to use cultivars similar to Stander with high levels of protein modification while MillerCoors prefers to use cultivars similar to Robust with moderate levels of malt modification. Mean wort protein of the progeny was intermediate to that of the two parents, while mean S/T and FAN of the progeny

was more similar to that of Stander. Mean wort color of the progeny was more similar to that of Robust.

Viscosity and wort ß-glucan are related to carbohydrate modification during malting. The values in this study for viscosity were generally within the desired values specified by the AMBA while the ß-glucan values were much higher than desired. It is not unusual in micro-malting to see higher than desired ß-glucan concentrations. In this study, the ß-glucan concentrations of the two parents did not differ significantly; yet, the nearly 60 μg g^{-1} difference between the two parents is considered meaningful by brewers. Beer brewed with malt having high concentrations of wort ß-glucan will lauter slower, which can be problematic.

Results for fermentable sugar are presented in Table 5. These sugars have been reported to be approximately 46% glucose, 9% maltose, 14% maltotriose and 2% fructose in a North American pilsner wort (Schwarz and Li, 2011). The AMBA has no set guidelines for desired levels of these sugars. The only significant difference found between the parents was for glucose, with Stander having a higher concentration of the sugar than Robust. Mean concentrations of maltotriose, maltose, and glucose of the progeny were more similar to that of Stander than Robust.

The activity of α-glucosidase, limit dextrinase, and ß-amylase are not typically measured by maltsters and the AMBA does not have specifications for them. I included measurement of these enzymes in my study to determine if malt made from Robust and Stander differ in levels of these enzymes and to map them if there is variability. Instead of measuring each of these enzymes individually, maltsters measure DP, which measures the total combined activity of the three enzymes. The enzyme that has the biggest impact on DP is ß-amylase, which was the only enzyme where significant differences between the two parents was found (Table 6).

Table 4. Mean barley and malt quality of Robust, Stander, and the Stander x Robust doubled haploid progeny based on the analyses of data from three environments in North Dakota (2011-2014).

	Robust	Stander	Population		
Trait	Mean		Mean	LSD[†]	CV[‡]
Protein (%)	14.3b[§]	13.4a	13.7	0.6	2.7
Barley color (L-value)	51.0a	50.6b	50.9	0.3	0.6
Malt Loss (%)	9.1a	9.1a	8.8	1.4	10.1
Extract (%)	76.3a	78.3b	77.7	0.9	0.7
α-amylase (20° DU)	59.7a	78.4b	68.5	9.4	8.5
DP (°ASBC)[¶]	179.6a	163.6a	178.5	21.3	7.4
DP/N (%)[††]	12.6a	12.2a	13.1	0.5	7.3
Wort protein (%)[§]	5.89a[‡‡]	6.37b	6.16	0.37	3.74
S/T (%)[‡‡]	41.4a	47.6b	45.2	2.9	4.1
Wort color (°SRM)	2.28a	2.81b	2.43	0.27	6.94
FAN (μg mg^{-1})[§§]	297.3a	339.7b	321.1	37.8	7.3
Viscosity (mPa s^{-1})	1.39a	1.39a	1.40	0.03	1.38
Wort ß-glucan (μg g^{-1})	274.3a	223.3a	222.9	72.0	20.1

[†]LSD=Least significant difference at P=0.05.
[‡]CV=Coefficient of Variation.
[§]Means for parents between columns followed by the same letter are not significantly different ($P \leq 0.05$) as determined by an F-test.
[¶]DP (°ASBC)=Diastatic Power measured in Degrees American Society of Brewing Chemists.
[††]DP/N=Diastatic Power divided by grain protein.
[‡‡]S/T=Ratio of wort protein to malt protein.
[§§]FAN=Free amino nitrogen

Table 5. Mean fermentable sugars of Robust, Stander, and the Stander x Robust doubled haploid progeny based on the analyses of data from three environments in North Dakota (2011-2014).

	Robust	Stander	Population		
Trait	Mean		Mean	LSD†	CV‡
Maltotriose(g/100mL)	0.94a§	0.79a	0.83	0.16	12.34
Maltose (g/100mL)	5.21a	5.36a	5.46	0.32	3.67
Glucose (g/100mL)	1.06a	1.36b	1.32	0.19	8.98
Fructose (g/100mL)	0.13a	0.13a	0.18	0.09	32.13

†LSD=Least significant difference at *P*=0.05.
‡CV=Coefficient of Variation.
§Means for parents between columns followed by the same letter are not significantly different ($P \leq 0.05$) as determined by an *F*-test.

Robust had significantly greater ß-amylase activity and numerically higher DP than Stander (Tables 4 and 6). Robust and Stander did not differ in ß-amylase thermostability, which is the ability of the enzyme to function at higher temperatures. However, this result cannot be assumed to be conclusive since it was only measured in malt from a single location.

Table 6. Mean α-glucosidase, limit dextrinase, ß-amylase, and thermostable ß-amylase of Robust, Stander, and the Stander x Robust doubled-haploid population based on the analyses of data from up to three environments in North Dakota (2011-2014).†

	Robust	Stander	Population		
Trait	Mean		Mean	LSD†	CV‡
α-glucosidase (Units/mL)	0.60a§	0.62a	0.61	-	-
Limit dextrinase (Units/kg)	372.4a	336.7a	410.6	109.3	9.6
ß-amylase (Units/g)	24.66b	19.22a	21.31	4.91	11.71
Thermostable ß-amylase (Units/g)	0.36a	0.12a	0.22	-	-

†Values for mean α-glucosidase and thermostable ß-amylase are based on data from one environment. Values for limit dextrinase and ß-amylase are based on data from three environments.
LSD=Least significant difference at *P*=0.05.
‡CV=Coefficient of Variation.
¶ß-amylase=ß-amylase and thermostable ß-amylase.
††Means for parents between columns followed by the same letter are not significantly different ($P \leq 0.05$) as determined by an *F*-test.

QTL Analyses

As done with the agronomic data, the QTL mapping software QTL Cartographer v. 2.5 (Basten et al. 1994, 2000; Wang et al. 2001) was used to analyze data from each environment separately. For this study, QTL were considered meaningful if they were detected in over 50% of environments. QTL were identified for each of the malt quality traits in every environment; however, only a few met the criteria of being meaningful.

Three QTL for barley protein were identified, but only two met the criteria of being identified in > 50% of the environments (Table 7 and Appendix Table A4). QTL for protein were located in chromosomes 4H and 5H-1, where Stander contributed to lower protein and Robust contributed to higher protein. In chromosome 4H, the QTL was identified in all environments between 8.31 to 10.41 cM and explained 11% of the variation in the population. The QTL in Chromosome 5H-1 was identified in two of the three environments between 0.01 to 2.01 cM and explains 7% of the population variation. Marquez-Cedillo et al. (2000) identified QTL in these chromosomes in a previous DH study where the QTL in chromosome 5H was suspected to hold a major QTL controlling grain protein content. Previous research by Shumny and Tokarev (1981) stated that a nitrate reductase enzyme that regulates nitrogen intake from plants may be in chromosome 5H. One important characteristic of grain protein in barley is that protein content seems to be influenced more from nitrogen availability than genetic aspects (Gubatz and Shewry 2011).

Four QTL for barley grain color (Table 7) were detected, one in chromosome 4H and three in chromosome 6H. Lines with the alleles from Robust had higher mean grain color. The QTL in chromosome 4H was detected between 6.81 and 9.41 cM and explained 4.5% of the population variation. In chromosome 6H, the first QTL was detected between 0.01 and 1.01 cM

and explained 6.5% of variation, the second was between 28.01 and 30.31 cM and explained 5.5% of variation, and the third was between 45.51 and 50.11 cM and explained 27% of the population variation. Lewis (2012), Pedraza-Garcia (2011) and de la Pena et al. (1999) identified QTL for kernel discoloration in a similar region of chromosome 6H between 45.51 and 50.11 cM.

One QTL each for malt loss and wort ß-glucan, and three for extract were identified (Table 8 and Appendix Table A5). The QTL for malt loss was detected in two of the three environments in chromosome 6H from 45.51 to 46.51 cM and it explained 7% variability in the population. However, even though the QTL was identified, one needs to be careful with applying this information for MAS because malt loss is highly dependent on each maltster's processes for producing malt. It might be interesting to analyze this trait in malt plants that can pilot malt larger amounts of seed to see if the QTL is still detected.

For extract (Table 8), arguably one of the most important quality traits in malt, QTL were identified in chromosomes 4H, 5H-3, and 6H. The QTL in chromosome 4H was detected in two of the three environments, was located at 5.21 cM, and explained an average 20% of the variability. The QTL in chromosome 5H-3 was detected in all three environments, located within the range of 0.01 to 2.01 cM, and explained an average of 21.3% of the variation in extract in the population. The third QTL was detected in two of the three environments in chromosome 6H at 0.01 cM. On average, the QTL explained 4.5% of the variation. Szucs et a*l*. (2009), Lewis (2012), and Pedraza-Garcia (2011) identified QTL for extract in chromosome 4H including extract. Additionally, chromosome 4H has been linked previously to plump kernels (Pedraza-Garcia, 2011), and this trait in turn directly affects the percent extract. Therefore, it is not surprising to find a QTL for extract in a similar region as one for plump kernels. Hayes et al.

(1993) also identified a QTL related to malt extract in chromosome 6H; however these were not in the same region.

One QTL for wort ß-glucan was found that met the criteria of being detected in > 50% of the environments (Table 8). The QTL was located in chromosome 5H-3 from 1.01 to 5.01 cM and it explained an average of 19.5% of the variation of ß-glucan in the population. Significant QTL for malt quality traits have been identified before in this chromosome, including for ß-glucan and extract (Szucs et al., 2009; Lewis, 2012).

Table 7. Quantitative trait loci for barley protein and barley color detected in >50% of environments using composite interval mapping analysis of the Robust x Stander doubled-haploid population.

Location[†]	Chromosome	Position (cM)[‡]	Left marker	Right marker	LOD[§]	Additive effect	R^2
----------Barley protein----------							
12NV	4H	8.31	SCRI_RS_137903	1_0627	4.03	-0.21	0.08
11MC	4H	8.91	1_0627	SCRI_RS_89959	4.68	-0.12	0.09
12FA	4H	10.41	SCRI_RS_89959	3_1148	8.35	-0.12	0.16
12FA	5H-1	0.01	2_0134	SCRI_RS_218201	4.19	-0.08	0.07
12NV	5H-1	2.01	2_0134	SCRI_RS_218201	3.47	-0.14	0.07
----------Barley color----------							
12NV	4H	6.81	SCRI_RS_194525	1_0010	4.64	-0.08	0.06
12FA	4H	9.41	SCRI_RS_89959	3_1148	2.73	-0.08	0.03
12NV	6H	0.01	SCRI_RS_237782	2_1521	6.40	-0.11	0.09
12FA	6H	1.01	2_1521	2_0315	3.52	-0.09	0.04
12FA	6H	28.01	SCRI_RS_231372	2_0745	3.32	0.14	0.05
12NV	6H	30.31	2_0745	3_1308	4.19	0.12	0.06
12FA	6H	45.51	2_0675	3_0857	19.65	-0.24	0.31
12NV	6H	50.11	SCRI_RS_175000	2_0904	14.29	-0.20	0.23

[†]Locations in North Dakota include 11MC=2011 McVille, 12FA=2012 Fargo, and 12NV=2012 Nesson Valley.
[‡]Position is that for the peak value in the QTL.
[§]Threshold values for each environment were determined by a permutation test with 1000 iterations at $P = 0.05$.

Table 8. Quantitative trait loci for malt loss, α-amylase, soluble protein over total protein, and ß-glucan detected in >50% of environments using composite interval mapping analysis of the Robust x Stander doubled-haploid population.

Location†	Chromosome	Position (cM)‡	Left marker	Right marker	LOD§	Additive effect	R^2
			----------Malt Loss----------				
12FA	6H	45.51	2_0675	3_0857	2.85	0.21	0.05
11MC	6H	46.51	3_0857	SCRI_RS_187343	4.23	0.38	0.09
			----------Extract----------				
12FA	4H	5.21	3_0605	1_0639	15.50	0.46	0.22
12NV	4H	5.21	3_0605	1_0639	11.58	0.39	0.18
11MC	5H-3	0.01	SCRI_RS_141226	1_0869	13.50	0.44	0.20
12FA	5H-3	0.01	SCRI_RS_141226	1_0869	16.97	0.50	0.25
12NV	5H-3	2.01	SCRI_RS_141226	1_0869	10.39	0.39	0.19
12FA	6H	0.01	SCRI_RS_237782	2_1521	2.52	0.17	0.03
12NV	6H	0.01	SCRI_RS_237782	2_1521	4.44	0.23	0.06
			----------Wort ß-Glucan----------				
12NV	5H-3	1.01	SCRI_RS_141226	1_0869	4.11	-10.05	0.08
12FA	5H-3	5.01	SCRI_RS_141226	1_0869	10.65	-38.89	0.31

†Locations in North Dakota include 11MC=2011 McVille, 12FA=2012 Fargo, and 12NV=2012 Nesson Valley.
‡Position is that for the peak value in the QTL.
§Threshold values for each environment were determined by a permutation test with 1000 iterations at $P = 0.05$.

Table 9. Quantitative trait loci for malt soluble protein, extract, wort color, and free amino nitrogen detected in >50% of environments using composite interval mapping analysis of the Robust x Stander doubled-haploid population.

Location[†]	Chromosome	Position (cM)[‡]	Left marker	Right marker	LOD[§]	Additive effect	R^2
			----------α-amylase ----------				
11MC	5H-3	0.01	SCRI_RS_141226	1_0869	3.13	2.39	0.05
12NV	5H-3	0.01	SCRI_RS_141226	1_0869	7.58	2.97	0.08
12FA	5H-3	2.01	SCRI_RS_141226	1_0869	8.95	3.51	0.14
12FA	6H	53.31	2_0744	2_0682	11.90	4.34	0.23
12NV	6H	53.31	2_0744	2_0682	23.42	6.53	0.38
			----------Wort protein----------				
12FA	5H-2	0.01	3_1023	SCRI_RS_236068	5.07	-0.1	0.06
11MC	5H-2	3.61	2_1202	SCRI_RS_228061	2.96	-0.08	0.04
11MC	5H-3	0.01	SCRI_RS_141226	1_0869	21.36	0.23	0.35
12NV	5H-3	0.01	SCRI_RS_141226	1_0869	26.4	0.29	0.45
12FA	5H-3	1.01	SCRI_RS_141226	1_0869	31.31	0.31	0.51
12FA	6H	45.51	2_0675	3_0857	4.14	0.09	0.04
12FA	6H	53.81	1_0040	2_0744	2.26	0.07	0.02
			----------S/T----------				
12FA	4H	4.71	SCRI_RS_9618	3_0605	4.26	0.68	0.04
11MC	4H	5.21	3_0605	1_0639	7.05	1.44	0.10
12FA	5H-2	0.01	3_1023	SCRI_RS_236068	3.35	-0.6	0.03
11MC	5H-2	3.61	2_1202	SCRI_RS_228061	2.40	-0.56	0.03
11MC	5H-3	0.01	SCRI_RS_141226	1_0869	18.79	1.76	0.31
12NV	5H-3	1.01	SCRI_RS_141226	1_0869	19.59	1.93	0.38
12FA	5H-3	2.01	SCRI_RS_141226	1_0869	32.9	2.40	0.54
11MC	6H	45.51	2_0675	3_0857	2.19	0.53	0.03
12FA	6H	45.51	2_0675	3_0857	5.52	0.79	0.06

Table 9. Quantitative trait loci for malt soluble protein, extract, wort color, and free amino nitrogen detected in >50% of environments using composite interval mapping analysis of the Robust x Stander doubled-haploid population (continued).

Location†	Chromosome	Position (cM)‡	Left marker	Right marker	LOD§	Additive effect	R2
			----------Wort Color----------				
11MC	5H-3	0.01	SCRI_RS_141226	1_0869	20.86	0.23	0.34
12FA	5H-3	0.01	SCRI_RS_141226	1_0869	22.14	0.16	0.35
12NV	5H-3	0.01	SCRI_RS_141226	1_0869	20.78	0.20	0.34
11MC	6H	49.11	SCRI_RS_175000	2_0904	4.07	0.09	0.06
12FA	6H	54.41	2_0744	2_0682	5.14	0.07	0.07
12NV	6H	54.41	2_0744	2_0682	4.63	0.09	0.06
			----------Free Amino Nitrogen----------				
12NV	5H-3	0.01	SCRI_RS_141226	1_0869	14.57	14.19	0.28
12FA	5H-3	3.01	SCRI_RS_141226	1_0869	34.16	19.72	0.57
11MC	6H	30.81	3_1308	3_1485	3.07	11.19	0.07
12FA	6H	38.11	3_0521	3_0361	4.02	5.25	0.04

†Locations in North Dakota include 11MC=2011 McVille, 12FA=2012 Fargo, and 12NV=2012 Nesson Valley.

‡Position is that for the peak value in the QTL.

§Threshold values for each environment were determined by a permutation test with 1000 iterations at $P = 0.05$.

Table 10. Quantitative trait loci for maltotriose, maltose, and glucose detected in >50% of environments using composite interval mapping analysis of the Robust x Stander doubled-haploid population.

Location[†]	Chromosome	Position (cM)[‡]	Left marker	Right marker	LOD[§]	Additive effect	R^2
			----------Maltotriose----------				
12FA	6H	53.31	12_10348	1_0040	9.63	-0.06	0.20
12NV	6H	60.61	1_0220	SCRI_RS_165945	9.88	-0.04	0.20
			----------Maltose----------				
12NV	4H	4.71	SCRI_RS_9618	3_0605	10.76	0.09	0.20
12FA	4H	9.41	SCRI_RS_89959	3_1148	5.09	0.07	0.09
			----------Glucose----------				
11MC	5H-3	0.01	SCRI_RS_141226	1_0869	7.79	0.06	0.16
12FA	5H-3	2.01	SCRI_RS_141226	1_0869	13.11	0.08	0.23
12NV	5H-3	3.01	SCRI_RS_141226	1_0869	11.47	0.07	0.22

[†]Locations in North Dakota include 11MC=2011 McVille, 12FA=2012 Fargo, and 12NV=2012 Nesson Valley.
[‡]Position is that for the peak value in the QTL.
[§]Threshold values for each environment were determined by a permutation test with 1000 iterations at $P = 0.05$.

Enzyme QTL analyses (Table 11) located significant loci in chromosomes 5H-3 and 6H. A QTL for ß-amylase in chromosome 6H from 45.41 to 49.11 cM was identified in two out of three environments. On average, it explained 5.5% of the variation. A QTL for limit dextrinase mapped to chromosome 5H-3 from 60.21 to 62.21 cM in two of the three environments. This QTL explained an average of 11% of the variation in the population. ß-amylase and limit dextrinase have been analyzed in different studies previously and QTL were identified in all chromosomes including 6H for ß-amylase. In limited research, limit dextrinase has been mapped to chromosome 7H. Both enzymes seem to be in similar regions as QTL associated with DP (Clancy et al., 2003).

Table 11. Quantitative trait loci for ß-amylase, and limit dextrinase in >50% of environments using composite interval mapping analysis of the Robust x Stander doubled-haploid population.

Location[†]	Chromosome	Position (cM)[‡]	Left marker	Right marker	LOD[§]	Additive effect	R^2
			----------ß-amylase----------				
12NV	6H	45.41	1_0910	2_0675	2.99	-0.96	0.06
11MC	6H	49.11	SCRI_RS_175000	2_0904	2.29	-0.82	0.05
			----------Limit Dextrinase----------				
12NV	5H-3	60.21	2_1108	12_20775	4.50	-38.58	0.14
12FA	5H-3	62.21	2_1108	12_20775	2.51	-29.39	0.08

[†]Locations in North Dakota include 11MC=2011 McVille, 12FA=2012 Fargo, and 12NV=2012 Nesson Valley.
[‡]Position is that for the peak value in the QTL.
[§]Threshold values for each environment were determined by a permutation test with 1000 iterations at $P = 0$.

Haplotype Development

Traits showing the greatest differences between parents and having strong SNP-trait relationships were barley protein, malt extract, α-amylase, S/T, wort color, and FAN. For all traits, including these traits, all alleles from Stander were represented with an A while the alleles from Robust were represented with a B. While there are different SNP-trait associations in

different chromosomes across the genome for each trait, the combination of specific alleles at different loci could allow for the creation of a haplotype or "genetic blue print" for any specific trait. Lines with the A allele at multiple loci for a trait may be better suited for ABI while lines with B alleles at the same loci may be more suited for MillerCoors. To determine if this is the case, haplotypes for barley protein, malt extract, alpha-amylase, S/T, wort color, and FAN were evaluated to determine if they actually separate genotypes into classes that would be more desirable for one of the brewing groups.

In a doubled-haploid population, each marker should segregate 1:1 for each allele. In a few cases in this experiment, such as for SNP 1_0869 in chromosome 5H-3, this is not the case. Segregation distortion was identified in the same region of chromosome 5H-3 by Pedraza-Garcia (2011) using a subset of Robust x Stander population. While use of double-haploids may reduce the length of time needed to reach homozygosity, lines developed with this process seem to be more prone to segregation distortion (R. Brueggeman, personnel communication, 2013). In the past, markers showing segregation distortion were often discarded by researchers; however; Zhang et al. (2010), stated that these markers can be used safely for MAS with no negative effects (Zhang et al., 2010).

Mean barley protein values were 13.4% for Stander and 14.3% for Robust (Table 12). Protein was lowest and with the highest frequency in the population when alleles at the loci 1_0627 and 3_1148 in chromosome 4H and 2_0134 in chromosome 5H were A; and the highest protein generally occurred when the alleles were all B at the same loci. The highest mean protein was observed in progeny with the haplotype BAB for the markers 1_0627, 3_1148, and 2_0134, respectively; yet, the haplotype was rare with an overall frequency of 0.02 in the population. Lines with the B allele at all three loci had mean protein of 13.8% and 25% of the lines in the

population had this genotype. Thus, it appears that reduced protein can be obtained by selecting against the B allele at all three loci.

Table 12. Haplotype for barley protein at three SNP loci.

	Markers[†]			Protein(%)	Frequency
	1_0627	3_1148	2_0134		
Stander	A	A	A	13.4	
Robust	B	B	B	14.3	
Population	A	A	A	13.4	0.20
	A	A	B	13.6	0.21
	A	B	B	13.6	0.03
	B	A	A	13.6	0.04
	B	B	A	13.7	0.24
	A	B	A	13.8	0.01
	B	B	B	13.8	0.25
	B	A	B	14.0	0.02

[†]Chromosome and position for three SNP loci on the 2011 OPA consensus map (Muñoz-Amatriaín et al., 2011) are chromosome 4H at 79.47 cM for 1_0627, chromosome 4H at 88.30 cM for 3_1148, and chromosome 5H at 95.11 cM for 2_0134.

Mean extracts were 78.3% for Stander and 76.3% for Robust. Table 13 presents the haplotypes with their mean values and frequency of occurrence for this most economically important malt quality trait at the loci 3_0605 in chromosome 4H, 1_0869 in chromosome 5H-3, and 2_1521 in chromosome 6H. For this trait, the population followed the same arrangements as the parents; the highest extract (78.4%) was found when the A allele was present at all loci with and lowest (77.0%) when B was present at all three loci. Thus, selection for the A allele at all three loci would be highly recommended for developing lines with the greatest malt extract.

Mean values for α-amylase were 78.4 for Stander and 59.7 for Robust. Table 14 has mean values for the parents and the population for one locus in chromosomes 5H-3 and two loci in chromosome 6H. The highest mean value in the population (74.7) had the haplotype ABA and the lowest mean extract (61.9) had the haplotype AAB; however, in both cases these haplotypes occurred infrequently in the population. The alleles present in the loci 2_0744 and 2_0682 in

chromosome 6H appeared to be most diagnostic in predicting alpha-amylase. In general, the highest mean alpha-amylase was observed when the A allele was present at both loci (0.49 frequency) and lowest when the B alleles were present in both loci (0.50 frequency). Selecting for the AA haplotype would identify lines that have the ABI profile for alpha-amylase. Thus, I suggest using only the two chromosome 6H loci for identifying individuals with the specific haplotypes for ABI or MillerCoors.

Table 13. Haplotype for malt extract (%) at three SNP loci.

	Markers[†]			Extract	Frequency
	3_0605	1_0869	2_1521		
Stander	A	A	A	78.3	
Robust	B	B	B	76.3	
Population	B	B	B	77.1	0.07
	B	B	A	77.3	0.06
	B	A	A	77.4	0.19
	B	A	B	77.4	0.21
	A	A	B	77.9	0.13
	A	B	A	78.1	0.08
	A	B	B	78.1	0.05
	A	A	A	78.4	0.20

[†]Chromosome and position for three SNP loci on the 2011 OPA consensus map (Muñoz-Amatriaín et al., 2011) are chromosome 4H at 56.22 cM for 3_0605, chromosome 5H-3 at 165.28 cM for 1_0869, and chromosome 6H at 2.24 cM for 2_1521.

Table 14. Haplotype for a-amylase (20^{o} DU) at three SNP loci.

	Markers			α-amylase (20^{o} DU)	Frequency
	1_0869	2_0744	2_0682		
Stander	A	A	A	78.43	
Robust	B	B	B	59.73	
Population	A	A	B	61.87	0.01
	B	B	B	62.24	0.14
	A	B	B	63.40	0.36
	A	A	A	73.76	0.36
	B	A	A	74.41	0.13
	A	B	A	74.65	0.01

[†]Chromosome and position for three SNP loci on the 2011 OPA consensus map (Muñoz-Amatriaín et al., 2011) are chromosome 5H-3 at 165.28 cM for 1_0869, chromosome 6H at 80.86 cM for 2_0744, and chromosome 6H at 84.47 cM for 2_0682.

Table 15 has mean values and haplotypes of wort protein for the parents and the population at five loci in chromosomes 5H-2, 5H-3 and 6H. The parents Robust and Stander are significantly different with values of 5.89% and 6.37%, respectively. The haplotype of the A allele at all five loci occurred in 15% of the population and the mean wort protein was 6.25%. The haplotype with the B allele at at five loci was observed in 7% of the population's lines and the mean wort protein was 5.95%. Based on the means and haplotypes, it appears that lines that meet the wort protein quality specifications for ABI and MillerCoors can be identified using these five loci.

Table 15. Haplotype for wort protein (%) at five SNP loci.

	Markers[†]					Wort protein	Frequency
	3_1023	2_1202	1_0869	2_0675	1_0040		
Stander	A	A	A	A	A	6.37	
Robust	B	B	B	B	B	5.89	
Population	A	B	B	B	B	5.61	0.01
	B	A	A	B	B	5.72	0.01
	A	A	B	A	A	5.89	0.06
	A	A	B	B	B	5.93	0.05
	B	B	B	B	B	5.95	0.07
	B	B	B	A	A	6.09	0.05
	A	A	A	B	B	6.10	0.15
	A	A	A	B	A	6.16	0.03
	B	B	B	B	A	6.18	0.01
	B	B	A	B	B	6.20	0.18
	A	A	A	A	A	6.25	0.15
	B	A	A	A	A	6.31	0.01
	B	B	A	A	A	6.32	0.16
	B	B	A	A	B	6.35	0.02
	B	B	B	A	B	6.36	0.01
	A	A	A	A	B	6.39	0.02
	A	B	A	A	A	6.44	0.01
	A	B	A	B	B	6.52	0.01
	B	B	A	B	A	6.55	0.01
	B	A	B	A	A	6.68	0.01

[†]Chromosome and position for five SNP loci on the 2011 OPA consensus map (Muñoz-Amatriaín et al., 2011) are chromosome 5H-2 at 4.15 cM for 3_1023, chromosome 5H-2 at 7.84 cM for 2_1202, chromosome 5H-3 at 165.28 cM for 1_0869, chromosome 6H at 53.54 cM for 2_0675, and chromosome 6H at 74.65 cM for 1_0040.

Table 16 has the mean values for S/T. The parents were significantly different for this trait; Stander had a value of 47.6% while Robust had 41.4%. The lowest mean S/T (40.1%) had a haplotype of AAABBBB, but only 1% of the lines had this haplotype. The highest S/T values was 49.66% with a AABABAA combination, but again with a low frequency of 0.01. If the markers 3_1023 and 2_1202 in chromosome 5H are not considered, the lines with the B allele at the remaining five loci have a mean S/T of 42.2%. Eight percent of the lines in the population

have this genotype. The lines with the A allele at the same five loci have a mean S/T of 46.5%. Sixteen percent of the population have this genotype. Based on these results, it appears that the markers 3_0605 and 1_0639 in chromosome 4H and 1_0869 in chromosome 5H-3, and markers 2_0675 and 3_0857 in chromosome 6H appear to be diagnostic. Selecting lines with the A allele at these five loci appears to identify lines that fit the ABI profile for S/T. Thus, the markers 3_1023 and 2_1202 should not be used for selection. To confirm this hypothesis, validation of the markers using a different set of lines must be done.

Mean wort color mean values were 2.81 ^{o}SRM for Stander and 2.28 ^{o}SRM for Robust (Table 17). Acceptable values for this trait according to the AMBA (2014) range from 1.8 to 2.5 ^{o}SRM. The loci evaluated for this trait that appear to be diagnostic are 1_0869 in chromosome 5H-3; and 2_0904, 2_0744, and 2_0682 in chromosome 6H. Lines with the haplotype of A at all loci had mean color of 2.57 ^{o}SRM. While lines with this haplotype did not have the highest wort color, the frequency of its occurrence in the population was high (0.38). The highest mean wort color, 2.68 ^{o}SRM, was found in lines with the haplotype AAAB; however, the frequency of this genotype in the population was only 0.01. Mean wort color of lines with B allele at all four loci was 2.22 ^{o}SRM. This haplotype occurred at a frequency of 0.14 in the population. Thus, selecting for the A allele at each locus should result in higher values for wort color.

Table 16. Haplotype for soluble protein over total protein (%) at seven SNP loci.

	Markers[†]							S/T[‡‡]	Frequency
	3_0605	1_0639	3_1023	2_1202	1_0869	2_0675	3_0857		
Stander	A	A	A	A	A	A	A	47.6	
Robust	B	B	B	B	B	B	B	41.4	
Population	A	A	A	B	B	B	B	40.1	0.01
	A	A	B	A	A	B	B	40.3	0.01
	B	B	A	A	B	B	B	41.6	0.03
	B	B	B	B	B	B	B	42.6	0.05
	B	B	A	A	B	A	A	43.2	0.03
	B	B	A	A	A	B	B	43.8	0.11
	A	A	B	B	B	A	A	44.3	0.03
	A	A	A	A	B	A	A	44.5	0.03
	B	B	B	B	A	B	B	44.7	0.09
	B	B	B	B	A	A	B	45.1	0.01
	B	B	B	B	B	A	A	45.1	0.03
	A	A	B	B	B	B	B	45.4	0.03
	A	B	A	A	A	A	A	45.5	0.01
	A	A	A	A	B	B	B	45.6	0.03
	B	B	A	A	A	A	A	45.8	0.08
	A	A	A	A	A	B	B	46.0	0.06
	A	A	A	A	A	A	A	46.3	0.08
	A	A	B	B	A	B	B	46.3	0.09
	A	A	B	B	A	A	A	46.6	0.08
	A	B	A	A	A	B	B	46.6	0.01
	B	B	B	B	A	A	A	46.6	0.09
	B	B	B	A	A	A	A	47.2	0.01
	B	B	A	B	A	A	A	47.5	0.01
	A	A	A	B	A	B	B	48.0	0.01
	A	A	B	A	B	A	A	49.7	0.01

[†]Chromosome and position for seven SNP loci on the 2011 OPA consensus map (Muñoz-Amatriaín et al., 2011) are chromosome 4H at 56.22 cM for 3_0605, chromosome 4H at 76.09 cM for 1_0639, chromosome 5H-2 at 4.15 cM for 3_1023, chromosome 5H-2 at 7.84 cM for 2_1202, chromosome 5H-3 at 165.28 cM for 1_0869, chromosome 6H at 53.54 cM for 2_0675, and chromosome 6H at 61.19 cM for 3_0857.

Table 17. Haplotype for wort color (°SRM) at four SNP loci.

	Markers†				Wort color	Frequency
	1_0869	2_0904	2_0744	2_0682		
Stander	A	A	A	A	2.81	
Robust	B	B	B	B	2.28	
Population	B	B	B	B	2.22	0.14
	B	A	A	A	2.37	0.12
	A	B	B	B	2.40	0.36
	B	B	A	A	2.43	0.01
	A	B	B	A	2.53	0.01
	A	B	A	A	2.56	0.02
	A	A	A	A	2.57	0.34
	A	A	A	B	2.68	0.01

†Chromosome and position for four SNP loci on the 2011 OPA consensus map (Muñoz-Amatriaín et al., 2011) are chromosome 5H-3 at 165.28 cM for 1_0869, chromosome 6H at 72.17 cM for 2_0904, chromosome 6H at 80.86 cM for 2_0744, and chromosome 6H at 84.87 cM for 2_0682.

For FAN (Table 18), Stander had a mean of 339.7 µg mg^{-1} and Robust had a mean of 297.3 µg mg^{-1}. The highest mean FAN (329.4) was found in lines having the AAA haplotype for the locus 1_0869 in chromosome 5H-3 and the loci 3_1308 and 3_0521 in chromosome 6H, respectively. The lowest mean FAN (310.7) occurred in the progeny having the BBB at the same three loci. Thus, it appears that these three loci may be able to select for lines that may fit the desired FAN requirements for ABI or MillerCoors.

Table 18. Haplotype for free amino nitrogen (µg mg^{-1})at three SNP loci.

	Markers†			FAN (µg mg^{-1})	Frequency
	1_0869	3_1308	3_0521		
Stander	A	A	A	339.7	
Robust	B	B	B	297.3	
Population	B	B	B	310.7	0.12
	B	B	A	313.3	0.02
	A	B	A	315.3	0.03
	B	A	A	316.8	0.13
	A	B	B	318.7	0.31
	A	A	B	325.3	0.03
	A	A	A	329.4	0.36

†Chromosome and position for three SNP loci on the 2011 OPA consensus map (Muñoz-Amatriaín et al., 2011) are chromosome 5H-3 at 165.28 cM for 1_0869, chromosome 6H at 33.72 cM for 3_1308, and chromosome 6H at 41.55 cM for 3_0521.

Neither ABI or MillerCoors indicate desired levels of sugars or the enzymes ß-amylase or limit dextrinase in their malt. In looking at haplotypes that could group progeny similar to one of the parents, only the markers for maltose appeared to be successfully diagnostic. Tables 19 shows the means and frequencies of the haplotypes of markers for maltose. Similar tables for maltotriose, glucose, ß-amylase, and limit dextrinase appear in Appendix tables A9-A12. Mean maltose was 5.36 g/100mL for Stander and 5.21 g/100mL for Robust. The highest mean concentration of mean maltose in the progeny (5.54 g/100mL) was in those lines with the AB haplotype at the markers 3_0605 and 3_1148 in chromosome 4H. However, the frequency of this haplotype was was low at 0.06. Mean concentration of maltose in the lines with the AA haplotype at the two loci was 5.52 g/100mL. Mean maltose concentration of lines with the BB haplotype was 0.11 units lower. Thus, it appears that selecting for lines with the AA haplotype at both loci may be useful in identifying lines with greater concentrations of maltose.

Table 19. Haplotype for maltose (g/100mL) at two SNP loci.

	Markers[†]		Maltose (g/100mL)	Frequency
	3_0605	3_1148		
Stander	A	A	5.36	
Robust	B	B	5.21	
Population	B	A	5.40	0.08
	B	B	5.41	0.46
	A	A	5.52	0.40
	A	B	5.54	0.06

[†]Chromosome and position for two SNP loci on the 2011 OPA consensus map (Muñoz-Amatriaín et al., 2011) are chromosome 4H at 56.22 cM for 3_0605, and chromosome 4H at 88.3 cM for 3_1148.

Validation of Markers Selected for Haplotyping

To validate if the markers selected for haplotyping are capable of selecting lines with the malt quality desired by ABI or MillerCoors, lines from the University of Minnesota (UMN) included in the 2006-2009 USDA-NIFA Barley Coordinated Project (Barley CAP) were used. The UMN lines were used because Robust and Stander are both releases from the UMN. The markers identified using the Robust x Stander population would be validated as successful for MAS if they are able to successfully classify 2006-2009 Barley CAP lines into ones preferred by ABI or MillerCoors based on haplotype. Genotype data for the SNP markers I used and phenotype data for barley protein, malt extract, wort color, α-amylase, S/T, and FAN are available on the Triticeae Toolbox for Barley (https://triticeaetoolbox.org/barley/). The haplotype for Stander as it appears in the Triticeae Toolbox does not have the A allele at all loci and Robust does not have the B allele in all loci. Explanation of these discrepancies could be that different seed sources were used for genotyping or the genotype data in the two projects were scored differently. The latter reason is the mostly likely reason. When Heilman-Morales scored the population, she called all Robust alleles A, regardless of where they appeared in the GenomeStudio (htto://www.illumina.com/techniques/microarrays/array-data-analysis-

experimental-design/genomestudio.html) output. When they were scored for the Barley CAP, the allele calls were made based on their location on the output. Thus, a validation using the UMN Barley CAP data on T3 is not practical. However, even though the parents may have been scored differently in the Barley CAP and my project, the data I used were acceptable for the mapping work in my project.

CONCLUSIONS

Past efforts in breeding malting barley populations have created a narrow genetic background between many modern cultivars. A prime example of this are the cultivars utilized in this experiment. After phenotyping the DH population for agronomic and malt quality traits, and identifying QTL controlling these traits, a genetic haplotype was developed to try and help distinguish the differences between genotypes representative of ABI and MillerCoors.

After concluding the research, it is plausible to say that QTL controlling agronomic and malt quality traits can be identified in a very narrow cross. The small chromosome regions mapped can actually be key gene regions responsible for the agronomic and malt quality differences observed in the DH population's parent cultivars.

This study demonstrated significant differences between Robust and Stander agronomic and malt quality traits. Further research should be conducted for traits such as plant height, lodging, and stem breakage for the agronomic aspect and barley protein, extract, α–amylase, wort protein, S/T, wort color, FAN, glucose, maltose and β-amylase.

These traits were selected since they demonstrate differences between parents and can help us identify the "perfect marker" when utilizing MAS in the barley breeding program. Reducing plant height will help improve farmer harvest as well as reduced lodging and stem breakage since a very high percent of modern cultivated barley are still swathed before harvest due to poor stem strength. The malt quality traits are all of crucial importance when determining if the grain will be used for its original malting purpose or sold cheaper for feed. For example, high protein values, determined by AMBA, will immediately mark the grain as undesirable for malt. Extract is possibly one of the most important traits for malting companies since it determines the amount of end product they can obtain and is a basis for the trade of malt. α-

amylase and β-amylase are both enzymes that play key roles in the conversion of starch to sugars needed for the fermentation process. Maltose is the primary fermentable sugar in malt and further investigation is warranted to plausibly obtain genes that can increase its availability by possibly being related to starch content in the grain.

Now that QTL have been identified the next step is to identify if these areas in each chromosome contain actual useful genes affecting any of our economically important traits to clone and make markers good enough to influence straw strength, enzymatic activity, starch content, etc. A method to obtain the actual genes could be the use of fine mapping to identify the actual variants in the chromosome region under investigation.

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APPENDIX

Table A1. Quantitative trait loci for days to heading detected in < 50% of environments using composite interval mapping analysis of the Robust x Stander doubled-haploid population.

Location[†]	Chromosome	Position (cM)[‡]	Left marker	Right marker	LOD[§]	Additive effect	R^2
11NV	4H	6.21	1_0639	SCRI_RS_194525	7.29	-0.56	0.13
13FA	4H	6.21	1_0639	SCRI_RS_137903	5.41	-0.25	0.12
11MC	4H	7.31	1_0639	SCRI_RS_137903	5.39	-0.26	0.09
11NV	5H-3	0.01	SCRI_RS_141226	1_0869	3.43	0.38	0.06
14OS	5H-3	23.01	SCRI_RS_141226	1_0869	3.39	0.20	0.09
11MC	5H-3	46.01	3_0769	3_1352	2.66	0.24	0.04
11MC	5H-3	51.21	2_1108	12_20775	2.89	0.26	0.05
12OS	6H	1.01	2_1521	2_0315	4.05	-0.22	0.07
13FA	6H	19.01	2_1521	2_0315	3.02	-0.21	0.07
12FA	6H	43.41	3_0316	1_0910	3.19	-0.28	0.06
13FA	6H	45.41	1_0910	2_0675	7.62	-0.45	0.14

[†]Locations in North Dakota include 11NV=2011 Nesson Valley, 11MC=2011 McVille, 12FA=2012 Fargo, 12OS=2012 Osnabrock, 13NV=2013 Nesson Valley, 13FA=2013 Fargo, and 14OS=2014 Osnabrock.
[‡]Position is that for the peak value in the QTL.
[§]Threshold values for each environment were determined by a permutation test with 1000 iterations at $P = 0.05$.

Table A2. Quantitative trait loci for plant height detected in < 50% of environments using composite interval mapping analysis of the Robust x Stander doubled-haploid population.

Location[†]	Chromosome	Position (cM)[‡]	Left Marker	Right Marker	LOD[§]	Additive effect	R^2
14OS	6H	54.41	2_0744	2_0682	3.55	-0.65	0.06
11MC	6H	59.61	1_0220	SCRI_RS_165945	6.46	-0.93	0.12
13NV	6H	59.61	1_0220	SCRI_RS_165945	4.10	-1.48	0.06

[†]Locations in North Dakota include 11NV=2011 Nesson Valley, 11MC=2011 McVille, 12FA=2012 Fargo, 12OS=2012 Osnabrock, 13NV=2013 Nesson Valley, 13FA=2013 Fargo, and 14OS=2014 Osnabrock.
[‡]Position is that for the peak value in the QTL.
[§]Threshold values for each environment were determined by a permutation test with 1000 iterations at $P = 0.05$.

Table A3. Quantitative trait loci for yield detected in < 50% of environments using composite interval mapping analysis of the Robust x Stander doubled-haploid population.

Location[†]	Chromosome	Position (cM)[‡]	Left marker	Right marker	LOD[§]	Additive effect	R^2
12OS	1H	7.51	SCRI_RS_168172	1_0905	3.50	0.13	0.09
11MC	2H	1.01	2_0711	2_1220	2.58	0.07	0.06
11NV	3H	3.61	SCRI_RS_237894	SCRI_RS_128254	8.55	-0.14	0.13
11NV	4H	5.21	3_0605	1_0639	5.57	0.11	0.08
11NV	5H-3	55.21	2_1108	12_20775	2.27	0.12	0.05
13FA	6H	29.01	SCRI_RS_231372	2_0745	9.13	-0.12	0.19
11NV	6H	34.81	3_1485	3_0358	7.72	-0.13	0.12

[†]Locations in North Dakota include 11NV=2011 Nesson Valley, 11MC=2011 McVille, 12FA=2012 Fargo, 12OS=2012 Osnabrock, 13NV=2013 Nesson Valley, 13FA=2013 Fargo, and 14OS=2014 Osnabrock.
[‡]Position is that for the peak value in the QTL.
[‡]Threshold values for each environment were determined by a permutation test with 1000 iterations at $P = 0.05$.

Table A4. Quantitative trait loci for barley moisture, protein, and color detected in < 50% of environments using composite interval mapping analysis of the Robust x Stander doubled-haploid population.

Location[†]	Chromosome	Position (cM)[‡]	Left marker	Right marker	LOD[§]	Additive effect	R^2
			----------Moisture----------				
12FA	1H	0.01	2_0959	SCRI_RS_168172	3.83	0.10	0.08
11MC	5H-3	50.21	2_1108	12_20775	2.43	-0.06	0.05
12NV	6H	0.01	SCRI_RS_237782	2_1521	2.62	-0.08	0.06
			----------Protein----------				
11MC	6H	49.11	SCRI_RS_175000	2_0904	2.53	-0.09	0.05
			----------Color----------				
12FA	2H	0.01	2_0711	2_1220	2.98	-0.08	0.03

[†]Locations in North Dakota include 11MC=2011 McVille, 12FA=2012 Fargo, and 12NV=2012 Nesson Valley.
[‡]Position is that for the peak value in the QTL.
[§]Threshold values for each environment were determined by a permutation test with 1000 iterations at $P = 0.05$.

Table A5. Quantitative trait loci for malt loss, α-amylase, diastatic power over nitrogen, malt moisture, viscosity and ß-glucan detected in < 50% of environments using composite interval mapping analysis of the Robust x Stander doubled-haploid population.

Location[†]	Chromosome	Position (cM)[‡]	Left marker	Right marker	LOD[§]	Additive effect	R^2
			----------Malt Loss----------				
12NV	1H	0.01	2_0959	SCRI_RS_168172	2.92	0.20	0.06
12FA	1H	5.51	SCRI_RS_168172	1_0905	3.61	-0.27	0.07
12FA	5H-3	0.01	SCRI_RS_141226	1_0869	3.78	0.24	0.07
12FA	5H-3	77.81	12_20775	SCRI_RS_159536	2.46	-0.24	0.05
			----------α-amylase ----------				
12NV	4H	6.21	1_0639	SCRI_RS_194525	2.50	1.67	0.02
12NV	6H	31.81	3_1485	3_0358	2.55	1.89	0.03
12NV	6H	42.91	3_0317	3_0316	3.43	2.51	0.03
			----------Diastatic Power over Nitrogen----------				
11MC	4H	3.61	SCRI_RS_167844	12_31414	2.57	0.30	0.06
12FA	5H-3	48.71	2_1162	SCRI_RS_167850	2.31	-0.45	0.05
			----------Malt Moisture----------				
11MC	1H	0.01	2_0959	SCRI_RS_168172	3.30	-0.11	0.07
12FA	5H-2	0.01	3_1023	SCRI_RS_236068	2.64	0.07	0.06
11MC	6H	60.21	2_1108	12_20775	3.02	-0.13	0.08
			----------Viscosity----------				
12FA	5H-3	65.21	2_1108	12_20775	2.62	0.01	0.07
			----------ß-Glucan----------				
11MC	4H	0.01	3_0992	1_0371	4.29	7.76	0.07
12FA	4H	6.81	SCRI_RS_194525	1_0010	8.05	-22.33	0.13
11MC	4H	7.31	1_0010	SCRI_RS_137903	4.71	8.10	0.07
11MC	5H-3	4.01	SCRI_RS_141226	1_0869	16.51	17.89	0.34
12FA	5H-3	48.71	2_1162	SCRI_RS_167850	4.76	28.50	0.09

[†]Locations in North Dakota include 11MC=2011 McVille, 12FA=2012 Fargo, and 12NV=2012 Nesson Valley.

[‡]Position is that for the peak value in the QTL.

[§]Threshold values for each environment were determined by a permutation test with 1000 iterations at $P = 0.05$.

Table A6. Quantitative trait loci for malt loss, α-amylase, diastatic power over nitrogen, malt moisture, viscosity and ß-glucan detected in < 50% of environments using composite interval mapping analysis of the Robust x Stander doubled-haploid population.

Location[†]	Chromosome	Position (cM)[‡]	Left marker	Right marker	LOD[§]	Additive effect	R^2
			----------Soluble Protein----------				
11MC	4H	5.21	3_0605	1_0639	4.08	0.13	0.05
			----------Extract----------				
12FA	2H	0.01	2_0711	2_1220	2.53	-0.18	0.03
12FA	4H	0.01	3_0992	1_0371	11.03	0.40	0.16
11MC	4H	3.61	SCRI_RS_167844	12_31414	13.36	0.44	0.20
			----------Wort Color----------				
11MC	5H-2	0.01	3_1023	SCRI_RS_236068	2.83	-0.08	0.04
12FA	6H	45.51	2_0675	3_0857	8.73	0.09	0.12
12NV	6H	45.51	2_0675	3_0857	6.89	0.10	0.09
			----------Free Amino Nitrogen----------				
12FA	4H	4.11	12_31414	SCRI_RS_9618	5.69	6.25	0.06
12FA	5H-2	0.01	3_1023	SCRI_RS_236068	3.20	-4.65	0.03

[†]Locations in North Dakota include 11MC=2011 McVille, 12FA=2012 Fargo, and 12NV=2012 Nesson Valley.

[‡]Position is that for the peak value in the QTL.

[§]Threshold values for each environment were determined by a permutation test with 1000 iterations at $P = 0.05$.

Table A7. Quantitative trait loci for maltose, glucose and fructose detected in < 50% of environments using composite interval mapping analysis of the Robust x Stander doubled-haploid population.

Location[†]	Chromosome	Position (cM)[‡]	Left marker	Right marker	LOD[§]	Additive effect	R^2
			----------Maltose----------				
12NV	3H	3.01	SCRI_RS_202154	1_1516	3.51	0.05	0.06
11MC	5H-3	60.21	2_1108	12_20775	2.08	-0.10	0.07
12NV	6H	53.31	12_10348	1_0040	2.78	-0.04	0.05
			----------Glucose----------				
12FA	6H	45.41	1_0910	2_0675	13.10	0.09	0.20
12FA	6H	55.41	2_0744	2_0682	13.15	0.08	0.21
			----------Fructose----------				
12FA	1H	0.01	2_0959	SCRI_RS_168172	5.29	-0.03	0.10
12FA	5H-3	32.81	1_0869	SCRI_RS_169845	2.43	-0.02	0.04

[†]Locations in North Dakota include 11MC=2011 McVille, 12FA=2012 Fargo, and 12NV=2012 Nesson Valley.

[‡]Position is that for the peak value in the QTL.

[§]Threshold values for each environment were determined by a permutation test with 1000 iterations at $P = 0.05$.

Table A8. Quantitative trait loci for alpha glucosidase, ß-amylase and limit dextrinase detected in < 50% of environments using composite interval mapping analysis of the Robust x Stander doubled-haploid population.

Location[†]	Chromosome	Position (cM)[‡]	Left marker	Right marker	LOD[§]	Additive effect	R^2
			----------Alpha Glucosidase----------				
11MC	5H-3	0.01	SCRI_RS_141226	1_0869	8.68	0.05	0.18
11MC	6H	25.01	SCRI_RS_231372	2_0745	2.53	0.03	0.05
			----------ß-amylase----------				
12NV	5H-3	0.01	SCRI_RS_141226	1_0869	3.93	-0.69	0.08
12FA	5H-3	18.01	SCRI_RS_141226	1_0869	4.45	-1.24	0.15
11MC	5H-3	48.71	2_1162	SCRI_RS_167850	3.41	-1.35	0.07
11MC	5H-3	61.21	2_1108	12_20775	5.76	-3.32	0.34
			----------Limit Dextrinase----------				
12NV	1H	0.01	2_0959	SCRI_RS_168172	2.51	-20.94	0.05
12NV	6H	49.11	SCRI_RS_175000	2_0904	3.31	-19.10	0.07

[†]Locations in North Dakota include 11MC=2011 McVille, 12FA=2012 Fargo, and 12NV=2012 Nesson Valley.
[‡]Position is that for the peak value in the QTL.
[§]Threshold values for each environment were determined by a permutation test with 1000 iterations at $P = 0.05$.

Table A9. Haplotype for maltotriose at two SNP loci.

	Markers[†]		Maltotriose	Frequency
	12_10348	1_0220		
Stander	A	A	0.79	
Robust	B	B	0.94	
Population	A	A	0.80	0.46
	A	B	0.85	0.03
	B	A	0.82	0.04
	B	B	0.86	0.48

[†]Chromosome and position for two SNP loci are chromosome 6H at 53.3 cM for 12_10348, chromosome 6H at 59.6 cM for 1_0220, and chromosome 6H at 38.1 cM for 3_0521.

Table A10. Haplotype for glucose at two SNP loci.

	Markers†	Glucose	Frequency	
	1_0869			
Stander	A		1.36	
Robust	B		1.06	
Population	A		1.34	0.73
	B		1.28	0.27

†Chromosome and position for one SNP locus is chromosome 5H-3 at 32.8 cM for 1_0869.

Table A11. Haplotype for ß-amylase at two SNP loci.

	Markers†		Beta amylase	Frequency
	2_0675	2_0904		
Stander	A	A	19.22	
Robust	B	B	24.66	
Population	A	A	20.67	0.45
	A	B	21.27	0.05
	B	A	21.87	0.02
	B	B	21.80	0.49

†Chromosome and position for two SNP loci are chromosome 6H at 45.5 cM for 2_0675, and chromosome 6H at 51.8 cM for 2_0904.

Table A12. Haplotype for limit dextrinase (Units kg^{-1}) at two SNP loci.

	Markers†		Limit Dextrinase	Frequency
	2_1108	12_20775		
Stander	A	A	336.7	
Robust	B	B	372.4	
Population	A	A	401.9	0.75
	A	B	449.6	0.07
	B	A	428.5	0.11
	B	B	442.5	0.07

†Chromosome and position for two SNP loci are chromosome 5H-3 at 50.2 cM for 2_1108, and chromosome 5H-3 at 68.8 cM for 12_20775.