IDENTIFICATION OF MOLECULAR MARKERS FOR MARKER-ASSISTED SELECTION OF MALTING QUALITY AND ASSOCIATED TRAITS IN BARLEY

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Identification of Molecular Markers for Marker-Assisted Selection of Malting Quality and Associated Traits in Barley

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ABSTRACT

Barley (*Hordeum vulgare* L.) is one of the most important cereal crops in North Dakota, which ranks second amongst all states for barley production in the United States. Barley is used for the production of malt, which is used for brewing beer. The malting and brewing industries set strict standards for malt quality; yet, determining malt quality of experimental barley lines is very expensive. For this reason, quality is typically determined at the latter stages of the breeding program, resulting in rejection of many genotypes after large investments for agronomic performance, disease resistance, and end-use quality evaluations have occurred. High quality malt cultivars must possess numerous genetically controlled characteristics. This limits the effectiveness of phenotypic selection for malt quality. The use of marker-assisted selection (MAS) may enable breeders to eliminate lines with undesirable traits earlier in the breeding process, reducing costs, and improving genetic gain. In spite of the large number of mapped QTLs, few examples exist in the literature in which QTL analysis and MAS have been applied to the genetic improvement of malting barley. This research was initiated to identify robust marker-trait associations for malting quality, disease resistance, and agronomic traits utilizing genome-wide association mapping of selected NDSU two-rowed lines. Our research successfully identified numerous marker-trait associations for the traits evaluated to be used for MAS to improve the North Dakota State University barley breeding program.

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INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the most important cereal crops in North America, especially in North Dakota, which ranks second in barley production in the United States (US) over the last five years (2010-2014) (USDA-NASS, 2015, http://quickstats.nass.usda.gov/). A major use of barley in the US is production of malt, which is used for brewing beer. High quality malting barley is important to meet the brewing needs of major breweries such as MillerCoors and Anheuser-Busch InBev (Lewis, 2012), as well as an emerging craft brewing industry.

Determining malt quality of experimental barley lines is an important component of malting barley breeding programs. However, due to the high cost of obtaining malt data and large seed amount requirements, many malt quality assessments are measured later in the breeding process, resulting in the rejection of many late-stage breeding lines on which resources have already been invested (Richard Horsley, personal communication, 2011).

It's difficult to develop high quality malt cultivars due to the complex range of genetically-controlled traits required to meet maltsters and brewers specifications (Briggs 1998; Fox et al., 2003). This limits the effectiveness of phenotypic selection to improve malt quality and increases the interest of breeders in using molecular markers (Laido et al., 2009). Numerous quantitative trait loci (QTL) associated with malt quality have been reported, as summarized by Zale et al. (2000), Fox et al. (2003), and Ullrich (2011). Nevertheless, in spite of the large number of mapped QTLs, few examples exist in the literature in which QTL analysis and marker-assisted selection (MAS) have been applied to the genetic improvement of malting barley (Laido et al., 2009). This failure to successfully apply MAS in malt quality improvement has been due to lack of markers that are effective across different genetic backgrounds (Ullrich,

2011). A comprehensive approach to detect QTL across diversified germplasm might be a way forward to identify markers that can be broadly deployed in malt quality improvement programs. Whole genome association mapping across a wider range of genotypes provides an alternative tool to bi-parental mapping studies. At the same time, this method may capture previously undetected genomic regions associated with malt quality.

This dissertation research was initiated to identify robust molecular markers associated with disease resistance, agronomic performance, and malt quality across the genetic backgrounds of North Dakota State University's (NDSU) barley germplasm. The ultimate goal is to enable NDSU barley researchers to utilize MAS for these complex traits during early stages of cultivar development; thus, improving barley breeding efficiency for these traits. The specific objectives of this research were to: (1) identify marker-trait associations (MTAs) for disease resistance agronomic performance, and malt quality traits using genome-wide association mapping of selected NDSU lines included in the 2006-2009 USDA-CSREES-NRI Barley Coordinated Project (Barley CAP), and (2) identify SNP markers that are candidates for use in MAS of specific traits.

CHAPTER 1. LITERATURE REVIEW

Importance, Market and Production of Barley

Barley is one of the major cereal grains in the world. It is ranked fourth worldwide in production (123,477,192 metric tons) and area harvested (47,892,680 ha) after maize (*Zea mays* L.), rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.). (Food and Agriculture Organization (FAO) of the United Nations. 2012). The major uses of barley are animal feed (55-60%), a premium source of malt (30-40%), seed (5%), and health foods for human consumption (2-3%) (Ullrich, 2011).

North American brewers use both six-rowed and two-rowed barley cultivars, which have distinct malting and brewing characteristics. Europeans brewers use little to no six-rowed barley, preferring two-rowed barley with its higher starch to husk ratio and it malty flavor (Ogle, 2006; Goldammer, 2008). The American Malting Barley Association (AMBA) (American Malting Barley Association, 2014), a non-profit trade association located in Milwaukee, WI, provides US malting barley breeders with specific quality parameters a new cultivar must possess before its members will consider it for use (http://ambainc.org/media/AMBA_PDFs/Pubs/Guidelines_for_Breeders.pdf; verified 24 May 2015).

Qualitative and Quantitative Traits in Barley

Agronomic, morphological, physiological, chemical, and barley quality characteristics vary widely due to the effects of genotype, environment, and their interaction. Agronomic and quality traits were important for barley domestication (Hayes et al., 2002). With domestication and selection the genetic base of the crop narrowed, bringing uniformity, but reducing genetic

variation to handle crop diseases and abiotic stress. The genetic variation amongst many modern cultivars is limited (Hussain, 2006).

Key Disease Traits

Fungal diseases are a principal limitation for achieving high grain yields of barley. These diseases affect barley production directly, reducing grain weight and germination, and indirectly by reducing photosynthesis. Key diseases of barley in North Dakota include spot blotch (caused by *Cochliobolus sativus* (Ito & Kuribayashi) Drechs. ex Dastur), spot form net blotch (caused by *Drechslera teres* f. sp. *maculata* Smedeg.), net form net blotch (caused by *Drechslera teres* f. sp. *teres* (Sacc.) Shoemaker), leaf rust (caused by *Puccinia hordei* Otth), and Fusarium head blight (caused by *Fusarium graminearum* Schwabe) (Mathre, 1997, Liu et al., 2012, Gutierrez, 2015). The development of cultivars with durable genetic resistance is a high priority for breeding programs and a key component of integrated pest management strategies. Using MAS with a broad range of informative QTLs should greatly enhance the rate of improvement for disease resistance (Gutierrez, 2015).

Greenhouse Experiments

Spot form net blotch

Spot form net blotch (SFNB) is a common worldwide disease in barley production areas. Its incidence is increasing because of the constant use of no-till or minimum cultural practices (McLean et al., 2009). Yield loss for SFNB has increased and become severe in the last few years (Liu et al., 2012). Spot form net blotch is a form of net blotch with dark brown round to elongated lesions that develop into large irregular patches. The lesion and patches are surrounded by necrosis or a chlorotic halo.

Net form net blotch

Net form net blotch (NFNB) is a common worldwide disease of barley that can adversely affect malting quality through a reduction of kernel plumpness and decreased malt extract (Liu et al., 2012; McLean et al., 2009). Again, minimum- or no-till cropping practices enhance the disease threat by increasing inoculum spread from prior host-crop residue. Usually, the symptoms of NFNB are small circular brown lesions that develop into narrow dark brown lesions with longitudinal and transverse lesions creating a net "pattern" within the leaf veins for which the disease was originally named. A chlorotic halo surrounds the net pattern. When severely affected, the lesions coalesce and the leaves die. Stems and kernels also can be infected. Crop rotation and conventional tillage are cultural practices used to reduce pathogen survival (Jordan and Allen, 1984). Furthermore, fungicides can be used to control net blotch, but extensive repetition use may result in fungicide resistance developing in the pathogen. Mathre (1997) demonstrated both qualitative and quantitative types of NFNB resistance in barley.

Spot blotch

The causal organism of spot blotch (SB) can cause root rot, SB, and kernel blight. The outcome of all three diseases when levels are high is reduced kernel size and weight (Mathre, 1997). The initial symptoms of SB are visualized by small round to oblong brown blotches with a chlorotic border. The SB pathogen can survive in the host seed, plant debris, and in the soil (Kiesling, 1985). The elimination of inoculum sources is important to control the disease. The use of crop rotation and fungicides may not be cost effective; thus, the best way to control SB is through the use of resistant cultivars.

Leaf rust

The pathogen causing leaf rust (LR) in barley is an obligate parasite and infection results in small chlorotic lesions on the leaves and the development of light orange-brown circular spore masses surrounded by a bleached or yellow halo on upper leaf surfaces. Leaf rust also can infect awns and glumes. Late sown barley and alternate hosts provide opportunities for leaf rust isolates to undergo sexual hybridization, resulting in new virulence types (Clifford, 1985). Spore dissemination can reach long distances. Early sowing as a cultural practice is used to reduce crop damage by enabling earlier plant development prior to the onset of the disease. Elimination of infected volunteer plants also can reduce the source of inoculum.

Field Experiments

Fusarium head blight and deoxynivalenol

Fusarium head blight (FHB) challenges barley growers worldwide. The most serious component of this disease is a mycotoxin produced by *F. graminearum* known as deoxynivalenol (DON) (McMullen et al., 1997). Infection with FHB is of considerable concern to growers and end users as it reduces overall quality of the harvested grain. Fusarium head blight also has a negative impact on malt quality. Fungal growth and mycotoxin production have been shown to continue during germination in the malting process (Schwarz et al., 1995).

Resistance to FHB is complex and confounded with subtle agronomic trait differences that may influence the expression of the disease in some environments but not others. For example, heading date, plant height, and spike morphological traits have been associated with FHB severity (Zhu et al., 1999; Canci et al., 2004; Horsley et al., 2006; Lamb et al., 2009). Breeders have also sought genetic solutions to reduce or detoxify the mycotoxin. As with resistance to FHB, a spike morphology trait (*Vrs1* locus controlling row type) and a heading date

QTL located in chromosome 2H have been associated with reduced DON concentration (Massman, 2011). In combating FHB, genome-wide association mapping studies (GWAS) have identified potential genetic solutions (Massman, 2011). As always, breeders need to combine resistance for key diseases like FHB, with key agronomic and quality traits that will make them acceptable to growers and command premiums from end-users such as the malting industry (Muñoz-Amatriaín, 2014).

Agronomic Traits

Most agronomic traits are quantitative in nature, influenced by many components of plant physiology, plant architecture, and the environment. This provides challenges to breeders in assembling the necessary collection of genes, many having small effects, into single cultivars. According to a GWAS analysis by Pasam (2012), genetically complex agronomic traits in spring barley include heading date, plant height, lodging resistance, plants m^{-2}, spikes m^{-2}, kernels per spike, spike density, thousand grain weight, starch content, crude protein content, and yield. There are interactions between these and other traits that breeders must consider when making selections. Day length and vernalization responses are examples of traits that many cereal grain breeders have selected for in temperate environments to ensure that crops are adapted to specific regions (Wang et al., 2010a). Furthermore, heading date and length of grain fill, which may determine geographic adaptation of cultivars, have important impacts on grain yield in crop species. Castro et al. (1997) demonstrated the importance of the length of the grain-filling period on yield and harvest index, noting that in cultivars having similar time periods from emergence to physiological maturity, earlier anthesis and longer grain fill period were positively associated with yield and harvest index.

Grain yield can be impacted by environmental conditions that influence the expression of several measurable components including spikes m^{-2} area and/or changes in the number of grains $spike^{-1}$. Additionally, reduction of plant height can be used to reduce yield losses arising from lodging; therefore, increasing harvest index (Bezant et al., 1997).

Field Experiments

Yield

From a genetic perspective, yield is an extremely complex phenomenon, influenced by multitudes of associated traits controlling plant morphology as well as physiological interactions and biochemical pathways (Berdahl et al., 1972; Flood et al, 2001; Sarrafi et al., 1987; Tungland et al., 1987, Yap and Harvey, 1972). From a macro perspective, yield traits can be divided into two interrelated groupings; those driving yield potential and those enabling yield realization. The yield potential of cereal crops is primarily under genetic control and associated with plant traits such as photosynthetic capacity, stand establishment, productive tillering, spikes m^{-2}, days to heading, fertility, and grain fill period. Yield realization depends not only on yield potential, but the ability of the cultivar to take advantage of favorable environments and defend itself in the presence of adverse environmental conditions, pests, and diseases.

Wang et al. (2014) sought to identify QTL for barley grain yield across six environments in China. The result was identification of numerous QTL, but the significance of these QTL varied across environments. There were no large effect QTL identified in two or more environments and only one minor QTL was identified in two environments. Wang et al. (2014) also found that many significant yield QTL were located in similar positions to QTL associated with for plant height. When plant height was used as a covariate in the analysis, many of the identified yield QTL became insignificant.

Hayes et al. (1993) conducted a QTL analysis using the six-rowed barley cross 'Steptoe' × 'Morex'. Fourteen QTL for yield were mapped to all seven chromosomes based on sixteen locations of phenotypic data. In follow-up studies by Romagosa et al. (1996, 1999) and Han et al. (1999), only five of these QTL, residing in chromosomes 2H, 3H, 5H, and 6H, were confirmed. In Spain, Mansour et al. (2014) utilized recombinant inbred lines from the cross 'Orria' x 'Plaisant' to identify SNPs associated with yield QTL in chromosomes 1H (44.6 cM), 2H (54.1 cM), 5H (14.8 cM), and 7H (58.2 cM).

When discussing yield-limiting factors, scientists often discuss the relative importance of "source versus sink". Source relates primarily to the production of carbohydrates through the process of photosynthesis, versus sink, which is the plant's ability to store photosynthetic products and their derivatives in various plant parts, including seed. Increasing photosynthetic capacity of leaves is one of the most important approaches to increase crop biomass (Horton, 2000). Most photosynthesis in barley occurs in the top three leaves on the stem, especially the flag leaf, which is the primary source of carbohydrate production (Sicher, 1993). Several investigators have found that during grain filling, sink capacity, rather than source, limits the yield potential of barley (Gallagher et al., 1975; Dreccer et al., 1997; Bingham et al., 2007). Therefore, increasing sink capacity by increasing the number of grains per unit area and/or their weight is a worthy breeding objective (Abeledo et al., 2002). Increasing the number of spikes per unit area and/or the number of grains per spike are two approaches for increasing sink capacity in barley (Locatelli et al., 2013). QTL associated with net photosynthetic rate in barley have been studied in two doubled haploid (DH) populations (Wójcik-Jagła et al., 2013). According to Jiang et al. (2006), stomatal conductance significantly affected net photosynthetic rate, and is key to assessing photosynthetic limitations in barley.

Another factor impacting yield in many barley production areas is drought. Barley tolerance to drought conditions has been extensively studied. The complexity of drought, and the fact that drought can occur at different plant stages, provides an indication that strong QTL x environment interactions are possible (Li et al., 2001; Teulat and Borries, 2001). Fan et al. (2015), using leaf wilting as an index for drought tolerance, identified QTL controlling drought tolerance in chromosomes 2H and 5H. The QTL in chromosome 2H was located a similar position as a QTL impacting days to heading identified by Wang et al. (2010a).

Kernels per spike

Over the past several decades, the average number of kernels per unit area has increased, while grain size has remained relatively stable (Abeledo et al., 2003, Abeledo et al., 2002). According to Abeledo (2003), genetic improvement has been primarily associated with an increase in spikes m^{-2} rather than grain number $spike^{-1}$. Modern cultivars possess more spikes $unit^{-1}$ area than their predecessors. Successful breeding efforts to increase grains m^{-2} through increasing the number of spikes $m\text{-}^{2}$ have been reported in barley (Martiniello et al., 1987; Wych and Rasmusson, 1983). While evidence that number of kernels $spike^{-1}$ in barley has not changed with newer cultivars (Wych and Rasmusson, 1983; Bulman et al., 1993; Jedel and Helm, 1994), increased number of kernels $spike^{-1}$ in wheat (*Triticum aestivum* L.) have been noted (Slafer and Andrade, 1989; Calderini et al., 1995).

Spike length

Spike length is an important trait that has been correlated with barley yield. The magnitude of the correlation, however, has been variable. Singh et al. (1987) found spike length to have high and positive impact on yield in barley, while Gonzalez et al. (1999) reported a positive but non-significant correlation between the traits.

Islamovic et al. (2013) identified QTL for spike length and spike angle using a recombinant inbred line (RIL) population (142 lines) from the cross 'Falcon' x 'Azhul'. QTL for spike length were found in chromosomes 2H, 3H, and 4H; and QTL controlling spike angle were found in chromosomes 3H and 5H. Ren et al. (2104) used a DH population of 122 lines from the cross 'Huaai 11' x 'Huadamai 6' to identify two significant QTL for spike length in chromosomes 2H and 7H. Wang et al. (2014) identified four QTL for spike length in chromosomes 1H, 2H, 5H, and 7H.

Days to heading

Flowering in barley occurs shortly before the spike emerges from the plant. The number of days to heading is a key trait for barley and other cereal crops. It is directly associated with the adaptation of cultivars to specific environments, reproductive fitness, and the alignment of flowering and grain fill periods with environmentally optimal growing periods (Karsai et al., 2008). Due to its impact on grain filling period, the number of days to heading has been correlated with yield (Bezant et al., 1996). Its clear association with adaptation and yield potential has made this trait a key target of researchers for genetic analysis (Laurie, 1997). Castro et al. (1997) demonstrated in barley cultivars having similar time intervals from emergence to physiological maturity, that harvest index and yield were negatively correlated with time to anthesis (days to heading) and positively correlated with the duration of the grain-filling period.

Five QTL in chromosomes 1H, 2H, 5H, and 7H were detected for days to heading in a study using 182 DH lines from cross TX9425 x 'Naso Nijo' (Wang et al., 2014). These five QTL explained about 50% of the total genetic variation, but all showed significant interactions with environment.

Pasam et al. (2012) found a total of 34 significant SNPs associated with 19 QTL impacting days to heading. Some of these QTL were located in genomic regions previously reported to harbor major genes, including *HvFT3*, *PpdH1*, *HvFT4*, *eps2*, *HvGI*, *HvCO3*, *HvFT1* and *HvCO1*. In a genome-wide associate study, SNPs impacting days to heading were located within 2 cM of *PpdH1* in chromosome 1H. Mansour et al. (2014) used RILs from the cross of Orria x Plaisant to identify QTL for days to heading in chromosomes 2H (5.0 cM), 5H (14.8cM), and 7H (58.2 cM).

Plant height

Plant height is a critical factor to monitor while breeding agricultural crops. Optimizing plant height is critical to maximize yield potential and harvest index while minimizing potential yield losses due to plant lodging (Bezant et al., 1996). Lodging in cereals can occur in high yield environments or due to adverse environmental conditions such as heavy wind, rain, or hail (Berry, 2004). Grain yield losses of between 28-65% have been reported as a result of lodging (Sisler and Olsen, 1951; Stanca et al., 1979; Jedel and Helm, 1991; Sameri et al., 2009). In contrast, taller plant height can benefit yields in severe drought environments that restrict vegetative development, as found in experiments conducted in the Mediterranean region (von Korff et al., 2008). Utilizing MAS to select early generation lines having optimal height to match the target environment could greatly improve breeding efficiency.

In barley, plant height is controlled by a combination of qualitative genes and modifiers (Tang et al., 2007). This includes genes that enable dwarf or semi-dwarf phenotypes. While dwarfing can reduce yield potential, attaining semi-dwarf cultivars is a goal of many barley breeders because semi-dwarves often have improved standability, which can result in reduced fungal disease development in the spikes and improved mechanical harvestability, while

maintaining or improving harvest index and yield potential (Zhang, 2003; Stanca et al., 1979; Sameri et al., 2009).

Dwarfing or semi-dwarfing genes have been described in barley. These genes include *uzu*, *sdw1*, *ari*, *denso*, *br*, *cud*, *ert*, *lzd*, *mnd*, *nld*, *sid*, *sld*, *dwf* and *bdwd1* (Sears et al., 1981; Franckowiak, 1987; Franckowiak and Pecio, 1992; Zhang and Zhang, 2003; Ren et al., 2010). There is a cluster of semi-dwarfing genes in chromosome 3H that includes the *uzu1*, *sdw1* and *denso* genes (Tsuchiya, 1972, Barua et al., 1993). Another QTL with the semi-dwarf trait has been reported in chromosome 7H (Yu et al., 2010). Pasam et al. (2012) identified a QTL, designated QTL4_PHT, in chromosome 2H having the same mapping position as *sdw3*, which plays a major role in the gibberellin-insensitive dwarfing barley phenotype (Gottwald et al., 2004). The presence of the *sdw1* gene has been confirmed in North American and Australian barley cultivars (Hellewell et al., 2000; Jia et al., 2009; Ren et al., 2010). The *denso* gene has been widely used in European barley cultivars, while the *uzu* gene has been used by breeders in China, Japan, and Korea.

The *sdw1* gene, which is allelic to *denso* and *uzu*, is associated with short awn and spike length, plus high grain density, resulting in undesirable twisted heads, indirectly affecting yield and kernel plumpness (Wang et al, 2010b). In addition, three alleles at the *sdw1* locus have been found to delay heading (Hellewell et al., 2000). *Uzu1* has been shown to be associated with temperature and/or day length sensitivity.

QTL with quantitative impacts on plant height have been reported in all seven chromosomes (Backes et al., 1995; Kjaer et al., 1995; Hori et al., 2003; Pillen et al., 2003; Sameri et al., 2006; von Korff et al., 2006; Baghizadeh et al., 2007; Wang et al., 2010b). Sameri et al. (2006) evaluated the genetics of reduced culm length. They identified QTL from the

cultivar ‘Kanto Nakate Gold’ impacting culm length, including one designated qCUL in chromosome 7HL that primarily impacts the length of the third and fourth culm internodes. Furthermore, Ren et al. (2010) performed a QTL analysis for seven plant height components using 122 DH lines derived from the cross ‘Huaai 11’ x ‘Huadamai 6’. They described a recessive dwarfing gene, *btwd1*, mapped to the long arm of chromosome 7H and a total of 20 QTL mapped to chromosomes 2H, 3H, 5H, 6H, and 7H. Of these, 11 QTL were detected in all three years of the study. Mansour et al. (2014) used RILs from the cross of Orria x Plaisant to identify QTL for plant height in chromosomes 2H (3.9 cM and 33.0 cM), 4H (33.0 cM), 6H (25.2 cM), and 7H (87.1 cM).

Wang et al. (2014) used 182 DH lines from the cross TX9425 and ‘Naso Nijo’ to identify nine QTL for plant height. Most had a small allelic effect or were only found in one environment. The strongest of the QTL identified was QPh.NaTx-1H in chromosome 1H, which explained 10.8% of genetic variance. The QTL QPh.NaTx-2H in chromosome 2H explained 6.7% of genetic variation. A major QTL, QPh.NaTx-7H, found in chromosome 7H, explained 23.2% of the genetic variance. The analysis of Wang et al. (2014) also demonstrated the impact of trait correlations on QTL analysis. As an example, several yield and kernel weight QTL became either less impactful or non-significant in specific location analyses when plant height was used as a covariate. In contrast, the QTL for plant height identified in chromosome 7H showed no significant effects on other agronomic traits or yield components.

Stem breakage

Strong stem strength is critical to ensuring growers are able to maximize the yield potential of the cultivars they purchase. Lodging slows mechanical harvesting and increases the potential for combine damage from rocks. Published results on QTL or genes controlling stem

breakage in barley are lacking. A study on stem structure suggests that barley stem strength, like that of other crops, is more closely correlated to the stem's cylindrical wall thickness rather than stem diameter (Berry et al., 2006).

Deciduous awn

Deciduous awns are lemma awns that break off from the spike before the end of the growing period. Information on the genetic control of deciduous awns is limited. A single QTL for deciduous awns was detected in chromosome 6H in a set of doubled haploid lines created from the cross 'Robust' x 'Stander' (Lewis, 2012). This QTL was detected in all environments where the trait was measured.

Morphological Traits

Field Experiments

Rachilla hair length

Rachilla length is a common trait used in barley for taxonomic differentiation (Nilan, 1964). A recessive gene for short rachilla length name *srh* has been mapped to the long arm of chromosome 5H about 26.8 cM distal from the smooth awn gene *raw1* (Kleinhofs et al., 1993). Costa et al. (2001) identified the SSR marker Bmag0223 associated with the *srh* locus in chromosome 5H at 59.4 cM. Javaid et al. (2009) confirmed this, finding the *srh* locus flanked on chromosome 5H by the Bmag0223 SSR marker and the CAPS marker k06288KU. More recently, Waugh et al. (2010) utilized SNPs to identify a QTL associated with the *srh* locus located at 87 cM.

Awn barbing type

The lemma awns of barley and other cereals possess stomata that fix CO_2 (Grundbacher, 1963). Their proximity to the grain and position above the canopy provides them with excellent access to low angle sunlight, CO_2 exchange, and rapid photosynthate translocation to nearby grains. Awns remain photosynthetically active throughout the grain-filling period. Lemma awns in barley vary greatly in their presence and length. These morphological differences are under relatively simple genetic control and allow barley breeders many options for selection (Johnson et al., 1975). Robertson et al. (1941) reported a single gene providing a 3 (rough):1 (smooth) F_2 ratio. The symbol *Rr* was assigned to this gene. Griffee (1925) and Johnston and Aamodt (1935) reported two dominant genes, *R* for rough awns and *Rt* for semi-smooth awns, resulting in an F_2 ratio of 12 rough, 3 semi-smooth, and 1 smooth. Everson and Shaller (1955) reported an association between higher yield and the presence of semi-smooth awns in a segregating population, which was attributed to linkage.

Castro et al. (1997) clearly states the importance of agronomic traits to cultivar adaptation. I included the measurement of key agronomic traits in my study to identify potential linkages to malt quality, agronomic traits, and disease resistances.

Barley Malting Traits and Factors Impacting Its Success

Malt quality is a complex trait determined by numerous parameters and is the term used to describe a collection of seed characteristics considered key factors for producing high quality malt for the brewing industry (Beattie et al., 2010). These include barley kernel characteristics (kernel plumpness, germination, kernel color, and protein), malt factors (total protein and malt loss), measures of malt modification (wort β-glucan, fine-coarse extract difference, Kolbach index, turbidity, wort viscosity, soluble protein, malt extract, and free amino nitrogen; FAN), and

malt enzyme activity (α-amylase and diastatic power; DP). These traits are evaluated on only the most advanced lines in a barley breeding program because of the high expense involved with their measurement (Beattie et al., 2010).

High quality malting barley exhibits uniform germination, needs shorter steeping times, and has relatively low protein levels in the extract (Schwarz and Horsley, 1997). Six-rowed barley typically has superior enzyme characteristics over two-rowed barley, which is crucial in production of beers with adjunct. Six-rowed barley typically has higher protein content than two-rowed barley and therefore, lower fermentable sugar content (Schwarz and Horsley, 1997). High protein barley is best suited for animal feed; however, barley with protein up to 13.5% protein can be used in American lager-style adjunct beers.

Barley malting, which is essentially the controlled germination of barley grain, is a complex process. It begins with a step called mashing, which entails mixing milled barley grain with supplementary grains and water, then heating the mixture. During the mashing process, enzymes in the mixture break down the grains, resulting in the dissolution of cell walls and the degradation of carbohydrates and proteins into sugars and amino acids, providing the necessary substrates for fermentation (Pollock, 1962, Burger and La Berge, 1985, and Bamforth and Barclay. 1993). A process known as lautering separates the clear liquid, known as wort, from the residual grain.

The success of the barley malting process and quality depends on numerous factors, including kernel characteristics and environmental factors. Environmental factors can include moisture availability, temperatures, diseases, seed handling conditions, and grain storage conditions (Schwarz and Li, 2011). Kernel characteristics, which result from a combination of genetic and environmental factors, include moisture content, test weight, thousand-kernel weight,

kernel plumpness, grain protein, and barley color. Another key component is an array of enzymes needed to successfully complete the malting process, including α-amylase, β-amylase, β-glucanase, and the endo- and exo-proteinases. The ability of the malt to convert starch into fermentable sugars through the action of the amylase enzymes is collectively known as DP (Burger and La Berge, 1985; Schwarz and Li, 2011).

Field Experiments – Laboratory Evaluation

Kernel weight, test weight and kernel plumpness

Kernel weight, test weight and kernel plumpness are weight-related measurements used to assess barley grain quality. All have been positively correlated with malt extract yield (Schwarz and Li, 2011). Kernel weight is typically expressed on a moisture free basis in mg $kernel^{-1}$ or g 1000^{-1} kernels. An associated trait is kernel plumpness, measured in percentage, which is sometimes used in place of kernel weight in the USA. It is determined by running a grain sample over of series of sieves with rectangular openings measuring 19.0 x 2.8-mm, 19.0 x 2.4-mm, and 19.0 x 2.0-mm. Kernels remaining on the top of the top two sieves (19.0 x 2.8-mm and 19.0 x 2.4-mm) are considered plump, while those that pass through both are considered thin (Schwarz and Li, 2011). According to the American Malting Barley Association (American Malting Barley Association, Inc., 2014), all malt two-rowed barley should have a minimum of 90% plump kernels and less than 3% thin kernels. Schwarz and Li (2011) suggest that a desirable ratio of plump to thin kernels is 9:1 and that seed lots with less than 85% plump kernels may be discounted or rejected.

Moisture determinations, measured as the percent of water in the grain, are usually conducted to enable other quality traits to be expressed on a dry-weight basis (Burger and La Berge, 1985). They also help buyers ensure that the grain can be safely stored.

Kernel color

A desirable kernel color for malting barley is typically a bright, light yellow straw color (Schwarz and Li, 2011). Buyers often examine kernel color and may refuse lots that may have been impacted by pre-mature harvest, disease, or weathering. Color brightness is usually measured as an *L*-value from the tristimulus color scale (Shellhammer, 2009; Schwarz and Li, 2011). These values can help evaluators recognize hull discoloration due to weathering and microbial damage (Burger and La Berge, 1985, Schwarz and Li, 2011). Samples with poor color values often display uneven germination and poor malting characteristics.

Wort color

Resulting wort color is measured with a spectrophotometer at 430 nm using a standard reference method. This method is used by brewers to measure color intensity (darkness) of a beer or wort (Schwarz and Li, 2011), with desirable levels falling between 1.4 and 2.1 (American Malting Barley Assn, Inc., 2014).

Protein content

Protein content is a final quality factor for malting barley grain. Currently, the most commonly used protein measurement technique is near-infrared (NIR) spectroscopy (Schwarz and Li, 2011). Determination of nitrogen by the Kjeldahl procedure is an alternative approach. According to the American Malting Barley Association (American Malting Barley Association, Inc., 2014), the ideal protein level for all malt two-rowed malting barley is less than 12%. Higher protein levels have been associated with lower levels of fermentable extract, but higher DP (Schwarz and Li, 2011, Eagles et al., 1995). This suggests difficulty in combining strong DP with higher levels of fermentable extract. Higher protein also has been associated with

processing problems, including uneven germination rates, longer steep times, increased wort and beer color, and haze formation in the beer (Burger and La Berge, 1985).

Soluble protein

Soluble protein is measured as a percentage in the resulting wort. A spectrophotometer at 430 nm is used to measure wort soluble protein. Values less than 5.30% are considered desirable for all malt two-rowed barley (American Malting Barley Association, Inc., 2014). High values may result in excessive wort color.

Ratio of wort soluble protein to total protein

A ratio of wort soluble protein to total protein (S/T), also known as the Kolbach Index, is calculated and expressed as a percentage. It is a direct measurement of proteolysis (Schwarz and Li, 2011). The American Malting Barley Association recommends that this ratio fall between 38-45% for all malt two-rowed barley. Ratios below this range are considered under-modified while those over the range are considered over-modified, having higher wort color and malt loss than desired (American Malting Barley Association, Inc., 2014).

Malt extract

Malt extract is a defined as the amount of soluble material obtained in the wort following the mashing process, as conducted in a standard ASBC laboratory procedure. Carbohydrates represent approximately 90 to 92% of the wort soluble materials, about half being maltose. Amino acids, peptides and hydrolysis products from nucleic acids comprise the remainder. Higher malt extract is one of most economically valued attributes desired by brewers. The fermentation limit is impacted by many factors including total fermentable sugars, malting enzyme levels, and free amino acids (Schwarz and Li, 2011). For all malt two-rowed barley, the

American Malting Barley Association set the acceptable level of extracts on a dry-weight basis at greater than 81% (American Malting Barley Association, Inc., 2014).

Wort color

Wort color is analyzed using a spectrophotometer, and is specific to the color desired in the final beer product. As an example, wort used to make pale yellow or golden colored beers can be measured using a wavelength of 430 nm (Schwarz and Li, 2011). Other wavelengths are used for dark or red-hued beers. Wort color is typically expressed as 10 times the optical absorbance at 430 nm, using a 127-mm (0.5-in) cuvette, with acceptable color values ranging from 1.4 and 2.1.

Wort clarity

Wort clarity is usually determined by visual inspection, classified as clear, slightly hazy, or hazy. Wort viscosity is measured in centipoises (cP), a unit of dynamic viscosity. It is measured in comparison to water at 20°C. Viscosities higher than 1.5 cP are considered undesirable since the lautering process is slowed. Soluble, high molecular weight ß-glucans are primary contributors to high wort viscosity.

Diastatic power

Another key attribute of malt is its diastatic power, which represents the malt's ability to convert starch into fermentable sugars. This is thought to be primarily associated with the levels of β-amylase, with additional impacts from α-amylase, limit dextrinase, and α-glucosidase (Schwarz and Li, 2011). Alpha-amylase breaks random α-1,4 bonds along the starch polysaccharide chain, while β-amylase splits alternate α-1,4 interglucose bonds, liberating maltose from the non-reducing ends of starch and dextrin chains (Burger and La Berge, 1985;

Schwarz and Li, 2011). Limit dextrinase degrades or hydrolyzes the α-1,6 glycosidic bonds in amylopectin and branched dextrins (McCafferty, 2004). Diastatic power is represented in accordance with the procedures of the American Society of Brewing Chemists (ASBC), with acceptable levels being in excess of 150°ASBC. Brewers may have specific preferences for β-amylase levels, depending on the mouth feel they desire in their beer.

Beta-glucans

Beta-glucans also impact the brewing process. β-glucans are found in the cell walls of the endosperm and can impact the viscosity and filtering during the brewing process (Blake et al., 2011; Schwarz and Li, 2011). An acceptable range for β-glucans is less than 100 mg L^{-1} for all malt two-rowed barley (American Malting Barley Association, Inc., 2014). Efforts to select barley cultivars with reduced β-glucans through identification of those with less endosperm cell wall material has been undertaken (Greenberg and Whitmore, 1974; Bendelow, 1975; Morgan, 1977; Allison et al., 1978; Anderson et al., 1978; Bamforth and Martin, 1981). Another approach to reduce β-glucan levels has been to identify cultivars with higher levels of β-glucanase (Prentice and Faber, 1981).

Free amino acid levels

Free amino acid levels are measured in the malt using FAN analysis (Schwarz and Li, 2011). According to the American Malting Barley Association, FAN levels from 140-190 mg L^{-1} are desirable for all malt two-rowed barley (American Malting Barley Association, Inc., 2014). Lower levels can slow fermentation speed or stimulate high diacetyl levels.

Genetics of Malt Quality

Malt quality is a high priority for barley breeders, but extremely complex to select for from a genetic perspective. For that reason, selection for malting quality traits has traditionally been completed late in the breeding cycle, when sufficient grain is available to conduct micro-malting and malting quality analyses (Mather et al., 1997).

Utilizing MAS for malt quality traits would enable earlier selection of experimental lines possessing key alleles that are critical to their acceptance by the industry and reduce costly yield testing of lines lacking the necessary genetic package. Mapping of QTL controlling malt quality traits began more than 20 years ago. Early studies in North America used the 'Steptoe' x 'Morex' and 'Harrington' x TR306 mapping populations (Hayes et al., 1993; Kasha and Kleinhofs, 1994). Marquez-Cedillo et al. (2000) utilized the Steptoe x Morex, Harrington x TR306, and Harrington x Morex populations to identify a QTL associated with the *Amy2* locus in chromosome 7H and the *hordein* loci in chromosome 1H. Gao et al. (2004) used the Steptoe x Morex population to identify a QTL region in the short arm of chromosome 4H for malt extract, DP, wort β-glucan, and α-amylase; and Han et al. (2004) mapped several QTL to chromosome 7H for malt extract, α-amylase, and DP.

In more recent studies, Muñoz-Amatriaín et al. (2010) used germplasm from the University of Minnesota barley-breeding program and 1,524 SNP markers to identify 49 genes associated with malt quality traits. In research done at North Dakota State University, Pedraza-Garcia (2011) and Lewis (2012) identified QTL in chromosomes 5H and 6H associated with FAN; QTL in chromosomes 2H and 5H associated with wort color; QTL in chromosome 6H associated with soluble protein and Kolbach Index; and QTL in chromosomes 4H, 5H, and 6H associated with fermentable sugars.

Molecular Marker and Quantitative Trait Loci Mapping in Barley

General Mapping

In the past, most molecular markers in breeding programs have been primarily used to identify and incorporate simply inherited traits. Today, MAS is being extended to quantitatively inherited traits and to allow pyramiding of multiple traits (Sebastian et al., 2010). Prior to the introduction of molecular markers, breeders relied on morphological traits to evaluate cultivars for commercial potential and to distinguish cultivars for the purpose of attaining intellectual property protection (Patra and Chawla, 2010). Later, biochemical identifiers, such as isozymes, were added to the breeder's toolbox. Starting in the late 1980s, the development of DNA markers including RFLPs, RAPDs, SSRs, DArT, and SNPs allowed breeders the capability of tracking individual genes or genetic segments (Jones et al., 1997; Wenzl et al., 2004; Ullrich, 2011). High throughput low-cost genotyping systems developed in the past few years have increased the number of genotypes and markers per genotype that can be analyzed. Molecular markers have proven valuable to differentiate the variation that exists in elite barley gene pools. This information has been used to plan crosses in a way that ensures maximization of genetic variability in breeding programs (Malysheva-Otto et al., 2006). Genetic mapping is used to identify markers tightly linked to the genes that affect QTL. Marker-trait associations help scientists identify putative gene positions within the chromosome or linkage groups. With low marker density, markers tend to represent genetic segments containing numerous genes. As saturation increases, breeders are able to find markers more closely linked with specific genes (Jannink and Walsh, 2002).

Bi-Parental Mapping

A traditional approach used to discover QTL involves creating bi-parental populations, using two parents with trait variations, in which individual lines in the population are segregating for traits of interest. The goal of QTL analysis is to identify genetic polymorphisms in segregating populations that are associated with different trait alleles. Doubled-haploid or RILs are typically used for QTL studies. Since markers typically represent polymorphisms in DNA flanking genes of interest, it is not uncommon for them to be informative on some breeding populations but not others; leading to differing results being reported across studies (Meyer et al., 2004). Romagosa et al. (1999) pointed to differential QTL significance for yield in the Steptoe x Morex DH population. Han et al. (1997) utilized QTL located in two different chromosomes to select for malting quality in two different populations. They found differential results between the populations as one of the QTL had an impact on malt quality in the first population, but not the second. Marker-assisted selection is most effective when the QTL are associated with alleles having large phenotypic effects that are consistent across populations.

Association Mapping

Association mapping is an alternative to traditional bi-parental QTL mapping that uses recombination events from many genetic backgrounds. Association mapping has advantages over traditional linkage mapping including (i) increased mapping resolution, (ii) reduced research time, and (iii) ability to detect greater allele numbers (Yu and Buckler, 2006). Association mapping relies on the assumption that genes recently entering the germplasm pool are associated with unique flanking DNA sequences from the source that can be used to identify marker-trait associations (MTAs). The main objective is to find the causative SNPs or SNP's in LD associated with key traits, exploiting the natural variation found in a species, landraces, or in

cultivars from multiple breeding programs. Association mapping is powerful because it enables the discovery of associations of broad application (Zhu et al., 2008). For quantitative traits, multiple loci may contribute to the phenotype, with each accounting for only a small amount of the variation. Common alleles that have consistent impacts on phenotypic expression across members of the population can be discovered using association mapping. A negative aspect of association mapping is that false-positive associations (Type I errors) between SNPs and trait alleles can occur because of population structure.

Linkage Disequilibrium

Linkage disequilibrium mapping in plants detects and locates QTL by the strength of the correlation between a trait and a marker. The theory behind estimating linkage disequilibrium via association mapping is that the extent of disequilibrium can be measured over generations. Over multiple generations of random mating, the correlation between trait and marker will decrease as a function of the genetic distance between the two. For association mapping to be successful, it is important to have good marker coverage across the genome. It also is helpful to know the impact of a locus on trait expression. If the impact of a locus is large, the QTL-trait correlation will be large and persist even at larger distances between the QTL markers and the trait loci with which they are associated (Kraakman et al. 2004). Larger distances between QTL positions and the trait gene reduce the power to detect markers linked to the small effect QTLs if the linkage decays rapidly (Mackay and Powell, 2007).

Marker-Assisted Selection

Thomas (2003) estimated that yield improvements in barley breeding programs were approximately 1% per year. Some breeding programs are employing larger population sizes and using DH's to enhance breeding progress. Increasing breeding program size to improve

selection and development of cultivars with desirable agronomic, yield, and malt quality traits raises the cost of the breeding programs as yield trial numbers and winter nursery resources increase. To improve the selection for quantitative traits, breeders need to find efficient tools and strategies to reduce this cost. The use of molecular marker technology can contribute to crop improvement, help identify QTL and be used to build linkage maps (Hayes et al., 1993).

Marker-assisted selection can be more efficient than extensive phenotypic testing to incorporate donor genes (foreground selection) in some cases. With adequate genomic coverage, it also can be used for background selection of recurrent parent alleles to eliminate undesired flanking donor DNA that may otherwise result in linkage drag. Foreground selection requires a tight linkage between the trait of interest and its flanking markers. Background selection necessitates genotyping with a larger number of markers that cover the whole genome (Haussmann et al., 2004).

Previous work shows that SNP markers can be used effectively for genetic assessment of barley lines (Comadran et al., 2011). Recent linkage maps in barley used SNPs identified in ESTs, developed from sequencing unigenes from several barley accessions, and exploiting the GeneChip (Close et al., 2004). Bioinformatic approaches have been used to identify and assign genes to linkage maps. The Illumina GoldenGate SNP assay allowed for utilization of SNPs for mapping (Gupta et al., 2008). These additional SNP markers are increasing coverage density, which improves the capability of MAS.

Genetic and Environmental Effect on Malt and Nutritional Quality Traits

For selection, QTL information alone may be insufficient to identify markers for complex traits. Genotype by environment (GxE) interactions can complicate the identification and use of molecular markers for improvement of complex quantitative traits (Zhu et al., 2008). Likewise,

different alleles and allelic interactions may impact malt quality across different germplasm pools. Sebastian et al. (2010) evaluated the use of QTL for yield improvement in soybean (*Glycine max* L.) and concluded that these interactions suggest that context-specific selection may be required to fully realize the value of molecular markers for quantitative traits that are influenced by genetic background.

The use of molecular makers has a great potential to enhance the efficiency of barley breeding, and elucidate the genetics behind many of the complex traits that breeders are expected to combine in elite cultivars. Major advancements in marker technologies and the information analysis systems that have been developed to interpret results are making this possible. In addition, the cost associated with marker technology use continues to drop, making their use as supplements or substitutions for phenotypic screening attractive. This dissertation research was focused on identifying MTAs that could be implemented in the NDSU barley breeding program for MAS, allowing for more efficient and effective selection for malting quality traits, key disease resistance traits, and selected agronomic traits.

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CHAPTER 2. UNDERSTANDING THE GENETIC ARCHITECTURE OF IMPORTANT FOLIAR DISEASES AND RELATED TRAITS IN NORTH DAKOTA GERMPLASM USING GENOME-WIDE ASSOCIATION MAPPING

Abstract

Developing disease resistance is a key objective for barley (*Hordeum vulgare* L.) breeding programs to maximize yield potential. The incorporation of marker-assisted selection (MAS) could dramatically enhance selection efficiency, increasing the rate of genetic gain, and reduce the cost of cultivar development. The objective of this research was to identify markers using genome wide association mapping (GWAS) that could be used to improve barley resistance to several key diseases utilizing MAS. The mapping populations used were two- and six-rowed barley mapping panels, consisting of a subset of North Dakota State University (NDSU) breeding lines from the 2006-2009 USDA-NIFA Barley Coordinated Agricultural Project. The lines were phenotyped in a controlled greenhouse environment for resistance to two isolates of the pathogen causing spot form net blotch [*Drechslera teres* f. sp. *maculata* Smedeg]; and single isolates of the pathogens causing net form net blotch [*Drechslera teres* f. sp. *teres* (Sacc.) Shoemaker], spot blotch [*Cochliobolus sativus* (Ito & Kuribayashi) Drechs. ex Dastur] and leaf rust [*Puccinia hordei* Otth]. Another key trait screened in field experiments was resistance to accumulation of the mycotoxin deoxynivalenol (DON) produced by *Fusarium graminearum* Schwabe [teleomorph *Gibberella zea* (Schwein)]). The breeding lines were screened using 3,072 SNPs from barley oligo pool assay platforms (BOPA1 and BOPA2). Genome wide association analyses successfully identified both novel and previously reported QTL in both mapping panels associated with the disease resistance traits. Others require further validation to establish their utility for MAS.

Introduction

Barley (*Hordeum vulgare* L.) is one of the major cereal grains in the world. It is ranked fourth worldwide in production (123 million metric tons) and area harvested (47 million ha) after maize (*Zea mays* L.), rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) (Food and Agriculture Organization (FAO) of the United Nations, 2012). In the USA, an average of 4.1 MMT of barley was harvested from 1.1 M ha^{-1} annually between 2010 and 2014. The major uses of barley are animal feed (55-60%), a premium source of malt for brewers (30-40%), seed (5%), and health foods for humans (2-3%) (Ullrich, 2011). From 2010 to 2014, Idaho, North Dakota and Montana were the largest producers of barley in North America with average annual production levels 1.09 MMT, 0.88 MMT and 0.85 MMT, respectively (Source: USDA\NASS http://quickstats.nass.usda.gov/results/332F67B1-86D4-3983-A7C3-B5874A82DE9C).

Several foliar diseases reduce yield or end-use quality of barley around the world. While cultural practices and use of fungicides help to control disease levels, the most environmentally friendly and cost-effective approach for controlling foliar diseases is the use of the resistant cultivars. Most resistance traits are quantitative in nature and require multiple alleles to provide tolerance to cultivars (Castro et al., 2012; Dracatos et al., 2014; Zhou et al., 2014; Wang et al., 2015; Gutiérrez et al., 2015). The identification of quantitative trait loci (QTL) associated with host plant resistance to diseases is important to enable introgression of multiple alleles at different loci using marker-assisted selection (MAS) in a breeding program. The use of MAS in these situations can reduce the time and cost of the breeding process that is used to develop resistant cultivars. In this study, I sought to identify QTL associated with resistance to the foliar diseases spot form net blotch (SFNB; caused by *Drechslera teres* f. sp. *maculata* Smedeg), net form net blotch (NFNB; caused by *Drechslera teres* f. sp. *teres* (Sacc.) Shoemaker), spot blotch

(SB; caused by *Cochliobolus sativus* (Ito & Kuribayashi) Drechs. ex Dastur), and leaf rust (LR; caused by *Puccinia hordei* Otth), which cause yield losses in North Dakota.

Developing resistant SFNB barley cultivars is complex because of the rapid evolution of virulent pathotypes (McLean et al., 2009 and 2012). McLean et al. (2014) observed that SFNB resistance had been studied mostly using bi-parental populations and that resistance was controlled primarily by few major genes, such as *Rpt6* in chromosome 5H and *Rpt4* in chromosome 7H (Williams et al., 1999; Manninen et al., 2006; McLean et al., 2009; Cakir et al., 2011; Wang et al., 2015). Williams et al. (1999) found that *Rpt4* explained 80% of the variation in the SFNB reaction using the 'Galleon' x 'Haruna Nijo' mapping population. Williams et al. (2003) found the same QTL to be responsible for SFNB seedling resistance in five additional lines. Liu et al. (2011), Cakir et al. (2011) and Wang et al. (2015) described lesser effect loci controlling SFNB. In a doubled-haploid (DH) population, Friesen et al. (2006) identified a large effect QTL conferring resistance for SFNB in the long arm of chromosome 4H between markers M47-P40-260 and HVM67, which explained 64% of the phenotypic variation. Furthermore, Wang et al. (2015) discovered several QTL influencing SFNB resistance at both seedling and adult stages in Australian breeding lines using association mapping (AM).

Net form net blotch is a significant pathogen in the north central region in the USA (Liu and Friesen, 2010) causing yield losses of up to 40% (Mathre, 1997). Several studies reported a seedling resistance QTL called *QRpt6* in chromosome 6H (Steffenson et al., 1996; Manninen et al., 2000; Cakir et al., 2003; Friesen et al., 2006; Abu Qamar et al., 2008; Grewal et al., 2008). Cakir et al. (2003) and Manninen et al. (2000) found that *QRpt6 explained* 83% and 65% of the phenotypic variation in disease severity, respectively, in the populations they screened. Cakir et al. (2011) provide a summary of QTL identified in different populations for resistance to NFNB.

For seedling resistance, they reported QTL in chromosomes 2H (140.5cM) and 4H (161.1cM). For adult plant resistance, they identified a QTL in chromosome 5H at 49.3cM. For QTL controlling both seedling and adult resistance, they found loci in chromosomes 2H (62.7cM), 3H (76.6cM), and 6H (19.9cM). At least one QTL has been found that confers resistance to SFNB and NFNB. Grewal et al. (2008) concluded that the QTL *QRpt* 7 found in the 'CDC Dolly'/ TR251 mapping population for NFNB resistance was in the same location as *Rpt4*, which confers seedling SFNB resistance.

Spot blotch can cause yield losses ranging from 10 to 30% in susceptible cultivars, and may be higher if the environment is favorable for disease development (Fetch and Steffenson, 1994; Arabi and Jawhar, 2003; Kumar et al., 2007). Quantitative trait loci for SB resistance have been found in all chromosomes (Gutiérrez, 2015; Castro et al., 2012; Roy et al., 2010). A QTL with a large effect in chromosome 1H, which may be the *Rcs6* locus for seeding SB resistance, was identified in multiple studies (Gutiérrez, 2015; Zhou and Steffenson, 2013b; Castro et al., 2012; Fetch et al., 2008; Bilgic et al., 2006; Steffenson et al., 1996). Bilgic et al. (2005) identified a lesser-effect QTL in the same region as the *Rcs5* locus in chromosome 7H. Drader (2010) identified five candidate genes for *Rcs5*. Four were associated with wall-associated kinases and one with a leucine-rich repeat.

Under favorable conditions, leaf rust can cause yield losses greater than 60% and reduce grain quality (Clifford, 1985). Based on seedling resistance studies, Qi et al. (1998) determined that the inheritance of rust resistance is complex. A key strategy for breeding for leaf rust resistance is to have uniform and durable resistance against several pathotypes, rather than specific pathotypes (Park, 2008). Franckowiak et al. (1997) and Jin et al. (1994) identified more than 20 major race-specific resistance genes in cultivated and wild barley; however, no single

gene was effective in controlling all pathotypes. Quantitative trait loci for leaf rust resistance have been mapped in chromosomes 2H, 3H, 4H, 5H, and 7H (Marcel et al., 2007; Cakir et al., 2011; Castro et al., 2012; Dracatos et al., 2014). Marcel et al. (2007) used the 'Steptoe' x 'Morex' population to identify a QTL in chromosome 7H, described as *Rhpq8*, that is related it to the defense gene homologue *HvNR-F1*. Franckowiak et al. (1997) mapped the *Rph1* gene to the short arm of chromosome 2H and the *Rph9* gene to chromosome 5H, about 26.1 cM distal from the *raw1* locus (smooth awns) or 9.3 cM from the *Est9* locus (esterase 9).

Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe [teleomorph *Gibberella zea* (Schwein)]), also referred to as scab, is a serious disease of barley, primarily impacting the spikes. Symptoms include brownish-tan lesions on the glumes, bleaching of the spike, and the presence of a salmon-pink spore clusters. *F. graminearum* produces the mycotoxin deoxynivalenol (DON), which is toxic to animals, including humans (Steffenson, 2003). Even low levels of the disease can result in severe grain price discounts due to the presence of DON. Fungicides do not consistently control FHB and the resulting mycotoxins (Horsley et al., 2006a). Since FHB is a saprophyte, crop rotation is recommended as a control mechanism (Perkowski et al., 1995). Partial genetic resistance to FHB has been discovered (Choo, 2009). Typically, six-rowed barley is more susceptible than two-rowed barley. FHB severity and DON accumulation have been negatively associated with plant height, days to heading, spike angle, and relative spike density (Urrea et al., 2002; Ma et al., 2000; de la Pena et al., 1999; Zhu et al., 1999; Mesfin et al., 2003; Dahleen et al., 2003).

Barley with high DON has been shown to have a reduced malt yield. In addition, β-glucan content and viscosity are decreased, while soluble nitrogen, FAN levels and wort color are increased (Schwarz, 2003; Schwarz et al., 2002). Malt affected by FHB can change the beer

flavor and cause excessive foam release (beer gushing) when bottles are opened (Schwarz, 2003; Wolf-Hall, 2007).

Reviews on breeding for foliar diseases of barley have been written by Kleinhofs and Han (2002), Chelkowski et al. (2003), and Weibull et al. (2003). Molecular mapping research of foliar disease resistance genes and QTL has been summarized by Graner (1996).

Many QTL for these major diseases have been identified using mostly bi-parental populations. However, these populations are only effective for identifying QTL from the phenotypic diversity generated in the cross, which often represents only a small fraction of the phenotypic variation in a species (Myles et al., 2009). Furthermore, different QTL segregate in different bi-parental mapping populations and the identified QTL often are not consistent across mapping populations (Holland, 2007). To overcome this difficulty, association mapping can be used as an alternative to bi-parental populations, where the use of diverse germplasm sources including breeding lines, cultivars, landraces, and wild sources are used to increase the map resolution, and provide more targets for MAS (Zhou and Steffenson, 2013a; Gutiérrez et al. 2015). Identification of QTL in a breeding program has the advantage of identifying candidates that are more likely to be valuable for MAS in that specific breeding program. With this, the objective of this research was to identify markers using genome wide association mapping that can be used for MAS by the NDSU barley-breeding program.

Materials and Methods

Sample Selection

The two-rowed and six-rowed mapping panels for this study were selected from 768 NDSU breeding lines (384 six-rowed and 384 two-rowed) used in the USDA-NIFA funded Barley Coordinated Agricultural Project (CAP). Lines in the panels were selected such that

maximum genotypic variation was captured. To identify these lines, 768 NDSU Barley CAP lines were screened with 52 SNP markers (BOPA set) that were randomly distributed across all the seven chromosomes based on the linkage map of Close et al. (2009). The data were subjected to analysis using the program STRUCTURE (Pritchard et al., 2000) to divide the genotypes into subpopulations. An admixture model with a 100,000 burn-in period and 500,000 interactions for each of the 10 replicates was used. The number of subpopulations was based on the first significant *P-value* between adjacent comparisons as described in McClean et al. (2012) and implemented in Ghavami et al. (2011), Iqbal et al. (2012), and Patel et al. (2013). From the selected number of sub-populations, lines were selected if the membership coefficient (q_i) was ≥ 0.95 for two-rowed lines and ≥ 0.97 for six-rowed lines, such that overall diversity of the population was maintained.

Greenhouse Experiments

Evaluations of both two-rowed and six-rowed barley panels were conducted as separate experiments. A randomized complete block design with three replicates was used for each of the disease screening experiments.

Spot form net blotch

Two isolates of spot form net blotch from diverse regions in North Dakota were evaluated in separate experiments. Isolates 12LT606 from Langdon, ND and 12DP101 from Dickinson, ND were utilized. The isolates were named SFNB-L and SFNB-D, respectively. The inoculum was prepared by growing previously dried plugs from SFNB cultures that had been stored at -20^{o}C on V8PDA (150 mL V8 juice, 10 g Difco potato dextrose agar, 3 g calcium carbonate, and 10 g agar L^{-1}) for 4-6 d in the dark at room temperature. The cultures were then moved to light for 24 h at room temperature, and then back to dark again for 24-48 h at 15-17°C.

Spores were collected by flooding plates with sterile distilled water, gently rubbing plate surfaces with a rubber policeman to release spores, and pouring the suspension through two layers of cheesecloth. Spore concentration was adjusted to 2,000 spores mL^{-1}.

Plants for inoculation were grown in 21-cm-tall x 38-mm-diameter Ray Leach UV stabilized Cone-tainers (Stuewe & Sons, Inc., Tangent, OR) filled with #1 Sunshine mix (Sun Gro Horticulture, Canada) potting media amended with controlled-release water-soluble fertilizer (Osmocote 14-14-14; Scott's Company, Marysville, OH) at a rate of 1 g $Cone\text{-}tainer^{-1}$ applied at sowing. Three seeds of each line were sown in a Cone-tainer and there were two Cone-tainers per line. Resistant and susceptible genotypes, BCN10 and 'Pinnacle', respectively, were included as checks to which other experimental lines were compared in order to obtain a uniform disease rating. Each Cone-tainer rack contained 30 lines. A border of 'Robust' was sown in Cone-tainers that surrounded the perimeter of the other Cone-tainers in the rack to minimize the "border effect". Cone-tainers were kept in a greenhouse at room temperature (20°C±5) with a 14-h photoperiod, under 430W Agrosun lights (Hydrofarm; Petaluma, CA).

Plants were inoculated at the 2-3 leaf stage in the greenhouse, two weeks after sowing, with aqueous spore suspensions containing two drops of Tween 100 mL^{-1} of solution, using an atomizer pressurized sprayer. Approximately 100 mL of spray suspension was applied at (55 kPa) per rack of 98 Cone-tainers until the inoculum began to bead up and run off of leaf surfaces. To enhance infection, inoculated plants were kept at 100% relative humidity in a mist chamber (Phytotronics, Inc; Earth City, MO.). Mist was applied for 30 sec every 4 min, under light, for 22-24 h. After inoculation and misting was completed, plants were moved back to the greenhouse, and plants were rated seven days later. The second leaf was rated using a disease lesion type rating scale of 1 to 5, as described by Nerpune et al. (2015), where 1 is a resistant

reaction with pinpoint lesions and no surrounding chlorosis and 5 is a susceptible reaction with coalescing necrotic and chlorotic regions covering greater than 70% of the leaf surface. Results for the isolates were analyzed separately.

Net form net blotch

The NFNB isolate ND89-19 was used to produce inoculum. The inoculum was grown for 12-14 d in the dark at room temperature and then moved to light for one day. The spores were collected by flooding plates with sterile distilled water, gently rubbing plate surfaces with a rubber policeman to release spores, and pouring the suspension through two layers of cheesecloth. Spore concentration was adjusted to 7,000 spores mL^{-1}.

Plants for inoculation were grown as described for SFNB screening, except the resistant and susceptible check genotypes were ND-B112 and 'Hector', respectively. Cone-tainers were grown in the greenhouse at a room temperature of 20°C±5 with a 14-h photoperiod under 430W Agrosun lights. Two-week old seedlings were inoculated with an aqueous spore suspension containing two drops of Tween 500 mL^{-1} of solution. The spray solution was applied with an atomizer-pressurized sprayer. Approximately 100 mL of spray solution was applied at 55 kPa to each rack of 98 Cone-tainers until the solution began to bead up and run off the leaf surfaces. To enhance infection, inoculated plants were kept in a mist chamber. Plants were misted for 24 sec every 12 min in the dark for 22-24 h with 100% relative humidity. After inoculation and misting were completed, plants were allowed to dry slowly with lights on, then moved back to the greenhouse. Plants were rated 7 d later for disease infection response (IR) using a 1-10 rating scale, where 1 = resistant and 10 = susceptible (Tekauz, 1985). The evaluation consisted of taking the highest and lowest IR observed. The highest IR was multiplied by 2/3 and the lowest

IR was multiplied by 1/3 to give the predominant and less predominant score and both values were summed to provide the final rating.

Spot blotch

The SB isolate used for screening was SB85-F pathotype 1 (Valjavec-Gratian and Steffenson, 1997). The inoculum was grown under 40-watt cool florescent lights for 12 h and in dark for 12 h, for 10-12 d. Spores were collected by flooding plates with sterile distilled water, gently rubbing plate surfaces with a rubber policeman to release spores, and pouring the suspension through two layers of cheesecloth. Spore concentration was adjusted to 8,000 spores mL^{-1}.

Plants for inoculation were grown as described for SFNB screening, except the resistant and susceptible check genotypes were ND-B112 and ND5883, respectively. Cone-tainers were kept in a greenhouse at room temperature (20 °C±5) with a 14-h photoperiod under 430W Agrosun lights. Two-week old seedlings (two to three-leaf stage) were inoculated with an aqueous spore suspension containing 2 drops of Tween 500 mL^{-1} of solution. The spray solution was applied with an atomizer pressurized sprayer. Approximately100 mL of spray solution was applied at 5 kPa $rack^{-1}$ of 98 Cone-tainers until the inoculum beaded and ran off the leaves. To enhance infection, inoculated plants were kept in a mist chamber. Plants were misted for 24 sec every 12 min, in the dark, for 22-24 h with 100% relative humidity. Next, plants were allowed to air dry slowly with lights on and moved back to the greenhouse. Disease ratings were collected on plants after 7 d using a 1-9 rating scale, where 1 = resistant and 9 = susceptible, as described by Fetch and Steffenson (1999). The evaluation consisted of taking the highest and lowest IR observed. The highest IR was multiplied by 2/3 and the lowest IR was multiplied by 1/3, to give

the predominant and less predominant score and both were summed to provide the final rating. Then the mean of the three replicates was used in the statistical and AM analysis.

Leaf rust

Leaf rust race 8 was used to inoculate and screen plants for reaction to the pathogen. 'Estate' and 'Moore' were the resistant and susceptible genotypes, respectively, used to make disease rating comparisons and to provide a uniform disease rating and to ensure a correct time of inoculation (Miller and Lambert, 1955). Rust spores were stored in a -80^{O}C freezer, then taken out and heat shocked for 9 min at 46^{O}C. They were then placed in an 80% manganese sulfate solution for 3.5 h.

Plants for inoculation were grown as described for SFNB screening. Cone-tainers were kept in a greenhouse at room temperature (20 °C±5) with a 14-h photoperiod, under 430W Agrosun lights. Seven-day old seedlings were inoculated with a spore suspension having a pore concentration adjusted to 6.5 mg spores mL^{-1}. Spores were applied with Soltrol 170 oil using a vacuum pump, with a pressure of 30 kPa. Inoculated plants were kept in a mist chamber with mist applied for 24 sec every 12 min, in the dark for 18 h, and 100% relative humidity. Lights were then turned on and plants were allowed to air dry for 4 h and moved back to the greenhouse. Plants were evaluated 14 d later.

A rating scale from 0 to 4 was used for the disease infection type (IT) evaluations, using a modification of the IT scale for wheat developed by Stakman et al. (1962). Like Stakeman's scale, the one used is based on primarily on uredial size as described by Miller and Lambert (1955). Another modification of the scale was described and utilized by Zhou et al. (2014). The rating scale in my study was composed by categorical seedling IT scores that were then converted to a numerical data suitable for use in marker associations analyses. ITs labeled as (c,

n, ; , 0, -1, 1, +1, -2, 2, +2, -3, 3, +3 and 4) were converted to the following numerical scale as (0, 0, 0, 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 5). Where "c" stands for chlorosis and "n" for necrosis. The IT "0" means a fleck with no sporulation. An IT of "1" was associated with small uredinial size plus distinct necrosis. The IT "2" was given for plants with larger uredinial size plus presence of distinct chlorosis. The 0.5 scale adjustments represent small differences in uredinial sizes like "-" and "+" signals. After recording the most frequent IT scores for the individual leaf sample basis, these numerical disease scores were weighted in order to represent the correct proportion of the ITs evaluated.

Calculation of the final scores is summarized in Table 2.1, which is a slight modification of the one used for scoring African stem rust (caused by *Puccinia graminis* Pers.:Pers.)

Table 2.1. Formula used in transforming barley seedling leaf rust categorical infection type (IT) data into numeric data for the four ITs leaf rust scores for individual plants.

Multiplier for respective ITs				
Most prevalent IT	Second most prevalent IT	Third most prevalent IT	Next most prevalent IT	Formulae for numeric score
A† × 100 %‡	-	-		A
A × 75 %	B × 25 %	-		0.75A + 0.25B
A × 60 %	B × 30 %	C × 10 %	-	0.6A + 0.3B + 0.1C
A x 40%	B x 30 %	C x 20 %	D x 10%	0.4A + 0.3B + 0.2C + 0.1D

†A, B, C or D represent numeric values from 0.0 to 5.0 for the most prevalent IT, second most prevalent IT and next most prevalent IT, respectively, which were assigned to categorical ITs. Categorical IT "0" was coded as 0.0; IT ";" as 0.0, IT "1-" as 0.5, IT "1" as 1.0, IT "1+"as 1.5, IT "2-" as 2.0, IT "2+" as 2.5, IT "2+" as 3.0, IT "3-" as 3.5, IT "3" as 4.0, IT "3+" as 4.5, IT "4" as 5.0.

‡Barley commonly exhibits mesothetic reactions, i.e., a mixture of different IT on the same leaf. The multiplier after A, B, C and D were weighted, reflecting the general proportions of the most frequent IT, second most frequent IT, third most frequent IT and forth most frequent IT.

pathotype TTKSK resistance in barley (Zhou et al., 2014). If only one infection type was identified for a line, the score value was multiplied by 1.0 and used as the final disease score. If two, three and four infection types were identified, then the frequency of them in order of prevalence were multiplied by 0.75 and 0.25; 0.60, 0.30, and 0.10; and 0.40, 0.30, 0.20, and 0.10, respectively, and the sum calculated was used as the final numeric disease score. The mean numeric score from the two replicates was averaged to determine the adjusted mean of each line to use in the AM analysis.

Field Experiments

Six-character environment code designations were used for presenting the results of analyses as follows: the first two digits represent the year (11=2011; 12=2012 and 13=2013); the next two characters are for the locations (NV= Nesson Valley; OS = Osnabrock and LA= Langdon); and the last two digits represent the row type of experiment (57= two-rowed type). Field screening was limited to the two-rowed panel.

Deoxynivalenol accumulation

In order to collect data on DON accumulation, the experimental materials were sown in the NDSU Fusarium head blight nursery at Osnabrock, ND in 2012 and Langdon, ND in 2012 and 2013. Entries were assigned to experimental units (hills) using the repeated augmented block experimented design (repeating four checks every 20 entries), as described by Horsley et al., (2006b). Each augmented block experiment was repeated twice at an environment each year. The grain-spawn inoculation method used was the same as described by Urrea et al. (2002). The DON content was determine in the NDSU Barley and Malt Quality Laboratory of Dr. Paul Schwarz and the threshold of detection was 0.5 µg g^{-1} (Schwarz, 1995).

Foliar diseases

At some loctions there was more than one foliar disease on a leaf and it was difficult to discern which diseeasae was causing the damage; thus, a general "foliar disease" score was accessed. Foliar disease (FD) severity was evaluated using a 1-9 scale (1 = no/low disease damage 9 = severe disease damage). This trait was evaluated in environments where foliar diseases occurred.

Statistical Analysis

Greenhouse Experiments

The GLM procedure of SAS 9.3 (SAS Institute Inc., 2004) was used to analyze the disease data for each pathogen. The phenotypic data were adjusted based on the least squares means for each experiment independently. Further correlation between the traits was estimated using the CORR procedure in SAS 9.3. The correlation value was considered significantly different from zero at $p \leq 0.05$.

Field Experiments

Foliar disease resistance for the two-rowed panel experiment was evaluated in three environments where the disease was present. In 2011 and 2013, scores were recorded in Nesson Valley, ND (11NV57, 13NV57). In 2012, the panel was evaluated at Osnabrock, ND (12OS57).

Statistical analyses for foliar disease traits were conducted using the MLM procedure of SAS 9.3 using Proc Mixed method type 3 (SAS Institute Inc., 2004). Analyses were conducted for each individual environment. The phenotypic data means were adjusted based on the least squares means (LSMeans) for each entry, within environments.

Genotypic Data

The USDA-ARS Molecular Genotyping Laboratory of Dr. Shiaoman Chao previously obtained genotypic data for the CAP breeding lines utilized in this study. Data were obtained from the Hordeum Toolbox database Website (http://hordeumtoolbox.org/). The SNP data used in the present study were those from the first of two barley oligo pool assay platforms (BOPA1 and BOPA2) (Close et al., 2009) containing allele-specific oligos for a set of 3,072 SNPs that were used to genotype all Barley CAP barley breeding lines.

Association Mapping Analyses

Association mapping analyses were done separately for each disease.

Imputation and Minor Allele Frequency

To improve the power of association mapping, imputation analyses were performed to estimate the missing data. The software FastPHASE v. 1.3 (Scheet and Stephens, 2006) was used to impute missing genotype data using the default parameters of the software. Only markers having a Minor Allele Frequency (MAF) > 0.05 were considered for further analyses.

Population Structure, Kinship and Best Model

Principal components that can be used to control for population structure were estimated using the PRINCOMP procedure in SAS 9.3 (SAS Institute, Inc. 2011). The principal components (PCs) that explained 25% (PC_{25}) of the cumulative variation were used in the regression model to control for population structure. The DISTANCE procedure in SAS 9.3 (SAS Institute, Inc. 2011) was used to calculate the relatedness by state between the breeding lines using the Gower's similarity coefficient. The results of this analysis were used as the kinship matrix to control for relatedness.

To calculate the *P-value* of the marker trait-associations, four linear regression models were employed: Naïve, PC, Kinship, and PC + Kinship using the MIXED procedure in SAS (SAS Institute, Inc. 2011). The best linear model for each trait was identified using the method suggested by Mamidi et al. (2011), which is based on the estimation of mean square difference (MSD) values between the observed and the expected *P-value*s.

Results

Marker-Trait Associations

Greenhouse Experiments

The linear regression models used to calculate the *P*-value of the marker-trait associations (MTAs) amongst the Naïve, PC, Kinship (K), and PC+ K analyses varied by environment. The best models were identified using the mean square difference (MSD) method of Mamidi et al. (2011) for each environment-trait combination as highlighted in Appendix table A1. The models with the minimum MSD for each trait were used for the association analyses.

The marker-trait associations (MTAs) significant at $P \leq 0.001$ ($-\text{Log}_{10} \geq 3.0$) and residing within 5 cM of each other were considered as belonging to the same QTL. I considered SNPs as candidates for MAS based on the following criteria: (1) they were associated with QTL that met the criteria previously explained and (2) the SNPs were linked to representative chromosomes or not linked. When multiple SNPs were potential candidates for the same QTL, I selected one per QTL, giving preference to SNPs that didn't detect heterozygotes (Table 2.5).

Field Experiments

The method to select the model used for the association mapping analyses is the same as described for greenhouse experiments. Marker-trait associations significant at $P \leq 0.01$ ($-\text{Log}_{10}$

≥2.0), residing within 5 cM of each other, and detected in > 50% of the environments (two) were considered as belonging to the same QTL. SNPs were considered SNPs as candidates for MAS if the associated MTAs were detected in at least 50% of the environments and the mean -Log_{10} P-values of the MTAs were ≥ 3.0.

Sample Selection

Structure analysis of 384 genotypes resulted in a peak at K=10 for two-rowed and K=9 for six-rowed breeding lines based on the Wilcoxon test. Individuals were assigned to sub populations based on membership coefficient (*qi*) greater than 0.95 and 0.97 for the two-rowed and six-rowed lines, respectively. Based on these criteria, a total of 81 two-rowed and 84 six-rowed lines were selected for inclusion in this study. However, due to missing phenotypic data for a few lines, the total numbers of lines for which I had disease resistance screening data were 80 and 81 individuals for the two-rowed and six-rowed panels, respectively.

Greenhouse Experiments

The association mapping panels exhibited substantial phenotypic diversity for all leaf spot diseases evaluated. All the disease scores were normally distributed with a *P-value* <0.010 for the Kolmogorov-Smirnov test (Table 2.2). In general, mean disease ratings for the two-rowed and six-rowed mapping panels were similar, except for NFNB. For this disease, the six-rowed panel, with a mean rating of 7.11, was much more susceptible than the two-rowed panel, with a mean rating of 3.73 (Table 2.2). In general, the distribution of phenotypes ranged from resistant to susceptible across the breeding lines for each mapping panel (Table 2.2, Appendix figure A1). However, none of the lines in the six-rowed panel showed resistance to NB.

In the two-rowed panel, a significant correlation was found between SFNB-L and SFNB-D ($r = 0.60$; $p < 0.0001$), between SFNB-L and NFNB ($r = 0.36$; $p=0.0009$), and between NFNB

and SB (r = 0.30; p=0.0059) (Table 2.3). Correlations values between the diseases were similar for the six-rowed panel. In this panel, a significant correlation was found between SFNB-L and SFNB-D (r = 0.53; p <0.0001), between NFNB and SB (r = 0.34; p=0.0018), and between SFNB-D and LR (r = 0.24; p = 0.03). The higher values for the correlation between SFNB-L and SFNB-D are not unexpected because the isolates are from the same species. Likewise, the lower but significant correlation values for the other disease comparisons suggest that there may be

Table 2.2. Phenotypic data descriptions for four foliar diseases (greenhouse experiments) evaluated in this study.

Disease[†]	Minimum	Maximum	Mean	SD[‡]	%CV[§]	*P*-value of K-S test[¶]	Evaluated score range	Cut off score[††]
Two-Rowed								
SFNB-L	1.33	3.83	2.99	0.43	14.26	<0.010	1 to 5	2.5
SFNB-D	2.17	3.83	3.12	0.37	11.81	<0.010	1 to 5	2.5
NFNB	1.78	7.78	3.73	1.64	43.94	<0.010	1 to 10	3.0
SB	2.56	7.11	3.68	0.81	22.09	<0.010	1 to 9	3.5
LR	0.00	3.96	2.45	1.35	55.02	<0.010	0 to 4	2.0
Six-rowed								
SFNB-L	2.17	3.67	2.97	0.34	11.41	<0.010	1 to 5	2.5
SFNB-D	2.17	3.83	2.92	0.35	11.84	<0.010	1 to 5	2.5
NFNB	3.89	9.33	7.75	0.78	10.03	<0.010	1 to 10	3.0
SB	2.33	5.44	3.15	0.58	18.40	<0.010	1 to 9	3.5
LR	0.55	4.38	3.52	0.57	16.08	<0.010	0 to 4	2.0

[†]SFNB-L = Spot form net blotch Langdon Isolate, SFNB-D = Spot form net blotch Dickinson isolate, NFNB = Net form net blotch, SB = Spot blotch, LR = Leaf rust, respectively.

[‡]SD = Standard deviation;

[§]CV = Coefficient of variation

[¶]K-S = Kolmogorov-Smirnov

[††]Cutoff score below which the genotype is considered tolerant.

Table 2.3. Simple linear Pearson correlations among four foliar diseases of barley in the two-rowed and six-rowed mapping panels.

†	Two-rowed mapping panel‡				
	SFNB-L	SFNB-D	NFNB	SB	LR
SFNB-L		<0.0001	0.001	0.414	0.542
SFNB-D	0.609***		0.219	0.410	0.464
NFNB	0.363***	0.139		0.006	0.862
SB	0.093	0.093	0.305**		0.230
LR	-0.069	-0.083	-0.020	-0.136	
	Six-rowed panel				
	SFNB-L	SFNB-D	NFNB	SB	LR
SFNB-L		<.0001	0.821	0.224	0.357
SFNB-D	0.534***		0.873	0.671	0.030
NFNB	-0.025	0.018		0.002	0.803
SB	0.137	-0.048	0.341**		0.576
LR	0.104	0.242	-0.028	-0.063	

†SFNB-L = Spot form net blotch Langdon Isolate, SFNB-D = Spot form net blotch Dickinson isolate, NFNB = Net form net blotch, SB = Spot blotch, LR = Leaf rust, respectively.

‡Probability values appear above the diagonals and correlation coefficients appear below the diagonals in the table.

*, **, and *** significant at the $P \leq 0.05$ $P \leq 0.01$ and $P \leq 0.001$ levels, respectively.

some genetically controlled attributes that provide partial resistance across these pathogenic species.

Field Experiments

Significant genotypic variation was detected for DON and foliar disease traits (Appendix table A3). Within environments, the mapping panel entries exhibited a range of diversity for both

traits evaluated in the field. In general, the mean barley and field disease and DON traits mapping panel was similar to that of the mean of the three checks (Table 2.4).

Above average precipitation and temperatures throughout the growing season in 2011 in Nesson Valley may have resulted in more genotypic diversity for foliar diseases than expressed other environments. In 2012, all locations had excellent growing conditions, with favorable temperatures, adequate rainfall, and no insect pressure, but the DON genotypic diversity at Osnabrock, ND was lower than the other environments and years. Significant genotypic variation was detected for DON and foliar disease traits based on the combined ANOVA across environments (Table 2.4).

Table 2.4. Mean, minimum, maximum, and standard deviation for deoxynivalenol and foliar disease traits for the two-rowed mapping panel and checks (Conlon, Pinnacle, and Tradition) across three environments in North Dakota, 2011-2013.

Traits		Number of environments[†]	Mean[‡]	Minimum	Maximum	Standard deviation
Deoxynivalenol	Mapping panel[†]	3	11.6a	2.9	38.7	3.0
	Avg. checks		13.3a	8.6	22.1	4.0
Foliar disease	Mapping panel	3	3.2a	1.5	5.3	0.7
	Avg. checks		2.8a	1.8	5.2	0.9

[†]Mapping panel is the mean of 81 NDSU cultivars and breeding lines.

[‡]Means for a trait followed by the same letter are not different at $P = 0.05$ as determined using an *F*-protected LSD.

Genotypic Data, Imputation and MAF

I used the same iSelect consensus genetic map to predict the known chromosomal locations of the SNPs used in this study Muñoz-Amatriaín et al. (2014).

The *P*-values of the MTAs for the analyses using the Naïve, PC, K, and PC+ K models varied by environment. The best models were identified using the MSD method of Mamidi et al. (2011) for each disease trait assessed in greenhouse experiments (Appendix table A1) and for

each trait-environment combination for DON and foliar diseases (Appendix table A2). The models with the minimum MSD for each trait were used for the association analyses.

Of the 3,072 SNPs comprising BOPA1 and BOPA2, 91.8% had known chromosomal locations. For the two-rowed and six-rowed panels, 2.0% and 3.3% of the missing genotype data were imputed, respectively. Furthermore, 1,605 of the SNPs in the two-rowed panel and 1,624 SNPs in the six-rowed panel had minor allele frequencies (MAF) > 5% and were used for subsequent analyses of both mapping panels. Of the total 1,605 two-rowed and 1,624 six-rowed polymorphic markers, 1,500 (93.5%) and 1,133 (89.6%), respectively, had known chromosome positions. The mapped markers were evenly distributed across the seven barley chromosomes. The analysis of the two-rowed panel revealed that 11.3%, 16.6%, 12.7%, 10.9%, 15.8%, 11.5% and 14.8% of the polymorphic SNPs were distributed in chromosomes 1H, 2H, 3H, 4H, 5H, 6H and 7H, respective. For the six-rowed panel, 10.5%, 17.6%, 11.7%, 11.5%, 15.1%, 10.8% and 12.3% of the polymorphic SNPs were distributed in chromosomes 1H, 2H, 3H, 4H, 5H, 6H and 7H, respectively.

Population Structure

Principal components were estimated using PRINCOMP in SAS 9.3 to control the impacts of population structure on the association mapping analyses. A total of four and two PCs were obtained that explained 25% of the cumulative variation for two-rowed and six-rowed mapping panels, respectively.

Marker Trait Associations for Greenhouse Evaluation Traits

Greenhouse Experiments

Spot form net blotch (SFNB)

In the screening of the two-rowed mapping panel for SFNB using the isolate from Langdon, 12 MTAs were found across all chromosomes except 5H (Table 2.5, Figure 2.1). There were also four MTAs associated with unmapped markers. One SNP, 11_21521 in chromosome 6H at 3.11 cM, met my criteria and was selected as a candidate for MAS of SFNB (Table 2.5).

Using the two-rowed panel and the isolate SNFB-D, 12 MTAs associated were found across all chromosomes and four with unmapped markers (Table 2.5, Figure 2.1). Unfortunately, none of them met the criteria as a candidate for MAS. In comparing the results for QTL across both isolates, there was one common chromosomal region associated with resistance/tolerance to both isolates in chromosome 6H, positioned between 2.27 and 3.11 cM. Additionally, two unlinked MTAs were identified using both isolates. The SNPs were 11_11208 and 12_30116. However, none of them met the MTA criteria for use in MAS.

Using the six-rowed mapping panel and the Langdon isolate for SFNB, 12 MTAs were found in chromosomes 1H, 2H, 4H, and 7H, and with unmapped markers (Table 2.5, Figure 2.3). Considering all markers, two SNPs were selected as candidates for MAS. The SNPs are 12_30277 located in chromosome 1H at 135.56 cM, and 11_20511, located in chromosome 2H at 120.80 cM.

In the screening of the six-rowed panel using the SFNB isolate from Dickinson, 11 significant MTAs were associated with resistance in chromosomes 1H, 2H, 6H and 7H (Table 2.5, Figure 2.3). Of the 11 MTAs, two remained as significant based on the -log10 (≥3.0) and one was selected as a candidate for MAS. The SNP is 11_21229 and it is located in chromosome

7H at 128.36 cM. In the six-rowed panel, there were no chromosomal regions that had SNPs associated with resistance to both SFNB isolates.

In comparing the results from the two-rowed and six-rowed panels, there were only three regions in common that contained significant SNPs. These regions include chromosome 1H at 0.75-1.51 cM, when the Langdon isolate was used on the two-rowed panel and the Dickinson isolate was used on the six-rowed panel, chromosome 2H at 120.80-125.46 cM, when the Dickinson isolate was used on the two-rowed panel and the Langdon isolate was used in the six-rowed panel, and in chromosome 7H at 144.45 cM, when the Langdon isolate was used on both panels. The two- and six-rowed panels had no respective SNPs in similar regions meeting the selection criteria for use in MAS.

Net form net blotch (NFNB)

Using the two-rowed mapping panel, there were 17 significant MTAs for NFNB in chromosomes 4H and 6H (Table 2.5, Figure 2.1). Of the 17 SNPs, 13 remained as significant meeting the criteria of -log10 (≥3.0) and five were selected as candidates for MAS based the additional criterion. The SNPs selected as MAS candidates belong to three chromosome regions; one in chromosome 4H at 50.40 cM, and two in chromosome 6H at 51.41 cM and 55.94-60.23 cM.

Using the six-rowed panel, 10 significant MTAs were found for resistance to NFNB in chromosome 4H and two more were unmapped (Table 2.5, Figure 2.3). Four were selected as candidates for MAS based on the selection criterion. These SNPs mapped to three regions in chromosome 4H at 48.50, 55.63 and 82.42 cM; and one in an unlinked chromosome region.

Only one QTL for resistance to NFNB was found in similar regions using the two- and six-rowed mapping panels. This QTL was in chromosome 4H at 50.40 cM.

Spot blotch (SB)

Using the two-rowed mapping population, 11 significant MTAs were found, located in chromosomes 3H, 4H, 5H and 7H (Table 2.5, Figure 2.2). Four were selected as candidates for MAS based on the criterion. These SNPs belong to two QTL regions in chromosome 3H, one region in chromosome 4H and one on chromosome 7H. The SNPs selected in chromosome 3H were 11_10559 at 24.99 cM and 12_30170 at 80.89 cM. The other SNPs were 11_20013 in chromosome 4H at 123.29 cM and 11_20162 in chromosome 7H at 31.75 cM.

Twelve significant MTA for SB were found in chromosomes 1H, 4H, 5H, 6H and 7H using the six-rowed panel and one more was unmapped (Table 2.5, Figure 2.4). Of these 13 SNPs, five were selected as a candidate for MAS based on the criterion. These SNPs belong to QTL regions in chromosomes 1H (11_10764 at 40.99 cM), 4H (11_20762 at 98.55 cM), 5H (11_10557 at 147.4 cM), 6H (12_30057 at 121.22 cm) and 7H (12_30219 at 34.82 cM). In comparing significant MTAs found using both mapping panels, there were none in common.

Leaf rust (LR)

Using the two-rowed mapping panel, 16 significant MTAs for LR were found in chromosomes 2H, 4H, 5H, 6H, and 7H, and an unmapped marker (Table 2.5, Figure 2.2). Of the 16 SNPs, two were selected as a candidate for MAS based on the criterion. These QTL were located in chromosome 2H at 39.1 cM (12_20326) and chromosome 6H at 24.36 cM (11_10868).

Using the six-rowed mapping panel, 13 significant MTAs associated with LR were found in chromosomes 1H, 6H, and 7H plus several unmapped markers (Table 2.5, Figure 2.4). Of these 13 significant SNPs, four were selected as candidates for MAS based on the criterion. These are located in chromosomes 1H, 6H, and 7H at 45.13 cM, 65.03 cM. 86.44 cM,

respectively, with the fourth SNP being unlinked. In comparisons of significant MTAs found using the two- and six-rowed mapping panels, there were no significant SNPs or QTL regions in common.

Marker Trait Associations for the Field Disease and Related Traits

Field Experiments

Deoxynivalenol (DON)

For DON, one QTL was found in chromosome 6H at 0 cM in two out of three environments (Table 2.6). One SNP associated with QTL, 11_10496, met the criteria and was selected as a candidate for MAS for DON levels (Table 2.7).

Foliar disease (FD)

For foliar disease, two QTL were found in chromosome 5H at 103.72-108.18 cM and 145.35 cM, and an additional QTL in chromosome 6H at 91.79-96.73 cM in two out of three environments (Table 2.6). However, the associated SNPs did not the criterion as candidates for MAS (Table 2.7).

Table 2.5. Significant associations between single nucleotide polymorphism (SNP) markers and resistance to major barley leaf diseases detected in the 80 two-rowed and 81 six-rowed barley breeding lines based on greenhouse screening.

					Allele A		Allele B		Heterozygote		
Panel/ Disease†	SNP marker	Chromosome	Position (cM)	-Log $_{10}$ (P-value)	No. of lines	Mean	No. of lines	Mean	No. of lines	Mean	Candidate SNP for MAS
Two-rowed SFNB-L											
	12_30715	1H	1.51	2.09	48	3.1	29	2.8	4	2.9	
	12_30781	2H	8.57	2.42	73	3.0	7	2.7	1	3.6	
	12_31205	2H	86.63	2.12	58	3.0	22	3.1	1	2.3	
	11_11533	2H	87.33	2.12	58	3.0	22	3.1	1	2.3	
	11_10900	2H	101.78	2.43	21	3.0	58	3.0	2	2.4	
	11_20715	2H	133.94	2.15	9	2.6	72	3.0			
	12_10014	3H	168.40	2.09	21	3.0	58	3.0	2	3.5	
	11_20089	4H	123.29	2.18	66	2.9	15	3.2			
	11_21521	6H	3.11	3.04	68	2.9	11	3.2	2	3.5	YES
	11_10165	6H	16.97	2.46	10	3.2	70	3.0	1	3.6	
	11_20691	7H	0.00	2.08	69	3.1	10	2.6	2	2.6	
	11_21363	7H	144.45	2.37	68	3.1	12	2.6	1	2.7	
	11_20044	Unlinked		2.88	23	3.3	57	2.9	1	2.2	
	12_11208	Unlinked		2.57	9	2.7	72	3.0			
	12_30116	Unlinked		2.57	72	3.0	9	2.7			
	12_30118	Unlinked		2.61	67	3.1	14	2.7			
Two-rowed SFNB-D											
	12_30241	1H	20.82	2.37	23	2.9	54	3.2	4	3.1	
	11_10446	2H	125.46	2.40	36	3.1	43	3.1	2	3.9	
	12_31161	3H	148.94	2.25	71	3.2	10	2.9			
	11_20515	4H	101.62	2.60	36	3.1	42	3.2	3	2.5	
	12_31023	5H	4.96	2.53	10	2.9	71	3.2			
	12_30977	5H	6.40	2.29	9	3.0	71	3.2	1		
	11_10669	6H	2.27	2.93	42	3.0	36	3.3	3		
	12_30956	6H	129.38	2.89	63	3.2	18	2.9			
	11_20537	6H	129.38	2.70	62	3.2	19	2.9			

Table 2.5. Significant associations between single nucleotide polymorphism (SNP) markers and resistance to major barley leaf diseases detected in the 80 two-rowed and 81 six-rowed barley breeding lines based on greenhouse screening (continued).

					Allele A		Allele B		Heterozygote		
Panel/ Disease†	SNP marker	Chromosome	Position (cM)	-Log $_{10}$ (P-value)	No. of lines	Mean	No. of lines	Mean	No. of lines	Mean	Candidate SNP for MAS
Two-rowed SFNB-D	11_21528	7H	46.19	2.36	26	3.0	54	3.2	1		
	11_20885	7H	74.52	2.52	56	3.2	25	2.9			
	12_31120	7H	74.52	2.52	25	2.9	56	3.2			
	12_11208	Unlinked		2.40	9	2.9	72	3.2			
	12_11254	Unlinked		2.33	56	3.2	25	2.9			
	12_30116	Unlinked		2.40	72	3.2	9	2.9			
	12_31203	Unlinked		2.59	27	2.9	53	3.2	1		
Six-rowed SFNB-L											
	11_21140	1H	126.00	2.86	13	2.9	69	3.0	1	2.2	
	12_30277	1H	135.56	3.87	46	3.1	35	2.9	2	2.4	YES
	12_30517	1H	135.56	3.31	38	3.0	44	3.0	1	2.2	
	12_10746	1H	138.31	2.60	25	2.9	56	3.0	2	2.4	
	12_30231	1H	138.31	2.60	25	2.9	56	3.0	2	2.4	
	11_11133	2H	58.24	2.54	5	2.6	77	3.0	1	2.1	
	11_20511	2H	120.80	3.53	56	3.1	27	2.8			YES
	12_10395	4H	24.59	2.74	5	3.4	76	3.0	2	2.4	
	11_10132	4H	24.59	2.49	77	3.0	5	3.4	1	2.2	
	12_31362	4H	73.57	2.55	74	3.0	8	3.0	1	2.2	
	12_31166	7H	144.45	2.55	44	2.9	38	3.0	1	2.2	
	12_30502	Unlinked		2.47	24	2.7	59	3.1			
Six-rowed SFNB-D											
	11_11223	1H	0.75	2.11	70	3.0	12	2.6	1	2.6	
	11_21067	1H	1.51	2.11	70	3.0	12	2.6	1	2.6	

Table 2.5. Significant associations between single nucleotide polymorphism (SNP) markers and resistance to major barley leaf diseases detected in the 80 two-rowed and 81 six-rowed barley breeding lines based on greenhouse screening (continued).

					Allele A		Allele B		Heterozygote		
Panel/ Disease†	SNP marker	Chromosome	Position (cM)	-Log $_{10}$ (P-value)	No. of lines	Mean	No. of lines	Mean	No. of lines	Mean	Candidate SNP for MAS
Six-rowed											
SFNB-D	11_20112	2H	15.15	2.14	77	2.9	6	3.3			
	11_10214	2H	93.50	2.36	28	3.0	55	2.9			
	11_10939	6H	33.74	2.18	35	2.8	48	3.0			
	12_10605	7H	64.80	2.13	58	3.0	23	2.7	2	3.2	
	12_10267	7H	68.46	2.25	28	2.7	53	3.0	2	3.2	
	11_20092	7H	110.99	2.82	22	2.7	61	3.0			
	11_21229	7H	128.36	3.10	76	3.0	7	2.5			YES
	12_11279	7H	128.36	3.10	76	3.0	7	2.5			
	11_10182	7H	128.36	2.21	5	2.5	78	3.0			
Two-rowed											
NFNB											
	11_21389	4H	36.37	3.24	58	3.7	22	3.7	1	7.3	
	12_30605	4H	50.40	3.16	73	3.5	7	6.1	1	4.7	YES
	11_10262	4H	55.63	2.45	7	5.5	74	3.6			
	12_30569	6H	51.41	3.73	44	4.5	36	2.8	1	5.4	YES
	12_30473	6H	52.75	3.73	36	2.8	44	4.5	1	5.4	
	11_20835	6H	55.94	6.42	47	4.6	34	2.4			
	11_10227	6H	55.94	6.42	47	4.6	34	2.4			
	11_10377	6H	55.94	4.03	21	5.3	57	3.1	3	4.7	YES
	12_31178	6H	55.94	4.03	57	3.1	21	5.3	3	4.7	
	12_30857	6H	56.48	6.42	47	4.6	34	2.4			YES
	12_30144	6H	56.48	6.42	47	4.6	34	2.4			
	11_10189	6H	60.23	5.53	36	2.6	44	4.6	1	6.1	
	11_20058	6H	60.23	5.53	36	2.6	44	4.6	1	6.1	
	11_21310	6H	60.23	5.53	44	4.6	36	2.6	1	6.1	YES
	12_30346	6H	60.23	2.79	43	3.0	36	4.5	2	4.9	
	11_10635	6H	60.23	2.27	40	3.0	39	4.4	2	5.2	
	11_10781	6H	64.36	2.78	35	4.5	46	3.2			

Table 2.5. Significant associations between single nucleotide polymorphism (SNP) markers and resistance to major barley leaf diseases detected in the 80 two-rowed and 81 six-rowed barley breeding lines based on greenhouse screening (continued).

					Allele A		Allele B		Heterozygote		
Panel/ Disease[†]	SNP marker	Chromosome	Position (cM)	-Log $_{10}$ (P-value)	No. of lines	Mean	No. of lines	Mean	No. of lines	Mean	Candidate SNP for MAS
Six-rowed NFNB											
	12_30488	4H	48.50	5.80	48	8.8	35	6.3			YES
	11_10942	4H	48.50	3.98	76	7.9	7	6.7			
	12_30450	4H	50.40	5.80	35	6.3	48	8.8			
	11_20363	4H	55.63	3.98	76	7.9	7	6.7			YES
	12_30060	4H	55.63	3.98	76	7.9	7	6.7			
	12_31297	4H	55.63	3.98	76	7.9	7	6.7			
	12_10088	4H	55.63	3.98	76	7.9	7	6.7			
	12_30620	4H	65.05	3.16	47	8.5	36	6.7			
	11_20670	4H	80.79	6.43	57	7.7	25	8.0	1	3.9	
	11_10724	4H	82.42	6.45	26	8.0	56	7.7	1	3.9	YES
	12_30503	Unlinked		5.80	48	8.8	35	6.3			
	12_30655	Unlinked		6.60	46	7.9	36	7.7	1	3.9	YES
Two-rowed SB											
	11_10559	3H	24.99	6.92	70	3.5	11	4.9			YES
	12_31346	3H	76.98	4.51	9	3.6	71	3.6	1	7.2	
	11_20362	3H	78.53	5.74	54	3.7	25	3.5	2	6.6	
	11_20597	3H	78.53	5.55	53	3.7	26	3.5	2	6.6	
	11_10047	3H	78.53	5.22	59	3.7	20	3.5	2	6.6	
	12_30170	3H	80.89	6.08	43	3.5	35	3.7	3	6.2	YES
	11_10387	4H	119.84	4.41	45	3.6	35	3.6	1	7.2	
	12_31422	4H	120.58	4.31	36	3.7	44	3.6	1	7.2	
	11_20013	4H	123.29	4.53	65	3.8	15	3.2	1	7.3	YES
	11_11456	5H	127.96	4.66	66	3.6	14	3.7	1	7.2	
	11_20162	7H	31.75	8.16	6	5.6	75	3.5			YES

Table 2.5. Significant associations between single nucleotide polymorphism (SNP) markers and resistance to major barley leaf diseases detected in the 80 two-rowed and 81 six-rowed barley breeding lines based on greenhouse screening (continued).

					Allele A		Allele B		Heterozygote		
Panel/ Disease[†]	SNP marker	Chromosome	Position (cM)	-Log $_{10}$ (P-value)	No. of lines	Mean	No. of lines	Mean	No. of lines	Mean	Candidate SNP for MAS
Six-rowed SB											
	11_10764	1H	40.99	4.40	72	3.0	10	3.9	1	3.6	YES
	12_30336	1H	41.76	2.81	72	3.1	10	3.8	1	3.6	
	11_10526	1H	47.47	3.19	6	4.0	77	3.1			
	12_31467	1H	47.47	2.57	75	3.1	6	4.0	2	2.9	
	11_20762	4H	98.55	3.53	5	4.6	78	3.1			YES
	12_10666	4H	98.55	2.80	44	2.4	38	4.0	1	3.8	
	11_11200	5H	117.47	3.17	50	3.0	33	3.4			
	11_10557	5H	147.4	4.63	6	4.2	77	3.1			YES
	12_30057	6H	121.22	4.87	36	4.2	47	2.3			YES
	12_30219	7H	34.82	3.46	37	4.2	45	2.3	1	3.9	YES
	12_30879	7H	61.32	3.08	72	3.0	11	3.9			
	12_30880	7H	61.32	3.08	11	3.9	72	3.0			
	12_30877	Unlinked		2.36	9	4.0	74	3.1			
Two-rowed LR											
	12_20326	2H	39.1	5.07	11	0.4	69	2.8	1	2.3	YES
	11_10919	2H	39.1	3.69	14	1.2	66	2.7	1	2.8	
	12_30657	2H	39.1	2.51	52	2.7	27	1.9	2	3.3	
	11_11505	2H	49.07	2.61	60	2.8	20	1.4	1	2.9	
	11_20674	2H	51.75	2.20	67	2.3	13	3.4	1	-0.4	
	12_30691	2H	51.75	2.20	13	3.4	67	2.3	1	-0.4	
	11_10422	2H	52.47	2.20	13	3.4	67	2.3	1	-0.4	
	11_20929	2H	52.47	2.20	67	2.3	13	3.4	1	-0.4	
	11_10733	2H	54.95	2.20	66	2.3	14	3.3	1	-0.4	
	11_20748	2H	56.28	2.32	59	2.3	21	3.1	1	-0.6	

Table 2.5. Significant associations between single nucleotide polymorphism (SNP) markers and resistance to major barley leaf diseases detected in the 80 two-rowed and 81 six-rowed barley breeding lines based on greenhouse screening (continued).

					Allele A		Allele B		Heterozygote		
Panel/ Disease†	SNP marker	Chromosome	Position (cM)	-Log $_{10}$ (P-value)	No. of lines	Mean	No. of lines	Mean	No. of lines	Mean	Candidate SNP for MAS
Two-rowed											
LR	11_10247	4H	0.00	2.60	27	2.0	50	2.8	4	0.7	
	11_10622	5H	103.01	2.31	30	1.8	49	2.8	2	2.8	
	11_10868	6H	24.36	3.48	17	1.5	63	2.8	1	-0.1	YES
	11_20307	7H	9.84	2.62	5	0.2	76	2.6			
	12_31127	Unlinked		2.81	59	2.8	20	1.4	2	3.0	
	12_20775	Unlinked		2.69	74	2.4	6	3.1	1	-0.2	
Six-rowed											
LR											
	12_10314	1H	45.13	3.63	7	2.6	76	3.6			YES
	11_11329	6H	0	3.63	76	3.6	7	2.6			
	11_11483	6H	63.27	3.63	7	2.6	76	3.6			
	12_11475	6H	63.27	3.63	76	3.6	7	2.6			
	11_11261	6H	65.03	3.63	7	2.6	76	3.6			YES
	11_10124	6H	65.03	3.63	76	3.6	7	2.6			
	12_31277	6H	118.35	3.00	76	3.6	5	2.5	2	3.5	
	12_10979	7H	43.38	3.06	37	2.7	45	4.2	1	3.0	
	11_20042	7H	86.44	3.00	10	3.7	72	3.5	1	1.4	YES
	12_20641	Unlinked		3.63	76	3.6	7	2.6			
	12_10491	Unlinked		3.63	76	3.6	7	2.6			YES
	12_30908	Unlinked		3.00	72	3.6	11	3.0			
	12_30939	Unlinked		3.22	72	3.6	11	3.0			

†SFNB-L = Spot form net blotch Langdon isolate, SFNB-D = Spot form net blotch Dickinson isolate, NFNB = Net form net blotch, SB = Spot blotch, LR = Leaf rust, respectively.

Table 2.6. Summary of the most significant QTL identified in 50% of the environments in the 80 two-rowed panel for deoxynivalenol levels and foliar disease resistance based on field experiments.

Traits	QTL	Chromosome	Position (cM)	Number of significant environments	Ave -log (10)	Candidate SNP for MAS
Deoxynivalenol	1	6H	0	2 out of 3	3.08	YES
Foliar disease	2	5H	103.72-108.18	2 out of 3	2.86	
	3		145.35	2 out of 3	2.30	
	4	6H	91.79-96.73	2 out of 3	2.65	

Table 2.7. Summary of the most significant SNP markers identified in 50% of the environments in the two-rowed panel for deoxynivalenol level ad foliar disease resistance based on field experiments.

Traits names†	Chromosome	Position (cM)	Sig markers	QTL	Environments	-Log $_{10}$ (P-value)	Candidate SNP for MAS§
Deoxynivalenol	6H	0.00	11_10496	1	12LA57	3.2026	YES
			11_11329	1	12OS57	2.9535	
Foliar disease	5H	103.72	11_21421	2	13NV57	3.0700	
		103.92	11_10414	2	13NV57	3.2833	
		105.22	12_30098	2	13NV57	3.1664	
		106.09	11_20018	2	12OS57	2.0541	
		108.18	11_21314	2	13NV57	3.3696	
			11_21321	2	12OS57	2.3772	
			12_30855	2	13NV57	2.6825	
		145.35	11_11092	3	12OS57	2.2475	
				3	13NV57	2.3527	
	6H	91.79	12_31235	4	12OS57	2.9266	
		94.73	11_10595	4	13NV57	2.7702	
		96.73	11_10734	4	13NV57	2.2588	

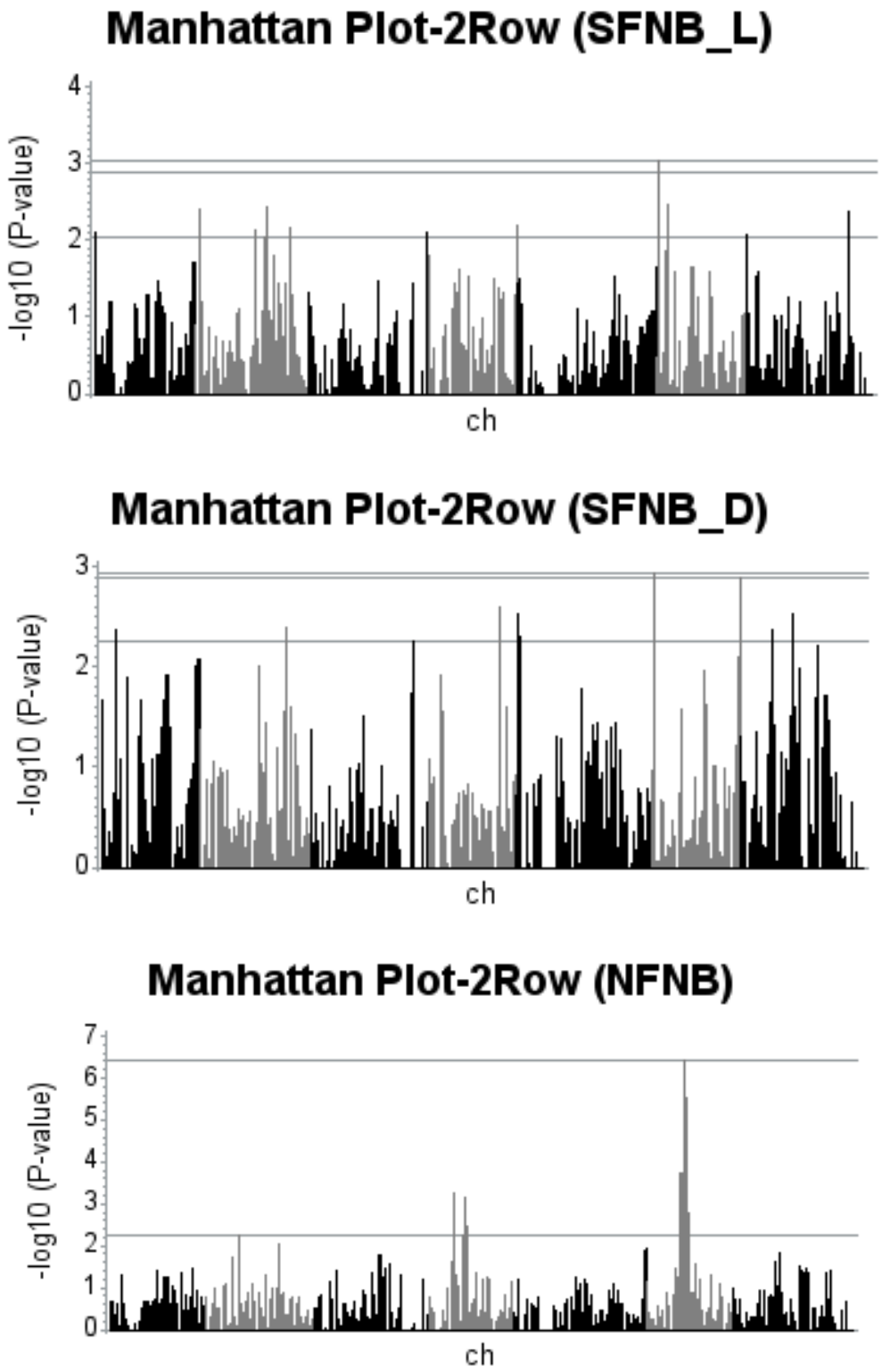

Figure 2.1. Manhattan plots of the studied traits for two-rowed mapping panel (SFNB-L = spot form net blotch, Langdon isolate; SFNB-D = spot form net blotch, Dickson isolate, NFNB = net form net blotch).

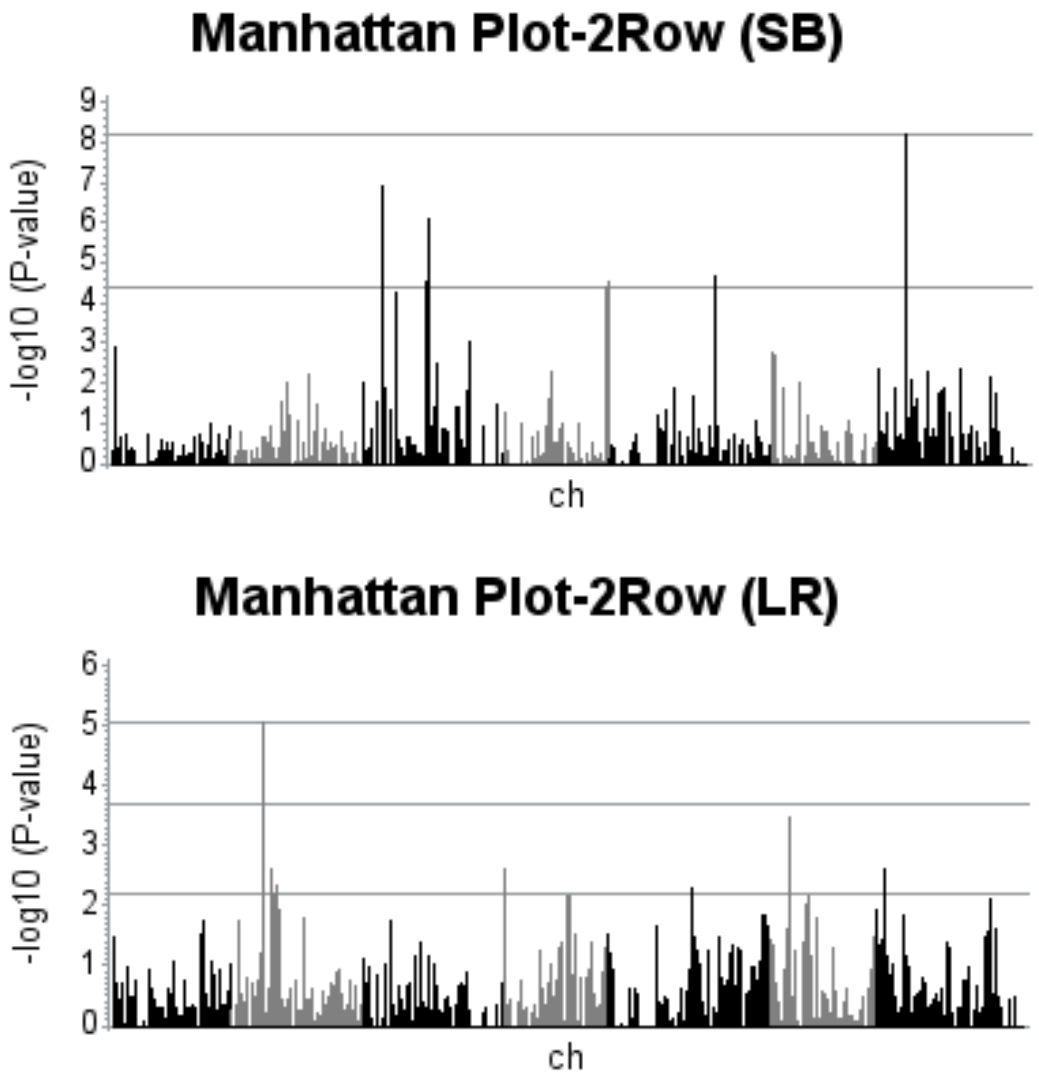

Figure 2.2. Manhattan plots of the studied traits for two-rowed mapping panel (SB = spot blotch, LR = leaf rust).

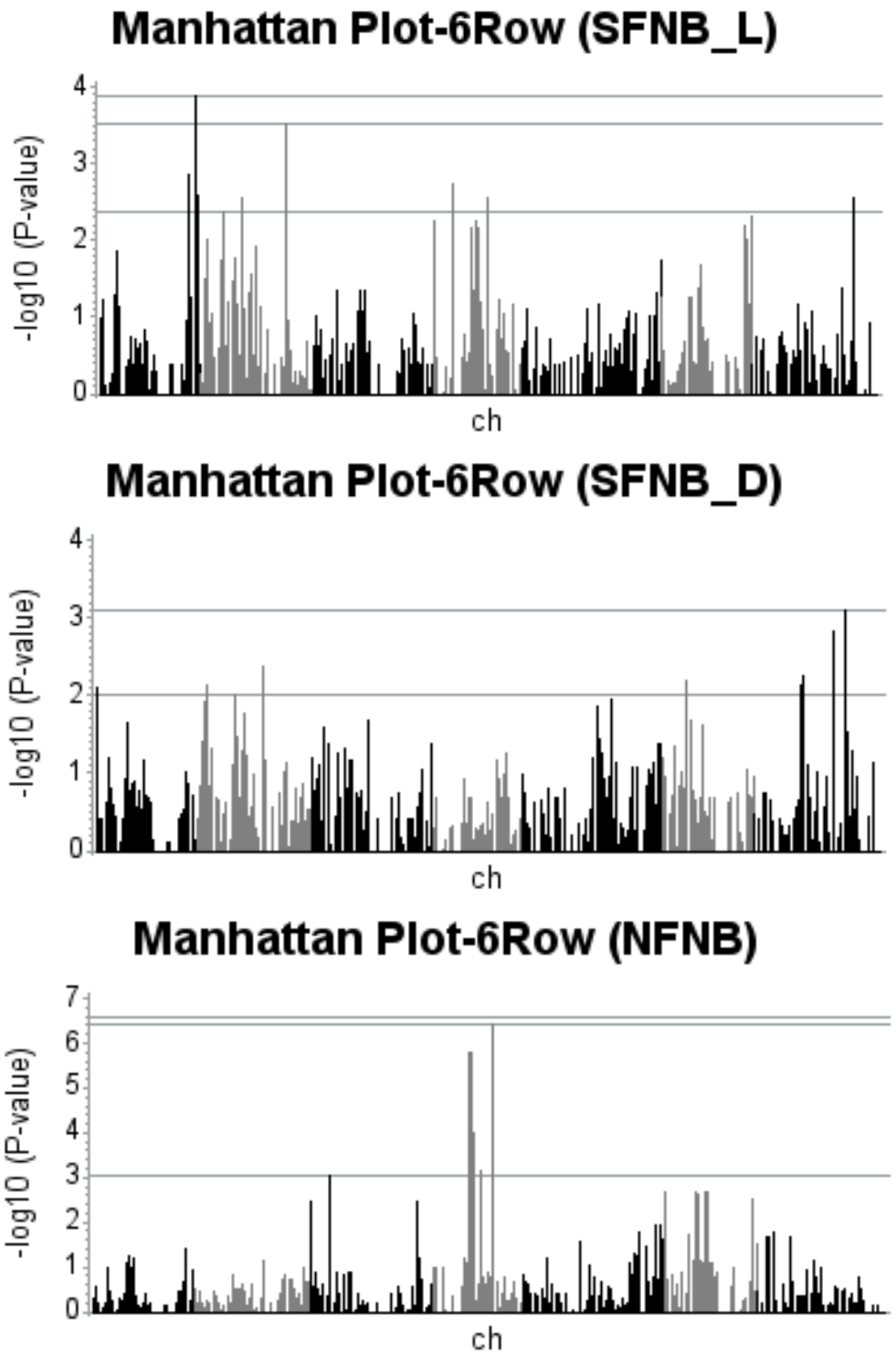

Figure 2.3. Manhattan plots of the studied traits for six-rowed mapping panel (SFNB-L = spot form net blotch, Langdon isolate; SFNB-D = spot form net blotch, Dickenson isolate; NFNB = net form net blotch).

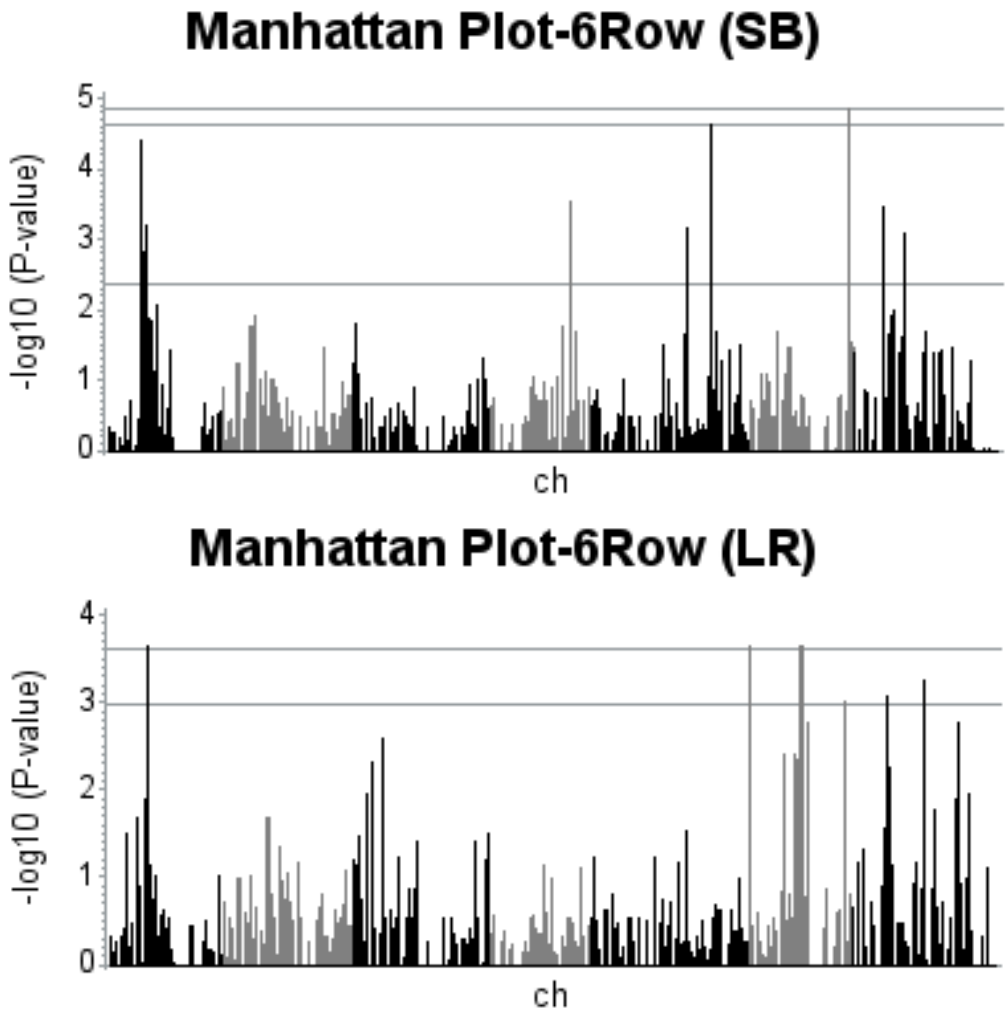

Figure 2.4. Manhattan plots of the studied traits for six-rowed mapping panel (SB = spot blotch, LR = leaf rust).

Discussion

The successful development of malting barley cultivars with resistance to multiple diseases requires the introgression of resistance alleles that function in the target genetic background that are free of linkage to undesirable traits. Success ensures that deployment of host plant resistance continues to be a key component of integrated pest management to manage foliar diseases. Breeding for disease resistance in plants is often difficult because resistance can be inherited both qualitatively and quantitatively, and resistance genes can lose their effectiveness

over time due to changes in pathogen populations. Past progress has relied on parent building after fixing genes for resistance or by exploiting individual segregating populations using phenotypic selection. For several diseases, markers now allow breeders to track resistance alleles across broad arrays of breeding lines within the program, thereby reducing the need for expensive and sometimes unreliable phenotypic screening. An approach to find QTL across diversified germplasm is a way forward to identifying markers that can be broadly deployed simultaneously for several traits in improvement programs. Genome wide association studies across a range of genotypes provide an alternative tool to bi-parental mapping studies (Kraakman et al., 2004) and are also important for the improvement of breeding programs of self-pollinated crops (Kraakman et al. 2004, 2006; Hayes and Szücs, 2006; Roy et al., 2010; Bradbury et al., 2011, Gutiérrez, 2015). At the same time, GWAS may also capture previously undetected genomic regions associated with traits of interest. This research has successfully identified SNP markers across NDSU genetic backgrounds associated with disease resistance traits that may enable plant breeders to utilize MAS or genomic selection for these complex traits during early stages of cultivar development, improving barley breeding efficiency for these traits, and enabling development of broad-spectrum resistance lines to manage susceptibility to multiple diseases in barley.

I generated mapping panels comprised of NDSU two- and six-rowed barley lines derived from the 2006-2009 Barley CAP. The representative mapping panels were built following the procedure described by Negeri (PhD dissertation, NDSU 2009) that selects a mapping panel of breeding lines that maximizes diversity among lines, with limited diversity in each of the subpopulations. The criterion for selection was based on the subpopulation membership coefficient of a line in an inferred subpopulation cluster (Mamidi et al., 2013). Studies sampling

advanced breeding material could be severely limited by the effects of inbreeding and/or selection (Visioni et al., 2013). However, by sampling based on subpopulation structure, I inevitably introduced sources of population structure that could be confounded in mixed regression models.

I used four mixed models (Comadran et al., 2009, Gurung et. al., 2011) that control for the confounding effect of population structure and population relatedness, which can cause false positives (Myles et al., 2009). This is necessary given that the breeding programs sampled used have strong population structure in itself. I chose the best model for interpreting association mapping results based on the least MSD as described by Stich et al. (2008). Furthermore, I selected the cutoff for significance based on the bootstrap approach proposed and implemented in Mamidi et al. (2014) and Gurung et al. (2014). This method takes the extreme tail of distribution of *P-value*s from an empirical distribution. I chose this approach over a cutoff *P-value* because it is dependent on the distribution of phenotype, the variation explained by the marker, structure and relatedness of the population, and heritability of the trait. With this, the *P-value* cutoff will vary from trait to trait. The percentile *P-value* cutoffs derived from empirical distribution were all within the 0.01 error level, and the significant markers were distributed in many chromosomes. This is expected given the complex nature of these disease traits.

Additionally, to minimize the markers that could be used for validation and MAS, while narrowing the QTL region and selecting for large-effect QTL, I selected MTAs significant at $P \leq 0.001$ ($-\text{Log}_{10} \geq 3.0$) and residing within 5 cM of each other, which were considered as belonging to the same QTL. Additionally, I considered SNPs as candidates for MAS that met strict MTA criteria.

The majority of the QTL I identified are considered novel and have not been identified in previous mapping studies. This could be because of the limited genetic background of the bi-parental populations in which previous QTL were identified. However, several of the QTL identified in my study reside in regions similar to those identified earlier by other scientists. Based on different genetic maps, however, it is hard to determine if they are exactly the same.

One of the significant SNPs for SFNB resistance evaluated using the Langdon isolate, the QTL in chromosome 4H in the six-rowed panel could be the same QTL identified earlier by Grewal et al. (2008), which was named as *QRpts4* and explained 21% of the phenotypic variation in their bi-parental cross. Despite the possibility that marks *QRpts4*, this SNP didn't meet my criteria to use as a candidate for MAS.

In screening for SFNB resistance using the Dickinson isolate and the two-rowed panel, I found a QTL in chromosome 6H in a similar region as one identified by Grewal et al. (2008) that was named *QRpts6* (75-78cM). Again, this SNP didn't meet my criteria to use as a candidate for MAS.

Furthermore, another QTL I found in chromosome 7H using the six-rowed panel was in the similar region as the QTL *QRpt7* identified by Grewal et al. (2008). Again, this SNP didn't meet the criteria to use as a candidate for MAS.

For NFNB resistance detected using the two-rowed mapping panel, the QTL in chromosome 4H could be the QTL *QRpts4* identified by Grewal et al. (2008) and the QTL in chromosome 6H could be the *Rpt5* locus identified by Manninen et al. (2006).

Using the six-rowed mapping panel for NFNB resistance, the second QTL in chromosome 6H could be the QTL *QRpts6* identified by Grewal et al. (2008). However, this SNP didn't meet my criteria for its use as a candidate for MAS.

For SB resistance, I detected a QTL using the two-rowed panel in chromosome 3H in a similar region (80.9 cM) as a QTL identified by Gutiérrez et al. (2015) using Latin American germplasm at 81 cM, designated Rcs-qtl-3H-4-6. The QTL in chromosome 7H (31.75 cM) is likely the same QTL identified by Zhou and Steffenson (2013b) in US breeding genotypes at 31.7 cM. They named the QTL Rcs-qtl-7H-11_20162.

Using the six-rowed panel for screening SB resistance, the QTL in chromosome 1H is in the same region as a QTL identified by Zhou and Steffenson (2013b). They concluded that the QTL Rcs-qtl-7H-11_20162 lies at the *Rcs5* locus described by Drader (2010) in the Steptoe/Morex bi-parental mapping population.

For the two-rowed LR resistance, four QTL were identified in chromosomes 2H, 4H, 5H and 7H. All of the QTL appear to be novel, except the one in chromosome 2H at 39.1cM, which might be an allele previously reported and designated *Rhp15* (Chicaiza, 1996; Franckowiak et al., 1997). Weerasena et al. (2004) identified a co-dominant marker *for Rph15* about 25.2cM distal from the centromere that co-segregated with the AFLP marker P13M40.

In screening for LR resistance using the six-rowed panel, the QTL in chromosome 7H is in a similar region as the QTL identified by Marcel et al. (2007), having an allele designation of *Rhpq8*. This QTL is related to a defense gene homologue with WBE101 (HvNR-F1). Despite this apparent association, this SNP didn't meet the criteria as a candidate for MAS.

I identified one significant QTL for DON levels based on field experiments. This QTL and its associated SNPs, one of which was recommended as a MAS candidate, was located on chromosome 6H at 0.0 cM. I believe that this is a novel QTL for DON levels. Massman et al, (2011) found QTL on 6H associated with Fusarium head blight and DON levels. However, these were located at positions 42-67 cM (DON and Fusarium head blight) and 124-127 cM (Fusarium

head blight). Dahleen et al. (2003) reported a QTL associated with low DON levels and later heading dates.

A high number of polymorphisms can help us to better understand and dissect the genetics of resistance and to identify novel genomic regions linked with resistance. This could be done using the emerging new generation sequencing technologies such as genotyping by sequencing (GBS) and resequencing. These technologies provide a deeper coverage of the genome and can increase the number of polymorphisms identified, which might help identify SNPs within casual genes or close by, or even the casual variant itself. For example Mamidi et al. (2014) identified QTLs having non-synonymous substitutions in the gene *FRE1* that have a major role in iron homeostasis.

Many of SNPs associated with disease resistance in this study based on greenhouse screening have potential to be used to enhance the NDSU barley breeding program through MAS. However, the allelic combinations need additional research to validate the markers I detected across additional genetic backgrounds and to ensure that those identified based using greenhouse screens are applicable to resistance demonstrated in the field. Furthermore, those QTL and SNPs identified using seedling assays need to be confirmed as conferring resistance in adult plants where protection is required beyond the seedling stage.

Detecting QTL is important to utilization of MAS in breeding programs, as well as for pyramiding genes. This is especially true when a breeder's goal is to pyramid resistance genes for multiple diseases into single commercial cultivars to provide strong and durable resistance. It is important to confirm the expression level of the traits detected by GWAS through additional field testing, and in germplasm having different backgrounds (Williams et al., 2003). A positive result from this study is that the NDSU barley breeding program now has the wealth of

information required to build it's own "SNP-chip" for future screening of early generations in the breeding program. All of the SNPs found meeting our criterion as MAS candidates can be used for further validation studies.

NDSU breeders have been selecting against susceptible genotypes over many years. For this reason, some of the favorable alleles are present at a high frequency in the program. Some disease resistance traits such as SFNB, are genetically complex, and require more specific studies to increase the precision and find markers for AM. The accuracy of the phenotypic evaluation is critical to have consistently good data for further studies.

Conclusion

Significant marker-trait associations for the SFNB, NFNB, SB and LR using the two-rowed and six-rowed mapping panels were identified. Many of the QTLs coincided in chromosomal regions where QTL had been previously detected using bi-parental and GWAS methodologies. In fact, several of the MTAs found were previously reported by others; thus, validating the effectiveness of this study. Novel putative QTLs identified in the present study provide additional genomic regions that may be associated with disease resistance. The markers identified here should also be validated in other genetic backgrounds to further establish their utility for MAS or use directly in MAS if that was recommended. Additionally, a greater number of markers in the areas where MTAs were detected help narrow QTL regions and can ensure minimal recombination between genes of interest and the associate SNPs that reside adjacent to them.

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CHAPTER 3. QTL IDENTIFICATION OF AGRONOMIC AND MORPHOLOGICAL TRAITS IN NORTH DAKOTA GERMPLASM USING ASSOCIATION MAPPING

Abstract

Developing malting barley (*Hordeum vulgare* L.) quality cultivars with high yield and superior agronomic characteristics is a key objective of the North Dakota State University (NDSU) barley breeding program. In addition to prescribed trait characteristics for malting quality, growers expect new cultivars to provide high stable yields and possess other traits such as lodging resistance and appropriate plant height to enable ease of harvesting. The objective of this research was to identify marker-trait associations for key agronomic traits using a genome-wide association mapping approach, using selected NDSU two-rowed lines included in 2006-2009 USDA-NIFA-NRI Barley Coordinated Project. Based on strict criteria including significance across the majority of test environments, I identified QTL-associated SNPs that should be good candidates for use in marker-assisted selection (MAS) for favorable spike length, resistances to stem breakage and deciduous awns, rachilla hair length, and awn barbing type. For yield and for several other agronomic traits, no QTL-associated SNPs were identified that met my criterion for MAS. Such QTL were more environmentally specific or significant in a minority of environments. My results highlight the challenge of yield enhancement in the NDSU breeding pool due to the absence of major QTL impacting yield across multiple environments and low correlations between yield and other traits for which selection could be applied.

Introduction

Barley (*Hordeum vulgare* L.) is one of the oldest crops in the world and is considered a model crop for agricultural development and scientific study. Barley has three main uses: the raw material for malt that is used in brewing, livestock and poultry feed, and a source of healthy food

for human consumption. (Ullrich, 2011). In recent years, barley has been the fourth most widely grown cereal crop globally (FAOSTAT: http//faostat.fao.org/site/330/defaut.aspx). There is a need to increase crop production of all major food crops by 60% to provide adequate supplies for the growing world population, which is projected to reach nine billion of people in 2050 (http://faostat3.fao.org). In the USA, Canada, Europe and Russia, barley area sown is declining, being replaced by more profitable crops such as corn (*Zea mays* L.) and soybean (*Glycine max* L.) (http://faostat3.fao.org/browse/Q/QC/E, FAO 2015).

Barley breeding programs need to evaluate large numbers of experimental lines in order to identify adapted cultivars having desirable traits. For this reason, the cost of conducting breeding programs is high, especially for yield trials and malt quality analyses. A primary goal of the North Dakota State University (NDSU) breeding program is to develop adapted cultivars with improved grain yield, malt quality, and disease resistant for regional growers. Despite ongoing efforts, challenges remain because many of the agronomic, disease, and quality traits have a complex or quantitative inheritance.

One of the tools modern breeding programs used to understand the genetic basis of complex traits is linkage analysis, which detects quantitative trait loci (QTL) that control phenotypic variation. Bi-parental mapping populations created by crossing two parents that are polymorphic for a specific trait have been used to study linkage disequilibrium (LD). One restriction of using bi-parental populations is the small number of recombinant events that occur, resulting in lower QTL mapping resolution (Flint-Garcia et al., 2003; Zhu et al. 2008).

With the advent of more cost-effective, high throughput sequencing and genotyping methods, association mapping (AM) has become a more attractive approach for QTL mapping in plants (Atwell et al., 2010). This method uses information from ancestral recombination events

that occur in the population and takes into account all major alleles present in a population to identify significant marker-trait associations (MTAs) (Muñoz-Amatriaín et al., 2014).

The use of AM and marker-assisted selection (MAS) should help NDSU barley researchers understand the underlying genetic control of agronomic traits and develop cultivars in a more efficient and effective way that accelerates genetic gain and reduces the costs associated with expensive phenotyping. The first objective of research covered in this chapter was to identify QTL using genome-wide association mapping (GWAS) for agronomic and morphological traits in the NDSU two-rowed barley training population. A second objective was to identify QTL that may be candidates for MAS. Ultimately, the success of barley production relies upon genetically controlled and environmentally influenced cultivar attributes that influence harvestable yield, including numerous agronomic and morphological traits discussed herein and evaluated in this study.

Materials and Methods

Experimental Population, Experimental Design and Phenotyping

The NDSU two-rowed mapping panel of 81 lines and checks (Tradition, Colon, and Pinnacle) was used for this research. The selection of individuals that comprise the mapping panel is described in Chapter 2. Field experiments were conducted at a minimum of two locations in each of three years. Eighty-one two-rowed lines from the NDSU breeding program were assigned to experimental units at each location using a 9 x 10 rectangular lattice design. Each entry was replicated two times at each location. Nine adapted malting barley cultivars ('Lacey', 'Rasmusson', 'Rawson', 'Robust', 'Tradition', 'Stellar-ND', 'Quest', 'Conlon', and 'Pinnacle') were included as checks. In 2011, the breeding lines were evaluated under rainfed conditions at the McVille, ND research site and under irrigated conditions near Ray, ND at the

Nesson Valley research site. The phenotypic data collected from the McVille yield trial in 2011 was limited to days to heading (both experiments) due to heavy rains that saturated soils following heading. In 2012 and 2013, the breeding lines were evaluated under rainfed conditions at the Fargo and Osnabrock, ND research sites and under irrigated conditions at Nesson Valley. Each experimental unit consisted of seven 2.43-m rows spaced 19.1 cm apart, and with the total plot area of 3.72 m^2. A seeding rate of 2.47 million seeds ha^{-1} was used at the rainfed sites and 3.71 million seeds ha^{-1} was used at the irrigated site.

Agronomic Traits

The following agronomic traits were evaluated as follows:

Number of days to heading

The number of days to heading was recorded as the number of days after 31 May when 50% of the spikes were completely emerged from 50% of the plants in an individual plot.

Plant height

Plant height was the distance in centimeters from the ground to the tip of the spikes, excluding the awns, taking as an average of two measurements per plot.

Stem breakage

Stem breakage severity was evaluated at harvest maturity using a 1 to 5 scale (1 = no stem breakage and 5 = severe stem breakage).

Deciduous awns

Deciduous awns were evaluated using a 1 to 5 scale (1 = intact awns and 5 = absence of awns).

Yield

Yield data were collected at maturity. Grain was harvested using a plot combine, dried in a forced-air dryer to approximately 135 g kg^{-1} moisture, de-awned, and cleaned. The weight of each plot was recoreded in grams and the clean yield data expressed in (kg ha^{-1}).

Number of kernels per spike

Data were collected from five random spikes sampled from each plot from two locations each year. Number of kernels per spike (KS) was measured by counting the number of kernels per spike.

Spike length

Data were collected from five random spikes sampled from each plot from two locations each year. Spike length was determined by measuring the length of the spike, excluding the awns in mm.

Morphological Traits

Data on morphological data also were collected from five random spikes sampled from each plot from two locations each year. Data were collected on awn barbing and rachilla hair length.

Rachilla hair length

Rachilla hair length was evaluated using a stereoscope to classify the hairs as either 1 = long or 2 = short.

Lemma awn barbing type

Lemma awn barbing type was evaluated by touching the awn along its length that is attached to the kernel and rating as either 1=smooth, 1.5=semi-smooth or 3=rough.

Statistical Analysis

Statistical analyses for all phenotypic traits were conducted using the MLM procedure of SAS 9.3 using Proc Mixed method type 3 (SAS Institute Inc., 2004). Analyses were conducted for each individual environment. Phenotypic data means were adjusted based on the least squares means (LSMeans) for each entry, within environments. A combined analysis across locations was conducted for all traits demonstrating uniform variances and residuals across locations using the LSMeans from the individual environments. *F*-tests for the combined analyses were considered significant at $P \leq 0.05$ and mean separation in the combined analyses was done using an *F*-protected LSD at $P = 0.05$.

Pearson correlations (Steel and Torrie, 1980) between the traits, based on LSMeans from each environment, were calculated using the CORR procedure in SAS 9.3 (SAS Institute Inc., 2004). Correlations values were considered significantly different from zero at $P \leq 0.05$. Principal component analysis (PCA) was performed using SAS 9.3 to create a biplots to visualize the relationships (correlation and direction) amongst quality traits (SAS Institute Inc., 2004).

Six-character environment code designations used for presenting the results of analyses were created as follows: the first two digits represent the year (11=2011; 12=2012 and 13=2013); the next two characters are for the locations (MC = McVille; NV= Nesson Valley; FA= Fargo, OS = Osnabrock and LA= Langdon); and the last two digits represent the row type of experiment (57= two-rowed type).

Genome Wide Association Mapping Analyses and Marker-Trait Association

Descriptions of methods used for handling the genotypic data, AM analyses, marker-trait associations (MTAs), and procedures are the same as described in Chapter 2.

Results

Phenotyping and Phenotypic Correlations

Unfavorable growing conditions, including above average precipitation and temperatures throughout the growing season in 2011 in Nesson Valley may have reduced starch accumulation in the kernels as described by Wallwork et al. (1998). In 2012, all locations had excellent growing conditions, and in 2013 the Fargo location experienced below average precipitation and high temperatures, and a grasshopper infestation that likely reduced yield potential. Significant genotypic variation was detected for most agronomic traits based on the combined ANOVA across environments (Table 3.1).

Thirteen of the 36 pairs of traits had correlation values that were significantly different from zero $P \leq 0.05$ (Table 3.2). Moderately strong correlations ($r \geq 0.65$) were found for days to heading vs. kernels per spike ($r = 0.73$) and kernels per spike vs. spike length ($r = 0.65$). The association between yield and plant height has been well documented in barley, including the identification of QTL impacting both traits by Wang et al. (2014). However, in my study the two traits were not significantly correlated (Table 3.2).

The agronomic trait panel biplot (Figure 3.1) shows the relationship amongst traits. The angle of the trait vectors represents the correlation between the traits while the length of the trait vector measures the variation of the trait. Traits that are correlated and have arrows facing the same general direction tend to be positively correlated, as between yield and stem breakage (ST), which are significantly and positively correlated. Those that are negatively correlated, such as

Table 3.1. Mean, minimum, maximum, and standard deviation for the two-rowed mapping population and checks (Conlon, Pinnacle, and Tradition) as grown in up to seven environments in North Dakota, 2011-2013.

Traits		Number of environments†	Mean	Minimum	Maximum	Standard deviation
Yield (kg ha^{-1})	Mapping panel	6	3783.7a‡	2374.9	4755.2	446.6
	Avg. checks		3722.1a	3579.1	4501.5	284.3
Kernels spike^{-1}	Mapping panel	5	21.6a	17.2	28.9	0.8
	Avg. checks		32.7b	19.4	61.3	18.5
Spike length (mm)	Mapping panel	5	74.2a	59.5	91.2	2.4
	Avg. checks		75.0a	67.3	80.2	3.7
Days to heading (days after 31 May)	Mapping panel	7	29.3a	24.9	35.1	2.9
	Avg. checks		29.0a	26.0	31.0	1.4
Plant height (cm)	Mapping panel	6	72.9a	58.4	83.6	3.4
	Avg. checks		76.3a	73.0	83.6	3.0
Stem breakage (1-5§)	Mapping panel	4	2.0a	0.8	4.6	0.1
	Avg. checks		2.4a	1.6	3.5	0.7
Deciduous awns (1-5¶)	Mapping panel	3	2.1a	1.1	4.7	0.4
	Avg. checks		2.0a	1.7	2.3	0.2
Rachilla Hair length	Mapping panel	5	1.73a	1.0	2.0	0.4
	Avg. checks		1.50b	1.5	1.5	0.0
Awn Barbing type	Mapping panel	5	1.67a	1.0	2.0	0.4
	Avg. checks		2.00b	2.0	2.0	0.0

†Number of environments where data were collected.
‡Means for a trait followed by the same letter are not different at $P = 0.05$ as determined using an *F*-protected LSD.
§A score of 1= no stem breakage at harvest maturity and 5 = severe stem breakage at harvest maturity.
¶A score of 1 = no deciduous awns at harvest maturity and 5 = severe deciduous awns at harvest maturity.

Table 3.2. Phenotypic correlations for barley and agronomic traits† across several environments for the NDSU two-rowed mapping panel.

	YD	KS	SL	HD	HT	ST	DA	AB	RH
YD	1‡	0.0844	0.4864	0.1538	0.2904	0.0383	0.1222	0.2763	0.0165
KS	-0.19417	1	<.0001	<.0001	0.0028	0.6125	<.0001	0.0011	0.0743
SL	-0.07894	**0.64704**	1	0.0002	<.0001	0.089	0.0786	0.9755	0.0213
HD	-0.16095	**0.72888**	**0.40334**	1	0.2313	0.0003	<.0001	0.2483	0.0053
HT	0.11965	**0.33016**	**0.43743**	0.13535	1	0.009	0.0683	0.6233	0.9506
ST	**0.23213**	-0.05748	0.19141	**-0.39535**	**0.29024**	1	0.0388	0.2057	0.5252
DA	-0.17424	**0.46006**	0.19780	**0.50027**	0.20486	**-0.23156**	1	0.1493	0.0718
AB	-0.12318	**0.35929**	0.00349	0.13059	0.05575	0.14301	0.16269	1	0.4831
RH	**-0.26737**	0.20064	**0.25716**	**0.30918**	-0.00703	-0.07207	0.20238	0.07954	1

†YD = yield, KS = number of kernels per spike, SL = pike length, HD = days to heading, HT = plant height, ST = stem breakage, DA = deciduous awns, RH = rachilla hair length, and AB = awn barbing type.

‡Probability values appear above the diagonals and correlation coefficients appear below the diagonals in the table. Correlation values in bold font are significant at $P \leq 0.05$.

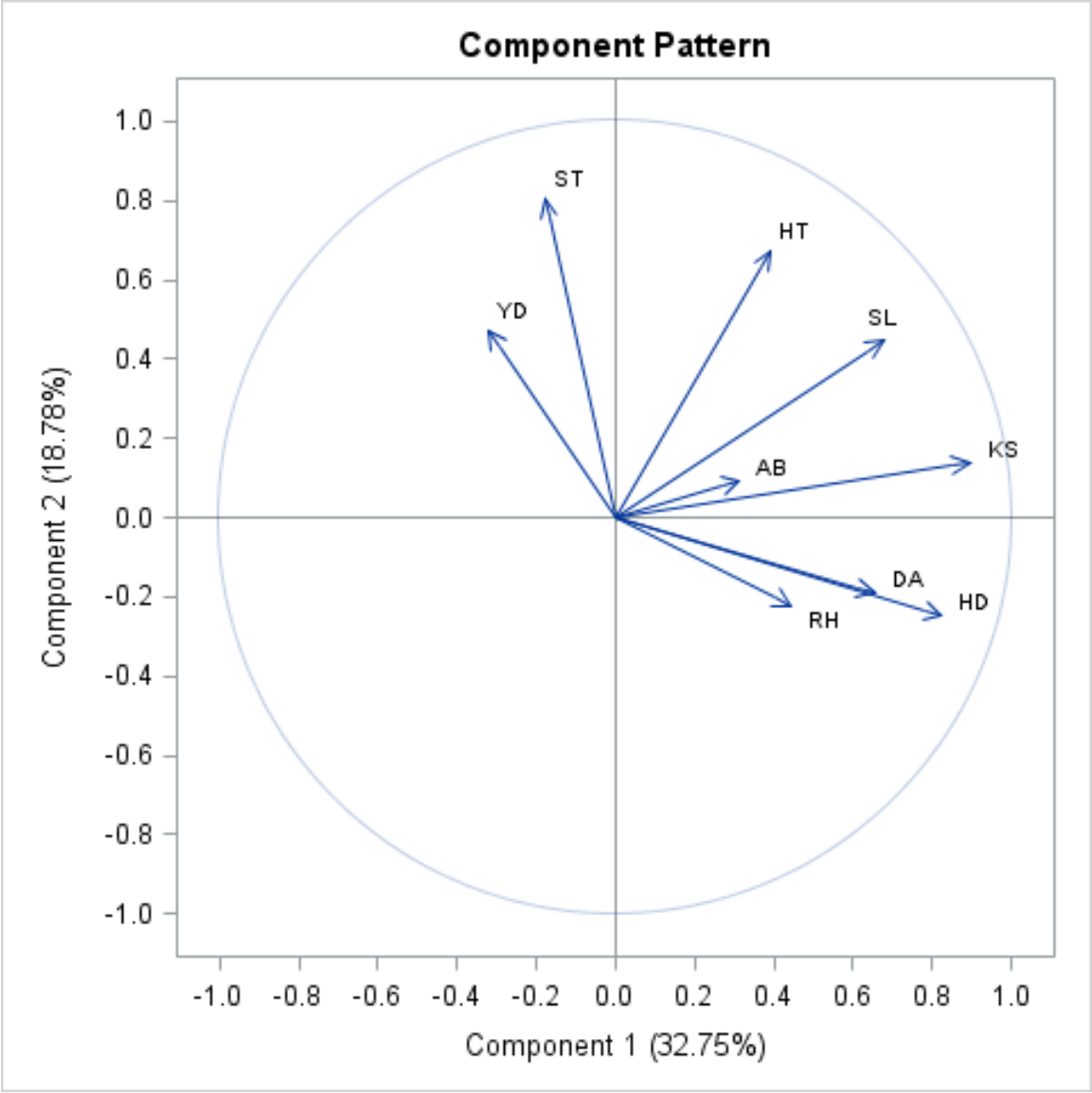

Figure 3.1. Biplot of the two-rowed malting panel phenotypic data showing relationship between agronomic traits. YD = yield, KS = number of kernels per spike, SL = spike length, HD = days to heading, HT = plant height, ST = stem breakage, DA = deciduous awns, RH = rachilla hair length, and AB = awn barbing type.

yield and rachilla hair length, have arrows facing generally opposite directions. Angles between traits approaching 90^{o}, such as that between yield and spike length, are indicative of low correlation.

Marker-Trait Associations

The linear regression models used to calculate the *P*-value of the MTAs amongst the Naïve, principle component (PC), Kinship (K), and PC+ K analyses varied by environment. The best models were identified using the mean square difference (MSD) method of Mamidi et al. (2011) for each environment-trait combination as highlighted in Appendix table A4. The models with the minimum MSD for each trait were used for the AM analyses. Marker-trait associations significant at $P \leq 0.01$ ($-\text{Log}_{10} \geq 2.0$), residing within 5 cM of each other, and detected in > 50% of the environments were considered as belonging to the same QTL. Additionally, I considered SNPs as candidates for MAS if the associated MTAs were detected in at least 50% of the environments and the mean $-\text{Log}_{10}$ *P*-values of the MTAs across environments were ≥ 3.0.

Field Experiments

Yield

No QTL or MTAs were detected for yield in any chromosome based on the selection criteria.

Number of kernels spike^{-1}

No QTL or MAS were identified for kernels spike^{-1} based on the selection criteria.

Spike length

For spike length, three QTL were identified in chromosome 5H, with associated SNPs located at 2.81 cM, 87.35 cM, and in a range between 151.36 and 152.79 cM, respectively (Table 3.3). The first QTL was significant in three out of five environments and the other two were significant in four out of five environments. Amongst these, only SNP 12_31221, associated with

the QTL at 151.36-152.79 cM, met the qualifications to be a candidate for MAS of spike length (Table 3.4, Appendix table A5).

Days to heading

For days to heading, QTL were identified in chromosome 3H at 78.53cM, in chromosome 7H in the range of 107.11 to 110.99 cM, and a third that was unlinked (Table 3.3). None, however, had SNPs that met the criteria to be used for MAS of days to heading (Table 3.4, Appendix table A5).

Plant height

Three QTL were identified for plant height. They were located in chromosome 4H in a range from 3.74 to 5.55 cM, chromosome 6H at 118.35 cM, and a third QTL for plant height was unlinked (Table 3.3). While the QTL in chromosome 6H was significant in four out of six locations, it did not meet the criteria as a candidate for MAS of plant height since none of the MTA had mean $-\text{Log}_{10}$ *P*-values ≥ 3.0 across environments (Appendix table A5).

Stem breakage

For stem breakage, two QTL were identified. They were found in chromosome 2H at 0 cM and in chromosome 4H at 106.03 cM (Table 3.3). The QTL in chromosome 2H had no associated SNPs that met the criteria to be candidates for MAS; however, the SNP 11_20974 in chromosome 4H was significant in three out of four environments and had a mean $-\text{Log}_{10}$ *P*-value across environments of 3.59. Thus it is a candidate for use in MAS for resistance to stem breakage (Table 3.4, Appendix table A5).

Deciduous awns

Seven QTL were identified for deciduous awns that were significant in two of the three environments where data were collected. Two were identified in chromosome 1H (35.45 cM and 59.71 cM), two in chromosome 5H (103.72 cM and from 180.71-182.88 cM), and three that were unlinked (Table 3.3). For these QTL, five associated SNPs met the requirements to be considered candidates for MAS (12_30820 and 12_30821 in chromosome 1H at 59.71 cM; 11_21421 in chromosome 5H at 103.72; and two unlinked SNPs, 12_30916 and 12_30944) (Table 3.4, Appendix table A5).

Rachilla hair length

For rachilla hair length, QTL significant in all five environments were identified in chromosomes 4H (40.35 cM) and 5H (99.56cM). An additional unlinked QTL also was identified (Table 3.3). Of these, the SNP 11_20526 on 5H at 99.56 cM and the unlinked SNP 12_30129 met my requirements as candidates for MAS of rachilla hair length (Table 3.4, Appendix table A5).

Awn barbing type

Four QTL were identified for awn barbing that were significant in four or more of the five environments where this trait was phenotyped (Table 3.3). Each was represented by a SNP meeting my criterion as candidates for MAS. These were SNPs 12_30933 in chromosome 1H at 6.03 cM, 11_11273 in chromosome 5H at 111.68 cM, 12_11298 in chromosome 5H at 123.33 cM, and 11_20245 in chromosome 7H at 12.42 cM (Table 3.4, Appendix table A5).

Table 3.3. Summary of the QTL identified in more than 50% of the environments using the NDSU two-rowed mapping panel and candidate SNPs for MAS.

Traits	Chromosome	Position (cM)	Number of significant environments	Mean –Log 10 (P-value)	Candidate SNP for MAS
Spike length	5H	2.81	3 out of 5	2.62	
Spike length	5H	87.35	4 out of 5	2.74	
Spike length	5H	151.36-152.79	4 out of 5	3.08	12_31221
Days to heading	3H	78.53	4 out of 7	2.20	
Days to heading	7H	107.11-110.99	5 out of 7	2.73	
Days to heading	Unlinked		4 out of 7	2.56	
Plant height	4H	3.74-5.55	3 out of 6	2.67	
Plant height	6H	118.35	4 out of 6	2.23	
Plant height	Unlinked		3 out of 6	2.25	
Stem breakage	2H	0	3 out of 4	2.72	
Stem breakage	4H	106.03	3 out of 4	3.59	11_20974
Spike angle	2H	0	3 out of 4	2.59	
Spike angle	4H	106.03	3 out of 4	2.64	
Deciduous awns	1H	35.45	2 out of 3	2.74	
Deciduous awns	1H	59.71	2 out of 3	3.45	12_30820 12_30821
Deciduous awns	5H	103.72	2 out of 3	4.13	11_21421
Deciduous awns	5H	180.71-182.88	2 out of 3	2.87	12_30769
Deciduous awns	Unlinked		2 out of 3	4.04	12_30916
Deciduous awns	Unlinked		2 out of 3	4.04	12_30944
Deciduous awns	Unlinked		2 out of 3	3.87	12_31279
Rachilla hair length	4H	40.36	5 out of 5	2.76	
Rachilla hair length	5H	99.56	5 out of 5	3.28	11_20526
Rachilla hair length	Unlinked		5 out of 5	4.44	12_30129
Awn barbing type	1H	6.03	5 out of 5	3.89	12_30933
Awn barbing type	5H	111.68	5 out of 5	4.80	11_11273
Awn barbing type	5H	123.33-123.52	5 out of 5	3.60	12_11298
Awn barbing type	7H	12.42	4 out of 5	3.23	11_20245

Table 3.4. Candidate single nucleotide polymorphisms (SNPs) for marker-assisted selection (MAS) of barley agronomic traits in NDSU two-rowed barley.

Traits	Chromosome	Position (cM)	Number of significant environments	Mean -Log_{10} (*P*-value)	SNPs
Spike Length	5H	152.79	3 out of 5	3.19	12_31221
Stem Breakage	4H	106.03	3 out of 4	3.59	11_20974
Deciduous awns	1H	59.71	2 out of 3	3.15	12_30820
Deciduous awns	1H	59.71	2 out of 3	3.70	12_30821
Deciduous awns	5H	103.72	2 out of 3	4.13	11_21421
Deciduous awns	5H	182.16-182.88	2 out of 3	3.05	12_30769
Deciduous awns	Unlinked		2 out of 3	4.04	12_30916
Deciduous awns	Unlinked		2 out of 3	4.04	12_30944
Deciduous awns	Unlinked		2 out of 3	3.87	12_31279
Rachilla hair length	5H	99.56	5 out of 5	3.28	11_20526
Rachilla hair length	Unlinked		5 out of 5	4.44	12_30129
Awn barbing type	1H	6.03	5 out of 5	3.89	12_30933
Awn barbing type	5H	111.68	5 out of 5	4.80	11_11273
Awn barbing type	5H	123.33	5 out of 5	3.60	12_11298
Awn barbing type	7H	12.42	4 out of 5	3.23	11_20245

Discussion

Improved agronomic performance is critical for successful barley production. Understanding the genetic control of these traits and enabling their improvement through MAS should improve the efficiency and efficacy of breeding operations, as well as increase the rate of genetic gain for these traits.

Phenotypic Variation

Phenotypic variation for all traits analyzed indicates there is ample opportunity for further selection and genetic gain. An interesting finding for my mapping population was the relative

lack of correlation of yield with other traits, despite significant variation for these traits. Examples in the literature where yield was related with other traits includes the correlations between plant height and yield previously reported by Wang et al. (2014) and between kernels spike^{-1} and yield as reported by Locatelli (2013). In the present study, only stem breakage and rachilla hair length had significant correlations with yield; yet, these relationships were relatively weak (r = 0.23* and R=-0.26*, respectively). Additionally, while the relationship between yield and stem breakage can be explained, no causal relationship between yield and rachilla hair length is intuitively obvious.

Marker-Trait Associations

Field Experiments

Yield

Two QTL for yield were identified in chromosome 1H based on MTA's. However, the associated SNPs, while residing with 5 cM of each other and detected in more than 50% of the test environments, did not meet the criterion for MAS candidates because they were not significant at $P \leq 0.01$ ($-\text{Log}_{10} \geq 2.0$). Therefore, no QTL or associated SNPs were identified as MAS candidates for yield based on criteria I used in this study. This is similar to findings by Wang et al. (2014), who found that yield QTL significance varied across environments and no QTL that were significant at more than a single environment. They also found that many of their putative yield QTL were associated with plant height, an association based on correlation analysis that did not exist in my experiments. Furthermore, my results differed from those of Hayes et al. (1993), who identified fourteen QLT for yield, including five that were confirmed in follow-up studies by Romagosa et al. (1996, 1999) and Han et al. (1999). The research in their studies used the Steptoe x Morex mapping population or additional Steptoe x Morex DH lines

not included as part of the original mapping population. This cross represents one where a feed barley (Steptoe) adapted to the Northwest US was crossed to a malting barley (Morex) adapted to the Midwest US. Because the two cultivars are adapted for very different growing regions and are very diverse in their germplasm base, discovery of multiple QTL for yield and other traits is not surprising. Entries for the NDSU mapping panel are generally adapted for production in the Midwest US and were selected from 384 total lines so diversity was maximized in the panel. Thus, it is not surprising to find fewer QTL in the relatively adapted NDSU two-rowed panel than the wide-cross represented by the Steptoe x Morex population. Other factors that may have limited the number of yield QTL detected in my study include SNP x environment interactions that occurred due to different environmental and abiotic stresses, including heat and drought stress that impacted yield realization.

Number of kernels spike^{-1}

There were a few MTA associated with two QTL for the number of kernels per spike in chromosome 2H; however, the associated SNPs were not considered as MAS candidates since they were not significant at $P \leq 0.01$ ($-\text{Log}_{10} \geq 2.0$). The lack of correlation between kernels spike^{-1} and yield suggests that the value of the SNP would have had limited value for improving yield potential in the NDSU breeding pool. This is consistent with the viewpoint of Abeledo et al. (2003), who indicated that spikes m^{-2}, not grain number per spike, was primarily responsible for yield improvements in modern cereal cultivars.

Spike length

The SNP 12_31221 in chromosome 5H at 151.36-152.79 cM qualified as a candidate for MAS for spike length. This QTL appears to be novel, not associated with the QTL in chromosomes 2H, 3H and 4H reported by Islamovic et al. (2013), nor the QTL in chromosome

5H (79.5 cM) reported by Wang et al. (2014). However, again, the lack of correlation between spike length and yield in my study suggests that this SNP may have limited utility for yield enhancement.

Days to heading

For days to heading, none of the QTL-associated SNPs were candidates for MAS; however, I did find some QTL that were reported previously in the literature. The QTL I found in chromosomes 3H and 7H differ from those identified by Wang et al. (2014); however, the QTL identified in chromosome 3H was in the same region at one reported by Daba (2015) at 74.0 cM. This QTL is within 10 cM of the photoperiod-related genes *HvG1* and *HvFT2* (Wang et al. 2010). The QTL identified in chromosome 7H between 107.11 and 110.99 cM is in the same region as one identified by Pasam et al. (2012), who reported its location as 104.78 cM. It is different than the QTL on 7H at 58.2 cM reported by Mansour et al. (2014) and a second reported at between 89.8 and 94.3 cM by Daba (2015). These putative confirmations with the findings of other research teams may be a reason to further assess these QTL as potential candidates for MAS. Regardless of the outcome, no significant correlations existed between days to heading and yield, suggesting that manipulating days to heading with MAS will not significantly alter yield potential in the NDSU two-rowed breeding program.

Plant height

Three QTL were detected for plant height; however, none of the associated SNPs met my criterion for use in MAS. None of the QTL identified in this experiment were associated with the cluster of semi-dwarfing genes in chromosome 3H that have been characterized by Tsuchiya (1972) and Barua et al. (1993), nor the QTL identified in chromosomes 1H (Wang et al., 2014); 2H (Pasam et al., 2012; Wang et al. 2014, Mansour et al., 2014), 3H (Pasam et al., 2012;

Locatelli et al., 2013); 4H (Mansour et al, 2014); or 7H (Yu et al., 2010; Wang et al. 2014; Mansour et al., 2014). The QTL identified in chromosome 6H at 118.35 cM is in a similar region as one identified by Pasam et al. (2012) at 124.85 cM. Ren et al. (2014) and Mansour et al. (2014) also reported QTL impacting plant height and culm length in chromosome 6H, but their reported locations (64.9 cM and 25.2 cM, respectively) indicates that they are likely not the same QTL in chromosome 6H found in my study. While none of the QTL associated SNPs identified in my study met the criterion for MAS, it is fortunate that plant height is a relatively simple and straightforward trait to assess through phenotypic evaluations. Additionally, based on the correlation analysis using data from the mapping panel in multiple environments, plant height in the NDSU two-rowed germplasm base is relatively independent of yield.

Stem breakage

Stem breakage at harvest is an undesirable trait for barley growers because it makes direct combining of the crop very difficult. The growers have to drive their combines slower and lower the combine headers closer to the ground to harvest the broken down grain. The risk with lowering the combine headers is that they may accidentally pick up or hit rocks in the field. The SNP, 11_20974 in chromosome 4H at 106.3 cM is a candidate for MAS of stem breakage. I believe this to be a novel QTL based on the lack of QTL analysis for stem breakage in the existing literature. Despite the positive correlation between stem breakage and yield, indicating that stem breakage increases as yield increases, this QTL was not identified in the yield analysis, indicating the potential to utilize MAS to improve stem breakage without negatively impacting yield.

Deciduous awns

A deciduous awn is one that breaks off the lemma awn prior to harvest. This trait is undesirable because the lemma is often torn or "skinned" when the awn breaks off. Grain with 5% or more skinned kernels is discounted when it is sold. Numerous QTL were identified that impact the presence of deciduous awns in my study. Among these, five SNPs met my criterion for use in MAS, which suggests excellent opportunities to manipulate this trait. The associated SNPs associated with deciduous awns in chromosomes 1H and 5H appear to be novel. The only prior published report regarding QTL for deciduous awn presence identified a locus in chromosome 6H (Lewis, 2012), which was not found in our research. Her research used a biparental cross between the six-rowed cultivars 'Stander' and 'Robust'

Rachilla hair length

Rachilla hair length is a morphological trait that is used regularly for distinguishing between different barley cultivars. This trait can be easily assessed in the field or laboratory using a small hand magnifying lens or even the naked eye. An excellent SNP candidate for MAS was identified for rachilla hair length in chromosome 5H at 99.56 cM. This occupies a region similar to a QTL identified by Waugh et al. (2010), which was located at 87 cM, and is associated with the *srh* locus that controls rachilla hair length.

Awn barbing type

Awn barbing is another morphological trait that is easy to assess in the field and can be simply used to differentiate between barley cultivars. Most growers prefer smooth or semi-smooth awns because they are associated with being less "itchy" (Martin Hochhalter, personal communication, 2015). For awn barbing type, SNPs associated with four QTL in chromosomes

1H, 5H, and 7H met my criterion for them to be candidates for MAS. To my knowledge, these are novel, being the first SNPs identified for awn barbing type. The QTL identified in chromosome 5H at 123.33 cM may represent the rough/smooth awn locus (*raw1*), which has been reported (wheat.pw.usda.gov/ggpages/bgn/26) to be 26.8 cM distal from the *srh* locus.

In conclusion, I was able to successfully identify SNPs suitable for MAS associated with three agronomic traits: spike length, stem breakage, deciduous awns, and two morphological traits: rachilla hair length, and awn barbing type. While the potential for pre-selecting experimental lines with the most desirable versions of these traits early in the breeding process using MAS is high, many of these traits can be easily assessed phenotypically in the F_2 generation.

Detecting SNPs meeting my criterion for MAS for most of the agronomic traits was challenging, reinforcing the notion that many agronomic traits, including yield, are very complex and impacted by interactions with environments as found by Romagosa et al. (1999). For numerous agronomic traits, significant MTAs were identified in individual environments, or fewer than 50% of the test environments. This supports the concept of environment specific associations between agronomic traits and the genes that impact them as previously reported by other researchers (Sebastian et al. 2010; Wang et al., 2014). For agronomic traits, breeding teams will need to weigh the benefits of remaining with relatively strict criteria for selecting MAS candidates that have been proposed herein, or to loosen the criterion and utilize a broader set of environment-specific SNPs for cultivar development. Likewise, genomic selection should be considered as a breeding strategy for selecting favorable genotypes for these genetically complex agronomic traits.

Conclusion

Significant marker-trait associations for the spike length, stem breakage, deciduous awns, rachilla hair length, and lemma awn barbing type using the two-rowed mapping panel were identified. Many of the QTLs coincided in chromosomal regions where QTL had been previously detected using bi-parental and GWAS methodologies. In fact, several of the MTAs found were previously reported by others; thus, validating the effectiveness of this study. Novel putative QTLs identified in the present study provide additional genomic regions that may be associated with agronomic and morphological traits. Markers identified as candidates can be used to implement MAS in the NDSU program. Others with lower significance levels or that are environment-specific, may be good candidates for further research validation to establish their potential utility for MAS. Additionally, identifying a greater number of markers in the areas where MTAs were detected could help narrow QTL regions and can ensure minimal recombination between genes of interest and the associate SNPs that reside adjacent to them.

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CHAPTER 4. ASSOCIATION MAPPING OF 13 MALT QUALITY TRAITS IN NORTH DAKOTA BARLEY GERMPLASM

Abstract

Developing cultivars with superior malting quality characteristics is a key objective of the North Dakota State University (NDSU) barley (*Hordeum vulgare* L.) breeding program. Released cultivars are expected to possess characteristics associated with acceptable malting quality as specified by the American Malting Barley Association. Breeding for malting quality is challenging due to its complex specifications and numerous traits that impact the final result. Many of these component traits are quantitatively inherited and correlated, sometimes unfavorably, with each other. Phenotypic screening for malting quality is expensive. As a result, malting quality analysis is typically performed late in the breeding process, and many experimental lines are discarded due to unacceptable malt quality, after considerable expense has been made conducting multi-year yield testing. The key objective of this research was to identify marker-trait associations (MTAs) for two-rowed barley malting quality that could provide NDSU breeders with the tools necessary to implement marker-assisted selection (MAS), enabling earlier and more effective selection for malting quality. A genome-wide association mapping approach was taken, using selected NDSU breeding lines. I successfully identified numerous MTAs and SNP marker combinations for several traits evaluated, such as wort ß-glucan, diastatic power, free amino nitrogen, wort color, wort protein, and α-amylase, and identified the most promising combinations based on consistency across environments for further validation and use in MAS implementation.

Introduction

One of the priorities of barley (*Hordeum vulgare* L.) breeders is to develop and release improved cultivars with superior malt quality characteristics desired by the malting and brewing industries. Malt quality profiles required by these industries vary by company (Lewis, 2012); however, most large US maltsters and brewers require that the barley they use for processing fall within the quality specifications established by the American Malting Barley Association, Inc. (AMBA). These specifications are set to ensure that high quality two-rowed and six-rowed malting barley is available for the AMBA's member companies. (http://ambainc.org/media/AMBA_PDFs/Pubs/Production/Guidelines_June_2014.pdf; verified 3 June 2015). A summary of the grain quality and malt quality traits desired in new US malting barley cultivars is presented in Table 4.1.

While malt quality is an important priority in barley breeding programs, it is complex to breed for because it involves numerous traits that are expensive to phenotype (Burger and LaBerge 1985 and Mohammadi et al. 2015). Determination of malt quality requires that the dry barley grain be steeped in water until kernel moisture reaches about 45%, then germinated under controlled conditions for four to five days, and finally dried or kilned under a specific schedule of drying temperatures that result in the finished malt maintaining most of its enzyme activity.

A substantial number of experimental lines are required for evaluation in a breeding program to identify the rare few that meet all malt quality requirements. Traditionally, evaluation for malt quality traits occurs near the end of the breeding process, F_6 or later, after preliminary selection for yield, agronomic, and disease resistance traits have already occurred. This is due to

Table 4.1. Acceptable barley quality and malt quality parameters for two- and six-rowed malt barleys specified by the American Malting Barley Association.

	Six-Rowed	Adjunct Two-Rowed	All Malt Two-Rowed
Measures made on barley			
Plump kernels (on 6/64)	> 80%	> 90%	> 90%
Thin kernels (thru 5/64)	< 3%	< 3%	< 3%
Germination (4ml 72hr. GE)	> 98%	> 98%	> 98%
Protein	≤ 13%	≤ 13%	≤ 12%
Skinned and broken kernels	< 5%	< 5%	< 5%
Measures made on malt			
Total protein	≤ 12.8%	≤ 12.8%	≤ 11.8%
Kernels retained (on 7/64 screen)	> 60%	> 70%	> 75%
Measures of malt modification			
Wort ß-glucan (ppm)	< 120	< 100	< 100
Fine-coarse extract difference	< 1.2	< 1.2	< 1.2
Soluble/total protein	42-47%	40-47%	38-45%
Turbidity (NTU)	< 10	< 10	< 10
Viscosity (absolute cP)	< 1.5	< 1.5	< 1.5
Measures made on congress wort			
Soluble protein	5.2-5.7%	4.8-5.6%	< 5.3%
Extract (fine grind db)	> 79%	> 81%	> 81%
Color (°ASBC)	1.8-2.5	1.6-2.5	1.6-2.8
Free amino nitrogen (ppm)	>210	> 210	140-190
Measures of malt enzymes			
Diastatic power (°ASBC)	> 150	> 120	110-150
α-amylase (20^{o} DU)	> 50	> 50	40-70

†Adapted from material posted by the American Malting Barley Association (http://ambainc.org/media/AMBA_PDFs/Pubs/Production/Guidelines_June_2014.pdf).

the high expense, time, and labor requirements for evaluating malt quality traits. A challenge with this approach is that resources are spent testing numerous experimental lines in yield trials, only to discard a significant percentage of them late in the breeding process due to unacceptable malt quality. To ensure an acceptable percentage of late stage lines meet malt quality specifications, many breeders limit their parents for crossing to lines demonstrating suitable quality characteristics, which in turn limits genetic gain for this group of traits (Han et al., 1997; Muñoz-Amatriaín et al., 2010).

An alternative approach that could result in lower overall breeding costs per released cultivar is to use marker-assisted selection (MAS) to facilitate enrichment of early generation breeding populations with favorable alleles for malt quality. Marker-assisted selection could also facilitate a broadening of the germplasm base without fear of significantly reducing the number of advanced breeding lines with acceptable malt quality.

To enable MAS, mapping of quantitative trait loci (QTL) controlling malt quality traits has been a high priority of barley researchers for over 20 years. Many of the early studies in North America used the 'Steptoe' x 'Morex' and 'Harrington' x TR306 mapping populations (Hayes et al., 1993; Kasha and Kleinhofs, 1994). An example of using these populations to identify QTL associated with specific malting traits is the work done by Marquez-Cedillo et al. (2000), which utilized the Steptoe x Morex, Harrington x TR306, and Harrington x Morex populations to identify a QTL associated with the *Amy2* locus in chromosome 7H and the *hordein* loci in chromosome 1H. Likewise, Gao et al. (2004) used the Steptoe x Morex population to identify a QTL region in the short arm of chromosome 4H for malt extract, diastatic power (DP), wort β-glucan, and α-amylase. Han et al. (2004) mapped several QTL to chromosome 7H for malt extract, α-amylase, and diastatic power.

In more recent studies using microarrays and expressed sequence tags (ESTs), control of six malt quality traits was estimated to be under the control of between 11 and 102 genes (Lapitan et al., 2009). Muñoz-Amatriaín et al. (2010) used germplasm from the University of Minnesota barley-breeding program and 1,524 SNP markers to identify 49 genes associated with malt quality traits. In research done at North Dakota State University, Pedraza-Garcia (2011) and Lewis (2012) identified QTL in chromosomes 5H and 6H associated with free amino nitrogen (FAN); QTL in chromosomes 2H and 5H associated with wort color; QTL in chromosome 6H associated with soluble protein and Kolbach Index; and QTL in chromosomes 4H, 5H, and 6H associated with fermentable sugars.

A limitation of many mapping studies conducted to date is that they used bi-parental populations, and may therefore be population specific. Many barley research teams have reported that QTL detected for a given trait can vary from population to population and across different environments. This is supported by findings by Marquez-Cedillo et al. (2000), where QTL controlling malt extract were identified in chromosomes 1H and 2H in a bi-parental North American population, but in chromosomes 1H and 5H in Australian and Canadian cultivars. Other potential issues of using bi-parental populations is that alleles may be fixed, and therefore, not identifiable in such populations (Dekkers and Hospital, 2002; Yu and Buckler, 2006). Association mapping methods that utilize a diverse population of lines from our NDSU breeding program would allow for identification of QTL that are segregating within our germplasm. Comadran et al. (2011) used association mapping to identify QTL for yield and yield related traits and Mohammadi et al. (2015) used association mapping to identify QTL for malt quality.

The overall objective of this study was to use a mapping panel comprised of NDSU two-rowed cultivars and breeding lines to identify QTL controlling barley and malt quality traits.

Information from this research will allow for identification of SNP markers that are candidates for use for MAS in the NDSU barley-breeding program.

Materials and Methods

Experimental Population, Experimental Design and Phenotyping

The NDSU two-rowed mapping panel of 81 lines and checks ('Tradition', 'Colon', and 'Pinnacle') were used for this research. The selection of individuals that comprise the mapping panel is described in Chapter 2.

Grain and Malting Quality Traits

Prior to analyzing the samples for malt quality, harvested grain from the cultivars Tradition, Conlon, and Pinnacle from experiment locations was evaluated for barley protein content and kernel plumpness to determine which locations would be submitted for malting. This was done because of the expense, time, and labor required for malting. Only locations having grain with acceptable protein ($\leq$ 13.5%) and kernel plumpness ($\geq$ 80.0%) for the selected cultivars were submitted. Grain protein was determined in the NDSU Barley and Malt Quality Laboratory of Dr. Paul Schwarz using a Foss Infratec 1241-grain analyzer (Foss; Eden Prairie, MN), which uses near-infrared spectroscopy (NIR). Kernel plumpness was tested in the same laboratory using a Sortimat (Pfeuffer, Germany). Kernels remaining on top of a 19.0 x 2.8-mm rectangular-slotted sieve were considered plump.

Grain from five environments in North Dakota was deemed suitable for malting and submitted to the USDA–ARS Cereal Crops Research Unit (USDA-ARS-CCRU) located in Madison, WI for malting and malt quality analyses. The environments were 2011 Nesson Valley, 2012 Fargo, 2012 Nesson Valley, 2013 Fargo, and 2013 Nesson Valley. Samples submitted for

malting were bulked across replicates within an environment. For the malting process, 170g (dry-weight basis, db) samples of each entry were steeped for 24-48 h, depending on their average kernel weight. The steep consisted of 4 h in water followed by 4 h in air, repeated for the 24-48 h total steep time at 16°C, with a targeted steep-out moisture of 45%. Samples were adjusted to 45% moisture at steep-out, as needed, and transferred to germinators. Next, the samples were germinated for 120 h at 17°C and > 98% humidity. The samples were turned for 3 min, every 30 min, during germination to prevent root matting. Sample moisture was checked and adjusted to 45% once during the germination period. Finally, samples were kilned for 24 h as follows: 10 h at 49°C, 4 h at 54°C, 3 h at 60°C, 2 h at 68°C, and 3 h at 85°C. There were 30 min temperature ramps between kilning stages.

Quality data collected on each entry included kernel weight (mg), kernel plumpness (%), barley color (0-100 scale), malt extract (% db), wort color (°ASBC), barley protein (% db), wort protein (% db), soluble/total protein ratio (%), DP (°ASBC), α-amylase (20°C DU), wort β-glucan (mg L^{-1}), and FAN (mg L^{-1}). The methods used for determining the measurements by the USDA-ARS-CCRU can be found at http://www.ars.usda.gov/SP2UserFiles/Place/50900500/barleyreports/CY%20METHODS.pdf (verified 3 June 2015) and are based on the official methods of the American Society of Brewing Chemists (http://methods.asbcnet.org/toc.aspx; verified 3 June 2015).

Statistical Analysis

The statistical analyses for all phenotypic traits were done using the MLM procedure of SAS 9.3 using Proc Mixed method type 3 (SAS Institute Inc., 2004). Locations were considered the replicates in the analysis because entries were combined across replicates before sending to the USDA-ARS-CCRU for malting. *F*-tests were considered significant at $P \leq 0.05$.

Phenotypic correlation coefficients among traits were determined using the CORR procedure in SAS 9.3 (SAS Institute Inc., 2004). Trait means across environments were used for the analysis and correlations values were considered significant at $P \leq 0.05$. Principal component analysis (PCA) was done with the PRINCOMP procedure of SAS 9.3 to create a biplots to visualize the relationships (correlation and direction) amongst quality traits. Again, trait means across environments were used for the analysis.

Genome Wide Association Mapping Analyses, marker-trait association

Methods of analyses are the same as described in Chapter 2.

Results

Phenotyping and Correlations

Significant genotypic variation was detected for all barley and malt quality traits (Table 4.2, Appendix table A7). However, since the quality samples were bulked across replicates for each environment, it was not possible to determine if there was a significant G x E interaction for any of the traits. For this reason, I chose to present my findings on a single environment basis. In general, the mean barley and malt quality of the mapping panel was similar to that of the mean of the three checks, except for barley color, grain protein, wort ß-glucan, DP, wort protein and FAN. Compared to the mean of the checks for these traits, the mapping panel generally had darker kernels; lower grain protein, wort ß-glucan, and DP; and higher wort protein and FAN.

Many traits exhibited significant correlations, which were calculated using means across environments (Table 4.3). While many of the 66 correlation values were significantly different from zero ($P \leq 0.05$), very few were ≥ 0.70, a value I consider moderately strong. Correlations $\geq$

0.70 included plump kernels vs. 1000-kernel weight, wort color vs. S/T, wort protein vs. S/T and FAN, and S/T vs. FAN. None of these relationships were unexpected.

The malt panel biplot (Figure 4.1) based on the first principal component (PC1) and the second principal component (PC2) from the PCA of all barley and malt quality traits shows the relationship amongst the traits. The angle of the trait vectors represents the correlation between the traits and the length of the vector is indicative of the variation for the trait. Traits that are correlated have arrows facing the same general direction, while those that are negatively correlated have arrows facing generally opposite directions. For example, kernel plumpness, kernel weight, and malt extract are all positively correlated with each other, but negatively correlated with DP.

The biplot is also useful for visualization of traits into groups. I grouped traits into those related to grain quality, carbohydrate modification, enzymatic activity, and protein modification. Traits related to grain quality include barley color, grain protein, and kernel weight, and plumpness. Barley color and grain protein are located closely together in the upper half of the plot near the origin for the first PC. Kernel weight and plumpness are located closely together in the bottom right quadrant and the opposite direction of the other barley quality traits. The opposite direction of the two pairs of traits is expected. High protein lines from the NDSU barley-breeding program often have brighter kernel color, but tend to have thinner, low-weight kernels. The traits I included in the carbohydrate modification group are wort ß-glucan and malt extract. Wort ß-glucan had negative values for both PC1 and PC2, while malt extract had a positive value for PC1 and a negative value for PC2. The opposite direction of the PC1 values is due to the negative relationship between the two traits. As malt extract increases, wort ß-glucan typically decreases. The traits included in the enzymatic activity group are DP and α-amylase

activity. The biplot in Figure 4.1 have the traits at nearly a 90° angle from each other, which is reflective of traits with a low correlation. While DP is a measure of multiple starch hydrolyzing enzymes that includes α-amylase and ß-amylase, it is ß-amylase that has the greatest impact on the magnitude of DP. The final group of protein modification includes the traits of wort color, wort protein, S/T, and FAN. The direction of the arrow for all four traits is in the positive direction of PC1 and the low angle of difference between the arrows is indicative of correlated traits.

Table 4.2. Phenotypic data statistics for the NDSU two-rowed mapping panel and the three check cultivars (Tradition, Conlon, and Pinnacle) across five environments in North Dakota, 2011-2013.

Traits	Entries	Mean	Minimum	Maximum	SD[†]
Grain quality					
Barley color (0-100)	Mapping panel[‡]	43.2a[§]	35.2	56.6	5.80
	Avg. 3 checks	50.6b	45.1	40.6	5.06
Barley protein (%)	Mapping panel	13.7a	12.2	15.8	0.61
	Avg. 3 checks	14.2b	13.6	12.5	0.97
Kernel weight (mg)	Mapping panel	39.3a	31.9	46.2	1.54
	Avg. 3 checks	38.9a	36.4	31.3	4.36
Kernel plumpness (%)	Mapping panel	92.1a	76.9	98.2	2.51
	Avg. 3 checks	93.8a	90.2	84.9	4.69

Table 4.2. Phenotypic data statistics for the NDSU two-rowed mapping panel and the three check cultivars (Tradition, Conlon, and Pinnacle) across five environments in North Dakota, 2011-2013 (continued).

Traits	Entries	Mean	Minimum	Maximum	SD†
Carbohydrate modification					
Wort ß-glucan (mg L^{-1})	Mapping panel	257.0a	54.0	706.0	19.86
	Avg. 3 checks	323.0b	206.0	142.0	101.5
Malt extract (%)	Mapping panel	78.9a	76.1	81.9	0.59
	Avg. 3 checks	79.2a	78.2	77.7	0.83
Enzymatic activity					
α-amylase (20° DU)	Mapping panel	90.9a	68.5	117.0	4.27
	Avg. 3 checks	90.8a	86.4	82.2	4.30
Diastatic power (°ASBC)	Mapping panel	135.0a	88.0	188.0	10.68
	Avg. 3 checks	236.0b	175.0	138.0	52.83
Protein modification					
Wort color (°ASBC)	Mapping panel	2.50a	1.70	4.0	0.23
	Avg. 3 checks	2.40a	2.30	2.2	0.08
Wort protein (%)	Mapping panel	5.81b	4.70	7.50	0.29
	Avg. 3 checks	5.50a	5.38	5.24	0.13
Soluble/total protein (%)	Mapping panel	43.7a	35.8	55.0	1.01
	Avg. 3 checks	43.2a	41.1	39.6	1.90
Free amino nitrogen (mg L^{-1})	Mapping panel	246.0a	184.0	357.0	5.89
	Avg. 3 checks	230.0a	216.0	207.0	12.22

†SD = Standard Deviation
‡Mapping panel is the mean of 81 NDSU cultivars and breeding lines.
§Means for a trait followed by the same letter are not different at $P = 0.05$ as determined using an *F*-protected LSD.

Table 4.3. Phenotypic correlations for barley and malt quality traits across five environments for the NDSU two-rowed mapping panel.

	Bclr[†]	Prt	Kwt	Plmp	Bgl	Ext	Alpha	DP	Wrtclr	Wrtprt	S/T	FAN
Bclr	1	0.0102	0.5183	0.0642	0.4829	0.6205	0.6817	0.7065	0.0012	0.1012	0.9601	0.4164
Prt	**0.29**	1	0.0681	0.0049	0.1328	0.0003	0.4720	0.0004	0.1130	0.0002	0.0725	0.0168
Kwt	-0.07	-0.21	1	<.0001	0.7480	0.0841	0.9388	0.0031	0.0066	0.0355	0.0009	0.0195
Plmp	-0.21	**-0.31**	**0.70**	1	0.3022	0.0003	0.5566	0.1031	0.2510	0.1428	0.0019	0.0526
Bgl	-0.08	-0.17	0.04	0.12	1	0.1172	<.0001	0.1232	0.1108	<.0001	<.0001	<.0001
Ext	-0.06	**-0.39**	0.19	**0.40**	-0.18	1	<.0001	0.0199	00040	0.0006	<.0001	<.0001
Alpha	-0.05	-0.08	0.01	0.07	**-0.59**	**0.47**	1	0.7239	0.0054	<.0001	<.0001	<.0001
DP	0.04	**0.39**	**-0.33**	-0.18	-0.17	**-0.26**	-0.04	1	0.7034	0.8417	0.0375	0.5229
Wrtclr	-0.13	-0.10	-0.15	0.03	**0.36**	0.06	0.0007	0.44	1	0.0059	0.0267	0.0182
Wrtprt	0.18	**0.40**	**0.24**	0.17	**-0.57**	**0.37**	**0.53**	0.02	**0.34**	1	<.0001	<.0001
S/T	0.01	-0.20	**0.36**	**0.34**	**-0.51**	**0.66**	**0.63**	**-0.23**	**0.53**	**0.81**	1	<.0001
FAN	0.09	**0.27**	**0.26**	**0.22**	**-0.52**	**0.48**	**0.54**	-0.07	**0.42**	**0.96**	**0.86**	1

[†]Bclr = barley color, Prt = barley protein, Kwt = kernel weight, Plmp = kernel plumpness, Bgl = wort β-glucan, Ext = malt extract, Alpha = α-amylase, DP = diastatic power, Wrtclr = wort color, Wrtprt = wort protein, S/T = soluble/total protein ratio, and FAN = free amino nitrogen.

[‡]Probability values appear above the diagonals and correlation coefficients appear below the diagonals in the table. Correlation values in bold font are significant at $P \leq 0.05$.

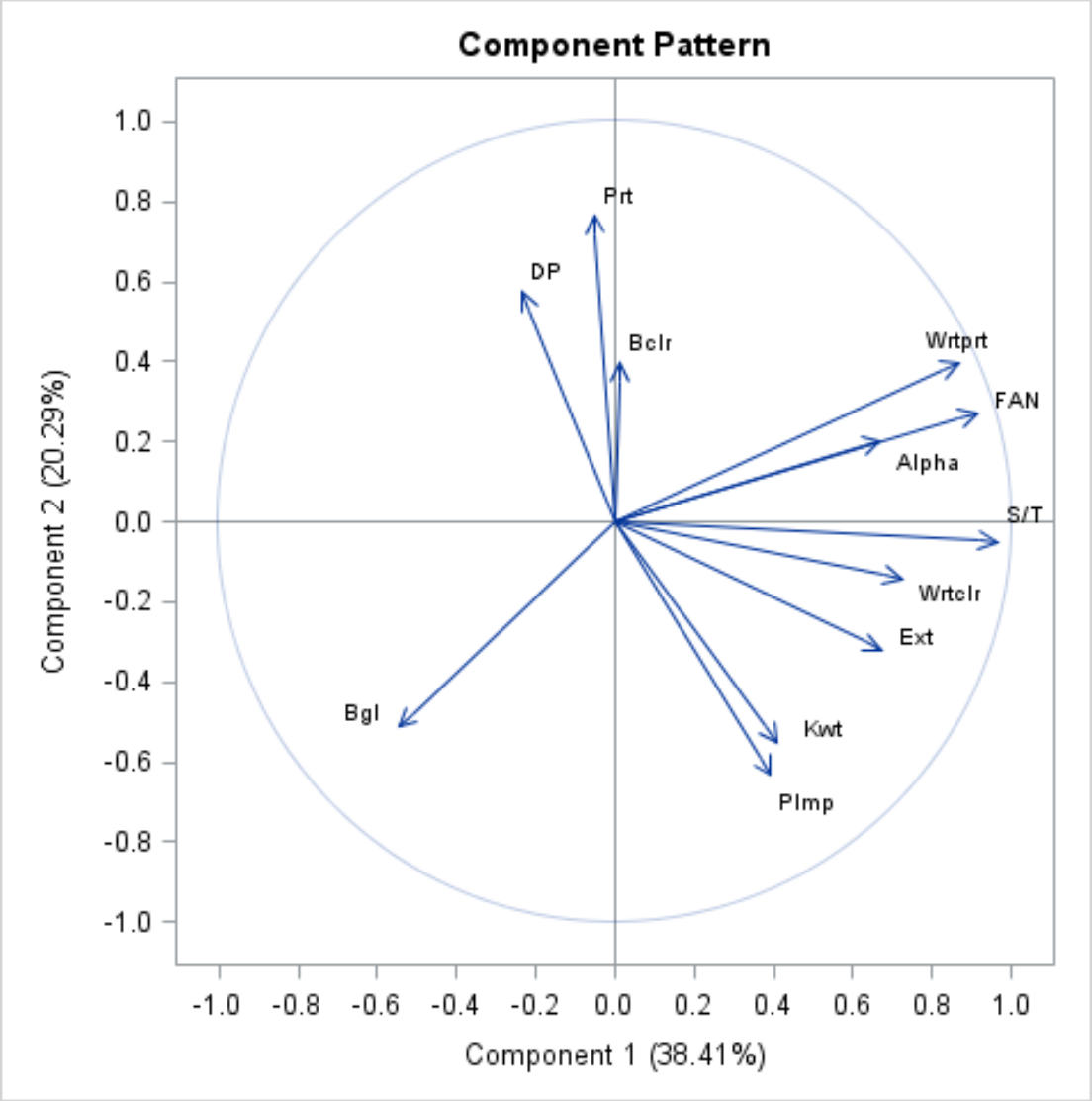

Figure 4.1. Biplot of the two-rowed malting panel phenotypic data showing relationship between quality traits. Bclr = barley color, Prt = barley protein, Kwt = kernel weight, Plmp = kernel plumpness, Bgl = wort β-glucan, Ext = malt extract, Alpha = α-amylase, DP = diastatic power, Wrtclr = wort color, Wrtprt = wort protein, S/T = soluble/total protein ratio, and FAN = free amino nitrogen.

Marker-Trait Associations

Field Experiment – Laboratory Evaluation

The *P*-values of the marker-trait associations (MTAs) for the analyses using the Naïve, principle component (PC), Kinship (K), and PC+ K models varied by environment. The best models were identified using the mean square difference (MSD) method described in Mamidi et al. (2011) for each environment-trait combination as highlighted in Appendix table A6. The models with the minimum MSD for each trait were used for the association analyses. Marker-trait associations significant at $P \leq 0.01$ ($-\text{Log}_{10} \geq 2.0$), residing within 5 cM of each other, and detected in > 50% of the environments were considered as belonging to the same QTL. Additionally, I considered SNPs as candidates for MAS if the associated MTAs were detected in > 50% of the environments and the mean $-\text{Log}_{10}$ values of the MTAs ≥ 3.0. Finally, because many of the quality traits are correlated, I presented the results by chromosome so QTL for multiple traits mapping to a similar region could be visualized.

Chromosome 1H

Quantitative trait loci were detected for barley color and wort ß-glucan. The QTL for barley color was detected in three of the five environments, ranging from 17.26 - 20.89 cM (Appendix table A8 and Table 4.4); however, none of the MTAs for any SNP were detected in more than three environments. Thus, there are no SNPs that are candidates for MAS of barley color in this chromosome.

For wort ß-glucan concentration, a QTL ranging from 47.47 - 50.00 cM was detected in four of the five environments (Appendix table A8 and Table 4.4). Within this region, three of the SNPs had associations significant in three or more environments, but only the SNPs

11_11367 and 11_21219 had mean -Log_{10} values > 3.0 (Table 4.5). Both of these SNPs map to the same position in chromosome 2H at 66.70 cM and are candidates for MAS of wort ß-glucan.

Chromosome 2H

Two QTL were detected for malt extract in chromosome 2H (Appendix table A8 and Table 4.4). The first QTL was located in the region of 21.61 - 26.53 cM and it was detected in three of the five environments. The second QTL was located from 95.64 – 98.59 cM and was detected in four of the five environments. Unfortunately, neither QTL had specific SNPs detected in more than two environments; thus, there are no candidate SNPs for MAS of malt extract in chromosome 2H.

Chromosome 3H

Quantitative trait loci for DP, wort protein, FAN, and grain protein were detected in chromosome 3H (Appendix table A8 and Table 4.4). The QTL for DP mapped to the position 28.44 cM and significant MTAs were detected in three of the five environments. The SNP 12_30284 was associated with DP in each of the three environments and all three MTAs had a -Log_{10} value > 3.0. Thus, the SNP 12_30284 is a candidate for MAS of DP (Table 4.5).

The QTL for wort protein and FAN mapped to the region 47.09 - 51.73 cM in chromosome 3H (Appendix table A8 and Table 4.4). Significant MTA were detected in three of the five environments for both traits; yet there were no SNPs with MTAs for wort protein in more than two environments. Thus, there are no candidates for MAS of wort protein. The SNPs 11_10380, 11_21109, and 12_30680 were found to be associated with FAN in three of the five environments. Mean -Log_{10} across environments was 3.10 for each SNP; thus, all three are candidates for MAS of FAN (Table 4.5).

The QTL for grain protein was located in the region from 162.15 – 167.77 cM (Appendix table A8 and Table 4.4). No SNP was associated with the trait in more than two environments; thus, there is no candidate for MAS of grain protein in chromosome 3H.

Chromosome 4H

A total of eight QTL were found in chromosome 4H (Appendix table A8 and Table 4.4). There was one each for α-amylase, wort ß-glucan concentration, S/T, kernel plumpness, FAN, wort protein, wort color, and DP. The QTL for FAN, wort protein, and wort color were located in the same region.

Four significant MTAs for α-amylase were detected in the QTL from 51.3 – 55.63 cM; however, none of the SNPs were associated with the trait in more than two environments (Appendix table A8 and Table 4.4). Thus, these are no candidates for MAS to select for this QTL.

A QTL for wort ß-glucan concentration was detected in the region from 65.05 to 65.80 cM (Appendix table A8 and Table 4.4). The SNPs 11_11224, 12_30620, and 12_31515 at 65.05 cM and 12_30455 at 65.80 cM each had associations detected in four of the five environments and the mean $-\text{Log}_{10}$ value was 4.41 for each SNP. Thus, these SNPs are all candidates for MAS of wort ß-glucan (Table 4.5).

A QTL for S/T detected at 77.31 cM had significant MTAs identified in three of the five environments; however, only the SNP 11_21332 had associations in three or more environments (Appendix table A8 and Table 4.4). Nonetheless, because the mean $-\text{Log}_{10}$ value of MTA was <3.0, the SNP 11_21332 was not considered a candidate for MAS of S/T.

A QTL for kernel plumpness was detected from 111.07 – 111.66 cM; however, none of the MTAs for any SNP were detected in more than two environments (Appendix table A8 and

Table 4.4). Thus, none of the SNPs in this region were considered candidates for MAS of kernel plumpness.

Quantitative trait loci for FAN, wort protein, and wort color were detected at 119.09 cM (Appendix table A8 and Table 4.4). Marker-trait associations for the SNP 11_20272 were found in four of the five environments for FAN, all environments for wort protein, and three of the environments for wort color. Furthermore, the mean -Log_{10} values were 3.80 for FAN, 3.29 for wort protein and 3.28 for wort color; thus, SNP 11_20272 is a candidate for MAS of FAN, wort protein and wort color (Appendix table A8 and Table 4.4).

The last QTL detected in chromosome 4H was one for DP in the region of 120.58 – 123.29 cM (Appendix table A8 and Table 4.4). Significant MTAs were detected in four of five environments and mean -Log_{10} value for them was 3.14. The SNPs 11_20013 and 11_20089 located at 123.29 cM met the criteria of having MTAs detected in three or more environments and having a mean -Log_{10} value >3.0; thus, these two SNPs are candidates for MAS of DP (Table 4.5).

Chromosome 5H

A total of six QTL were identified in chromosome 5H (Appendix table A8 and Table 4.4). There were three for α-amylase, and one each for S/T, kernel weight, and malt extract. The QTL for kernel weight and malt extract were located in the same region of the chromosome.

The first QTL for α-amylase was located in the region from 33.09 – 34.25 cM (Appendix table A8 and Table 4.4). The SNP 12_10530 at 33.09 cM had MTAs detected in three environments and mean -Log10 > 3.0; therefore, it is a candidate SNP for MAS of the α-amylase QTL in this region (Table 4.5). The second QTL for α-amylase was located in the region from 80.61 – 85.21 cM; however, none of the SNPs associated with α-amylase in this region were

detected in more than two environments (Appendix table A8 and Table 4.4). Thus, there are no candidates in this region for MAS of α-amylase. The final QTL for α-amylase in chromosome 5H was detected in the region from 194.64 - 196.85 cM (Appendix table A8 and Table 4.4). The SNP 12_30382 was associated with α-amylase in three environments, but the mean -Log10 value < 3.0. Therefore, this and the other SNPs in this QTL are not candidates for MAS of α-amylase.

A QTL for S/T was detected in the region from 64.04 – 67.54 cM (Appendix table A8 and Table 4.4). None of the MTAs in the region were detected in more than two environments; so no SNPs in this QTL are candidates for MAS of S/T.

In the region from 132.48 – 137.16 cM, QTL were found for kernel weight, malt extract, and kernel plumpness. While MTAs for SNPs associated with kernel weight and malt extract were detected in three environments, the mean $-Log_{10}$ values for both traits were < 3.0. Thus, there are no candidate SNPs for MAS of kernel weight, malt extract, and kernel plumpness in this region.

Chromosome 6H

A QTL for DP was detected at 97.39 cM (Appendix table A8 and Table 4.4). The SNP 12_30144 was associated with the trait in four environments; however, the mean $-Log_{10}$ value was < 3.0. Therefore, there are no candidates for MAS of DP in chromosome 6H.

Chromosome 7H

Quantitative trait loci for α-amylase, wort ß-glucan, and DP were detected (Appendix table A8 and Table 4.4). The QTL for α-amylase and wort ß-glucan were at 29.82 cM and the QTL for DP was in the region from 138.17 – 141.76 cM. The SNPs 12_30780 and 12_31351 were each associated with α-amylase in three environments and had a mean $-Log_{10} \geq 3.0$. Thus, both are candidates for MAS of α-amylase (Table 4.5). The SNP 12_31351 was associated with

wort ß-glucan in three environments; however the mean -Log10 < 3.0. Therefore, this SNP is not a candidate for MAS of wort ß-glucan. The QTL for DP was detected in four environments; however, MTA's were found in no more than two environments for any of the SNPs in the region. Thus, there are no candidates for MAS of DP in this QTL.

Table 4.4. Summary of identified QTL, including the number of significant environments and mean -log10 *P*-values.

Chromosome	Position (cM)	Quality area	Trait	Number of significant environments	Ave Log_{10} (*P*-value)
1H	17.26-20.89	Grain Quality	Barley color	3 out of 5	
	47.47-50.00	Carbohydrate modification	Wort ß-glucan	4 out of 5	2.55
	66.7			3 out of 5	3.36
2H	21.61-26.53	Carbohydrate modification	Malt extract	3 out of 5	
	95.64-98.59			4 out of 5	
3H	28.44	Enzymatic activity	Diastatic power	3 out of 5	3.40
	51.73	Protein modification	Free amino nitrogen	3 out of 5	3.10
	47.09-51.73	Protein modification	Wort protein	3 out of 5	
	162.15-167.77	Grain Quality	Barley protein	3 out of 5	
4H	51.30-55.63	Enzymatic activity	α-amylase	4 out of 5	
	65.06-65.8	Carbohydrate modification	Wort ß-glucan	4 out of 5	4.41
	77.31	Protein modification	Soluble/Total protein	3 out of 5	
	111.07-111.66	Grain Quality	Kernel plumpness	3 out of 5	
	119.09	Protein modification	Free amino nitrogen	4 out of 5	3.79
	119.09	Protein modification	Wort protein	5 out of 5	3.29
	119.09	Protein modification	Wort color	4 out of 5	3.28
	120.58-123.29	Enzymatic activity	Diastatic power	4 out of 5	3.17

Table 4.4. Summary of identified QTL, including the number of significant environments and mean -log10 *P*-values (continued).

Chromosome	Position (cM)	Quality area	Trait	Number of significant environments	Ave Log_{10} (*P*-value)
5H	33.09-34.25	Enzymatic activity	α-amylase	4 out of 5	3.08
	64.04-67.54	Protein modification	Soluble/Total protein	3 out of 5	
	80.86-85.21	Enzymatic activity	α-amylase	3 out of 5	
	132.48-137.16	Grain Quality	Kernel weight	3 out of 5	
	135.72-137.16	Carbohydrate modification	Malt extract	4 out of 5	2.14
	194.64-196.85	Enzymatic activity	α-amylase	3 out of 5	2.89
6H	97.39	Enzymatic activity	Diastatic power	4 out of 5	2.90
7H	29.82	Enzymatic activity	α-amylase	3 out of 5	3.01
	29.82	Carbohydrate modification	Wort ß-glucan	3 out of 5	2.88
	138.17-141.76	Enzymatic activity	Diastatic power	4 out of 5	

Table 4.5. Candidate single nucleotide polymorphisms (SNPs) for marker-assisted selection of barley and malt quality traits.

Chromosome	**Position (cM)**	**Quality area**	**Trait**	**Number of significant environments**	**Mean - Log_{10} (*P*-value)**	**Number of candidate SNPs for MAS**	**SNPs**
1H	66.70	Carbohydrate modification	Wort ß-glucan	3 out of 5	3.36	2	11_11367 11_21219
3H	28.44	Enzymatic activity	Diastatic power	3 out of 5	3.40	1	12_30284
3H	51.73	Protein modification	Free amino nitrogen	3 out of 5	3.10	3	11_10380 11_21109 12_30680
4H	65.06-65.8	Carbohydrate modification	Wort ß-glucan	4 out of 5	4.41	4	11_11224 12_30620 12_31515 12_30455
4H	119.09	Protein modification	Free amino nitrogen	4 out of 5	3.79	1	11_20272
	119.09	Protein modification	Wort protein	5 out of 5	3.29	1	11_20272

Table 4.5. Candidate single nucleotide polymorphisms (SNPs) for marker-assisted selection of barley and malt quality traits (continued).

Chromosome	Position (cM)	Quality area	Trait	Number of significant environments	Mean - Log_{10} (*P*-value)	Number of candidate SNPs for MAS	SNPs
	119.09	Protein modification	Wort color	4 out of 5	3.28	1	11_20272
4H	120.58-123.29	Enzymatic activity	Diastatic power	4 out of 5	3.17	2	11_20013
							11_20089
5H	33.09-34.25	Enzymatic activity	α-amylase	4 out of 5	3.08	1	12_10530
7H	29.82	Enzymatic activity	α-amylase	3 out of 5	3.01	2	12_30780
							12_31351

Discussion

Barley malt is one of the major components of beer. With environmental challenges, genetic complexity of barley and malt quality traits, and variable specifications desired by different maltsters and brewers for specific products, the ability of barley breeders to effectively select for specific quality parameters is critical. NDSU's malting barley-breeding program is focused on improved malting quality that meets the needs of the malting and brewing industries, while meeting the productivity and agronomic demands of the growers.

Phenotypic Variation

There was significant phenotypic variation amongst genotypes grown in my experiments suggesting ample room for further selection for the 13 traits analyzed. For barley color, barley protein, and wort protein, relatively small but significant differences were detected between the means of the mapping panel and the malting quality checks, but within or extremely close to AMBA standards for the respective traits. The check mean for DP was 43% higher than the mean of the mapping population, indicating a high hurdle for future improvement; yet, the mapping panel mean of 135 ^{o}ASBC still met AMBA specifications. Increasing the probability that growers will harvest malting quality barley, even when adverse environmental conditions occur is a high priority breeding goal. In some of the environments (Table 4.2), the range of means for the mapping panel lines was outside of the malting barley quality standards set by the AMBA (American Malting Barley Association, Inc. 2014). For example, the grain protein levels for all experimental lines in the 2011 and 2012 Nesson Valley experiments were above the desired maximum level of 13.0%. I believe that excessive rain and ground saturation, which resulted in stunted plant growth and impacted kernel development, was a key causal factor.

Correlations

Quality traits are correlated by their nature because of associations with common biochemical pathways, such as hydrolysis of starch, proteins, and cell walls by enzymes that are synthesized de novo or liberated during malting (Pauli et al, 2015). Many of the observed phenotypic correlations in my study agreed with prior reports about such associations. For example, Schwarz and Li (2011) detected positive correlations between kernel weight and plumpness with malt extract. In my research, kernel weight and plumpness had a correlation of $r = 0.69$ ($P < 0.0001$). As I indicated earlier, I considered correlation values ≥ 0.70 as moderately strong. Correlations meeting this criterion included plump kernels vs. 1000-kernel weight, wort color vs. S/T, wort protein vs. S/T and FAN, and S/T vs. FAN. The strong relationships between these pairs of traits are not surprising. The correlation between plump kernels and 1000-kernel weight is related to kernel size, and the correlations between wort color vs. S/T, wort protein vs. S/T and FAN, and S/T vs. FAN involve traits that are all related to protein modification. A key selection criterion for malting barley breeders is selection of genotypes that have high levels of protein modification.

Marker-Trait Associations

My research identified numerous QTL impacting various traits associated with barley and malt quality. Despite limiting my reported findings to MTAs that were significant at $P \leq 0.01$ ($-\text{Log}_{10} \geq 2.0$), a high number of QTL were identified across environments for most traits. In total, I found 24 QTL that were detected in > 50% of the environments. These QTL were located in all seven chromosomes (Table 4.4).

Four chromosome regions had QTL for multiple traits impacting malt quality. The region in chromosome 3H from 47.09 – 51.73 cM had QTL for FAN and wort protein in three out of

five environments. This genetic association aligns with the significant phenotypic correlation (r = 0.96) between these traits. The region in chromosome 4H at 119 cM had QTL for the traits FAN, wort protein, and wort color. While these three traits are all positively associated with protein modification, only the correlation between FAN and wort protein was strong. The correlations between FAN and wort color, and wort color and wort protein were ≤ 0.42. In chromosome 5H at the region of 132.48 – 137.16 cM, I found QTL for kernel weight and malt extract. While kernel plumpness has been associated with higher extract previously (Schwarz and Li, 2011), the phenotypic correlation between these two traits in my research was not significantly different from zero (r = 0.19, P > 0.05). The final region with coincidental QTL was in chromosome 7H at 28.82cM. The QTL for α-amylase and wort ß-glucan were identified in three of the five environments. Phenotypically, there was a moderate negative relationship between the two traits (r =-0.59, P ≤0.05). The causality of the relationship between wort ß-glucan and α-amylase is not understood; however, the situation where selecting for reduced wort ß-glucan can result in increased α-amylase should not be problematic in developing new malting barley cultivars.

Eleven QTL in five chromosomes met the criteria of having specific SNPs that are candidates for MAS. I believed that detection of associated MTAs in > three environments and a mean $-\log_{10}$ value ≥ 3.0 was considered a good cutoff because it offered a compromise that would result in a reasonable number of strong candidates for further validation. Future validation is proposed using NDSU breeding lines that were not part of the mapping panel. There were three QTL regions for α-amylase, two each for wort ß-glucan, DP, and FAN, and one each for wort protein and wort color. There were two regions that had coincidental QTL, including one in chromosome 3H for FAN and α-amylase and one for FAN, wort protein, and wort color in

chromosome 4H. The number of SNPs to be used for MAS of specific QTL ranged from one to four.

The QTL for wort ß-glucan in chromosomes 1H and 4H; DP in chromosome 3H; and α-amylase, FAN, wort protein, wort color in chromosome 4H appear to be unique to the NDSU germplasm base as they have not been identified in other mapping studies. This is not surprising when the uniqueness of the NDSU two-rowed germplasm is considered. The program was started in 1974 with a goal to develop two-rowed barley adapted for the hot and dry conditions often experienced in western North Dakota. Most of the original lines were feed barley lines with drought resistance. The release of 'Bowman' in 1984, a feed barley cultivar, was the first release from the program (Franckowiak et al., 1985). Beginning in the early 1980's, a new priority added to the program was to develop cultivars desirable for adjunct (e.g. rice or corn) brewing. Barley for this use needs to have a similar malt quality profile as Midwest six-rowed malting barley, which has high levels of enzyme activity and protein modification. The favorable alleles for these traits were introduced to the NDSU two-rowed germplasm by using Midwest six-rowed barley lines as parents. Materials introduced later into the germplasm pool include feed barley accessions from China for disease resistance in the 1990's, and two-rowed malting barley breeding lines from Canada and Germany in the 2000's. Thus, the NDSU two-rowed germplasm base represents a unique pool that incorporates drought-resistant two-rowed feed barley lines, Midwest six-rowed malting barley lines, and Canadian and European two-rowed malting barley lines.

Four QTL identified in the present study were identified previously. Two of these QTL were found by Daba (2015), whose research included landraces from Ethiopia; and breeding lines from the Ethiopian National breeding program, ICARDA, and NDSU. The QTL for FAN

in chromosome 3H was identified in the same region as one found by Daba (2015). The QTL for DP in chromosome 4H is in the same region as a QTL for this trait found in multiple studies (Mohammadi et al., 2015; Igartua et al., 2002; Zale et al., 2000) and the *Bmy1* locus that controls ß-amylase, which is the enzyme that comprises the main component of DP. The QTL for α-amylase in chromosome 5H was mapped to the same region as a QTL for this trait identified by Daba (2015). The QTL for α-amylase in chromosome 7H was in the same region as one found for this trait by Igartua et al. (2002).

Even though my research identified SNPs that can be used for MAS associated with malt quality, breeding for malt quality will continue to be challenging because these traits are the result of numerous independent traits, many of which are complexly inherited. This appears to be particularly true for traits associated with kernel size/plumpness and its correlated trait malt extract. Despite these challenges, the identification of associated QTL that may be candidates for MAS provides the breeders the tools that may increase genetic gain towards the goal of improved malt quality. Traits where MAS may not be successful because of smaller effects, as indicated by lower $-\log_{10}$ values, but still identified in > 50% of the environments may be candidates for improvement using genomic selection.

Conclusion

Significant marker-trait associations for wort β-glucan, α-amylase, diastatic power, wort color, wort protein and free amino nitrogen using the two-rowed mapping panel were identified. Many of the QTLs coincided in chromosomal regions where QTL had been previously detected using bi-parental and GWAS methodologies. Several of the MTAs found were previously reported by others; thus, validating the effectiveness of this study. Novel putative QTLs identified in the present study provide additional genomic regions that may be associated with

disease resistance. SNPs meeting our robust criteria as MAS candidates can be used to increase the efficiency of the NDSU barley-breeding programs, enabling selection of malting quality traits earlier in the breeding process, and ensuring that experimental lines reaching the yield testing phase have a higher probability of meeting malting quality standards. The markers identified here should also be validated in other genetic backgrounds to further establish their utility for MAS. Additionally, identifying a greater number of markers in the areas where MTAs were detected help narrow QTL regions may enable the identification of additional SNPs that would minimize recombination between genes of interest and the associated SNPs that are used for MAS.

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CHAPTER 5. SUMMARY AND CONCLUSIONS

Barley (*Hordeum* vulgare L.) is an important cereal crop used for the production of malt, which is used for brewing beer. The malting and brewing industries purchase malting barley on an identity preserved basis. To maintain cultivar purity, malting barley in the major growing areas of the US is typically grown under contract. When producers sell their barley, they need to specify the cultivar they are selling so the buyer can segregate cultivars in storage. The maltster must know the cultivar they are processing because their large brewing customers typically know specifically what cultivars and the proportion they occupy in their brewing blends.

Development of new malting barley cultivars with improved disease resistance, agronomic performance, and malt quality are key to the success of malting barley breeding programs, including the North Dakota State University (NDSU) barley breeding program. A limiting factor in developing improved cultivars is that many disease resistance, agronomic performance, and malting quality traits are genetically complex and expensive to phenotype. The use of marker-assisted selection (MAS) could accelerate genetic gain for disease, agronomic and malting quality traits and reduce breeding time and costs.

Most prior molecular marker mapping research has been based on bi-parental crosses that provide markers targeted at specific populations. My research targeted a broad range of germplasm representing NDSU breeding program lines. The dissertation was divided into three chapters focusing on disease resistance, agronomic and morphological, and malting quality traits.

The purpose of each chapter was to identify quantitative trait loci (QTL) using genome-wide association mapping (GWAS) for disease resistance, agronomic, morphological and quality traits in the NDSU barley training population. Following that, and perhaps most importantly, I identified QTL-associated single nucleotide polymorphisms (SNPs) that could be candidates for

use in MAS. My research successfully identified 62 SNP candidates that could be deployed for MAS in the NDSU barley breeding program.

Disease Resistance Traits

A total of 29 candidate SNPs for MAS were identified for foliar disease resistance. The diseases covered by these included spot blotch (caused by *Cochliobolus sativus* (Ito & Kuribayashi) Drechs. ex Dastur), spot form net blotch (caused by *Drechslera teres* f. sp. *maculata* Smedeg.), net form net blotch (caused by *Drechslera teres* f. sp. *teres* (Sacc.) Shoemaker), and leaf rust (caused by *Puccinia hordei* Otth). Additionally, a SNP for MAS of reduced deoxynivalenol accumulation (produced by *Fusarium graminearum* Schwabe) in barley grain was identified. The identified markers will provide a great arsenal for breeders to quickly impact the efficiency of improving resistance to key diseases faced by North Dakota barley producers.

Agronomic and Morphological Traits

A total of nine SNP candidates for MAS were identified for agronomic traits, plus six for morphological traits. The traits were spike length, stem breakage, deciduous awns, rachilla hair length, and awn barbing type. Unfortunately, identifying MAS candidates for yield, and the correlated traits kernel weight and kernel plumpness, was elusive. For these traits, no associated SNP markers were identified that occurred in greater than 50% of the test environments. I believe that this was due to environmental interactions with the underlying alleles that these markers represent.

Quality Traits

A total of 16 SNP candidates for MAS were identified for wort ß-glucan, free amino nitrogen, diastatic power, α-amylase, wort protein, and wort color. A single SNP, 11_20272 located in chromosome 4H at 119.09 cM impacted three different quality traits: wort color, wort protein, and DP. Several markers were identified that should aid breeders in their efforts to select for improved malting quality at earlier stages in their programs and to ensure that experimental lines entering late stage yield testing have a higher probability of meeting malting quality standards.

A summary list with all candidate SNPs for all disease resistance, agronomic and related traits, and malt quality traits can be found in Table 5.1.

Conclusions

This research was successful in identifying numerous QTL and associated SNPs that should provide NDSU scientists an opportunity to enhance genetic gain and breeding efficiency for several key disease resistance, agronomic and malting quality traits using MAS, bringing trait enhancements in elite cultivars to growers more rapidly. The next step in the process is to validate the utility of the SNPs using NDSU barley germplasm that was not part of the mapping panels.

Table 5.1 Summary of all candidate SNPs for all disease resistance, agronomic and related traits, and malt quality traits.

Row Type	Traits Area	Traits	Chromosome	Position (cM)	Candidate SNP for MAS
Two-rowed	Agronomic	Awn barbing type	1H	6.03	12_30933
Six-rowed	Diseases	Spot blotch	1H	40.99	11_10764
Six-rowed	Diseases	Leaf rust	1H	45.13	12_10314
Two-rowed	Agronomic	Deciduous awns	1H	59.71	12_30820
Two-rowed	Agronomic	Deciduous awns	1H	59.71	12_30821
Two-rowed	Quality	Wort ß-glucan	1H	66.70	11_11367
Two-rowed	Quality	Wort ß-glucan	1H	66.70	11_21219
Six-rowed	Diseases	Spot form net blotch - Langdon	1H	135.56	12_30277
Two-rowed	Diseases	Leaf rust	2H	39.10	12_20326
Six-rowed	Diseases	Spot form net blotch - Langdon	2H	120.80	11_20511
Two-rowed	Diseases	Spot blotch	3H	24.99	11_10559
Two-rowed	Quality	Diastatic power	3H	28.44	12_30284
Two-rowed	Quality	Free amino nitrogen	3H	51.73	12_30680
Two-rowed	Quality	Free amino nitrogen	3H	51.73	11_10380
Two-rowed	Quality	Free amino nitrogen	3H	51.73	11_21109
Two-rowed	Diseases	Spot blotch	3H	80.89	12_30170
Six-rowed	Diseases	Net form net blotch	4H	48.50	12_30488
Two-rowed	Diseases	Net form net blotch	4H	50.40	12_30605
Six-rowed	Diseases	Net form net blotch	4H	55.63	11_20363
Two-rowed	Quality	Wort ß-glucan	4H	65.06	11_11224
Two-rowed	Quality	Wort ß-glucan	4H	65.06	12_30620
Two-rowed	Quality	Wort ß-glucan	4H	65.06	12_31515
Two-rowed	Quality	Wort ß-glucan	4H	65.80	12_30455
Six-rowed	Diseases	Net form net blotch	4H	82.42	11_10724
Six-rowed	Diseases	Spot blotch	4H	98.55	11_20762
Two-rowed	Agronomic	Stem breakage	4H	106.03	11_20974
Two-rowed	Quality	Free amino nitrogen	4H	119.09	11_20272
Two-rowed	Quality	Wort color	4H	119.09	11_20272
Two-rowed	Quality	Wort protein	4H	119.09	11_20272
Two-rowed	Quality	Diastatic power	4H	123.29	11_20089
Two-rowed	Quality	Diastatic power	4H	123.29	11_20013
Two-rowed	Diseases	Spot blotch	4H	123.29	11_20013
Two-rowed	Quality	α-amylase	5H	33.09	12_10530
Two-rowed	Agronomic	Rachilla hair length	5H	99.56	11_20526
Two-rowed	Agronomic	Deciduous awns	5H	103.72	11_21421
Two-rowed	Agronomic	Awn barbing type	5H	111.68	11_11273
Two-rowed	Agronomic	Awn barbing type	5H	123.33	12_11298
Six-rowed	Diseases	Spot blotch	5H	147.40	11_10557
Two-rowed	Agronomic	Spike length	5H	152.79	12_31221
Two-rowed	Agronomic	Deciduous awns	5H	182.88	12_30769

Table 5.1 Summary of all candidate SNPs for all disease resistance, agronomic and related traits, and malt quality traits (continued).

Row Type	Traits Area	Traits	Chromosome	Position (cM)	Candidate SNP for MAS
Two-rowed	Diseases	Deoxynivalenol	6H	0.00	11_10496
Two-rowed	Diseases	Spot form net blotch - Langdon	6H	3.11	11_21521
Two-rowed	Diseases	Leaf rust	6H	24.36	11_10868
Two-rowed	Diseases	Net form net blotch	6H	51.41	12_30569
Two-rowed	Diseases	Net form net blotch	6H	55.94	11_10377
Two-rowed	Diseases	Net form net blotch	6H	56.48	12_30857
Two-rowed	Diseases	Net form net blotch	6H	60.23	11_21310
Six-rowed	Diseases	Leaf rust	6H	65.03	11_11261
Six-rowed	Diseases	Spot blotch	6H	121.22	12_30057
Two-rowed	Agronomic	Awn barbing type	7H	12.42	11_20245
Two-rowed	Quality	α-amylase	7H	29.82	12_31351
Two-rowed	Quality	α-amylase	7H	29.82	12_30780
Two-rowed	Diseases	Spot blotch	7H	31.75	11_20162
Six-rowed	Diseases	Spot blotch	7H	34.82	12_30219
Six-rowed	Diseases	Leaf rust	7H	86.44	11_20042
Six-rowed	Diseases	Spot form net blotch - Dickinson	7H	128.40	11_21229
Two-rowed	Agronomic	Deciduous awns	Unlinked		12_30916
Two-rowed	Agronomic	Deciduous awns	Unlinked		12_30944
Two-rowed	Agronomic	Deciduous awns	Unlinked		12_31279
Six-rowed	Diseases	Leaf rust	Unlinked		12_10491
Six-rowed	Diseases	Net form net blotch	Unlinked		12_30655
Two-rowed	Agronomic	Rachilla hair length	Unlinked		12_30129

APPENDIX

Table A1. Mean square difference for four models used to identify the best regression model for four different foliar diseases phenotyped on a two-rowed and six-rowed mapping panel for the greenhouse experiments.

Disease[†]	Naïve	PC[‡]	Kinship	PC + Kinship
Two-rowed				
SFNB-L	0.0507	0.0020	0.0003	***0.0002***[§]
SFNB-D	0.0285	0.0033	***0.0004***	0.0007
NFNB	0.0424	0.0076	0.0027	***0.0015***
SB	0.0010	0.0054	0.0009	***0.0001***
LR	0.0108	0.0007	***0.0004***	0.0007
Six-rowed				
SFNB-L	0.0154	0.0310	***0.0020***	***0.0020***
SFNB-D	0.0296	0.0295	***0.0016***	***0.0016***
NFNB	0.0023	0.0174	0.0041	***0.0011***
SB	0.0089	0.0281	0.0022	***0.0016***
LR	0.0089	0.0070	***0.0009***	***0.0009***

[†]SFNB-L = spot form net blotch, Langdon isolate; SFNB-D = spot form net blotch, Dickinson isolate; NFNB = net form net blotch, SB = spot blotch, LR = leaf rust.
[‡]PC = Principal component
[§]Bold and italicized numbers indicate the lowest MSD and best-fit model for each disease.

Table A2. Mean square difference for four models used to identify the best regression model for Deoxynivalenol and foliar disease from field experiments.

Field Traits	Environments	Naïve	PC[‡]	Kinship	PC + Kinship
Two-rowed					
Deoxynivalenol	12LA57	0.0035	0.0030	0.0009	***0.0005***[§]
	12OS57	0.0085	0.0045	***0.0001***	0.0002
	13LA57	0.0004	***0.0001***	0.0049	0.0007
Foliar disease	11NV57	0.0096	0.0126	***0.0002***	0.0005
	12OS57	0.0071	***0.0004***	0.0006	0.0106
	13NV57	0.0430	***0.0002***	0.0003	0.0015

[†]Field traits
[‡]PC = Principal component
[§]Bold and italicized numbers indicate lowest mean square deviation (MSD) and best-fit model for each field disease and related trait

Table A3. Phenotypic data statistics for the genotype component in the combined environment analysis for deoxynivalenol and foliar disease traits on the two-rowed experiments.

Traits	Environments	Mean‡	Minimum	Maximum	Standard deviation
Deoxynivalenol					
	ND_12LA57	12.31	3.81	42.36	7.17
	ND_12OS57	6.17	1.85	16.70	2.93
	ND_13LA57	16.45	3.00	57.07	12.23
	Mapping panel†	11.64	2.89	38.71	2.99
	Avg. checks	13.31	8.64	22.10	3.96
Foliar disease					
	FD_11NV57	4.44	2.02	6.88	1.15
	FD_12OS57	2.14	0.99	4.56	0.87
	FD_13NV57	3.06	1.49	4.49	0.59
	Mapping panel	3.21	1.50	5.31	0.67
	Avg. checks	2.81	1.81	5.16	0.94

† Mapping panel is the mean of 81 NDSU cultivars and breeding lines.

Table A4. Mean square difference for four models used to identify the best regression model.

Quality traits†	Environments	Naive	PC	Kinship	PC + Kinship
Yield	11NV57	0.017020070	0.003073937	0.002238764	**0.000984611**
	12FA57	0.042268943	0.004772499	**9.49E-05**	0.000168661
	12NV57	0.039183619	0.005899465	8.59E-05	**0.000079469**
	12OS57	0.066288577	0.001438952	0.00055587	**0.000256027**
	13FA57	0.005377083	0.007426026	0.002016886	**0.001001677**
	13NV57	0.029131743	0.011962738	0.002381396	**0.002250335**
Plants m^{-2}	12FA57	0.01001234	0.005680359	0.000225506	**0.000202634**
	12OS57	0.012597338	**8.67E-05**	0.000526774	0.001499744
Spikes m^{-2}	12FA57	0.001999726	0.002558712	**0.001355106**	0.824106151
Kernels spike^{-1}	11NV57	0.076633628	0.003789927	**0.000878641**	0.001111693
	12FA57	0.063861452	0.006183806	0.000325783	**0.000322108**
	12OS57	0.06591808	0.007268922	**0.000343826**	0.00143479
	13FA57	0.079657231	0.005267495	0.002208198	**0.00067902**
	13NV57	0.076244467	0.009262287	0.019550992	**0.001607441**

Table A4. Mean square difference for four models used to identify the best regression model (continued).

Quality traits†	Environments	Naive	PC	Kinship	PC + Kinship
Spike length	11NV57	0.046350185	0.000128405	0.000332645	**0.000118876**
	12FA57	0.039334653	0.003521455	**0.000145892**	0.000218932
	12OS57	0.029558451	0.003613649	**0.000356428**	0.000514706
	13FA57	0.043757222	0.001687664	0.000222572	**0.000067432**
	13NV57	0.048111905	0.005856972	**0.000079553**	0.000118386
Rachis nodes spike^{-1}	11NV57	0.076770105	0.003724529	**0.000786228**	0.001064108
	12FA57	0.064266313	0.006227532	0.000254107	**0.000208002**
	12OS57	0.065520484	0.006171099	**0.000472853**	0.00138009
	13FA57	0.081408421	0.004952508	0.002216311	**0.000601984**
	13NV57	0.076166483	0.00940327	0.018494229	**0.001607657**
Spike density	11NV57	0.062633238	0.007438607	0.000588051	**0.000335549**
	12FA57	0.050001813	0.00110521	**0.000179826**	0.000534196
	S12OS57	0.061470286	0.002641122	**0.00007659**	0.000692419
	13FA57	0.062081558	0.002526598	**0.000289378**	0.000547527
	13NV57	0.063461229	0.004557251	0.000242299	**0.000115958**
Days to heading	11MC57	0.062161768	0.008765955	**0.000143913**	0.000373148
	11NV57	0.08139623	0.009015096	0.000572029	**0.000309936**
	12FA57	0.065096451	0.011238102	0.001667832	**0.001231401**
	12NV57	0.079122247	0.010948545	0.003955448	**0.000191826**
	12OS57	0.071221533	0.009181201	**0.000235757**	0.001283927
	13FA57	0.0582789	0.01675316	**0.002739519**	0.003919775
	13NV57	0.069648276	0.009989262	0.000190924	**0.000177068**

Table A4. Mean square difference for four models used to identify the best regression model (continued).

Quality traits†	Environments	Naive	PC	Kinship	PC + Kinship
Plant height	11NV57	0.009301743	0.000421283	0.000133853	**0.000112967**
	12FA57	0.042626679	0.001721783	**0.000294079**	0.000434109
	12NV57	0.003053261	0.000320969	0.000299378	**2.60E-05**
	12OS57	0.004852888	**0.000257589**	0.00040911	0.007225508
	13FA57	0.040647588	0.000467784	**0.000253705**	0.000346233
	13NV57	0.048971515	0.002923836	0.00021327	**0.000114532**
Lodging	12FA57	0.020820864	0.008384405	**0.001536817**	0.001565827
	12OS57	0.011041025	0.001122459	0.001538538	**0.000187022**
Stem breakage	12FA57	0.003318083	0.004088252	**0.00041278**	0.000664451
	12NV57	0.012213694	0.001267541	0.000578387	**0.000192638**
	12OS57	0.008675242	**0.000143722**	0.000213265	0.00037431
	13FA57	0.013849454	0.001425759	0.000804858	**0.000286973**
Spike angle	11NV57	0.01135386	**0.000265364**	0.002940375	0.002146833
	12FA57	0.004770074	0.006368038	**0.000633211**	0.000651455
	12NV57	0.010320222	0.000244302	**0.000209645**	0.001293044
	13FA57	0.012701885	0.009855437	**0.000810229**	0.000980092
Deciduous awns	11NV57	0.037275553	0.003774861	**0.000923508**	0.001029551
	12OS57	0.029641137	0.000542882	**0.000435249**	0.000560471
	13FA57	0.056113206	0.002591229	**0.002059927**	0.002094196
Awn barbing type	11NV57	0.050112	0.012876871	**0.000782329**	0.002241824
	12FA57	0.050112	0.012876871	**0.000782329**	0.002241824
	12OS57	0.050112	0.012876871	**0.000782329**	0.002241824
	13FA57	0.050112	0.012876871	**0.000782329**	0.002241824
	13NV57	0.052208989	0.012468574	**0.000777596**	0.002182803

Table A4. Mean square difference for four models used to identify the best regression model (continued).

Quality traits†	Environments	Naive	PC	Kinship	PC + Kinship
Rachilla hair length	11NV57	0.034265135	0.011491184	0.001797055	**0.001718644**
	12FA57	0.034265135	0.011491184	0.001797055	**0.001718644**
	12OS57	0.034265135	0.011491184	0.001797055	**0.001718644**
	13FA57	0.034265135	0.011491184	0.001797055	**0.001718644**
	13NV57	0.034265135	0.011491184	0.001797055	**0.001718644**

†Quality traits.
‡Bold and italicized numbers indicate lowest mean square deviation (MSD) and best-fit model for each disease trait.

Table A5. Summary of the most significant SNP markers identified in 50% of the environments in the NDSU two-rowed mapping panel.

Trait names†	Chromosome	Position (cM)	Significant markers	Environments	-Log_{10} (*P*-value)	Candidate SNP for MAS§
Spike length	5H	2.81	11_20553	13FA57	3.094555096	
				13NV57	2.287791820	
			12_30543	12FA57	2.110640005	
				13FA57	3.614422680	
				13NV57	2.006207494	
		87.35	11_20645	11NV57	2.154998688	
				12OS57	4.504097829	
				13FA57	2.147662499	
				13NV57	2.144266336	
		151.36	12_30183	11NV57	1.959823168	
				12OS57	3.743340212	
				13FA57	2.450109402	
				13NV57	2.891871960	
		152.79	12_30795	12OS57	3.990193995	
				13NV57	3.156791959	
			12_31221	11NV57	2.010342225	X

Table A5. Summary of the most significant SNP markers identified in 50% of the environments in the NDSU two-rowed mapping panel (continued).

Trait names[†]	Chromosome	Position (cM)	Significant markers	Environments	-Log$_{10}$ (*P*-value)	Candidate SNP for MAS[§]
Spike length	5H	152.79	12_31221	12OS57	4.329127285	
				13NV57	3.228417590	
Days to heading	3H	78.53	11_10047	11MC57	2.135755047	
				12FA57	2.423691920	
				12OS57	2.105209458	
				13NV57	2.120955338	
			11_20597	11MC57	2.157642443	
	7H	107.11	12_31261	11MC57	3.743531215	
				13NV57	2.629404685	
		110.99	11_10853	11MC57	1.923546037	
				12NV57	2.057691832	
				12OS57	3.184247838	
				13FA57	2.174591157	
			11_20092	12OS57	2.711276038	
			11_20385	12OS57	3.423975904	
	Unlinked		12_30285	11MC57	2.791944870	
				11NV57	2.384401543	
				12OS57	2.710986668	
				13FA57	2.357051091	
Plant height	4H	3.74	11_10409	12FA57	3.443490173	
				12OS57	2.107474618	
			11_21228	11NV57	2.313030203	
				12OS57	2.363944387	
		5.55	11_11345	12FA57	3.102218239	
	6H	118.35	11_10645	11NV57	2.362198441	

Table A5. Summary of the most significant SNP markers identified in 50% of the environments in the NDSU two-rowed mapping panel (continued).

Trait names†	Chromosome	Position (cM)	Significant markers	Environments	-Log$_{10}$ (*P*-value)	Candidate SNP for MAS§
Plant height	6H	118.35	11_10645	12NV57	2.019270604	
				13FA57	2.177631622	
				13NV57	2.374876549	
	Unlinked		12_30967	11NV57	2.208038103	
				12OS57	2.340997330	
				13FA57	2.208260690	
Stem breakage	2H	0.00	11_11346	12FA57	2.829139518	
				13FA57	2.983512879	
			11_20498	13FA57	2.460430931	
			11_20609	12OS57	2.623693265	
	4H	106.03	11_20974	12FA57	3.856223198	X
				12OS57	2.948859715	
				13FA57	3.975674451	
Deciduous awns	1H	35.45	11_10814	11NV57	2.381465138	
				12OS57	3.096594179	
		59.71	11_10552	11NV57	2.452435382	
			12_30820	11NV57	3.846999324	X
				13FA57	3.545285295	
			12_30821	11NV57	3.846999324	X
				13FA57	3.545285295	
	5H	103.72	11_21421	11NV57	2.421634665	X
				13FA57	5.834134421	
		180.71	12_30494	12OS57	2.678846468	
		182.16	12_30504	12OS57	2.678846468	
			12_30769	12OS57	3.133837177	X
				13FA57	2.977554389	

Table A5. Summary of the most significant SNP markers identified in 50% of the environments in the NDSU two-rowed mapping panel (continued).

Trait names†	Chromosome	Position (cM)	Significant markers	Environments	-Log$_{10}$ (*P*-value)	Candidate SNP for MAS§
Deciduous awns	Unlinked		12_30916	11NV57	2.591355803	X
				13FA57	5.490405862	
			12_30944	11NV57	2.591355803	X
				13FA57	5.490405862	
			12_31279	11NV57	2.441571808	X
				13FA57	5.303998147	
Awn barbing type	1H	6.03	12_30933	11NV57	3.492940615	X
				12FA57	5.633260469	
				12OS57	3.492940615	
				13FA57	3.492940615	
				13NV57	3.339685325	
	5H	111.68	11_11273	11NV57	4.532273534	X
				12FA57	5.294269512	
				12OS57	4.532273534	
				13FA57	4.532273534	
				13NV57	5.125140169	
		123.33	12_11298	11NV57	2.790574427	X
				12FA57	6.937297295	
				12OS57	2.790574427	
				13FA57	2.790574427	
				13NV57	2.711964172	
			11_20127	12FA57	4.704013102	
	7H	12.42	11_20245	11NV57	2.739351193	X
				12FA57	4.713694010	
				12OS57	2.739351193	
				13FA57	2.739351193	

Table A5. Summary of the most significant SNP markers identified in 50% of the environments in the NDSU two-rowed mapping panel (continued).

Trait names†	Chromosome	Position (cM)	Significant markers	Environments	-Log10 (*P*-value)	Candidate SNP for MAS§
Rachilla hair length	4H	40.36	12_10063	11NV57	2.755984574	
				12FA57	2.755984574	
				12OS57	2.755984574	
				13FA57	2.755984574	
				13NV57	2.755984574	
	5H	99.56	11_20526	11NV57	3.289350041	X
				12FA57	3.289350041	
				12OS57	3.289350041	
				13FA57	3.289350041	
				13NV57	3.289350041	
	Unlinked		12_30129	11NV57	4.442454303	X
				12FA57	4.442454303	
				12OS57	4.442454303	
				13FA57	4.442454303	
				13NV57	4.442454303	

†Quality traits
‡-Log10 (*P*-value)

Table A6. Mean square differences for four models used to identify the best regression model for the two-rowed mapping panel.

Quality traits†	Environments	Naive	PC	Kinship	PC + Kinship
Grain quality					
Barley color					
	MC_11NV57	0.007731824	0.000463387	0.000304789	**0.000104323**‡
	MC_12FA57	0.007097219	0.009227584	0.000596141	**0.000318113**
	MC_12NV57	0.054276383	0.003158702	**0.000392792**	0.000870228
	MC_13FA57	0.022221532	0.000227842	0.00042948	**8.89E-05**
	MC_13NV57	0.005520971	0.004939994	**0.000302303**	0.000627797
Barley protein					
	MP_11NV57	0.071584182	0.013665573	0.000692742	**0.000542633**
	MP_12FA57	0.059650761	0.025854384	**0.001327349**	0.002088161
	MP_12NV57	0.060215931	0.01354739	0.000385365	**0.000299288**
	MP_13FA57	0.032359718	0.013710108	0.00022054	**0.000125105**
	MP_13NV57	0.014419637	0.01638676	**0.000227719**	0.000572733
Kernel weight					
	MK_11NV57	0.058567375	0.00042517	**0.00027544**	0.000301035
	MK_12FA57	0.069285523	0.001101163	0.000486158	**0.000386689**
	MK_12NV57	0.025718074	0.001608252	0.001363258	**0.00052866**
	MK_13FA57	0.031875347	**0.000116109**	0.001378808	0.000139693
	MK_13NV57	0.016637294	0.006705979	0.00037067	**6.31E-05**
Kernel plumpness					
	MU_11NV57	0.057811296	0.001157276	**0.000310329**	0.001175295
	MU_12FA57	0.026507783	**0.000330623**	0.000433577	0.059257305
	MU_12NV57	**0.00025242**	0.000884895	0.000416279	0.000795216
	MU_13FA57	0.02739013	0.001891453	**0.000136634**	0.000392563
	MU_13NV57	0.034931976	**0.000300962**	0.000500033	0.000406836

Table A6. Mean square differences for four models used to identify the best regression model for the two-rowed mapping panel (continued).

Quality traits†	Environments	Naive	PC	Kinship	PC + Kinship
Carbohydrate modification					
Wort ß-glucan					
	MB_11NV57	0.045645352	0.004225965	0.000290373	**0.000161307**
	MB_12FA57	0.051088824	0.001125284	**0.000245485**	0.000576558
	MB_12NV57	0.037627948	0.002732459	**0.000080133**	0.000222126
	MB_13FA57	0.039157106	0.001890783	0.002735353	**0.001804273**
	MB_13NV57	0.038657447	0.003794161	**9.27E-05**	0.00026538
Malt extract					
	ME_11NV57	0.006009115	0.00626934	0.000450874	**0.000241369**
	ME_12FA57	0.025703426	0.003259076	0.000805178	**0.000266628**
	ME_12NV57	0.00681775	0.005168864	0.000191139	**0.000165989**
	ME_13FA57	0.007616616	0.000444165	**0.00025453**	0.000492184
	ME_13NV57	0.041250293	0.002625947	**0.000152386**	0.000494254
Enzymatic activity					
α-amylase					
	MA_11NV57	0.058632262	0.007282073	**0.000648271**	0.000904191
	MA_12FA57	0.02865711	0.005820289	**0.00033328**	0.000412529
	MA_12NV57	0.056661318	0.009001508	**0.00055363**	0.00066883
	MA_13FA57	0.075997398	0.008684181	0.000534949	**0.000169413**
	MA_13NV57	0.021215873	0.005622289	0.000816881	**0.000481174**
Diastatic power					
	MI_11NV57	0.022609914	0.002009983	0.000548117	**0.000137523**
	MI_12FA57	0.021765843	0.002686789	**0.000128076**	0.00017126
	MI_12NV57	0.034286047	0.000344206	0.000703874	**0.000100565**
	MI_13FA57	0.002395529	**0.000178034**	0.000208659	0.007919195
	MI_13NV57	0.016220844	0.001584191	0.001920026	**0.000718882**

Table A6. Mean square differences for four models used to identify the best regression model for the two-rowed mapping panel (continued).

Quality traits†	Environments	Naive	PC	Kinship	PC + Kinship
Protein modification					
Wort color					
	MO_11NV57	0.004514315	**0.000149767**	0.000163014	0.000177828
	MO_12FA57	0.046591383	**0.000570094**	0.002334953	0.000748222
	MO_12NV57	0.013521012	**0.000249043**	0.000297891	0.00038203
	MO_13FA57	0.033524297	**0.000190784**	0.000461354	0.116250454
	MO_13NV57	0.013975198	0.000793833	0.000305212	**0.000106993**
Wort protein					
	MT_11NV57	0.045767473	0.000404697	**0.000199994**	0.000513668
	MT_12FA57	0.032591387	0.004209368	**0.000184166**	0.000184308
	MT_12NV57	0.056038514	0.001144619	**0.000489134**	0.000554064
	MT_13FA57	0.071114078	0.002214679	0.000405455	**8.76E-05**
	MT_13NV57	0.014403956	0.001475395	0.000190045	**0.000169616**
Soluble/total protein					
	MS_11NV57	0.012309389	0.002337919	**0.000507305**	0.000509567
	MS_12FA57	0.02285389	0.0056089	0.003221707	**0.001499436**
	MS_12NV57	0.02932701	0.003439196	**0.000377661**	0.000548344
	MS_13FA57	0.022220436	0.004625066	0.000155933	**3.31E-05**
	MS_13NV57	0.002204515	0.000474231	0.000293066	**0.000184948**
Free amino nitrogen					
	MF_11NV57	0.032456447	0.000619536	0.000784396	**0.000475711**
	MF_12FA57	0.038103327	0.001146021	0.000425305	**0.000111687**
	MF_12NV57	0.02609102	0.001068116	0.000913259	**0.000806568**
	MF_13FA57	0.034763767	0.002836879	0.000294191	**0.000166755**
	MF_13NV57	0.013734353	0.000519066	**9.97E-05**	0.00011592

†Quality traits

‡Bold and italicized numbers indicate lowest mean square deviation (MSD) and best-fit model for each disease trait.

Table A7. Phenotypic data statistics and F-value for the genotype component in the combined environment analysis for the two-rowed mapping panel.

Traits †	Environments	Minimum	Maximum	Mean	SD‡	CV§
Grain quality						
Barley Color						
	MC_11NV57	27.00	44.00	32.21	2.83	8.79
	MC_12FA57	49.00	72.00	57.05	4.30	7.53
	MC_12NV57	26.00	46.00	34.09	3.61	10.61
	MC_13FA57	45.00	77.00	57.71	6.04	10.47
	MC_13NV57	29.00	44.00	35.09	3.50	9.97
	Ave Genotypes	35.20	56.60	43.23	5.80	
	Ave 3 checks	45.13	40.60	50.60	5.06	11.22
Barley protein						
	MP_11NV57	13.70	17.20	15.43	0.84	5.46
	MP_12FA57	11.80	15.70	13.55	0.91	6.73
	MP_12NV57	13.50	17.00	14.79	0.84	5.70
	MP_13FA57	10.90	13.90	12.30	0.68	5.54
	MP_13NV57	11.30	15.20	12.58	0.79	6.28
	Ave Genotypes	12.24	15.80	13.73	0.61	
	Ave 3 checks	13.64	12.52	14.22	0.97	7.11
Kernel weight						
	MK_11NV57	33.10	46.20	39.38	2.55	6.48
	MK_12FA57	32.90	46.40	39.76	2.50	6.29
	MK_12NV57	29.20	43.80	37.93	2.44	6.43
	MK_13FA57	25.80	42.80	34.93	3.01	8.61
	MK_13NV57	38.40	51.70	44.42	2.88	6.49
	Ave Genotypes	31.88	46.18	39.28	1.54	
	Ave 3 checks	36.35	31.32	38.92	4.36	11.99
Kernel plumpness						
	MU_11NV57	93.30	99.80	98.59	1.32	1.34
	MU_12FA57	80.40	96.40	91.41	3.57	3.90

Table A7. Phenotypic data statistics and F-value for the genotype component in the combined environment analysis for the two-rowed mapping panel (continued).

Traits †	Environments	Minimum	Maximum	Mean	SD‡	CV§
Kernel plumpness	MU_12NV57	64.50	98.80	87.14	5.92	6.79
	MU_13FA57	53.20	96.40	86.29	7.30	8.46
	MU_13NV57	93.20	99.50	97.07	1.37	1.41
	Ave Genotypes	76.92	98.18	92.10	2.51	
	Ave 3 checks	90.19	84.88	93.78	4.69	5.20
Carbohydrate modification						
Wort ß-glucan						
	MB_11NV57	60.00	639.00	264.51	133.61	50.51
	MB_12FA57	52.00	741.00	290.44	149.37	51.43
	MB_12NV57	38.00	525.00	186.43	90.84	48.73
	MB_13FA57	47.00	879.00	246.24	136.58	55.47
	MB_13NV57	71.00	744.00	297.26	138.56	46.61
	Ave Genotypes	53.60	705.60	256.98	19.86	
	Ave 3 checks	206.00	142.40	323.00	101.45	49.25
Malt extract						
	ME_11NV57	75.60	80.70	78.00	1.17	1.50
	ME_12FA57	76.30	81.90	79.03	1.33	1.68
	ME_12NV57	73.10	80.30	77.35	1.19	1.54
	ME_13FA57	76.30	83.30	79.37	1.53	1.93
	ME_13NV57	79.00	83.40	80.75	1.01	1.25
	Ave Genotypes	76.06	81.92	78.90	0.59	
	Ave 3 checks	78.20	77.70	79.16	0.83	1.06
Enzymatic activity						
α-amylase						
	MA_11NV57	73.60	131.40	104.41	12.51	11.98
	MA_12FA57	62.90	119.50	88.08	12.34	14.01
	MA_12NV57	71.70	127.20	94.44	12.86	13.61
	MA_13FA57	69.00	112.10	89.06	9.91	11.13
	MA_13NV57	65.20	94.90	78.31	7.37	9.41

Table A7. Phenotypic data statistics and F-value for the genotype component in the combined environment analysis for the two-rowed mapping panel (continued).

Traits †	Environments	Minimum	Maximum	Mean	SD‡	CV§
α-amylase	Ave Genotypes	68.48	117.02	90.86	4.27	
	Ave 3 checks	86.35	82.24	90.82	4.30	4.98
Diastatic power						
	MI_11NV57	104.00	221.00	163.83	25.53	15.59
	MI_12FA57	61.00	177.00	112.81	21.24	18.83
	MI_12NV57	107.00	206.00	151.79	20.63	13.59
	MI_13FA57	70.00	146.00	109.35	17.57	16.07
	MI_13NV57	99.00	186.00	139.59	19.89	14.25
	Ave Genotypes	88.20	187.20	135.47	10.68	
	Ave 3 checks	175.20	137.60	235.60	52.83	30.15
Protein modification						
Wort color						
	MO_11NV57	1.60	5.30	3.07	0.70	22.87
	MO_12FA57	1.40	2.70	1.98	0.35	17.48
	MO_12NV57	1.60	3.20	2.11	0.33	15.62
	MO_13FA57	2.10	5.20	3.04	0.64	21.15
	MO_13NV57	1.80	3.70	2.49	0.42	16.68
	Ave Genotypes	1.70	4.02	2.54	0.23	
	Ave 3 checks	2.31	2.22	2.38	0.08	3.50
Wort protein						
	MT_11NV57	5.40	9.00	6.91	0.73	10.50
	MT_12FA57	4.30	7.30	5.57	0.62	11.21
	MT_12NV57	4.70	7.50	5.83	0.58	9.95
	MT_13FA57	4.40	6.70	5.32	0.46	8.62
	MT_13NV57	4.70	7.00	5.41	0.49	9.03
	Ave Genotypes	4.70	7.50	5.81	0.29	
	Ave 3 checks	5.38	5.24	5.50	0.13	2.41

Table A7. Phenotypic data statistics and F-value for the genotype component in the combined environment analysis for the two-rowed mapping panel (continued).

Traits †	Environments	Minimum	Maximum	Mean	SD‡	CV§
Soluble/total protein						
	MS_11NV57	37.00	59.30	46.10	4.63	10.05
	MS_12FA57	33.50	54.00	42.34	4.84	11.44
	MS_12NV57	33.60	50.00	40.47	3.87	9.57
	MS_13FA57	36.90	54.70	44.92	4.27	9.50
	MS_13NV57	38.10	56.90	44.51	3.54	7.95
	Ave Genotypes	35.82	54.98	43.67	1.01	
	Ave 3 checks	41.06	39.56	43.20	1.90	4.63
Free amino nitrogen						
	MF_11NV57	186.00	378.00	256.05	36.30	14.18
	MF_12FA57	156.00	344.00	226.55	38.99	17.21
	MF_12NV57	183.00	347.00	240.18	34.21	14.24
	MF_13FA57	182.00	354.00	250.43	32.37	12.93
	MF_13NV57	215.00	364.00	258.76	29.82	11.52
	Ave Genotypes	184.40	357.40	246.39	5.89	
	Ave 3 checks	216.27	207.40	230.20	12.22	5.65

†Quality traits
‡SD = Standard Deviation
§CV = Coefficient of Variation

Table A8. Summary of the most significant SNP markers identified in three or more out of five environments in the two-rowed panel.

Chromosome	Quality area	Traits names†	Position (cM)	Significant markers	Environments	-Log_{10} (*P*-value)	Candidate SNP for MAS§
1H							
	Grain quality	Barley color	17.26	11_10775	13NV57	1.906392007	
			20.82	12_30241	13FA57	2.122372306	
			20.89	11_10873	11NV57	2.076872116	
	Carbohydrate modification	Wort ß-glucan	47.47	12_30683	11NV57	3.481786617	
					13FA57	6.132480642	
				12_31467	11NV57	3.481786617	
					13FA57	6.132480642	
			50.00	11_20047	11NV57	2.804541339	
					12NV57	2.559165919	
					13NV57	2.273884403	
			66.70	11_11367	11NV57	2.647996776	X
					12NV57	3.186923780	
					13FA57	4.249899864	
				11_21219	11NV57	2.647996776	X
					12NV57	3.186923780	
					13FA57	4.249899864	

Table A8. Summary of the most significant SNP markers identified in three or more out of five environments in the two-rowed panel (continued).

Chromosome	Quality area	Traits names†	Position (cM)	Significant markers	Environments	-Log$_{10}$ (*P*-value)	Candidate SNP for MAS§
2H							
	Carbohydrate modification	Malt extract	21.61	12_10777	13NV57	2.143715078	
			26.53	11_10216	11NV57	1.913664665	
					12FA57	2.037246562	
			95.64	11_20080	12FA57	2.127113642	
					12NV57	3.123254051	
			96.82	12_10649	12NV57	3.561698835	
				11_11307	13FA57	2.175336514	
			98.59	11_20086	13NV57	2.283205892	
3H							
	Enzymatic activity	Diastatic power	28.44	12_30284	12FA57	4.017881702	X
					12NV57	3.171081344	
					13NV57	3.024862968	
	Protein modification	Wort protein	47.09	11_20356	13FA57	2.137158361	
			48.63	11_20719	13FA57	2.137158361	
				11_21189	13FA57	2.137158361	
				12_30737	13FA57	2.137158361	
				12_30474	13FA57	2.137158361	

Table A8. Summary of the most significant SNP markers identified in three or more out of five environments in the two-rowed panel (continued).

Chromosome	Quality area	Traits names†	Position (cM)	Significant markers	Environments	-Log_{10} (*P*-value)	Candidate SNP for MAS§
3H	Protein modification	Wort protein	51.73	11_10380	12FA57	2.162472052	
					12NV57	2.345611697	
				11_21109	12FA57	2.162472052	
					12NV57	2.345611697	
				12_30680	12FA57	2.162472052	
					12NV57	2.345611697	
	Protein modification	Free amino nitrogen	51.73	11_10380	12FA57	3.446352138	X
					12NV57	3.242328297	
					13FA57	2.616285646	
				11_21109	12FA57	3.446352138	X
					12NV57	3.242328297	
					13FA57	2.616285646	
				12_30680	12FA57	3.446352138	X
					12NV57	3.242328297	
					13FA57	2.616285646	
	Grain quality	Barley protein	162.15	12_30767	13NV57	2.351045832	
			167.77	11_10893	11NV57	3.390195857	
					12FA57	2.299247631	

Table A8. Summary of the most significant SNP markers identified in three or more out of five environments in the two-rowed panel (continued).

Chromosome	Quality area	Traits names[†]	Position (cM)	Significant markers	Environments	-Log_{10} (*P*-value)	Candidate SNP for MAS[§]
4H							
	Enzymatic activity	α-amylase	51.30	11_20496	13FA57	2.356058849	
			53.50	12_30427	13FA57	2.356058849	
			55.63	12_30995	11NV57	3.446071848	
				11_10527	13NV57	2.194043555	
	Carbohydrate modification	Wort ß-glucan	65.05	11_11224	11NV57	4.229653978	X
					12NV57	4.081708011	
					13FA57	6.878718876	
					13NV57	2.454448720	
				12_30620	11NV57	4.229653978	X
					12NV57	4.081708011	
					13FA57	6.878718876	
					13NV57	2.454448720	
				12_31515	11NV57	4.229653978	X
					12NV57	4.081708011	
					13FA57	6.878718876	
					13NV57	2.454448720	
			65.80	12_30455	11NV57	4.229653978	X

Table A8. Summary of the most significant SNP markers identified in three or more out of five environments in the two-rowed panel (continued).

Chromosome	Quality area	Traits names†	Position (cM)	Significant markers	Environments	-Log$_{10}$ (*P*-value)	Candidate SNP for MAS§
4H	Carbohydrate modification	Wort ß-glucan	65.80	12_30455	12NV57	4.081708011	
					13FA57	6.878718876	
					13NV57	2.454448720	
	Protein modification	Soluble/total protein	77.31	11_11004	13FA57	1.971194357	
				11_21332	11NV57	2.369420382	
					12NV57	2.801560940	
					13FA57	1.941489357	
				11_21353	13FA57	1.971194357	
				12_30136	13FA57	1.971194357	
				12_31231	13FA57	1.971194357	
	Grain quality	Kernel plumpness	111.07	12_31138	12FA57	3.194793869	
					13FA57	5.314126292	
			111.66	12_11233	13NV57	2.157873157	
	Protein modification	Free amino nitrogen	119.09	11_20272	12FA57	4.988937305	X
					12NV57	2.978982840	

Table A8. Summary of the most significant SNP markers identified in three or more out of five environments in the two-rowed panel (continued).

Chromosome	Quality area	Traits names†	Position (cM)	Significant markers	Environments	-Log10 (*P*-value)	Candidate SNP for MAS§
4H	Protein modification	Free amino nitrogen	119.09	11_20272	13FA57	3.635563081	
					13NV57	3.571655888	
		Wort protein			11NV57	2.526577427	X
					12FA57	3.135456348	
					12NV57	2.739802542	
		Wort Color			13FA57	4.270268405	
					13NV57	3.775613799	
					11NV57	3.381847685	X
					12NV57	4.285374384	
					13NV57	2.165906323	
				12_30873	12FA57	2.490639802	
					13NV57	3.174858117	
	Enzymatic activity	Diastatic power	120.58	12_31422	11NV57	3.248753623	
					12FA57	2.524640977	
			123.29	11_11019	11NV57	3.022225791	
					12FA57	3.039160633	
					12NV57	2.714926512	

Table A8. Summary of the most significant SNP markers identified in three or more out of five environments in the two-rowed panel (continued).

Chromosome	Quality area	Traits names†	Position (cM)	Significant markers	Environments	-Log$_{10}$ (*P*-value)	Candidate SNP for MAS§
4H	Enzymatic activity	Diastatic power	123.29				
					13FA57	1.927751989	
				11_20013	11NV57	4.694205962	X
				11_20013	12FA57	3.585223633	
					12NV57	3.847375055	
					13FA57	2.352716606	
				11_20089	11NV57	5.155400711	X
					12FA57	4.302293022	
					12NV57	4.441646688	
					13FA57	2.967023275	
				12_30824	11NV57	3.022225791	
					12FA57	3.039160633	
					12NV57	2.714926512	
					13FA57	1.927751989	
				12_30825	11NV57	3.022225791	
					12FA57	3.039160633	
					12NV57	2.714926512	
					13FA57	1.927751989	

Table A8. Summary of the most significant SNP markers identified in three or more out of five environments in the two-rowed panel (continued).

Chromosome	Quality area	Traits names†	Position (cM)	Significant markers	Environments	-Log_{10} (*P*-value)	Candidate SNP for MAS§
5H							
	Enzymatic activity	α-amylase	33.09	12_10530	12FA57	3.563721238	X
					12NV57	3.187939231	
					13NV57	2.483071797	
			34.25	12_10499	12FA57	3.564023201	
					13FA57	2.207491088	
	Protein modification	Soluble/total protein	64.04	12_30745	12FA57	2.547473256	
			67.54	11_21275	13FA57	1.876528844	
					13NV57	2.038855390	
	Enzymatic activity	α-amylase	80.61	11_21133	12FA57	2.904914481	
					12NV57	2.410488040	
				12_10634	12FA57	2.904914481	
			85.21	11_20246	12FA57	2.750421636	
					13NV57	3.010090278	

Table A8. Summary of the most significant SNP markers identified in three or more out of five environments in the two-rowed panel (continued).

Chromosome	Quality area	Traits names†	Position (cM)	Significant markers	Environments	-Log$_{10}$ (*P*-value)	Candidate SNP for MAS§
5H	Grain quality	Kernel weight	132.48	12_11472	12NV57	2.279979718	
			132.63	11_10705	13FA57	2.950879278	
			135.72	11_10783	13NV57	2.055990779	
			137.16	11_11080	13FA57	2.311479919	
					13NV57	2.100527319	
				11_21241	12NV57	3.502049715	
			137.16	12_30930	12NV57	2.255644460	
					13FA57	2.190905067	
					13NV57	2.019264761	
				12_31237	12NV57	3.502049715	
	Carbohydrate modification	Malt extract	135.72	11_10783	11NV57	2.248312305	
					13FA57	2.231765062	
					13NV57	1.942565590	
			136.43	12_30668	11NV57	2.186739100	
					13FA57	2.294781223	
			136.43	12_30869	11NV57	2.186739100	
					13FA57	2.294781223	

Table A8. Summary of the most significant SNP markers identified in three or more out of five environments in the two-rowed panel (continued).

Chromosome	Quality area	Traits names†	Position (cM)	Significant markers	Environments	-Log$_{10}$ (*P*-value)	Candidate SNP for MAS§
5H	Carbohydrate modification	Malt extract	137.16	11_11080	11NV57	2.011345429	
					13FA57	2.278782243	
			137.16	11_21241	13FA57	2.051471834	
			137.16	12_30930	12NV57	3.697793575	
			137.16	12_31237	13FA57	2.051471834	
	Carbohydrate modification	Kernel plumpness	137.16	12_31237	12NV57	2.892127050	
5H	Enzymatic activity	α-amylase	194.64	12_30382	12NV57	2.359512560	
					13FA57	3.622022787	
					13NV57	2.690704283	
			195.42	11_20402	13FA57	3.376530516	
			196.12	12_10322	13FA57	2.764263848	
			196.85	12_31123	13FA57	2.764263848	
6H							
	Enzymatic activity	Diastatic power	97.39	11_20531	13NV57	2.514674246	
				12_31044	11NV57	2.691350208	

Table A8. Summary of the most significant SNP markers identified in three or more out of five environments in the two-rowed panel (continued).

Chromosome	Quality area	Traits names†	Position (cM)	Significant markers	Environments	-Log$_{10}$ (*P*-value)	Candidate SNP for MAS§
6H	Enzymatic activity	Diastatic power	97.39	12_31044	12FA57	3.469792103	
					12NV57	3.476997281	
					13NV57	1.951392800	
				12_31048	12NV57	3.087558035	
				12_31049	11NV57	2.691350208	
					12FA57	3.469792103	
					12NV57	3.476997281	
					13NV57	1.951392800	
7H	Enzymatic activity	α-amylase	29.82	12_30780	11NV57	2.621284689	X
					12FA57	3.214564879	
					12NV57	3.228160907	
7H	Enzymatic activity	α-amylase		12_31351	11NV57	2.461596813	X
					12FA57	3.304658529	
					12NV57	3.219385122	

Table A8. Summary of the most significant SNP markers identified in three or more out of five environments in the two-rowed panel (continued).

Chromosome	Quality area	Traits names[†]	Position (cM)	Significant markers	Environments	-Log_{10} (*P*-value)	Candidate SNP for MAS[§]
7H	Carbohydrate modification	Wort ß-glucan		12_30780	12NV57	2.707700435	
				12_31351	12FA57	2.420663031	
					12NV57	3.409390861	
					13FA57	2.800610233	
	Enzymatic activity	Diastatic power	138.17	12_30380	13FA57	1.757634029	
			139.72	11_10885	13NV57	1.994639104	
			140.21	11_10454	12FA57	2.339217444	
			141.76	11_20139	12NV57	3.177721791	
				12_30761	12NV57	3.177721791	

[†]Quality traits;
[‡]-Log_{10} (*P*-value)

a) Two-rowed panel

b) Six-rowed panel

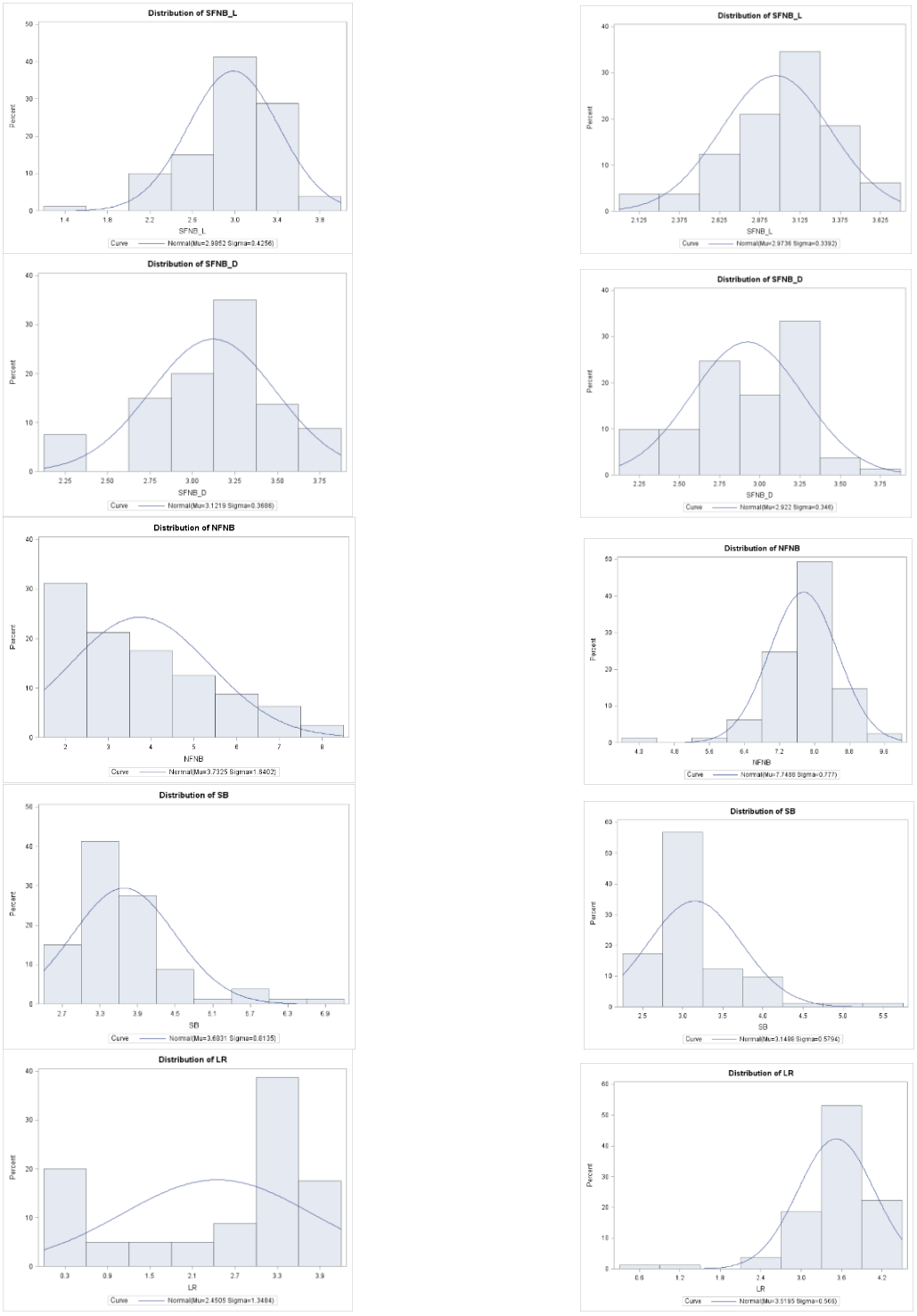

Figure A1. Phenotypic distribution of the studied traits for both mapping panels (SFNB-L = spot form net blotch, Langdon isolate; SFNB-D = spot form net blotch, Dickenson isolate, NFNB = net form net blotch, SB = spot blotch, LR = leaf rust) in greenhouse experiments.