GENETIC DIVERSITY AND GENOME-WIDE ASSOCIATION MAPPING OF AGRONOMIC, DISEASE RESISTANCE, AND QUALITY TRAITS IN BARLEY ACCESSIONS FROM ETHIOPIA, ICARDA, AND THE US

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

DOCTOR OF PHILOSOPHY

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ABSTRACT

Plant breeding is a dynamic process that incorporates new germplasm to introduce genetic variation. Knowledge gained from genetic diversity studies and identification of potentially useful germplasm is critical for efficiently utilizing these new materials for breeding program. Linkage disequilibrium (LD), diversity, and association mapping analyses in barley (*Hordeum vulgare* L.) were done using a mapping panel that included Ethiopian landraces, and cultivars and breeding lines from the Ethiopian, ICARDA, and NDSU breeding programs. LD decayed within 10 to 20 cM in the mapping panel and large proportions of unlinked loci were found to have large LD estimates, indicating that factors other than linkage contributed to LD. Diversity analyses using phenotypic data and molecular markers indicated that the mapping panel was highly structured according to spike row-type, geographic origin, and breeding history; thus, accounting for population structure and familial relatedness was crucial for association analyses. Comparison of the four models (Naïve, P, K, and P+K) indicated that the P+K model is the best model for the current mapping panel. The genome-wide association study (GWAS) identified 94 QTL for 14 agronomic and disease resistance traits; and 145 QTL for 11 malt and grain quality traits. Association mapping of agronomic and disease resistance traits identified six photoperiod related loci (*Ppd_H1*, *HvFT4*, *HvGI*, *HvFT2*, *HvCO2*, and *HvCO1*) and one vernalization-related locus (*VRN-H1*) for days to heading, one semi-dwarf locus (*sdw3*) for plant height, and four resistance loci (*Rrs1*, *Rrs15, rpt.k* and *rpt.r*). The largest number of QTL for malt and grain quality traits was detected in chromosome 5H, followed by chromosome 7H. QTL for malt and grain quality traits were mapped near the *Hor1*, *Hor2*, *Upg2*, *Dor4*, *Ltp1*, *Amy1*, and *Amy2* loci. Several unique QTL were identified in the ICARDA and NDSU accessions, with the NDSU materials having the favorable marker genotypes. These regions could be useful to the Ethiopian breeding program for improving malt quality. The current study indicated that association mapping provided useful tool to identify QTL for several traits simultaneously. Because the QTL had small effect and distributed across the genome, genome selection may be warranted for improving these traits.

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DEDICATION

This dissertation is dedicated to my late mother, Shashitu Negeri, who shaped me from the very beginning. She gave me all the chances of formal education in the expense of her life. Thank you mother for all you did to me and I hoped you see this day.

PREFACE

When my advisor Dr. Richard D. Horsley and I decided to work on this project four years ago, a major goal was to obtain results that would benefit both the Ethiopian and NDSU barley breeding programs. Extensive characterization in every aspect from agronomic performance to quality and mapping of QTL in a different genetic background was the top priority. The current study included germplasm sampled from Ethiopian landraces as well as breeding lines and cultivars from the Ethiopian, International Center for Agricultural Research in the Dry-land Areas (ICARDA), and North Dakota State University (NDSU) breeding programs. The landraces from Ethiopia have been acknowledged for their huge diversity resulting from the varied, but distinct agro-ecology, topography, and socioeconomic dissimilarities of the farming community. The landraces have been widely used by breeders around the globe to incorporate resistance genes for several diseases. The Ethiopian barley-breeding program focuses on improving barley for different crowing conditions, seasons, and end used including food and malt. The ICARDA breeding program is also focusing on developing cultivars for semi-arid and high rainfall areas around the world. Unlike these programs that are breeding for vast areas, the NDSU breeding program has been striving to improve agronomic performance and malt quality for a specific region, the upper Midwest US. Regardless of the location of a breeding program, the sharing of germplasm provides new alleles that can be exploited by another breeding program.

Though conducting experiments in two continents and importing germplasm and samples from Ethiopia were challenging, it was a nice experience to work on all-encompassing evaluation of barley genotypes from Ethiopia, ICARDA, and NDSU. It was also a good opportunity to work on disease screening, malt quality analysis, and DNA genotyping, which I only participated in a few times in my past careers.

I managed to record large amounts of information on barley adaptation attributes, yield and yield components, disease resistance in the field and greenhouse, and malt and grain quality traits. The study covered diversity and population structure analyses using both phenotypic and molecular marker data. I detected several genomic regions with QTL that putatively control different agronomic, disease resistance, and quality traits. While others had identified many of the QTL previously, I did find new QTL unique to one or more of the genotype groups I used.

This dissertation is divided into five chapters. Chapter I provides an overall literature review on the current state of knowledge for the information pertinent to this dissertation. Chapter II provides the results on the study of the phenotypic diversity and structure of the mapping panel. Chapter III reports on the genome-wide association study (GWAS) of agronomic and disease resistance traits, and Chapter IV reports on the GWAS of malt and grain quality traits. The final chapter provides an overall summary of the research conducted and provides recommendations on how to apply the identified genomic regions for germplasm improvement in the Ethiopian and NDSU barley breeding programs.

Finally, I really want to thank the contributions from friends in Ethiopia and NDSU, for which the successes of this project would not have been possible.

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GENERAL INTRODUCTION

Searching for useful germplasm for a breeding program is indispensable for sustained crop improvement. Improving breeding strategies and efficiencies in a continuous basis is also equally important. Thus, plant breeders typically look for germplasm that has favorable alleles that are lacking in their own breeding program. They usually opt to introduce new techniques and technologies to improve the breeding process.

Barley germplasm from Ethiopia usually is tall and has weak straw. Thus, obtaining germplasm that can be used to improve plant height and lodging resistance is of utmost importance for the Ethiopian breeding program. In the area of malt quality, the Ethiopian barley-breeding program has been making improvements in their most recent releases. However, the variability for these traits in their current germplasm base is inadequate to make larger gains. Thus, searching for germplasm that can be used as parents to improve malt quality traits in the Ethiopian breeding program is crucial. Despite its weaknesses in straw strength and malt quality, the germplasm from Ethiopia, especially landraces, has been extensively used in breeding programs throughout the world as sources of alleles for disease resistance. The North Dakota State University (NDSU) barley-breeding program has not utilized barley germplasm from Ethiopia for over 40 years because of its poor malt quality.

Finding germplasm that can be useful to improve a trait lacking in a breeding program alone is not sufficient. It is also equally important to understand how breeding programs can efficiently utilize the germplasm in their program. Getting insight on the genetic basis of these traits is critical to design an appropriate breeding strategy. One way to accomplish this is through determining the effect and position of quantitative trait loci (QTL). Breeders should know which QTL are fixed in the breeding program and which others are lacking. The current study utilized genome-wide association study (GWAS) to identify QTL across different germplasm and those that are unique to a certain germplasm group.

A goal of this study is to determine the genetic diversity in barley germplasm from Ethiopia, and lines and cultivars from the Ethiopian, ICARDA, and NDSU breeding programs for different agronomic, disease resistance, and malt and grain quality traits, as well as to detect QTL that can be utilized in Ethiopian and NDSU breeding program. To achieve these ends, experiments were conducted in the field in Ethiopia (Bekoji, Koffele, and Holetta) and USA (Fargo, Langdon, and Osnabrock), greenhouse, and

laboratory (malting and brewing laboratory). In the meantime, the accessions were genotyped using SNP markers that were generated using genotyping-by-sequencing (GBS) methods. This dissertation is organized in five chapters, with three of them presenting the results from different experiments.

Chapter I provides an overall literature review on the current state of knowledge for the information pertinent to this dissertation. In chapter II, the results from experiments to determine the diversity and population structure for the mapping panel are described. To this end, different descriptive statistics and multivariate procedures were applied. Ultimately, potentially useful germplasm were identified for Ethiopian and/or NDSU breeding programs. Chapter III was devoted to the discussion of results on molecular marker based diversity analysis, understanding the extent of linkage disequilibrium (LD), and identification of QTL for agronomic and disease resistance traits. Just like Chapter III, Chapter IV concentrated on identification of QTL, but this time for malt and grain quality traits. In chapter III and IV, the discussion of unique QTL identified in specific germplasm groups was also included. Finally, the general summary and conclusions, which includes my recommendations for utilizing this research and for future research is presented as Chapter V.

CHAPTER I: LITERATURE REVIEW

Taxonomy and Evolution of Barley

Barley (*Hordeum vulgare L.*) belongs to the Tribe *Triticeae* and grass family *Poaceae* along with rice (*Oryza sativa*), wheat (*Triticum aestivum* L.), oat (*Avena sativa* L.), maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L.), and some lawn and pasture grasses (Bothmer and Jacobsen, 1985; Kellogg, 2001; Bothmer et al., 2003). Barley is in the *genus Hordeum,* which consists of 32 *species* and 45 *taxa* including *diploid* (2n = 2x = 14), *tetraploid* (2n = 4x = 28), and *hexaploid* (2n = 6x = 42) cytotypes (Bothmer et al., 2003). All species in the *genus Hordeum* have three one-flowered spikelets at a rachis node, where the two lateral spikelets are fertile in six-rowed barley and sterile in two-rowed barley. Moreover, there is also biological diversity in the *genus Hordeum* in growth habit (i.e. annual and perennials) and mode of reproduction (i.e. inbreeding and self-incompatible) (Bothmer et al., 2003). Based on the gene pool concept, cultivated barley and *Hordeum vulgare ssp. spontaneum* are in the primary gene pool while *Hordeum bulbosum* is the only species in the secondary gene pool (Bothmer et al., 2003). All the other species in the genus *Hordeum* are in the tertiary gene pool. Cultivated barley and *Hordeum vulgare ssp. spontaneum* have good chromosome paring and also similarity in isozyme patterns (Bothmer and Jacobsen, 1985).

The genome sizes and structures of cereal species have been shaped by evolutionary events such as genome duplications, chromosomal translocations, chromosomal breakage and fusions, and polyploidy (Bolot et al., 2009). *Poaceae* is considered to be monophyletic and all grasses belonging to it may have evolved from a single ancestor (Pourkheirandish and Komatsuda, 2007; Bolot et al., 2009). The current cereal genomes were shaped from a five-chromosome ancestor via a 12-chromosome intermediate ancestor (Bolot et al., 2009). Initially, whole genome duplication in the five-chromosome ancestor some 50-70 million years ago was followed by two inter-chromosomal translocations and fusions that resulted in the n = 12 intermediate ancestral chromosomes. For wheat and barley, a subsequent five chromosomal fusion in the n = 12 chromosome ancestor led to n = seven chromosomes (Bolot et al., 2009). Barley is believed to have diverged from wheat approximately 11.6 million years ago and further diversification occurred since then into several sub-species (Chalupska et al, 2008).

Domestication and Dissemination of Barley

Plant domestication is the outcome of a selection process that leads to increased adaptation of plants to cultivation and utilization by humans. According to Bothmer et al. (2003), there are two dominant theories for barley domestication. The first theory is that the two-rowed type was directly derived from *Hordeum vulgare ssp. spontaneum* in the Fertile Crescent in southwest Asia. Supportive to this theory, archaeological remains of two-rowed barley with sporadic six-rowed elements were found in Ali Kosh in southern Iran some 9000 BP (Pourkheirandish and Komatsuda, 2007). The second theory is the hypothesis of two independent centers of origin. With the discovery of six-rowed barley with a brittle rachis in western China in the 1930s, it was firmly believed that six-rowed barley evolved from *Hordeum acriocrithon* (Bothmer et al., 2003). Some phylogenetic studies using markers closely linked to the *btr1*/*btr2* genes (genes controlling rachis brittleness) indicated that cultivated barley consists of two geographic types, western and eastern types (Pourkheirandish and Komatsuda, 2007; and references cited therein), supporting the two independent domestication hypotheses of barley proposed by Takahashi in 1955 (Pourkheirandish and Komatsuda, 2007). According to the review by Burger et al. (2008), consensus about domestication of barley has not been reached.

Additionally, there are other reports suggesting other areas of domestication for barley in countries like Ethiopia (Orabi et al., 2007; and references therein), Morocco (Molina-Cano et al., 1999; references cited therein), and Tibet (reference cited in Molina-Cano et al., 1999). However, the most favored theory among barley scientists at present is a single evolutionary line from *Hordeum vulgare ssp. spontaneum* to cultivated two-rowed barley, which was then changed to a six-rowed spike in cultivated barley by mutation during the domestication process (Bothmer et al., 2003; Pourkheirandish and Komatsuda, 2007; Newman and Newman, 2008).

Domestication of barley was associated with gradual accumulation of traits that facilitated agricultural production (Bothmer et al., 2003; Pourkheirandish and Komatsuda, 2007). The three key traits vital in the domestication of barley are non-brittle rachis, six-rowed spike, and naked caryopsis (Pourkheirandish and Komatsuda, 2007). The non-brittle rachis facilitated efficient harvest without loss of grains in the cultivated barley. However, the brittle rachis along with rough awn was crucial for natural dispersal in the wild types. The most important non-brittle rachis genes for barley domestication were *btr1*

and *btr2*, which were mapped to chromosome 3H (Bothmer et al., 2003; Pourkheirandish and Komatsuda, 2007).

The selection for six-rowed barley can potentially result in three times the number of seeds compared to two-rowed barley and ultimately increased grain yield (Bothmer et al., 2003; Pourkheirandish and Komatsuda, 2007). According to Pourkheirandish and Komatsuda (2007), there are at least five independent loci controlling the six-rowed spike phenotype in barley (*vrs3*, *vrs1*, *vrs4*, *vrs5* or *int-c*, and *vrs2*), which were mapped to chromosomes 1H to 5H, respectively. Of these loci, the *vrs1* allele located in chromosome 2HL in the recessive homozygous form was found in all six-rowed barley cultivars while the dominant form was found in wild and two-rowed barley lines. According to Franckowiak and Lundquist (2012), the *vrs1* locus is a multiple allelic series with incomplete dominant allele interactions based on the size and shape of lateral spikelets. They also stated that alleles at this locus modify development of the lateral spikelets and the associated lemma awn, where the *vrs1.a* allele is present in most six-rowed cultivars and produces well-developed lateral spikelets. The locus *vrs5* or *int-c*, which was also observed in cultivars, modifies the degree of fertility in lateral spikelets and produces an intermediate spike type (Bothmer et al., 2003; Pourkheirandish and Komatsuda, 2007; Franckowiak and Lundqvist, 2012). The mutations at the *vrs2*, *vrs3*, and *vrs4* loci were detected only in induced mutant lines (Pourkheirandish and Komatsuda, 2007). Hence, the *vrs1* and *vrs5* or *int-c* loci seem important in the conversion of the two-rowed morphology to six-rowed morphology during the domestication process of barley.

The long arm of chromosome 7H contains the locus for the naked caryopsis, which is controlled by a single recessive gene (*nud*) (Bothmer et al., 2003; Pourkheirandish and Komatsuda, 2007). The *nud1.a* mutant, which is expressed in all environments, results in lemma and palea that thresh free of the hull at maturity (Franckowiak and Lundquist, 2012). Remains of naked kernels have been found in Ali Kosh about 8000 BP (Pourkheirandish and Komatsuda, 2007). In addition to the above traits, reduced dormancy is an important trait in the cultivation of barley and is required in cultivars (Bothmer et al., 2003). In the GrainGenes database (http://wheat.pw.usda.gov/GG2/Barley/), five QTLs were reported for dormancy in chromosomes 1H, 4H, 5H, and 7H. In a review of loci controlling dormancy, Pourkheirandish and Komatsuda (2007) summarize that seed dormancy is a quantitative trait that is affected by several genes, with two of the most common loci (*SD1* and *SD2)* located in chromosome 5H. Romagosa et al.

(1999) as in Pourkheirandish and Komatsuda (2007) indicated that *SD1* is epistatic to *SD2* at the early-ripening stages, but they seem to act additively at later ripening stages.

Currently, barley is cultivated throughout the world (FAO, 2015). Thus, the important question may be how and when barley was disseminated throughout the world. Migration of people with crop seeds led to a major diversification and adaptation of crops to new areas, and hence barley is now virtually found worldwide (Bothmer et al., 2003). According to Bothmer et al. (2003), the first route of dissemination of barley was believed to be to Greece, Iran, India, Ethiopia, and North Africa about 8000 BP. Then, barley arrived in Spain 7000 BP, and to North Germany and South Scandinavia 6000 BP. Bothmer et al. (2003) also pointed out that barley disseminated to Eurasia and China some 4000 and 3000 BP, respectively. It was in the 17th century that barley was successfully introduced to Canada and USA by the first Governor Samuel de Champlain and early settlers of USA, respectively (Horsley and Harvey, 2011).

Mutations at some loci facilitated adaptability of barley to different regions, which in turn lead to distribution of barley throughout the world. Mutations that were important in dissemination of barley include those for reduced vernalization requirement and photoperiod insensitivity (Pourkheirandish and Komatsuda, 2007; and references cited therein). According to a review by Pourkheirandish and Komatsuda (2007), three genes control reduced vernalization: *Sgh1* or *Vrn-H2*, *Sgh2* or *Vrn-H1*, and *Sgh3* or *Vrn-H3*, which are located in chromosomes 4H, 5H and 7H, respectively. Mutations in these loci have enabled development of the spring growth habit. According to Wang et al. (2010), vernalization or low temperature upregulates *Vrn-H1*, which in turn down regulates the dominant flowering repressor *Vrn-H2* and allows long day induction of *Vrn-H3*. The *Vrn-H3* gene accelerates subsequent stages of floral development, including expression of the flowering gene *HvFT1*. The recessive allele for *Vrn-H2* and dominant alleles for *Vrn-H1* and *Vrn-H3* are required for the reduced vernalization requirement.

Photoperiod insensitivity interacts with vernalization to determine flowering time (Pourkheirandish and Komatsuda, 2007), which is important for adaptation of barley to different growing conditions. The common gene controlling the flowering time under long-day conditions (about 13 to 16 h of light) is *Ppd-H1*, which is located in the short arm chromosome 2H (Pourkheirandish and Komatsuda, 2007). The wild type accessions have the dominant allele for this locus while the cultivated barley lines have the dominant

or recessive forms. The second major photoperiod response gene (*Ppd-H2*) was mapped to the long arm of chromosome 1H, which affects flowering time under short day conditions (less than 12 h of light). Actually, Wang et al (2010) discussed seven photoperiod related genes including *Ppd-H1* and *Ppd-H2*.

Diversity in Barley

Genetic diversity studies can be used to determine the extent of variability in a breeding program and to identify parental lines for hybridization and introgression of desirable genes into the available genetic base (Mohammadi and Prasanna, 2003; Chakravorty et al., 2013). Moreover, genetic diversity studies are important to identify core collections for conservation (Mohammadi and Prasanna, 2003). Mohammedi and Prasanna (2003) extensively reviewed methodologies useful in diversity studies, including measures of genetic distance (similarity statistics), multivariate procedures (cluster analysis, principal component analysis, principal coordinate analysis, and multidimensional scaling), types of data for multivariate analysis (if individual or combined data should be used), and the different tests to identify true clusters.

Some 466,531 barley accessions have been preserved in about 199 locations throughout the world (http://www.fao.org/docrep/013/i1500e/i1500e12.pdf). Among these conservation centers, the largest collection of barley accessions is conserved in the Plant Gene Resources of Canada (PGRC) with 40,031 barley accessions. Large numbers of barley accessions are also conserved in USA, with the International Center for Agriculture Research in Dry-land Areas (ICARDA), and Ethiopia (Institute of Biodiversity Conservation), with 29,874, 26,679, and 16,388 accessions, respectively. In these three conservation centers, landraces comprise the largest proportion of the collections (56%, 67%, and 94%, respectively). Generally, there are massive genetic resources for barley breeders to use in their breeding programs.

Bothmer et al. (2003) provides a detailed review of the diversity of barley at the molecular level, including isozymes, hordeins, and molecular markers. For isozymes, at least 60 loci were reported with average allele frequency of 3.3 alleles per locus. Among the 60 isozymes, 47 of them are localized to the seven chromosomes of barley and the *EST1* locus is the most polymorphic isozyme locus with 15 alleles. Hordeins are storage proteins of barley and comprise a group of proteins similar in biological function, but differentiated to various degrees in structure and chemical properties (Bothmer et al., 2003). The hordeins

can be differentiated by electrophoresis (*SDS-PAGE*, *IEF*, starch gels) and high-performance liquid chromatography (*HPLC*). Four hordein loci (*Hor1, Hor2*, *Hor4*, and *Hor5*) have been localized to the short arm of chromosome 1H, while *Hor3* is located in the long arm of the same chromosome (Bothmer et al., 2003). The *hordein* loci *Hor1* and *Hor2* have been the subject of many diversity studies due to their variety and their quantitatively greater expression. Diversity in hordeins is greater than that in isozymes. However, in both isozyme and hordein loci, the diversity is greater for wild relatives compared to cultivated barley (Bothmer et al., 2003).

Bothmer et al. (2003) attributed the differentiation of cultivated barley during the dissemination process to such forces as natural selection in heterogeneous seed sources, mass selection by the local farming community, outcrossing to other species of *Hordeum*, and the founder effect. On the basis of morphological diversity, Vavilov distinguished two principal centers where the diversification of cultivated barley occurred (Graner et al., 2003). The first is in Ethiopia, which is especially rich in forms of hulled barley. The second is in Southeast Asia (including China, Japan, and regions adjoining Tibet), where naked, short awned, awnless, or hooded spike types are dominant.

Ethiopia has been recognized as one of the twelve Vavilovian centers of diversity of crops in the world (Vavilov, 1951); with diversity in agro-ecological and climatic features, as well as cultural diversity of the people are the contributing factors. Barley has great diversity in Ethiopia, including numerous unique forms of accessions such as deficient and irregular types (Harlan, 1969). In one study by Zemede (2000), greater diversity in Ethiopian barley was reported with respect to different traits, including morphological types (>60), hordein groups (>40), biochemical composition, disease-resistant genes, protein and lysine content, and molecular markers. Farmers in different regions of Ethiopia grow different local cultivars (landraces) mainly for food and sometimes for production of homemade malt. Zemede (2000) reports there are studies that have identified accessions with high lysine and protein content in Ethiopian landraces, which are important nutritional quality traits. Furthermore, Lance and Nilan (1980) reported that barley β-glucan content ranged from 1.8 % to 6.4% for 543 barley accessions of Ethiopia origin in the USDA National Small Grains Collection.

Barley Production and Utilization

Barley is among the principal 'founder crops' in the Near East some 8,000 to 10,000 BP (Bothmer and Jacobsen, 1985). It has wider adaptation than other cereal crops, with its cultivation extending from tropical zones to the Arctic Circle as well as from sea level to about 4500 m (Bothmer and Jacobsen, 1985). The wide distribution of barley is fostered by its general tolerance to cool, drought, alkaline, and salty conditions (Edney, 1996).

Barley ranks fourth in total production of cereals after maize, rice, and wheat in the USA and the world (Figure 1.1). Barley is the fifth most important cereal crop in Ethiopia, with about 10.5% of cereal production exceeded by maize, wheat, teff (*Eragrostis tef* (Zuccagni) Trotter teff), and sorghum (CSA, 2007). The rate of increase in total production of barley over years has been well below that of other cereals, particularly compared to maize (Figure 1.1). This can probably be attributed to improved maize hybrids and more research funding for maize. The top 10 barley-producing countries in the world contribute about 58.5% of the barley production (Table 1.1). The USA sits in 10th place with average production of about 4.2 million tons. In the Africa continent, the three largest producers of barley are Morocco, Ethiopia, and Algeria with average production (over 2010 to 2013) of about 2.2, 1.8, and 1.4 million tons, respectively (FAO, 2015). In these African countries, barley is mainly used as human food.

Barley was used for human consumption some 10,000 years BC and its use in alcoholic beverage dates back to 3,000-5,000 BC (Edney, 1996). Bothmer et al. (2003) also point out that barley was initially used as a source of human food and animal feed. Currently, about 75% of barley production globally goes to livestock feed and malting is the second important use of barley, with about 20% of the total world barley production (Edney, 1996; Newman and Newman, 2008). However, barley assumes an important nutritional role for humans in many of the countries of South and East Asia, as well as North and East Africa (Newman and Newman, 2008). There is a renewed interest worldwide in barley for food because of its health benefits, which are associated with its higher levels of dietary fiber (Zemede, 2000). The importance of β-glucan (soluble fiber) in barley depends on the ultimate use of the grain. For barley cultivars meant for malting and poultry feed, reduction of barley β-glucan is a primary objective while increased soluble β-glucan is important in barley intended for human food (Schmitt and Wise, 2009). The fiber is effective in lowering serum cholesterol and can reduce the risk of heart disease as determined in

animal and human clinical trials (Newman and Newman, 2008). They also found that barley slows down absorption of sugar and reduces the risk of developing type 2 diabetes. Generally, waxy and hulless types are high in β-glucan (Lee et al., 1997; Jaby El-Haramein, 2005), which makes them preferable for human food.

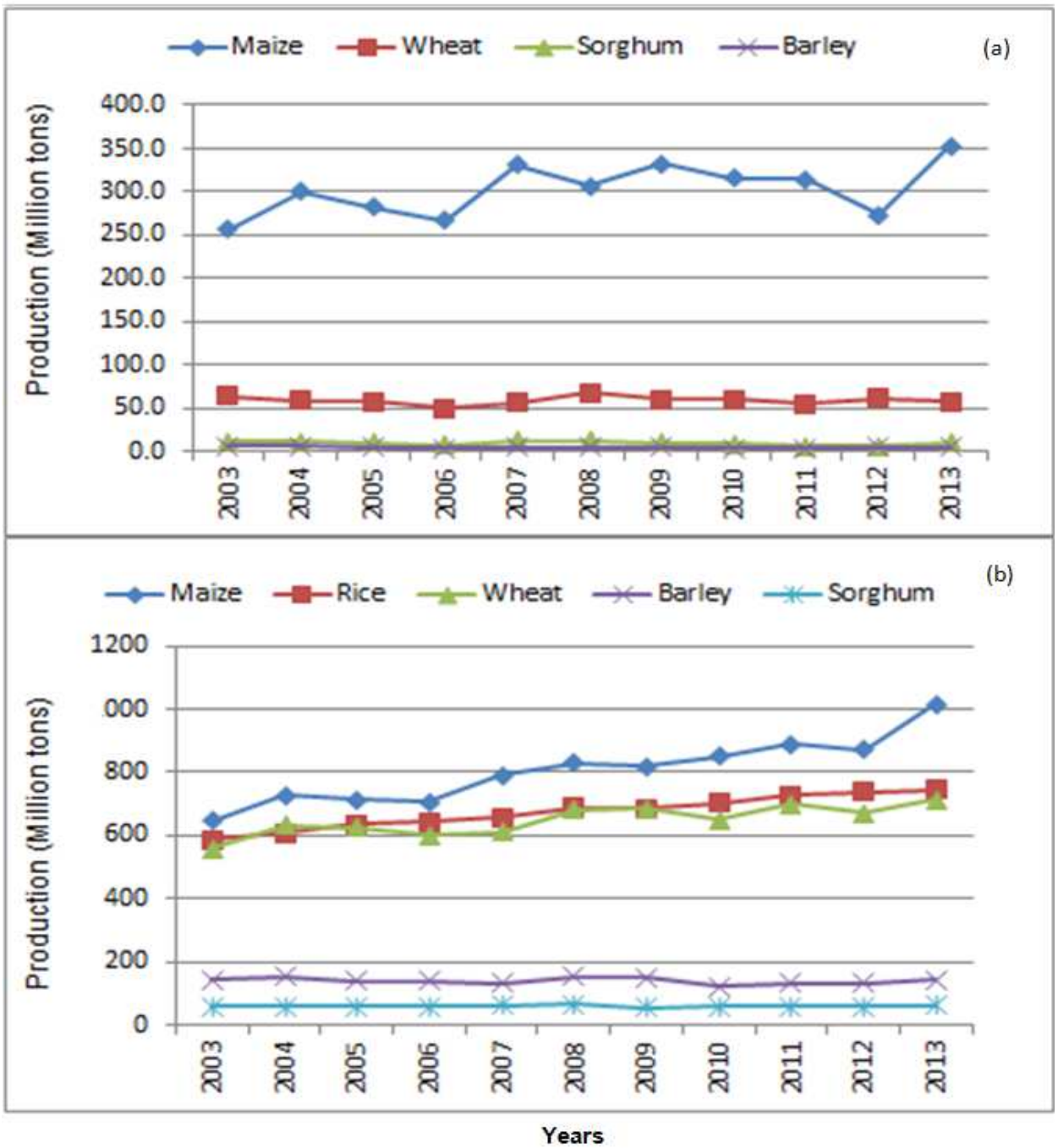

Figure 1.1. Barley production (in million tons) in relation to other cereals in: (a) USA and (b) Ethiopia (source: FAO, 2015).

Table 1.1. Top 10 barley producing countries in the world in total production (million tons).

Countries	2010	2011	2012	2013	Mean
Russia	8.4	16.9	14.0	15.4	13.7
Germany	10.4	8.7	10.4	10.3	10.0
France	10.1	8.8	11.3	10.3	10.1
Canada	7.6	7.8	8.0	10.2	8.4
Ukraine	8.5	9.1	6.9	7.6	8.0
Spain	8.2	8.3	6.0	10.1	8.1
Turkey	7.3	7.6	7.1	7.9	7.5
Australia	7.3	8.0	8.2	7.5	7.7
UK	5.3	5.5	5.5	7.1	5.8
USA	3.9	3.4	4.8	4.7	4.2

(FAO, 2015)

Barley is preferred for malt production compared to other cereals because of some beneficial features, include its attached hull, which helps in filtration in the brewing process; a firm kernel during germination; its contribution of unique flavors to alcoholic beverages; and it's being a good source of fermentable sugars and hydrolytic enzymes (Edney, 1996). As mentioned above, high levels of wort β-glucan are undesirable for malt barley. This is because β-glucan impacts malt extract and creates problems of filtration and haze formation in beer (Steiner et al., 2012). Besides its economic and health benefits, barley is also useful as a model crop for genetic analysis due to the existence of large numbers of genetic polymorphisms, its diploid inheritance, high degree of self-pollination, and relatively small number *(2n* =14) of large chromosomes (Newman and Newman, 2008).

Quantitative Trait Loci (QTL) Mapping

A quantitative trait locus (QTL) is a polymorphic site contributing to the genetic variability of a quantitative trait (Mackay and Powell, 2006). Geldermann introduced the concept of QTL in 1975 to describe those regions of the genome underlying a continuous trait (Cavanagh et al., 2008). Estimation of the positions and effects of QTL is of prime importance for marker-assisted selection (MAS). In order to understand QTL mapping, it is crucial to know the molecular marker systems and the methods that can be used for mapping QTL.

Molecular Marker System

A genetic marker is defined as a chromosomal landmark that allows tracing a specific region of DNA (Semagn et al., 2006). Genetic markers and the genes they mark are close together in the same chromosome that tend to stay together in each generation. Genetic markers are grouped into three broad classes (Semagn et al. 2006): (1) those based on visually assessable traits, such as morphological and

agronomic traits; (2) those based on a gene product (biochemical markers), such as isozymes and proteins (*hordeins*); and (3) those relying on a DNA assay (DNA markers). DNA markers are further classified into three broad classes (Gupta et al., 2001): (1) the first generation molecular markers, including restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), and their modifications; (2) the second generation molecular markers, including simple sequence repeats (SSR), amplified fragment length polymorphism (AFLP), and their modified forms; and (3) the third generation molecular markers, including single nucleotide polymorphisms (SNP). Genome-wide scans using genotyping platforms like the Illumina GoldenGate Bead Arrays and the diversity arrays technology (DArT) have been successfully utilized in barley (Sreenivasulu et al., 2008).

Several maps have been developed for barley using cytogenetic techniques (mainly using trisomics), isozymes, morphological markers, and a range of DNA markers including SSR, DArT, and SNPs (Hussain, 2006; Wenzl et al., 2006; Varshney et al., 2007; Suzcs et al., 2009; Munoz et al., 2011). According to Gupta et al. (2001), SNPs are more abundant in plant systems compared to the human genome on which they were initially applied. Close et al. (2009) selected 3,072 SNPs to fill two 1,536-SNP "production" assays (BOPA1 and BOPA2). Examination of USA breeding materials with these SNP markers provided excellent coverage and sensitivity for detection of minor alleles (Close et al., 2009). Most importantly, SNPs provide the ultimate form of molecular markers because a nucleotide base is the smallest unit of inheritance (Edwards et al., 2007). Edwards et al. (2007) estimated the SNP frequency in barley to be one SNP every 27 to 240 bp. Even if SNPs at any particular site can in principle involve four different nucleotide variants, they are generally biallelic in nature. Edwards et al. (2007) pointed out that SNP markers are abundant in the genome and low in mutation rate. However, the abundance of SNP markers in the genome compensates for their biallelic nature. These features make SNPs excellent markers in studying genome evolution, map-based positional cloning, studying complex genetic traits, genetic mapping, detection of marker-trait associations, and assessment of genetic relationships between individuals (Edwards et al., 2007).

Elshire et al. (2011) developed a genotyping-by-sequencing (GBS) approach that is suitable for population studies, germplasm characterization, breeding, and trait mapping in diverse organisms. GBS is the latest application of next-generation sequencing protocols for the purposes of discovering SNPs in a

variety of crop species and populations (Spindel et al., 2013). The GBS procedure reduces cost per sample by sequencing only subsets of genomic regions targeted by restriction enzymes (Elshire et al., 2011). The low cost of GBS makes it an attractive means of saturating mapping and breeding populations with a high density of SNP markers. The value of sequencing restriction site-associated genomic DNA (i.e., RAD tags) for high density SNP discovery and genotyping was first demonstrated by Baird and coworkers in 2008 (Elshire et al., 2011). Alternatively, a series of polymerase chain reactions (PCR) can also be used instead of restriction enzymes to sample specific regions of the genome to sequence. The bioinformatics is the big barrier of widespread use of GBS because it is often accompanied by a high number of erroneous SNP calls that are not easily diagnosed or corrected (Spindel et al., 2013).

Molecular markers serve a variety of purposes relevant to crop improvement and genetic study. The applications include QTL mapping (Jones et al., 1997; Gupta et al., 2001; Sreenivasulu et al., 2008; Edwards et al., 2007; Wang et. al., 2012), genetic diversity analysis (Mohammadi and Prasanna, 2003), population structure studies (Wang et. al., 2012), and phylogenetic and comparative genomics analyses (Whitkus et al., 1992). Molecular markers are also useful tools to overcome linkage drag and background genetic effect problems associated with utilization of landraces and wild types in crop improvement (Hussain, 2006). In crop breeding, molecular markers are useful in MAS methods, such as F_2 enrichment, marker-assisted back crossing, marker-assisted recurrent selection (Bernardo and Charcosset, 2006), and genomic selection (Bernardo, 2009; Bernardo, 2010; Bernardo, 2013; Massman et al, 2012).

Methodological Review in QTL Mapping

Two commonly used QTL mapping approaches are linkage mapping and association mapping (Abdurakhmonov and Abdukarimov, 2008; Cavanagh et al., 2008; Sreenivasulu et al., 2008). The linkage mapping approach utilizes a bi-parental mapping population segregating for the trait(s) of interest whereas association mapping utilizes a well-chosen natural population of lines, accessions, or cultivars referred to as the “mapping panel”. Both linkage analysis and association mapping rely on co-inheritance of functional polymorphisms and neighboring DNA variants (Zhu et al., 2008), ultimately identifying genotype-phenotype associations that lead to discovery of QTL that are responsible for phenotypic variation (Abdurakhmonov and Abdukarimov, 2008; Zhu et al., 2008; Myles et al., 2009). The three basic requirements to map QTL are a genetic map of variable markers, a population with which to follow the

segregation of these markers, and trait measurements on individuals of the population (Slate, 2005). The following few sections will first discuss facts about linkage disequilibrium and then the two QTL mapping approaches (linkage and association mapping approaches).

Linkage disequilibrium (LD)

Linkage equilibrium (LE) and linkage disequilibrium (LD) are important terms to describe linkage relationships in population genetics (Abdurakhmonov and Abdukarimov, 2008). Linkage equilibrium is the random association of alleles at different loci in a population. On the contrary, LD is the non-random association of alleles at different loci in a population. Linkage disequilibrium does not necessarily imply genetic linkage, and it can occur between physically unlinked loci (Flint-Garcia, 2003; Mackay and Powell, 2006; Abdurakhmonov and Abdukarimov, 2008). Linkage refers to the correlated inheritance of loci due to their physical connection on a chromosome whereas LD refers to the correlation of alleles in a population (Flint-Garcia, 2003). Generally, LD forms the basis for the construction of genetic maps and the localization of genetic loci for a variety of traits (Hussain, 2006). Association mapping particularly relies on LD decay (Mackay and Powell, 2006).

Theoretically, the degree of LD between two loci is a function of recombination (θ) and time in generations (t) since the origin of a new mutation (Oraguzie et al., 2007), which can be given in the following expression.

$$D_t = (1-\theta)^t * D_0,$$

Where θ is the recombination fraction and D_0 and D_t represent LD in time at generations 0 and t, respectively. Thus, LD will tend to be smaller when two loci are located further apart (i.e.; when θ is large) and will decrease through time as a result of recombination. Linkage disequilibrium decays quicker at higher recombination frequencies (Mackay and Powell, 2006).

Mutation and recombination are two important factors that impact LD, where mutations create polymorphisms that are in LD while recombination breaks down LD (Oraguzie et al., 2007). Tight linkage may lead to high levels of LD (Flint-Garcia, 2003). Other factors affecting LD include selection, migration, and admixtures (Mackay and Powell, 2006; Oraguzie et al, 2007), all which increase the level of LD. In a large random mating population with no linkage, selection, mutation, or migration, two or more polymorphic loci will be in linkage equilibrium (Flint-Garcia, 2003).

Different selection methods have different effects on LD and polymorphisms (Mackay and Powell, 2006). Regions of increased LD and reduced polymorphisms can indicate a history of directional selection. Plant breeders usually practice directional selection in the cultivar development process, where selection is for higher or lower values of the trait of interest (Acquaah, 2007). Balancing selection, which is also called stabilizing or optimum selection, can lead to greater LD and increased polymorphisms (Mackay and Powell, 2006). Two additional ways that selection affects LD are hitchhiking and epistatic selection (Oraguzie et al., 2007). Hitchhiking rapidly swaps an entire haplotype that flanks a favored variant to high frequency or even fixation. Epistatic selection keeps together combinations of alleles at two or more loci that are located on the same or different chromosomes (i.e.; co-adapted gene complexes). However, epistatic selection needs to be very strong to maintain allelic associations at the scale of megabases in the face of substantial recombination.

The concept of LD was first described by Jennings in 1917 and Lewtonin developed its quantification value (*D*) in 1964 (Abdurakhmonov and Abdukarimov, 2008). Linkage disequilibrium is statistically estimated using different statistics (Abdurakhmonov and Abdukarimov, 2008; Flint-Garcia, 2003; Oraguzie et al, 2007), of which the two most common (*D'* and r^2) ones described by Flint-Garcia (2003) are discussed here. Considering two loci with alleles A and a for the first locus and B and b for the second, the allele frequencies are π_A, π_a, π_B, and π_b, respectively. The observed haplotype frequencies for the two loci are π_{AB}, π_{Ab}, π_{aB}, and π_{ab}. Thus, LD is calculated as the difference between the observed (example π_{AB}) and expected ($\pi_A * \pi_B$) haplotype frequencies, which is denoted as *D*.

$$D = \pi_{AB} - \pi_A * \pi_B$$

The measure *D* is dependent on allele frequencies and hence a standardized LD measure would be useful for comparisons across loci with different frequencies (Oraguzie et al, 2007). The most frequently used measure of LD is r^2, which is given as in the following formula:

$$r^2 = \frac{D^2}{\pi A * \pi B * \pi a * \pi b}$$

The distinction between the above statistics lies in the scaling of the difference between the observed and expected haplotype frequencies (Flint-Garcia, 2003). Alternatively, LD statistics can be calculated as normalized LD (*D'*).

$$D' = \frac{D^2}{min(\pi A * \pi b, \pi a * \pi B)}, \text{ for } D > 0;$$

$$= \frac{D^2}{min(\pi A * \pi B, \pi a * \pi b)}, \text{ for } D < 0.$$

The above two equations for *D'* are scaled for *D* based on the observed allele frequencies so that *D'* will range between -1 and 1.

Oraguzie et al. (2007) discussed differences between the two methods of calculating LD (*D'* and r^2). *D'* is useful for comparisons across loci with different frequencies while r^2 has more reliable sampling properties than |*D'*|. Additionally, *D'* is affected more by mutational history while r^2 is affected by both recombination and mutation history. Oraguzie et al. (2007) pointed out that r^2 is preferred for assessing the extent of LD for association genetic studies.

Graphical displays of pairwise LD between two loci are very useful to understand the LD patterns measured using a large number of molecular markers (Abdurakhmonov and Abdukarimov, 2008). There are two common ways to visualize the extent of LD between pairs of loci, the LD decay plot and the LD color-code triangle (Flint-Garcia, 2003; Abdurakhmonov and Abdukarimov, 2008). The LD decay plot can be used to visualize the rate at which LD decays with genetic or physical distance. The LD color-code triangle is effective to visualize the linear arrangement of LD between polymorphic sites within a gene or loci along a chromosome.

The resolution of association mapping is a function of how quickly LD decays over distance (Myles et al, 2009), which justifies the need for determining the structure of LD in association analysis. LD decay depends on different factors such as the mating system, genetic diversity, and the way selection takes place in the functional variants (Abdurakhmonov and Abdukarimov, 2008; Myles et al, 2009). Abdurakhmonov and Abdukarimov (2008) observed that LD decays over shorter distances in cross-pollinated crops and in a population comprised of diverse materials. For instance, Kraakman et al. (2004) reported that LD extended over a distance of at least 10 cM in barley using 236 AFLP markers, which is actually large compared to other species such as sugar beet (*Beta vulgaris* L.) and maize. They attributed the high LD in their study to the fact that barley is a highly inbreeding species. Thus, it is crucial to make due consideration when selecting or sampling individuals that will comprise the mapping panel in association studies.

Linkage (family) mapping

Even if the focus of this review is association mapping, I will briefly discuss linkage (family) mapping. Linkage mapping is a commonly employed QTL mapping method to explain phenotypic variation in terms of simple changes in DNA sequence in experimental populations created by bi-parental crosses (Myles et al., 2009). Linkage mapping involves six general steps (Abdurakhmonov and Abdukarimov, 2008): (1) developing an experimental population (F_2, doubled-haploid, backcross, near-isogenic lines, and recombinant-inbred lines), (2) phenotyping (collecting data on traits) across environments, (3) genotyping using markers that identify polymorphisms in the parents, (4) constructing linkage maps using molecular markers, (5) statistically correlating phenotypic data with positioned markers, and (6) identifying QTL regions affecting a trait of interest.

Some of the advantages of linkage mapping include identification of low frequency functional alleles and application when there is a strong relatedness problem (Myles et al., 2009). Linkage analysis is based on few recombination opportunities within families of known ancestry, and hence results have relatively low resolution (Zhu et al., 2008). The bi-parental mapping population samples only a small portion of phenotypic diversity existing in the species (Abdurakhmonov and Abdukarimov, 2008), which enables only few alleles to be evaluated simultaneously. Furthermore, linkage mapping is very costly, as it requires longer research time. In fact, the precision of linkage mapping depends on the genetic variation covered by the mapping population, the size of the mapping population, and the number of marker loci used (Abdurakhmonov and Abdukarimov, 2008).

Association (population) mapping

Association mapping involves searching for genotype-phenotype correlations (i.e. marker-trait associations) in unrelated individuals taken from a natural population (Myles et al., 2009). The method results in localization of QTL based on the strength of the correlation between mapped genetic markers and traits of interest (Mackay and Powell, 2006). Decay of LD is the basis for association mapping (Mackay and Powell, 2006). Association mapping can lead to the most effective utilization of ex-situ conserved natural genetic diversity (Abdurakhmonov and Abdukarimov, 2008).

The association mapping panel could be composed of three types of populations, namely; germplasm bank collections, elite breeding materials, and synthetic populations (Breseghello and

Sorrells, 2006). Each of these populations has their own limitations and benefits. Germplasm bank collections usually contain maximum diversity and the LD can decay within a short distance. Because the germplasm bank collections have high heterogeneity within individual accessions, the trait data recording and genotyping can be complicated. Hence, it is crucial to be careful during phenotyping and genotyping. Germplasm bank collections may also be unadapted to the experimental locations, which may result in large amounts of missing data. Elite breeding materials may not have problems of heterogeneity and adaptation to the target environment; however, population structure is a potentially big problem that can lead to false positives if not handled properly. Additionally, LD usually decays over long distances in populations comprised of elite materials. In synthetic materials, there may be mild or no population structure; hence, the power to detect QTL in synthetic populations is maximized and the risk of false associations is minimized.

Family-based linkage (FBL) mapping is a special case of association mapping in which the mapping population is established from a small number of founders (Mackay and Powell, 2006). Cavanagh et al. (2008) also discussed the importance of Multi-parent Advanced Generation Inter-cross (MAGIC) populations derived from elite breeding germplasm for gene-trait analysis in crop species. For FBL mapping, careful selection of parents with phenotypic extremes is required to ensure high levels of segregation for most QTL of the trait(s) of interest (Mackay and Powell, 2006). In maize, 25 diverse inbred lines were crossed to B73 to create a nested association mapping (NAM) population (McMullen et al., 2009). They recognized this population for high power and resolution through a joint linkage-association analysis by capturing the best features of both approaches. Currently, NAM populations are being developed for barley in the USA involving the collaboration of different parties, including the barley-breeding program at North Dakota State University (personal observation). I also developed a NAM population by crossing 40 genotypes to a single common female parent, where all the parents were selected from Ethiopian (landrace and breeding materials), ICARDA, and NDSU accessions used in this study.

Association mapping has the general steps shown in Figure 1.2. The selection of germplasm to be included in a mapping panel and the control of false positives are two important steps that need due consideration when one applies association mapping approach for QTL mapping.

Currently, there is increasing interest in association mapping due to two main driving forces (Zhu et al., 2008). The first is the development of high-throughput genome sequencing and genotyping technologies to obtain large amounts of marker data across large number of accessions. Genotyping approaches like Illumina's Genome Analyzer (Myles et al., 2009) and genotyping-by-sequencing (Elshire et al. 2011) are among the technologies developed in recent years. According to Close et al. (2009), the possible methodologies to access genes are genome sequencing and SNPs developed using cDNA (mainly ESTs) and sequenced PCR amplicons. The second factor driving use of association mapping is the development of statistical methodologies that alleviate the issue of false positives (Zhu et al., 2008). Some of the methods to control false positives, including the transmission disequilibrium test (TDT), genomic control, structured association, logistic regression, eigenstrat, and haplotype analysis were discussed in detail by Mackay and Powell (2006).

Mixed linear model (MLM) approaches that account for population structure and/or unequal relatedness have been demonstrated as improved methods (Zhang et al., 2010). In the MLM, population structure is fitted as a fixed effect and kinship among individuals is fitted as a random effect. Population stratification can be inferred as population structure (Q-matrix) from the software STRUCTURE (Pritchard et al, 2010), P-matrix from principal component analysis, or M-matrix form nonmetric multidimensional scaling (Gutiérrez et al., 2011). Similarly, the possible kinship estimation methods include kinship matrices (K) from TASSEL (Bradbury et al, 2007; Ramdoss et al, 2011), K obtained by the efficient mixed model association (EMMA) method (Kang et al, 2008), K obtained from the software SPAGeDi (Hardy and Vekemans. 2002), and K matrix generated with JMP Genomics (SAS Inc., Cary, NC). Understanding which combinations of fixed (population structure) and random (kinship) effects need to be utilized in association mapping is crucial. Myles et al (2009), referring to four papers published from 2006 to 2008, stated that the K and K+Q mixed models are useful for controlling false positives.

Association mapping can be done in two ways, the candidate gene approach and the whole-genome association study (GWAS) (Myles et al., 2009). In the candidate gene approach, genetic markers around a locus thought to be involved in a phenotype are used to test their association with the phenotype. This approach was widely used in identifying disease resistance genes in humans. However, the understanding that a researcher can get in the genetic control of a particular trait is limited because

many genes controlling the trait can remain undetected. GWAS involves covering the whole genome with markers so functional alleles will be in LD with at least one of these markers (Myles et al., 2009). SNP markers have great potential for GWAS because they are abundant across the genome (Edwards et al., 2007).

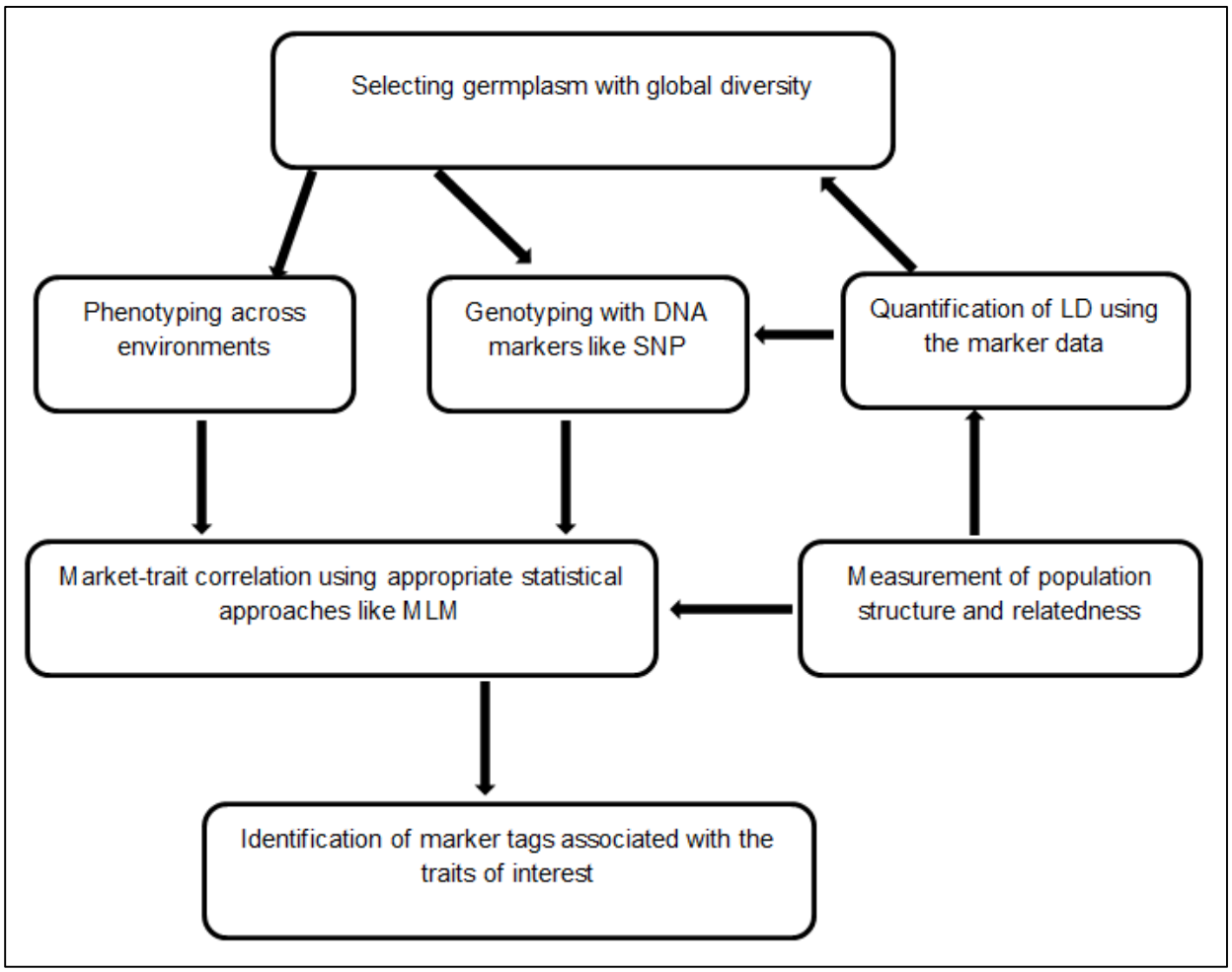

Figure 1.2. General outline of association mapping technique (taken from Abdurakhmonov and Abdukarimov, 2008).

One of the major concerns in association mapping and linkage mapping is the statistical power and the control of false positive associations or type I error rate (Muller et al, 2011). False positive associations occur when a significant QTL is declared where none really exists. In addition to population structure, false positives occur in relation to multiple comparison tests (Muller et al, 2011). Some of the correction procedures for false positives due to multiple comparison tests are the Bonferroni correction and permutation test for linkage mapping, and false discovery rate (FDR) for both linkage and association

mapping approaches (Muller et al., 2011). Muller et al. (2011) also proposed a general method to control genome-wide type I error rate (GWER).

Association mapping can be used to identify or confirm QTL. Identification of significant marker-trait associations (MTAs) in regions that had not been implicated before suggests new QTL (Kraakman et al., 2006). Such new QTL can reflect that the QTL was not polymorphic in the previous populations or markers were missing in the QTL region in the previous studies. Association mapping is efficient in identifying novel or new genes for important traits that can subsequently be validated in specific bi-parental crossing populations. Kraakman et al. (2004) examined the association between markers and traits in three ways: (1) significance of MTAs, (2) LD profiles over chromosomes, and (3) MTAs found in other QTL studies.

Individual MTAs detected by GWAS usually account for small portions of the explained variability and heritability of the trait (Myles et al., 2009). This is partly due to the existence of large numbers of low frequency alleles that cannot be detected with the GWAS approach. Myles et al. (2009) suggested applying linkage mapping approach to detect such low frequency alleles. Generally, it may be important to combine a family and association mapping approaches in QTL mapping efforts.

Mapped QTL for Different Traits in Barley

Yield and Yield-Related Traits

Grain yield improvement is the primary objective in many cereal-breeding programs (Welsh, 1981) and it can be defined in terms of the product of three yield components, i.e.; number of spikes per unit area, number of kernels per spike, and kernel weight (Nickell and Grafius, 1969). Currently, there is growing interest in the application of molecular marker information closely related to important traits in breeding programs. The first RFLP marker map for barley developed two decades ago was ultimately used to map agronomic, quality, and disease resistance traits (Sreenivasulu et al., 2008). Hussain (2006) reviewed several articles on QTL mapping in barley and discussed chromosomal location of 16 agronomic traits. They reported QTL on all seven chromosomes for grain yield based on 15 papers. According to review by Sreenivasulu et al. (2008), 1000-kernel weight and kernel number per spike were mapped to all chromosomes except 1H and 7H, respectively. The other yield component, spike number

per unit area, was mapped to chromosome 3H. For plant height and days to heading, they reported several QTL in all seven chromosomes.

Using 146 two-rowed current commercial spring barley cultivars in Europe, Kraakman et al. (2004) observed two most significantly QTL for yield (1H: 7.4 cM and 3H 19.5 cM). These two regions were also identified in linkage mapping mainly using North American barley crosses like 'Blenheim'/'Kym' and 'Steptoe'/'Morex'. They hypothesized that the coincidence may be due to the resemblance of North American and European materials as barley was initially introduced to North America from Central, Northern, and Eastern Europe.

Xue et al. (2010) did linkage analysis for yield and yield components under waterlogged and well drained conditions using 156 doubled-haploid lines derived from the cross 'Yerong' (waterlogging-tolerant) x 'Franklin' (waterlogging-sensitive). Using a genetic linkage map of 496 DArT, 80 AFLP, and 28 microsatellite markers, they identified 31 QTL for kernel weight, grains per spike, spikes per plant, spike length, and grain yield, with individual QTL explaining 4.7% to 55.3% of the phenotypic variability. Interestingly, most of those QTL with larger effects were detected in the same region of chromosome 2H, indicating tight linkage or pleiotropic effects of the gene(s) controlling the traits. They also identified some unique QTL under waterlogging conditions, which implied that different markers might be use in selecting cultivars under such conditions.

Disease Resistance Traits

Selection for disease resistance has been equally important like improving crops for yield and yield components. Diseases can affect yield and quality of the product as well as leaving mycotoxins on the grain. Fusarium head blight (FHB; incited by *Fusarium graminearum* Schwabe), leaf scald (incited by *Rhynchosporium secalis* (Oudem.) J. J. Davis, the net form net blotch (incited by *Drechslera teres* (Sacc.) Shoemaker) and spot blotch (incited by *Cochliobolus sativus* (Ito & Kuribayashi) Drechs. ex Dastur) are among the major fungal diseases in barley (Hussain, 2006), with resistance to each by one to 14 genes.

Fusarium head blight is an economically important disease that can cause significant reductions in yield and quality in wheat and barley (Ma et al., 2000; Dahleen et al., 2003). The U.S. Government Accounting Office (GAO) estimated total losses due to FHB in the upper-Midwest USA exceeded $200 million from 1993-1997 (U.S. GAO, 1999). Nganje et al. (2001) estimated losses of $136 million in the

same region from 1998-2000. Therefore, FHB is a major target for breeding and several QTL mapping projects have been done on this disease (Ma et al., 2000; Dahleen et al., 2003; Mesfin et al., 2003; Canci et al., 2004; Hori et al, 2005; Hori et al, 2006; Sato et al., 2008; Yu et al., 2010; Massman et al., 2011). In each of these studies, three to 14 QTL were identified across all chromosomes. Additionally, all studies reported QTL on chromosome 2H. Similarly, three to 37 QTL were identified for deoxynivalenol (DON) accumulation in different studies across all chromosomes except 4H (Ma et al., 2000; Dahleen et al., 2003; Mesfin et al., 2003; Canci et al., 2004; Yu et al., 2010; Massman et al., 2011). Many of the QTL identified for FHB coincided with QTL for DON (Ma et al., 2000; Dahleen et al., 2003; Mesfin et al., 2003), days to heading (Dahleen et al., 2003; Mesfin et al., 2003), plant height (Dahleen et al., 2003), and the *Vrs1* locus (Mesfin et al., 2003).

Leaf scald is one of the most severe diseases of barley in the highlands of Ethiopia where precipitation is high and temperature is low during the cropping season. Yield losses can range from 21% to 67%, and it also affects grain quality and ultimately the price paid for the grain (Kiros et al., 2004; Zhan et al., 2008). Overall, several QTL for leaf scald have been mapped to all seven chromosomes, except 5H, in at least 18 mapping populations (Williams et al., 2001; Jensen et al., 2002; Bjørnstad et al., 2002; Shtaya et al., 2006; Li and Zhou, 2011; Hofmann et al., 2013). Particularly, resistance genes for leaf scald clustered in the short arms of chromosomes 1H, 3H, 6H and 7H; and in the centromeric region and the long arm of chromosome 3H (Zhan et al. 2008). The most interesting result in each of these studies is that all identified at least one QTL for resistance to leaf scald on chromosome 3H. Therefore, the QTL in chromosome 3H could be the target for marker-assisted selection (MAS) for leaf scald resistance.

Net form net blotch constitutes a serious constraint to barley production worldwide and can cause significant yield losses of up to 50% and negatively impact grain quality (Adawy et al., 2013). Several QTL mapping studies were conducted in barley for net form net blotch, with one to 14 QTL per study identified across all seven chromosomes (Ma et al., 2004; Emebiri et al., 2005; Manninen et al., 2006; Grewal et al., 2008; Qamar et al., 2008; Gupta et al., 2010; Gupta et al., 2011; Grewal et al., 2012; Adawy et al., 2013). A QTL in chromosome 6H was commonly reported in all studies; thus, it seems that the QTL in this chromosome could be potential targets for MAS. For the spot form of net blotch (incited by

Drechslera teres f. maculata Smedeg.), one QTL was reported in chromosome 5H (Manninen et al., 2006) and four more were found in chromosomes 1H, 2H, 4H, and 6H (Grewal et al., 2012).

Until a virulent pathotype was reported in the late 1990s (Zhou and Steffenson, 2013), spot blotch had been effectively controlled in Midwest USA six-rowed barley through the deployment of durable resistance for over 50 years. The source of resistance traces back to the breeding line NDB112. A similar level of durable resistance to spot blotch has not been reported in two-rowed barley in the Midwest USA (Grewal et al., 2012). Thus, spot blotch remains a target for breeding and QTL mapping. Several QTL for spot blotch resistance have been identified in all seven chromosomes of barley for either seedling or adult plant resistance (Bilgic et al, 2005; Bilgic et al, 2006; Bovill et al., 2010; Castro et al, 2012; Grewal et al., 2012). Applying an association-mapping approach using 318 diverse wild barley accessions, Roy et al. (2010) identified 13 QTL for spot blotch, with each explaining 2.3 to 3.9% of the phenotypic variation. Similarly, Zhou and Steffenson (2013) identified three QTL in chromosomes 1H, 3H, and 7H using an association-mapping approach with 3,072 breeding lines from 10 U.S barley breeding programs. In all of these studies, chromosomes 3H and 7H were identified as important carriers of QTL for spot blotch. Thus, QTL on these chromosomes may be candidates for manipulation using MAS.

Malt and Grain Quality Traits

Several important issues may be raised in relation to QTL mapping for malt quality traits, including: (1) how the malting and brewing processes affect malt quality traits, (2) major components of malt quality, (3) if malt quality traits are targets for DNA marker-based selection and the reasons, (4) if malt quality traits are the subject of QTL mapping studies, (5) if the reported QTL have efficiently utilized in breeding programs, and (6) recommendations on how to utilize the detected QTL in breeding programs. The focus of this review is to discuss in some detail the points mentioned above.

Understanding the malting process is important for comprehending the biochemical changes that occur in barley during malting. The malting process involves three major steps: steeping, germination, and kilning (Schuster, 1962; Burger and LaBerg, 1985). Steeping is immersion of cleaned barley into water so the moisture content reaches to 42-46% (Schuster, 1962). The purpose of steeping is to create favorable conditions for germination and the activation/synthesis of enzymes. Additionally, steeping can remove dust and microorganisms from the grain. Germination is the growth of the embryo and coincident

modification of the contents of the endosperm (Schuster, 1962). Modification involves high levels of degradation of the endosperm cell wall and protein matrix, but with minimum degradation of starch granules (Igartua et al., 2002). Finally, the green malt is kilned in a two-step process, i.e., drying and curing (Schuster, 1962; Burger and LaBerg, 1985). Kilning is important to stabilize desirable properties arisen during the course of germination, preserve the malt for safe storage, and introduce flavor, aroma, color, and friability to the finished malt.

The endosperm cell wall of barley consist in 75% of (1-3,1-4)-β-D-glucans, 20% of arabinoxylan, 2% of cellulose, and 2% of glucomannan (Jamar et al., 2011). At the starting stage of germination, the decomposition of high molecular weight materials in the endosperm cell wall is done with enzymes present in barley (Schuster, 1962; Jamar et al., 2011). These enzymes include (1-3,1-4)-β-glucanases, (1-3)-β-glucanases, β-glucosidases, and the β-glucane exohydrolases to degrade (1-3,1-4)-β-glucans While (1-4)-β-xylan endohydrolase, arabinofuranosidase and β-xylosidase to degrade Arabinoxylans (Jamar et al, 2011). The starch-degrading enzymes such as α-amylase and β-amylase are synthesized or released during the germination process. These hydrolytic enzymes have important roles in the mashing step of brewing. Finally, the barley is modified, which results in increased sugar, dextrins, amino acids, and nucleic acid breakdown products (Burger and LaBerge, 1985).

The brewing process has five major steps (Burger and LaBerge, 1985): mashing, lautering, boiling and hoping, fermentation, and lagering. Mashing is a combined extraction and enzymatic process designed to maximize production of fermentable materials. It is often considered the most important step in the brewing process. Initially, crushed malt is combined with brewing water at 35-50^{o}C to consistency of a thin porridge and allowed to stand for 20-30 min (protein rest). Then, hot gelatinized rice or corn grits can be added as adjuncts and starch conversion is allowed at 65-70^{o}C for 15-20 min (conversion phase). Finally, the temperature is increased to 80^{o}C to inactivate the bulk of the enzymes. The enzymes activated or synthesized in the malting process are utilized in the mashing step of the brewing process to hydrolyze the different components of the malt and adjunct. Lautering is filtration of hot mash to separate the wort from spent grains (Berger and LaBerge, 1985). The resulting wort is transferred to boiling kettles and hops are added. This step is important to isomerize the α-acid of hops to iso-α-acid to produce the desirable bitterness in the beer. It is also important for sterilization and concentration of the wort. The

bitter wort is transferred to fermentation tanks, inoculated with brewer's yeast, and allowed to ferment at low temperature (12-15^{o}C) for 5 to 8 d. The extracted sugars from the mashing step are fermented by yeast to produce CO_2 and alcohol. Finally, the beer is transferred to the lagering cellar and stored at nearly 0^{o}C for 8 to 21 d to promote physical stability and flavor of the beer.

The malting properties of barley are influenced by over 30 physical, chemical, and biochemical traits, which exhibit quantitative inheritance and are affected by environmental conditions under which the barley is grown, stored, and malted (Edney, 1996; Igartua et al, 2002; and references therein). Malt quality generally reflects the extent to which barley malt provides nutrition to yeast during fermentation and essential flavor components for beer (Fox et al., 2003). Proper balance of starch and protein is crucial for yeast nutrition, which is influenced by genes involved in plant growth and development, and their interactions with the environment. The conversion of starch to fermentable carbohydrate is catalyzed by α-amylase, β-amylase, limit dextrinase, and α-glucosidase (Igartua et al., 2002). Some of the most important malt quality traits are malt extract, wort viscosity, Kolbach index, wort β-glucan concentration, fermentability, diastatic power (DP), α-amylase activity, free amino nitrogen (FAN), friability, and β-glucanase activity (Fox et al., 2003). Generally, malt quality traits are grouped into three categories (Igartua et al., 2002): (1) those measured on grain such as kernel size, kernel plumpness, kernel weight, and protein content; (2) those measured on malt solutions such as malt extract, wort β-glucan, wort viscosity, activity of starch degrading enzymes, proteolytic activity, wort soluble protein, Kolbach Index, FAN, and wort color; and (3) indirect measures of malt quality such as milling energy for extract and milling energy loss for modification. Actually, the first group can be grouped separately as grain quality traits even if they impact malt quality traits.

Malt quality traits have been one of the most important targets for QTL mapping and marker-based selection for several reasons (Gutiérrez et al., 2011). In the first place, several genes determine a large number of interrelated malt quality traits. Malt quality traits are also affected by different environmental factors, and hence selection for lines with the best quality can be complicated. Another important reason is that many of the component malt quality traits are only measured with good accuracy in the later period of the breeding cycle. Finally, the malting process and quality analyses require time and

are expensive. Thus, breeders have devoted a lot of efforts in the identification of QTL associated with malt quality traits.

QTL for malt quality traits have been summarized in different review papers (Zale et al., 2000; Igartua et al., 2002; Fox et al., 2003; Hussain et al., 2006; Szucs et al., 2009) and the GrainGenes database (http://wheat.pw.usda.gov/GG2/Barley/). The mapping populations for malt quality traits are mainly generated from crosses of germplasm from North America, Europe, Australia, and Asia. Zale et al. (2000) summarized 156 QTL for 19 malt quality traits, with 84% of them being coincidental. For several malt quality traits, QTL were reported in all chromosomes. For instance, malt extract (Zale et al., 2000; Fox et al., 2003; Gutiérrez et al., 2011), DP (Fox et al., 2003; Szucs et al., 2009), α-amylase (Fox et al., 2003; Szucs et al., 2009; Gutiérrez et al., 2011), barley and wort β-glucan (Zale et al., 2000; Fox et al, 2003; Hussain, 2006; Szücs et al., 2009; Wei et al., 2009; Gutiérrez et al., 2011; Kim et al., 2011; Zhou et al., 2012), barley protein content (Fox et al., 2003); and proportion of plump kernels (Zale et al., 2000) were mapped to all the seven barley chromosomes. The major QTL for barley β-glucan reported by Kim et al. (2011) explained for 44.4% of phenotypic variability and was positioned near to the waxy locus (*wx*) and *HVM4* in chromosome 7H.

Wort viscosity mapped to all chromosomes except 1H (Zale et al., 2000; Fox et al., 2003), soluble protein to all chromosomes except 6H (Zale et al., 2000; Fox et al., 2003), Kolbach Index to all chromosomes except 6H and 7H (Hussain, 2006), and FAN to all chromosomes except 1H and 6H (Emebiri et al, 2004; Islamovic et al., 2014). Additionally, QTL were reported in all chromosomes except 5H for β-amylase (Zale et al., 2000; Hussain, 2006; Wei et al., 2009). β-amylase is the key enzyme involved in the production of the fermentable sugar maltose, which is utilized by yeast during fermentation (Fox et al., 2003). The *Bmy1* locus, which is located in chromosome 4H, is the major grain form of β-amylase in barley (Guerin et al., 1992). According to GrainGenes database (http://wheat.pw.usda.gov/GG2/Barley/), the *Bmy1* is positioned at 143.4 cM in chromosome 4H.

One of the most interesting aspects of QTL analyses for malt quality traits is that many of these QTL shared common chromosomal regions. For instance, malt extract QTL were almost always coincidental with component traits such as carbohydrate hydrolyzing enzymes (Zale et al., 2000). QTL for DP were often associated with QTL for α-amylase and/or β-amylase activity. QTL for malt quality traits

also coincided with loci associated with hordeins, grain character, spike character, plant morphology, and plant height (Beecher et al., 2002). They found that five QTL impacting grain hardness were located in chromosomes 1H, 4H, 5H, and 7H; and the QTL for grain hardness in chromosomes 1H and 4H were associated with DP and grain protein, respectively. Mather et al. (1997) identified QTL for grain hardness, fine-grind extract, and extract viscosity in a similar region in chromosome 5H. In another study by Wei et al. (2009), QTL for albumins and protein content were detected in the short arm of chromosome 3H. Pleiotropic effects or gene clusters could be possible reasons for such coincidence of QTL for different traits in the same chromosomal region (Zale et al., 2000).

According to Igartua et al. (2002), QTL for malt quality traits are concentrated in chromosome 1H between the *Hor1* and *Hor2* loci (13.8-23.8 cM) and around the *Glb1* locus (68.1 cM), in chromosome 2H near the *vrs1* locus (92.6 cM), in chromosome 3H near the *Ugp2* (25.2-36.0 cM) and *Dor4* (50.2 cm) loci, in chromosome 4H near the *Bmy1* locus (143.4 cM), in chromosome 5H near the *ACt8B* (0-5 cM) and *Ltp1* (44.6 cM) loci, in chromosome 6H near the *Amy1* locus (88.1 cM), and in chromosome 7H near the *Amy2* locus (881.1cM). Generally, chromosome 5H contains the most QTL for malt quality traits (Hussain, 2006). Similarly, 58 of the 184 QTL compiled in the GrainGenes database are located in chromosome 5H (http://wheat.pw.usda.gov/GG2/Barley/). Using the Oregon Wolfe Barley (OWB) population, Szűcs et al. (2009) summarized map locations of 154 malt quality trait-related QTL, with 40 of them mapped to chromosome 5H. Recently, Mohammadi et al. (2015) found the largest number of MTAs in chromosome 5H using GWAS with lines from USA barley breeding programs.

Although several QTL have been identified, applying them to cultivar development has been difficult. This is because the identified QTL were frequently background specific and the parents of mapping populations were often exotic or one of the parents lacked malt quality (Gutiérrez et al., 2011). In many cases, the favorable alleles for major QTL were already fixed in relevant germplasm. Because QTL for malt quality traits are distributed across the genome, selection should be done carefully (Zale et al., 2000). The magnitude of QTL effects can serve as an important criterion. Widely conserved QTL regions may be targets for selection to maintain malting quality, whereas selection for unique QTL regions is important to gain new improvements (Zale et al., 2000). In most cases, the genomic selection scheme best fits for grain and malt quality traits since they are distributed across the genome.

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CHAPTER II: PHENOTYPIC DIVERSITY AND POPULATION STRUCTURE IN BARLEY ACCESSIONS FROM ETHIOPIA, ICARDA, AND NDSU

Abstract

Genetic diversity studies are important in a breeding program to identify parental lines and in genetic resource conservation to identify a core collection. A total of 264 genotypes sampled from Ethiopian landraces, and cultivars and breeding lines from the Ethiopian, International Center for Agricultural Research in Dry-land Areas (ICARDA), and North Dakota State University (NDSU) breeding programs were utilized in this study. Data on 17 agronomic and disease resistance-related traits, and 13 malt and grain quality traits were recorded. Simple descriptive statistics and multivariate approaches such as principal component and cluster analyses were applied to assess the genetic diversity and population structure. The analysis of variance indicated significant genotypic main and genotype x environment interaction effects for almost all the traits evaluated. However, the genotype x environment interactions was mainly due to changes in magnitude rather than crossover types of interactions. Generally, the diversity analysis indicated that the population was highly structured according to spike row-type, geographic origin, and breeding history. Since the population is highly structured, appropriate statistical models will be needed when this population is used for association mapping. Three to four principal components (PCs) in principal component analysis (PCA) accounted for most of the variation (77.3-81.3%). The most related traits were included in the same PC, implying that results from PCA could give clues as to the relationship among traits. Though variability existed within and among clusters, useful germplasm clustered together. Some ICARDA lines could be useful for the Ethiopian and NDSU breeding programs for the improvement of several agronomic traits. These lines were clustered in CL2.2 at Bekoji and CL3 at Koffele. The NDSU materials are important sources of germplasm for the improvement of reduced plant height, lodging resistance, low deoxynivalenol (DON) content, and several malt quality traits. The barley accessions from Ethiopia could be exploited for leaf scald and net form net blotch resistance particularly in NDSU breeding program if the need arise. The study also indicated that photoperiod related genes controlled days to heading in NDSU lines.

Introduction

Genetic diversity studies can be important tools to identify diverse parental lines for hybridization and introgression of desirable genes into elite germplasm (Chakravorty et al., 2013). Plant breeding is a dynamic process in which one needs to be aware of the genetic variability in their germplasm and that in other's germplasm in order to sustain continued improvement. Genetic diversity studies are also useful to identify accessions for a core collection. The rationale of making a core collection is to balance the cost of conservation with the goal of capturing maximum genetic variability in the species. Genetic diversity studies provide a useful tool to reduce redundancy of germplasm samples while capturing sufficient genetic variability that represents a species.

Ethiopia has been recognized as one of the twelve Vavilovian Centers of Diversity of crops in the world (Vavilov, 1951), and is known particularly for the great genetic diversity of barley (*Hordeum vulgare* L.) and tetraploid wheat (*Triticum turgidum L.* subsp. *durum* (Desf.) Husn.) (Harlan, 1969). The major reasons for such sizable genetic diversity in Ethiopia include diverse agro-ecological and climatic features, as well as the cultural diversity of the people (Vavilov, 1951). It is believed that barley was introduced to Ethiopia some 8000 BP (Bothmer et al., 2003). Since then, Ethiopian farmers have selected types that fit their environment based on maturity, suitability for local food preparation, and use in animal feed. Additionally, farmers residing in a single location usually relied on several alternative landraces that could be used for the preparation of different local foods.

The institute of Biodiversity Conservation (IBC) of Ethiopia has over 65,000 accessions of more than 120 plant species, including >15,000 accessions of barley (Adugna, 2011). The majority of the conserved barley accessions (66.9%) in the collection are landrace collections from Ethiopia, while 7.2% of the accessions are donated germplasm from nine countries worldwide, and 25.9% are accessions of unknown origin. Approximately 93% of the barley accessions originated and housed in the IBC are not unique as they are duplicated in several international collections. Ethiopian barley germplasm has been exploited to improve disease resistances to barley yellow dwarf virus, barley stripe mosaic virus, powdery mildew (incited by *Erysiphe graminis* DC. f. sp. *hordei* Em. Marchal), leaf rust (incited by *Puccinia hordei* Otth), and loose smut (*Ustilago tritici* (Pers.) Rostr.); and high lysine content (Adugna, 2011 and references therein; Spies et al., 2012; Munoz et al., 2014). In a diversity study by Spies et al. (2012),

European cultivars were clustered with Ethiopian landraces. They speculated that the reason for such a cluster is that Ethiopian germplasm was used preferentially by European breeding programs as the source of major disease resistance (R) genes.

The barley breeding programs of North Dakota State University (NDSU) and the International Center for Agricultural Research in the Dry-land Areas (ICARDA) generate breeding lines and cultivars based on their particular breeding objectives. The NDSU breeding program primarily focuses on developing two-rowed and six-rowed malt barley cultivars adaptable to the northern Great Plains of the USA. The ICARDA breeding program develops cultivars for diverse agro-ecologies, including arid and high rainfall regions. Similarly, the Ethiopian barley-breeding program focuses on developing cultivars for diverse agro-ecologies and growing seasons, as well as different end uses including malt and human food. The difference in the growing environments for barley and the breeding objectives for these three programs leads to development of diverse barley genotypes.

Genetic variability can be assessed using univariate methods that measure dispersion, including calculation of population variances, the coefficients of variability (CV), and range estimates. Mahammadi and Prasanna (2003) provide a detailed review of four multivariate techniques (cluster analysis, principal component analysis, principal coordinate analysis, and multi-dimensional scaling) to studying genetic diversity. Comparisons of mean differences among sub-populations that are created based on certain criteria can also be used to understand the extent of genetic diversity in a population.

As part of a QTL mapping effort, I conducted several field experiments in Ethiopia, particularly at Bekoji and Koffele, as well as in North Dakota, USA particularly at Fargo, Langdon, and Osnabrock to collect data on agronomic, disease resistance, and grain and malt quality traits of Ethiopian landraces, and breeding lines and cultivars from the Ethiopian, ICARDA, and NDSU breeding programs. The research discussed in this chapter describes the phenotypic diversity and population structure of the aforementioned genotypes. These results will be utilized ultimately to identify germplasm that may be of use to the Ethiopian or NDSU breeding programs.

Materials and Methods

Experimental Materials and Environments

A total of 264 genotypes were utilized in this study. Out of these, 134 of them were evaluated in all the experiments and the rest were evaluated in at least one experiment. These genotypes included two-rowed and six-rowed lines. The experimental materials were sampled from four sources of barley germplasm: landraces from Ethiopia, and cultivars and breeding lines from the Ethiopian, ICARDA, and NDSU breeding programs. As shown in Table 2.1, field experiments were conducted at nine environments. Three of the locations (Bekoji, Koffele, and Holetta) are in Oromia, Ethiopia and the fourth location (Fargo) is in North Dakota, USA. In this chapter, the results from all environments except the experiment at Holetta in 2012 are discussed. The numbers of genotypes tested at each environment (location-year combinations) for each group are given in Table 2.1. In 2011 at Bekoji and Koffele, entries were assigned to experimental units using an augmented block design (Federer, 1993) with 11 incomplete blocks. For the experiments in 2012 and 2013, entries were assigned to experimental units using a 14 x 14 simple lattice design. The plot size and seeding rate for all experiments in Ethiopia were 1.2-m x 2.5-m and 125 kg ha-1, respectively. For the experiments at Fargo, the seeding rate was 2.47 million seeds per hectare and the plot size was 1.5-m x 2.4-m.

Descriptions of Data Recorded

Agronomic and disease resistance data

Data on 14 agronomic and disease resistance traits were collected in field experiments and the data recording procedures are described below.

Days to heading: The number of days from sowing to the time when 50% of the plants in the plot had 50% of the spikes emerged above the flag leaf. The days to heading was recorded in all the environments.

Days to Maturity: The number of days from sowing to when 75% of the spikes in the plot had reached physiological maturity. Physiological maturity was reached when the peduncle had lost its green color. The days to maturity was recorded only in the environments in Ethiopia.

Plant Height (cm): The average height of two to five randomly selected plants at maturity from the ground level to the tip of the spike, excluding the awns. The plant height was recorded in all environments.

Lodging (%): Visual scoring of the percentage of plants lodged in the plot. The lodging score was recorded in most environments in Ethiopia.

Stand (%): Visual scoring of the percentage of plot area covered by the plants. The stand score was recorded in most environments in Ethiopia.

Leaf scald, caused by *Rhynchosporium secalis (Oudem.) J. J. Davis*: A 0 to 9 rating scale was used, where zero indicates no infection and nine indicates highly susceptible. The leaf scald score was recorded in most environments in Ethiopia.

Net Blotch, caused by Drechslera teres (Sacc.) Shoemaker: A 0 to 9 rating scale was used, where zero indicates no infection and nine indicates highly susceptible. The net blotch score was recorded in most environments in Ethiopia.

Number of tillers per plant: The average number of tillers per plant, counted on five randomly selected plants in the middle rows of each plot. The number of tillers per plant was recorded in the environments in Ethiopia.

Number of spikes per plant: The average number of spike-bearing tillers per plant counted on five randomly selected plants in the middle rows of each plot. The number of spikes per plant was recorded in the environments in Ethiopia.

Number of kernels per spike: The average number of kernels per main tiller spike, counted on two to five randomly selected plants in the middle rows of each plot. The number of kernels per spike was recorded in all the environments

Spike length (cm): The average length of the main tiller spike, excluding the awns, of five random plants in the middle rows of each plot. The spike length was recorded in the environments in Ethiopia.

Thousand-kernel weight (g): The weight of two 250-kernel samples and multiplied by two for the experiments in Ethiopia. The weight of two 100-kernel samples and multiplied by five for the experiments in Fargo. The thousand-kernel weight was recorded in all environments.

Table 2.1. The number of genotypes of each group tested at the nine environments

	Environment†								
Group‡	BE11	KO11	BE12	FA12	HO12	KO12	BE13	FA13	KO13
Landrace_2R	11	11	5	7	5	5	5	7	5
Landrace_6R	74	74	48	43	48	48	48	38	48
Ethiopian_2R	37	37	25	29	25	25	25	29	25
Ethiopian_6R	10	10	7	5	7	7	7	4	7
ICARDA-2R	39	39	39	37	39	39	39	37	39
ICARDA-6R	43	43	42	34	42	42	42	34	42
NDSU_2R	1	1	12	12	12	12	12	24	12
NDSU_6R	1	1	14	12	14	14	14	20	14
Total	216	216	192	179	192	192	192	193	192

†BE11 = Bekoji in 2011, KO11 = Koffele in 2011, BE12 = Bekoji in 2012, FA12 = Fargo in 2012, HO12 = Holetta in 2012, KO12 = Koffele in 2012, BE13 = Bekoji in 2013, FA13 = Fargo in 2013, and KO13 = Koffele in 2013.
‡Landrace=Ethiopian landraces, Ethiopian=cultivar or breeding lines from Ethiopia, ICARDA=breeding lines from the International Center for Agricultural Research in Dry-land Area, NDSU=cultivars and breeding lines from North Dakota State University, 2R=two-rowed, and 6R=six-rowed.

Hectoliter weight (kg hL^{-1}): The weight of a hectoliter of seed measured using a Sinar Ap 6060 Moisture Analyzer (Sinar Technology, Camberley, UK) for the experiments in Ethiopia. Hectoliter weight was recorded for experiments at Fargo by weighing the grain in a half-liter container to get g $0.5L^{-1}$ and converting the result to kg hL^{-1} unit. The hectoliter weight was recorded in all environments.

Grain yield (t ha^{-1}): Weight of cleaned grain harvested in each plot and reported in tons per hectare. The grain yield was recorded in all environments.

Greenhouse disease testing

Two hundred sixty-two of the 264 genotypes were tested in the greenhouse in 2014 for resistance to one isolate of the net form of net blotch (ND 89-19) and one isolate of the spot form of net blotch (ND 111), caused by *Drechslera teres* f. *maculata* Smedeg. Two lines with known response to each disease were used as checks. For net form net blotch, 'Hector' was used as the susceptible check and NDB112 was the resistant check. For spot form net blotch, 'Pinnacle' was the susceptible check and BNC127 was the resistant check.

Up to three seeds of each entry were sown in 3.8 x 21-cm Ray Leach "containers" (Stuewe & Sons, Corvallis, OR) containing #1 Sunshine mix (3:1 peat moss: perlite) (Sun Gro, Bellevue, WA). A

total of 15 racks, each holding 98 cones, were used for each disease. Each genotype was replicated three times and the checks appeared twice in each rack. The outer cones of each rack were sown with susceptible checks of the respective diseases to avoid any "boarder" effects on the genotypes tested. The three replicates for each disease were sown on three consecutive days. A suspension of pathogen spores was sprayed on the plants on the twelfth day after sowing. The spore count was about 5000 spores mL^{-1} for net form net blotch and about 2000 spores mL^{-1} for spot form net blotch. For each rack, 100 mL of the spore solution was sprayed to a uniform coverage of all the plants in the rack. For net form net blotch, the plants were kept in the dark in a humidity chamber for 20 h. Plants inoculated with spot form net blotch were kept in lighted humidity chambers for 20 h. To maintain 100% humidity, water was applied as a mist for 30 sec every 4 min for both of the diseases.

Following incubation, the plants were returned to the greenhouse for disease development. Seven days after inoculation, disease scoring was done on two plants in each cone. The net form net blotch infection rate (IR) was record on the second leaf using the 1-10 rating scale described by Tekauz (1985). Infection rates below 5 were considered as resistant and those 5 and above were considered susceptible. For spot form net blotch, a five-point scale was used (1-5), where IRs below 2.5 were considered resistant and IRs 2.5 and above were considered susceptible (Iowa State University, 2006-2010). The results from the three replicates were compared before discarding the plants so genotypes with inconsistent results could be looked at again.

Deoxynivalenol (DON) content

DON was determined for grain samples harvested from the NDSU Fusarium Head Blight nursery in Langdon in 2012 and 2013, and Osnabrock in 2012. Genotypes sown in the nurseries were assigned to experimental units once, with four checks repeated every 20 entries. Genotypes were inoculated with *F. graminearum* using the grain-spawn method described by Urrea et al. (2002). DON content (μg g^{-1}) was determined using method Barley-11 of American Society of Brewing Chemists (ASBC, http://www.asbcnet.org/MOA/toc.aspx) and as described by Schwarz et al. (1995). The threshold for DON detection was 0.5 μg g^{-1}.

Grain and malt quality traits

All barley grain and malt data were collected in the NDSU Barley and Malt Quality laboratory of Dr. Paul Schwarz. Methods with the prefix Barley, Malt, Wort, or Beer followed by a number refer to specific methods of the American Society of Brewing Chemists (ASBC, http://www.asbcnet.org/MOA/toc.aspx). Kernel assortment to determine percent plump and thin kernels was done using the SORTIMAT (Pfeuffer, Kitzengen, Germany) according to method Barley-2C. The method required a 100 g sample to be loaded onto the machine and shaken for two minutes. Kernels retained on a sieve with 0.24 x 1.9-cm slotted openings were classified as plump. Kernels passing through the sieve were classified as thin. Data on barley protein content was recorded on cleaned samples using an Infratec 1241 Grain Analyzer (Foss Tecator, Hillrod, Denmark) according to the method Barley-7D.

Once the grain quality traits were recorded, samples were prepared for malting by sorting the grain into size grades. Hulled kernels retained on a sieve with 0.24 x 1.9-cm slotted openings were used for malting. For hulless barley genotypes, kernels retained on a sieve with 0.20 x 1.9-cm slotted openings were also used for malting. Prior to malting, pilot steeping was done on 10 g (dry basis; db) samples to determine the time required for each genotype to reach 46% moisture. Next, 80 g (db) of grain from each sample was weighed out and malted using the methods described in Karababa et al. (1993).

Finished malt moisture was determined using 5 g of ground malt as specified in method Malt-3. Wort was prepared using 50 g of ground malt as specified in method Wort-1. Data collected on wort were malt extract, diastatic power (DP), α-amylase, wort color, free amino nitrogen (FAN), wort viscosity, wort soluble protein, and wort β-glucan. The methods to obtain the results were Malt-4 for malt extract, Malt-6 for DP, Malt-7 for α- amylase, Beer-10 for wort color, Wort-12 for FAN, Wort-13 for wort viscosity, Wort-17 for wort soluble protein, and Wort-18 for wort β-glucan. The Kolbach Index was calculated as the ratio of wort soluble protein to total barley protein. Additionally, ratio of DP to total barley protein content was also calculated.

The barley β-glucan was recorded using samples taken from field experiments in 2012 at Bekoji and Fargo. The grain β-glucan was extracted applying the acid extraction technique. The quantification of grain β-glucan was done using the plate reader as described in Schmitt and Wise (2009).

Statistical Procedures

Analysis of variance (ANOVA)

Individual environment and combined analyses of variance were done using the GLM procedure of SAS 9.4 (Cary, NC) according to the experimental design used to assign treatments to experimental units. Mean separation of the groups for each trait was done using the Means statement and LSD option of SAS. *F*-tests and differences between means were considered significant at $P \leq 0.05$. Least square (LS) means were used in further analyses, including calculating group or cluster means, principal component analyses, and cluster analyses.

Descriptive statistics

The data for each location were described using the mean and range estimates that were estimated using the means procedure of SAS 9.4 (SAS Inc., Cary, NC). The meanand range values also were estimated for different groups of genotypes using origin-row combination or clusters as the grouping variable.

Principal component and cluster analyses

Principal component analysis (PCA) was done using LS means of genotypes (from a single environment or appropriate combinations of environments) with SPSS 13.0 (Chicago, IL). The criteria for the number of principal components (PC) to extract were: (1) Eigen values > 1, (2) individual principal component explaining > 10% of the total variation, (3) the cumulative explained variation > 70%, and (4) interpretability of the results using the loading values. The communality values also were used to determine which traits to include in the PCAs. The plot of the first two PCs was done where spike row-type or barley origin-spike row-type combinations were used as grouping variables using SPSS 13.0 (Chicago, IL). Where necessary, the plot of the first and third PCs was also done.

Cluster analyses were done two ways for each data set: (1) using LS means for genotypes and (2) using the loading values for genotypes in the most significant (or important) PCs from PCA. The resulting clusters from the two analyses were compared. The MANOVA considering clusters as the factor variable was used to determine the most appropriate number of clusters (Mahammadi and Prasanna, 2003). The cluster number with the maximum *F*-value in the MANOVA was used as the most suitable number of clusters. To test significance differences among the clusters and to generate box plots for each

trait, cluster memberships created using the most important principal components were used as grouping variables in the SAS GLM procedure (SAS Inc., Cary, NC)

Results

Analysis of Variance

Generally, significant ($P \leq 0.01$) year x genotype interactions were observed for most of the agronomic traits recorded at Bekoji and Koffele (Table 2.2), and Fargo (Table 2.3). The exceptions were number of tillers per plant, number of spikes per plant, spike length, and hectoliter weight at Bekoji; days to maturity, plant height, leaf scald, number of tillers per plant, and number of spikes per plant at Koffele; and number of kernels per spike and hectoliter weight at Fargo. The significant interactions indicate that the genotypes were responding differently in the different growing seasons in each of the environments. The genotype main effect was significant for all traits at Bekoji (Table 2.2), Koffele (Table 2.2), and Fargo (Table 2.3) as well as seedling resistance to net form net blotch and spot form net blotch (Table 2.4), which suggests variability exits among the genotypes for each of the traits.

Traits such as disease ratings, number of tillers per plant, and number of spikes per plant were shown to have higher percent CVs (Table 2.2 and Table 2.4), indicating the impact of micro-environmental variation across the field that affects these traits. Sintayehu and Ketema (2011) also reported higher CV values for number of tillers per plant and number of spikes per plant in a study at Bekoji. Generally, the CV values were smaller in the Fargo experiments compared to those at Bekoji and Koffele (Table 2.2 and 2.3). This fact indicates that there may be less plot-to-plot variation at Fargo.

In the combined ANOVA for grain and malt quality traits for Bekoji (2011 and 2012), the environment was significant ($p \leq 0.05$) for all traits (Table 2.5). Similarly, the genotypic main effects were significant ($p \leq 0.01$) for all traits. The CV values ranged from 2.0% for malt extract to 31.3% for β-glucan. The CV values for β-glucan, FAN, DP, and α-amylase were relatively large, implying that the change in relative rank between entries in the two years for these traits was larger as compared to that of the other traits.

Additionally, barley protein content was determined on grain harvested in Bekoji in 2011 and 2012; Fargo in 2012 and 2013; and in Koffele in 2012. Proportion of plump kernels was also determined on grain harvested in Bekoji in 2011 and 2012; and Fargo in 2012. The barley β-glucan was determined

on grain samples taken from field experiments in 2012 at Bekoji and Fargo. The ANOVA was performed using these data and is presented in Table 2.6. The genotype and environment effects for barley protein content were highly significant ($p \leq 0.01$). Similarly, both the genotype and environment effects were significant ($p \leq 0.01$) for proportion of plum kernels and barley β-glucan.

Table 2.2. Pertinent mean squares, results of F-tests, and percent coefficients of variation (%CV) from the combined analyses of variance of agronomic traits collected from experiments conducted at Bekoji and Koffele, Ethiopia. (2011-2013).

Traits†	Bekoji				Koffele			
	Gen	Year x Gen	Error	%CV	Gen	Year x Gen	Error	%CV
DH	83.5**	11.8**	7.1	3.2	121.7**	34.4**	20	5.4
DM	58.6**	21.1**	10.6	2.4	62.1**	47.3	46.5	4.9
PLH	795.7**	104.4**	59.2	7.5	751.9**	164.0	150.8	11.9
SC	13.7**	2.8**	1.2	23.4	8.7**	2.8	2.3	45.2
NB	2.8**	0.9**	0.3	35.6	4.7**	2.0**	1.3	34.6
NTP	7.4**	4.8	5.2	28.2	8.4**	5.8	5.2	37.7
NSP	6.1**	4.0	4.3	26.7	8.0**	4.9	4.7	39.1
NKS	380.4**	62.3**	21.8	11.7	814.9**	182.7**	93.6	20.8
SL	2.7**	0.8	0.6	10.7	5.5**	3.7**	1.5	16.7
TKW	176.7**	21.3**	16.8	9.7	121.7**	33.9**	19.7	12.3
HLW	26.1**	6.4	6.2	4.2	25.6**	16.2**	8.9	5.2
YLD	3.7**	0.9**	0.6	20	3.7**	2.6**	1.4	36.2

** Significant at $P \leq 0.01$ levels of significance, respectively.
†DH = days to heading, DM = days to maturity, PLH = Plant height, SC = Scald, NB = Net blotch, NTP =Number of tillers per plant, NSP =Number of spikes per plant, NKS = Number of kernels per spike, SL = Spike length, TKW = thousand-kernel weight, HLW = Hectoliter weight, and YLD = Grain yield.

Table 2.3. Pertinent mean squares, results of *F*-tests, and percent coefficients of variation (%CV) from the combined analyses of variance across two years for agronomic traits collected from experiments conducted at Fargo (2012 and 2013).

Traits	Genotype	Year x Genotype	Error	CV (%)
Days to heading	111.9**	5.7**	2.9	3.0
Plant height	140.2**	160.8**	21.1	8.1
Number of kernels per spike	375.7**	20.9	31.0	16.3
Thousand-kernel weight	66.2**	8.7**	5.7	5.9
Hectoliter weight	19.3**	4.2	2.8	2.7
Grain yield	3.0**	0.36**	0.13	13.6

** Significant at $P \leq 0.01$ levels of significance, respectively.

Table 2.4. Pertinent mean squares, results of *F*-tests, and percent coefficients of variation (%CV) from the analysis of variance of data collected in greenhouse experiments for two foliar diseases.

Traits	Genotype	Error	CV (%)
Net form Net blotch	8.3**	0.7	19.0
Spot form Net blotch	1.1**	0.2	13.4

** Significant at *P*≤0.01 levels of significance, respectively.

Table 2.5. Pertinent mean squares, results of F-tests, and percent coefficients of variation (%CV) from the combined analyses of variance of malt quality traits collected from experiments conducted at Bekoji, Ethiopia (2011 and 2012).

Traits	Environment	Genotype	Error	CV (%)
Malt extract	198.6**	7.3**	2.3	2.0
Diastatic power	360.3**	1003.0**	433.6	20.8
α-amylase	715.3**	142.1**	36.5	17.4
Wort color	2.9**	0.06*	0.0	12.5
Free amino nitrogen	53650.7**	1509.4**	1102.6	23.6
Viscosity	0.10**	0.05**	0.0	9.5
Soluble protein	2.4**	0.5**	0.2	13.9
β-glucan	1730380.7**	60355.3**	27409.6	31.3
Kolbach index	12666.9**	26.3**	11.2	14.3

*, ** Significant at P≤0.05 and P≤0.01 levels of significance, respectively.

Table 2.6. Pertinent mean squares, results of F-tests, and percent coefficients of variation (%CV) from the analysis of variance of grain quality traits from barley grown at Bekoji in 2011 and 2012, Koffele in 2012, and Fargo in 2012 and 2013.

Traits	Environment	Genotype	Error	CV (%)
Protein content†	672.1**	5.8**	1.8	10.3
Plump kernels‡	14350.9**	440.6**	155.4	17.1
Barley β-glucan§	36.3**	0.59**	0.35	13.2

** Significant at P≤0.01 levels of significance.

†Analysis for protein content was done using samples taken from Bekoji 2011 and 2012, Fargo 2012 and 2013, and Koffele 2012 field experiments.

‡Analysis for protein content was done using samples taken from Bekoji 2011 and 2012, Fargo 2012 and 2013, and Koffele 2012 field experiments.

§Analysis for barley β-glucan was done using samples taken from Bekoji 2012 and Fargo 2012 field experiments

The significance of interaction effects needs further dissection to identify the root cause of the interaction. The interaction could be significant due to differences in the magnitude of means from different environments or a true interaction, which is the differential response of genotypes in the different environments. Further dissection of the significant interaction effects using two-dimensional plots revealed that they were mainly due to differences in magnitude (data not shown). Thus, I concluded it was acceptable to use means or least square (LS) means across environments for the principal component and cluster analyses.

Comparison of Location and Group Means

Agronomic and disease resistance traits

There was more than three weeks difference in days to heading between the Ethiopian locations and Fargo (Table 2.7), which may reflect the effects of the differences in daylight length and temperature during the growing season at the different sites. Interestingly, the NDSU lines were very early in heading as compared to the rest of the entries when grown at Fargo and were found to be late or comparable in heading to the Ethiopian lines when grown in Ethiopia (Table A.1). The range values for each location indicate a higher variability for days to heading among the genotypes at Koffele and Fargo compared to those at Bekoji (Table 2.7). Additionally, there was large genetic variability in days to heading within the landrace, Ethiopian, and ICARDA groups at all the three locations (Table A.1).

Table 2.7. Mean and range estimates for different agronomic and grain quality traits at each location.

Traits†	Bekoji (2011 to 2013)		Koffele (2011 to 2013)‡		Fargo (2012 and 2013)	
	Mean	Range	Mean	Range	Mean	Range
DH	81	67 - 91	84	62 - 103	57	44 - 71
DM	133	116 - 146	140	127 - 162	-	-
PLH	105.0	70.0 - 134.9	107	64.9 - 139.1	57	35 - 79
LODG	5.3	0 - 45	32.4	0 - 98	-	-
ST	83	53 - 95	72	48 - 95	-	-
SC	4.6	0.8 - 8.7	3.5	0.3 – 9.0	-	-
NB	1.8	0.4 - 7.7	3.4	0.4 - 8.8	-	-
NTP	8	3.4 - 13.5	7	2.1 - 14.5	-	-
NSP	7	3.1 - 11.6	6	2.2 - 14	-	-
NKS	39	13.6 - 56.6	48	17.7 - 83.3	36	17 - 66
SL	6.9	4.2 - 9.5	7.1	3.6 - 10.6	-	-
TKW	42.3	28.5 - 68	36.5	22.5 - 57	40.3	27.7 - 53.3
HLW	59.9	51.3 - 74.8	57.7	48.9 - 71.3	59.6	48.5 – 75.0
YLD	4.2	1.4 – 7.0	3.8	0.8 - 8.1	2.7	0.1 - 4.2
BPC	11.4	9.3 - 14.6	14.5	11.0 – 19.0	14.4	9.3 - 19.1
PL	76.5	22.4 - 98.7	83.9	61.6 - 100.0	62.3	12.1 - 96.2

†DH = days to heading, DM = days to maturity, PLH = Plant height, LODG = Lodging, ST= Stand, SC = Scald, NB = Net blotch, NTP =Number of tillers per plant, NSP =Number of spikes per plant, NKS = Number of kernels per spike, SL = Spike length, TKW = 1000-kernel weight, HLW = Hectoliter weight, YLD = Grain yield, BPC = protein content, and PL = Plumpness.
‡The mean, standard error, and range values for BPC and PL at Koffele were estimated from a single year data.

There was a 7 d difference between mean days to maturity at Bekoji and Koffele, with more than 30 days difference between the earliest and latest maturing individual genotypes at both locations (Table 2.7). When looking at the mean days to maturity of the different groups, the means ranged from 130 d for the two-rowed landraces to 138 d for the six-rowed NDSU lines at Bekoji; and from 138 d for the two-

rowed NDSU lines to 143 d for the six-rowed landraces at Koffele (Table A.2). There was also large variability for days to maturity among the genotypes at both locations.

On average, the genotypes were tallest at Koffele (107 cm) and experienced more lodging, with mean lodging of 32.4%, ranging from 0 to 98% (Table 2.7). The NDSU lines were significantly ($p<0.05$) shorter than all the landrace and Ethiopian groups (Table A.3) and hence experienced almost no lodging (Table A.3). All the groups were shown to be more or less similar in plant height at Fargo (Table A.3). It is possible that the reduced height of the Ethiopian genotypes and landraces in Fargo might be due to their poor adaptation in this environment. Overall, plant height and lodging severity exhibited large variability within each group.

Leaf scald and net blotch are among the major barley diseases in Ethiopia, causing up to 31.5% and 59.0% of yield loss, respectively (Bekele et al., 2011a&b). The environment at Bekoji was more conducive for leaf scald and that of Koffele was more favorable for net blotch (Table 2.7). Comparisons of leaf scald scores between genotypes at Bekoji indicated that the NDSU lines were more susceptible and the landraces and genotypes from Ethiopian and ICARDA breeding programs tended to be moderately resistant (Table A.5). With the exception of six-rowed Ethiopian breeding lines, all of the remaining groups contained genotypes with a range of leaf scald scores, extending from resistant to susceptible. As a group, the six-rowed Ethiopian breeding lines were resistant to moderately resistant to leaf scald.

Overall, the NDSU and ICARDA lines were more resistant to net blotch compared to the Ethiopian breeding lines and landrace groups, particularly at Koffele (Table A.6). Generally, most of the genotypes in the Ethiopian breeding line and landrace groups were susceptible to net blotch in field experiments. Thus, even though landraces are often included as one parent in most of the crosses made by the Ethiopian breeding programs, they cannot be relied on to provide any genes for resistance to local pathotypes of net blotch in Ethiopia.

Quite different results for net form net blotch resistance were found when the genotypes were evaluated in the greenhouse using an isolate from North Dakota. There is a higher percentage of landraces and Ethiopian breeding lines classified as resistant compared to the ICARDA and NDSU breeding lines (Table 2.8). With the exception of the six-rowed NDSU lines, all the other groups had wider variability for net form net blotch scores. The group mean of seedling resistance to spot form net blotch

indicated that all the groups were within the susceptible range (>2.5). The limited number of genotypes classified as resistant came from the six-rowed landraces, two-rowed ICARDA, and two-rowed NDSU groups (Table 2.8).

Table 2.8. Mean and range for net form net blotch (NFNB) and spot form net blotch (SFNB) of different group of barley estimated from seedling test conducted in greenhouse in 2014.

Groups†	Net form net blotch (1-10)		Spot form net blotch (1-5)	
	Mean	Range	Mean	Range
12	3.6	2.4-5.1	3.9	3.0-4.3
16	3.7	2.3-6.4	3.5	2.3-4.3
22	3.8	2.0-6.7	3.6	2.7-4.5
26	3.4	2.5-5.0	3.2	2.7-4.3
42	4.6	2.4-7.6	3.3	2.3-4.0
46	5.3	3.0-7.8	3.3	2.5-4.3
52	4.5	2.1-8.0	3.0	2.0-3.5
56	7.3	6.0-7.9	3.0	2.7-3.5

†12 = Two-rowed landrace, 16= Six-rowed landrace, 22 = Two-rowed Ethiopian lines, 26 = Six-rowed Ethiopian lines, 42= Two-rowed ICARDA lines, 46= Six-rowed ICARDA lines, 52 = Two-rowed NDSU lines, and 56 = Six-rowed NDSU lines.

The mean concentration of DON detected in the 2012 samples obtained from the Langdon and Osnabrock FHB nurseries were higher than that detected in the Langdon samples in 2013 (Table 2.9). Generally, the NDSU and ICARDA breeding lines were associated with lower mean DON compared to the Ethiopian genotypes (landraces and breeding lines), particularly in 2012 at both locations. However, the higher variability for DON content within landrace and Ethiopian groups may suggest that there may be a few lines with lower DON accumulation (Table 2.9). Overall, 43 genotypes had relatively low DON accumulation, with the majority being in the NDSU groups (Table A.14). Additional research will be needed to confirm their appropriateness as parents to develop cultivars with low DON accumulation.

The number of tillers per plant, thousand-kernel weight, and hectoliter weight were the lowest at Koffele, while spike length and the number of kernels per spike were the highest at this location (Table 2.7). The low thousand-kernel weight and hectoliter weight at Koffele may partly stem from the high amount of lodging (Table 2.7). Comparisons of the different groups within each location indicated that the two-rowed groups produced relatively more tillers per plant (Table A.7), had longer spikes (Table A.9), heavier kernels (Table A.10), and greater hectoliter weight (Table A.11), but fewer numbers of kernels per spike (Table A.8). Generally, thousand-kernel weight and numbers of kernels per spike are negatively correlated as reported elsewhere in the literature (Nickel and Grafius, 1969; Grafius and Okoli, 1974;

Tesema and Hailu, 1992; Kjaer and Jensen, 1996; Yan and Wallace, 1995; Sintayehu and Ketema, 2011).

Table 2.9. Mean and range for deoxynivalenol (DON) of different group of barley estimated from data generated on samples from Langdon in 2012 and 2013, and Osnabrock in 2012.

Groups†	2012 Langdon		2012 Osnobrack		2013 Langdon	
	Mean	Range	Mean	Range	Mean	Range
12	47.4	9.9 -140.8	56.9	26.1 -114.4	6.6	1.7 -23.8
16	65.5	10.1 - 244.3	70.5	4.0 -192.7	14.8	0.8 -79.0
22	35.3	7.8 -100.6	31.8	3.2 -86.1	13.2	0.6 -57.6
26	61.5	6.0 -139.8	55.3	18.6 -180.6	10.6	2.0 -24.0
42	20.9	2.5 -57.6	23.5	3.0 -58.0	21.2	2.5 -82.8
46	40.0	4.1 -101.5	56.8	11.5 -170.7	31.5	0.2 -86.5
52	17.0	6.8 -27.2	21.7	14.7 -28.7	11.0	1.5 -36.3
56	21.2	17.2 -25.1	42.0	34.6 -49.3	19.7	0.3 -44.5

†12 = Two-rowed landrace, 16= Six-rowed landrace, 22 = Two-rowed Ethiopian lines, 26 = Six-rowed Ethiopian lines, 42= Two-rowed ICARDA lines, 46= Six-rowed ICARDA lines, 52 = Two-rowed NDSU lines, and 56 = Six-rowed NDSU lines.

On average, the two-rowed NDSU and ICARDA lines produced higher numbers of tillers per plant compared to the other two-rowed groups at Bekoji (Table A.7). At Koffele, however; the two-rowed NDSU lines produced lower numbers of tillers per plant compared to the other two-rowed groups. For number of kernels per spike (Table A.8), the six-rowed ICARDA and NDSU groups had more kernels in a spike (47-50 kernels) compared to the six-rowed Ethiopian breeding lines and landraces at Bekoji (43-45 kernels). At Koffele, mean number of kernels per spike were slightly higher for six-rowed Ethiopian and landrace groups (60 kernels) compared to six-rowed ICARDA and NDSU lines (58 kernels). The two-rowed landraces produced higher number of kernels per spike compared to all the other two-rowed groups particularly at Bekoji (Table A.8).

The two-rowed and six-rowed NDSU lines were associated with lower thousand-kernel weight compared to the other six-rowed and two-rowed groups, respectively, at Bekoji and Koffele (Table A.10). At Fargo, the two-rowed NDSU lines were shown to be equivalent or better in thousand-kernel weight to the other groups, while the six-rowed NDSU and ICARDA lines were found to have lower thousand-kernel weight compared to the six-rowed Ethiopian lines. Generally, there were no significant differences within the two-rowed and six-rowed groups for hectoliter weight at Bekoji and Koffele (Table A.11). However, the mean hectoliter weight values for two-rowed landraces was significantly ($p<0.05$) lower than those for the

other two-rowed groups at Fargo. Even if non-significant compared to the other groups, the six-rowed landraces had numerically lower hectoliter weight values. These results indicate that the Ethiopian landraces appear to have poor adaptation to the environmental conditions in Fargo. Overall, wide range estimates for all the groups for numbers of tillers per plant, numbers of kernels per spike, spike length, thousand-kernel weight, and hectoliter weight are indicative of the high variability within each group for these traits (Table A.7 to A.11).

Mean yield was greatest at Bekoji (4.2 t ha^{-1}) and lowest at Fargo (2.7 t ha^{-1}) (Table 2.7). There is large difference in the length of the growing season in Ethiopia vs. Fargo. As a general matter of fact, the longer growing period results in longer vegetative development and grain filling periods. This can often lead to more kernels per spike, higher grain weight, and ultimately higher grain yields. Thus, the difference in the length of growing season between the Ethiopian locations and Fargo may partly explain the differences in grain yield between the two regions. The grain yield difference between Bekoji and Koffele (0.4 t ha^{-1}) can be partly explained by the differences in stand establishment and lodging severity. In general, lower stand establishment and higher lodging were observed at Koffele than Bekoji (Table 2.7).

In addition to mean yield difference among test locations, there was also large variability among and within the different groups in yield at each location (Table A.12). The mean yield for the groups ranged from 2.0 t ha^{-1} (two-rowed NDSU lines) to 5.3 t ha^{-1} (six-rowed Ethiopian lines) at Bekoji; from 1.9 t ha^{-1} (two-rowed NDSU lines) to 4.9 t ha^{-1} (six-rowed Ethiopian lines) at Koffele; and from 1.4 t ha^{-1} (six-rowed Ethiopian lines) to 3.8 t ha^{-1} (six-rowed NDSU lines) at Fargo. The rank of the groups in yield changed between the Ethiopian locations (Bekoji and Koffele) and Fargo. The low grain yield of the NDSU groups in the Ethiopian experiments could stem from their poor seedling establishment (Table A.13). The variability in grain yield was not restricted among groups, with each group having wide range estimates (Table A.12).

Grain and malt quality traits

Prior to malting, percent germination was determined to ensure there was no residual dormancy. Germination of almost all entries was greater than 95%. With respect to barley protein content, results at Bekoji were quite different from those obtained at Koffele and Fargo (Table 2.7). Bekoji had mean protein

of 11.4% with a range of 9.3 to 14.6%, whereas Koffele had mean of 14.5% with a range of 11.0 to 19.0%, and Fargo had mean of 14.4% with a range of 9.3 to 19.1%. The lowest mean protein content at Bekoji was recorded for two-rowed NDSU breeding lines (10.1%) while the highest mean protein content was recorded for the two-rowed Ethiopian breeding lines and six-rowed NDSU breeding lines, with mean of 11.5% (Table 2.10). However, there was large variability within each group for barley protein content, particularly within the two-rowed landraces (9.4-13.1%), two-rowed Ethiopian breeding lines (9.9-13.4%), and six-rowed ICARDA breeding lines (9.0-14.1%).

On average, the mean proportion of plump kernels across locations ranged from 62.3% at Fargo to 76.5% at Bekoji (Table 2.7). The relatively low proportion of plump kernels at Fargo was mainly due to the poor performance of most Ethiopian breeding lines and landraces probably because of their late maturity and overall poor adaptation. In 2012 at Bekoji, both six-rowed and two-rowed Ethiopian breeding lines, and two-rowed ICARDA breeding lines generally had high proportions of plump kernels (81.8-85.8%), while both two-rowed and six-rowed NDSU breeding lines (62.7-68.9%), landraces (67.8-77.6%), and six-rowed ICARDA lines (73.7%) were associated with low proportions of plump kernels (Table 2.10). Again, there was a wide range of variability among the genotypes within each group including landrace and NDSU groups. For instance, the range in the proportion of plump kernels for six-rowed landraces ranged from 30.5% to 97.0% and 22.4% to 95.2% for two-rowed NDSU lines.

In 2012 at Bekoji, the highest mean malt loss (10.0%) was recorded for two-rowed NDSU lines while the lowest values (7.5%) were recorded for six-rowed ICARDA lines (Table 2.10). Generally, malt loss is attributed to moisture loss, respiration loss, and removal of rootlets from the finished malt. Though a maltster wants to minimize the malt loss, the low levels of malt loss of some lines in this trial may be due to their non-adaptation for malting. These types of lines would have less than desired levels of malt modification; hence, less malt loss. In this study, malt loss was positively correlated with α-amylase (r = 0.545), malt extract (r=0.434), soluble protein (r = 0.369), and wort color (r = 0.333); and negatively correlated with protein content (r = -0.351), β-glucan (r = -0.523), and wort viscosity (r = -0.456) (Table A.15). These results again imply that genotypes with greater modification have greater malt loss.

Table 2.10. Mean and range for grain protein, proportion of plump kernels, and malt loss of different group of barley grown at Bekoji, Ethiopia in 2012.

Group	Protein content (%)		Plump Kernel (%)		Malt Loss (%)	
	Mean	Range	Mean	Range	Mean	Range
Two-rowed landrace	11.1	9.4 - 13.1	77.6	59.4 - 98.2	8.5	6.8 - 9.9
Six-rowed landrace	10.6	8.9 - 12.5	67.8	30.5 – 97.0	7.9	5.7 - 10.5
Two-rowed Ethiopian lines	11.5	9.9 - 13.4	85.6	54.3 – 99.0	7.7	5.8 - 10.5
Six-rowed Ethiopian lines	11.1	10.1 - 12.1	81.8	68.1 - 97.2	8.4	6.2 - 11.1
Two-rowed ICARDA lines	11.2	9.9 - 13.1	85.8	52.2 - 99.2	8.1	5.9 - 10.6
Six-rowed ICARDA lines	10.8	9.0 - 14.1	73.7	47.3 - 95.6	7.5	5.2 - 10.5
Two-rowed NDSU lines	10.1	9.4 - 11.1	62.7	22.4 - 95.2	10.0	7.2 - 12.2
Six-rowed NDSU lines	11.5	10.4 - 13.3	68.9	48.2 - 93.7	8.4	6.8 - 10.7

α-amylase is one of the important enzymes that converts starch to fermentable sugars (Igartua et al., 2002). The mean α-amylase activity for genotypes grown in 2012 at Bekoji ranged from 32.4 ^{o}DU for six-rowed landraces to 63.7 ^{o}DU for two-rowed NDSU lines (Table 2.11). Generally, both two-rowed and six-rowed NDSU lines (with mean of 63.7 ^{o}DU and 55.3 ^{o}DU, respectively) were shown to be significantly ($p \leq 0.05$) higher in α-amylase activity compared to all the other groups (Table 2.11). In fact, the range estimates for the groups indicated that some genotypes from the six-rowed landrace, and both two-rowed and six-rowed Ethiopian and ICARDA breeding lines had α-amylase activity > 50 ^{o}DU, and hence each group had some genotypes meeting the minimum desired level by American Malting Barley Association (AMBA) (http://ambainc.org/media/AMBA_PDFs/Pubs/Production/Guidelines_June_2014.pdf; accessed 27 Jan 2015).

For DP, the six-rowed NDSU breeding lines had the highest mean (168.7 ^{o}ASBC) whereas the six-rowed Ethiopian breeding lines had the lowest (81.5 ^{o}ASBC) (Table 2.11). However, the range values indicated that there were genotypes within all groups, except the two-rowed landraces and six-rowed Ethiopian breeding lines that met the AMBA's minimum desired value for DP of 110 ^{o}ASBC (http://ambainc.org/media/AMBA_PDFs/Pubs/Production/Guidelines_June_2014.pdf; accessed 27 Jan 2015).

Diastatic power (DP) is often correlated with protein content, but in this study the relationship between these two traits was weak (r = 0.386) (Table A.15). The lower than expected correlation between DP and protein in this study may have occurred because many of the genotypes utilized in this study were not selected for or utilized for malting. To remove the confounding effect of protein content on DP, the new variable of the ratio of DP to protein is often calculated by malting barley breeders and used for making comparisons between genotypes. Even though the relationship between DP and protein was

weak in this study, I still calculated the ratio. In the present study, the ratio ranged from 7.3 % for six-rowed Ethiopian breeding lines to 14.8 % for six-rowed NDSU breeding lines (Table 2.11). Both six-rowed and two-rowed NDSU groups were significantly ($p \leq 0.05$) higher in DP and the ratio of DP to barley protein content compared to all the other groups. However, the range values for each of the groups indicated that there was larger variability within groups for all enzyme components of malt quality.

Malt extract is to brewers as grain yield is to farmers. Malt extract is the amount of soluble material from malt that can be fermented and used for producing beer (Berger and LaBerge, 1985). The higher the malt extract, the less malt a brewer needs to use. Thus, breeders are always striving to improve malt extract levels in new cultivars. In the current study, mean malt extract of NDSU two-rowed barley genotypes was significantly higher ($p \leq 0.05$) than that of the other groups, except the NDSU six-rowed group (Table 2.12). The mean malt extract of the two-rowed and six-rowed NDSU genotypes was greater than 79.0%. In fact, each of the two-rowed NDSU lines had malt extract greater than 79.0%. The desired levels of malt extract by AMBA for six-rowed and two-rowed barley genotypes are > 79.0% and > 81.0%, respectively (http://ambainc.org/media/AMBA_PDFs/Pubs/Production/Guidelines_June_2014.pdf; accessed 27 Jan 2015). Because the NDSU groups are entirely malting types while most of the non-NDSU groups are food or feed types, the malt extract of NDSU genotypes is expected to be much higher than that of the other genotypes. Nonetheless, within each of the non-NDSU groups, there was large variability for malt extract and at least some genotypes could be found with malt extract >79.0%.

Table 2.11. Mean and range for α-amylase, diastatic power (DP), and ratio of DP to barley protein content (DPN) of different groups of barley grown at Bekoji, Ethiopia in 2012.

Group	α-amylase (°DU)		DP (°ASBC)		DPN (%)	
	Mean	Range	Mean	Range	Mean	Range
Two-rowed landrace	33.1	23.5-45.8	89.1	56.0-105.8	8.2	5.6-10.6
Six-rowed landrace	32.4	15.3–52.0	86.1	53.2-151.2	8.3	5.5- 6.8
Two-rowed Ethiopian lines	34.8	21.6–60.0	103.2	60.8-180.9	8.9	5.5-13.9
Six-rowed Ethiopian lines	36.1	19.9-52.9	81.5	51.2–107.0	7.3	5.1-9.5
Two-rowed ICARDA lines	39.9	21.4–53.0	103.6	51.1-144.6	9.2	5.0-12.7
Six-rowed ICARDA lines	35.4	17.7-53.7	101.1	40.5-162.7	9.4	3.7-15.1
Two-rowed NDSU lines	63.7	47.4-87.5	126.1	92.8-164.9	12.5	9.8-15.4
Six-rowed NDSU lines	55.3	48.1-64.7	168.7	132.7-196.5	14.8	12.5-18.5

Soluble protein is important for yeast nutrition during fermentation, for beer foam stability, and can impact beer color. The correlation between soluble protein and wort color was moderate and positive (r = 0.429) (Table A.15). According to the AMBA, the minimum desired soluble protein levels for six-rowed and two-rowed malt barley genotypes are 5.2-5.7% and 4.8-5.6%, respectively

(http://ambainc.org/media/AMBA_PDFs/Pubs/Production/Guidelines_June_2014.pdf; accessed 27 Jan 2015). Mean soluble protein values for all groups were less than the targets of the AMBA (Table 2.12). However, the two-rowed and six-rowed NDSU groups had mean soluble protein content of 4.3% and 4.6%, respectively; which were significantly higher than those for the other groups. The range values indicated that some genotypes within six-rowed NDSU lines had soluble protein values > 5.2%. According to Schwarz and Horsley (1995), the total extract can be partitioned into two soluble components, soluble protein extract and the carbohydrate extract. About 5.3 to 5.8% of the total malt extract for NDSU groups was contributed by the soluble protein fraction while soluble protein contributed 4.0 to 4.5% of the total malt extract in the non-NDSU groups.

The present study revealed that the NDSU lines had higher values for the Kolbach Index than observed in the other groups. Kolbach Index is the ratio of soluble protein in the wort to the barley protein content and is referred to as S/T by USA maltsters and brewers (Table 2.12). The desired S/T values by AMBA are 42-47% and 38-45%, respectively, for six- and two-rowed barley. Accordingly, only the mean of two-rowed NDSU group meet the desired level for S/T. Like the other grain and malt quality traits, there was genetic variability for S/T in each of the groups and hence there were some genotypes within each group, except two-rowed landraces and six-rowed ICARDA lines, meeting the desired level for S/T.

Table 2.12. Mean and range for malt extract, soluble protein, and ratio of soluble to total protein (S/T) of different groups of barley grown at Bekoji, Ethiopia in 2012.

Group	Malt extract		Soluble protein (%)		S/T (%)	
	Mean	Range	Mean	Range	Mean	Range
Two-rowed landrace	76.6	73.9-80.1	3.3	2.4-4.4	31.6	23.6-37.7
Six-rowed landrace	77.0	72.7-81.8	3.1	2.1-4.5	29.4	20.2-42.5
Two-rowed Ethiopian lines	76.7	71.1-81.6	3.4	2.4–5.0	30.0	19.7-42.5
Six-rowed Ethiopian lines	76.3	73.0-79.5	3.3	2.1-4.8	29.2	19.9-40.3
Two-rowed ICARDA lines	77.5	72.1-80.0	3.5	2.5-4.5	31.1	20.4-38.7
Six-rowed ICARDA lines	76.1	71.1-81.5	3.1	2.4-4.0	29.0	20.5-37.2
Two-rowed NDSU lines	80.6	79.2-82.2	4.3	3.8-5.1	42.9	36.7-50.3
Six-rowed NDSU lines	79.2	77.8-80.5	4.6	3.7-5.7	39.9	35.4-46.1

Free amino nitrogen is necessary for yeast nutrition during fermentation. In the present study, the highest mean FAN was observed for the six-rowed NDSU group (218 mg L^{-1}). This value was significantly higher than mean FAN of all groups except the two-rowed NDSU group (Table 2.13). In turn, mean FAN of the two-rowed NDSU group was significantly higher than that of all remaining groups, except the two-rowed ICARDA group. AMBA indicates that desired FAN values for two-rowed and six-rowed are 140-190

and > 210 mg L^{-1}, respectively. Thus, the mean values of FAN were within the desired levels for all the two-rowed groups except the two-rowed landrace. However, only the mean of six-rowed NDSU lines met the desired level of all the six-rowed groups. The wide range estimates associated with each group (Table 2.13) indicate that at least some genotypes from each group, except the six-rowed ICARDA lines, meet the desired FAN levels specified by AMBA.

Wort color can be used to determine the contribution of malt to final beer color. The color of the beer will be dependent on the types and quantities of the different types of malts used to make a beer. The AMBA indicates that desired wort colors are 1.6-2.5 °ASBC and 1.8- 2.5 °ASBC for two-rowed and six-rowed malt, respectively. The higher the wort color value, the darker the beer color. In general, wort color of the NDSU breeding lines was greater than that of the remaining groups (Table 2.13). However, there were two-rowed landraces that had wort color values comparable to that of the NDSU lines. Wort color was correlated positively with soluble protein (r= 0.429) and FAN (r= 0.315) (Table A.15), indicating that soluble protein and FAN are associated with wort color. Because NDSU lines had higher values for soluble protein and FAN, it is not surprising that the NDSU genotypes tended to have the highest wort color values.

Table 2.13. Mean and range for free amino nitrogen and wort color of different groups of barley grown at Bekoji, Ethiopia in 2012.

Group	Free Amino Nitrogen (mg L^{-1})		Wort color (°ASBC)	
	Mean	Range	Mean	Range
Two-rowed landrace	138.5	94.3 - 166.2	2.0	1.7 - 2.3
Six-rowed landrace	147.5	74.9 - 293.0	1.7	1.3 - 2.7
Two-rowed Ethiopian lines	164.9	86.8 - 282.2	1.7	1.4 - 1.9
Six-rowed Ethiopian lines	155.7	92.2 - 226.5	1.6	1.3 - 1.9
Two-rowed ICARDA lines	172.2	108.6 - 278.5	1.7	1.2 - 2.2
Six-rowed ICARDA lines	138.8	77.6 - 206.0	1.7	1.4 - 2.2
Two-rowed NDSU lines	194.8	131.3 - 241.0	2.1	1.7 - 2.3
Six-rowed NDSU lines	218.3	162.3 - 318.5	1.9	1.6 - 2.3

The concentration of wort β-glucan and viscosity are important parameters for the brewer to determine problems that might impact lautering (i.e. wort filtration) or “mouth-feel” of the beer. Desirable levels for β-glucan are < 120 mg L^{-1} for six-rowed malt and < 100 mg L^{-1} for two-rowed malt. In general, the mean wort β-glucan of the NDSU two-rowed and six-rowed groups (135.6-153.4 mg L^{-1}) were significantly lower (p≤0.05) than the mean values for the other groups (Table 2.14). In fact, mean differences of >200 mg L^{-1} for β-glucan between the NDSU and the other groups are considered extremely large. High levels of wort β-glucan are indicative of malt that is under modified (Igartua et al.,

2002), which can negatively impact malt extract, wort separation, and beer filtration (Schwarz and Horsley, 1995). The current study also indicated that β-glucan was negatively correlated with malt extract (r = -0.549) and wort soluble protein (r = -0.604) (Table A.15). Though mean β-glucan concentration for all groups was higher than the desired level specified by AMBA, there were some genotypes associated with lower β-glucan values (<100 ppm), within two-rowed and six-rowed ICARDA and NDSU groups, and within the six-rowed Ethiopian breeding lines (Table 2.14). In the case of barley β-glucan, all the groups were closer to each other except two-row ICARDA and six-rowed Ethiopian breeding lines (Table 2.14). However, the NDSU (4.1-4.3%) and landrace (4.2-4.4%) groups were slightly lower than ICARDA (4.8-5.1%) and Ethiopian (4.5-4.8%) breeding lines. Comparison of wort and barley β-glucan indicated that the bulk of barley β-glucan degraded during the malting process in the NDSU breeding lines.

Wort β-glucan positively correlated with wort viscosity (r = 0.712) in this study (Table A.15). This moderately strong correlation suggests that wort β-glucan content is one of the major contributing factors to high wort viscosity. Igartua et al. (2002) indicated that wort β-glucan can impact wort viscosity, which can cause problems in lautering during brewing and beer stability during storage. At least some NDSU lines were shown to have acceptable level desired by AMBA for wort viscosity (Table 2.14), which is <1.5.

Table 2.14. Mean and range for β-glucan and wort viscosity of different groups of barley grown at Bekoji, Ethiopia in 2012 and Fargo in 2012.

Groups	Wort β-glucan (mg L-1)		Wort Viscosity (mPa.s)		Barley β-glucan (%)†	
	Mean	Range	Mean	Range	Mean	Range
Two-rowed landrace	458	183 - 654	1.7	1.5 - 1.9	4.2	3.7-4.8
Six-rowed landrace	486	57 - 763	1.8	1.5 - 2.4	4.4	3.7-5.6
Two-rowed Ethiopian lines	497	122 - 762	1.8	1.5 - 2.3	4.5	3.7-5.8
Six-rowed Ethiopian lines	454	226 - 710	1.8	1.6 - 2.4	4.8	4.4-5.2
Two-rowed ICARDA lines	420	103 - 770	1.7	1.5 - 2.4	5.1	3.8-6.1
Six-rowed ICARDA lines	477	87 - 766	1.7	1.5 - 2.1	4.6	3.8-5.5
Two-rowed NDSU lines	136	18 - 283	1.5	1.4 - 1.7	4.1	3.5-4.6
Six-rowed NDSU lines	153	54 - 345	1.5	1.4 - 1.6	4.3	4.1-4.6

†Means and ranges for barley β-glucan was estimated using both Bekoji and Fargo in 2012 while means and ranges for wort β-glucan and Wort Viscosity are estimated using data from Bekoji in 2012.

Principal Component Analysis

The first four principal components (PCs) for agronomic and disease resistance traits in the Bekoji analysis had Eigen values greater than 1.0, and they collectively accounted for 81.3% of the variability in the original data (Table 2.15). Three traits with load scores > |0.75| were yield (0.931), leaf scald (-0.850), and stand (0.815) in the first PC (PC1), which accounted for 32.5% of the total variation

among the genotypes. These results suggest that the highest yielding genotypes had greater stand establishment and resistance to scald. The second PC for Bekoji accounted for 25.4% of the total variation and traits with load score >|0.75| were hectoliter weight (0.808), number of kernels per spike (-0.804), thousand-kernel weight (0.839), and plump kernels (0.794). Thus, genotypes with high positive load values in the second PC (PC2) had higher hectoliter weight, thousand-kernel weight, and plump kernels; but lower numbers of kernels per spike. These traits generally separated the six-rowed genotypes from the two-rowed ones (Figure 2.1a), where the two-rowed genotypes were plotted to the positive side with respect to PC2 axis and the six-rowed barley genotypes were plotted to the negative side of the axis. Days to heading (0.881) and days to maturity (0.903) were the only traits with load scores >|0.75| for the third PC and lodging (0.904) was the only trait in the fourth PC with a load score >|0.75|.

Table 2.15. Eigen values, explained variation, communality values, and Eigen vectors in PCA for Bekoji, Ethiopia estimated using LS means over three years (2011-2013).

Parameters		PC1†	PC2	PC3	PC4
Eigen Values		3.6	2.7	1.6	1.0
Individual variation explained (%)		32.5	24.5	15.0	9.3
Cumulative variation explained (%)		32.5	57.0	72.0	81.3
Traits	Communalities	Load scores			
Grain yield	0.875	0.931	0.087	-0.029	-0.010
Scald	0.753	-0.850	-0.148	-0.091	0.009
Stand	0.823	0.815	0.061	0.186	0.347
Plant Height	0.846	0.583	0.108	0.230	0.665
Hectoliter weight	0.722	0.090	0.808	0.241	-0.056
Number of kernels per spike	0.789	0.285	-0.804	0.246	0.009
Thousand-kernel weight	0.835	0.319	0.839	-0.122	0.122
Plump kernels	0.716	0.244	0.794	-0.095	-0.127
Days to heading	0.876	0.178	-0.211	0.881	0.150
Days to maturity	0.853	0.022	0.055	0.903	-0.182
Lodging	0.849	0.021	-0.107	-0.142	0.904

†PC1 = First principal component, PC2 = Second principal component, PC3 = Third principal component, and PC4 = Fourth principal component.

Referring to Figure 2.1c, the PC1 successfully separated the NDSU breeding lines from the other groups at Bekoji; however, neither PC1 nor PC2 successfully differentiated between the landraces, Ethiopian breeding lines, and ICARDA breeding lines. Generally, NDSU genotypes were aligned on the negative side of the PC1 axis while the other groups were on the positive side of the axis. The negative PC1 values for NDSU lines were associated with low grain yield, poor seedling establishment, and susceptibility to leaf scald as compared to genotypes from the other groups at Bekoji. The load score for plant height in PC1 was also positive (0.583) though it was < 0.75. The negative PC1 score for NDSU genotypes can also occur because the NDSU genotypes are shorter compared to genotypes in the other

groups. Thus, the NDSU genotypes could be useful as sources of genes to develop short statured cultivars that are more resistant to lodging.

At Koffele, the first four PCs had Eigen values > 1.0 and accounted for 77.3% of the variation (Table 2.16). PC1 successfully differentiated between the two-rowed and six-rowed genotypes (Figure 2.1b), which explained 31.1% of the total variation. Three traits had load values > |0.75|, number of spikes per plant (0.870), number of tillers per plant (0.866), and grain yield (0.761) (Table 2.16). The numbers of tillers and spikes per plant are usually different in two-rowed and six-rowed genotypes. Additionally, the NDSU lines were separated from ICARDA lines along PC1, where NDSU materials were plotted on the negative side of the axis (Figure 2.1d). Generally, the NDSU lines were relatively lower yielding and had fewer numbers of tillers and spikes per plant than the ICARDA genotypes. PC2 explained 23.1% of the variation and two traits had load values >|0.75|, plant height (0.901) and lodging (0.841). PC2 generally differentiated the ICARDA and NDSU genotypes from the Ethiopian breeding lines and landraces (Figure 2.1d). The ICARDA and NDSU lines were generally on the negative side of the axis with respect to PC2, while the Ethiopian breeding lines and landraces were on the positive side. Thus, these results indicate that the Ethiopian breeding lines and landraces were generally taller and lodged more than the NDSU and ICARDA lines. Spike length and days to maturity were the only traits in PC3 and PC4, respectively, with load values >|0.75|.

Table 2.16. Eigen values, explained variation, communality values, and Eigen vectors in PCA for Koffele, Ethiopia estimated using LS means over three years (2011-2013).

Parameters		PC1†	PC2	PC3	PC4
Eigen Values		3.4	2.5	1.3	1.2
Individual variation explained (%)		31.1	23.1	11.8	11.3
Cumulative variation explained (%)		31.1	53.2	66.0	77.3
Traits	Communalities		Load score		
Number of tillers per plant	0.836	0.866	0.065	0.266	-0.105
Number of spikes per plant	0.830	0.870	0.067	0.210	-0.157
Grain yield	0.743	0.761	0.102	-0.379	0.098
Thousand-kernel weight	0.706	0.673	-0.341	0.367	0.037
Stand	0.607	0.663	0.310	-0.099	0.248
Plant height	0.831	0.278	0.841	0.051	0.208
Lodging	0.817	0.016	0.901	-0.010	-0.060
Number of kernels per spike	0.804	-0.389	0.429	-0.662	0.173
Spike length	0.752	0.012	0.170	0.846	0.080
Days to heading	0.739	-0.121	0.454	0.020	0.719
Days to maturity	0.845	0.076	-0.107	-0.006	0.910

†PC1 = First principal component, PC2 = Second principal component, PC3 = Third principal component, and PC4 = Fourth principal component.

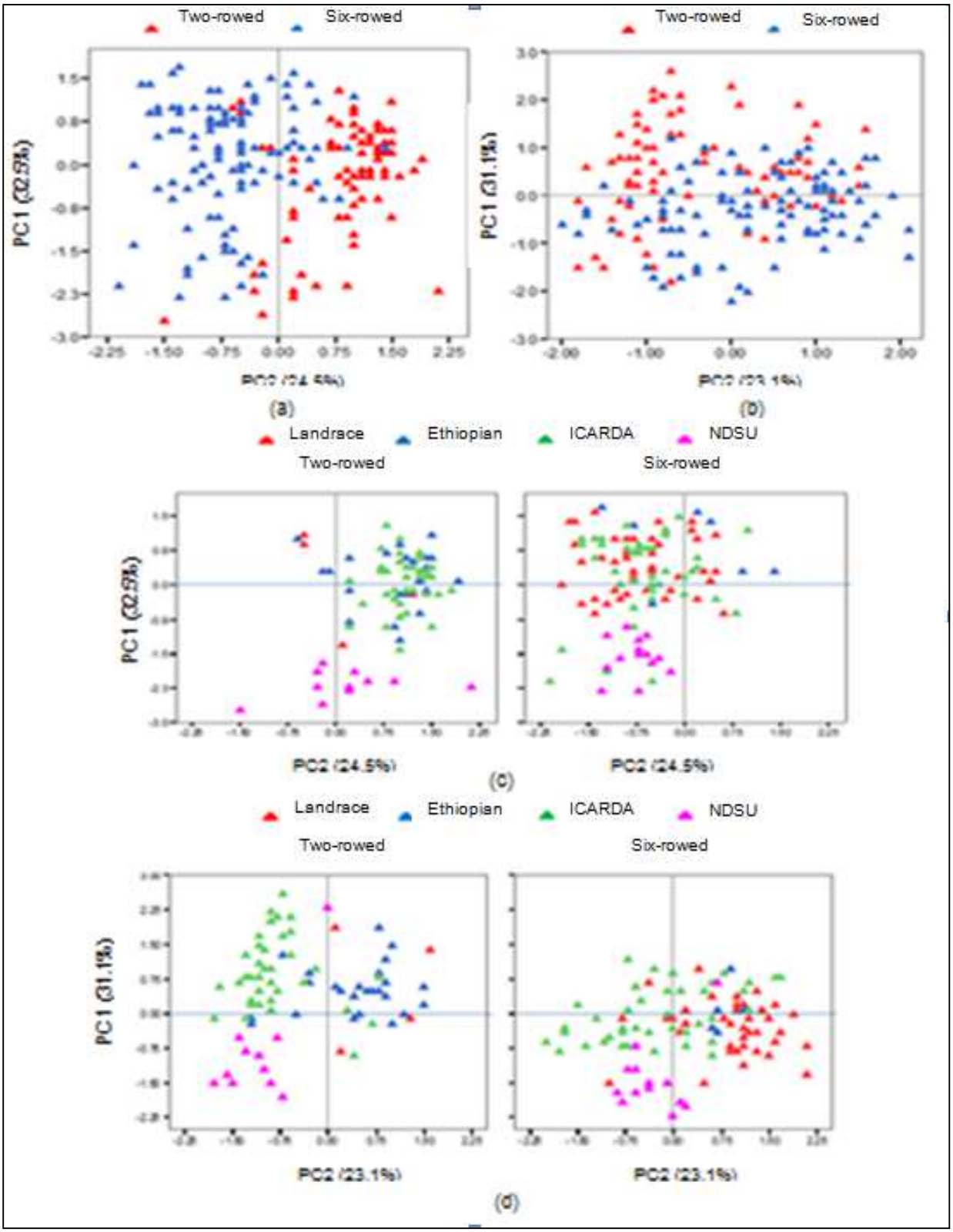

Figure 2.1. Biplot of PC1 by PC2: (a) grouping based on row-type at Bekoji, (b) grouping based on row-type at Koffele, (c) grouping based on source of the genotype for each row-type separately at Bekoji, and (d) grouping based on source of the genotype for each row-type separately at Koffele.

For grain and malt quality traits obtained from the Bekoji experiment in 2012, the first three PCs had Eigen values > 1.0, and collectively accounted for 78.3% of the total variation (Table 2.17). PC1 explained 39.5% of the variation and was composed of four traits that had load values >|0.75|, soluble protein (0.920), α-amylase (0.866), β-glucan (-0.815), and DP (0.774). Three of these four traits are associated with malt modification. The concentration of β-glucan is associated with endosperm cell wall modification. Referring to Figure 2.2b, PC1 successfully separated NDSU breeding lines from the other groups. Both the two-rowed and six-rowed NDSU genotypes were plotted to the positive side of the PC1 axis. In general, the NDSU barley genotypes had higher values for α-amylase, DP, and soluble protein, and lower values for β-glucan than genotypes in the other groups.

PC2 explained 23.3% of the total variation and had three traits with load values >|0.75|, proportion of plump kernels (0.963), proportion of thin kernels (-0.913), and thousand-kernel weight (0.821). Each of these three traits has a relationship with kernel size. The second PC generally differentiated the two-rowed genotypes from six-rowed genotypes (Figure 2.2a). The six-rowed genotypes were on the negative side of the PC2 axis, with the two-rowed genotypes located on the positive side of the axis. Thus, the two-rowed barley genotypes generally had high proportions of plump kernels and thousand-kernel weight, but minimum proportions of thin kernels compared to the six-rowed barley genotypes.

Table 2.17. Eigen values, explained variation, communality values, and Eigen vectors in PCA for Bekoji for malt and grain quality traits in 2012.

Parameters		PC1†	PC2	PC3
Eigen Values		4.3	2.6	1.7
Individual variation Explained (%)		39.5	23.3	15.5
Cumulative Variation explained (%)		39.5	62.9	78.3
Traits	Communalities	Load scores		
Thousand-kernel weight	0.753	-0.252	0.821	0.122
Barley protein content	0.927	0.185	0.097	0.940
Plump kernels	0.929	0.029	0.963	-0.038
Thin kernels	0.841	-0.078	-0.913	0.046
α-amylase	0.784	0.866	-0.017	-0.186
β-glucan	0.801	-0.815	0.115	0.352
Diastatic power	0.718	0.774	-0.166	0.303
Free amino nitrogen	0.604	0.720	0.127	0.263
Malt extract	0.785	0.556	0.194	-0.662
Soluble protein	0.869	0.920	0.075	0.135
Wort viscosity	0.602	-0.703	0.219	0.246

†PC1 = First principal component, PC2 = Second principal component, and PC3 = Third principal component.

Only one trait had a load value >|0.75| in PC3, which was barley protein content (0.940). The next highest load value in PC3 was that for malt extract (-0.662). Previous research found a relatively strong relationship between protein content and malt extract (Burger and LaBerge, 1995; Igartua et al., 2002; Fox et al., 2003). In the current study, I observed a negative correlation (r=-0.424) between barley protein content and malt extract (Table A.15). The NDSU two-rowed lines typically had lower protein content and higher malt extract (Figure 2.3). Most of the six-rowed NDSU genotypes were plotted to the positive side with respect to PC3, indicating that they may be generally lower in malt extract and higher in protein content compared to the two-rowed NDSU genotypes. Some of the ICARDA genotypes and a few Ethiopian breeding lines and landraces had satisfactory malt quality for some traits. Additionally, some of two-rowed and six-rowed ICARDA genotypes and six-rowed landrace genotypes had higher malt extract with low protein content and a relatively good combination of other malt quality traits (Figure 2.3).

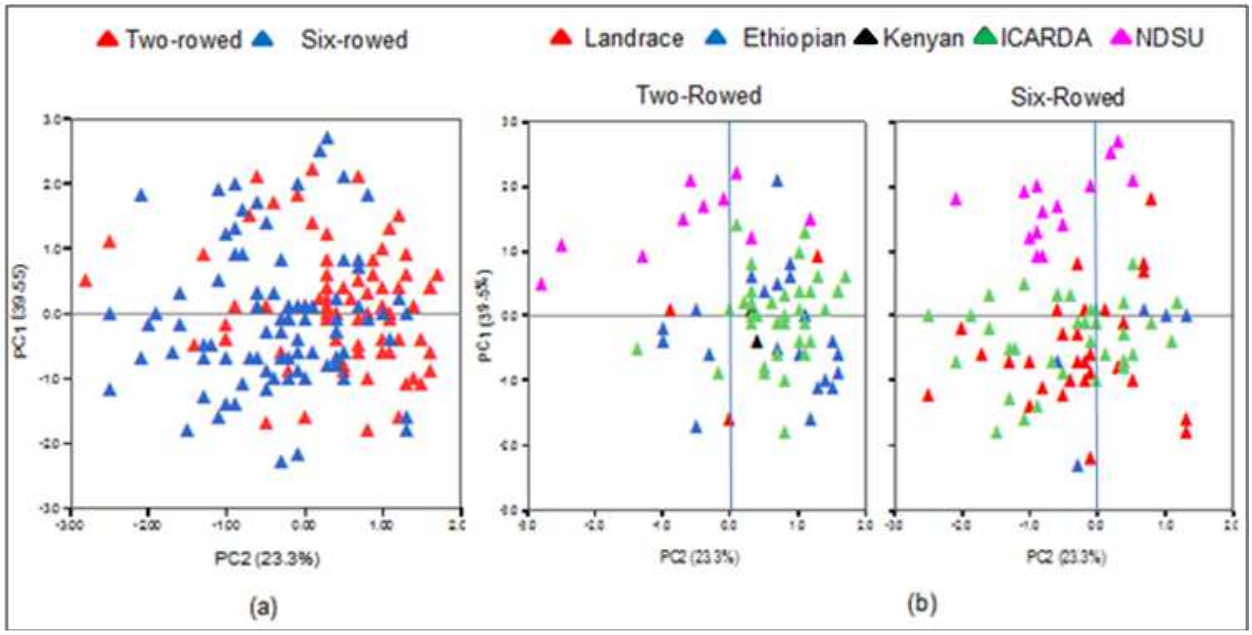

Figure 2.2. Biplot of PC1 by PC2: (a) grouping based on row-type for grain and malt quality traits at Bekoji in 2012, and (b) grouping based on source of the genotype within each row-type separately for grain and malt quality traits at Bekoji in 2012.

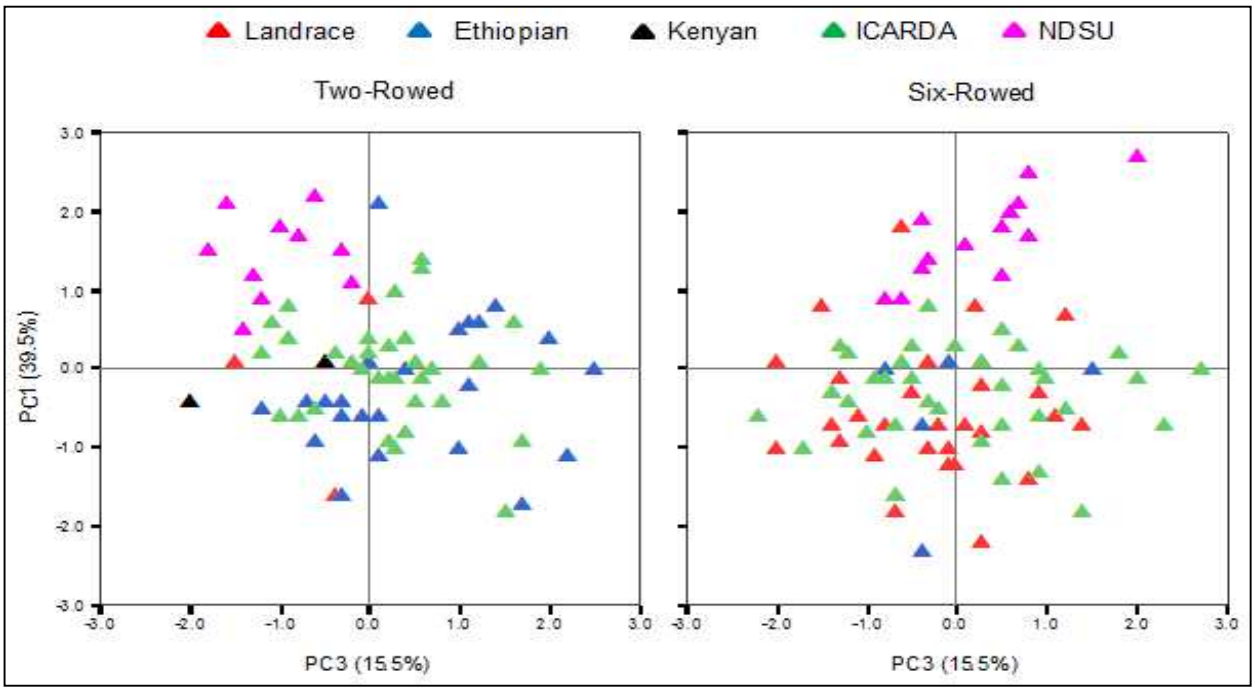

Figure 2.3. Biplot of PC1 by PC3: (a) grouping based on row-type, and (b) grouping based on source of the genotype for row-type for grain and malt quality traits at Bekoji in 2012.

Cluster Analysis

Because the results of cluster analyses based on mean phenotypic data and loading scores of genotypes in the extracted principal components from PCA were more or less the same, the cluster analyses results based on principal components were selected as the most suitable and will be the basis of my discussion.

The dendogram based on the cluster analysis of the agronomic data from Bekoji is given in Figure 2.4. The 189 genotypes included in this analysis were grouped into four major clusters. The threshold for defining clusters was a semi-partial R^2 of 0.10. Three of the clusters could also be further divided into sub-clusters. The threshold for defining the sub-cluster was a semi-partial R^2 of 0.05. The first cluster (CL1) was comprised of 29 genotypes mainly coming from the NDSU breeding program (14 six-rowed and 11 two-rowed). Genotypes in this cluster were characterized as having late heading and maturity; and relatively poor stand establishment, low grain yield, and susceptibility to scald (Figure 2.5). Favorable characteristics of genotypes in cluster CL1 included shorter plant height and better resistance to lodging, which could make them suitable for the improvement of reduced plant height and lodging resistance in the Ethiopian breeding program.

The second cluster has two sub-clusters, with the first sub-cluster (CL2.1) having only three six-rowed ICARDA lines and the second sub-cluster (CL2.2) consisting of 44 genotypes (Figure 2.4). The major features of the entries in CL2.1 were their low grain yield, number of tillers per plant, seedling establishment, hectoliter weight; shorter spike length; and high susceptibility to leaf scald (Figure 2.5). While these genotypes have many of the same characteristics of the genotypes in CL1, they had fewer days to heading and maturity compared to genotypes in all the other clusters. Sub-cluster CL2.2 is mainly comprised of two-rowed (29) and six-rowed (9) genotypes from the ICARDA breeding program. This sub-cluster contained genotypes that had relatively high grain yield, tillering capacity, and stand establishment; moderately earlier heading and maturity dates; high thousand-kernel and hectoliter weights; moderately shorter and stronger straw; and moderate resistance to leaf scald. Generally, this sub-cluster contained potentially promising genotypes that may be useful to the Ethiopian breeding program for enriching the genetic variability.

The third main cluster had two sub-clusters (Figure 2.4). The first sub-cluster (CL3.1) included 29 genotypes from multiple origins, including six-rowed landraces, and two-rowed and six-rowed ICARDA and Ethiopian breeding lines. In general, genotypes in this cluster had moderate grain yield, tillering, and stand establishment; high thousand-kernel and hectoliter weights; and resistance to leaf scald (Figure 2.5). The genotypes also had greater days to heading and maturity, and were relatively taller but had low lodging. However, drawing conclusions on lodging based on results from Bekoji alone is not warranted because overall lodging levels were lower at Bekoji than Koffele. Sub-cluster CL3.2 contained 32 genotypes, with the majority of them being six-rowed ICARDA lines (18) and six-rowed landraces (10). The major features of this cluster were high grain yield, tillering, numbers of kernels per spike, and stand establishment; medium plant height and low lodging; and moderate resistance to leaf scald. Even though the genotypes in this sub-cluster yielded well, they had lower thousand-kernel and hectoliter weights. They were also greater days to heading and maturity. It is possible that the high grain yield may be due to large number of spikes per plant and number of kernels per spike, and later maturity. The low thousand-kernel and hectoliter weights could be due to the fact that this sub-cluster is dominated by six-rowed genotypes.

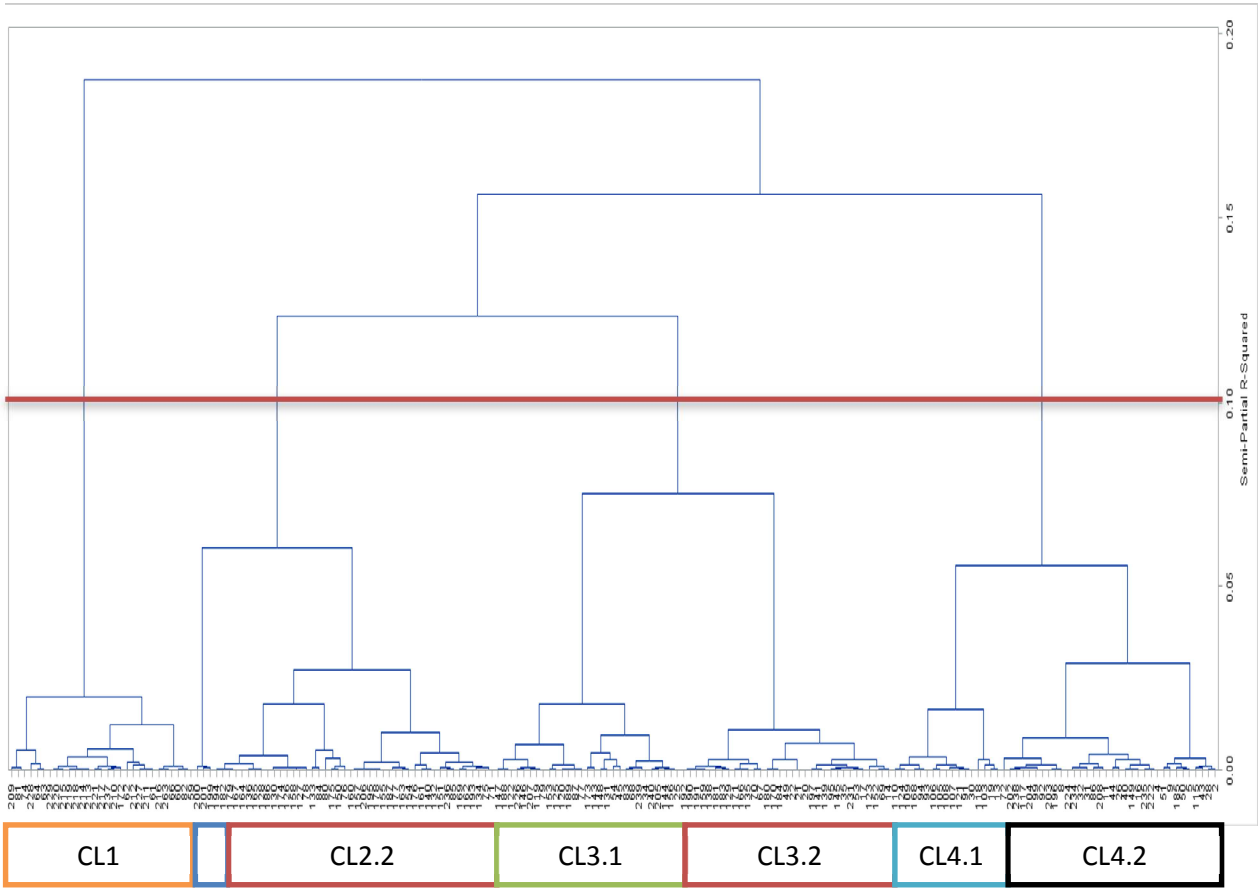

Figure 2.4. Cluster dendogram based on four significant principal components (where the PCA was constructed from 11 agronomic traits collected at Bekoji over three years -2011 to 2013).

Just like clusters 2 and 3, the fourth cluster was subdivided into two sub-clusters (Figure 2.4). The first sub-cluster (CL4.1) included 18 genotypes, with 12 of them being two-rowed Ethiopian breeding lines. This cluster was mainly characterized by entries with average days to heading and maturity; moderately resistant to scald; relatively moderate grain yield with high tillering capacity and stand establishment; high thousand-kernel and hectoliter weights; and longer spikes (Figure 2.5). However, this sub-cluster had taller genotypes with relatively high lodging for the location. Sub-cluster CL4.2 included 34 genotypes, with 22 of them being six-rowed landraces and six being six-rowed ICARDA genotypes. The major features of this sub-cluster included high yield, tillering, and stand establishment; moderate maturity; moderate thousand-kernel and hectoliter weights; and moderately resistance to leaf scald. However, genotypes in this sub-cluster tended to be taller, have relatively high lodging for the location, and shorter spikes.

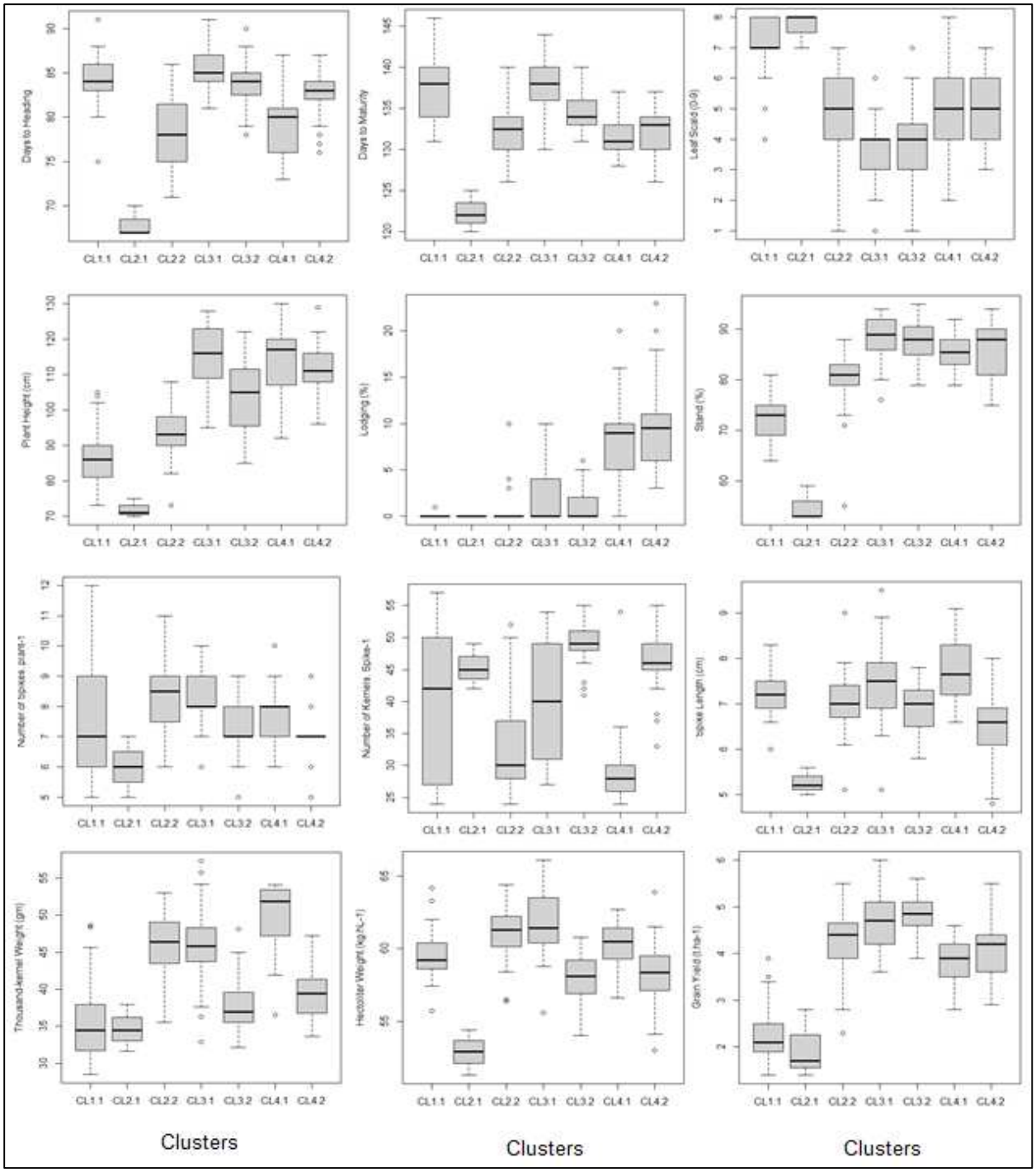

Figure 2.5. Mean and variability of the seven clusters generated based on the four significant principal components for 11 agronomic traits recorded on Bekoji experiments for three years (2011- 2013).

For Koffele, the cluster dendogram revealed four major clusters for 185 genotypes at a threshold of 0.10 for the semi-partial R^2 value (Figure 2.6). Two of the clusters could be further subdivided into two sub-clusters each at a semi-partial R^2 of 0.05 (Figure 2.6). The first cluster contained a total of 62 genotypes. The cluster was further subdivided into sub-clusters CL1.1 and CL1.2. Sub-cluster CL1.1 was

comprised of 24 barley genotypes, of which 18 of them were NDSU breeding lines (11 six-rowed and 7 two-rowed). This sub-cluster was characterized by genotypes having low grain yield, poor stand establishment, and relatively poor tillering (Figure 2.7). The positive aspects of the genotypes in this sub-cluster were shorter plant height, low lodging, and longer spikes. Sub-cluster CL1.2 included 38 genotypes, with 32 of them being six-rowed and two-rowed ICARDA breeding lines, two-rowed Ethiopian breeding lines, and six-rowed landraces. Each of these groups contributed about six to 11 genotypes. Genotypes in sub-cluster CL1.2 tended to have moderate grain yield, stand establishment, and numbers of tillers; late heading and maturity dates; medium plant height and lodging resistance; and moderate resistance to net blotch.

The second cluster (CL2) included 27 genotypes, of which 21 were six-rowed ICARDA breeding lines (Figure 2.6). The genotypes in this group had above average grain yield, average stand establishment, and relatively shorter plant height and hence experienced low levels of lodging (Figure 2.7). However, genotypes in this cluster were late in days to maturity, poor in tillering, shorter in spike lengths, and low in thousand-kernel and hectoliter weights.

The third cluster (CL3) was comprised of 32 genotypes, with 23 of them being the two-rowed ICARDA breeding lines (Figure 2.6). Genotypes in this cluster tended to have high grain yield, numbers of tillers, and stand establishment; shorter plant height and low lodging; resistance to net blotch; above average thousand-kernel and hectoliter weights; average spike length; and fewer days to heading and maturity (Figure 2.7). This cluster corresponded to the sub-cluster CL2.2 in the Bekoji analysis. Like cluster CL2.2 at Bekoji, the CL3 at Koffele included lines that may be beneficial in providing favorable alleles that may be deficient in the Ethiopian breeding germplasm.

The fourth cluster was sub-divided into two sub-clusters, CL4.1 and CL4.2 (Figure 2.6). Sub-cluster CL4.1 was comprised of 23 genotypes, with 11 of them being two-rowed Ethiopian breeding lines. The remaining lines were from each of the remaining groups, with one to three genotypes from each group. The genotypes in sub-cluster CL4.1 tended to have fewer days to heading and maturity; moderate resistance to net blotch; longer spikes; average grain yield and stand establishment; and above average thousand-kernel and hectoliter weights, and tillering capacity (Figure 2.7). The genotypes in this sub-cluster also tended to be taller in plant height and be more susceptible to lodging. Sub-cluster CL4.2

consisted of 41 genotypes, with 23 and 10 of them being six-rowed landraces and six-rowed ICARDA breeding lines, respectively. The genotypes in this sub-cluster tended to have average grain yield, stand establishment, numbers of tillers, and days to heading and maturity. However, they were more susceptible to net blotch compared to genotypes in the other clusters and tended to have shorter spikes, low thousand-kernel and hectoliter weights, and taller plants with weaker straw. The low thousand-kernel weight could be due to that the cluster was dominated by six-rowed genotypes.

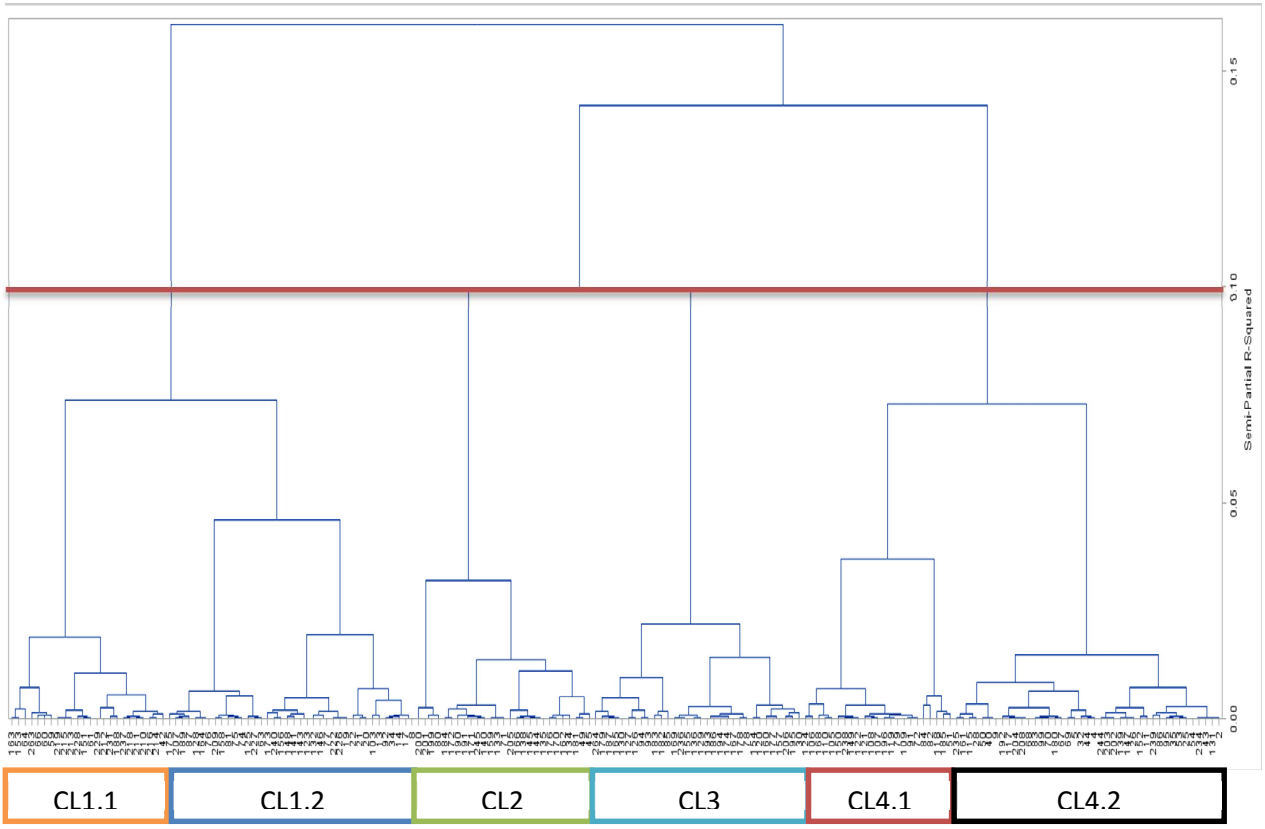

Figure 2.6. Cluster dendogram based on four significant principal components (where the PCA was constructed from 11 agronomic traits collected at Koffele over three years -2011 to 2013).

For the nine malt and two grain quality traits from the 2012 Bekoji experiment, the cluster dendogram was based on genotype load scores from the first three PCs (Figure 2.8). There were four major clusters for 160 genotypes at a semi-partial R^2 of 0.10. Two of the cluster could be further divided into two sub-clusters, each at a semi-partial R^2 of 0.05. The first cluster had two sub-clusters. Sub-cluster CL1.1 consisted of 12 genotypes; with five being two-rowed Ethiopian breeding lines and three being six-rowed ICARDA breeding lines. The remaining four genotypes were six-rowed genotypes from ICARDA breeding lines, landraces, and Ethiopian breeding lines groups, with each contributing one to two

genotypes. Overall, the genotypes in this sub-cluster tended to have lower than the minimum required levels for α-amylase (<40 °DU), malt extract (<79.0%), and soluble protein (<5.2%); and higher than maximum required values for β-glucan (>120 ppm) and wort viscosity (>1.5 cP) (Figure 2.9). However, the positive features of genotypes in this sub-cluster included higher proportions of plump kernels (about 90%) and thousand-kernel weight (47.0 g); and acceptable levels for barley protein content (12.5%), DP (125 °ASBC), FAN (210), and wort color (1.7 °ASBC). These results indicate that the endosperms of the genotypes in this sub-cluster were under modified with the current malting method.

Sub-cluster CL1.2 was comprised of 43 genotypes, with 25 being two-rowed ICARDA breeding lines and nine being two-rowed Ethiopian breeding lines (Figure 2.8). The remaining genotypes included six-rowed and two-rowed landraces, and six-rowed ICARDA and Ethiopian breeding lines. Just like CL1.1, the genotypes in this sub-cluster tended to have undesirable malt quality (Figure 2.9). They had lower than the minimum required values for α-amylase (<40 DU), DP (<110 °ASBC), malt extract (<79.0%), and soluble protein (<5.2%); and higher than maximum required values for β-glucan (>120 ppm) and wort viscosity (>1.5 cP). Some of the beneficial features of genotypes in CL1.2 were higher proportions of plump kernels (> 90%) and thousand-kernel weight (47.0 g); and acceptable levels for barley protein content (11.0%), FAN (160), and wort color (1.7 °ASBC).

There were 15 genotypes in the second cluster (CL2), with 11 being six-rowed NDSU lines (Figure 2.8). Genotypes in this cluster generally had acceptable malt quality, with higher means for α-amylase (55 DU), DP (165 °ASBC), malt extract (>79 %), FAN (235), and wort color (1.5 °ASBC); and acceptable values for barley protein content (11.5%) and wort viscosity (1.5 cP). Even if the soluble protein (4.7 %) and β-glucan (175 ppm) values were beyond the acceptable values, the genotypes in this cluster were found to have better values for these two traits compared to all the other groups. Two of the negative features in this cluster were low proportions of plump kernels (about 70%) and thousand-kernel weight (about 32.0 g). The low proportion of plump kernels and thousand-kernel weight could stem from poor adaptability of the NDSU genotypes to conditions at Bekoji, including susceptibility to leaf scald. Because many of the genotypes in this cluster had acceptable malt quality for multiple traits, they could be important sources of favorable alleles to improve malt quality, particularly for the Ethiopian breeding program.

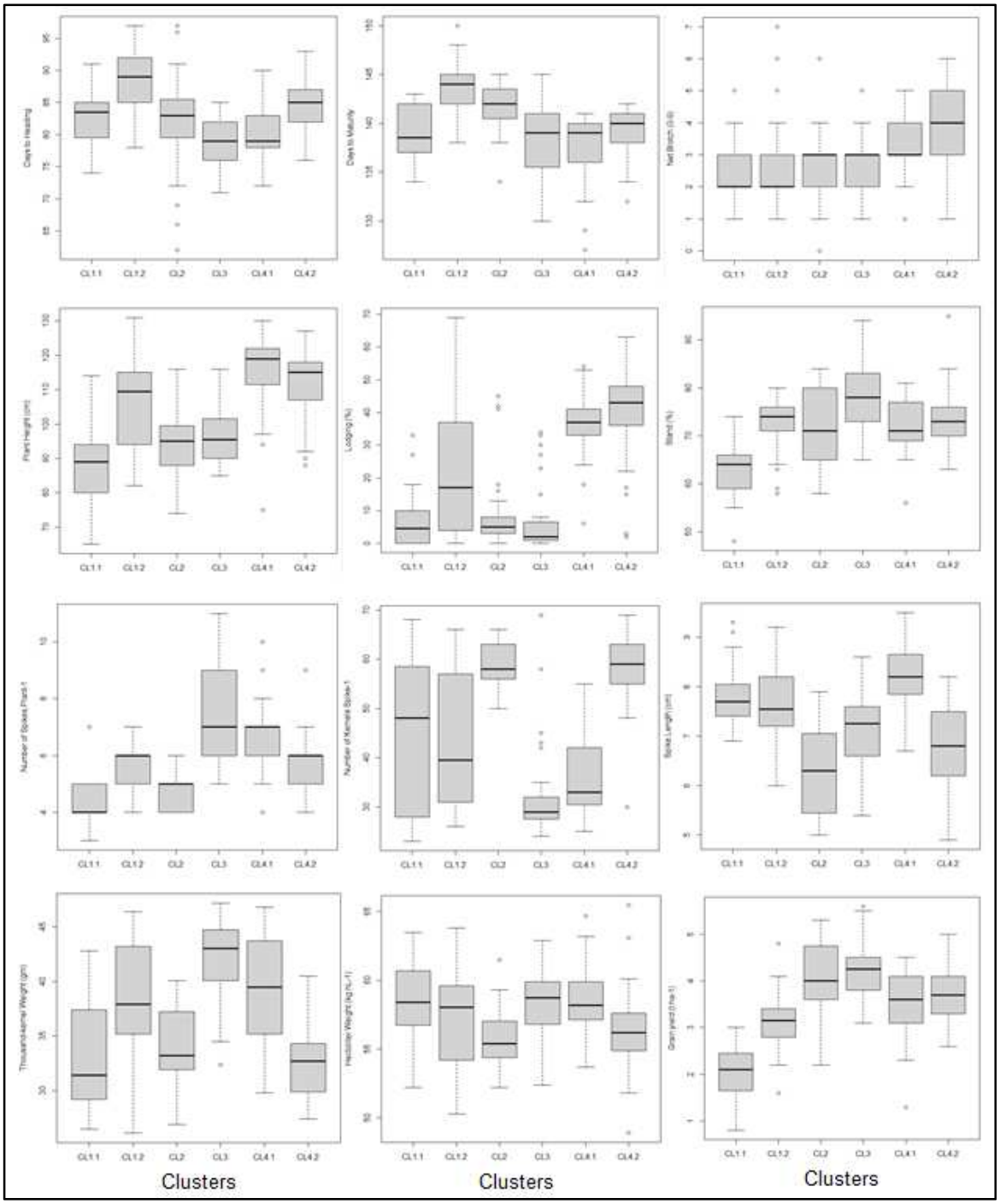

Figure 2.7. Mean and variability of the seven clusters generated based on the four significant principal components for 11 agronomic traits recorded on Koffele experiments for three years (2011- 2013).

The third cluster (CL3) had 39 genotypes and was mainly composed of 18 ICARDA lines (14 six-rowed and four two-rowed), nine NDSU lines (seven two-rowed and two six-rowed), and eight six-rowed landraces (Figure 2.8). Generally, this cluster had positive attributes for some malt and grain quality traits

such as low protein content (9.9%); acceptable values for α-amylase (45 DU) and wort color (1.8 °ASBC); and marginally acceptable levels for thousand-kernel weight (39 g), proportions of plump kernels (80%), and FAN (150) (Figure 2.9). On the other hand, the soluble protein (3.3%) was very low, which may be related to the low barley protein. Additionally, the wort viscosity (1.6 cP) and β-glucan (about 300 ppm) were higher than the maximum desired levels. The DP (104) and malt extract (78.8 %) were slightly lower than the minimum desired values (110 °ASBC and 79.0, respectively).

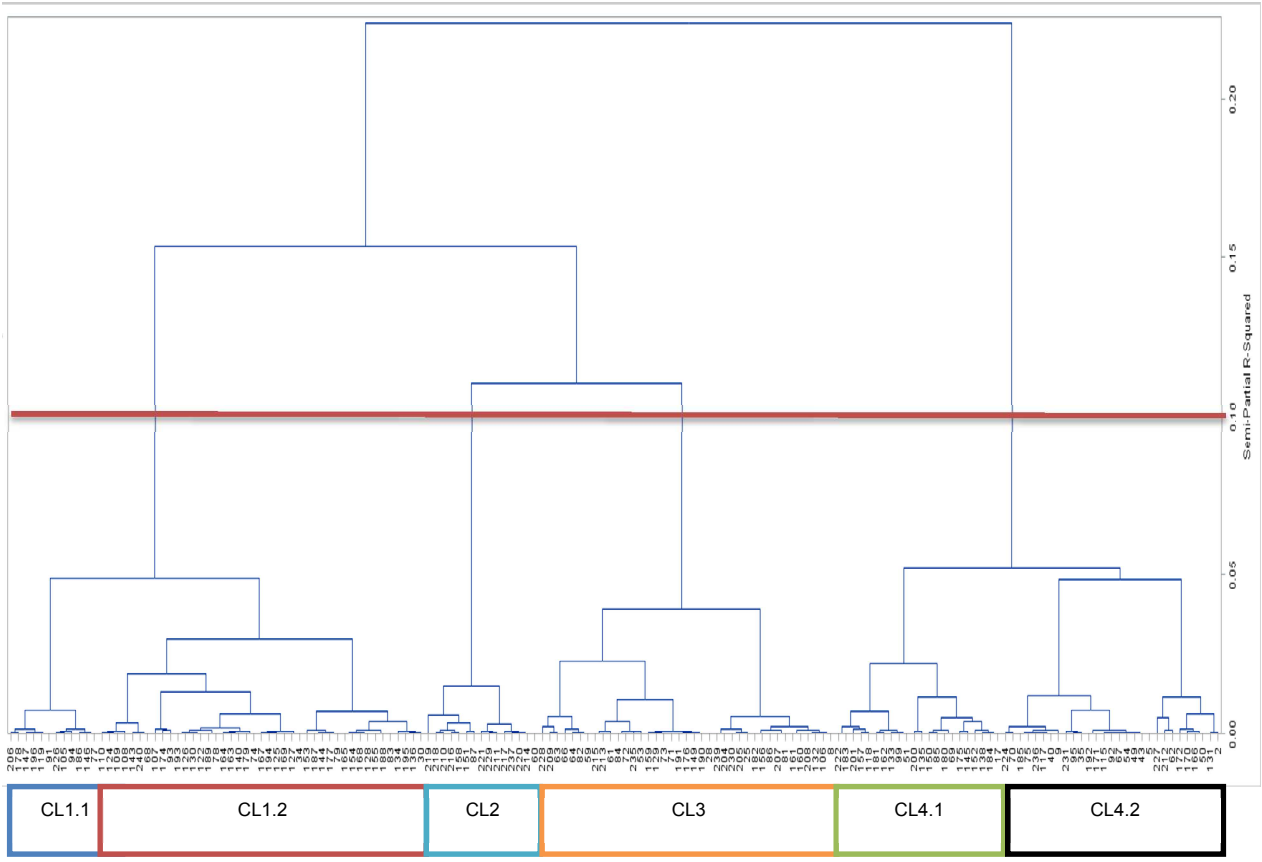

Figure 2.8. Cluster dendogram based on three significant principal components constructed from 11 grain and malt quality traits collected on samples from Bekoji experiments in 2012.

The fourth cluster had two sub-clusters; with sub-cluster CL4.1 containing 22 genotypes and sub-cluster CL4.2 containing 29 genotypes (Figure 2.8). Sub-cluster CL4.1 included 13 ICARDA lines (11 six-rowed and two two-rowed genotypes), five six-rowed landraces, and four two-rowed Ethiopian breeding lines. In general, genotypes in this sub-cluster had less than desirable proportions of plump kernels, α-amylase, DP, malt extract, and soluble protein; and higher than desired β-glucan content and wort viscosity (Figure 2.9). The thousand-kernel weight, protein content, FAN, and wort color were marginally acceptable for cluster CL4.1. Sub-cluster CL4.2 included genotypes from all groups. However, the major

contributing groups were six-rowed landraces (11 genotypes) and six-rowed ICARDA breeding lines (eight genotypes) (Figure 2.8). Generally, this sub-cluster was characterized by poor malt quality performance (Figure 2.9), including less than desired levels for thousand-kernel weight, proportions of plump kernels, α-amylase, DP, malt extract, FAN, and soluble protein; and higher than desired levels of β-glucan and wort viscosity. However, the grain protein content and wort color of the genotypes in this sub-cluster were within the desired range.

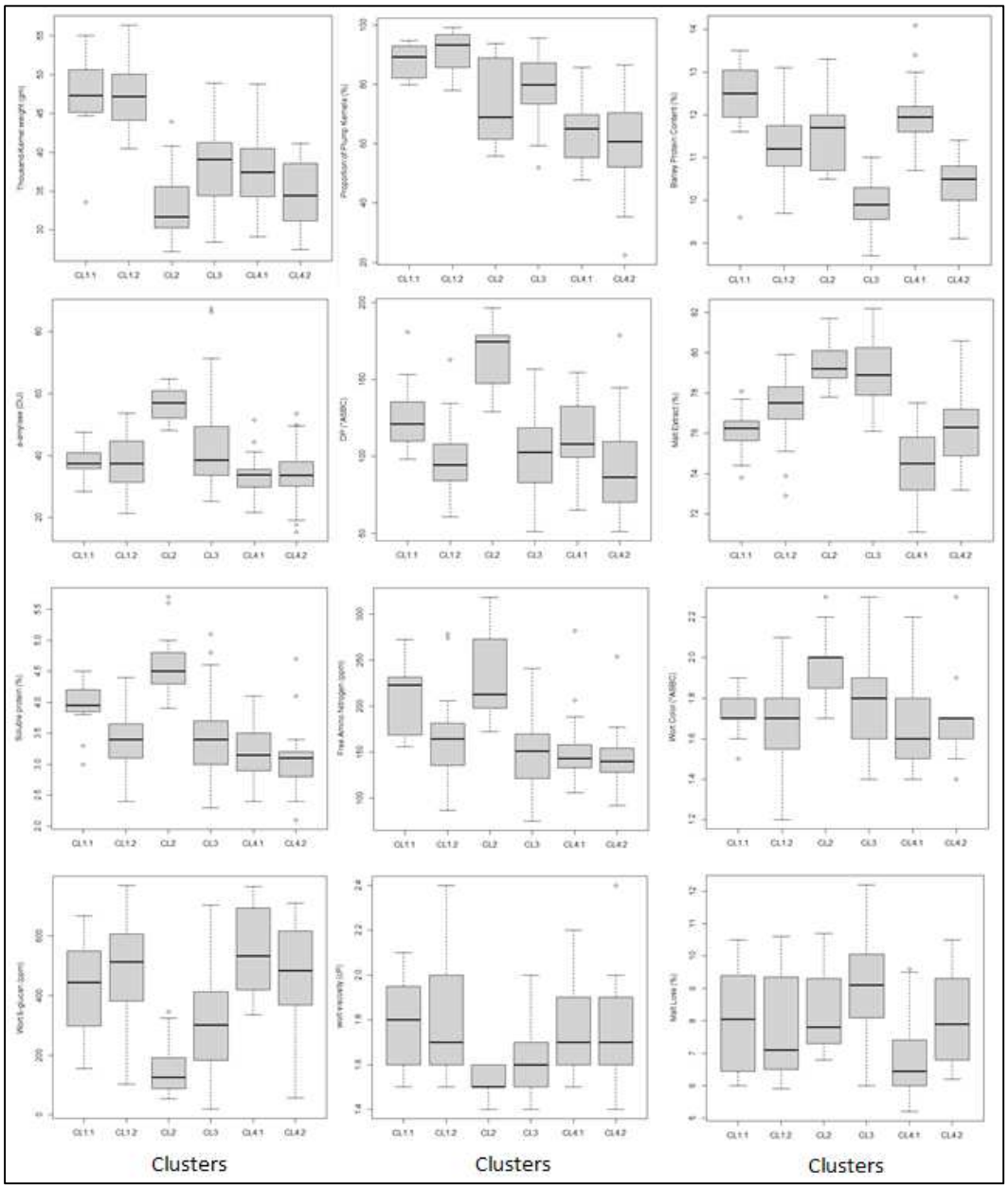

Figure 2.9. Mean and variability of the six-clusters generated based on the three significant principal components for 11 grain and malt quality traits on samples taken from Bekoji 2012 experiment.

Discussion

An important goal of genetic diversity and population structure studies is to determine the level of variability for economically important traits in the available germplasm and to identify potentially useful germplasm for a breeding program. Breeding programs are usually searching for new alleles to sustain continued improvement and to incorporating alleles lacking in their breeding germplasm. This study presented a wide-ranging characterization of diversity of agronomic performance and disease resistance as well as grain and malt quality traits in barley genotypes sampled from Ethiopian landraces, and breeding lines and cultivars from the Ethiopian, ICARDA, and NDSU breeding programs. Breeders sometimes consider maximum genetic distance among parents to be used in hybridization in order to capture the benefits of transgressive segregation.

Because of the greatly different growing conditions at each of the locations, I chose to perform ANOVA's combined across years for each location separately. Bekoji lies at 7°37'N, 39°18'E and at an elevation of 2780 MASL. (Berhane et al., 1996). The site has mean minimum and maximum temperatures of 7.5 °C and 15.4 °C, respectively, with average annual rainfall of 1024 mm. Koffele lies at 7°04'27''N, 38°46'45''E and at an elevation of 2660 MASL (Tamene et al., 2013). The mean minimum and maximum temperatures are 7.1°C and 18.0°C, respectively, with average annual rainfall of 1211 mm. Koffele generally has a longer growing season (about five months) compared to Bekoji (four to four-and-half months). Fargo has long day length (>14 h) between the months of May and August (http://weatherspark.com/averages/30234/Fargo-North-Dakota-United-States). However, the locations in Ethiopia have 12 h daylight year-round. Fargo generally experiences very high temperature during the growing season compared to the Ethiopian locations. The warm season at Fargo lasts from 17 May to 17 September, with an average daily high temperature above 20.6°C (http://weatherspark.com/averages/30234/Fargo-North-Dakota-United-States). These differences among the test locations likely led to large performance difference in the agronomic, disease resistance, and quality traits.

In the analysis of variance, I generally found significant year x genotype interactions for most of the traits studied. However, the interaction effects were always due to differences in magnitude of the means from different environments rather than differential responses of the genotypes in different

environments. The genotypic effects were also significant for all traits considered, indicating that variability existed among the genotypes for each of the traits studied. Even if large variability exists within and among groups for all traits, specific groups were found to harbor more favorable alleles for one or more of the traits.

Photoperiod (day length) is one of the most important factors determining days to flowering in barley (Lauriel, 1997; Karsai et al., 2008). However, days to flowering also are impacted by temperature, with the number of days decreasing significantly with increasing temperature (Karsai et al., 2008). As mentioned previously, Fargo has long days (>14 h) between the months of May and August (http://weatherspark.com/averages/30234/Fargo-North-Dakota-United-States); however, the locations in Ethiopia have 12 h daylight almost throughout the year. Fargo generally experiences warmer temperatures compared to the Ethiopian sites during the growing season. Days to flowering/heading in barley are controlled by three groups of genes, photoperiod-related, vernalization-related, and earliness *per se* genes (Samari et al., 2011). In the current study, NDSU lines had the fewest day to heading at Fargo, but more days to heading compared to the other barley groups at the Ethiopian locations. These observations suggest that photoperiod-related genes might be involved in controlling the heading behavior of NDSU lines. In fact, this proposition was further strengthened by the results of QTL mapping for days to heading, which is discussed in the next chapter.

Semi-dwarf cereals have long been considered as important component in intensive agriculture (Kuczyńska et a., 2013). Among the test locations, Koffele was where the genotypes became taller and experienced more lodging, which may be related to the fertile land and high rainfall received at Koffele during the growing season. At Koffele, the NDSU lines were significantly shorter and experienced almost no lodging compared to the landrace and Ethiopian breeding lines groups. This makes NDSU lines potentially useful germplasm for the Ethiopian breeding program to access alleles for short-stature and lodging resistance. In Fargo, the NDSU lines were undistinguishable from Ethiopian breeding lines and landraces in plant height, which could be related to the lack of adaptability of the Ethiopian breeding lines and landraces in Fargo. This, in turn, suggests that utilization of Ethiopian accessions (landraces and breeding lines) to introduce one or more important traits needs due consideration.

Generally, Bekoji was more conducive for leaf scald development. Comparisons of leaf scald scores at Bekoji revealed that NDSU lines were more susceptible while the landrace and Ethiopian breeding lines groups had genotypes that were moderately resistant. With exception of the six-rowed Ethiopian breeding lines, all the other groups were composed of resistant and susceptible genotypes for leaf scald. The resistance to leaf scald in most of the landrace and Ethiopian groups was likely enhanced due to the co-existence of the pathogen and barley genotypes over an extended period of time. Leaf scald is not considered a problem in most years in the upper Midwest USA, so breeding for resistance is not a priority in the NDSU barley-breeding program (Dr. Richard D. Horsley, personal communication, 2015).

Koffele was more favorable for net blotch development. The NDSU and ICARDA lines were more resistant compared to Ethiopian breeding lines and landrace groups at Koffele. Surprisingly, the seedling test for net form net blotch in the greenhouse indicated that the Ethiopian breeding lines and landraces were more resistant compared to ICARDA and NDSU groups when inoculated with an isolate from North Dakota. Overall, the six-rowed NDSU lines were entirely susceptible to net form net blotch, which could be due to that they share a common susceptible parent in their pedigree. In the case of spot form net blotch, all four of the groups had a mean severity rating within the susceptible category (>2.5). Only a few genotypes from the six-rowed landraces, and two-rowed ICARDA and NDSU breeding lines were relatively resistant (scores < 2.5). Hence, it is crucial to make further greenhouse evaluations of these moderately resistant genotypes to verity their resistance to spot form net blotch.

The NDSU and ICARDA breeding lines were generally associated with low mean DON accumulation compared to the Ethiopian breeding lines and landrace groups, particularly in 2012 at Langdon and Osnabrock. Overall, 43 genotypes had relatively low DON accumulation, with the majority being from NDSU breeding program. Because FHB is not among the major diseases in the Ethiopian highlands, the Ethiopian breeding materials have not been actively selected for resistance to FHB and low DON concentration in the grain (personal observation). Research in Ethiopia needs to be conducted to determine if FHB and DON are a problem in the Bekoji and Koffele growing regions. If the disease is a problem, I suggest further assessment of the NDSU lines with low DON accumulation to determine their appropriateness for use by the Ethiopian breeding program as parents.

Generally, grain yield performance of genotypes depends on their adaptation to specific environments. For instance, the genotypes from Ethiopia (both breeding lines and landraces) performed well in Ethiopian locations whereas the NDSU lines were superior when they were grown in Fargo. This is not surprising because several adaptation traits are selected either by breeders or nature at the respective regions. Interestingly, 23 two-rowed ICARDA lines performed acceptably well for most of the agronomic and disease resistance traits in both Ethiopian locations and Fargo. Thus, it seems that these ICARDA lines could potentially be utilized in both the Ethiopian and NDSU breeding programs to improve agronomic performance and disease resistance.

Depending on the end use of the grain, increased levels of barley protein content can be desirable or undesirable. Higher grain protein is required for food and feed barley (Cai et al., 2013), while low to moderate grain protein concentrations are required for malt barley cultivars (http://ambainc.org/media/AMBA_PDFs/Pubs/Production/Guidelines_June_2014.pdf; accessed 27 Jan 2015). Barley with high protein content negatively impacts the processing of the barley into malt, and malt into finished beer. Because barley protein content was by far lower at Bekoji compared to that at Koffele and Fargo, the samples from Bekoji were utilized for malt production. The two-rowed NDSU lines had lower mean protein content than the two-rowed Ethiopian and six-rowed NDSU breeding lines at Bekoji. Generally, the NDSU genotypes were superior for all malt quality traits recorded as compared to the other genotypes. In fact, the majority of the NDSU lines met the desired levels for most quality traits specified by the AMBA. These differences in malt quality traits are attributed to the fact that all landraces and most of the Ethiopian and ICARDA breeding lines are food types while the NDSU lines were specifically developed for the malting and brewing industries. The NDSU lines may be a source of favorable malt quality alleles for the Ethiopian breeding program. However, it is crucial to pay attention to some of the adaptation problems of NDSU lines to the Ethiopian highlands, like poor stand establishment and susceptibility to leaf scald.

Many of the agronomic and end-use quality traits evaluated in this study are correlated; thus, some multivariate approaches may provide additional help in explaining the relationships between the variables. One such multivariate method is PCA. In PCA, a new set of uncorrelated variables called PCs are derived with the hope that the large number of original interrelated variables can be reduced by using

the first few PCs that explain a large proportion of the variation. One criterion used to determine which PCs to retain is an Eigen value > 1.0.

The two multivariate approaches utilized in the current study, principal component and cluster analyses, revealed that the genotypes evaluated were clustered according to spike row-type, geographic origin, and breeding history. Being the current population is highly structured, it is crucial to apply appropriate statistical models when this population is used in association mapping in the subsequent chapters. In the current study, three to four PCs were sufficient to account for the large proportions (77.3-81.3%) of the variability in the original data. The most related traits were placed in the same principal component (PC) and hence the results from PCA provided clues as to the relationship among traits. Among all the clusters, cluster CL2.2 at Bekoji and cluster CL3 at Koffele had 23 of the same two-rowed ICARDA lines that showed acceptable performance for most agronomic and disease resistance traits. Breeders in Ethiopia should consider these genotypes in barley improvement, particularly for development of improved food barley cultivars. Additionally, the cluster analysis for grain and malt quality traits clearly indicated NDSU lines to have superior malt and grain quality. Thus, the NDSU breeding lines can be utilized in Ethiopian breeding programs to develop malt barley cultivars.

In conclusion, the current study revealed the pattern of population structure and genetic diversity of barley genotypes sampled from landraces as well as breeding lines and cultivars from Ethiopian, ICARDA, and NDSU breeding programs. The study also identified potential germplasm for improvement of agronomic and quality traits particularly for the Ethiopian barley-breeding program.

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CHAPTER III: GENOME-WIDE ASSOCIATION STUDY FOR AGRONOMIC AND DISEASE RESISTANCE TRAITS IN BARLEY ACCESSIOS FROM ETHIOPIA, ICARDA, AND NDSU

Abstract

Determining the position and effect of quantitative trait loci (QTL) is crucial in the application of marker-based breeding. This study was conducted to determine the position of QTL for different agronomic and disease resistance traits in barley (*Hordeum vulgare* L.) using association mapping. Three genome-wide association analyses were done for 14 agronomic and disease-resistant traits using 186 to 256 accessions, which includes Ethiopian landraces and breeding lines and cultivars from the Ethiopian, ICARDA, and NDSU breeding programs. Molecular marker-based diversity and population structure analyses revealed three major groups, each representing Ethiopian (landraces and breeding lines), ICARDA, and NDSU materials, with each group further clustered according to row-type. Similar to the results obtained using phenotypic data, the clustering pattern based on molecular marker data followed row-type, geographic origin, and breeding history. Linkage disequilibrium (LD) decayed over distances of 10 to 20 cM and a large number of unlinked or loosely linked markers were shown to have high LD estimates ($r^2 \geq 0.1$), which may imply that factors other than linkage contributed to LD. The genome-wide association mapping identified 94 QTL for the 14 traits using three different methods of analyses. Particularly, three regions in chromosome 2H (4.7-12.1, 125.0-134.3 and 163.2-169.7 cM), two regions in chromosome 6H (27.3-38.0 and 93.4-106.8 cM), and one each in chromosomes 1H (38.5-46.5 cM) and 7H (89.8-94.3 cM) were the locations where QTL were concentrated. These regions could be targets for fine-mapping or marker-based breeding efforts. Of the 94 QTL identified, >50% of them were coincidental with known genes or QTL reported in the literature for the respective trait. Overall, the association mapping identified QTL for days to heading in the region where six photoperiod related genes (*Ppd_H1*, *HvFT4*, HvGI, *HvFT2*, *HvCO2*, and *HvCO1*) and one vernalization-related gene (*VRN-H1*) are located. Additionally, a QTL for plant height was located in the same region as the semi-dwarf gene *sdw3*, and QTL for disease resistance was located in the same region as the resistance genes *Rrs1* and *Rrs15* for leaf scald, and *rpt.k* and *rpt.r* for net form net blotch.

Introduction

Barley is among the 15 plant species providing the majority of the human diet (Xu, 2010; however, the majority of barley production worldwide goes to livestock feed (about 75%) and malting (about 20%) (Edney, 1996; Newman and Newman, 2008). Because the amount of grain produced per unit area is important for both these uses, improving grain yield is the number one priority in cereal breeding programs (Welsh, 1981). Several traits impact grain yield, including adaptation traits such as days to heading and maturity, lodging resistance, and seedling establishment. The yield components of barley are number of tillers per plant or unit area, number of kernels in a spike, and grain weight.

Reduced time and cost required to develop cultivars is an important gauge for the efficiency of a breeding program. Hence, breeders are open to applying new techniques and technologies that can increase their programs' efficiencies. Two technologies receiving attention are marker-assisted section (MAS) and genomic selection (GS). Estimation of the positions and effects of QTL and associated markers is crucial for MAS. Mapping QTL for economically important traits is mainly done using bi-parental linkage analysis (linkage mapping) or association mapping (Abdurakhmonov and Abdukarimov, 2008; Cavanagh et al., 2008; Sreenivasulu et al., 2008).

Association mapping involves searching for genotype-phenotype associations in unrelated individuals to localize QTL with respect to mapped genetic markers (Mackay and Powell, 2006; Myles et al., 2009). The resolution of QTL mapping depends on LD decay over distance (Mackay and Powell, 2006; Myles et al, 2009). Thus, estimation of LD and assessing the structure of LD across the genome is important. Two common statistics to quantify LD are *D'* and r^2 (Flint-Garcia, 2003). The LD patterns along the genome are usually assessed as LD decay and color-code triangle plots (Flint-Garcia, 2003; Abdurakhmonov and Abdukarimov, 2008). Breseghello and Sorreles (2006) and Laido et al. (2014) used the 95% percentile of the square root transformed r^2 estimates from unlinked (inter-chromosomal) marker pairs as the critical value beyond which LD is caused by linkage. According to Laido et al. (2014) and referenced therein, the level of linkage between marker-pairs can be defined in four classes: (1) tightly linked (within 10 cM apart), (2) moderately linked (10-20 cM apart), (3) loosely linked (20-50 cM apart), and (4) unlinked or independent (≥ 50 cM apart).

Population structure and familial relatedness often results in higher levels of false positives in association mapping. Currently, there is wide interest in using association mapping due to improvements in genotyping technology and statistical methodologies that minimize the issues of false positives (Zhu et al., 2008). Several statistical approaches for association mapping have been developed, including different mixed linear models (MLM). The MLM are regarded as improved approaches that can simultaneously account for population structure and unequal relatedness among individuals (Zhang et al., 2010). According to Myles et al. (2009) and references therein, the K (kinship) and K+Q (kinship + structure) mixed linear models are useful for controlling against false positives. In the MLM, population structure is fitted as a fixed effect and kinship among individuals is fitted as a random effect. The selection of the best approach for a specific mapping population and trait may be important. Some of the techniques pertinent to comparing association mapping models include the observed vs. expected probability plot (Stich et al., 2008) and mean square difference (MSD) estimates of observed and expected *p*-values (Stich et al., 2008; Mamidi et al. 2011). Comparisons of the number of significant marker-trait associations (MTAs) in each model are also helpful.

Three important decision tools to detect “true” MTAs discussed by Kraakman et al. (2004) are the significance of MTAs, LD profiles over chromosomes, and MTAs coincidence with the previously reported QTL. Another important consideration in the identification of QTL is grouping significant markers in a genomic region in such a way that significant MTAs within a short distance (say 5-10 cM) are delineated as a single QTL (Laido et al., 2014). In fact, the LD decay plot can be utilized in setting the distance within which significant MTAs can be considered as a single QTL. To increase the level of confidence of identified QTL, assessing MTA genomic regions in multiple association mapping analyses across environments or conditions can be useful. Detection of specific MTAs in multiple analyses provides more evidence to declare that MTA as a meaningful or “true” QTL for the trait. Thus, it is crucial to collect phenotypic data from multiple environments and conduct association-mapping analyses for each environment.

In this chapter, data on agronomic and disease related traits recorded in field and greenhouse experiments are utilized. Determination of the genetic basis of these traits in different populations could have great contributions to the development of efficient breeding strategies in crop improvement

programs. Traits such as days to heading plays critical role in the adaptation of cultivars to different environments and production practices (Wang et al., 2010). In the current study, barley genotypes sampled from Ethiopian landraces and lines and cultivars from the Ethiopian, ICARDA, and NDSU breeding programs were utilized. The objectives of this chapter are: (1) to determine genetic diversity and structure of the mapping population composed of accessions from Ethiopia, ICARDA, and NDSU, (2) to determine the pattern of genome-wide LD in the whole population and different groups representing different spike row types and origin of barley, (3) to identify MTAs or QTL for 14 agronomic and disease resistance traits in the aforementioned population, and (4) to define specific genomic regions that can potentially be used in crop improvement programs for agronomic and disease resistance traits.

Materials and Methods

Description of the Mapping Population and Data Recorded

A total of 262 genotypes were utilized in this study. The genotypes included two-rowed and six-rowed genotypes from four sources of barley germplasm: Ethiopian landraces and lines and cultivars from the Ethiopian, ICARDA, and NDSU breeding programs.

Phenotype data for genome-wide association analyses were collected on 12 agronomic and disease resistance traits phenotyped in field experiments. The agronomic traits were days to heading and maturity, plant height, lodging percentage, number of spikes per plant, number of kernels per spike, spike length, thousand-kernel weight, hectoliter weight, and grain yield. Data were also collected on disease severity of natural infections of leaf scald (incited by *Rhynchosporium secalis* (Oudem.) Magnus) and net blotch (incited by *Pyrenophora teres* Dreschs). The descriptions of the traits collected are given in greater detail in Chapter II. Moreover, data for disease resistance of seedlings inoculated with *Pyrenophora teres* f. *teres* Drechsler (net form net blotch; NFNB) and *Pyrenophora teres* f. *maculata* (spot form net blotch; SFNB) were recorded in greenhouse experiments in 2014. The details of the procedures for inoculation, disease development, and infection rate (IR) scoring are presented in chapter II. The numbers of genotypes used for association mapping analyses varied for each trait (Table 3.1).

Table 3.1. The number of genotypes and compositions of the populations utilized for association mapping of different agronomic and disease resistance traits in each environment.

Trait†	N‡	Composition of the population
NB and SC	186	53 Landraces, 81 ICARDA lines, 29 Ethiopian lines, 21 NDSU lines, and 2 Kenyan cultivars
DH and PLH	209	65 Landraces, 81 ICARDA lines, 35 Ethiopian lines, 26 NDSU lines, and 2 Kenyan cultivars
TKW	235	85 Landraces, 82 ICARDA lines, 42 Ethiopian lines, 24 NDSU lines, and 2 Kenyan cultivars
DM, LODG, NSP, NKS, SL, and HLW	237	85 Landraces, 82 ICARDA lines, 42 Ethiopian lines, 26 NDSU lines, and 2 Kenyan cultivars
YLD	238	85 Landraces, 82 ICARDA lines, 42 Ethiopian lines, 27 NDSU lines, and 2 Kenyan cultivars
NFNB and SFNB	256	85 Landraces, 82 ICARDA lines, 42 Ethiopian lines, 45 NDSU lines, and 2 Kenyan cultivars

†NB = Net blotch, SC = Scald, DH = days to heading, PLH = Plant height, TKW = thousand-kernel weight, DM = days to maturity, LODG = lodging percentage, NSP =Number of spikes per plant, NKS = Number of kernels per spike, SL = Spike length, HLW = Hectoliter weight, YLD = Grain yield, NFNB = Net form net blotch recorded in the green house in 2014, and SFNB = Spot form net blotch recorded in the green house in 2014.
‡N indicates the number of genotypes in the mapping population for each trait.

Experimental Locations and Years

Field experiments were conducted at three locations in Ethiopia (Bekoji, Koffele, and Holetta) and one location in the USA (Fargo, ND). Data were collected in three growing seasons (2011, 2012, and 2013) at Bekoji and Koffele; two growing seasons (2012 and 2013) in Fargo; and one growing season (2012) in Holetta. Thus, phenotypic data from up to nine environments (location-year combinations) are available for association mapping.

Genotyping and Marker Selection

A total of 262 barley accessions were genotyped using the genotyping-by-sequencing (GBS) procedure using the Ion Torrent ® PGM system (Life technologies, Grand Island, NY) in Dr. Robert Brueggeman's laboratory in the Department of Plant Pathology, NDSU. Marker information such as polymorphic information content (PIC), minor allele frequency (MAF), heterozygosity, and allele diversity was estimated using the allele procedure of SAS 9.4 (SAS Inc., Cary, NC). The markers were selected based on marker quality score > 1000, MAF > 5%, missing data < 10%, and heterozygosity < 50%. The SNP markers meeting these criteria were used in association mapping, LD, and genetic diversity

analyses. The estimated chromosomal positions of the selected SNP markers were attributed to map positions based on Munoz et al. (2011) and the Gbrowse function on the *Hordeum Toolbox* (http://hordeumtoolbox.org/cgi-bin/gbrowse/tht/). The selected markers were localized to the seven chromosomes of barley using JMP Genomics (SAS Inc., Cary, NC). The missing genotypic and phenotypic data were imputed using the mean of five of the nearest neighbors identified with Euclidean distance (Cover and Hart, 1967) in Tassel 5.2 (Cornel University, Ithaca, NY).

Marker-based Genetic Diversity Analysis

The identical-by-descent (IBD) relationship matrix among the 256 genotypes was generated using JMP Genomics (SAS Institute Inc., Cary, NC). The matrix was used to produce heat maps and cluster dendograms. The cluster memberships based on SNP markers were assessed and compared with the clustering based on phenotypic data presented in Chapter II.

Linkage Disequilibrium (LD) and LD Decay

The patterns of LD using r^2 values between any pair of SNP markers were determined using JMP Genomics (SAS Institute Inc., Cary, NC). The LD decay was assessed for the whole population and sub-groups based on row-type and origin of barley (i.e.; geographic origins and breeding programs). The extent of LD in some specific regions of the genome, such as genomic regions with significant MTAs, was assessed if required. LD decay with genetic distances in cM between pairs of loci was assessed using the LD plot generated using nonlinear regression in JMP Genomics (SAS Institute Inc., Cary, NC). The 95% percentile of the square root transformed r^2 estimates from unlinked marker pairs was used as the critical value beyond which LD is caused by linkage (Breseghello and Sorreles, 2006; Laido et al., 2014).

Association Mapping Procedures

Four models were applied for genome-wide MTA analysis, namely naive, P, K, and P+K using JMP Genomics (SAS Institute Inc., Cary, NC). The descriptions for the models used in this study are presented in Table 3.2. For all 14 traits, association analyses were done utilizing all of the genotypes. Additionally, two more analyses were done for days to heading, plant height, lodging, leaf scald, net blotch, grain yield, net form net blotch, and spot form net blotch. These two analyses were based on sub-samples from the original population, the first including only Ethiopian accessions (landraces and breeding lines) and the second including only ICARDA and NDSU breeding lines.

Table 3.2. The descriptions of the association mapping models' components used in this study for agronomic and disease resistance traits.

Name	Model†	Description
Naïve	$y = x\alpha + \varepsilon$	Model with no control for population structure and familial relatedness.
P	$y = x\alpha + p\beta + \varepsilon$	Model with control only for population structure using the first four dimensions of multidimensional scaling generated in JMP Genomics.
K	$y = x\alpha + kv + \varepsilon$	Model with control only for familial relatedness using IBD relationship matrix generated in JMP Genomics.
P+K	$y = x\alpha + p\beta + kv + \varepsilon$	Model with control for both population structure using the first four dimensions of multidimensional scaling and familial relatedness using IBD relationship matrix generated in JMP Genomics.

†*y* is the response vector for phenotypic values of each trait, *α* is the vector of fixed effects related to SNP marker effects, *β* is the vector of fixed effects related to population structure, *v* is the vector of random effects related to familial relatedness, and *ε* is a vector of the residual effects. *x* denotes the genotypes at the marker, *p* denotes the four dimensions from the multidimensional scaling generated in JMP Genomics, and *k* is the relationship matrix generated in JMP genomics.

For presentation of the results, one of the four models was selected based on the following criteria. The models were compared using the plot of observed vs. expected *p*-values to identify the "best" model among the four as described in Stich et al. (2008). Additionally, the mean square difference (MSD) of observed vs. expected *p*-values were calculated for each model using the formula described in Mamidi et al. (2011). The model with the smallest MSD values (i.e.; approaching zero) was considered as the "best" model. The numbers of significant MTAs were counted for each trait in all the environments for the four models to aid in comparison of the models.

Selection of meaningful MTAs for discussion was based on two criteria. For traits with phenotypic data from multiple locations, MTAs needed to be significant at $p \leq 0.05$ at a single location, and significant at a minimum of 50% of the experiments. For disease traits phenotyped in the greenhouse, MTAs were considered significant at $p \leq 0.01$. Significant MTAs within 10 cM distance for each trait were considered a single QTL and reported in a range of distance associated with the markers shown to be significant. Locations of meaningful QTL were compared with the locations of known genes or previously reported QTL for the trait. I utilized the genetic maps of Wenzel et al. (2006) for DArT markers, Varshney et al. (2007) for SSR markers, and Munoz et al. (2011) for SNP markers to make comparisons of my results with those reported in the literature. I also used Szucs et al. (2009) in some of the cases.

Results and Discussions

Distribution of Markers on Chromosomes

A total of 357 SNP markers identified DNA polymorphisms using GBS in the 262 genotypes. Of which, 226 were selected based on a marker quality score > 1000, MAF > 5%, missing data < 10%, and heterozygosity < 50%. The distribution of the 226 markers in the seven chromosomes, with map positions attributed to genetic map of Munoz et al. (2011), is shown in Figure 3.1. The 226 markers covered 1058 cM on the seven barley chromosomes (Table A.16) and the average distance between adjacent markers was 4.8 cM (with a minimum of 4.2 cM for chromosomes 5H and 6H and a maximum of 5.7 cM for chromosomes 1H and 7H). Across the genome there were also 26 regions with gaps between adjacent markers of >10 cM (Table A.16), which could limit the ability to detect MTAs in those regions. Chromosome 5H had the greatest number of markers (43) and chromosome 1H had the fewest (23) (Table A.17).

Figure 3.1. Distribution of the SNP markers used in the association analysis and marker based genetic diversity study.

Marker-based Genetic Diversity and Population Structure Analysis

The heat map and cluster dendogram based on the relationship matrix for the 256 genotypes and 226 SNP markers are presented in Figure 3.2. Generally, the 256 genotypes were grouped into three major clusters, which further subdivided into 10 sub-clusters of genotypes. The first major cluster had three sub-clusters (CL1.1, CL1.2, and CL1.3), which were mainly composed of landraces and Ethiopian breeding lines (91 accessions, 96.8%). Sub-cluster CL1.1 was the largest, having 60 genotypes and 76.7% of them being six-rowed landraces. Sub-cluster CL1.2 had 20 genotypes, with the majority being six-rowed landraces (nine accessions) and two-rowed Ethiopian breeding lines (six accessions). Sub-cluster CL1.3 had 14 genotypes, all of them being two-rowed Ethiopian breeding lines. The second major cluster was comprised of five sub-clusters, CL2.1 to CL2.5. The majority of the genotypes in these sub-clusters came from the ICARDA breeding program (65.8 %). Sub-cluster CL2.1 included 11 genotypes, with seven of them being six-rowed landraces. Forty-two genotypes belonged to sub-cluster CL2.2, with 59.5% of them being six-rowed and two-rowed ICARDA genotypes. There were 17 genotypes in sub-cluster CL2.3, with 14 of them being six-rowed ICARDA lines. Seventeen of the 19 genotypes in sub-cluster CL2.4 were six-rowed landraces, Ethiopian breeding lines, and ICARDA genotypes. Sub-cluster CL2.5 had 31 genotypes, with 26 of them being two-rowed ICARDA genotypes. The third major cluster was composed of two sub-clusters (CL3.1 and CL3.2), which included a high proportion of the NDSU genotypes (about 83.3%). Eighteen of the 20 accessions in sub-cluster CL3.1, and 17 of the 22 accessions in sub-cluster CL3.2 were NDSU genotypes. Sub-cluster CL3.1 was mainly two-rowed genotypes and sub-cluster CL3.2 was predominantly six-rowed.

In the heat map presented in Figure 3.2, the red diagonal line indicates the perfect relationship of a genotype with itself. The blocks in different colors along the diagonal indicate the relationship among the genotypes clustered together. For instance, the genotypes in CL2.1, CL3.1, and CL3.2 were shown to be more related compared to genotypes in the other clusters. The heat map also indicated that the NDSU genotypes were unrelated to the landraces and Ethiopian genotypes (Figure 3.2). This is expected because the NDSU program has never used germplasm from Ethiopia. There was some level of similarity between CL3.1 and CL3.2, which are both from NDSU breeding program. Overall, the current mapping

panel was highly structured on spike row-type, geographic origin, and breeding history; which indicates the need for applying appropriate association mapping models to control against false positives. The clustering based on SNP markers was also in line with the clustering patterns based on agronomic and quality traits reported in Chapter II. Spike morphology (two-rowed vs. six-rowed), geographic origin, domestication or breeding history, growth habits and vernalization requirements (winter vs. spring) were the major line of sub-division for barley in previous studies (Varshney et al, 2012; Pauli et al. 2014; Matthies et al., 2014).

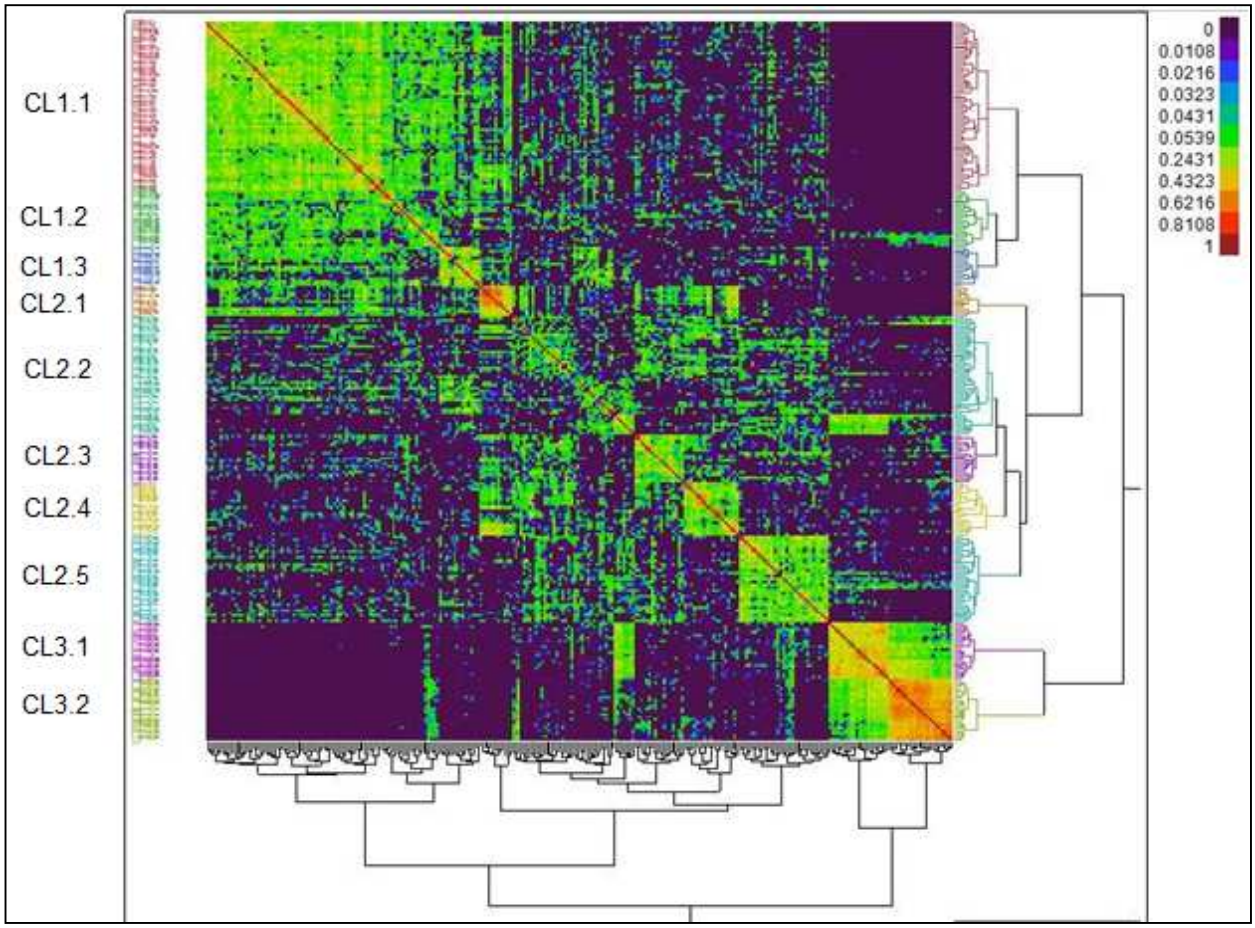

Figure 3.2. Relationship among 256 genotypes and dendogram generated using identical-by-descent (IBD) coefficient estimated based on 226 SNP markers.

Linkage Disequilibrium (LD) and LD Decays

About 12.3% of the LD estimates (r^2) in the whole population were ≥ 0.1 (Table 3.3). Of these, only 32.6% were between marker pairs within 20 cM distance and the majority (67.4%) were between markers separated by >20 cM. According to Laido et al. (2014) and references therein, markers within 20 cM distance are considered tightly or moderately linked while those with >20cM apart are considered

loosely linked or independent. Generally, the results from the current study indicated that a large proportion of the marker-pairs with LD ≥ 0.1 were for those marker-pairs >20 cM apart, implying that factors other than linkage were also contributing to the LD. According to Abdurakhmonov and Abdukarimov (2008), long stretches of LD between unlinked loci could exist as a result of factors such as selection, population structure, and relatedness. They also indicated that long stretches of LD could lead to high numbers of significant MTAs. Wang et al. (2012) reported large proportions of significant LD (88.6%) for inter-chromosomal markers (i.e., marker pairs located on different chromosomes) and they attributed this to the existence of high population structure in their mapping population. However, when they did separate LD estimates for winter- and spring-type barley genotypes, they found a highly reduced proportion of inter chromosomal LD (10.8% and 18.9% for spring- and winter-types, respectively). In general, the proportion of LD ≥ 0.1 for marker-pairs > 20 cM was not reduced in separate LD analyses for different row-type and origin groups (Table 3.3). The only exceptions were for the Ethiopian and ICARDA genotypes. Overall, the results for LD in the current study indicated factors other than linkage contributed to LD. This in turn suggests the need for handling the population stratification and relatedness properly to reduce the likelihood of false positives in association analyses.

Table 3.3. The number of marker-pair LD estimates in two categories of r^2 (≥ 0.1 vs. < 0.1) and in two categories of distance between pair of markers (≤ 20 cM vs. > 20 cM).

LD (r^2)	Distance (cM)	Two-rowed	Six-rowed	Ethiopian	ICARDA	Landrace	NDSU	Whole
≥ 0.1	Sub-total	642	1460	394	218	698	928	902
	≤ 20	256	382	206	144	210	332	294
	> 20	386	1078	188	74	488	596	608
< 0.1	Sub-total	6704	5886	6332	7128	6448	5828	6444
	≤ 20	1470	1344	1398	1582	1474	1288	1432
	> 20	5234	4542	4934	5546	4974	4540	5012
Total		7346	7346	6726	7346	7146	6756	7346

The LD decay plots (Figure 3.3) indicated that LD decayed within 10 cM across all genotypes as well as the six-rowed and landrace groups. However, LD extended up to 15 cM in two-rowed germplasm, and 15-18 cM in the Ethiopian and NDSU breeding lines, and 18-20 cM in the ICARDA breeding lines. Abdurakhmonov and Abdukarimov (2008) stated that the extent of LD in barley varies between 10 and 50 cM. The difference in LD decay in the whole germplasm and separate analyses for the different groups in the current study can imply the existence of population structure and unequal relatedness in the entire

association panel. Hence, proper control for population structure and familial relatedness is needed in the association analyses.

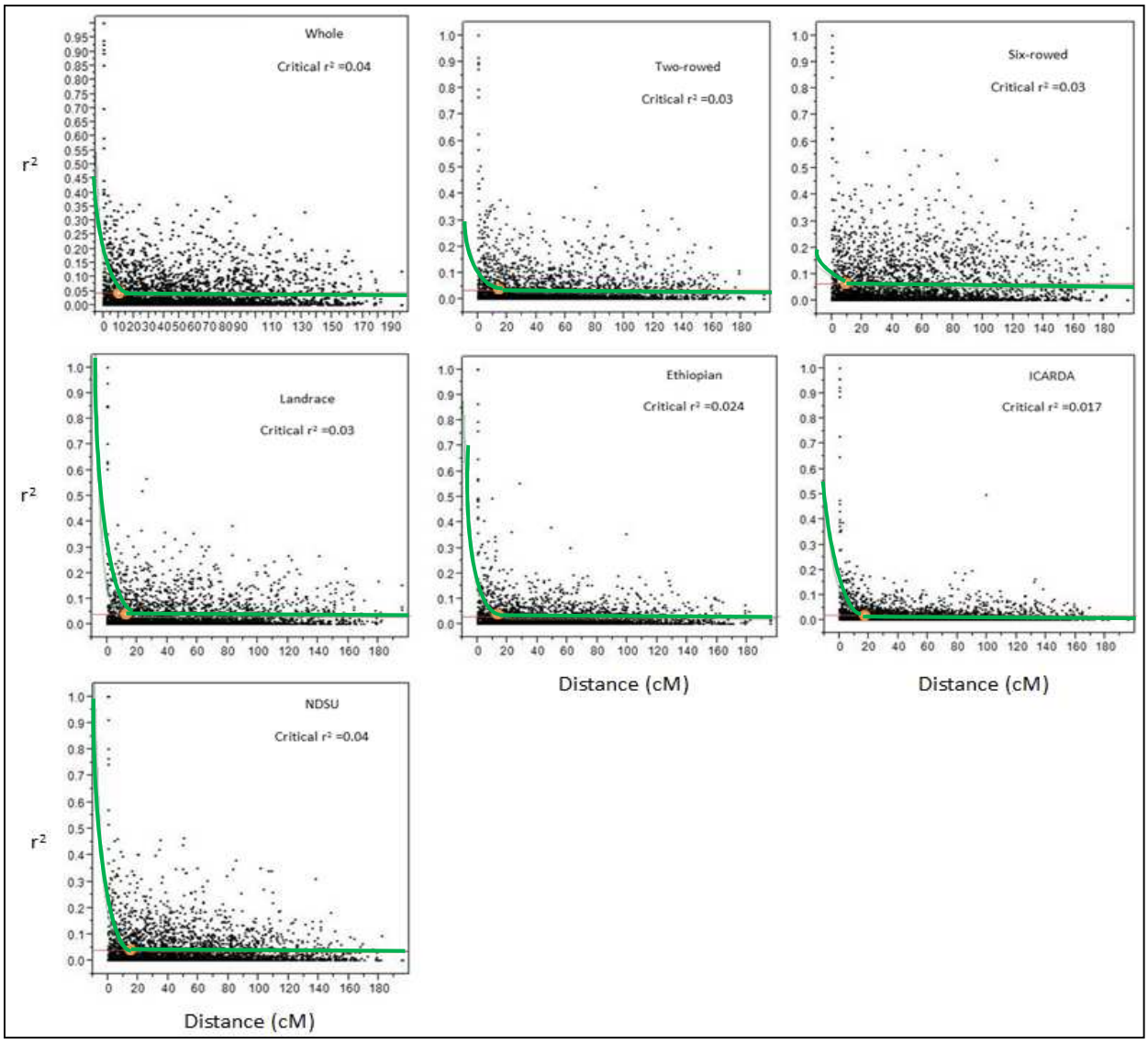

Figure 3.3. Linkage disequilibrium (LD) decay for the whole germplasm and six different groups of germplasm (two based on row type and four based on source of the germplasm).

QTLs for Agronomic and Disease Resistant Traits

Three analyses based on different groups of genotypes were done for each trait. The first analysis used all genotypes of the mapping population, the second analysis used only the Ethiopian landraces and Ethiopia breeding lines, and the third analysis used only genotypes from the ICARDA and NDSU breeding programs. The purpose of the analyses using subsets of genotypes was to determine if

there were QTL that were unique to only one of the subgroups. It is these unique QTL that can be utilized for crop improvement by the breeding programs without them.

Across the three methods of analyses, a total of 94 MTAs or QTL were detected for 12 traits phenotyped in field experiments and two disease resistance traits phenotyped in the greenhouse (Table 3.4 to 3.13). The number of QTL varied among chromosomes; with the highest number in chromosome 2H (24) followed by chromosome 5H (19). The fewest number of QTL were observed in chromosome 4H (6). The 94 QTL were positioned in 41 genomic regions. Of these, 26 regions had significant QTL for more than one trait (Table A.18) and collectively accounted for 84% of the QTL. Overall, three regions in chromosome 2H (4.7-12.1, 125.0-134.3, and 163.2-169.7 cM), two regions in chromosome 6H (27.3-38.0 and 93.4-106.8 cM), and one each in chromosome 1H (38.5-46.5 cM) and 7H (89.8-94.3 cM) were the locations where QTL were concentrated. The details of the association mapping results for each trait are discussed in the next three sub-sections.

Days to heading and maturity, plant height, and lodging

A model is considered best if the line of observed *p*-values is close or overlaps the diagonal line of the expected *p*-values in the observed vs. expected *p* plots. The results of these plots (Figure 3.4 a-d) indicated that the K and P+K mixed models were the best models for days to heading and maturity, plant height, and lodging. The MSD estimates for days to heading in the naïve, P, K, and P+K models were 0.075, 0.006, 0.003, and 0.002, respectively; implying that P+K model was the best for days to heading compared particularly to naïve and P (Table A.18). Similar results for the MSD estimates were obtained for days to maturity, plant height, and lodging (Table A.18). As expected, the number of significant MTAs detected for the four traits decreased as the model for the association mapping analyses became more restrictive (Figure 3.5), suggesting that control for both population structure and familial relatedness or familial relatedness alone reduced the likelihood of false positives for the four traits. Therefore, discussions of the MTAs for days to heading and maturity, plant height, and lodging are based on the results of the analyses using the P+K model.

Across all the three methods of analyses, 12 genomic regions had QTL for days to heading on chromosomes 2H, 3H, 5H, 6H, and 7H (Table 3.4). The greatest number of QTL was detected in chromosome 2H (5 QTL among the three analyses). Four of these QTL (4.7, 20.5, 130.4-134.3, and

163.2 cM) were commonly identified in two of the three analyses. Additionally, one of the two QTL for days to heading in chromosome 3H (167.3 cM) was also detected in two analyses.

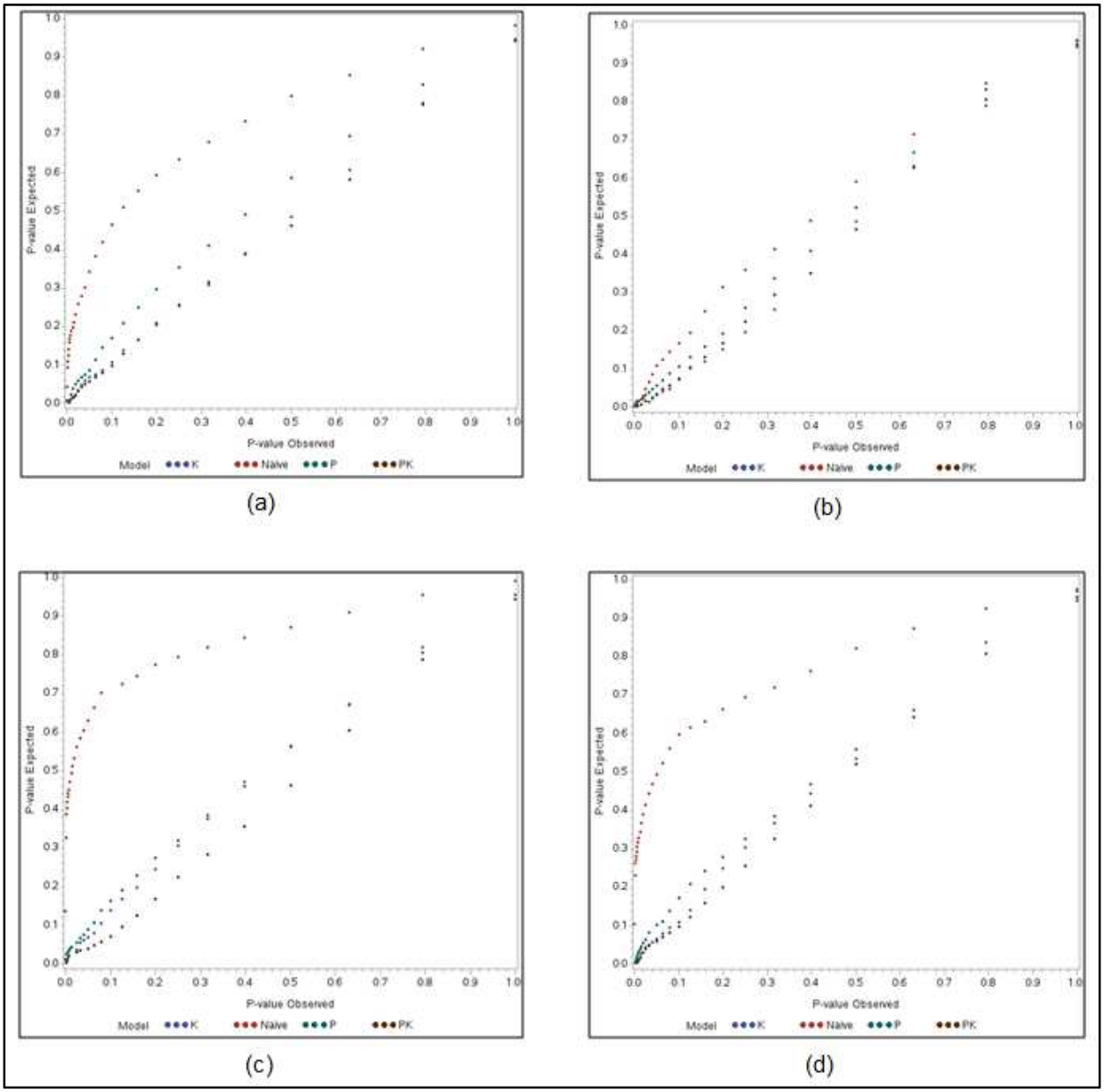

Figure 3.4. Plots of observed vs. expected p-values of four association mapping models for: (a) days to heading (DH), (b) days to maturity (DM), (c) plant height (PLH), and (d) lodging (LOD).

Overall, the genetics of days to heading has been studied extensively (Boyd et al., 2003; Wang et al., 2010; Samari et al., 2011; Compoli et al., 2012; Pauli et al., 2014). The genes controlling flowering time in barley are divided into three categories: photoperiod-related genes, vernalization-related genes, and earliness *per se* genes (Samari et al., 2011). The variations in response to day length (photoperiod)

and low temperature (vernalization) are important factors determining adaptation of genotype to different environments and farming practices (Wang et al., 2010).

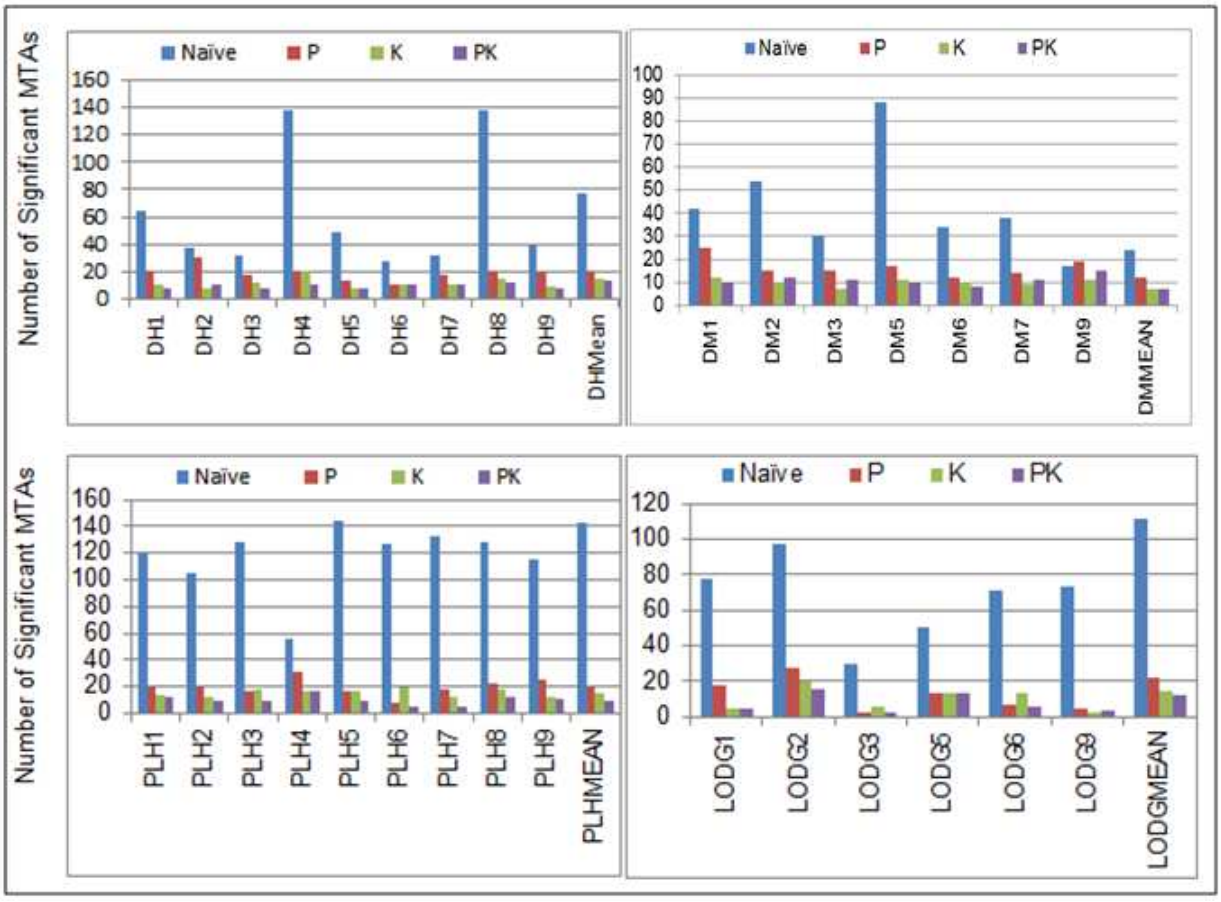

Figure 3.5. The number of significant marker-trait associations (MTAs) for days to heading and maturity, plant height, and lodging. The numbers associated with the trait abbreviations in X-axis indicate the environments: Env1 = Bekoji in 2011, Env2 = Koffele in 2011, Env3 = Bekoji in 2012, Env4 = Fargo in 2012, Env5 = Holetta in 2012, Env6 = Koffele in 2012, Env7 = Bekoji in 2013, Env8 = Fargo in 2013 and Env9 = Koffele in 2013.

Most of the significant QTL for days to heading in the current study are in regions where known genes or previously identified QTL were found. Significant QTL were detected in chromosome 2H at the positions of 4.7, 20.5, 54.9, 130.4-134.3, and 163.2 cM (Table 3.4). Of these, the QTL at 4.7 cM was in a similar region as one reported by Mansour et al. (2014). Furthermore, the QTL at 20.5 cM was located in a similar region as the *HvFT4* gene and a QTL reported previously for days to heading (Pasam et al., 2012; Boyd et al., 2003). According to Faure et al. (2007), the flowering time (*FT*) genes are involved in the transition of plants from the vegetative stage to floral development (double ridge stage), which occurs during the second week under long days and during the fourth week under short days. An expression

study by Faure et al. (2007) indicated higher expression of *HvFT4* after three to four weeks under short days, which suggests that *HvFT4* gene could be involved in the promotion of flowering under short days. The QTL at 54.9 cM was in a similar region as a QTL identified for days to heading reported previously (Wang et al, 2010; Pasam et al., 2012), which could be the *Ppd-H1* (*Eam1*) gene (Wang et al., 2010). The *Ppd-H1* (*Eam1*) confers a strong photoperiod response in some genotypes; under long-day conditions the dominant form reduces the number of days to heading by 7-8 d (Franckowiak, and Lundquist, 2012).

Of the two QTL in chromosome 3H, the one detected at 74.0 cM in the analysis using the ICARDA and NDSU accessions was within 10 cM of two photoperiod-related genes (*HvGI* and *HvFT2*) (Wang et al. 2010). The second significant QTL in chromosome 3H (167.3 cM) was in a region where Negeri (2009) and Wang et al. (2010) also reported a QTL for days to heading. Two QTL were identified in chromosome 5H for days to heading (Table 3.4). The QTL at 138.2 cM identified in the analysis using the landrace and Ethiopian breeding lines was in a similar region as the vernalization related gene *VRN-H1* (Wang et al., 2010). Vernalization upregulates *VRN-H1*, which in turn upregulates expression of *VRN-H3* and down regulates expression of *VRN-H2* (dominant flowering repressor) (Wang et al., 2010).

Two QTL were detected in chromosome 6H. The QTL identified in the analysis using the landrace and Ethiopian breeding lines at 38.0 cM (Table 3.4) was located in a similar region as a QTL reported by Pasam et al. (2012). The second QTL (85.9 cM), which was detected in the analysis using ICARDA and NDSU genotypes, was in a similar region as the photoperiod related gene *HvCO2* reported by Wang et al. (2010). The QTL detected for days to heading in chromosome 7H (89.8-94.3 cM) in the analysis using all genotypes was in a similar region as the photoperiod-related gene *HvCO1* (Wang et al., 2010), and where Pasam et al. (2012) and Negeri (2009) also reported a QTL for days to heading. In summary, the results from the present study found QTL for days to heading in regions where six photoperiod-related genes (*Ppd-H1*, *HvFT4*, HvGI, *HvFT2*, *HvCO2*, and *HvCO1*) and one vernalization-related gene (*VRN-H1*) had been previously mapped.

Days to maturity is not a widely studied trait in barley, the focus has been on days to flowering or heading. In the current study, the only association mapping analysis done used all genotypes. Significant QTL were detected in chromosomes 2H, 3H, 4H, and 6H (Table 3.4). Each of these regions was coincidental with regions having QTL for days to heading (Table A.19). It is unclear from the present

study to determine if the coincidental location is due to pleiotropic effects or tight linkage of the genes. Additionally, comparison of the locations of previously identified QTL with those found in the present study is difficult because of the differences in the collinearity of the different maps.

A total of 11 genomic regions were identified with QTL for plant height in chromosomes 1H, 2H, 3H, 5H, 6H, and 7H across the three ways of analyses (Table 3.5). Four of these QTL, particularly two in chromosome 2H (34.2-44.0 cM and 51.0 cM) and one each in chromosomes 3H (167.3 cM) and 7H (89.8-94.3 cM), were detected in at least two of the three methods of analyses.

Four QTL were detected in chromosome 2H for plant height (Table 3.5). The significant QTL at 34.2-44.0, 51.0-54.9, and 123.0-134.3 cM corresponded to QTL identified in previous studies (Yu et al., 2010; Pasam et al., 2012; Mansour et al., 2014). Giang et al. (2010) mapped the *sdw3* gene between two markers (MWG2287 and cMWG658) in chromosome 2H, which were located at 64.9 cM and 66.9 cM, respectively (Suzcs et al., 2009). That would place the QTL in chromosome 2H at 51.0-54.9 cM in the present study within 10-15 cM of the *sdw3* gene. The short culm 1 (*hcm1*) gene is commonly found in USA cultivars (Kuczyńska et al., 2013). However, no MTA were detected for plant height around this locus, which is located in chromosome 2H at 84.2 cM (http://avena.pw.usda.gov/cgi-bin/graingenes/report.cgi?class=locus;name=hcm1).

Kuczyńska et al. (2013) described 17 semi-dwarf genes in barley, including the *denso* and *sdw1* genes, which are responsible for reducing plant height in the majority of modern barley cultivars. According to GrainGenes database (http://wheat.pw.usda.gov/), these two genes are located in chromosome 3H at 35.6 cM and 122.0 cM, respectively; which would place the QTL detected in the present study in chromosomes 3H (163.2-169.7 cM) more than 30 cM from both genes. Thus, it is likely that none of the genotypes utilized in this study have the height reducing form of the allele of these two genes. However, other studies detected QTL for plant height (Pasam et al., 2012; Locatelli et al., 2013) in a similar region as the QTL I detected in chromosome 3H (163.2-169.7 cM).

Table 3.4. Significant markers for days to heading (DH) and maturity (DM) according to the P+K model using all genotypes or subgroups of genotypes.

Trait	Group†	Chromosome	Position‡	SNP	NLP§	%R^2	Significant¶
DH	Whole	2H	4.7	11_10326_121	1.4-1.9	2.1-3.1	5 out of 10
	ICARDA/NDSU	2H	4.7	11_10326_121	1.4-2.7	4.0-8.7	7 out of 10
DH	Whole	2H	20.5	12_10777_62 - 12_10777_61	1.4-2.7	2.1-4.6	9 out of 10
	ICARDA/NDSU	2H	20.5	12_10777_62	1.3-2.4	3.8-7.6	8 out of 10
DH	Whole	2H	54.9	11_21005_121	1.4-2.1	2.0-3.4	6 out of 10
DH	ICARDA/NDSU	2H	130.4	11_10429_121 - 11_20141_89	1.5-2.5	4.2-8.3	9 out of 10
	Ethiopian	2H	134.3	12_10739_61	1.31-1.9	4.0-6.5	5 out of 10
DH	Whole	2H	163.2	11_20943_123	1.4-1.6	1.9-2.5	7 out of 10
	ICARDA/NDSU	2H	163.2	11_20943_123- 11_10085_121	1.4-2.3	4.0-7.5	5 out of 10
DH	ICARDA/NDSU	3H	74.0	11_10373_121	1.5-2.1	4.5-6.6	6 out of 10
DH	Whole	3H	167.3	12_20198_69	1.4-3.6	2.0-6.5	9 out of 10
	ICARDA/NDSU	3H	167.3	12_20198_69	1.4-3.3	3.9-11.1	9 out of 10
DH	Ethiopian	5H	138.2	11_20100_121	1.7-2.6	5.7-9.2	5 out of 10
DH	ICARDA/NDSU	5H	157.6	12_30162_61	1.33-2.0	3.8-6.2	6 out of 10
DH	Ethiopian	6H	38.0	11_10427_121	1.4-2.8	4.5-10.1	8 out of 10
DH	ICARDA/NDSU	6H	85.9	11_20745_79	1.5-3.8	4.5-12.9	6 out of 10
DH	Whole	7H	89.8-94.3	11_20083_121 - 12_30026_61	1.31-3.0	1.9-5.2	5 out of 10
DM	Whole	2H	17.2-20.5	11_10943_121 - 12_10777_62	1.4-2.3	1.9-3.3	4 out of 8
DM	Whole	3H	167.3	12_20198_69	2.3-4.9	3.3-8.0	4 out of 8
DM	Whole	5H	160.9	11_20829_121	1.4-2.5	1.8-3.7	5 out of 8
DM	Whole	6H	97.4-106.8	11_20972_121 - 11_30151_61	1.5-1.8	2.0-2.5	4 out of 8

†Genotypes used for association mapping. Group whole = all genotypes, Ethiopian = landraces and breeding lines from Ethiopia, and ICARDA/NDSU = ICARDA and NDSU breeding lines.

‡The map distance for SNP markers is according to Munoz et al. (2011).

§NLP = -log10(p)

¶Number of environments where significant marker-trait associations were detected.

Table 3.5. Significant markers for plant height according to the P+K model using all genotypes or subgroups of genotypes.

Chromosome	Group†	Position‡	SNP	NLP§	%R^2	Significant¶
1H	ICARDA/NDSU	45.2-53.4	12_30110_61-11_20997_121	1.5-2.3	4.3-7.3	5 out of 10
2H	Whole	44.0	12_30432_61	1.31-2.7	1.9-4.6	6 out of 10
	Ethiopian	34.2-44.0	11_21304_121-12_30432_61	1.4-2.0	4.5-6.8	5 out of 10
2H	Whole	54.9	11_21005_121	1.4-2.8	2.0-4.8	8 out of 10
	Ethiopian	51.0-54.9	12_30703_61-11_21005_156	1.6-2.8	5.0-10.0	6 out of 10
2H	Ethiopian	125.0-134.3	11_10128_121-12_10739_47	1.33-2.2	4.1-7.8	7 out of 10
2H	Ethiopian	163.2-169.7	11_20943_123-11_10085_121	1.4-2.3	4.3-7.9	6 out of 10
3H	Whole	167.3	12_20198_69	1.7-3.6	2.7-6.5	5 out of 10
	ICARDA/NDSU	167.3	12_20198_69	1.5-3.4	4.4-11.4	5 out of 10
5H	Whole	33.6	11_20845_121-11_20845_124	1.5-2.5	2.3-4.8	4 out of 10
5H	ICARDA/NDSU	50.5-59.7	12_30538_61-11_10641_121	1.31-2.5	3.7-8.1	6 out of 10
5H	ICARDA/NDSU	173.5-181.2	12_30504_61-12_31352_61	1.5-2.4	4.4-7.7	6 out of 10
6H	Whole	70.5	11_11483_121	1.31-2.2	2.3-3.6	7 out of 10
7H	Whole	89.8-94.3	11_20083_121-12_30026_61	1.4-3.4	2.1-6.0	8 out of 10
	Ethiopian	89.8-94.3	11_20083_121-12_30026_61	1.31-1.9	4.1-6.4	7 out of 10
	ICARDA/NDSU	94.3	12_30026_61	1.34-2.3	3.8-7.2	7 out of 10

†Genotypes used for association mapping. Group whole = all genotypes, Ethiopian = landraces and breeding lines from Ethiopia, and ICARDA/NDSU = ICARDA and NDSU breeding lines.

‡The map distance for SNP markers is according to Munoz et al. (2011).

§NLP = -log10(p)

¶Number of environments where significant marker-trait associations were detected.

Table 3.6. Significant markers for lodging according to the P+K model using all genotypes or subgroups of genotypes.

Chromosome	Group†	Position‡	SNP	NLP§	%R^2	Significant¶
1H	ICARDA/NDSU	12.9	12_30588_61	1.4-2.1	4.1-6.6	5 out of 7
1H	ICARDA/NDSU	53.4-59.3	11_20997_121-12_30304_61	1.6-2.4	4.6-7.8	5 out of 7
2H	ICARDA/NDSU	51.0-54.9	12_30703_61-11_21005_156	1.7-2.8	5.0-9.1	5 out of 7
5H	Ethiopian	123.8	12_30067_61	1.7-3.0	4.4-8.5	4 out of 7
5H	ICARDA/NDSU	181.2-189.2	12_31352_61-12_10322_74	1.7-2.5	5.0-8.2	4 out of 7
6H	ICARDA/NDSU	27.3-33.0	11_20315_81-12_31485_61	1.4-4.4	3.8-5.1	4 out of 7
6H	ICARDA/NDSU	55.7-65.8	SCRI_RS_176650_61-12_10758_61	1.4-2.3	3.9-7.5	4 out of 7

†Genotypes used for association mapping. Group whole = all genotypes, Ethiopian = landraces and breeding lines from Ethiopia, and ICARDA/NDSU = ICARDA and NDSU breeding lines.

‡The map distance for SNP markers is according to Munoz et al. (2011).

§NLP = -log10(p).

¶Number of environments where significant marker-trait associations were detected.

Three QTL regions were detected in chromosome 5H for plant height (Table 3.5), with the one at 50.5-59.7 cM being located in a similar region as a QTL reported by Pasam et al. (2012). Pasam et al. (2012) and Mansour et al. (2014) reported a QTL in the same region as one I identified in chromosome 6H (70.5 cM). Only one QTL was detected in chromosome 7H (89.8-94.3 cM). It was detected in all three analyses and it is located in similar region as one found by Yu et al. (2010) and Mansour et al. (2014).

In the current study, seven QTL for lodging were consistently expressed in at least 50% of the environments in chromosomes 1H, 2H, 5H, and 6H (Table 3.6). Six of the QTL were detected only in the analysis that included the ICARDA and NDSU genotypes; the other QTL was detected only in the analysis using the Ethiopian landraces and breeding lines. Because these QTL are detected in only one of the analyses using subgroups, it is possible that these QTL may be useful to the subgroup in which they are not detected. This is especially true if the loci in the subgroup are fixed with the unfavorable alleles. Excessive plant height is a contributing factor to lodging severity in barley. Referring to Tables 3.5 & 3.6, four QTL for lodging coincide with significant QTL for plant height in chromosomes 1H (45.2-59.3 cM), 2H (51.0-54.9 cM), 5H (173.5-189.2 cM), and 6H (55.7-70.5 cM). The co-localization of QTL for lodging and plant height may strength the thought that plant height influences lodging. Two QTL corresponding to those detected in chromosomes 2H (51.0-54.9 cM) and 6H (55.7-65.8 cM) also have been found by Tinker et al. (1996) and Hayes et al. (1993), respectively.

Yield and yield related traits

The observed vs. expected *p*-value plots for yield and major yield components are presented in Figure 3.6 a-f. As with the other traits, the K and P+K models were the most suitable models for yield and yield related traits, and the low MSD estimates for the P+K model indicated that this was the most appropriate model to use (Table A.18). Likewise, the number of significant MTAs decreased with the addition of factors to the model (Figure 3.7), indicating that using the P+K model reduced the likelihood of false positives.

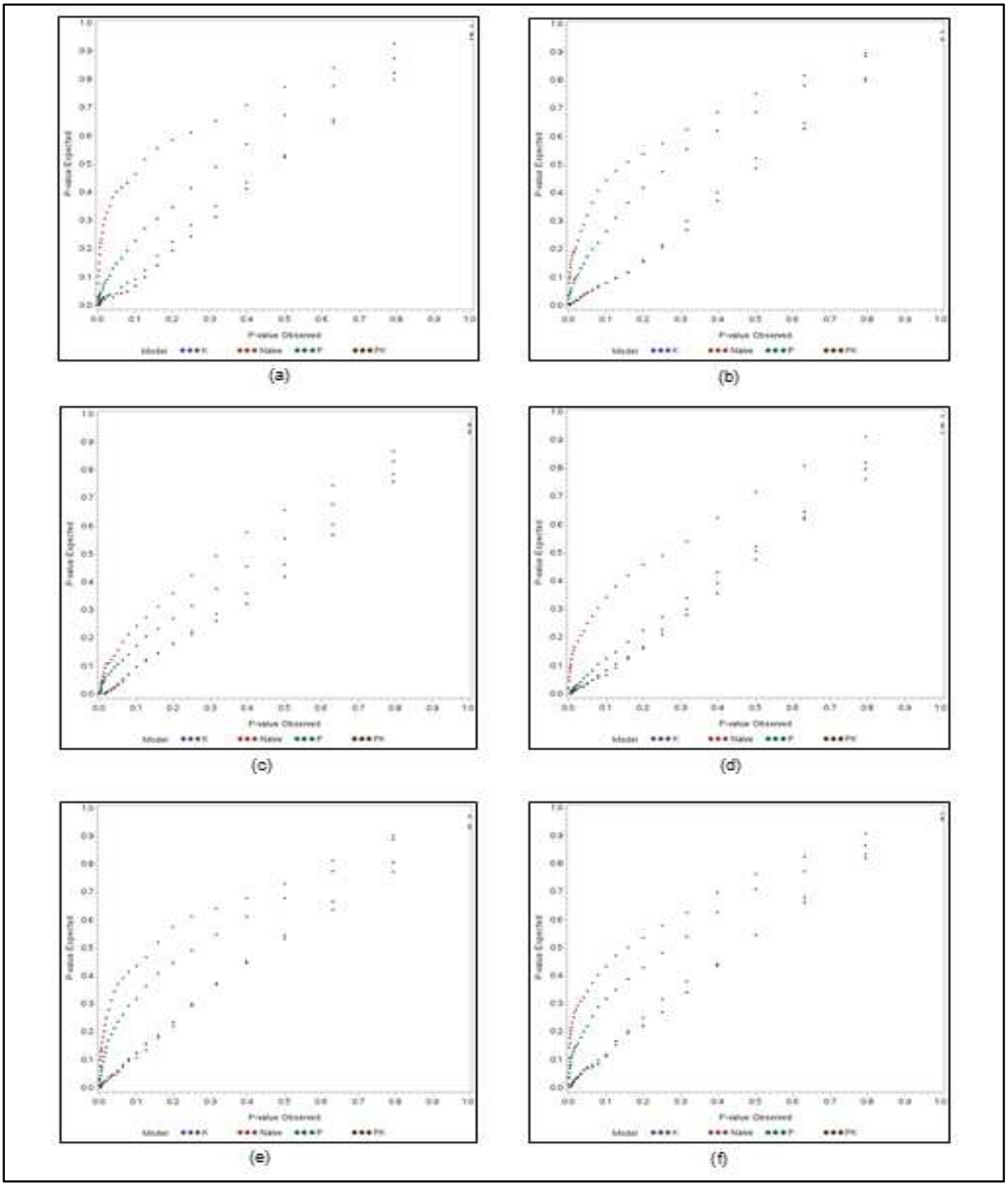

Figure 3.6. Plot of observed vs. expect p-values of four association mapping models for six traits: (a) number of spikes per plant, (b) number of kernels per spike, (c) spike length, (d) thousand-kernel weight, (e) hectoliter weight, and (f) grain yield.

Three QTL were detected for number of spikes per plant in chromosome 2H in the analysis utilizing all genotypes (Table 3.7). In the GrainGenes database (http://wheat.pw.usda.gov/GG2/Barley/),

four consensus QTL were reported for number of spikes per plant in chromosomes 2H, 4H, 6H, and 7H. The QTL in chromosome 2H at 101.7-105.6 cM was positioned within 12 cM from one of the QTL for number spikes per plant reported by Kjaer and Jensen (1996). Two of the QTL not identified in previous studies could represent QTL unique to the materials utilized in this study. Four regions with significant QTL were identified for number of kernels per spike (Table 3.7). These regions were located in chromosomes 2H (4.7 cM and 63.6-68.1 cM), 3H (146.3 cM), and 5H (88.1 cM). All of the significant QTL for number of kernels per spike overlapped with the QTL regions for other traits including, number of spikes per plant and thousand-kernel weight (Table A.19), implying pleotropic effect or linkage of genes controlling these traits. Actually, these three traits are major yield components and usually show high association. One of the QTL in chromosome 2H (63.3-68.1 cM) was in a similar region as a QTL for number of kernels per spike found by Locatelli et al. (2013).

For spike length, three QTL were identified in chromosomes 2H (130.4 cM), 6H (55.7 cM), and 7H (94.3 cM) (Table 3.7). Varshney et al. (2012) previously reported QTL for spike length in chromosome 2H; however, none of them correspond to QTL detected in the current study. Thus, the QTL I detected may be unique in the materials utilized in the current study. In the case of hectoliter weight, two QTL were detected in chromosomes 5H and 7H (Table 3.7). Both the regions also included QTL for other traits, including grain yield. One of the two QTL in chromosome 5H (162.0-165.3 cM) was in a similar region as one reported by Tinker et al. (1996).

In the analysis utilizing all genotypes, nine QTL were identified for thousand-kernel weight in all chromosomes except 7H (Table 3.8). Five of these QTL regions overlapped with QTL for number of kernels per spike, spike length, and grain yield (Table A.19). Three QTL for thousand-kernel weight were detected in chromosome 2H (4.7, 63.6-68.1, and 134.3-139.3 cM) and all were in similar regions as previously mapped QTL (Table 3.8). The QTL at the QTL at 63.6-68.1 cM was in a similar region as a QTL identified by multiple studies (Comadran et al., 2011; Pasam et al., 2012; Rode et al., 2012; Locatelli et al., 2013), and the QTL at 134.3-139.3 cM mapped to a similar region as one reported by Pasam et al. (2012). Of the two significant QTL for thousand-kernel weight in chromosome 4H, the one at 19.5 cM corresponded to a QTL reported previously (Comadran et al., 2011; Pasam et al. 2012; Wang et al., 2012). Similarly, the QTL identified in chromosome 6H (98.7 cM) mapped to similar region as a QTL

reported by Pasam et al. (2012). The QTL for thousand-kernel weight in the current study not identified previously could represent unique QTL found in the current association panel.

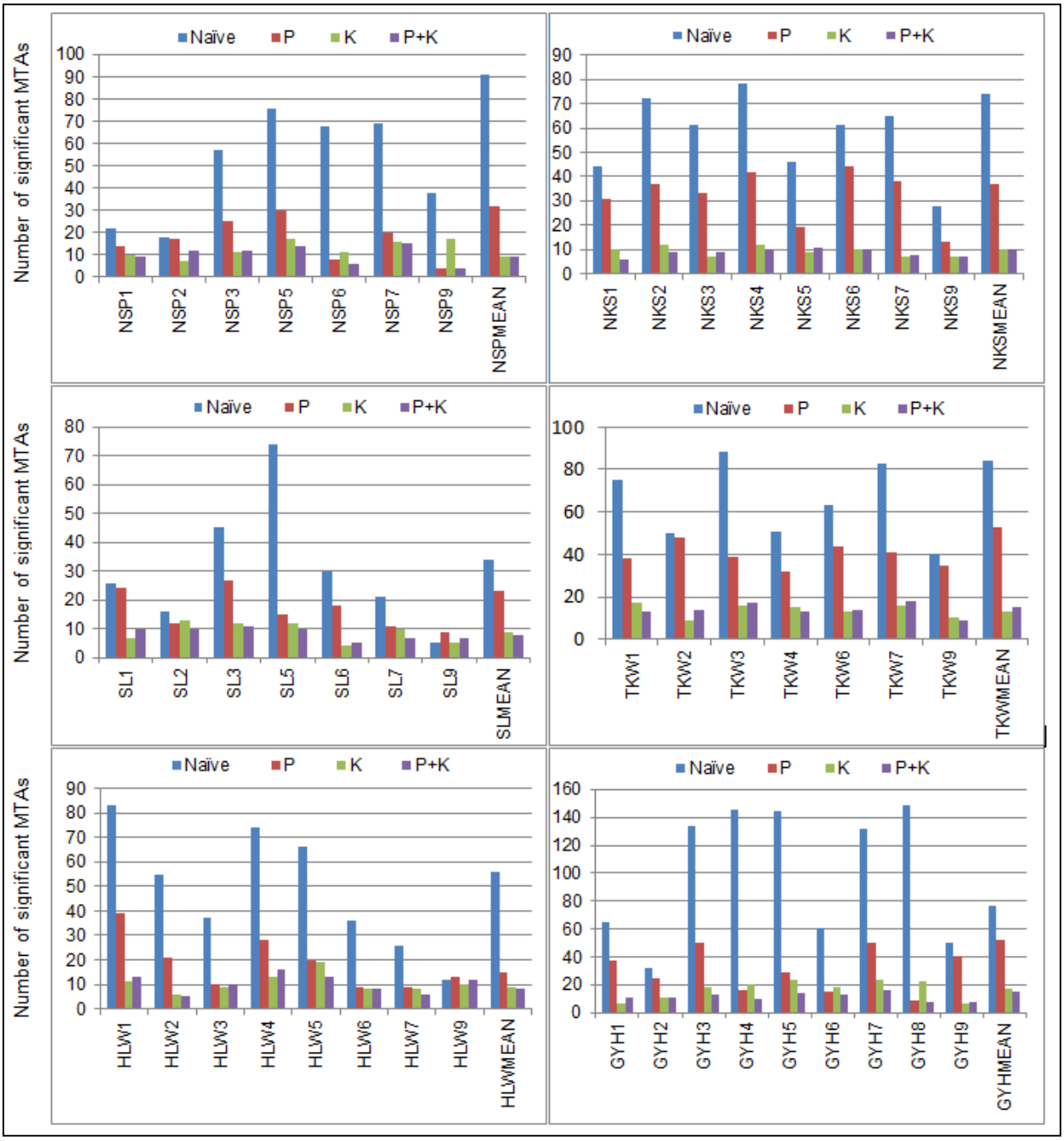

Figure 3.7. The number of significant markers-trait associations (MTAs) in the four models for number of spikes per plant (NSP), number of kernels per spike (NKS), spike length (SL), thousand-kernel weight (TKW), hectoliter weight (HLW), and grain yield (YLD). The numbers associated with the trait abbreviations in X-axis indicate the environments: Env1 = Bekoji in 2011, Env2 = Koffele in 2011, Env3 = Bekoji in 2012, 1= Env4 = Fargo in 2012, 5 = Env5 = Holetta in 2012, 6 = Env6 = Koffele in 2012, 7 = Env7 = Bekoji in 2013, 8 = Env8 = Fargo in 2013, and 9 = Env9 = Koffele in 2013.

Table 3.7. Significant markers for number of spikes per plant (NSP), number of kernels per spike (NKS), spike length (SL), and hectoliter weight (HLW) according to the P+K model using all genotypes.

Trait	Chromosome	Position†	SNP	NLP‡	%R^2	Significant§
NSP	2H	12.1-20.5	12_30631_40 - 12_10777_62	1.5-2.1	2.0-3.1	4 out of 8
NSP	2H	101.7-105.6	12_10936_61 - 11_10214_121	1.4-2.3	1.7-3.3	4 out of 8
NSP	2H	169.7	11_10085_121 - 11_10085_121	1.4-2.1	1.8-3.0	5 out of 8
NKS	2H	4.7	11_10326_121	1.5-2.5	2.0-3.6	8 out of 9
NKS	2H	63.6-68.1	12_10485_61 - 12_10099_61	1.6-2.0	2.1-2.9	4 out of 9
NKS	3H	146.3	11_20085_121	1.31-2.8	1.7-4.2	7 out of 8
NKS	5H	88.1	11_10518_121	1.5-1.7	1.9-2.2	5 out of 9
SL	2H	130.4	11_10429_121	1.4-2.6	1.9-3.8	4 out of 8
SL	6H	55.7	SCRI_RS_176650_51	1.4-1.9	1.8-2.6	4 out of 8
SL	7H	94.3	12_30996_61	1.5-1.9	2,0-2.7	4 out of 8
HLW	5H	162.0-165.3	11_20826_121 - 11_10869_121	1.31-2.2	1.7-3.3	5 out of 9
HLW	7H	94.3	12_30026_61 - 11_21448_121	1.5-2.2	1.9-3.3	5 out of 9

†The map distance for SNP markers is according to Munoz et al. (2011).

‡NLP = -log10(p).

§Number of environments where significant marker-trait associations were detected.

Across the three methods of analyses, seven QTL were identified for grain yield in all seven chromosomes (Table 3.9). The QTL regions in chromosomes 3H (11.0 cM) and 7H (89.8-94.3 cM) also included QTL for other traits (Table A.18). The QTL for grain yield detected in the Ethiopian accessions (landraces and breeding lines) in chromosome 1H (38.4-46.5 cM) mapped to a similar region as a QTL for grain yield reported by Mansour et al. (2014). The QTL detected in the ICARDA and NDSU genotypes in chromosome 4H (78.1-86.7 cM) was comparable to a QTL reported previously (Locatelli et al., 2013). The QTL identified in chromosome 7H (89.8-94.3 cM) was in a similar region as a QTL reported by Comadran et al. (2011) and Berger et al. (2013).

Table 3.8. Significant markers for thousand-kernel weight according to the P+K model using all genotypes.

Chromosome	Position†	SNP	NLP‡	%R^2	Significant§
1H	20.3	11_20712_121	1.3-3.1	1.7-4.8	4 out of 8
2H	4.7	11_10326_121	1.6-2.2	2.1-3.1	5 out of 8
2H	63.6-68.1	12_10485_61 - 12_10099_61	1.5-2.4	2.0-3.6	5 out of 8
2H	134.3-139.3	12_10739_47 - 11_20141_89	1.31-2.0	1.7-2.9	5 out of 8
3H	11.0	11_21398_121	1.5-2.7	1.9-4.1	6 out of 8
4H	19.45	11_10223_121	1.7-3.5	2.4-5.5	4 out of 8
4H	92.4	12_31246_54	1.5-2.6	2.0-3.9	6 out of 8
5H	64.8	11_21309_121	1.5-2.2	2.1-3.1	7 out of 8
6H	98.7	12_30698_61	1.31-1.7	1.7-2.4	4 out of 8

†The map distance for SNP markers is according to Munoz et al. (2011).
‡NLP = -log10(p).
§Number of environments where significant marker-trait associations were detected.

Table 3.9. Significant markers for grain yield according to the P+K model using all genotypes or subgroups of genotypes

Chromosome	Group†	Position‡	SNP	NLP§	%R^2	Significant¶
1H	Ethiopian	38.4-46.5	12_10314_61 - 11_20810_121	1.33-2.0	3.2-5.2	7 out of 10
2H	Ethiopian	130.4-134.3	11_10429_121 - 12_10739_61	1.4-2.3	3.4-6.3	6 out of 10
3H	Whole	11.0	11_21398_106 - 12_30818_61	1.31-2.2	1.7-3.2	5 out of 10
4H	ICARDA/ NDSU	78.1-86.7	11_10606_81 - 11_11004_121	1.34-2.1	3.8-6.3	6 out of 10
5H	Whole	171.6-181.2	12_30494_61 - 12_31352_61	1.31-2.6	1.7-3.8	5 out of 10
	ICARDA/ NDSU	173.5-181.2	12_30504_61 - 12_31352_61	1.4-2.2	3.8-6.9	5 out of 10
6H	Ethiopian	27.3-33.0	11_20315_81 - 12_31485_61	1.4-2.3	3.4-6.2	5 out of 10
7H	Whole	89.8-94.3	11_20083_121 - 12_30026_61	1.5-3.4	2.0-5.3	6 out of 10

†Genotypes used for association mapping. Group whole = all genotypes, Ethiopian = landraces and breeding lines from Ethiopia, and ICARDA/NDSU = ICARDA and NDSU breeding lines.
‡The map distance for SNP markers is according to Munoz et al. (2011).
§NLP = -log10(p).
¶Number of environments where significant marker-trait associations were detected.

Disease resistance and related traits

Similar to the traits discussed previously, the observed vs. expected p-value plots indicated that the K and P+K models were the most appropriate for the association analyses of the four disease traits (leaf scald and net blotch in field evaluations, and net form net blotch and spot form net blotch in greenhouse evaluations) (Figure 3.8 a-d). Additionally, the smallest MSD estimates were obtained for the P+K and K models in the four traits (Table A.18) and P+K model provided smallest number of significant MTAs (Figure 3.9). Thus, the MTA results obtained using the P+K model will be the basis for the discussion in this section.

Across the three methods of analyses, 15 QTL were detected for leaf scald (SC) in all chromosomes except 1H (Table 3.10). The QTL in chromosomes 3H (167.3 cM), 4H (112.9-116.7 cM), 5H (121.7-123.8 cM), and 7H (57.6 cM) were identified in at least two of the methods of analysis. Given that only one of the five QTL (chromosome 4H at 112.9-116.7 cM) identified in the Ethiopian genotypes coincided with QTL found in two or more methods of analyses (Table 3.10), I conclude that the other four QTL are unique to Ethiopian genotypes. The Ethiopian accessions CIho 2222 and CIho 668 (not utilized in this study) were documented to be resistant to leaf scald and were used in the development of mapping populations for leaf scald (Grønnerød et al., 2002; Patil et al., 2003).

Many of the 15 QTL I identified for leaf scald mapped to regions where QTL for this trait had been previously found. The QTL in chromosome 2H (163.2-169.7 cM) was in the same region as one reported by Looseley et al., (2012). Two QTL were mapped to chromosome 3H in the current study (Table 3.10). The QTL at 74.0 cM in chromosome 3H (Table 3.10) was located in the same region as the *Rrs1* gene and a QTL identified previously (Grønnerød et al., 2002; Patil et al., 2003, and Wang et al., 2014). Similarly, Korff et al. (2005) and Wang et al. (2014) identified QTL in the long arm of chromosome 3H as I did (167.3 cM). Furthermore, Wang et al. (2014) found QTL in similar region as the ones I found in chromosome 4H (78.1-85.1 cM and 112.7-116.7 cM). The QTL I detected in chromosome 6H at 33.0-38.0 cM was in a similar region as a QTL reported by Backes et al. (1995), and the ones at 93.4-100.7 cM and 119.6-127.8 cM corresponded to QTL reported by Wang et al. (2014) for leaf scald. Four QTL were identified in chromosome 7H (Table 3.10), with the QTL identified at 57.6 cM being in the same region as a QTL reported by Backes et al. (1995). The one at 83.4-89.8 cM in chromosome 7H was positioned in

similar region where Looseley et al. (2012) and Wang et al. (2014) identified QTL for leaf scald. Additionally, Wang et al. (2014) identified the *Rrs15* gene in chromosome 7H at 170.0-180.4 cM, which is in a similar region as a QTL I detected in chromosome 7H at 173.2 cM (Table 3.10).

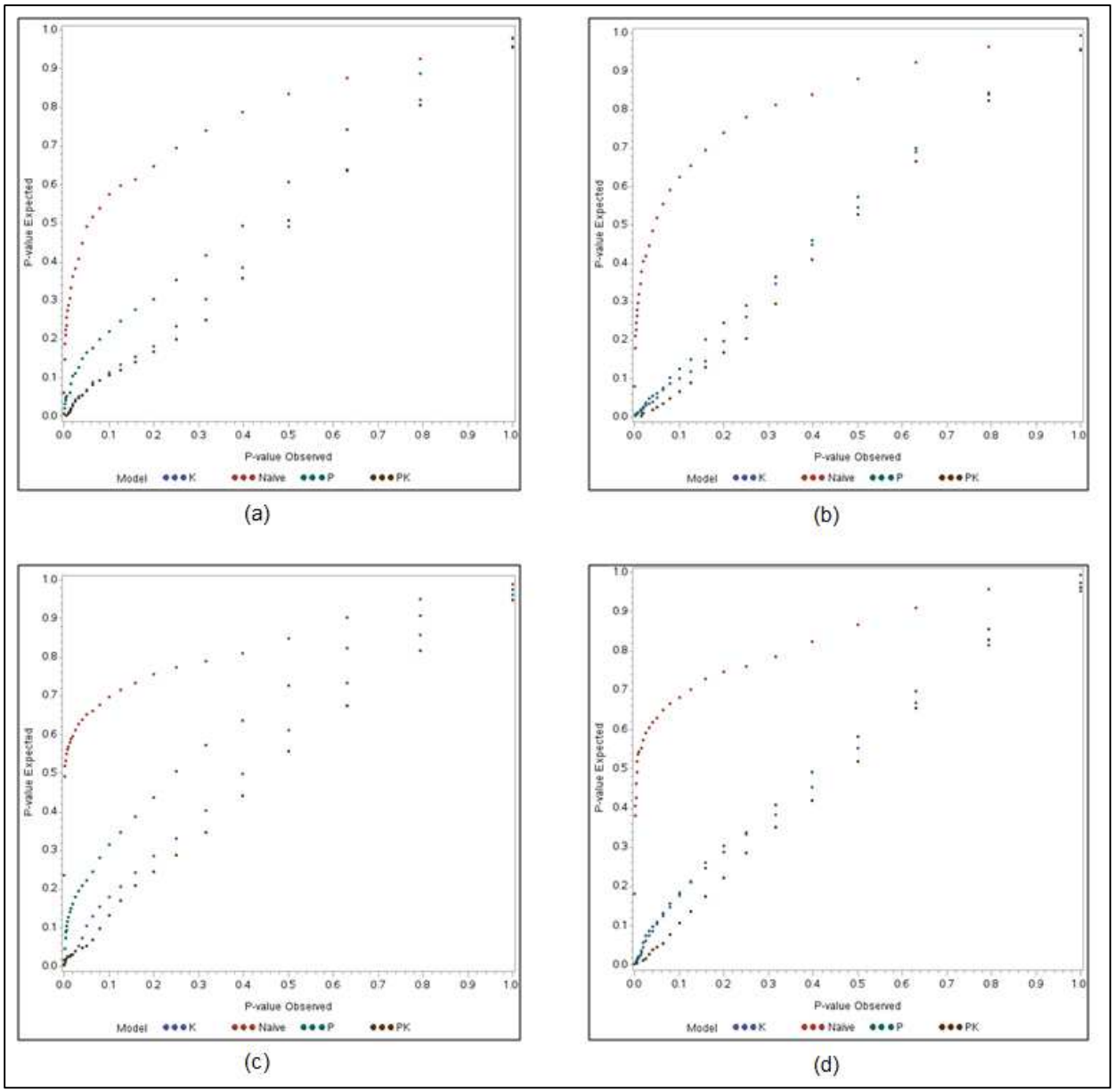

Figure 3.8. Plot of observed vs expect p-values for four traits: (a) leaf scald (SC), (b) net blotch (NB), (c) Net form net blotch (NFNB), and (d) Spot form net blotch (SFNB).

A total of eight QTL were identified for net blotch phenotyped in the field in all chromosomes except 4H in the three methods of analyses (Table 3.11). One QTL was identified in the analysis using the whole genotypes, three QTL in the the Ethiopian genotypes, and five QTL in the ICARDA and NDSU genotypes. The QTL identified in chromosome 1H for ICARDA and NDSU genotypes at 38.4-45.2 cM and

for the Ethiopian genotypes at 46.5-53.4 cM were considered as the same QTL. Two of the eight QTL in chromosome 5H (88.1-93.7 cM) and 6H (60.7-62.7 cM) coincided with QTLs identified in previous studies by Spaner et al. (1998) and Steffenson et al. (1996), respectively.

For net form net blotch (NFNB), six QTL were detected in chromosomes 2H, 3H, 5H, and 6H in the analyses using the whole genotypes and only the ICARDA and NDSU genotypes (Table 3.12). Three of the eight QTL, i.e.; in chromosomes 2H at 113.5 cM, 6H at 79.2 cM, and 6H at 98.7 cM were detected in the analyses using all genotypes and the analysis using the ICARDA and NDSU breeding lines.

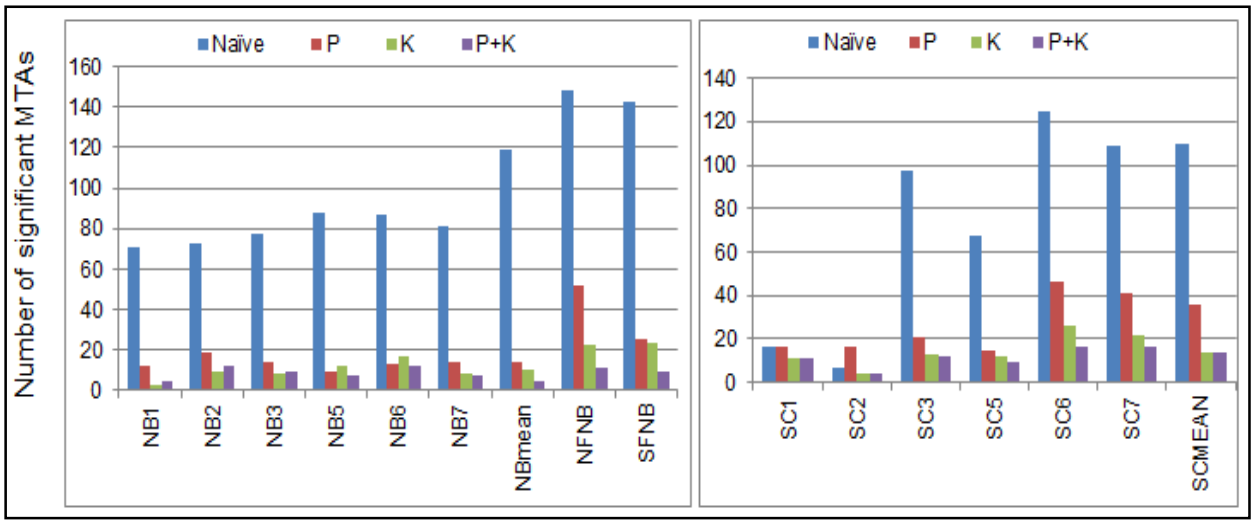

Figure 3.9. The number of significant markers-trait associations in the four models for net blotch (NB), net form net blotch (NFNB), spot form net blotch, and scald (SC). The numbers associated with the trait abbreviations in X-axis indicate the environments: 1 = Env1 = Bekoji in 2011, 2 = Env2 = Koffele in 2011, 3 = Env3 = Bekoji in 2012, 5 = Env5 = Holetta in 2012, 6 = Env6 = Koffele in 2012, and 7 = Env7 = Bekoji in 2013.

The QTL in chromosome 3H (69.9 cM) for NFNB was located in a similar region as a QTL identified by Konig et al. (2013). In chromosome 5H, the QTL identified at 93.7 cM mapped to a similar region as a QTL found by Grewal et al. (2008). Several QTL mapping studies for NFNB reported a QTL in chromosome 6H (Friesen et al., 2006; Grewal et al., 2008; Pierre et al., 2010; Grewal et al., 2012), which corresponds to the QTL I identified at 79.2 cM (Table 3.12). The marker HVM11 (88.5 cM) reported to associated with NFNB (Friesen et al., 2006) is located within 10 cM from both QTl I identified in chromosome 6H. The two linked NFNB resistance genes (*rpt.k* and *rpt.r*) mapped to 95.1 and 96.8 cM on chromosome 6H, respectively (Liu et al., 2010) could be the QTL detect at 98.7 cM in the current study.) The recessive forms of the two genes confer resistance to different pathotypes of NFNB Qamar et al. (2008.

Table 3.10. Significant markers for leaf scald according to the P+K model using all genotypes or subgroups of genotypes.

Chromosome	Group†	Position‡	SNP	NLP§	%R^2	Significant¶
2H	ICARDA/NDSU	163.2-169.7	11_20943_123 - 11_10085_121	1.37-2.5	4.1-8.5	5 out of 7
3H	Whole	74.0	11_10373_90	1.35-2.8	2.2-5.3	4 out of 7
3H	Whole	167.3	12_20198_69	1.6-1.9	2.7-3.4	4 out of 7
	ICARDA/NDSU	167.3	12_20198_69	1.32-2.0	3.9-6.5	4 out of 7
4H	ICARDA/NDSU	78.1-85.1	11_10606_81 - 11_10309_121	1.32-1.7	3.9-5.3	5 out of 7
4H	Whole	116.7	SCRI_RS_148330_61 - 11_20974_121	1.33-2.1	2.2-3.9	5 out 0f 7
	Ethiopian	116.7	11_20974_121	1.8-3.2	7.3-14.3	5 out of 7
	ICARDA/NDSU	112.9-116.7	11_10510_121 - 11_20974_121	1.4-2.7	4.3-9.4	4 out of 7
5H	Whole	121.7	11_20653_121 - 12_30067_61	1.31-2.4	2.1-4.5	4 out of 7
	ICARDA/NDSU	121.7-123.8	11_20653_121 -12_30067_61	1.6-3.6	5.0-13.0	5 out of 7
5H	Ethiopian	148.6-157.6	12_31221_61 - 12_30162_61	1.37-1.6	5.3-6.3	4 out of 7
5H	Ethiopian	162.0-168.4	11_20826_121 - 11_20536_121	1.38-2.3	5.4-9.9	5 out of 7
6H	Ethiopian	33.0-38.0	12_31485_61 -11_10427_121	1.4-2.4	5.6-10.2	4 out of 7
6H	ICARDA/NDSU	93.4-100.7	11_11246_121 - 11_10400_121	1.3-3.6	3.9-13.0	7 out of 7
6H	ICARDA/NDSU	119.6-127.8	12_30734_61 -11_10107_121	1.32-1.8	3.9-5.9	4 out of 7
7H	Whole	57.6	11_10050_121 - 12_30149_61	1.31-2.3	2.1-4.2	4 out of 7
	ICARDA/NDSU	57.6	11_10050_121 - 12_30149_61	1.4-2.3	4.1-7.8	4 out of 7
7H	Whole	83.4-89.8	12_10982_61 - 11_20083_121	1.32-4.0	2.2-8.1	5 out 0f 7
7H	Ethiopian	129.3-131.0	11_10182_121 -11_20185_121	1.36-3.2	5.3-14.6	4 out of 7
7H	Whole	173.2	11_20185_121	1.33-2.0	2.2-3.6	4 out of 7

†Genotypes used for association mapping. Group whole = all genotypes, Ethiopian = landraces and breeding lines from Ethiopia, and ICARDA/NDSU = ICARDA and NDSU breeding lines.

‡The map distance for SNP markers is according to Munoz et al. (2011).

§NLP = -log10(p).

¶Number of environments where significant marker-trait associations were detected.

Table 3.11. Significant markers for net blotch according to the P+K model analysis using all genotypes or subgroups of genotypes.

Chromosome	Group†	Position‡	SNP	NLP§	%R^2	Significant¶
1H	ICARDA/NDSU	38.4-45.2	12_10314_61 - 12_30110_61	1.32-1.8	3.9-5.6	4 out of 7
	Ethiopian	46.5-53.4	11_20810_133 - 11_20997_121	1.4-2.1	5.7-8.7	4 out of 7
1H	Ethiopian	119.8	11_10854_121	1.4-2.3	5.5-9.6	4 out of 7
2H	ICARDA/NDSU	130.4-139.3	11_10429_121 - 11_20141_89	1.4-1.9	4.1-6.4	4 out of 7
3H	ICARDA/NDSU	96.5-100.3	SCRI_RS_225641_61 - 12_31018_61	1.4-1.8	4.4-5.7	4 out of 7
5H	Whole	88.1-93.7	11_10518_121 - 11_11350_121	1.35-2.4	2.2-4.6	4 out of 7
5H	Ethiopian	123.8-125.1	12_30067_61 - 11_21247_119	1.6-3.1	6.5-4.0	5 out of 7
6H	ICARDA/NDSU	60.7-62.7	11_10377_124 - 12_11253_61	1.4-2.3	4.2-7.6	4 out of 7
7H	ICARDA/NDSU	131.0-135.9	11_20185_121 -11_10797_121	1.5-2.0	4.5-6.7	4 out of 7

†Genotypes used for association mapping. Group whole = all genotypes, Ethiopian = landraces and breeding lines from Ethiopia, and ICARDA/NDSU = ICARDA and NDSU breeding lines.

‡The map distance for SNP markers is according to Munoz et al. (2011).

§NLP = -log10(p).

¶Number of environments where significant marker-trait associations were detected.

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For spot form net blotch (SFNB), four QTL were identified on chromosomes 1H, 4H, and 7H using all three methods of analyses (Table 3.13). The QTL in chromosome 1H (46.5 cM) was detected in two of the three analyses (the whole genotypes and thr Ethiopian genotypes), and was in the same region where a QTL for NFNB was found by Grewal et al. (2012).

Table 3.12. Significant markers net form net blotch (NFNB) according to the P+K model analysis using all genotypes or subgroups of genotypes.

Chromosome	Group†	Position‡	SNP	NLP§	%R^2	Significant¶
2H	Whole	113.5	11_10398_121	2.3	3.0	1 out of 1
	ICARDA/NDSU	113.5	11_10398_121	2.54	7.0	1 out of 1
3H	ICARDA/NDSU	69.9	12_30009_61	2.15	5.7	1 out of 1
5H	Whole	29.9	11_10580_104	2.5	3.4	1 out of 1
5H	Whole	93.7	11_11350_121	2.6	3.5	1 out of 1
6H	Whole	79.2	11_20892_121	4.6	6.9	1 out of 1
	ICARDA/NDSU	79.2	11_20892_121	2.81	7.8	1 out of 1
6H	Whole	98.7	12_30698_61	3.3	4.8	1 out of 1
	ICARDA/NDSU	98.7	12_30698_61	2.27	6.1	1 out of 1

†Genotypes used for association mapping. Group whole = all genotypes, Ethiopian = landraces and breeding lines from Ethiopia, and ICARDA/NDSU = ICARDA and NDSU breeding lines.
‡The map distance for SNP markers is according to Munoz et al. (2011).
§NLP = -log10(p).
¶Number of environments where significant marker-trait associations were detected.

Table 3.13. Significant markers for spot form net blotch (SFNB) according to the P+K model for analysis on the whole accessions and two sub-sample groups.

Chromosome	Group†	Position‡	SNP	NLP§	R^2	Significant¶
1H	Whole	46.5	11_20810_121	2.4	3.2	1 out of 1
	Ethiopian	46.5	11_20810_121	2.28	6.3	1 out of 1
4H	ICARDA/NDSU	48.8	11_11180_121	2.84	8.0	1 out of 1
7H	Ethiopian	18.7	11_20495_121	2.03	5.5	1 out of 1
7H	Whole	148.3	12_20640_61	2.3	3.0	1 out of 1

†Genotypes used for association mapping. Group whole = all genotypes, Ethiopian = landraces and breeding lines from Ethiopia, and ICARDA/NDSU = ICARDA and NDSU breeding lines.
‡The map distance for SNP markers is according to Munoz et al. (2011).
§NLP = -log10(p).
¶Number of environments where significant marker-trait associations were detected.

Summary and Conclusions

The diversity and population structure analyses using SNP markers revealed that the mapping panel could be grouped according to spike row-type, geographic origin, and breeding history. The marker-based diversity analysis was in line with the diversity analyses based on phenotypic data discussed in chapter II. Past research by others also indicated that spike row-type, geographic origin, and breeding history are major factors for population structure in barley (Varshney et al, 2012; Pauli et al. 2014;

Matthies et al., 2014). The current mapping panel is highly structured and hence appropriate statistical models should be considered for the association mapping analyses.

The LD analyses indicated that LD decayed within 10 to 20 cM distance. About 12.3% of the LD estimates (r^2) in the whole population were ≥ 0.1; however, a large proportion (67.4%) of these estimates were between marker-pairs separated by >20 cM. These results suggest the importance of factors other than linkage, which again implies that population stratification and relatedness need to be controlled through the use of MLM in the association mapping analyses so the likelihood of false positives results is reduced.

Based on the results of the observed vs. expected *p*-values and MSD values for almost all the traits, the P+K and K models were shown to be the most appropriate. These results were not unexpected since the mapping panel was highly structured. The current study also confirmed that the application of MLM like P+K and K models was appropriate for identifying MTAs in highly structured mapping panels.

A goal of the association mapping analyses was to identify regions with significant QTL for important agronomic and disease resistance traits. For any program wishing to increase variability for a specific trait by introducing new alleles, it is important to be aware of which QTL are present in their germplasm base and what unique QTL are available in others' materials that can be brought into their program via crossing. Zale et al. (2000) suggested two ways of selecting QTL for malt quality traits in breeding programs, i.e.; selection for widely conserved QTL regions to maintain malting quality whereas selection for unique QTL regions to gain new improvements. Historically, breeding programs around the world have utilized landrace and breeding lines from Ethiopia as sources of alleles for disease resistance. Also, many breeding programs worldwide have used ICARDA breeding materials as parents or the source of selecting cultivars. In the last 40 years, the NDSU program has not utilized the Ethiopian landrace or lines from the Ethiopian and ICARDA breeding programs as parents in crossing. Likewise, the Ethiopian and ICARDA programs have rarely utilized NDSU breeding lines or cultivars as parents. Thus, the NDSU and Ethiopian breeding programs could benefit from each other if unique alleles can be identified in each other's materials.

Research in the present study identified QTL controlling agronomic and disease resistance traits in ≥ 50% of the environments where the traits were phenotyped. Analyses of the traits using subsets of

the mapping panel based on origin were able to identify unique genomic regions that could be exploited. Overall, 17 QTL unique to the Ethiopian accessions were found for seven traits (days to heading, plant height, lodging, grain yield, leaf scald, net blotch, and spot form net blotch). Just above half of these QTL were concentrated in chromosomes 2H (125.0-134.3 cM), 5H (123.8-125.1 cM and 148.6-168.4 cM), and 6H (27.3-38.0 cm). Similarly, a total of 34 QTL unique to the ICARDA and NDSU genotypes were found for eight traits (days to heading, plant height, lodging, grain yield, leaf scald, net blotch, net form net blotch, and spot form net blotch). More than half of these unique QTL were located in eight regions in chromosomes 1H (45.2-59.3 cm), 2H (163.2-169.7 cM), 3H (69.9-74.0 cM and 167.3 cM), 5H (178.5-181.2 cM), and 6H (55.7-65.8 cM, 79.2-85.9 cM, and 93.4-100.7 cM). Several QTL not found in previous studies were identified in the analysis that used all genotypes. Because most of the studies where these QTL were identified used genotypes from North America, Europe, East Asia, or Australia, it is possible that these QTL represent new sources of alleles that can be employed by these programs.

A total of 94 QTL for 12 traits phenotyped in field experiments and two disease traits phenotyped in the greenhouse were detected in this study using three groups of materials for the association analyses. Three regions in chromosome 2H (4.7-12.1, 125.0-134.3 and 163.2-169.7 cM), two regions in chromosome 6H (27.3-38.0 and 93.4-106.8 cM), and one each in chromosome 1H (38.5-46.5 cM) and 7H (89.8-94.3 cM) were the locations where QTL were concentrated. It is logical to consider these regions for fine mapping and molecular marker based breeding in barley.

The QTL detected in the current study correspond to the chromosomal positions of some known genes or QTL for the traits reported in the literature. Out of 12 QTL detected for days to heading, 10 of them were in similar regions where QTLs or known genes for days to heading were mapped. Overall, the association mapping putatively identified six photoperiod-related genes (*Ppd_H1*, *HvFT4*, HvGI, *HvFT2*, *HvCO2*, and *HvCO1*) and one vernalization-related gene (*VRN-H1*) for days to heading. Seven of the 11 QTL for plant height were also supported by previous research reports. My results found QTL for plant height near one of the plant height gene (*sdw3*) in chromosome 2H.

It is generally believed that plant height and lodging resistance are related traits in that taller plants are more susceptible to lodging. The QTL for these two traits overlapped in chromosome 1H (45.2-59.3 cM), 2H (51.0-54.9 cM), 5H (173.5-189.2 cM), and 6H (55.7-70.5 cM). For yield and the main yield

components, several QTL were detected in the current study. In the case of thousand-kernel weight, five of the nine QTL detected in chromosome 2H, 4H, and 6H were also reported in at least one past research report. For grain yield, three of the seven QTL, located in chromosomes on 1H, 4H, and 7H, are in similar regions as other QTL reported in the literature. The current study also found that 11 of the 15 QTL for leaf scald correspond to QTL reported previously, including the *Rrs1* locus in chromosome 3H and the *Rrs15* locus in chromosome 7H. Moreover, two of the eight QTL detected for net blotch, four of the six QTL detected for net form net blotch including *rpt.k* and *rpt.r* loci in chromosome 6H, and one of the four QTL detected for spot form net blotch in the current study were also reported in previous studies.

The QTL for reduced plant height and increased lodging resistance is especially important for the Ethiopian breeding program since materials from this program are typically taller and have weaker straw than desired. The four unique QTL for plant height and the six unique QTL for lodging identified in the ICARDA and NDSU breeding lines could be potential candidates for the improvement of reduced plant height and lodging resistance by the Ethiopian breeding program. The four QTL unique for leaf scald, two unique QTL for net blotch, and one unique QTL for spot form net blotch found in the Ethiopian accessions (landraces and breeding lines) could be utilized to improve the resistance in these leaf diseases by the NDSU breeding program.

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CHAPTER IV: GENOME-WIDE ASSOCIATION STUDY FOR GRAIN AND MALT QUALITY TRAITS IN BARLEY ACCESSIONS FROM ETHIOPIA, ICARDA, AND NDSU

Abstract

Malting is the second most important utilization of barley (*Hordeum vulgare* L.) grain, and malt quality traits are major targets for molecular breeding. Identification of genomic regions controlling phenotypic expression of malt quality traits is crucial. Genome-wide association mapping analyses were done for nine malt quality and two grain quality traits using 165-255 genotypes that included Ethiopian landraces and breeding lines and cultivars from the Ethiopian, ICARDA, and NDSU breeding programs. Grain from field experiments at Bekoji in 2011 and 2012 was utilized. Collectively in all the three analyses, 145 marker-trait associations (MTAs) or QTL were identified for the nine malt and two grain quality traits. I detected QTL for malt and grain quality traits near the *Hor1*, *Hor2*, *Upg2*, *Dor4*, *Ltp1*, *Amy1*, *Amy2,* and *Bmy1* loci but none near the *vrs1* locus that controls row-type. There was a gap in markers coverage near the *GLB1* and *vrs1* locus in the current study, which may be the reason why I did not detect QTL around the *vrs1* locus. I also identified unique QTL in NDSU genotypes that may be beneficial for improving malt quality of the Ethiopian breeding program's germplasm. The unique QTL were generally concentrated in two regions each in chromosome 5H (50.5-59.7 cM and 118.2-125.1 cM) and 7H (3.5-9.7 cM and 83.3-98.4 cM). Favorable alleles were also identified for each marker that was associated with improved malt quality traits. Overall, the favorable alleles of QTL identified in the analysis using ICARDA and NDSU accessions came from NDSU genotypes. Generally, haplotypes containing combinations of favorable genotypes at each marker locus resulted in good mean values of the trait. Further analysis of the regions of the genome unique to the NDSU genotypes using more marker coverage is important, so to make way for their utilization by Ethiopian breeding program.

Introduction

Malt is the second important use of barley. Barley is particularly preferred for malting compared to the other cereal crops because of its several benefits. It provides enzymes to break down different compontnes of the grain (endosperm cell wall, grain storage protein, and carbohydrate). The hask from barley grain could serve as filter bed during the brewing process. Furthermore, barley also provides nutrition to yeast as well as color and flavor components to the beer.

Malting barley quality traits are major targets for molecular breeding because of their complexity and the challenges they raise in developing improved cultivars (Igartua et al., 2002; Emebiri et al., 2009; Zhang et al., 2012). Malt quality is defined by over a dozen of traits; and barley cultivars used by maltsters and brewers must have the desired levels for all traits. The American Malting Barley Association (AMBA) in the USA provides barley breeders with specifications new cultivars must meet to be recommended for use by their members, which include the largest maltsters and brewers in the USA. A challenge in developing new cultivars is improving one or more traits while maintaining the desired levels of the others. Four challenges in cultivar development pointed out by Igartua et al. (2002) are: (1) maintaining or increasing kernel weight and plumpness while improving malt quality, (2) keeping grain protein content low in crosses between two-rowed and six-rowed parents, (3) decreasing grain protein content without reductions in diastatic power (DP), and (4) decreasing wort β-glucan concentration without excessive increases in the soluble to total protein ratio. Zhang et al, (2012) and Yang and Ham (2012) pointed out another concern, which is development of new cultivars with high levels of α-amylase in malt, which is desired by some adjunct brewers, that are resistant to pre-harvest sprouting. To overcome these and other challenges in developing improved cultivars, breeders have to adopt new techniques and technologies. Marker-assisted selection (MAS) could be one solution, which requires mapping QTL and identifying candidate regions for molecular breeding intervention. Generally, the important determinants of barley grain and malt quality exhibit quantitative variation, which are affected by genetic and environmental factors (Igartua et al., 2002). Several genomic regions distributed across the seven chromosomes are associated with malt and grain quality traits (Zale et al., 2000; Igartua et al., 2002; Mohammadi et al., 2015).

According to Fox et al. (2003), the most important malt quality traits are malt extract, wort viscosity, wort β-glucan, Kolbach index, free amino nitrogen (FAN), DP, α-amylase, friability, β-glucanase, and fermentability. In a review on the genetic control of malt quality traits, Igartua et al. (2002) provide a detailed account of endosperm modification during malting and how the different malt quality traits are related to this process. The malt modification process involves the degradation of starch, cell wall, and protein, which involves several cell wall, protein, and starch degrading enzymes. For instance, the conversion of starch to fermentable carbohydrate is catalyzed by α-amylase, β-amylase, limit dextrinase,

and α-glucosidase. Malt extract, which determines the quantity of beer that can be produced during fermentation, is often considered the most important malt quality trait (Islamovic et al., 2014). Thus, malting barley breeders consider increasing malt extract in new cultivars a priority. According to Emebiri et al. (2009) and references therein, malt extract has increased from 75% to 82% in 50 years (0.14% per year). Another group of malt quality traits receiving attention in barley breeding programs are those associated with wort protein, which is important for fermentation and providing essential flavor components (Fox et al., 2003). Two traits in this group include FAN and wort soluble protein. High levels of FAN and wort protein are crucial to ensure sufficient multiplication and growth of the yeast during the fermentation process (Islamovic et al., 2014).

Many barley-breeding programs around the world have used accessions from Ethiopia as parents in their crosses. Ethiopian landraces are known for several important traits, including resistance to powdery mildew (caused by *Erysiphe graminis* DC. f. sp. *hordei* Em. Marchal), leaf rust (caused by *Puccinia hordei* Otth), loose smut (caused by *Ustilago tritici* (Pers.) Rostr.), barley yellow dwarf virus (BYDV), and barley stripe mosaic virus (BSMV); and high lysine content (Adugna, 2011; Spies et al., 2012; Munoz et al., 2014). The Ethiopian Institute of Biodiversity Conservation (IBC) has conserved > 15,000 barley accessions, with nearly 67% being landraces collected in Ethiopia (Adugna, 2011). The diversity in Ethiopian barley landraces stems from the country's diverse agro-ecologies, diverse socio-cultural situations, and wide ranges of utilization of barley for food, feed, and alcoholic beverages. Even though Ethiopian landraces have been utilized extensively in crossing programs and direct cultivar development by the national Ethiopian barley-breeding program (Bayeh and Berhane, 2011), I have not found any literature that extensively presents results on the malt quality attributes of Ethiopian landraces or breeding lines.

Bayeh and Berhane (2011) briefly discussed the history of the Ethiopian barley-breeding program and indicated that organized barley research started in earnest in 1966. The period before the 1980s was characterized by breeding for high input areas. Later, landrace evaluation and introduction of exotic materials, mainly from the International Centers for Agricultural Research in Dry Area's (ICARDA) gained attention. Today, the Ethiopian barley-breeding program mainly focuses on improving yield and yield components; lodging resistance; resistance to scald (caused by *Rhynchosporium secalis* (Oudem.) J. J.

Davis), net blotch (caused by *Drechslera teres* (Sacc.) Shoemaker), leaf rust, Russian wheat aphid (*Diuraphis noxia* (Mordvilko)) and barley shoot fly (*Delia flavibasis* Stein); resistance to abiotic stresses (drought, water logging, and frost); and quality (malting, food, and feed quality) (Bayeh and Berhane, 2011). Generally, the barley-breeding program utilizes landrace collections, exotic introductions, and lines from local crossing programs to develop cultivars for diverse production systems, including late, early, and 'Belg' (short growing season with planting in February to March). As a result, the Ethiopian barley-breeding program's germplasm has maintained high levels of genetic diversity. The barley-breeding programs of North Dakota State University (NDSU) and ICARDA have unique germplasm that reflects their breeding objectives. The NDSU breeding program mainly focuses on developing two-rowed and six-rowed malt barley cultivars adaptable to the northern Great Plains of the USA while the ICARDA breeding program develops cultivars for diverse agro-ecologies, including dryland and high rainfall regions.

Mapping QTL for traits of breeding importance is a crucial precursor for MAS or molecular-marker based breeding. For locating malt quality QTL, different mapping approaches have been used, including bi-parental mapping populations (Emebiri et al., 2004; Zhou et al., 2012; Islamovic et al., 2014); association mapping (Cai et al., 2013; Matthies et al., 2014; Mohammadi et al., 2015); and fine mapping using chromosome substitution lines (Gao et al., 2004) and wild barley introgression lines (Schmalenbach and Pillen, 2009). Linkage and association mapping are the most commonly used QTL mapping approaches (Abdurakhmonov and Abdukarimov, 2008). Association mapping is now getting more attentions due to two driving forces, which are improvements in statistical models and improvements in genotyping technologies (Zhu et al., 2008). Use of mixed linear models helps in reducing excessive false positives associated with population structure and familial relatedness.

In this chapter, natural populations sampled from Ethiopian landraces, and cultivars and breeding lines developed by the Ethiopian, ICARDA, and NDSU breeding programs were used to identify QTL for malt and grain quality traits using association mapping. The objective of the study was to identify marker-trait associations for malt and grain quality traits, and thereby detect QTL that can be utilized by the Ethiopian breeding program. I also compared the identified QTL with the well-known genes controlling grain and malt quality traits and proposed candidate QTL that may be important in malt barley breeding.

Material and Methods

Description of the Mapping Panel and Data Recorded

The number of genotypes used in association mapping for the malt and grain quality traits varied according to the group of traits and experiments where the grain samples were obtained (Table 4.1). The genotypes included two-rowed and six-rowed lines from four sources of barley germplasm: landraces from Ethiopia, and cultivars and breeding lines from the Ethiopian, ICARDA, and NDSU breeding programs. Data for genome-wide association analyses were collected on nine malt and two grain quality traits. The malt quality traits were α-amylase activity, DP, soluble protein, Kolbach Index, FAN, wort color, β-glucan, wort viscosity, and malt extract. The grain quality traits were barley grain protein and proportion of plump kernels. All traits were measured according to the procedures of the American Society of Brewing Chemists (ASBC; http://www.asbcnet.org/MOA/toc.aspx) as described in chapter II.

Phenotypic data for malt quality traits were obtained from two experiments (Bekoji in 2011 and 2012). The least square (LS) means for all the traits in each individual environment were generated using GLM procedure of SAS statistical software (SAS Inc., Cary, NC), where the checks were repeated. The LS means for combined analyses were generated in PROC GLM of SAS (SAS Inc., Cary, NC) using LS mean values of genotypes in individual environments as replicates. For barley protein content, LS means of combined analysis were generated from five experiments (Bekoji in 2011 and 2013, Koffele in 2012, and Fargo in 2012 and 2013). In the case of proportion of plump kernels, the LS means for combined analysis were estimated from three experiments (Bekoji in 2011 and 2012, and Fargo 2012).

Selection of SNP Markers

A total of 226 SNP markers were selected using the following criteria: marker quality score >1000, a minor allele frequency (MAF) > 5%, missing data < 10% and heterozygosity < 50%. The missing phenotypic and genotypic data imputation was done using the mean of the five nearest neighbors identified using the Euclidean distance (Cover and Hart, 1967) in Tassel 5.2 (Cornel University, Ithaca, NY).

Table 4.1. The number of genotypes and compositions of the populations considered for association mapping of grain and malt quality traits.

Group of trait	N†	Composition of the population
Malt and grain quality traits on samples from Bekoji 2011	213	85 Landraces, 82 ICARDA lines, 42 Ethiopian lines, 2 NDSU lines, and 2 Kenyan cultivars
Malt and grain quality traits on samples from Bekoji 2012	178	48 Landraces, 72 ICARDA lines, 31 Ethiopian lines, 25 NDSU lines, and 2 Kenyan cultivars
Combined malt quality data	236	85 Landraces, 82 ICARDA lines, 42 Ethiopian lines, 25 NDSU lines, and 2 Kenyan cultivars
Grain quality traits on samples from Fargo 2012	165	43 Landraces, 70 ICARDA lines, 27 Ethiopian lines, 23 NDSU lines, and 2 Kenyan cultivars
Grain quality traits on samples from Fargo 2013	184	40 Landraces, 71 ICARDA lines, 29 Ethiopian lines, 42 NDSU lines, and 2 Kenyan cultivars
Grain quality traits on samples from Koffele 2012	183	48 Landraces, 81 ICARDA lines, 29 Ethiopian lines, 23 NDSU lines, and 2 Kenyan cultivars
Combined barley protein content	255	85 Landraces, 82 ICARDA lines, 42 Ethiopian lines, 44 NDSU lines, and 2 Kenyan cultivars
Combined proportion of plump kernels	236	85 Landraces, 82 ICARDA lines, 42 Ethiopian lines, 25 NDSU lines, and 2 Kenyan cultivars

†N indicates the number of genotypes used in each group of traits

Association Mapping Procedures

Four models were applied for genome-wide marker-trait association analysis, namely; naive, P, K, and P+K models using JMP Genomics (SAS Institute Inc., Cary, NC). The descriptions for the models used in this study are presented in Table 4.2. Finally, the discussions of results are based on the best of the four models. The selection of the best model was based on known criteria. The models were compared using plots of the observed vs. expected *p*-values as used in Stich et al. (2008). Additionally, the mean square difference (MSD) was calculated for each model using the formulae discussed in Mamidi et al. (2011). The model with the smallest MSD was considered the best, which reflects the good correspondence of the observed and expected *p*-values. The numbers of significant marker-trait association (MTAs) were counted for each trait in all the environments for the four models to aid in comparison of the models.

Table 4.2. The descriptions of the association mapping models' components used in this study for grain and malt quality traits.

Name	Model†	Descriptions
Naïve	$y = x\alpha + \varepsilon$	Model with no control for population structure and familial relatedness.
P	$y = x\alpha + p\beta + \varepsilon$	Model with control only for population structure using the first four dimensions of multidimensional scaling generated in JMP Genomics
K	$y = x\alpha + kv + \varepsilon$	Model with control only for familial relatedness using relationship matrix as IBD generated in JMP Genomics
P+K	$y = x\alpha + p\beta + kv + \varepsilon$	Model with control for both population structure using the first four dimensions of multidimensional scaling and familial relatedness using relationship matrix as IBD generated in JMP Genomics

†y is the response vector for phenotypic values of each trait, α is the vector of fixed effects related to SNP marker effects, β is the vector of fixed effects related to population structure, v is the vector of random effects related to familial relatedness, and ε is a vector of the residual effects. x denotes the genotypes at the marker, p denotes the four dimensions from the multidimensional scaling generated in JMP Genomics, and k is the relationship matrix generated in JMP genomics.

Three different genome-wide association study (GWAS) analyses were done for each trait in individual environments, the first utilizing all genotypes, the second considering only the Ethiopian genotypes (landraces and breeding lines), and the third using only the ICARDA and NDSU genotypes. The two analyses on the subgroups of genotypes are useful to identify unique QTL in each germplasm group. Marker-trait associations for each model in the individual location analyses were deemed significant at $p \leq 0.05$. For each trait, significant markers were assessed across environments and MTAs found to be significant in at least half of the environments were discussed in detail. Significant MTAs within 10 cM distance were considered as a single QTL and reported in a range of distance associated with the significant markers flanking the QTL. These QTL were compared with the known genes or previously reported QTL for the respective trait. In order to match the QTL in this study with those in the literature, I adopted the marker positions from the genetic maps of Wenzel et al. (2006) for DArT markers, Varshney et al. (2007) for SSR markers, and Munoz et al. (2011) for SNP markers. I also used the consensus map of Szucs et al. (2009) in some of the cases.

Results and Discussions

Comparison of Models

The association mapping approach that adheres to nominal α-level shows a uniform distribution of *p*-values (Stich et al., 2008). The distribution of the *p*-values for models can be assessed using plots of the observed vs. expected *p*-values (*P-P* plot). The plot for the P+K model had a more uniform distribution than the other models for all of the grain and malt quality traits evaluated (Figure 4.1 & 4.2), so I deemed it the best model in this study. Additionally, the smaller MSD values for P+K model supported this decision (Table A.20). These results were expected since the current mapping population is highly structured on the row-type, geographic origin, and breeding history of the lines. The utilization of mixed models in association mapping, such as the P+K model, is done with the goal of reducing the number of false-positives due to population structure and familial relatedness. In the current study, the number of significant MTAs for the P+K model was much lower than the number detected using the naïve model (Figure 4.3).

Association Mapping for Malt and Grain Quality Traits

The identification of unique QTL that do not express/exist in a target breeding germplasm is crucial in QTL mapping studies because those QTL can be utilized to introduce new variation. Zale et al. (2000) stated that selection for widely conserved QTL regions is important to maintain existing malting quality attributes whereas selection for unique QTL regions is important to make new improvements. I did three separate GWAS analyses, the first included genotypes from all regions, the second utilized a subset containing the Ethiopian breeding lines and landraces, and the third utilized a subset containing genotypes from the ICARDA and NDSU breeding programs. The analyses using all genotypes allowed for the identification of QTL that exist in the entire mapping panel; however, the analyses using the subsets allowed for identification of QTL that are unique to the subset and can be exploited. For example, unique QTL for disease resistance in the Ethiopian germplasm may be of interest to breeders at NDSU or ICARDA. Likewise, unique QTL for malt quality in the NDSU germplasm may be of interest to the Ethiopian breeding program.

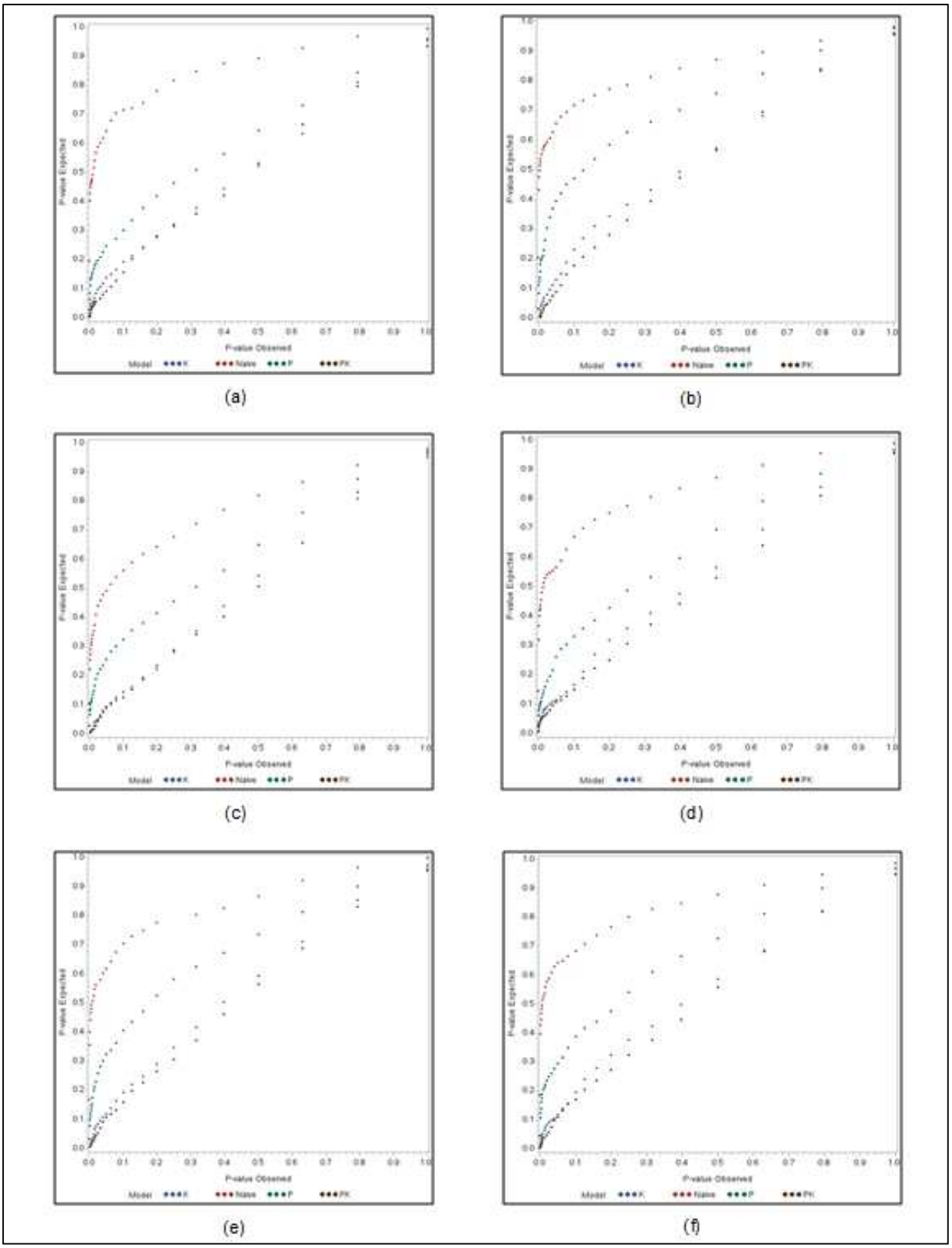

Figure 4.1. Plots of observed vs. expected p-values of four association mapping models for six malt quality traits: (a) α-amylase (AA), (b) diastatic power (DP), (c) malt extract (ME), (d) free amino nitrogen (FAN), (e) soluble protein (SP), and (f) Kolbach index (KI).

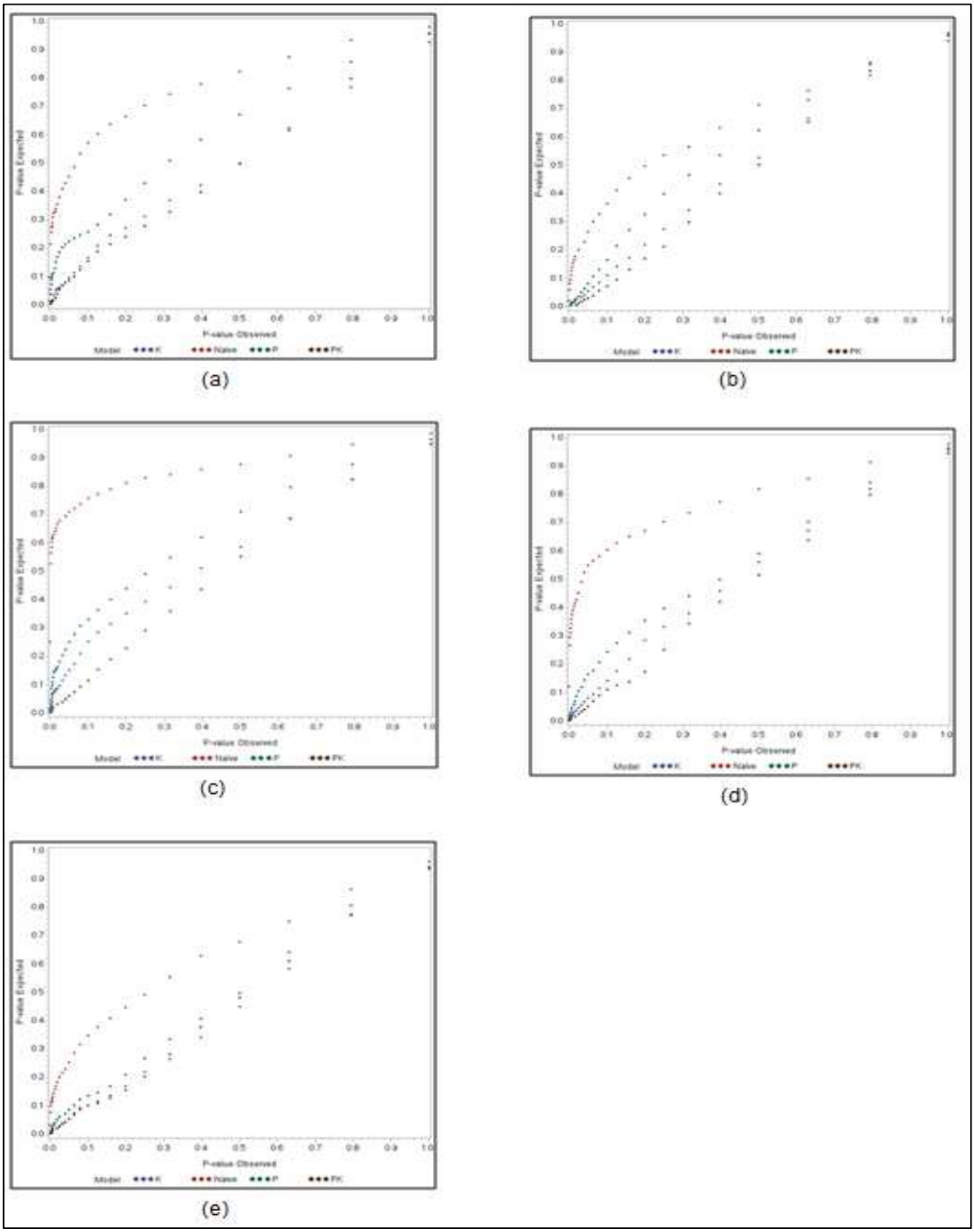

Figure 4.2. Plots of observed vs. expected p-values of four association mapping models for four malt and two grain quality traits: (a) wort color (WCO), (b) wort β-glucan (BG), (c) wort viscosity (VIS), (d) barley protein content (BPC), and (e) proportion of plump kernels (PL).

Figure 4.3. The number of significant marker-trait associations (MTAs) using four models for α-amylase (AA), diastatic power (DP), DP as a percent of grain protein (DPN), free amino nitrogen (FAN), soluble protein (SP), Kolbach index (KI), wort color (WCO), wort viscosity (VIS), wort β-glucan (BG), fine grind extract (ME), grain protein content (BPC), and proportion of plump kernels (PL). The numbers associated with the trait abbreviations in X-axis indicate the environments: Env1 = Bekoji in 2011, Env3 = Bekoji in 2012, Env4 = Fargo in 2012, Env6 = Koffele in 2012, and Env8 = Fargo in 2013.

Across the three groups of analyses, a total of 145 MTAs were identified in 49 genomic regions in all seven chromosomes for the nine malt quality and two grain quality traits, with the largest number concentrated in chromosome 5H (37) and the fewest in chromosome 1H (9) (Table 4.3 to Table 4.9). Thirty-five genomic regions contained QTL for more than one trait (Table A.21). Ten of these regions, i.e.; three regions each in chromosomes 5H (29.1-33.6, 50.5-59.7, and 167.4-173.5 cM) and 7H (3.5-9.7, 94.3-98.4, 121.4-135.9 cM); two regions in chromosome 3H (4.7-11.0 cM and 95.5-106.0 cM); and one region each in chromosome 2H (169.7 cM) and 4H (86.7-95.2 cM) were regions where QTL for malt and grain quality traits concentrated.

In chromosome 1H, nine QTL were identified for seven of the malt and grain quality traits evaluated (Table 4.3). These QTL were positioned in five regions (12.9-20.3, 45.2-53.4, 80.5, 122.3, and 138.7 cM); with each region observed to have QTL for one to three traits. Some of the QTL detected in chromosome 1H were observed in previous studies. Igartua et al. (2002) reported clusters of QTL for malt quality traits near the *Hor1* (13.8 cM) and *Hor2* (23.8 cM) loci in chromosome 1H, where QTL for wort color, β-glucan, and proportion of plump kernels were detected in the current study. The *Hor1* and *Hor2* loci code for C hordeins and B hordeins, respectively (Sogaard and Wettstein-Knowles, 1987); which are components of storage protein prolamine. The QTL for DP at 45.2-53.4 cM in chromosome 1H was in similar position to a QTL reported by Barr et al. (2003). The QTL for malt extract detected at 122.3 cM in chromosome 1H is in a similar region as a QTL reported previously for malt extract (Schmalebach and Pillen, 2009; Mohammadi et al., 2015).

Twenty-three QTL were detected in chromosome 2H for all the traits except α-amylase (Table 4.4), which were positioned in nine regions (4.7, 12.1-20.5, 29.7-34.2, 51.0-63.6, 101.7-105.6, 118.4, 130.4-142.7, 156.7-156.8, and 169.7 cM). QTL for one or more of the traits matched with QTL reported previously in many of these regions. At 12.1-20.5 cM in chromosome 2H, I detected QTL for soluble protein, FAN, wort viscosity, and malt extract. Zale et al. (2000) reported QTL in a similar region for wort viscosity and malt extract. A QTL for DP I identified in chromosome 2H at 29.7-34.2 cM was in a similar region as a QTL for DP identified by Zale et al. (2000). QTL for barley protein content and malt extract at 51.0-63.6 cM in chromosome 2H (Table 4.4) were in a similar region as a QTL for these traits identified by Cai et al. (2013) and Pauli et al (2015), respectively. Generally, excessive barley protein content is undesirable because it can lower malt extract level and cause problems with beer stability and viscosity (Igartua et al., 2002). However, some protein is needed to provide yeast nutrition, and enzymes are also proteins. A QTL was identified for DP at 118.4 cM in chromosome 2H in the current study, which correspond to QTL reported by Pauli et al (2015) at 113.5 cM. I identified QTL in the region of 130.4-142.7 cM related to protein modification (soluble protein, Kolbach Index, and FAN) and carbohydrate modification (β-glucan, wort viscosity, and malt extract). Emebiri et al. (2004) found a QTL for wort viscosity and Emebiri et al. (2004) and Matthies et al. (2014) found a QTL for malt extract in a similar region in chromosome 2H.

Table 4.3. Significant marker-trait associations for grain and malt quality traits in chromosome 1H according to the P+K model using all genotypes or subgroups of genotypes.

Position (cM)†	Trait	Group	SNP	NLP‡	%R^2	Significant§
12.9-20.3	Wort color	ICARDA/NDSU	12_30588_61	1.6-2.4	4.9-8.4	2 out of 3
	Wort β-glucan	Whole	12_30588_61	1.5-2.3	1.9-3.7	2 out of 3
		ICARDA/NDSU	12_30588_61	1.5-2.6	4.3-10.7	2 out of 3
	Plump kernels	Whole	11_20371_121 - 11_20712_121	1.42-2.3	2.5-3.4	2 out of 4
45.2-53.4	Diastatic power	ICARDA/NDSU	12_30110_61-11_20997_121	1.6-2.0	6.0-6.9	2 out of 3
	Wort color	Ethiopian	12_30343_61	1.4-2.4	5.6-11.0	2 out of 3
	Wort viscosity	ICARDA/NDSU	12_30343_61	1.9-2.3	5.7-9.4	2 out of 3
80.5	Diastatic power	Whole	11_20990_121	1.6-2.7	2.1-5.5	2 out of 3
122.3	Malt extract	Ethiopian	11_20908_121	2.4-2.4	6.5-6.7	2 out of 3
138.7	Soluble protein	Ethiopian	11_11105_121	1.5-1.8	3.8-4.6	2 out of 3

†The map distances for SNP markers are according to Munoz et al. (2011).

‡ NLP = -log10(p).

§ Number of environments where significant marker-trait associations were detected.

Table 4.4. Significant marker-trait associations for grain and malt quality traits in chromosome 2H according to the P+K model using all genotypes or subgroups of genotypes.

Position (cM)†	Trait	Group	SNP	NLP‡	%R^2	Significant§
4.7	Plump kernels	Whole	11_10326_121	2.14-2.2	3.2-4.5	2 out of 4
12.1-20.5	Soluble protein	Whole	12_30631_61-12_10777_61	1.4-2.7	1.8-4.5	3 out of 3
	Free amino nitrogen	Whole	12_30631_61-12_10777-62	1.31-2.2	1.9-3.5	2 out of 3
	Wort viscosity	Ethiopian	12_30631_40	1.5-1.8	3.9-7.9	2 out of 3
		ICARDA/NDSU	12_30631_61-12_10777_61	1.4-2.1	4.6-7.2	2 out of 3
	Malt extract	Ethiopian	11_10943_121	1.6-2.1	4.0-5.7	2 out of 3
29.7-34.2	Diastatic power	Whole	11_10891_121	1.5-2.6	2.1-5.2	2 out of 3
51.0-63.6	Malt extract	ICARDA/NDSU	12_10485_61	1.7-1.8	5.3-5.8	2 out of 3
	Barley protein	Whole	12_30703_61 -11_10325_121	1.36-2.5	1.6-3.4	4 out of 6
101.7-105.6	Wort β-glucan	ICARDA/NDSU	12_10936_61-11_10214_121	1.5-1.8	5.6-5.9	2 out of 3
118.4	Diastatic power	Ethiopian	12_30555_61	1.6	4.1	2 out of 3
130.4-142.7	Soluble protein	Ethiopian	12_31095_61-12_10739_61	1.4-2.5	3.4-7.0	3 out of 3
	Kolbach Index	Ethiopian	12_31095_61-12_10739_61	1.4-1.9	3.5-5.7	3 out of 3
	Free amino nitrogen	Ethiopian	12_31100_61	1.7-2.4	6.5-7.2	2 out of 3
	Wort β-glucan	Ethiopian	12_31100_61	2.3-3.2	6.2-9.4	2 out of 3
		ICARDA/NDSU	12_10739_47	1.9-2.2	5.8-7.8	2 out of 3
	Wort viscosity	ICARDA/NDSU	12_10739_47	1.9-2.4	5.8-8.5	2 out of 3
		Ethiopian	12_31100_61	3.1-3.9-	9.0-11.5	2 out of 3
	Malt extract	ICARDA/NDSU	11_20141_89	1.9-2.0	6.0-7.0	2 out of 3
154.7-156.8	Free amino nitrogen	Whole	12_30341_61	1.5-2.5	2.8-3.8	2 out of 3
	Wort color	Ethiopian	12_30352_61	1.5-1.7	3.7-4.3	2 out of 3
169.7	Diastatic power	ICARDA/NDSU	11_10085_121	2.2-2.9	7.8-9.8	3 out of 3
		Whole	11_10085_121	1.7-2.2	3.1-3.2	2 out of 3
	Soluble protein	ICARDA/NDSU	11_10085_121	1.6-2.2	4.9-8.9	2 out of 3
	Free amino nitrogen	ICARDA/NDSU	11_10085_121	1.3-2.2	3.8-9.0	2 out of 3
	Wort β-glucan	ICARDA/NDSU	11_10085_121	1.7-2.0	5.0-7.9	3 out of 3
		Ethiopian	11_10085_140	3.8-5.7	11.2-17.1	2 out of 3
	Wort viscosity	ICARDA/NDSU	11_10085_121	2.1-3.2	6.6-13.5	2 out of 3
		Ethiopian	11_10085_140	4.6-5.4	13.8-16.1	2 out of 3

†The map distances for SNP markers are according to Munoz et al. (2011).
‡NLP = -log10(p).
§Number of environments where significant marker-trait associations were detected.

In chromosome 3H, 27 QTL were detected for all malt and grain quality traits except proportion of plump kernels (Table 4.5). These QTL were clustered in eight regions (4.7-11.0, 32.9, 44.3, 51.0-58.6, 69.9, 95.5-106.0, 146.3, and 167.3 cM). QTL for enzymatic activity (α-amylase and DP), protein modification (Kolbach Index and FAN), and carbohydrate modification (β-glucan, wort viscosity, and malt extract) were detected at 4.7-11.0 cM. Sdeghi et al. (2013) reported a QTL for α-amylase in a similar region. In the current study, QTL were reported for soluble protein, Kolbach Index, FAN, malt extract, and barley protein content at 32.9 cM in chromosome 3H (Table 4.5). Igartua et al. (2002) found clusters of QTL for multiple malt quality traits in the same region, which is near the *Upg2* locus that codes for the enzyme UDP-glucose pyrophosphorylase (Meng et al, 2009). This enzyme produces UDP-glucose, which is essential for sucrose and polysaccharide synthesis. I also detected QTL for soluble protein, barley protein content, and proportion of plump kernels at 51.0-58.6 cM (Table 4.5), which is near another cluster of malt quality QTL found by Igartua et al. (2002). This region also is the location of the *Dor4* locus, which codes for seed dormancy. In the region at 96.5-106.0 cM in chromosome 3H, I identified QTL for six traits: α-amylase, DP, soluble protein, FAN, wort color, and β-glucan. QTL were reported in a similar region for α-amylase and wort β-glucan (Emebiri et al., 2004), and FAN (Islamovic et al., 2014). The QTL identified at 146.3 cM for α-amylase was found in a similar region as a QTL for the same trait reported by Zale et al. (2000). QTL for β-glucan and malt extract were identified at 167.3 cM (Table 4.5), which placed them within 11 cM distance from a QTL reported by Mohammadi et al. (2015) for malt extract.

The second fewest number of QTL (11) were found in chromosome 4H (Table 4.6), which were positioned in five regions (58.1-63.4, 86.7-92.4, 95.2-103.9, 116.7, and 144.4 cM). Of these regions, the one at 58.1-63.4 cM had QTL for DP, wort color, and malt extract. Emebiri et al. (2004) found a QTL for DP and Gao et al. (2004) found QTL for DP and malt extract in the same region. The position at 95.2-103.9 cM included QTL for wort viscosity and malt extract, which overlapped with QTL reported by Schmalenbach and Pillen (2009). A QTL for malt extract was also found at 116.7 cM (Table 4.6), where Zale et al. (2000) reported a QTL for malt extract in the same region. The last of the significant regions in chromosome 4H at 144.4 cM included a QTL for DP (Table 4.6), which was in line with one identified previously in multiple studies (Zale et al., 2000; Igartua et al., 2002; Mohammadi et al., 2015). The QTL at

this position could be associated with the *Bmy1* locus (Igartua et al., 2002; Guerin et al., 1992). The *Bmy1* locus controls the enzyme β-amylase, which is the main component of DP (Berger and LaBerge, 1985; Georg-Kraemer et al., 2001; Islamovic et al., 2014).

Chromosome 5H had the largest number of QTL (37), which were mapped to 10 regions (Table 4.7). The GrainGenes database (http://wheat.pw.usda.gov/GG2/Barley/), includes more than 100 QTL reported in papers before January 2008 for seven malt and grain quality traits, including α-amylase, DP, barley protein content, malt extract, Kolbach Index, β-glucan, and wort viscosity. The majority of these QTL were mapped in chromosome 5H.

Previous mapping studies for malt and grain quality traits detected QTL in the majority of the 10 regions in chromosome 5H detected in the present study. The first was positioned at 11.4-12.8 cM where QTL for FAN and malt extract were detected (Table 4.7). QTL for seven malt quality traits were mapped in the region positioned from 29.1-33.6 cM (Table 4.7). Previous studies identified QTL for soluble protein (Islamovic et al., 2014; Pauli et al, 2015), FAN (Matus et al., 2003; Emebiri et al., 2004; Pauli et al., 2015), and β-glucan (Matus et al., 2003; Islamovic et al., 2014) in the same region. QTL for six malt quality traits were found in the region from 50.5-59.7 cM (Table 4.7), where previous studies found QTL for α-amylase (Zale et al., 2000; Schmalebach and Pillen, 2009), DP (Zale et al., 2000; Islamovic et al., 2014), and wort viscosity (Zhou et al., 2012). Igartua et al. (2002) identified a cluster of QTL for malt quality traits near the *Ltp1* locus (47.3 cM) and near RFLP marker *ABC302* (68.8 cM) in chromosome 5H. The *Ltp1* locus codes for the lipid transport protein (Sogaard and Wettstein-Knowles, 1987), which likely impacts α-amylase/protease inhibitors. Of the three traits (α-amylase, soluble protein, and FAN) identified at 93.7 cM in chromosome 5H (Table 4.7), QTL were reported in previous studies in the same region for α-amylase (Zwickert-Menteur et al., 1996) and soluble protein (Matthies et al., 2014). I found four QTL in the region from 138.2-139.0 cM in chromosome 5H (Table 4.7), of which α-amylase (Zale et al., 2000; Matus et al., 2003), Kolbach Index (Zhou et al., 2012), and malt extract (Zale et al., 2000; Zhou et al., 2012; Matthies et al., 2014) were mapped in the same region previously. In the region from 167.4-173.5 cM, I detected QTL for α-amylase, DP, soluble protein, Kolbach Index, wort color, and wort viscosity (Table 4.7). Similarly, QTL were reported for α-amylase (Emebiri et al., 2004; Pauli et al., 2015), DP (Zale et al., 2000) and soluble protein (Matthies et al., 2014; Mohammadi et al., 2015) in the same region. In

the current study, a QTL for β-glucan was identified at 189.2 cM in chromosome 5H (Table 4.7), which was also detected in an association mapping study by Mohammadi et al. (2015) between 183.3 cM and 188.2 cM as well as by Pauli et al (2015) between 189.2 cM and 189.9 cM.

A total of 10 QTL were detected in chromosome 6H, which were positioned in five regions (27.3-33.0, 49.2, 55.7-65.8, 70.5-79.2, and 88.9-98.7 cM) (Table 4.8). Several matching QTL were also reported in previous studies. The region at 55.7-65.8 cM in chromosome 6H had QTL for DP and β-glucan (Table 4.8). In the same region, Zale et al. (2000), Islamovic et al. (2014), and Mohammadi et al. (2015) reported QTL for DP; and Zhou et al. (2012) found a QTL for β-glucan. In the region from 70.5-79.2 cM, I identified QTL for DP and FAN (Table 4.8). Previous studies also found QTL for DP in this region (Zale et al., 2000; Islamovic et al. (2014). The region from 88.9 to 98.7 cM included QTL for α-amylase, DP, and malt extract (Table 4.8). In previous studies, QTL for α-amylase (Zale et al., 2000; Mohammadi et al., 2015), DP (Islamovic et al., 2014), and malt extract (Schmalenbach and Pillen, 2009; Mohammadi et al., 2015) were reported in the same region. Igartua et al. (2002) reported that the *Amy1* locus (Sogaard and Wettstein-Knowles, 1987) mapped to this region (88.1 cM). *Amy1* is one of the two α-amylase isozymes, which hydrolyzes (1-4)-α-glycosidic bonds in amylose, amylopectin, and related oligosaccharides (Jensen et al., 2003; and references therein).

In chromosome 7H, 30 QTL were identified in seven regions (3.3-9.7, 18.7, 55.0, 83.4-89.8, 94.3-98.4, 109.4, and 121.4-135.9 cM) (Table 4.9). Soluble protein and Kolbach Index mapped to position of 18.9 cM, where Islamovic et al. (2014) also detected QTL for soluble protein. Five malt and grain quality traits mapped to the position at 55.0 cM in chromosome 7H (Table 4.9). Three of these traits, wort viscosity (Matthies et al., 2014), β-glucan (Pauli et al., 2015), and malt extract (Zale et al., 2000) mapped to the same region. I found QTL for protein modification traits (soluble protein, Kolbach Index, and FAN) and β-glucan in the region from 83.4-89.8 cM (Table 4.9). A QTL for β-glucan was found in a similar region in multiple studies (Han et al., 2004; Islamovic et al., 2014; Mohammadi et al., 2015). Furthermore, Igartua et al. (2002) reported a cluster of QTL for malt quality traits near the *Amy2* locus in chromosome 7H (88.1 cM). The two isozymes (*Amy1* and *Amy2*) differ in their isoelectric points (pI), stability in elevated temperature, and tendency to be inhibited by endogenous α-amylase inhibitor (Ajandouz et al., 1992; Jensen et al., 2003). *Amy2* has high *pI* (5.1-6.1) and high survival in kilning, but inhibited by α-

amylase inhibitor; and *Amy2* generally dominates in the malt (Ajandouz et al., 1992; Georg-Kraemer et al., 2001; Jensen et al., 2003). Seven QTL were detected in the region from 94.3-98.4 cM in chromosome 7H, one for enzymatic activity (α-amylase), four for protein modification traits (soluble protein, Kolbach Index, FAN, and wort color), and two for grain quality traits (barley protein content and proportion of plump kernels). Mather et al. (1996) and Barr et al. (2003) reported QTL for α-amylase while Emebiri et al. (2004) and Pauli et al. (2015) found QTL for barley protein content in the same region. A QTL for DP was identified at 109.4 cM (Table 4.9), which is in the same region where Mather et al. (1996) identified a QTL for DP.

The previous paragraphs in this section particularly discussed QTL that mapped to regions where previous studies had reported similar QTL. In addition to this work, an important goal of this research was to identify unique QTL for malt and grain quality traits, particularly in the ICARDA/NDSU accessions that can be utilized by the Ethiopian breeding program. In the current study, a total of 58 unique QTL were detected in the ICARDA/NDSU genotypes for the nine malt quality traits, with more than 50% of these found in chromosomes 5H (7 regions) and 7H (5 regions). Particularly, two regions each in chromosomes 5H (50.5-59.7 cM and 118.2-125.1 cM) and 7H (3.5-9.7 cM and 83.3-98.4 cM) contained QTL for several malt quality traits unique to the ICARDA and NDSU genotypes. Thus, focused marker-based malt barley breeding could be employed for these specific regions. Effects of each marker reflected how the traits were related (Table 4.10). For instance, SNP12_30538_61 showed negative mean effect for α-amylase, DP, soluble protein, and wort color, indicating that these traits were related positively. Generally, haplotypes containing combinations of favorable genotypes at each marker locus resulted in good mean values of the trait (data not shown). Conversely, haplotypes with combinations of unfavorable genotypes lead to poor quality for each trait. The NDSU genotypes were found to have the favorable haplotype, which is not surprising. In this study, the malt quality traits generally were controlled by several loci distributed across the genome, each with a small effect. Thus, a sort of genomic selection may be an appropriate strategy for improving malt and grain quality traits.

Table 4.5. Significant marker-trait associations for grain and malt quality traits in chromosome 3H according to the P+K model using all genotypes or subgroups of genotypes.

Position (cM)†	Trait	Group	SNP	NLP‡	%R^2	Significant§
4.7-11.0	α-amylase	Whole	11_21398_121-12_30818_61	1.9-2.6	3.0-4.0	3 out of 3
	Diastatic power	Whole	11_21398_121-12_30818_61	1.5-2.3	1.9-4.4	3 out of 3
	Kolbach Index	ICARDA/NDSU	12_30818_61	1.7-1.7	5.2-5.5	2 out of 3
		Whole	12_30818_61-11_21398_121	1.6-2.9	2.1-5.6	2 out of 3
	Free amino nitrogen	Whole	12_30818_61-11_20252_121	1.5-2.9	2.6-4.4	3 out of 3
	Wort β-glucan	Whole	11_21398_121-12_30818_61	1.7-2.0	2.4-3.7	2 out of 3
	Wort viscosity	Whole	11_21398_121-11_21398_124	1.5-2.2	2.6-3.5	2 out of 3
		ICARDA/NDSU	11_21398_124	1.8-3.2	5.5-3.5	2 out of 3
	Malt extract	Whole	11_21398_121- 12_30818_61	1.3-3.3	1.7-6.8	3 out of 3
	Barley protein	Whole	11_20252_121- 11_21398_121	1.6-2.3	2.9-4.3	3 out of 6
32.9	Soluble protein	Ethiopian	12_30571_61	1.9-2.1	5.1-5.8	2 out of 3
	Kolbach Index	Ethiopian	12_30571_61	2.3-3.0	6.4-8.7	2 out of 3
	Free amino nitrogen	Ethiopian	12_30571_61	1.8-1.9	4.8-5.1	2 out of 3
	Malt extract	Ethiopian	12_30571_61	2.3	6.3	2 out of 3
	Barley protein	Whole	12_30571_61	1.4-2.1	2.0-2.8	3 out of 6
44.3	Soluble protein	Ethiopian	SCRI_RS_127994_61	1.6-2.8	4.1-7.8	2 out of 3
	Free amino nitrogen	Ethiopian	SCRI_RS_127994_61	1.9-2.7	4.9-7.6	2 out of 3
51.0-58.6	Soluble protein	Whole	12_30818_61	1.5-2.3	2.0-3.3	2 out of 3
	Barley protein	Whole	12_30009_61	1.5-2.0	1.8-3.8	3 out of 6
69.9	Diastatic power	ICARDA/NDSU	12_30009_61	1.6-1.9	4.7-6.6	2 out of 3
96.5-106.0	α-amylase	Whole	12_31367_61-SCRI_RS_225641_61	1.8-2.1	2.4-4.1	2 out of 2
	Diastatic power	Whole	SCRI_RS_163092_61	1.4-2.0	2.0-2.8	2 out of 3
	Soluble protein	Whole	12_31018_61	1.8-2.0	2.5-3.8	2 out of 3
	Free amino nitrogen	Whole	SCRI_RS_225641_61-12_31018_61	1.4-2.6	1.8-3.8	2 out of 3
		Ethiopian	SCRI_RS_225641_61	1.7-2.0	4.4-5.2	2 out of 3
	Wort color	ICARDA/NDSU	SCRI_RS_225641_61-12_31367_61	1.7-1.9	5.8-7.6	2 out of 3
		Whole	12_31018_61-12_31018_61	1.4-2.5	1.8-4.9	2 out of 3
	Wort β-glucan	Whole	12_31367_61	1.6-3.5	2.1-7.2	2 out of 3
146.3	α-amylase	Ethiopian	11_20085_121	2.5-2.8	6.9-8.0	2 out of 3
167.3	Wort β-glucan	ICARDA/NDSU	12_20198_69	1.9-2.3	5.8-9.3	2 out of 3
		Whole	12_20198_69	2.4-2.7	4.0-4.1	2 out of 3
	Malt extract	ICARDA/NDSU	12_20198_69	1.7-1.9	5.1-7.5	2 out of 3

†The map distances for SNP markers are according to Munoz et al. (2011).
‡NLP = -log10(p).
§Number of environments where significant marker-trait associations were detected.

Table 4.6. Significant marker-trait associations for grain and malt quality traits in chromosome 4H according to the P+K model using all genotypes or subgroups of genotypes.

Position (cM)†	Trait	Group	SNP	NLP‡	%R^2	Significant§
58.1-63.4	Diastatic power	Ethiopian	11_10262_121	1.3-2.4	5.2-6.6	2 out of 3
		Whole	11_10262_121	1.9-2.0	2.8-3.6	2 out of 3
	Wort color	Ethiopian	SCRI_RS_189180_61	1.5-1.9	3.7-5.2	2 out of 3
	Malt extract	ICARDA/NDSU	SCRI_RS_189180_61	1.6-1.9	5.5-7.7	3 out of 3
86.7-92.4	Diastatic power	Ethiopian	12_31246_54	1.6-2.0	5.3-6.7	2 out of 3
	Soluble protein	Ethiopian	11_11004_121	1.6-2.1	6.5-5.6	2 out of 3
	Kolbach Index	Whole	11_11004_121	1.8-2.1	3.0-3.3	2 out of 3
	Wort viscosity	ICARDA/NDSU	12_31246_61	1.5-1.8	5.0-6.8	3 out of 3
95.2-103.9	Wort viscosity	Ethiopian	SCRI_RS_148330_61	2.0-2.3	5.2-6.4	2 out of 3
		Whole	SCRI_RS_148330_61	1.8-3.0	2.9-4.7	2 out of 3
	Malt extract	Ethiopian	11_20178_121	1.5-1.7	3.6-4.5	2 out of 3
116.7	Malt extract	Whole	11_20974_121	1.6-2.2	2.3-3.5	2 out of 3
144.4	Diastatic power	ICARDA/NDSU	11_11186_121	1.6-2.2	5.2-6.9	2 out of 3

†The map distances for SNP markers are according to Munoz et al. (2011).
‡NLP = -log10(p).
§Number of environments where significant marker-trait associations were detected.

Table 4.7. Significant marker-trait associations for grain and malt quality traits in chromosome 5H according to the P+K model using all genotypes or subgroups of genotypes.

Position (cM)†	Trait	Group	SNP	NLP‡	%R^2	Significant§
11.4-12.8	Free amino nitrogen	Whole	11_20010_121	1.4-2.2	2.3-3.2	2 out of 3
	Malt extract	ICARDA/NDSU	12_31094_61	1.6-1.7	4.7-6.7	2 out of 3
29.1-33.6	α-amylase	Whole	11_20845_124-11_20845_129	2.0-2.0	2.8-3.2	2 out of 3
	Diastatic power	Whole	11_20845_121-11_20845_129	1.7-2.4	2.4-4.7	2 out of 3
	Soluble protein	Whole	11_10621_121-11_20845_121	1.4-2.1	2.1-4.0	2 out of 3
	Kolbach Index	Whole	11_10621_121-11_208845_124	1.4-2.1	2.0-3.0	2 out of 3
	Free amino nitrogen	Whole	SCRI_RS_108416_61-11_20845_121	1.5-2.8	2.1-5.6	3 out of 3
	β-glucan	Whole	11_10621_121-11_20645_121	1.5-2.5	2.1-3.4	3 out of 3
	Wort viscosity	Whole	11_10580_79-11_20845_124	1.6-2.4	2.2-3.9	2 out of 3
		Ethiopian	11_20845_124	1.7-1.8	4.4-4.8	2 out of 3
50.5-59.7	α-amylase	Whole	12_30538_61- 11_10641_121	1.4-2.6	1.8-5.2	3 out of 3
		ICARDA/NDSU	12_30538_61	1.8-2.1	6.2-6.7	2 out of 3
	Diastatic power	Whole	12_30538_61-11_10641_121	1.4-2.5	1.8-4.9	2 out of 3
		ICARDA/NDSU	12_30538_61-11_10641_121	2.0-2.3	6.9-7.3	2 out of 3
	Soluble protein	Whole	12_30538_61-11_10641_121	1.31-2.9	1.8-6.0	2 out of 3
		ICARDA/NDSU	12_30538_61-11_10641_121	1.6-2.2	5.4-7.6	2 out of 3
	Free amino nitrogen	Whole	11_10641_121	3.1-3.8	4.8-7.9	2 out of 3
		ICARDA/NDSU	10641_121	2.1-2.1	6.7-7.5	2 out of 3
	Wort color	ICARDA/NDSU	12_30538_61	1.6-2.0	5.4-6.4	2 out of 3
	Wort viscosity	Whole	12_30538_61	1.8-2.4	2.5-3.9	2 out of 3
93.7	α-amylase	Whole	11_11350_121	2.1-2.9	4.1-4.4	2 out of 3
	Soluble protein	Whole	11_11350_121	1.9-3.0	3.5-4.7	2 out of 3
		ICARDA/NDSU	11_11350_121	1.6-2.7	6.2-8.9	2 out of 3
	Free amino nitrogen	Whole	11_11350_121	1.5-3.0	2.5-4.7	2 out of 3
107.2	Malt extract	ICARDA/NDSU	12_30456_61	1.5-2.0	4.5-7.8	2 out of 3

Table 4.7. Significant marker-trait associations for grain and malt quality traits in chromosome 5H according to the P+K model using all genotypes or subgroups of genotypes (continued).

Position (cM)†	Trait	Group	SNP	NLP‡	%R^2	Significant§
118.2-125.1	α-amylase	ICARDA/NDSU	12_30067_61	1.9-2.2	6.7-6.8	2 out of 3
	Free amino nitrogen	Whole	11_21247_119	1.4-2.6	2.4-3.9	2 out of 3
		ICARDA/NDSU	11_21247_119	1.8-2.6	5.9-8.7	2 out of 3
	β-glucan	ICARDA/NDSU	11_20653_121	1.8-2.4	6.2-7.6	2 out of 3
	Malt extract	ICARDA/NDSU	11_11507_56	1.7	5.2-5.3	2 out of 3
138.2-139.0	α-amylase	Whole	11_20551_105-11_20375_121	1.31-2.2	1.7-3.2	2 out of 3
		ICARDA/NDSU	11_20551_105-11_20375_121	1.6-1.7	5.6-6.3	2 out of 3
	Kolbach Index	Whole	11_20551_105	1.4-2.4	1.8-4.7	2 out of 3
	β-glucan	Whole	11_20551_105	1.5-2.1	2.0-4.0	2 out of 3
		ICARDA/NDSU	11_20551_105	1.5-1.6	4.7-6.1	2 out of 3
	Malt extract	Whole	11_20551_105	1.7-2.0	2.3-3.9	2 out of 3
157.6-162.0	Free amino nitrogen	Ethiopian	11_20826_121	1.9-1.9	5.1-5.1	2 out of 3
	Wort color	Ethiopian	11_20826_121	1.8-1.8	4.6-4.9	2 out of 3
	β-glucan	Ethiopian	12_30162_61-11_20829_121	1.5-1.7	6.4-7.1	2 out of 3
167.4-173.5	α-amylase	Ethiopian	11_10600_121 - 11_20536_121	1.6-2.1	6.5-5.7	3 out of 3
	Diastatic power	Ethiopian	11_20536_121	1.8-2.3	7.7-6.4	2 out of 3
	Soluble protein	Ethiopian	11_20536_121-12_30504_68	1.6-2.4	4.0-6.6	3 out of 3
	Kolbach Index	Ethiopian	11_20536_121-12_30504_68	1.6-1.8	4.2-7.9	2 out of 3
	Wort color	ICARDA/NDSU	12_30504_61	1.6-1.7	4.7-5.5	2 out of 3
	Wort viscosity	Ethiopian	12_30494_61-12_30504_68	1.5-1.6	6.0-6.8	2 out of 3
189.2	β-glucan	Ethiopian	12_10322_55	1.7-2.3	4.5-6.4	2 out of 3

†The map distances for SNP markers are according to Munoz et al. (2011).
‡NLP = -log10(p).
§Number of environments where significant marker-trait associations were detected.

Table 4.8. Significant marker-trait associations for grain and malt quality traits in chromosome 6H according to the P+K model using all genotypes or subgroups of genotypes.

Position (cM)†	Trait	Group	SNP	NLP‡	%R^2	Significant§
27.3-33.0	Free amino nitrogen	ICARDA/NDSU	11_10136_121-12_31485_61	1.7-1.8	6.4-7.1	2 out of 3
	Malt extract	Ethiopian	11_10136_121	1.4-2.2	5.5-6.0	2 out of 3
49.2	Diastatic power	ICARDA/NDSU	11_10462_121-SCRI_RS_186193_61	1.6-2.3	4.9-9.2	2 out of 3
55.7-65.8	Diastatic power	ICARDA/NDSU	12_11253_61-12_10758_61	1.31-2.6	4.1-11.0	2 out of 3
	β-glucan	Ethiopian	SCRI_RS_186193_61	1.5-1.5	3.7-3.8	2 out of 3
		ICARDA/NDSU	12_10758_61	2.3-2.4	7.5-9.7	2 out of 3
		Whole	12_10758_61	2.1-2.6	2.9-4.4	2 out of 3
70.5-79.2	Diastatic power	Whole	11_20892_121	1.4-2.3	2.1-3.3	2 out of 3
	Free amino nitrogen	ICARDA/NDSU	11_11483_121	1.7	5.0-5.7	2 out of 3
88.9-98.7	α-amylase	Whole	11_10220_12-12_30698_61	1.5-2.5	2.2-3.7	3 out of 3
	Diastatic power	ICARDA/NDSU	12_30698_61	1.7-2.0	5.8-6.1	2 out of 3
	Malt extract	ICARDA/NDSU	12_30698_61	1.9-2.6	6.0-10.9	2 out of 3
		Whole	12_30698_61	1.4-2.8	2.5-4.7	3 out of 3

†The map distances for SNP markers are according to Munoz et al. (2011).
‡NLP = -log10(p).
§Number of environments where significant marker-trait associations were detected.

Table 4.9. Significant marker-trait associations for grain and malt quality traits in chromosome 7H according to the P+K model using all genotypes or subgroups of genotypes.

Position (cM)†	Trait	Group	SNP	NLP‡	%R^2	Significant§
3.3-9.7	α-amylase	Whole	11_20710_121	1.8-2.3	3.3-4.2	2 out of 3
		ICARDA/NDSU	11_20710_121	1.5-1.6	4.8-5.1	2 out of 3
	Diastatic power	ICARDA/NDSU	11_10841_121	1.6-1.9	4.7-7.3	3 out of 3
	Soluble protein	ICARDA/NDSU	11_20710_121	1.8-2.0	5.5-6.7	2 out of 3
		Whole	11_20710_121	1.8-2.3	2.5-4.4	2 out of 3
	Kolbach Index	Whole	11_20710_121	2.0-2.5	3.7-3.8	2 out of 3
		Ethiopian	11_10841_121	1.8-2.0	4.6-8.9	2 out of 3
		ICARDA/NDSU	11_20710_121	1.7-2.1	5.6-6.6	2 out of 3
	Wort color	ICARDA/NDSU	11_20710_121	1.4-2.0	4.4-6.2	2 out of 3
	β-glucan	Whole	11_20710_121	1.5-2.6	2.2-3.8	3 out of 3
	Malt extract	Ethiopian	11_20710_121-11_10841_121	1.6-2.8	4.0-7.8	3 out of 3

Table 4.9. Significant marker-trait associations for grain and malt quality traits in chromosome 7H according to the P+K model using all genotypes or subgroups of genotypes (continued).

Position (cM)†	Trait	Group	SNP	NLP‡	%R^2	Significant§
18.7	Soluble protein	Ethiopian	11_20495_121	2.4-2.4	6.7-6.8	2 out of 3
	Kolbach Index	Ethiopian	11_20495_121	2.0-2.2	5.4-6.1	2 out of 3
55.0	β-glucan	ICARDA/NDSU	12_30880_61	1.6-1.7	4.8-5.5	2 out of 3
	Wort viscosity	ICARDA/NDSU	12_30880_61	1.33-2.6	4.9-7.6	3 out of 3
		Whole	12_30880_61	2.4-2.5	3.7-4.0	2 out of 3
	Malt extract	Ethiopian	12_30880_61	1.8-1.9	4.6-5.1	2 out of 3
	Barley protein	Whole	12_30880_61	2.11-2.5	3.1-4.2	2 out of 4
83.4-89.8	Soluble protein	Whole	12_10982_61	1.4-2.6	2.5-5.1	2 out of 3
	Kolbach Index	Whole	12_10982_61- 11_20083_121	1.5-3.1	2.1-5.5	2 out of 3
	Free amino nitrogen	ICARDA/NDSU	12_10982_61	1.7-2.3	5.5-7.5	2 out of 3
		Whole	12_10982_61	2.8-3.2	4.9-5.7	2 out of 3
	β-glucan	Whole	12_10982_61	1.7-2.7	3.8-5.5	2 out of 3
94.3-98.4	α-amylase	ICARDA/NDSU	12_30026_61 -SCRI_RS_112204_61	1.7-1.8	5.8-7.1	2 out of 3
	soluble protein	ICARDA/NDSU	SCRI_RS_112204_61	1.5-2.0	4.2-7.8	2 out of 3
	Kolbach index	ICARDA/NDSU	SCRI_RS_112204_61	1.5-2.2	4.8-9.2	3 out of 3
	Free amino nitrogen	ICARDA/NDSU	SCRI_RS_112204_61	1.3-2.9	3.7-2.3	2 out of 3
	Wort color	ICARDA/NDSU	12_30026_61	1.7-2.0	5.6-6.1	2 out of 3
	Barley protein content	Whole	SCRI_RS_112204_61	1.41-3.0	2.4-6.0	3 out of 6
	Proportion of plump kernels	Whole	12_30996_61	1.6-2.8	2.9-4.3	3 out of 4
109.4	Diastatic power	Whole	11_20824_121	1.4-2.6	1.8-4.4	2 out of 3
121.4-135.9	α-amylase	Ethiopian	11_10182_121 -11_10797_121	1.4-2.0	3.5-5.5	3 out of 3
	Diastatic power	Ethiopian	11_11243_100-11_20185_121	2.2-3.2	6.1-9.3	2 out of 3
	Soluble protein	Ethiopian	11_10182_121-11_20185_136	1.7-2.6	4.2-7.1	2 out of 3
	Kolbach Index	Ethiopian	11_10182_121-11_20185_121	1.6-2.2	4.0-6.1	2 out of 3
	Free amino nitrogen	Ethiopian	11_10182_121-11_10085_121	1.4-2.4	3.6-6.7	2 out of 3

†The map distances for SNP markers are according to Munoz et al. (2011).

‡NLP = -log10(p).

§Number of environments where significant marker-trait associations were detected..

Table 4.10. Mean effects and favorable genotypes of markers association with several malt quality traits in two regions of chromosome 5H (50.5-59.7 and 1118.2-125.1 cM)) and two regions of chromosome 7H (3.5-9.7 and 83.4-98.4 cM).

Traits	Chromosome	Locus	Position	Alleles (0/2)†	Mean effect (2-0)‡	Favorable allele
α-amylase	5H	12_30538_61	50.5	A/G	-8.5	AA
Diastatic power	5H	12_30538_61	50.5	A/G	-22.4	AA
Soluble protein	5H	12_30538_61	50.5	A/G	-0.4	AA
Wort color	5H	12_30538_61	50.5	A/G	-0.2	AA
α-amylase	5H	11_10641_121	59.7	G/A	6.8	AA
Diastatic power	5H	11_10641_121	59.7	G/A	16.4	AA
Free amino nitrogen	5H	11_10641_121	59.7	G/A	19.8	AA
Malt extract	5H	11_11507_56	118.2	G/A	2.1	AA
β-glucan	5H	11_20653_121	121.7	G/A	-140.4	AA
α-amylase	5H	12_30067_61	123.8	G/A	8.7	AA
Free amino nitrogen	5H	11_21247_119	125.1	C/A	42.5	AA
α-amylase	7H	11_20710_121	3.5	C/G	9.5	GG
Kolbach Index	7H	11_20710_121	3.5	C/G	4.4	GG
Soluble protein	7H	11_20710_121	3.5	C/G	0.6	GG
Wort color	7H	11_20710_121	3.5	C/G	0.2	GG
Diastatic power	7H	11_10841_121	9.7	A/G	-21.9	AA
Free amino nitrogen	7H	12_10982_61	83.4	G/C	41.0	CC
Wort color	7H	12_30026_61	94.3	G/A	0.2	AA
Kolbach Index	7H	SCRI_RS_112204_61	98.4	T/C	0.3	CC
Soluble protein	7H	SCRI_RS_112204_61	98.4	T/C	2.5	CC
Free amino nitrogen	7H	SCRI_RS_112204_61	98.4	T/C	19.4	CC

†0 = major (dominant) allele, and 2 = minor allele.

‡Mean difference between homozygous genotype for minor allele (2) and homozygous genotype for major allele (0).

Summary and Conclusions

Selection among germplasm introductions started the development of Ethiopian malt barley cultivars by local breeding programs. Later, crossing programs were started to create variability for malt barley. However, there is still shortage of genetic variability for different malt quality attributes in the Ethiopian breeding program. This suggests the need for identifying outside germplasm that may provide new variability for favorable alleles for the Ethiopian malt barley-breeding program. The NDSU barley-breeding program has had improvements in malt quality a priority since the mid-1940s and it likely has QTL that can be utilized by the Ethiopian breeding program for improving malt quality.

Four modles were compared to identify the best one for the current mapping panels. Just like the agronomic and disease resistance traits, the P+K and K models were the most appropriate ones for the current mapping panels for all the malt and grain quality traits. This is expected because the current mapping population is highly structure according to row-type, geographic origin, and breeding history. Hence, control for population structure and familial relatedness is crucial to reduce the level of false positives. A total of 145 QTL were identified for nine malt quality traits recorded on grain samples harvested and malted from two experiments, barley protein content on grain harvested from five experiments, and barley kernel plumpness on grain harvested from three experiments. Many of these QTL corresponded to previously reported QTL or known genes. Previous studies indicated the positions of malt quality traits in chromosome 1H near the *Hor1* and *Hor2* loci (13.8-23.8 cM) and the *GLB1* locus (68.1 cM); in chromosome 2H near the *vrs1* locus (92.6 cM); in chromosome 3H near the *Upg2* locus (25.2-36.0 cM) and the *Dor4* locus (50.2 cM); in chromosome 4H near the *Bmy1* locus (143.4 cM); in chromosome 5H near the *Ltp1* locus (44.6 cM); in chromosome 6H near the *Amy1* locus (88.1 cM); and in chromosome 7H near the *Amy2* locus (88.1 cM). Overall, the current association mapping study detected QTL for malt and grain quality traits near all of the aforementioned loci except the *GLB1* and *vrs1* loci. There was a gap in markers coverage near the *vrs1* locus in the current study, which may be the reason why I did not detect QTL around the *vrs1* locus.

The unique QTL found in ICARDA and NDSU genotypes may be useful to the Ethiopian breeding programs. In the current study, a total of 58 QTL in 26 genomic regions were detected that were unique to the ICARDA and NDSU accessions for nine malt quality traits. More than 50% of these QTL were found in

chromosomes 5H (7 regions) and 7H (5 regions). Particularly, two regions each in chromosome 5H (50.5-59.7 cM and 118.2-125.1 cM) and 7H (3.5-9.7 cM and 83.3-98.4 cM) contained QTL for several malt quality traits. Because multiple genomic regions, each with a small effect were impacting malt quality traits, it is important to consider genomic selection as a method for improving malt quality in the Ethiopian germplasm. Finally, I suggest that further studies be done to saturate the target regions in chromosomes 5H and 7H with more markers. Having more markers in these regions may increase the efficiency of enriching favorable alleles or haplotypes.

In conclusion, accounting for population structure and familial relatedness in association analysis is crucial to minimize the level of false positives. The study also indicated that genome-wide association mapping provides useful tool to detect a bulk of QTL for several malt quality traits simultaneously.

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CHAPTER V: GENERAL SUMMARY AND CONCLUSIONS

Knowledge gained from genetic diversity studies and dissection of the genetic basis of traits is a crucial initial step in crop improvement process. The current study was conducted with the following objectives (1) to determine the genetic diversity and population structure in an association mapping panel that includes barley genotypes from Ethiopian landraces, and breeding lines and cultivars from the Ethiopian, ICARDA, and NDSU breeding programs, (2) to determine the appropriate association mapping model for use with the mapping panel, (3) to identify QTL for several agronomic, disease resistance, and quality traits in the aforementioned barley genotypes and compare them with previously reported QTL or known genes, and (4) to make recommendations as how the Ethiopian and NDSU barley-breeding programs can apply the findings for their improvement efforts. In this general conclusion, the discussions follow the above-mentioned general objectives.

Both phenotypic and molecular marker data based genetic diversity and population structure analyses revealed diversity in the current population, which followed spike row-type, geographic origin of the genotype, and breeding history. These results were also in agreement with past reports as to the trend of genetic diversity and population structure of barley (Varshney et al, 2012; Pauli et al. 2014; Matthies et al., 2014). Understanding this pattern of genetic diversity in barley is important at least for two reasons, it helps in deciding where to look for important alleles to exploit in a breeding program and it also helps in designing an appropriate association mapping model. Though large variability existed within and among the genotype groups (i.e. all genotypes, landrace and Ethiopian breeding lines, or ICARDA/NDSU breeding lines), the favorable alleles for one or more of the traits were concentrated in a certain group. For instance, the NDSU materials were generally found to have superior malt quality attributes with shorter stature and low lodging severity. Hence, the Ethiopian breeding program can utilize NDSU materials to develop lodging resistance and good malt barley cultivars. However, NDSU lines were found to be poor in stand establishment, susceptible to scald, and ultimately low in grain yield when grown in Ethiopia. Hence, it is of utmost importance that the Ethiopian breeding program takes these issues into consideration if they use NDSU materials as parents. Finally, the disease resistances to leaf scald and net form net blotch found in the Ethiopian landraces and breeding materials could be of benefit to the NDSU breeding program.

One of the major issues in association mapping is the problem of population structure that can lead to high levels of false positives. As mentioned above, the current mapping panel was highly structured according to spike row-type, geographic origin, and breeding history. Additionally, the linkage disequilibrium (LD) decay analyses indicated that large proportions of unlinked or loosely linked loci showed high LD, which implies that factors other than genetic linkage are contributing to LD in this mapping panel. Supporting the above-mentioned facts, the comparison of the association mapping models revealed that control for population structure and familial relatedness with the P+K mixed linear model provided better results. In general, the application of mixed linear models that account for population structure and familial relatedness is crucial in highly structured mapping panel like the current one.

An important goal of a genome-wide association study (GWAS) is to delineate genomic regions containing significant QTL controlling agronomic, disease resistance, and quality traits of economic importance. Prior to introducing new alleles to a breeding program, breeders need to aware of which QTL are present in their germplasm base and what unique QTL are available in others' germplasm that may be helpful. Generally, the already fixed favorable alleles for a trait in a breeding program can be maintained by selecting for widely conserved QTL regions whereas new improvements can be made by selecting for unique QTL from other germplasm groups (Zale et al., 2000). Ethiopian barley genotypes have been utilized worldwide as sources of alleles for disease resistance. Likewise, many breeding programs worldwide have used barley germplasm from ICARDA. The NDSU breeding program has not utilized germplasm from Ethiopia or ICARDA in over 40 years because of their poor malt quality. Likewise, the Ethiopian and ICARDA programs have rarely utilized NDSU breeding lines or cultivars as parents. Thus, the NDSU and Ethiopian breeding programs could benefit from each other if unique alleles can be identified in each other's materials.

A total of 94 QTL for 12 traits phenotyped in field experiments and two disease traits phenotyped in the greenhouse were detected in this study using GWAS. Three regions in chromosome 2H (4.7-12.1, 125.0-134.3 and 163.2-169.7 cM), two regions in chromosome 6H (27.3-38.0 and 93.4-106.8 cM), and one each in chromosome 1H (38.5-46.5 cM) and 7H (89.8-94.3 cM) were the locations where QTL where concentrated for agronomic and disease resistance traits. Likewise, a total of 145 QTL were identified for

nine malt and two grain quality traits. Three regions each in chromosomes 5H (29.1-33.6, 50.5-59.7, and 167.4-173.5 cM) and 7H (3.5-9.7, 94.3-98.4, 121.4-135.9 cM), two regions in chromosome 3H (4.7-11.0 cM and 95.5-106.0 cM), and one region each in chromosomes 2H (169.7 cM) and 4H (86.7-95.2 cM) are where QTL for malt and grain quality traits were concentrated. It is logical to consider all of these regions as targets for fine mapping and molecular marker-based breeding in barley to improve agronomic, disease resistance, and malt and grain quality traits.

The QTL detected in the current study for agronomic and disease resistance traits correspond to the chromosomal positions of some known genes or QTL for the traits reported in the literature. The GWAS for days to heading identified QTL in the same regions as the location of six known photoperiod-related loci (*Ppd_H1*, *HvFT4*, HvGI, *HvFT2*, *HvCO2*, and *HvCO1*) and one vernalization-related locus (*VRN-H1*). The analysis of plant height identified one QTL in the same general region as the plant height locus *sdw3* in chromosome 2H. The current GWAS detected QTL in the same region as two leaf scald resistance genes (*Rrs1* in chromosome 3H and *Rrs15* in chromosome 7H) and two net form net blotch resistance genes (*rpt.k* and *rpt.r*) in chromosome 6H. Previous studies indicated the positions of malt quality traits in chromosome 1H near the *Hor1* and *Hor2* loci (13.8-23.8 cM) and the *GLB1* locus (68.1 cM), in chromosome 2H near the *vrs1* locus (92.6 cM), in chromosome 3H near the *Upg2* locus (25.2-36.0 cM) and the *Dor4* locus (50.2 cM), in chromosome 4H near the *Bmy1* locus (143.4 cM), in chromosome 5H near the locus *Ltp1* (44.6 cM), in chromosome 6H near the *Amy1* locus (88.1 cM), and in chromosome 7H near the *Amy2* locus. I detected QTL for malt and grain quality traits near all of the above-mentioned loci except the *GLB1* and *vrs1* loci. My study confirmed the important of GWAS as useful tool to identify known QTL and known genes, and new QTL for multiple traits of interest to barley improvement.

Because the barley germplasm in Ethiopian breeding program is typically taller and has weaker straw than desired, the four unique QTL for plant height and the six unique QTL for lodging in the ICARDA and NDSU accessions could be potential candidates for the improvement of reduced plant height and lodging resistance in the Ethiopian breeding program. Likewise, the four QTL unique for leaf scald, two unique QTL for net blotch, and one unique QTL for spot form net blotch found in the Ethiopian

accessions (landraces and breeding lines) could be utilized to improve the resistance in these leaf diseases by the NDSU breeding program.

Though some improvements have been made for malt quality traits in the Ethiopian breeding program, there is a shortage of genetic variability for different malt quality attributes. Thus, searching for germplasm that may benefit the Ethiopian malt barley-breeding program is of utmost importance. The NDSU barley-breeding program has made large improvements in malt quality traits in the last four decades and the germplasm from this breeding program can be a potential source of favorable alleles for malt quality traits that can be utilized by the Ethiopian breeding program. The identification of unique QTL that exist in the ICARDA and NDSU accessions is crucial. In the current study, a total of 58 QTL unique to ICARDA and NDSU accessions for the nine malt quality traits were detected in 26 genomic regions. More than 50% of these QTL were found in chromosomes 5H (7 regions) and 7H (5 regions). Particularly, two regions each in chromosome 5H (50.5-59.7 cM and 118.2-125.1 cM) and 7H (3.5-9.7 cM and 83.3-98.4 cM) contained QTL for several malt quality traits. All the favorable alleles for malt quality traits detected in these regions came from the NDSU genotypes. Because there are several genomic regions with QTL for malt quality having smaller effects, it is important to consider a genomic selection as a scheme for improving malt and grain quality traits. Finally, I suggest for the Ethiopian breeding program that they target regions in chromosome 5H and 7H for saturation with additional markers that could be used for fine-mapping the regions or improve the efficiency of genomic selection.

APPENDIX

Table A.1. Mean and range for days to heading of different group of barley estimated from data at Bekoji and Koffele combined over three years (2011-2013) and Fargo combined over two years (2012 and 2013).

Groups	Bekoji		Koffele		Fargo	
	Mean	Range	Mean	Range	Mean	Range
Two-rowed landrace	82	68-87	85	80-93	59	55-62
Six-rowed landrace	83	72-90	87	75-103	63	55-69
Two-rowed Ethiopian lines	79	71-88	83	74-95	57	48-66
Six-rowed Ethiopian lines	82	76-90	86	75-96	64	56-71
Two-rowed ICARDA lines	79	71-89	81	71-94	54	47-62
Six-rowed ICARDA lines	81	67-91	83	62-98	57	46-69
Two-rowed NDSU lines	83	76-87	81	74-90	51	44-56
Six-rowed NDSU lines	85	80-91	82	75-88	51	47-56

Table A.2. Mean and range for days to maturity of different group of barley estimated from data at Bekoji and Koffele combined over three years (2011-2013).

Groups	Bekoji		Koffele	
	Mean	Range	Mean	Range
Two-rowed landrace	130	116-136	143	139-151
Six-rowed landrace	132	119-143	142	127-155
Two-rowed Ethiopian lines	131	119-144	142	132-150
Six-rowed Ethiopian lines	133	126-140	142	139-147
Two-rowed ICARDA lines	133	127-141	140	130-150
Six-rowed ICARDA lines	134	120-144	141	129-162
Two-rowed NDSU lines	137	131-146	138	127-147
Six-rowed NDSU lines	138	134-142	139	134-144

Table A.3. Mean and range for plant height (cm) of different group of barley estimated from data at Bekoji and Koffele combined over three years (2011-2013) and Fargo combined over two years (2012 and 2013).

Groups	Bekoji		Koffele		Fargo	
	Mean	Range	Mean	Range	Mean	Range
Two-rowed landrace	110.9	96.5-130.9	116.0	89.5-133.1	58.9	55.6-61.5
Six-rowed landrace	112.3	91.7-130.9	114.8	71.5-139.1	60.1	34.7-70.6
Two-rowed Ethiopian lines	115.3	92.6-130.4	118.6	87.3-136.1	58.5	46.5-70.1
Six-rowed Ethiopian lines	118.6	107.3-134.9	118.2	107.3-135.1	57.5	37.6-67.8
Two-rowed ICARDA lines	97.0	78.6-124.6	96.5	82.2-119.1	51.3	41.0-61.7
Six-rowed ICARDA lines	96.7	70.0-123.4	98.8	73.9-127.6	55.7	47.8-62.5
Two-rowed NDSU lines	82.4	72.6-92.3	83.1	64.9-94.3	59.6	43.7-74.7
Six-rowed NDSU lines	89.2	79.4-104.9	90.2	77.5-110.9	61.9	50.0-78.5

Table A.4. Mean and range for lodging (%) of different group of barley estimated from data at Bekoji and Koffele combined over three years (2011-2013).

Groups	Bekoji		Koffele	
	Mean	Range	Mean	Range
Two-rowed landrace	12	3-25	57	20-98
Six-rowed landrace	9	0-45	54	4-98
Two-rowed Ethiopian lines	7	0-30	45	0-98
Six-rowed Ethiopian lines	10	0-40	52	18-95
Two-rowed ICARDA lines	1	0-9	8	0-43
Six-rowed ICARDA lines	2	0-15	15	0-48
Two-rowed NDSU lines	0	0-1	4	0-13
Six-rowed NDSU lines	0	-	7	0-16

Table A.5. Mean and range for Scald (0-9) of different group of barley estimated from data at Bekoji and Koffele combined over three years (2011-2013).

Groups	Bekoji		Koffele	
	Mean	Range	Mean	Range
Two-rowed landrace	4.8	2.7-8.7	3.7	2.1-9.2
Six-rowed landrace	4.1	1.5-8.7	3.1	0.6-17.5
Two-rowed Ethiopian lines	4.3	1.2-7.9	3.4	0.9-6.5
Six-rowed Ethiopian lines	3.0	1.4-4.8	2.1	1.2-2.6
Two-rowed ICARDA lines	4.4	0.8-7.4	3.3	0.3-7.3
Six-rowed ICARDA lines	4.6	1.4-8.2	3.6	0.7-8.0
Two-rowed NDSU lines	7.5	6.7-8.1	5.5	0.8-8.7
Six-rowed NDSU lines	7.4	6.6-7.7	6.8	4.2-8.7

Table A.6. Mean and range for net blotch (0-9) of different group of barley estimated from data at Bekoji and Koffele combined over three years (2011-2013).

Groups	Bekoji		Koffele	
	Mean	Range	Mean	Range
Two-rowed landrace	3.3	1.0-6.7	5.5	2.8-8.8
Six-rowed landrace	2.5	0.6-7.7	4.6	1.2-8.8
Two-rowed Ethiopian lines	1.6	0.7-2.5	3.2	1.1-5.8
Six-rowed Ethiopian lines	1.8	1.0-3.1	3.8	2.0-5.5
Two-rowed ICARDA lines	1.1	0.4-2.6	2.4	0.6-4.1
Six-rowed ICARDA lines	1.2	0.7-2.5	2.7	1.0-6.0
Two-rowed NDSU lines	1.1	0.8-1.4	1.9	1.0-3.2
Six-rowed NDSU lines	1.0	0.6-1.3	1.8	0.4-3.5

Table A.7. Mean and range for number of tillers per plant of different group of barley estimated from data at Bekoji and Koffele combined over three years (2011-2013).

Groups	Bekoji		Koffele	
	Mean	Range	Mean	Range
Two-rowed landrace	8	5-10	9	5-14
Six-rowed landrace	7	3-11	6	3-12
Two-rowed Ethiopian lines	8	4-10	8	5-15
Six-rowed Ethiopian lines	7	5-9	7	5-9
Two-rowed ICARDA lines	9	7-14	7	5-10
Six-rowed ICARDA lines	7	5-10	6	4-9
Two-rowed NDSU lines	9	7-12	6	3-10
Six-rowed NDSU lines	7	5-10	4	2-7

Table A.8. Mean and range for number of kernels per spike of different group of barley estimated from data at Bekoji and Koffele combined over three years (2011-2013).

Groups	Bekoji		Koffele	
	Mean	Range	Mean	Range
Two-rowed landrace	35	16-49	31	18-48
Six-rowed landrace	45	33-54	61	39-83
Two-rowed Ethiopian lines	29	14-48	34	26-52
Six-rowed Ethiopian lines	43	33-50	60	40-69
Two-rowed ICARDA lines	29	24-37	30	24-45
Six-rowed ICARDA lines	47	35-55	58	32-69
Two-rowed NDSU lines	27	24-31	30	23-38
Six-rowed NDSU lines	50	42-57	58	46-66

Table A.9. Mean and range for spike length (cm) of different group of barley estimated from data at Bekoji and Koffele combined over three years (2011-2013).

Groups	Bekoji		Koffele	
	Mean	Range	Mean	Range
Two-rowed landrace	6.8	5.2-8.0	8.0	6.9-10.6
Six-rowed landrace	6.6	4.2-9.2	6.7	3.6-8.7
Two-rowed Ethiopian lines	7.3	4.6-9.1	7.2	3.6-9.6
Six-rowed Ethiopian lines	6.9	5.6-8.2	6.8	3.6-8.6
Two-rowed ICARDA lines	7.4	6.5-9.5	7.4	5.4-9.3
Six-rowed ICARDA lines	6.6	4.8-8.1	6.7	5.0-8.8
Two-rowed NDSU lines	7.4	6.8-8.3	7.9	6.9-9.1
Six-rowed NDSU lines	7.1	6.0-7.5	7.7	6.9-8.8

Table A.10. Mean and range for thousand-kernel weight (g) of different group of barley estimated from data at Bekoji and Koffele combined over three years (2011-2013) and Fargo combined over two years (2012 and 2013).

Groups	Bekoji		Koffele		Fargo	
	Mean	Range	Mean	Range	Mean	Range
Two-rowed landrace	50.3	33.3-63.5	42.5	32.1- 57.0	41.5	30.9-47.2
Six-rowed landrace	40.1	32.6-54.9	33.2	24.4-45.8	37.7	31.4-46.6
Two-rowed Ethiopian lines	49.2	40.4-68.0	41.9	32.6-56.0	44.3	36.1-49.5
Six-rowed Ethiopian lines	44.0	33.8-55.7	37.2	26.7-48.4	39.5	37.3-42.5
Two-rowed ICARDA lines	47.9	40.0-53.0	42.4	34.7-47.2	44.4	35.7-53.3
Six-rowed ICARDA lines	37.7	31.6-48.4	34.0	26.9-43.5	36.3	27.7-46.3
Two-rowed NDSU lines	36.8	28.5-48.6	34.1	22.5-40.4	44.3	38.7-51.2
Six-rowed NDSU lines	32.1	29.6-36.3	28.7	25.6-32.1	33.7	30.3-6.0

Table A.11. Mean and range for hectoliter weight (kg hL-1) of different group of barley estimated from data at Bekoji and Koffele combined over three years (2011-2013) and Fargo combined over two years (2012 and 2013).

Groups	Bekoji		Koffele		Fargo	
	Mean	Range	Mean	Range	Mean	Range
Two-rowed landrace	60.1	54.8-63.5	58.0	53.7-60.3	55.3	48.5-59.3
Six-rowed landrace	59.1	53.0-74.8	57.2	48.9-71.3	57.1	51.7-71.7
Two-rowed Ethiopian lines	61.3	57.4-65.2	59.5	51.2-65.2	60.4	55.3-66.9
Six-rowed Ethiopian lines	60.0	55.9-62.3	56.2	51.8-60.4	59.1	57.3-63.7
Two-rowed ICARDA lines	61.9	56.5-64.4	58.4	53.1-62.1	62.2	56.4-65.8
Six-rowed ICARDA lines	58.5	51.3-63.6	56.0	50.3-60.1	58.0	52.1-75.0
Two-rowed NDSU lines	59.3	55.7-64.2	59.7	53.9-63.5	62.9	59.6-65.9
Six-rowed NDSU lines	59.3	57.4-62.0	57.8	48.9-63.1	60.5	54.8-62.9

Table A.12. Mean and range for grain yield (t.ha-1) of different group of barley estimated from data at Bekoji and Koffele combined over three years (2011-2013) and Fargo combined over two years (2012 and 2013).

Groups	Bekoji		Koffele		Fargo	
	Mean	Range	Mean	Range	Mean	Range
Two-rowed landrace	4.4	2.6-5.6	4.2	2.8-6.0	1.8	0.7 - 2.7
Six-rowed landrace	4.7	2.9-7.0	3.8	1.2-7.8	1.7	0.4 - 3.1
Two-rowed Ethiopian lines	4.0	2.8-5.1	4.4	2.5-8.1	2.3	0.8 - 3.3
Six-rowed Ethiopian lines	5.3	4.1-6.0	4.9	3.1-7.7	1.4	0.1 - 2.3
Two-rowed ICARDA lines	4.3	2.8-5.5	3.9	1.9-5.6	2.9	2.0 - 3.6
Six-rowed ICARDA lines	4.3	1.4-6.0	3.9	2.2-5.1	3.1	0.9 - 4.1
Two-rowed NDSU lines	2.0	1.6-2.5	1.9	0.8-3.9	3.6	2.9 - 4.1
Six-rowed NDSU lines	2.1	1.4-2.9	2.1	0.9-4.3	3.8	3.4 - 4.2

Table A.13. Mean and range for stand (%) of different group of barley estimated from data at Bekoji and Koffele combined over three years (2011-2013).

Groups	Bekoji		Koffele	
	Mean	Range	Mean	Range
Two-rowed landrace	87.2	76 - 94	76.6	64 - 85
Six-rowed landrace	86.8	73 - 95	72.9	48 - 84
Two-rowed Ethiopian lines	86.4	81 - 93	75.1	64 - 86
Six-rowed Ethiopian lines	87.4	79 - 94	73.9	68 - 82
Two-rowed ICARDA lines	82.2	73 - 93	74.4	56 - 86
Six-rowed ICARDA lines	81.3	53 - 93	70.7	56 - 84
Two-rowed NDSU lines	68.5	64 - 74	65.9	56 - 94
Six-rowed NDSU lines	73.3	64 - 81	65.9	50 - 95

Table A.14. Some of the genotypes with mean DON content less than 15 ppm at Langdon in 2012 (LA_12) and 2013 (LA_13) and at Osnobrack in 2012 (OS_12).

Code	Genotypes	Langdon in 2012	Osnabrock in 2012	Langdon in 2013	Mean
9	3335	17.8	17.3	4.2	13.1
16	25161	14.1	4.0	NA	9.0
59	2ND26333	NA†	NA	9.9	9.9
60	2ND27421	NA	NA	5.5	5.5
61	2ND27440	NA	NA	4.4	4.4
63	2ND29817	NA	NA	4.9	4.9
64	2ND29820	NA	NA	5.8	5.8
65	2ND29835	NA	NA	12.0	12.0
66	2ND29836	NA	NA	3.6	3.6
82	Conlon	6.8	14.7	12.3	11.3
91	EH 1487 SEL 1	8.7	20.5	5.5	11.6
92	EH 1487 SEL 2	8.5	20.8	5.3	11.5
102	EH 1517 SEL 3	7.8	23.9	0.6	10.8
125	EH1847/F4.2P.5.2	8.6	10.2	10.5	9.8
126	EMBSN 9303/05	2.5	6.8	2.5	3.9
130	EMBSN 9344/05	12.8	19.8	4.6	12.4
155	IBON 11/99	11.2	3.0	4.7	6.3
158	IBON 74/03	7.9	20.0	8.5	12.1
165	IBON 9075/05	16.7	13.0	3.1	10.9
167	IBON 9086/05	16.6	12.7	2.7	10.7
174	IBON 9104/06	5.8	7.0	12.1	8.3
175	IBON 9106/05	13.2	8.8	7.6	9.9
186	IBON-MRA 28/06	8.2	9.2	3.1	6.8
194	IBYT 925/06	12.4	6.4	4.1	7.6
207	MSEL/ND21117	2.7	11.7	5.4	6.6
212	ND25652	NA†	NA	11.1	11.1
213	ND26891	NA	NA	6.1	6.1
215	ND27245	NA	NA	10.4	10.4
216	ND29134	NA	NA	8.0	8.0
228	RAWSON	NA	NA	5.5	5.5
230	SABINI	5.1	23.7	5.2	11.3
248	2ND27705	NA	NA	10.0	10.0
249	2ND28071	NA	NA	10.0	10.0
251	2ND29827	NA	NA	2.5	2.5
252	2ND29990	NA	NA	3.7	3.7
254	2ND30002	NA	NA	3.7	3.7
255	ND30036	NA	NA	6.0	6.0
256	ND30067	NA	NA	0.3	0.3
257	ND30125	NA	NA	11.0	11.0
258	2ND30612	NA	NA	1.5	1.5
260	2ND30658	NA	NA	7.2	7.2
261	2ND30672	NA	NA	11.7	11.7
266	ND20493	4.4	11.7	6.3	7.5

†Data not available

Table A.15. Correlation coefficients among different malt and grain quality traits recorded on samples from Bekoji experiment in 2011 and 2012.

Traits†	AA	BG	DP	FAN	ME	SP	VIS	WCO	BPC	PL	ML
AA	1.000	-0.591	0.617	0.559	0.534	0.709	-0.499	0.362	-0.018	0.228	0.545
BG		1.000	-0.507	-0.506	-0.549	-0.604	0.712	-0.331	0.134	-0.040	-0.523
DP			1.000	0.508	0.254	0.661	-0.409	0.165	0.386	0.088	0.097
FAN				1.000	0.328	0.797	-0.373	0.315	0.302	0.228	0.219
ME					1.000	0.402	-0.285	0.169	-0.424	0.169	0.434
SP						1.000	-0.511	0.429	0.347	0.231	0.369
VIS							1.000	-0.396	0.035	0.063	-0.456
WCO								1.000	-0.004	-0.018	0.333
BPC									1.000	0.079	-0.351
PL										1.000	0.027
ML											1.000

† AA=α-amylase, BG= β-glucan, FAN= free amino nitrogen, ME= malt extract, SP = Soluble protein, VIS = wort viscosity, WC = Wort color, BPC = barley protein content, PL= proportion of plump kernels, and ML = malt loss.

Table A.16. Marker coverage, mean gap, and gaps counts ≥ 10 cM for the markers considered.

Chromosome	Start (cM)	End (cM)	Coverage (cM)	Gap (Mean, cM)	Number (Gap ≥ 10 cM)
1H	12.9	138.9	125.9	5.7	3
2H	4.7	169.7	164.9	4.5	2
3H	4.7	167.3	162.6	5.6	5
4H	19.4	146.5	127.0	4.7	3
5H	11.4	189.2	177.8	4.2	6
6H	1.0	142.2	141.2	4.2	2
7H	3.5	162.0	158.5	5.7	5
Total			1058.0	4.8	26

Table A.17. The Number of markers (N), Polymorphic Information Content (PIC), Heterozygosity (HET), and allele Diversity (DIV) for SNP markers in each chromosome.

Chromosome	N	PIC			HET			DIV		
		Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
1H	23	0.27	0.11	0.37	0.16	0.03	0.32	0.33	0.12	0.49
2H	38	0.28	0.02	0.38	0.16	0.01	0.43	0.35	0.02	0.50
3H	30	0.26	0.04	0.38	0.15	0.02	0.39	0.32	0.04	0.50
4H	28	0.27	0.08	0.38	0.17	0.04	0.37	0.34	0.08	0.50
5H	43	0.29	0.07	0.38	0.17	0.03	0.42	0.36	0.08	0.50
6H	35	0.25	0.06	0.37	0.15	0.02	0.41	0.31	0.06	0.50
7H	29	0.27	0.04	0.37	0.16	0.02	0.39	0.34	0.04	0.50
Overall	226	0.27	0.02	0.38	0.16	0.01	0.43	0.34	0.02	0.50

Table A.18. The mean square difference (MSD) estimated for the models in each of the traits

Trait	Naïve	P	K	P+K
Days to heading	0.075	0.006	0.003	0.002
Days to maturity	0.007	0.002	0.003	0.002
Plant height	0.159	0.004	0.003	0.003
Lodging	0.104	0.004	0.003	0.002
Number of spikes per plant	0.072	0.018	0.002	0.002
Number of kernels per spike	0.057	0.027	0.002	0.002
Spike length	0.017	0.004	0.005	0.002
Thousand-kernel weight	0.060	0.030	0.003	0.003
Hectoliter weight	0.036	0.002	0.003	0.002
Grain yield	0.060	0.030	0.003	0.002
Leaf scald	0.104	0.010	0.003	0.002
Net blotch	0.140	0.003	0.003	0.002
Net form net blotch	0.150	0.036	0.007	0.002
Spot form net blotch	0.149	0.006	0.004	0.002

Table A.19. Genomic regions shown to be associated with more than one agronomic and disease resistance traits.

No.	Chrom	Distance	Traits†
1	1H	12.9-20.3	LODG, TKW
2	1H	38.5-46.5	SFNB, NB, YLD, PLH
3	1H	53.4-59.3	PLH, NB, LODG
4	2H	4.7-12.1	DH, NSP, NKS, TKW
5	2H	12.1-20.5	DH, DM, NSP
6	2H	51.0-54.9	DH, PLH, LODG
7	2H	63.6-68.1	NKS, TKW
8	2H	125.0-134.3	DH, PLH, SL, TKW, YLD, NB
9	2H	163.2-169.7	DH, PLH, NSP, SC
10	3H	11.0	TKW, YLD
11	3H	69.9-74.0	DH, SC, NFNB
12	3H	167.3	DH, DM, SC
13	4H	78.1-86.7	YLD, SC
14	5H	29.9-33.6	PLH, NFNB
15	5H	50.5-64.8	PLH, TKW
16	5H	88.1-93.7	NKS, NB, NFNB
17	5H	121.7-125.1	LODG, SC, NB
18	5H	148.6-160.9	DH, DM, SC
19	5H	162.0-168.4	HLW, SC
20	5H	171.6-181.2	PLH, YLD
21	6H	27.3-38.0	DH, LODG, YLD, SC
22	6H	55.7-62.7	LODG, SL, NB
23	6H	79.2-85.9	DH, NFNB
24	6H	93.4-106.8	DM, TKW, SC, NFNB
25	7H	83.4-94.3	DH, PLH, SL, HLW, YLD, SC
26	7H	129.3-135.9	SC, NB

†LODG = lodging, TKW= thousand-kernel weight, SFNB = spot form net blotch, NB= net blotch, DH = days to heading, NSP = number of spikes per plant, NKS =number of kernels per spike, DM =days to maturity, PLH =plant height, SL =spike length, YLD= grain yield, SC =leaf scald, NFNB = net form net blotch, HLW= hectoliter weight.

Table A.20. The mean square difference (MSD) of observed and expected p-values for the four association mapping models.

Trait	Naïve	P	K	P+K	Trait	Naïve	P	K	P+K
AA	0.171	0.021	0.004	0.003	FEX	0.100	0.024	0.002	0.002
DP	0.158	0.067	0.008	0.005	BG	0.105	0.021	0.004	0.002
SP	0.156	0.052	0.007	0.004	VIS	0.109	0.010	0.004	0.002
KI	0.159	0.046	0.007	0.004	BPC	0.180	0.033	0.010	0.002
FAN	0.147	0.030	0.006	0.003	BP	0.031	0.002	0.003	0.002
WCO	0.038	0.011	0.002	0.002					

Table A.21. Genomic regions shown to be associated with more than one malt and grain quality traits.

No	Chromosome	Positions	Traits†
1	1H	12.9-20.3	BG, WCO, PL
2	1H	45.2-53.4	DP, WCO, VIS
3	2H	12.1-20.5	VIS, FAN, SP, ME
4	2H	51.0-63.6	BPC, ME
5	2H	130.4-134.3	FAN, KI, SP, BG, VIS
6	2H	139.3-142.7	ME, BG, FAN, VIS, FAN,
7	2H	154.7-154.7	FAN, WCO
8	2H	169.7	BG, DP, FAN, SP, VIS
9	3H	4.7-11.0	BG, DP, ME, FAN, BPC, KI, AA, VIS
10	3H	32.8	BPC, ME, FAN, KI, SP
11	3H	44.3	FAN, SP
12	3H	51.0-69.9	SP, BPC, DP
13	3H	96.5-106.0	FAN, AA, WCO, SP, DP, BG
14	3H	167.3	BG, ME
15	4H	58.1-63.4	ME, WCO, DP
16	4H	86.7-95.2	SP, KI, DP, VIS, ME
17	5H	11.4-12.8	FAN, ME
18	5H	29.1-33.6	FAN, VIS, BG, KI, SP, AA
19	5H	50.5-59.7	AA, DP, VIS, WCO, SP, FAN
20	5H	93.7	AA, FAN, SP
21	5H	118.2-125.1	ME, BG, AA, FAN
22	5H	138.3-139.0	AA, BG, ME, KI
23	5H	157.6-162.0	FAN, WCO, BG
24	5H	167.4-173.5	AA, DP, KI, SP, WCO, VIS
25	6H	27.3-33.0	FAN, ME
26	6H	49.2-55.7	DP, BG
27	6H	62.7-65.8	DP, BG
28	6H	70.5-79.2	FAN, DP
29	6H	88.7-98.7	DP, ME, AA
30	7H	3.5-9.7	BG, AA, KI, SP, WCO, ME, DP
31	7H	18.7	SP, KI
32	7H	55.0	BG, PL, ME, VIS
33	7H	83.4-89.8	FAN, SP, BG, KI
34	7H	94.3-98.4	AA, BP, WCO, BPC, FAN, KI, SP
35	7H	121.4-135.9	DP, FAN, KI, SP, AA

†AA= α-amylase activity, DP = diastatic power, SP= soluble protein, KI = Kolbach Index, FAN = free amino nitrogen, WCO= wort color, BG= β-glucan, VIS = wort viscosity, ME = malt extract, BPC = barley grain protein, and PL = proportion of plump kernels.