Genetic Characterization of Multiple Disease Resistance and Agronomical/Nutritional Traits in *Hordeum*

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Dedication

Lovingly dedicated to my parents and siblings, especially mother, for their support, encouragement and constant love during my early school years without knowing what education is; also for enduring my absence during the past eight years.

Abstract

Barley is an economically important crop plant whose yield and quality is affected by multiple diseases. Landrace and wild barley gene pools can be utilized to enhance disease resistance and nutrition in cultivated barley. To identify and map resistance loci for three important diseases (stem rust, spot blotch and Fusarium head blight [FHB]), and enhanced accumulation of two vital micronutrients (zinc and iron), genetic mapping was employed. The genetics of resistance to stem rust race TTKSK in Swiss landrace and wild barley accessions at the seedling stage was conducted through bi-parental mapping. Genetic analysis of $F_{2:3}$ families derived from these accessions revealed that a single gene that maps to chromosome 5HL—at or in close proximity to the complex stem rust resistance locus *rpg4/Rpg5* confers resistance. An association mapping approach was utilized to identify Quantitative Trait Loci (QTL) associated with disease resistance and zinc and iron concentration in 298 Ethiopian and Eritrean barley landraces genotyped with 7,842 single nucleotide polymorphism (SNP) markers. For stem rust race TTKSK, five seedling resistance loci were identified: one each on chromosome 2HS, 2HL, 3HL, 4HL, and 5HS. The ones on chromosomes 2HL and 4HL are novel, whereas the other three mapped to regions coincident with previously reported stem rust resistance QTL. For stem rust race MCCFC at the adult plant stage, one locus coincident with a known race TTKSK resistance QTL was identified on chromosome 5HL. For spot blotch, SNP markers located in close proximity with known adult plant spot blotch resistance QTL were found on chromosomes 2HL and 4HS in six-rowed barley landraces. For FHB, one common resistance QTL on chromosome 2HL, also associated with deoxynivalenol (DON) concentration, was identified. A locus mapping to a region of chromosome 4HL, known to contain QTL associated with DON, also was detected. The loci identified on chromosomes 2HL and 4HL associated with FHB and/or DON were not associated with heading date or plant height. Two novel loci associated with grain zinc concentration were identified on chromosomes 4HS and 6HL. For kernel weight, a known QTL region on chromosome 2HL was detected. The findings of this study will be useful to enhance disease resistance and nutrition in cultivated barley.

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Chapter 1

General Introduction - Overview of Genetic Mapping and Genetics of Disease Resistance and Nutritional Traits in Barley

1.1 General background

Barley (*Hordeum vulgare* ssp. *vulgare* L.) is one of the oldest cultivated crop plants and the fourth most important cereal crop in the world both in terms of quantity produced and area of cultivation (Schulte et al. 2009; http://faostat.fao.org). It is grown on every continent, except Antarctica, and occupies a land area of about 57 million hectares (Grando and Macpherson 2005) with an annual harvest of about 140 million tons. The five major barley producing countries with the highest farm gate value of the crop are France, United States, Australia, Russia, and China. In the United States, barley is grown in almost every state, but the most important production areas are in Idaho, Montana, North Dakota, Washington, and Arizona (2011 data from http://www.statista.com). Globally, approximately three-quarters of barley grain is used for livestock feed, 20% as malt in beverages, and the reminder for food products (http://www.agmrc.org). However, in some regions, barley is an important part of a staple diet, especially in Asia and the northern and Horn regions of Africa (Newman and Newman 2006). In addition to the grain, barley straw also is valuable as a direct livestock feed in the form of hay and silage, as a green manure, and also as roofing material for thatched houses in rural areas of Africa and South America. Barley is one of the most dependable crops and has a long history of cultivation in areas with extreme environmental conditions (Grando and Macpherson 2005).

Fossil evidence indicates that domestication and cultivation of barley dates back to the earliest times of agricultural activity around 10,000 years ago in the Fertile Crescent (Morrell et al. 2003; Zohary and Hopf 2000). A recent study revealed that barley domestication is polyphyletic with the Tibetan Plateau and vicinity being one of the centers of domestication (Dai et al. 2012). The only recognized progenitor of cultivated barley is wild barley [*Hordeum vulgare* ssp. *spontaneum* (C. Koch) Thell.] (Nevo et al. 1979). Dai et al. (2012) suggested that the split between the wild barleys in the Near East and those in Tibet occurred around 2.76 million years ago (MYA). Among many possible traits, wild barley was likely selected first for the non-shattering character because this allowed for permanent cultivation of the crop in early agriculture.

Taxonomically, barley belongs to the

- Division: Magnoliophyta
- Class: Liliopsida
- Order: Cyperales
- Family: Poaceae (or Gramineae)
- Subfamily: Pooideae
- Tribe: Hordeae
- Genus: *Hordeum*
- Species: *vulgare*.

Gaut (2002) hypothesizes that the Triticeae tribe formed by the closely related species of barley, bread wheat (*Triticum aestivum* L.), durum wheat (*Tritricum durum L.*), rye (*Secale cereale L.*) and their respective wild progenitors evolved about 12 MYA in the Pooideae subfamily of the Poaceae. Barley and wheat diverged approximately 11.6 million years before present and have diversified further into several subspecies since then (Chalupska et al. 2008). A recent intra-species genomic diversity study in barley and wheat varieties estimated that some haplotypes of barley varieties diverged more than 500,000 years ago (Wicker et al. 2009), long before domestication.

1.2 Barley genetics and molecular markers

Barley is a true diploid inbreeding species with seven large chromosomes (2n=14) that are designated 1H through 7H, according to their homologous relationship to other Triticeae linkage groups (Linde-Laursen 1997). Barley has a complex genome structure. The genome size is estimated to be 5.1 x 10^9 bp (Bennett and Smith 1976; Dolezel et al. 1998), which makes it one of the largest of the cereal crops. Compared to rice and humans, the barley genome is 12x and 2x larger, respectively. The barley genome is comprised of more than 80% repetitive DNA (Schulte et al. 2009; Wicker et al. 2009), which makes cloning of valuable genes by a map-based approach a challenging task. Due to these attributes, advances made in the structural genomics of model plant species and

other plants of low genome complexity have surpassed those in barley and other members of the Poaceae family.

Barley is considered as a model species for the genetics of the Triticeae tribe. It is one of the best studied crop plants having numerous genetic, cytogenetic, and genomic tools available. Moreover, barley has a rich resource of molecular tools that can enhance genetic and genomics research. In the last decade, more than 444,652 barley expressed sequence tags (ESTs) with 28,001 EST contigs and 22,937 EST singletons were developed (Close et al. 2008; http://www.harvest-web.org). In addition, barley has several thousand markers of various types, many marker-rich genetic maps, an Affymetrix GeneChip representing 22,439 genes (Close et al. 2004), and numerous bacterial artificial chromosome (BAC) libraries (Stein 2007; Yu et al. 2000) developed over the years. Many genomic studies were conducted in recent years on barley that advanced knowledge of the Triticeae in particular and of plant biology in general (summary in Schulte et al. 2009). Model studies emphasized barley grain maturation and germination, disease resistance towards biotrophic pathogens, response to abiotic and photoperiodic responses, and inflorescence morphology. Several databases have been developed and are integrated into "Barley Boulevard," which is a shortcut to "Barley Information in GrainGenes" and elsewhere (http://wheat.pw.usda.gov). Significantly, an integrated and ordered physical, genetic and functional sequence assembly of 4.98×10^9 bp of the barley genome (cv. Morex) was recently completed (Mayer et al. 2012).

Various types of markers are available in barley. Starting from domestication until recent decades, desirable barley accessions were being selected and advanced solely based on desirable phenotypic characters, including large kernels, lodging resistance, and disease resistance. Morphological markers are commonly used in conventional selection programs and also to assess genetic diversity and uniformity of barley cultivars (Allard 1992; Brown and Munday 1982). Isozyme markers also have been utilized in barley breeding and population genetic studies (Parzies et al. 2000). Recently, the advent of modern DNA-based markers has changed the sphere of barley genetics. DNA markers are more informative and thus preferred in marker-assisted selection (MAS) for plant

breeding and also for the map-based cloning of genes. Several types of molecular markers have been developed in barley using various approaches. These include restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), sequence-tagged sites (STS), microsatellites or simple sequence repeats (SSR), Diversity Array Technology (DArT), and single nucleotide polymorphism (SNP) markers (Close et al. 2009; Giese et al. 1994; Graner et al. 1991; Muñoz-Amatriaín et al. 2011; Qi and Lindhout 1997; Rostoks et al. 2005; Tragoonrung et al. 1992; Varshney et al. 2007; Wenzl et al. 2004). Szűcs et al. (2009) developed a 2,383 locus linkage map integrating multiple types of the above markers into a single map. For a comprehensive list of mapped molecular markers in barley, refer to "Interactive Maps on GrainGenes" (http://wheat.pw.usda.gov/ggpages/map_summary.html). Of these markers, only a fraction are utilized in MAS in barley breeding. Recent SNP markers are accessible in The Hordeum Toolbox (http://www.hordeumtoolbox.org/), which has now been migrated to The Triticeae Toolbox (T3) of the Triticeae Coordinated Agricultural Project (TCAP) (http://triticeaetoolbox.org/).

Most of the SNP markers were derived from ESTs developed from different tissues at different growth stages and under various biotic and abiotic stresses (Stein et al. 2007). Thus, the markers provide an unprecedented opportunity to study phenotypic traits that could be explained by genes expressed under these conditions. These markers are being used to link phenotypes and genotypes, characterize germplasm diversity and population structure, and conduct genome-wide association studies (GWAS) in barley. In the past, traits were mapped entirely through genetic linkage mapping using bi-parental populations. Schulte et al. (2009) indicate that at least 5,000 barley genes have been genetically mapped so far. However, in recent years, GWAS is becoming popular in the mapping of complex traits in plants, especially barley (for more details, chapters 3-5). The availability of a large number of markers that are densely populated throughout the genome and amenable to high-throughput genotyping is vital for GWAS to ensure the efficient detection of significant loci. In brief, barley has a rich genetic and genomic infrastructure to conduct basic and applied research of general importance.

1.3 Diversity in Ethiopian and Eritrean barley landraces

Genetic diversity is defined as "the amount of genetic variability among individuals in a single species, whether the species exists as a single interbreeding group or as a number of populations, strains, breeds, races, or subspecies" (Farnham 2005). Wild barley, like other wild species of crop plants, has been limited to nature-driven change in its genomic context. However, cultivated barley, including landraces, has been under continuous natural selection and human-driven evolution for the last 10,000 years—since the domestication of the crop. Over the historic course of its cultivation, barley has evolved with a high level of genetic diversity, much of which is captured in genebanks. Records indicate that more than 370,796 accessions of 31 different *Hordeum* species (including cultivated barley, *H. vulgare* ssp. *vulgare*) are in *ex situ* genebanks worldwide (van Hintum and Menting 2003). Of the 290,820 total accessions where germplasm type has been described, 221,000 (76%) are cultivated barley (landraces, breeding materials, and cultivars), with the remaining numbers being wild relatives and genetic stocks (Ullrich 2011). According to an FAO (1996) estimate, barley has the second largest number of *ex situ* accessions among crops after wheat.

Landraces are defined as "farmer's varieties" because they have been selected from early domesticates by farmers and maintained by them for generations as heterogenous individuals. They represent the largest component (44%) of *Hordeum* germplasm conserved in genebanks worldwide (Ullrich 2011). Landraces have been utilized and preserved through generations for their desirable agronomic traits. For instance, barley, most likely in the form of landraces, is believed to have been under cultivation in Ethiopia since 3000 B.C. (Gamst 1969; cited from Grando and Macpherson 2005). Today, landraces are still the main seed sources used by subsistence farmers in Ethiopia and Eritrea (Grando and Macpherson 2005) as modern breeding is in its infancy in the two countries. Barley production in Ethiopia represents about 18% of the total national cereal production (Lakew 1997), and landraces comprise more than 90% of the crop cultivated in Ethiopia (Hadado et al. 2010).

Although the precise area of ancient barley cultivation in Ethiopia is not known, it is likely that landraces were cultivated under a wide range of environmental conditions. Barley landraces grow in almost all regions of Ethiopia, from the warm dry environs of the lowlands to the cooler moist areas of the highlands. Today, they are cultivated from 1,400 m above sea level (masl) to over 4,000 masl (Asfaw 2000). It is reasonable to speculate that to survive in such diverse production areas, landraces would have undergone genome evolution in order to develop the cellular components to cope with such extreme environments. Developing adaptation mechanisms would ensure the continuous survival of progeny through the evolution of desirable traits. Such germplasm is probably rich in unique genes underlying phenotypic variation. Consistent with this hypothesis, Hadado et al. (2010) analyzed Ethiopia barley landraces from two growing seasons to examine selection for adaptation to different altitudes. They investigated molecular variation using microsatellite markers and found that landraces are constituted by highly variable local populations with large within-population diversity that varied along an altitudinal gradient.

The great variation and endemicity of barley forms in Ethiopia has been interpreted in different ways. Vavilov (1951) initially considered Ethiopia as the center of origin for barley based on morphological diversity, and subsequently as a center of diversity for the crop—due to the absence of wild barley in Ethiopia. In some cases, subsequent research has supported Vavilov's assessment of Ethiopia as a secondary center of diversity (Huffnagel 1961; Takahashi 1955). Cultivated barley has gone through several domestication events (Dai et al. 2012); thus, the East African region of Ethiopia and Eritrea might be one of the centers of cultivated barley domestication like Tibet. A comprehensive study similar to Dai et al. (2012) that includes barley accessions (landraces and available wild forms) from Ethiopia/Eritrea, the Near East and Tibet may provide an answer to this hypothesis. Ethiopian/Eritrean barleys comprise rare and unique genes, particularly those conferring resistance against various diseases. Novel genes for resistance to Barley Yellow Dwarf, powdery mildew, net blotch, scald, and loose smut have been identified in Ethiopian barley germplasm (Jørgensen 1992; Metcalfe et al. 1978; Qualset 1975; Wiberg 1974; Yitbarek et al. 1995; Zhang et al. 1987).

Different interpretations are presented in the literature about the level of genetic diversity in Ethiopian landraces. Early exploratory studies found diverse characters, including row type, kernel color, spike morphology (deficiens and irregulare) and short rachilla hair types, endemic to Ethiopian and Eritrean barley collections (Orlov 1929, 1931; Vavilov 1951; Ward 1962). These studies hypothesized the East African region (Ethiopia and Eritrea) as a possible "center of accumulation" of diverse morphologies and/or origin of barley. Bjørnstad et al. (1997) investigated the diversity of Ethiopia landraces by comparing them to modern cultivars from North America, Europe, and Jordan using RFLP markers. They found that Ethiopian germplasm is significantly less diverse than modern germplasm, but is genotypically very distinct. A study of morphological variation by Tolbert et al. (1979) found that Ethiopian barley landraces were not more diverse than barleys from Eastern Europe. Hadado et al. (2010) compared genetic diversity in a local collection of landraces from Ethiopia to those from Syria and Jordan. They showed that Ethiopian landraces possess lower genetic diversity, but this may due to the fact that they sampled from a narrower geographic area. Cross (1994) studied barley collections from different countries (including Ethiopia) and found a high level of diversity in phenotypic and biochemical traits. The Ethiopian landraces investigated in that study comprised a very distinct group. Likewise, a genome-wide molecular variance analysis using SNP markers on wild and cultivated barley accessions from the Western Mediterranean, Fertile Crescent, Ethiopia, and Tibet found that Ethiopian germplasm grouped into a distinct cluster (like in other studies), but had the lowest overall diversity (Arregui et al. 2013).

In summary, the great phenotypic variability of Ethiopian barley landraces may be due to other genomic regions not accounted for by the genetic markers used in these diversity analyses. The existing molecular markers used for genetic diversity studies were mostly derived from coding (expressed) regions of the genome such as ESTs. Even the SNPs developed through resequencing were derived mostly from cultivated barleys (i.e., modern cultivars), which have a different phenotypic diversity profile from Ethiopian/Eritrean barleys. Genome regions that may better explain the phenotypic diversity of Ethiopian/Eritrean barley are perhaps few in number or were not captured in

the existing barley molecular infrastructure. As such, transposable elements (TEs) at the regulatory or near the coding regions might have contributed to the distinct phenotypic diversity in barley landraces from Ethiopia/Eritrea. TEs were first discovered in maize (*Zea mays*) as being responsible for kernel color variation (McClintock 1948)—a trait also very prominent in Ethiopian/Eritrean barleys. Few studies have indicated the existence and contribution of TEs for insertion polymorphism in barley (Scherrer et al. 2005; Shirasu et al. 2000; Wicker et al. 2009). Wicker et al. (2009) hypothesized the effects of transposons on the expression of neighboring genes and their significant contribution to phenotypic variability. The number, arrangement and copy number of TEs may vary among various barley germplasm groups, including landraces from Ethiopia/Eritrea. Comparison of intergenic regions of these barley panels may reveal more about the possible role of TEs in generating variation.

Comparisons of genetic diversity depend on the type and number of markers used, type of trait assayed, and sample size of populations, among other factors. Orabi et al. (2007) characterized landrace and wild barley accessions from west Asia, north Africa and northern Europe, landrace accessions from Eritrea (240 lines), and landraces (23 lines) and wild accessions (2 lines) from Ethiopia using chloroplast and nuclear SSR markers. They found a high level of diversity with respect to chloroplast SSR markers, with Ethiopian/Eritrean landraces forming a separate group. This germplasm group also was genetically dissimilar from the other barley accessions studied with respect to both chloroplast and nuclear SSR markers. The authors hypothesized that wild barley from the Fertile Crescent might not be the progenitor of the barley cultivated in Eritrea (and Ethiopia), and an independent barley domestication might have taken place in the Horn of Africa that gave rise to Eritrean/Ethiopian barley. Arregui et al. (2013) studied whole-genome molecular variation in wild and cultivated barley accessions from the western Mediterranean, Fertile Crescent, Ethiopia, and Tibet using SNP markers. Characterizing some of the genomic regions containing domestication (e.g. photoperiod response) genes, and the quantitative trait locus for flowering time, the authors claimed that Ethiopia is a possible center of barley domestication, along with the western Mediterranean and Fertile Crescent. This was in line with earlier phenotype diversity-based claims of the East

African Plateau of Ethiopia and Eritrea as a possible center of origin of barley (Negassa 1985; Vavilov 1951). The Ethiopian/Eritrean barley landraces with a long history of existence might carry untapped alleles for disease resistance and other agronomic traits. The level of variation for disease resistance and other important traits in landrace and wild barley accessions collected from diverse agro-ecological zones could be exploited using traditional bi-parental mapping and a genome-wide genetic analysis approach.

1.4 Genetic linkage mapping and genome-wide association mapping

Broadly speaking, genomic locations (genes or quantitative trait loci [QTL]) underlying or associated with a trait of interest in an organism can be identified through two main genetic methods: linkage mapping (aka bi-parental mapping or QTL mapping) and genome-wide association mapping.

1.4.1 *Bi-parental mapping*

Bi-parental mapping is a classical genetics procedure employed to map agriculturally, economically and biologically important traits in many species of plants and animals. The procedure is a forward genetics approach of identifying the genetic variant contributing to a phenotype or trait and positioning it on the genome. To conduct linkage mapping, two accessions of a species contrasting for a target trait are crossed to develop progenies segregating for the phenotype under investigation.

Genetic linkage was first discovered by Bateson et al. (1905), while studying flower color and pollen shape in sweet pea (*Lathyrus odoratus* L.) plants. Through their findings, the researchers were able to observe deviations from Mendel's laws of independent assortment for the traits they were studying because of the presence of linkage. The underlying reasons behind genetic linkage were later explained by Morgan (1910) through his genetic study of *Drosophila melanogaster* Meigen, which led to theconstruction of the first genetic maps. Further linkage studies in pea (*Pisum sativum* L.) and *Drosophila* provided information regarding gene location on chromosomes and resulted in the concept of gene mapping. Since then, linkage mapping has become an

established method for mapping molecular markers on the genome and discovering markers linked to a gene or QTL controlling a target phenotype.

Thousands of morphological and molecular markers have been mapped through linkage analysis in barley (see section 1.2 above). So far, at least 5,000 barley genes have been genetically mapped (Schulte et al. 2009). These genes control morphological phenotypes, quality traits, and reaction to various biotic and abiotic stresses, among others. In the era of molecular biology, gene or QTL mapping is complemented with high throughput approaches of gene identification and availability of marker rich genetic maps. Despite the relatively low map resolution, high comparative cost, and extensive labor and time requirements for developing segregating populations, linkage mapping is still widely used in plant species lacking a whole-genome sequence and in plant breeding programs. Genetic mapping has been effectively utilized in barley for mapping many traits, including major disease resistance genes (e.g. Borovkova et al. 1995; Druka et al. 2000; Kilian et al. 1994; Steffenson et al. 2009).

1.4.2 *Genome-wide association mapping*

Genome-wide association study (GWAS; synonymous with association mapping and interchangbly used hereafter) or linkage disequilibrium (LD) mapping has been utilized in human genetics to dissect the genetic basis of complex inherited diseases (Risch 2000). GWAS are based on statistical tests for associations between genotypes (polymorphisms in nearby markers) and phenotypes to identify a candidate genetic locus underlying a trait. GWAS identify QTL by examining the marker-trait associations that can be attributed to the strength of LD between markers and functional polymorphisms across a set of diverse germplasm (Zhu et al. 2008).

There are two approaches for association mapping. In the GWAS approach, diverse individuals are genotyped with several thousand molecular markers, ideally positioned at regular intervals along the chromosomes. This approach requires genome-wide markers and involves complex statistical analysis (Hirschhorn and Daly 2005). In the candidate-gene approach, previously known genes are targeted for genotyping

(Pflieger et al. 2001) to find allelic variants that might underlie natural trait variation. Previously known genes are those whose function have been characterized or proposed to have a role in the phenotype of interest. The candidate gene approach relies on a hypothesis about the identity of the gene and as such is biased; however, the technique can be applied in species without a complete genome sequence (Gebhardt et al. 2007).

Unlike the bi-parental approach, which relies on linkage for gene or QTL detection in segregating progenies derived from two parents, GWAS can be applied for gene/QTL identification in a diverse set of natural populations, modern cultivars or wild progenitor species. Using large numbers of unrelated natural populations provides multiple alleles at each locus, increasing the power of QTL detection through association genetics. Bi-parental mapping populations can only be utilized to study phenotypes for which the parents have clear contrasting characters. A single representative population, once genotyped, could be used to investigate multiple phenotypes through GWAS (Breseghello and Sorrells 2006). GWAS is also becoming a method of choice to study genetic diversity in plants (Abdurakhmonov and Abdukarimov 2008).

GWAS is becoming popular for the analysis of genetic variants underlying a phenotype of interest mainly for the following reasons: (1) it can be applied to collections of diverse sets of genotypes often with unknown relationships (Malosetti et al. 2007), which makes it cost effective; (2) it has high-resolution power to detect QTL because it utilizes recombination events occurring over time (Aranzana et al. 2005); (3) it is amenable for high-throughput genotyping technologies for identifying and genotyping many available polymorphic DNA markers, and (4) it is facilitated by the recent development of rigorous statistical methods for doing marker-trait correlations. However, GWAS is not without limitations. For example, it is more prone to type I (false positive) and type II (false negative) errors than is bi-parental QTL mapping [see Breseghello and Sorrells (2006) for details].

The association mapping approach was first used in plant genetics by Thornsberry et al. (2001) in their study of loci underlying variation of flowering time and plant height

in maize. Since then, association mapping has been applied to many plants from model species to agricultural crops. For example, Ehrenreich et al. (2009) used a candidate gene approach to identify flowering time genes in *Arabidopsis*. In maize, Wilson et al. (2004) used the same approach to identify genes that might underlie natural variation in maize kernel composition and starch production. In cultivars of soft winter wheat, Breseghello and Sorrells (2006) detected markers significantly associated with kernel size on three chromosomes, which were in agreement with previous QTL studies. Using a genome wide approach, Skøt et al. (2005) identified three AFLP markers closely linked to each other within a major QTL explaining 70% of the total variation for heading date in natural populations of perennial ryegrass (*Lolium perenne* L.). In rice, 34 marker-trait associations were detected for stigma and spikelet characteristics (Yan et al. 2009). Agrama et al. (2007) also found markers associated with yield and yield components of rice cultivars in regions where QTL had previously been identified, confirming the potential of association mapping for QTL detection.

In barley, Kraakman et al. (2004) identified 18–20 markers that accounted for 40–58% of variation in mean yield and yield stability in regions where QTL for the respective traits were found earlier. Cockram et al. (2008) developed an approach to validate the feasibility of association mapping for identifying markers showing significant associations with the known genes (*VRN-H1* and *VRN-H2)* underlying seasonal growth habit in barley. Roy and colleagues (2010) identified 13 QTL for spot blotch resistance, explaining from 2.3 to 3.9% of the phenotypic variance, in wild barley accessions through a GWAS approach. The authors indicated that several of the QTL mapped to coincident regions where loci for spot blotch resistance were previously reported. A number of other GWAS for disease resistance in barley have been recently published. GWAS of contemporary US barley breeding germplasm led to the identification of QTL for resistance to Fusarium head blight (Massman et al. 2011), spot blotch (Zhou and Steffenson 2013a) and Septoria speckled leaf blotch (Zhou and Steffenson 2013b). Using GWAS, Pasam et al. (2012) detected several previously mapped as well as novel QTL for important agronomic traits of barley, including heading date, plant height, starch content and crude protein content in a spring barley collection.

Thus, association mapping is a valuable technique for studying marker-trait associations for disease resistance and other agronomic traits in representative samples of barley landraces, breeding lines, and other accessions.

GWAS are gaining popularity in the plant sciences due to the advent of high-throughput genotyping techniques and advances in statistical models amenable for handling large sets of marker data. Among other things, the success of GWAS depend on the rate of historical recombination (extent of linkage) across the genome or region underlying the trait of interest. The fundamental principle of the technique is that recombination is nil in the region between a marker and the QTL controlling a trait. In other words, there is tight linkage between a marker and the QTL underlying a trait. In some cases, a marker associated with a trait might be within a causal gene or QTL. In GWAS, the amount to which loci are linked is measured using LD, which is an important parameter affecting the outcome of GWAS. Structure within populations used for GWAS is another factor that affects the success of this analysis.

1.4.2.1 *Linkage disequilibrium*

Association mapping analysis is based on LD, which refers to the non-independence of alleles in haplotypes in a population (Gaut and Long 2003) or simply the nonrandom association of alleles at two or more loci (Flint-Garcia et al. 2003). Association mapping identifies QTL by examining the marker-trait associations that can be attributed to the strength of LD between markers and functional polymorphisms across a set of diverse germplasm (Zhu et al. 2008). LD is important for gene mapping because it takes advantage of close linkage (reduced recombination or high LD) between markers and causal variants (i.e. genes or QTL). Due to the long history of recombination, LD is reduced in natural or wild populations, and in out-crossing species. Thus, relatively large numbers of markers are required for GWAS in natural populations and out-crossing species. However, if a significant marker-trait correlation is detected, it is likely that the marker identified is close to, or within, a locus responsible for variation, increasing the precision of QTL mapping. LD distribution across the genome is affected by several

biological and evolutionary factors that affect mapping resolution. These factors include recombination, selection, mutation, mating system, type of germplasm, population size, population structure, and relatedness (Soto-Cerda and Cloutier 2012).

The concept of LD was first described by Jennings in 1917 and its quantification (*D*) was developed by Lewontin in 1964 (Abdurakhmonov and Abdukarimov 2008, as cited in Soto-Cerda and Cloutier 2012). If A and B are alleles at two loci, LD measure, *D* or *D′* (standardized version of D), would be the difference between the observed gametic frequencies of haplotypes and the expected gametic frequencies of haplotypes under linkage equilibrium.

$$D_{AB} = P_{AB} - P_A P_B$$

Where P_{AB} is the frequency of gametes carrying alleles A and B at two loci and P_A and P_B are the products of the frequencies of alleles A and B, respectively. D′ (Lewontin 1964) and r^2, the square of the correlation coefficient between two loci (Hill & Robertson 1968), are the two most utilized statistics for LD (Soto-Cerda and Cloutier 2012). For biallelic loci 1 (with alleles 'A' and 'a') and 2 (with alleles 'B' and 'b'), D' and r^2 are derived from the following formula:

$$D' = |D| / D_{max}$$

$$D_{max} = \min (P_A P_b, P_a P_B) \text{ if } D > 0;$$

$$D_{max} = \min (P_A P_B, P_a P_b) \text{ if } D < 0$$

$$r^2 = D^2 / P_A P_a P_B P_b$$

D is limited because its range is determined by allele frequencies; thus, it is not widely used. Both *D'* and r^2 have values that range between 0 and 1 that literally denote no LD and 'complete' LD between two loci, respectively.

If D'=1 between two biallelic loci, only three or fewer of the four possible haplotypes are present in the population. If all four haplotypes are detected, there has to have been either recombination or recurrent mutation (Hudson and Kaplan 1985). Although D' and r^2 are still allele frequency-dependent, the bias due to allele frequency is considerably smaller than in D, and for r^2 smaller than in D' (Ardlie et al. 2002). Currently, most LD mapping studies in plants use r^2 for LD quantification because it also provides information about the correlation between markers and QTL of interest (Flint-Garcia et al. 2003; Gupta et al. 2005). Typically, r^2 values of 0.1 or 0.2 are considered the minimum thresholds for significant associations between pairs of loci and to describe the maximum genetic or physical distance at which LD is significant (Zhu et al. 2008).

1.4.2.2 *Population structure*

Population structure is the presence of a systematic difference in allele frequency between subpopulations in a population due possibly to different ancestry, especially in the context of association studies (Kliegman 2012). Population structure is also referred to as population stratification and could be caused by selective mating between groups, often due to isolation followed by genetic drift of allele frequencies in each group. Systematic ancestry differences among subpopulations of an association mapping panel can cause confounding in GWAS, producing spurious associations: i.e. genotype-phenotype associations that are not genetically linked to any causative gene or QTL (Pritchard et al. 2000b). If not properly corrected, spurious associations due to heterogeneity of genetic backgrounds among individuals may lead to false positive and false negative results (Ziv and Burchard 2003).

Over the years, several analytical methods have been developed to minimize the effect of population stratification on GWAS. The three main types of approaches used for correcting population structure in association analyses are genomic control (GC), structured association, and principal components analysis (PCA). The GC method measures the extent of inflation due to population stratification or other confounders and makes corrections by shrinking the association-testing statistic at each marker by a

common factor (Devlin and Roeder 1999; Reich and Goldstein 2001; Zheng et al. 2006). However, this generalized adjustment of test-statistic will not maximize the power to detect true associations, depending on the genetic ancestry of individual markers (Epstein et al. 2007; Price et al. 2006). The structured association (Pritchard et al. 2000a,b) and PCA (Patterson et al. 2006; Price et al. 2006; Zhu et al. 2002) methods correct for stratification using methods that infer genetic ancestry from genotype data. The structured association approach uses subpopulation matrix information generated from a model-based clustering program such as STRUCTURE (Pritchard et al. 2000a) to compute association statistics stratifying by cluster. Inferring population structure using the STRUCTURE program is computationally intensive for large data sets. Alternatively, the use of major principal components (PCs), generated by PCA, as covariates is much less computationally intensive and has proven successful for accounting for structure in GWAS (Price et al. 2006).

All of the three above methods have limitations in that they are unable to account for other types of sample structure that may exist in the panel data sets. For example, these approaches are not efficient when family structure or cryptic relatedness is also present in addition to population structure (Price et al. 2010). Thus, modeling all possible genetic structures, depending on the population under investigation, is becoming necessary to increase the validity of GWAS results. In recent years, GWAS methods using mixed linear models (MLM) that incorporate the full covariance structure across individuals are being widely used (Yu et al. 2005). The MLM approach incorporates pairwise relatedness information of individuals (known as kinship matrix K) using genotype or pedigree information as a covariate in GWAS. A variation of the MLM called efficient mixed-model association (EMMA) can correct for a wide range of sample structures by explicitly accounting for pairwise relatedness between individuals using high-density markers to model the phenotype distribution (Kang et al. 2008). Though better at controlling spurious associations (Yu et al. 2005; Kang et al. 2008), these variance component approaches of MLM methods are not amenable for large data sets (Kang et al. 2010). Recently, faster variance component approaches that reduce the

computational time for analyzing large GWAS data sets have been developed (Kang et al. 2010).

In this dissertation, the EMMA approach was utilized to correct for sample structure (i.e., pairwise relatedness), by incorporating the kinship matrix K as a covariate in the GWAS model. The EMMA method was used because it has proven useful for correcting for population structure and genetic relatedness, as well as generating reliable results with reduced false positive inflations (Kang et al. 2008). Except for the analyses being computationally intensive for large datasets, the MLM implemented in EMMA has high statistical power to detect markers significantly associated with agriculturally important traits. Since its original development, the EMMA method has been successfully used in association mapping of various traits in several different organisms (Atwell et al. 2010; Brachi et al. 2010; Myles et al. 2011; Neumann et al. 2011; and many others).

1.5 Genetics of stem rust, spot blotch and Fusarium head blight resistance in barley

Despite its economic, ecological and scientific significance, several constraints hinder the production and productivity of barley worldwide. The major constraints include lack of improved varieties, shortage of farm inputs such as fertilizers, and a variety of abiotic (unsuitable climatic conditions) and biotic stresses. Of the biotic stresses, there are many diseases caused by parasitic fungi, viruses, bacteria, and nematodes that are capable of severely reducing barley yields globally. The major diseases of barley include leaf rust (*Puccinia hordei* Otth), powdery mildew [*Blumeria* [*Erysiphe*] *graminis* (DC) Merat f. sp. *hordei* Em. Marchal], stem rust (*Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & Henn.), spot blotch [*Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem. [teleomorph: *Cochliobolus sativus* (Ito and Kurib.) Drechsl. ex Dastur]], net blotch [*Drechslera teres* [Sacc.] Shoem. [teleomorph: *Pyrenophora teres* Drechsler]], Fusarium head blight (*Fusarium graminearum* Schwabe [teleomorph: *Gibberella zeae* (Schw.) Petch.]), smut (*Ustilago* spp.), bacterial leaf streak (*Xanthomonas translucens* pv. *translucens*), scald [*Rhynchosporium secalis* (Oudem.) J. J. Davis], ergot [*Claviceps*

purpurea (Fr.) Tul.], and Barley Yellow Dwarf (*Barley Yellow Dwarf Virus*) (Mathre 1997).

The effect of disease is prominent partly because cultivated barley has a narrow genetic base for resistance and other economically and agriculturally important traits. It is essential to address this gap of lower genetic diversity in cultivated barley through discovery of novel agronomic traits in barley landraces and wild progenitors and introgress them into elite breeding materials. Towards this end, the broad objectives of this dissertation were to:

(1) characterize the genetics of resistance to stem rust race TTKSK in landrace and wild barley accessions (chapter 2);
(2) analyze population structure of Ethiopian and Eritrean barley landrace germplasm, map genes/QTL conferring resistance against multiple fungal diseases (stem rust, spot blotch and Fusarium head blight) and those controlling agro-morphological traits correlated with Fusarium head blight through genome-wide association study (chapters 3 and 4); and
(3) characterize the diversity for micronutrient content (zinc and iron) in Ethiopian and Eritrean barley landrace germplasm and map the underlying QTL using association genetics (chapter 5).

Specific introductory information about the genetics of resistance to each of these diseases and also nutritional traits is presented below.

1.5.1 *Genetics of stem rust resistance*

Wheat stem rust (Figure 1.1), caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*), is a devastating disease of wheat and barley in many regions of the world (Roelfs et al. 1992). The rye stem rust pathogen (*P. graminis* Pers.:Pers. f. sp. *secalis* Eriks. & E. Henn.) (*Pgs*) is another less important fungus that also causes stem rust in barley. Very few stem rust resistance genes have been identified, mapped and characterized in barley. To date, at least eight stem rust resistance genes have been identified in different accessions of

barley. Most of these genes are race-specific in that they impart resistance against only specific *Pgt* races. The first stem rust resistance gene discovered in barley, *Rpg1* (acronym for Reaction to *Puccinia graminis* 1), was originally identified from barley lines Chevron (CIho 1111) and Peatland (CIho 5267) in the 1930s (Powers and Hines 1933; Sogaard and von Wettstein-Knowles 1987).

Of all identified *Pgt* resistance genes, only *Rpg1* has been extensively used in barley breeding programs throughout the Midwest United States and Canada (Steffenson 1992). It has protected barley from significant losses for more than 70 years (Steffenson 1992) and is effective against most pathotypes of *Pgt*, except a race known as QCCJ (Jin et al. 1994) and the African race TTKSK (Kleinhofs et al. 2009; Steffenson and Jin 2006). Race TTKSK (aka isolate Ug99) was detected in Uganda in 1998 and later found to be virulent for the most widely used *Pgt* resistance gene in wheat (*Sr31*) as well as many other commonly used resistance genes (Jin and Singh 2006; Pretorius et al. 2000). Since then, race TTKSK and its variants have spread to a number of other countries in Africa and also the Middle East (Mukoyi et al. 2011; Nazari et al. 2009; Pretorius et. 2012; Wanyera et al. 2006). Race TTKSK is capable of attacking over 70% of the world's wheat cultivars (Singh et al. 2008). Additionally, it also is widely virulent on barley, attacking over 97% of cultivars grown worldwide (Steffenson et al. 2012).

In addition to *Rpg1*, other *Pgt* resistance genes have been identified in different barley accessions. The *Pgt* resistance genes *Rpg2* and *Rpg3* and the *Pgs* resistance gene *rpgBH* confer resistant to moderately resistant (R-MR) infection types against different *Pgt* and *Pgs* pathotypes, respectively (Steffenson et al. 1993*;* Sun and Steffenson, 2005). The map locations of these genes are not known. The recessive *Pgt* resistance gene *rpg6* was identified from 212Y1, a barley line with an introgression of *H. bulbosum* L. chromatin. Apart from *rpg4* (for details, see chapter 1), *rpg6* is the only gene that provides resistance against race QCCJ (Fetch et al. 2009).

In addition to *Rpg1*, the stem rust resistance gene *Rpg5* is well characterized at the molecular level (reviewed by Kleinhofs et al. 2009). *Rpg5* and *rpg4* were discovered in

the barley breeding line Q21861 (PI 584766). These two genes are tightly linked and co-segregate with each other in the Q21861/SM89010 population (Borovkova et al. 1995). The *rpg4/Rpg5* complex locus maps to the long arm of barley chromosome 5H (Druka et al. 2000). With respect to gene action, *rpg4* is recessive, or at least partially so, and dependent on a functional *Rpg5* gene for full resistance against races QCCJ and TTKSK. In contrast, *Rpg5* functions as a dominant gene and provides resistance against *Pgs* isolate 92-MN-90 independent of *rpg4* (Brueggeman et al. 2009). Of the known *Pgt* resistance loci in barley, only the *rpg4*/*Rpg5* complex confers resistance against race TTKSK (Steffenson et al. 2009; this thesis chapter 2). Currently, no barley cultivars with *rpg4*/*Rpg5* have been released for the stem rust prone areas of the Midwest USA and Canada.

The *rpg4* gene is temperature sensitive being effective only at temperatures below 27°C with optimal expression at 18-20°C (Jin et al. 1994). Interestingly, barley accession Q21861, the original donor of *rpg4/Rpg5*, exhibited susceptible reaction types to race TTKSK infection in a 2009 barley screening nursery in Kenya (B. Steffenson, personal communication). This may have been due to elevated temperature conditions during the season as pathotype analyses revealed the presence of race TTKSK only (M. Rouse, personal communication). Elevated stem rust levels on line Q21861 from native *Pgt* races also were reported in Canada and Minnesota (W. Legge and A. Case, personal communication). Regardless, it is important to identify additional genes for resistance to race TTKSK in barley and transfer them into commercial cultivars before the pathogen makes its way to the Americas. It is just a matter of time before African stem rust spreads to other cereal-producing regions of the world (Hodson et al. 2012; Singh et al. 2008). Gene pyramiding across a wider agroclimatic region is a possible strategy to avoid or reduce the threat posed by TTKSK on barley and wheat production worldwide.

1.5.2 *Genetics of spot blotch resistance*

Spot blotch (Figure 1.2), caused by *Bipolaris sorokiniana*, is a common foliar disease of barley (Clark 1979) and wheat worldwide. In addition to spot blotch, *B.*

sorokiniana can cause other diseases on barley including root rot, seedling blight, and black point. Spot blotch development is favored by warm weather (above 20°C) and moist, humid conditions within the crop canopy during and after heading (Zillinsky 1983). Under such conditions, spot blotch can cause yield losses of about 35% in susceptible barley cultivars (Clark 1979). The disease can be controlled by fungicides and use of resistant cultivars, with the latter being the most cost effective and environmentally safe alternative. In North America, the deployment of resistant cultivars has been an effective strategy for over 40 years in six-rowed malting types (Bilgic et al. 2005). Several spot blotch resistance genes or QTL have been reported in barley. The studies showed that spot blotch resistance in barley is conferred by qualitative or quantitative acting loci (Arny 1951; Bilgic et al. 2005; Bilgic et al. 2006; Gonzalez Ceniceros 1990; Griffee 1925; Steffenson et al. 1996). The expression of some QTL is growth-stage dependent (Bilgic et al. 2005). More recently, Roy et al. (2010) identified 13 QTL for spot blotch resistance through GWAS in wild barley accessions, with seven of them mapping to unique chromosomal regions. Zhou and Steffenson (2013a) identified through GWAS several significant associations for spot blotch resistance in US barley breeding germplasm. The QTL on chromosomes 1H (*Rcs-qtl-1H-11_10764*), 3H (*Rcs-qtl-3H-11_10565*), and 7H (*Rcs-qtl-7H-11_20162*) were found to confer both seedling and adult plant resistance and together comprise the Midwest six-rowed durable resistant haplotype present in all Midwest six-rowed cultivars released since the 1960s.

1.5.3 *Genetics of Fusarium head blight resistance*

Fusarium head blight (FHB) (scab, ear blight) (Figure 1.3), caused primarily by *Fusarium graminearum* and also other *Fusarium* spp., is a devastating disease of barley and wheat in many countries with temperate climates (Parry et al. 1995; Ma et al. 2000). Although the disease caused periodic epidemics in the USA since the turn of the last century, it was in 1993 that FHB became one of the most destructive diseases of barley in the Upper Midwest region of the US and the Prairie Provinces of Canada (McMullen et al. 1997a; Tekauz et al. 2000). *Fusarium* infection is favored by higher levels of

precipitation, especially at heading, which also is critical for subsequent disease spread (McMullen et al. 1997b; Prom et al. 1999; Tekauz et al. 2000).

FHB infection affects yield and grain quality in several ways. Kernel infections may lead to completely destroyed kernels or lighter and shriveled grains. Such kernels would be lost during harvesting and processing (McMullen and Stack 1999). The kernels may also be discolored, further lowering the quality of the grain and thereby the market value of the crop. In addition, the *Fusarium* fungi produce mycotoxins. *F. graminearum* primarily produces deoxynivalenol (DON) in infected grain (Prom et al. 1999). DON is a trichothecene toxic to humans and other animals (Scott 1990). Barley grain with a DON content greater than 0.5 ppm is unacceptable to the malting and brewing industry (Steffenson 1998). Another phenomenon associated with FHB-infected barley grain is "gushing." Beer gushing, i.e. the overfoaming or explosion of beer from bottles, can occur when malt infected with *Fusarium* is used in beer production (Schwarz et al. 1996). Host resistance is the preferred method of choice for controlling FHB of barley (Steffenson 1998), but must be deployed in conjunction with other management strategies (residue management, crop rotation, application of fungicides) to achieve economic control. Resistance against FHB is complex and has low heritability in elite germplasm (Urrea et al. 2002). FHB resistance expression is variable and frequently poorly correlated with DON accumulation, a factor that adds further challenges to FHB resistance breeding (Rutkoski et al. 2012).

Major gene resistance to FHB has not been reported in barley. Germplasm screening efforts by different institutions identified only a few sources of partial FHB resistance, mostly in two-rowed barley. In general, six-rowed cultivars are more susceptible than two-rowed barleys (Tekauz and McCallum 1999). High kernel density may create a more conducive micro-environment for infection as six-rowed barleys usually exhibit higher levels of FHB severity and DON accumulation than two-rowed barleys (Steffenson et al. 1996). In barley, several QTL studies identified minor genes that act additively to confer resistance to *Fusarium*. Molecular markers associated with genes for FHB resistance, DON accumulation, and kernel discoloration were identified

on all seven chromosomes (Review by Kolb et al. 2001). Evaluation of materials for FHB resistance is laborious and prone to environmental fluctuations. Molecular markers have the potential to improve the efficiency of transfering FHB resistance into breeding materials. GWAS in contemporary US barley breeding germplasm was recently used to identify QTL for FHB resistance (Massman et al. 2011). From the previous evaluation of a global collection of 23,255 *Hordeum* accessions, Huang et al. (2013) characterized the haplotype of 78 accessions reported as resistant or moderately resistant. Previously, Ethiopian barley accessions with a moderate level of FHB resistance were identified (Dahl et al. 2009). In this dissertation, we have extended this effort to utilize GWAS for identifying QTL for FHB resistance and low DON concentration in a collection of Ethiopian/Eritrean barley landrace germplasm.

1.6 Nutritional value of barley and genetics of zinc and iron accumulation

Barley has been part of a sustainable source of food since pre-historic times of human evolution. Today, less than 2% of the barley grain produced is used directly for food in the United States and the European Union. However, it is major part of a staple diet in Central and West Asia (China & India), North and East Africa (Morocco & Ethiopia/Eritrea), and the mountainous regions of Andean countries in South America. In these countries, more than 60% of the barley produced is used directly for human consumption (Grando and Macpherson 2005; Newman and Newman 2006). In western countries, barley is gaining renewed attention as a "new" food due to its potential health benefit effects (Baik and Ullrich 2008; http://barleygenome.org). Barley grain contains about 65–68% starch, 10–17% protein, 4–9% β-glucan, 2–3% free lipids and 1.5–2.5% minerals (Czuchajowska et al. 1998; Izydorczyk et al. 2000; Quinde et al. 2004).

β-glucans are the most abundant constituents of the cell walls of cereal grains and are chemically referred to as (1→3,1→4)-β-D-glucans (Schmitt and Wise 2009). Specifically, in the starchy endosperm of the grain, they can contribute up to 70% by weight of the cell walls in barley, rye, and oat grain (Burton and Fincher 2009). Exccessively high levels of β-glucans have negative effects in the barley end uses for

malting/brewing. They cause a number of problems in the brewing process, including poor mash conversion, retarded and inefficient wort and beer filtration, and nonbacterial colloidal hazes in the final product (Han et al. 1995; Wei 2009). However, high β-glucan levels are desirable for human nutrition because of their cholesterol-lowering effect. The production of Barliv™, a commercial product containing barley β-glucans, resulted from the recognition of the importance of β-glucan in human health (Newton et al. 2011). The grain composition of amylase starch, β-glucans, protein, and phenolic compounds are dependent on barley genotype and environment as described in a recent review by Baik and Ullrich (2008). Grando and Macpherson (2005) also present an extensive review of the use of barley for food and perspectives for genetic improvement of the crop for human consumption in different countries.

Both genotype and environment can affect the β-glucan concentration in barley grain, but the genotype has a larger effect (Bamforth 1982). With regards to genotype effect, several mapping studies have identified loci implicated in β-glucan concentration of barley grain. A study by Powell et al. (1985) revealed that β-glucan concentration of barley grain is mainly controlled by genetic factors. It was estimated that β-glucan concentration of barley grain is controlled by two to three dominant genes (Greenberg 1977). Analysis of the Steptoe/Morex mapping population identified three large effect QTL for β-glucan concentration, one on chromosome 1H and two on chromosome 2H, each explaining 5–20% of the variation (Han et al. 1995). Molina-Cano et al. (2007) detected QTL associated with β-glucan concentration on chromosomes 1H, 5H, and 7H in the Beka/Logan doubled haploid (DH) population. Linkage mapping in the TR251/CDC Bold DH population identified a QTL on chromosome 7H, explaining up to 39% of the variation found for β-glucan concentration (Li et al. 2008). Additionally, a QTL for β-glucan on chromosome 4H, which accounted for 11% of the total phenotypic variation, was identified using a DH population from a cross of the barley cultivars CM72 and Gairdner (Wei et al. 2009).

The other nutritional benefits of barley include many vitamins and micronutrients. Barley grain contains numerous vitamins and minerals including niacin (vitamin B3),

thiamin (vitamin B1), calcium (Ca), iron (Fe), magnesium (Mg), phosphorous (P), potassium (Na), selenium (Se), zinc (Zn) and copper (Cu) (http://www.nal.usda.gov). Zn and Fe deficiencies are regarded as two of the "big five" micronutrient deficiencies in humans throughout the world, along with iodine, Se and vitamin A deficiencies (Genc et al. 2005). Micronutrient malnutrition, also termed as "hidden hunger," affects over two billion people throughout the world (Welch and Graham 1999; White et al. 2009). Zn, Fe, folate and vitamin A malnutrition were considered to be the most important global challenges at the Copenhagen Consensus 2008 conference (www. copenhagenconsensus.com). The problem of "hidden hunger" is more significant in resource-poor parts of the world where alternative food sources are scarce to supplement cereal grain-based food materials, which are inherently low in composition of micronutrients, especially if grown on soils deficient in such elements (Cakmak 2008).

It is possible to alleviate some of these micronutrient deficiencies through improving the dietary composition of locally grown food crops (Mayer et al. 2008). This could be achieved through the breeding of cultivars able to accumulate higher levels of micronutrients (Genc et al. 2005; Schachtman and Barker 1999; White et al. 2009). Towards this end, Lonergan et al. (2009) identified in a DH mapping population of barley several QTL contributing to total Zn concentration in the grain. These QTL were positioned on chromsomes 1H, 2H and 5H and explained 11 to 30% of the total variation for Zn concentration. Another report indicates the existence of genetic variation for Fe concentration in barley grain (Ma et al. 2004); however, it appears that no study has been done on the genetics of Fe accumulation in barley grain (Ghandilyan et al. 2006). In other cereal crops including wheat and rice, some progress is being made in mapping QTL for grain Fe concentration (Shi et al. 2008; Stangoulis et al. 2007; Tiwari et al. 2009), as a prerequisite to characterizing the genetic mechanisms involved in grain Fe accumulation and use of the underlying QTL in breeding.

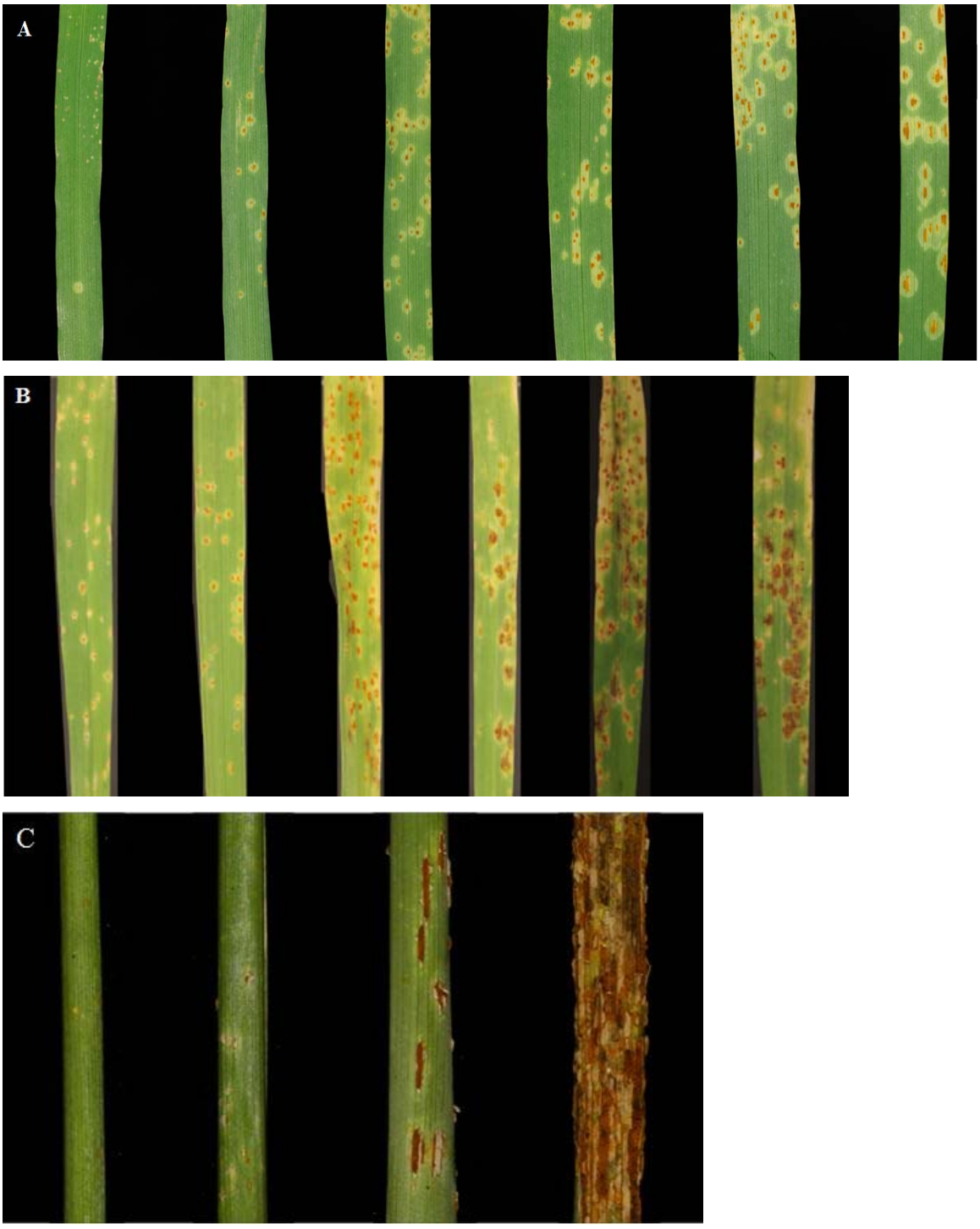

Figure 1.1. Diversity of stem rust infection types in Ethiopian and Eritrean barley landrace germplasm to races of *Puccinia graminis* f. sp. *tritici* (**A**) race TTKSK at the seedling stage, (**B**) race MCCFC at the seedling stage, and (**C**) race MCCFC at the adult plant stage.

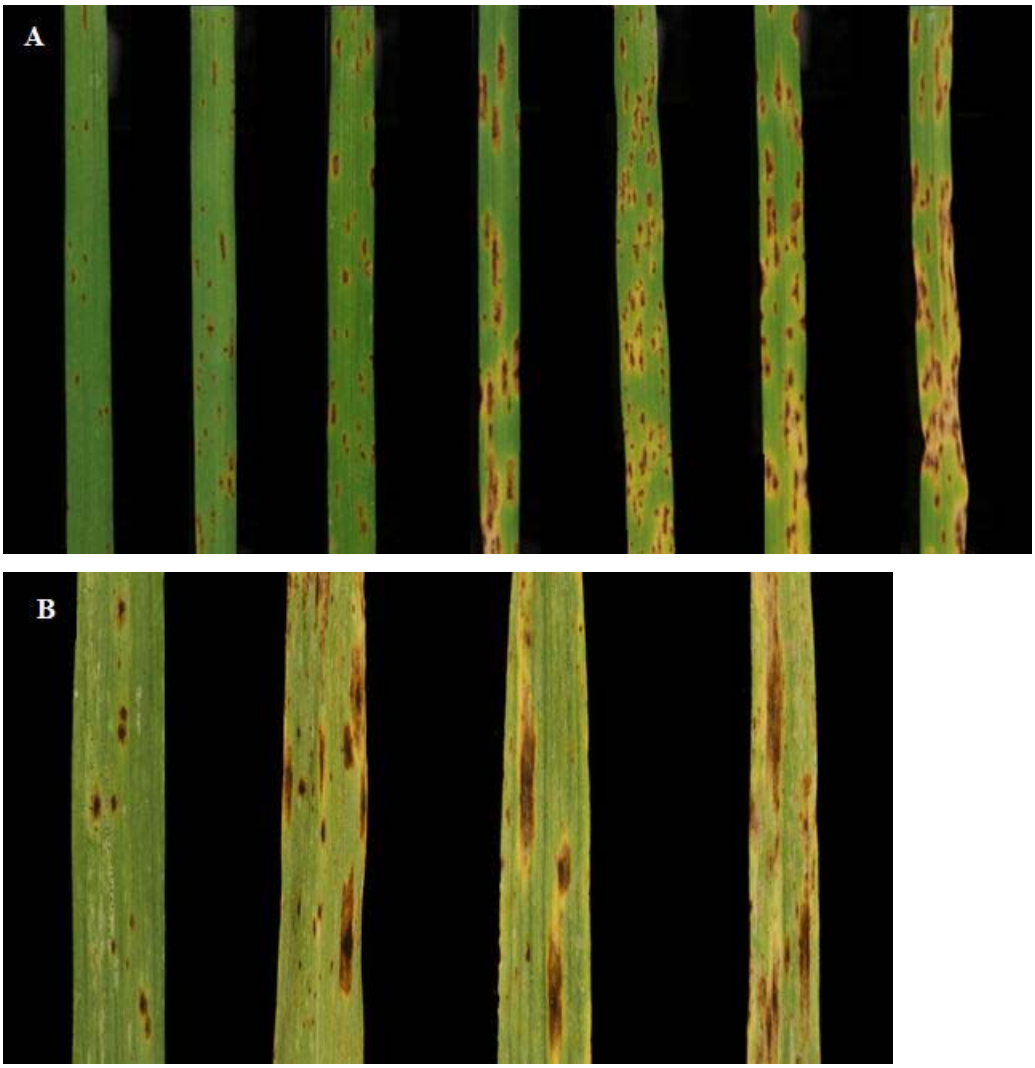

Figure 1.2. Diversity of spot blotch infection responses in Ethiopian and Eritrean barley landrace germplasm to *Cochliobolus sativus* at (**A**) the seedling stage and (**B**) adult plant stage.

Figure 1.3. Diversity of responses in Ethiopian and Eritrean barley landrace germplasm to *Fusarium graminearum.*

Chapter 2

Genetics of Resistance to Race TTKSK of *Puccinia graminis* f. sp. *tritici* in Landrace and Wild Barley Accessions

2.1 Introduction

Stem rust, caused by *Puccinia graminis* Pers.:Pers., is one of the most important diseases of wheat, barley, oat, and rye, owing to its ability to completely destroy crops in a short period of time over a large scale. Wheat stem rust, caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & Henn. (*Pgt*), attacks both wheat and barley in many regions of the world. The main effects of stem rust on wheat and barley include interruption of nutrient and water flow to the developing kernels resulting in shriveled grain, lower grain yields, and reduced grain quality (Stokstad 2007). Stems affected by rust infection are also weakened and are therefore susceptible to lodging and additional loss of grain (Roelfs et al. 1992). In barley, stem rust can impact malting quality by causing reductions in the germination and starch content of barley grain (Mwando et al. 2012).

The prevalence and severity of stem rust varies from year to year, depending on the amount of inoculum, weather conditions, and the interaction of host genotypes with specific *Pgt* races comprising the inoculum (http://ipm.illinois.edu/). The presence of ample inoculum over a wide area increases the risk of stem rust damage, and thus elevates the economic significance of the disease. *Pgt* has caused multiple widespread epidemics in the northern Great Plains region of the United States and Canada, with the most recent ones occurring in the 1930s and 1950s (Roelfs 1986). Stem rust is particularly problematic under warm weather conditions, although serious damage can occur on susceptible cultivars grown in diverse agro-climatic regions (Leonard and Szabo 2005). Losses due to stem rust in wheat have been greatly reduced in the Great Plains region of United States since the late 1950s due to the wide scale deployment of resistant, early maturing cultivars, and also the eradication of the alternate host, barberry (*Berberis vulgaris* L.) (Kolmer 2001; Roelfs 1982).

To minimize the risk of emergence of new races of *Pgt* and the subsequent damage they can cause, it is important to reduce inoculum levels and eliminate sexual recombination of the pathogen. Reducing inoculum levels will lessen the chances for

possible mutations for new virulences occurring in the pathogen population (Shafer and Roelfs 1985). Barberry was considered as a source of primary inoculum and a potential medium for the evolution of new races through sexual recombination. Since the eradication of barberry in the major cereal-producing regions of the Great Plains, the incidence of new *Pgt* races and epidemics has been very infrequent (Roelfs 1982). However, in 1998, a new race of *Pgt* designated as TTKSK (also known as isolate Ug99) was detected in Uganda (Pretorius et al. 2000) and later found to be virulent against the most widely used *Pgt* resistance gene in wheat (*Sr31*) as well as many other commonly used resistance genes (Jin and Singh 2006; Pretorius et al. 2000). Currently, race TTKSK threatens wheat and barley production worldwide. It is capable of attacking over 70% of the world's wheat cultivars (Singh et al. 2008). Additionally, it also is widely virulent on barley, attacking over 97% of cultivars grown worldwide (Steffenson et al. 2013). The mechanism contributing to the evolution of race TTKSK is not known.

Since its detection in Uganda, race TTKSK and its variants have spread to a number of other countries in Africa (Kenya, Ethiopia, Sudan, Tanzania, South Africa, Zimbabwe, Mozambique and Eritrea) and also in the Middle East (Yemen and Iran) (Mukoyi et al. 2011; Nazari et al. 2009; Pretorius et al. 2010; 2012; Visser et al. 2010; Wanyera et al. 2006; Wolday et al. 2011). Variants closely related to the original Ug99 isolate of race TTKSK from Uganda have been described. These isolates appear to be part of a clonal lineage sharing almost identical molecular fingerprints and were designated as the "Ug99 race group" by Hodson et al. (2012). Variants of the Ug99 race group are expected to spread to other cereal-producing regions of the world in the near future (Hodson et al. 2012; Singh et al. 2008).

The damaging effects of stem rust, including race TTKSK, can be mitigated by fungicide applications. However, the extra input costs and potential negative consequences of chemical treatments on the environment warrant the use of alternate methods. Repeated applications of a fungicide may lead to the development of fungicide-resistant pathogen strains and could influence the development of non-target diseases. Residual activity of fungicides also could pollute the soil and water. Some fungicides

persist undegraded for up to six months in soil and water environments (Bromilow et al. 1999; Yen et al. 2003). These chemicals may contaminate groundwater if leached from treated agriculture fields (Yen et al. 2003). Fungicides also may adversely affect the development of beneficial organisms, including mycorrhizal fungi and pollinating insects (Huntzinger et al. 2008; Murillo-Williams and Pedersen 2008; Sugavanam et al. 1994). Thus, host resistance is favored as a sustainable approach to control stem rust.

To date, eight stem rust resistance genes have been identified in different accessions of barley. The first stem rust resistance gene in barley, *Rpg1* (abbreviation for Reaction to *Puccinia graminis* 1), was identified from barley accessions Chevron (CIho 1111) and Peatland (CIho 5267) (Powers and Hines 1933; Shands 1939). *Rpg1* has been bred into every major malting cultivar in the northern Great Plains of the United States and Canada since the 1950s and has protected barley from serious losses for over 70 years (Steffenson 1992). However, the new African stem rust races like TTKSK are highly virulent for *Rpg1* and therefore threaten barley production in the Upper Midwest region (Steffenson and Jin 2006; Steffenson et al. 2007). The stem rust resistance genes *Rpg2* and *Rpg3* were identified from the accessions Hietpas-5 (CIho 7124) (Patterson et al. 1957) and PI 382313 (Jedel 1989; Jedel 1990), respectively, and like *Rpg1*, are not completely effective against race TTKSK (Steffenson et al. 2013). In another genetic study conducted with cultivar Peatland, Fox et al. (1995) identified *RpgU* in addition to *Rpg1*. The recessive stem rust resistance gene *rpg4* was identified in breeding line Q21861 (PI 584766) and confers resistance to QCCJ (Jin et al. 1994a), a race identified in North Dakota in 1989 and responsible for minor outbreaks of stem rust in the northern Great Plains in the early 1990s (Roelfs et al. 1991, Roelfs et al. 1993). More recently, a recessive stem rust resistance gene (*rpg6*) was identified in 212Y1, a barley line with an introgression of bulbous barley grass (*H. bulbosum* L.) chromatin (Fetch et al. 2009). Barley also is a host to a second *forma specialis* of *Puccinia graminis*: *P. graminis* f. sp. *secalis* (*Pgs*) (or the rye stem rust pathogen), and several resistance genes to this pathogen have been described. The dominant resistance gene *Rpg5* (initially designated *RpgQ*) was discovered in the breeding line Q21861 (Brueggeman et al. 2008; Sun et al. 1996). *Rpg5* is very tightly linked to *rpg4* and is located on the long arm of barley

chromosome 5H (Brueggeman et al. 2008; Druka et al. 2000). A recent study by Wang et al. (2013) identified two tightly linked loci required for *rpg4*-mediated resistance, one of which includes the *Rpg5* locus. The three genes at the *Rpg5* locus implicated in *rpg4*-mediated resistance are *Hv*Rga1 (a nucleotide binding site-leucine rich repeat (NBS-LRR) domain gene), *Rpg5* (an NBS-LRR-protein kinase domain gene) and *Hv*Adf3 (an actin depolymerizing factor-like gene) (Wang et al. 2013). The authors hypothesize that these genes follow the emerging pathogen recognition and resistance model that pairs of unrelated yet genetically linked resistance genes are required to function together against specific races or isolates of the corresponding pathogen. A recessive gene designated as *rpgBH* also confers resistance to rye stem rust and was described from Black Hulless (CIho 666) (Steffenson et al. 1984).

Of all identified stem rust resistance genes, only *Rpg1* has been extensively used in barley breeding programs in the United States and Canada (Steffenson 1992). *Rpg1* is effective against most races, except QCCJ and TTKSK (Kleinhofs et al. 2009; Steffenson and Jin 2006). A quantitative trait locus (QTL) with minor effect on chromosome 2H was shown to reduce infection by race TTKSK in line Q21861, but the largest effect identified by far in all environments was at the *rpg4/Rpg5* locus (Moscou et al. 2011). Using an association mapping approach in US barley breeding germplasm, Zhou (2011) detected a minor effect QTL conferring adult plant resistance at a chromosomal position coincident to the one identified by Moscou et al. (2011). No signal was detected at the *rpg4*/*Rpg5* region, indicating that the genes at this locus are likely not present in US barley breeding germplasm nor current commercial cultivars.

Given that race TTKSK and variants in its lineage can cause devastating losses in wheat and barley and are a potential threat to production worldwide (Hodson et al. 2012; Mwando et al. 2012; Singh et al. 2011; Steffenson et al. 2011), it is important to identify and genetically characterize new sources of resistance and transfer their resistance genes into commercial cultivars. Identification and utilization of additional TTKSK resistance genes in barley would help to avoid or reduce potential epidemics that could result from widespread distribution of race TTKSK and related variants. Though diversity for stem

rust resistance is limited in cultivated barley (Jin et al. 1994b), wild barley and landrace accessions are potential sources of novel disease resistance genes, including those conferring resistance to race TTKSK (Steffenson and Jin 2006; Steffenson et al. 2007). In fact, it has been long-recognized that wild and landraces of barley are rich sources of disease resistance and are valuable resources in resistance breeding (Fetch et al. 2003; Newton et al. 2011; Roy et al. 2010; Yun et al. 2005).

Towards this end, the screening of a worldwide collection of barley germplasm has been completed at the University of Minnesota to identify new sources of resistance to race TTKSK. This work has identified a number of sources of seedling resistance to race TTKSK in the *Hordeum* gene pool comprising cultivars, landraces, and wild barley accessions (Steffenson et al. 2013). Subsequent evaluations of a subset of these accessions at the Kenya Agricultural Research Institute (KARI) in Njoro, Kenya, a hot spot for African stem rust races, revealed several that also were resistant at the adult plant stage (B. Steffenson, unpublished). Some of the most resistant accessions included landraces (*Hordeum vulgare* ssp. *vulgare*) from Switzerland and also accessions of wild barley (*H. vulgare* ssp. *spontaneum*) from the Wild Barley Diversity Collection (WBDC) (Steffenson et al. 2007). Six of these resistant barley accessions were chosen for detailed study to elucidate the genetic basis of race TTKSK resistance prior to their use in breeding. Knowledge of the genetics of TTKSK resistance in these accessions can aid in the introgression of stem rust resistance into adapted or advanced breeding materials. Thus, the specific objectives of this research were to: (1) characterize the inheritance of resistance to race TTKSK in landrace and wild barley accessions at the seedling stage through bi-parental mapping; (2) map and determine the chromosomal locations of the TTKSK resistance gene(s); and (3) determine the allelic relationships among the resistance gene(s) in these accessions and the previously described *rpg4*/*Rpg5* complex locus.

2.2 Materials and Methods

2.2.1 *Plant materials*

Six *Hordeum* accessions exhibiting seedling and/or adult resistance to race TTKSK were crossed with the susceptible barley cultivar Steptoe (CIho 15229) to develop mapping populations for genetic analysis (Table 2.1). Four of the accessions (Hv501, Hv545, Hv602 and Hv612) were landraces originally collected from the alpine regions of eastern Switzerland (Canton Graubünden), a country previously known to be a source of stem rust resistant barley germplasm (Steffenson 1992). Seed was donated by the Station federale de recherches en production vegetale de Changins in Nyon, Switzerland, courtesy of Geert Kleijer. Selection of these four accessions from a total collection of 74 landraces was based on their genetic diversity as revealed by 12 simple sequence repeat (SSR) markers (P. Olivera, unpublished), geographic location within Graubünden, and their resistant stem rust phenotype. The two other accessions (WBDC213 and WBDC345) investigated were wild barleys collected from Samarkand and Kashkadarya provinces of Uzbekistan, respectively (Table 2.1). These accessions were part of the Wild Barley Diversity Collection (Steffenson et al. 2007), provided by the International Center for Agricultural Research in the Dry Areas in Aleppo, Syria, courtesy of Jan Valkoun. They were selected based on their genetic diversity with 558 Diversity Array Technology (DArT) and 2,878 single nucleotide polymorphism (SNP) markers (Roy at al. 2010; J. Roy and B. Steffenson, unpublished) and also their resistant stem rust phenotype.

Prior to crossing, all resistant accessions were purified by making a single plant selection based on their phenotype to stem rust. Crosses and population advancements (F_1 to F_3) were made in the greenhouse in 2008 and 2009. Individual F_3 families were derived from single F_2 plants. A total of 120 to 187 F_3 families from crosses between the resistant barley accessions and Steptoe were evaluated to stem rust in this study (Table 2.2).

2.2.2 *Planting, inoculation and incubation of plants*

Twenty-five to 35 plants of each F_3 family were evaluated at the seedling stage for response to race TTKSK. The tests were conducted inside the Biosafety Level-3

(BSL-3) Containment Facility at the University of Minnesota, St. Paul campus, during the winter months. F_3 families and the parents were planted in plastic pots (7.6 × 7.6 × 10.8 cm, l × w × h) filled with a 50:50 mix of steam-sterilized native soil and Metro-Mix® 200 (Sun Gro Horticulture, Quincy, MI), a growing media containing vermiculite, sphagnum peat moss, perlite, dolomitic limestone, and a wetting agent. The pots were placed into support trays for easy handling. Several replicates of the respective parents of each population were included in each experiment. Additionally, multiple replicates of the following classes of controls also were included in all evaluations to monitor the infection level and virulence of race TTKSK: (1) barleys with no known resistance genes (Steptoe, Hiproly [PI 60693], and 80-TT-30 [CIho 16130]); (2) barleys with *Rpg1*, a gene effective against many races, but not *Pgt*-TTKSK (Chevron [PI 38061], Morex [CIho 15773] and 80-TT-29 [CIho 16129]); (3) barleys with the gene complex *rpg4/Rpg5*, which is effective against races TTKSK and QCCJ (line QSM20 and Q21861 [PI 584766] with the additional gene of *Rpg1*); and (4) susceptible wheat controls of Line E (PI 357308) and McNair 701 (CItr 15288). F_2 plants from the crosses were not evaluated because of space limitations for growing this generation to the F_3 within the BSL-3 facility.

After planting, all pots were watered and fertilized with Osmocote® controlled release fertilizer 14-14-14 (Scott's Company, Marysville, OH) (1.4 g/pot) and Peters Dark Weather fertilizer 15-0-15 (Scott's Company) (ca. 40 g/liter at 1/16 dilution). Populations derived from wild barley accessions were kept at 4°C for two weeks to facilitate uniform emergence and growth prior to inoculation. All populations were initially kept in a greenhouse at 19-22°C with a 16 hr photoperiod (supplemented by 400 W high-pressure sodium lamps emitting a minimum of 300 µmol photons $s^{-1}m^{-2}$). When the plants began to emerge from the soil, they were brought into the BSL-3 facility for the remainder of the experiment. Inside the BSL-3 greenhouse, plants were grown at 20-22°C and 75% RH with a 14 hr photoperiod (supplemented by 400 W high-pressure sodium lamps emitting a minimum of 300 µmol photons $s^{-1}m^{-2}$). Stocks of rust isolate 04KEN156/04 of race TTKSK (Steffenson et al. 2009) were utilized in this experiment.

This rust isolate was initially increased on a susceptible wheat host (McNair701), collected, desiccated in a 20% RH chamber, and stored in tubes at −80°C until needed.

On the day of inoculation, the tubes of stored urediniospores were heat-shocked in a water bath at 45°C for 10 min and rehydrated in an 80% RH chamber for one hour. A small sample of urediniospores was then sprayed onto a water agar plate to assess the germination rate. After two hours of incubation in the dark at room temperature, the germination rate was checked by scanning random fields on the plate using a compound microscope. Urediniospores were weighed and placed in gelatin capsules (size 00, Capsuline, Inc., Pompano Beach, FL) containing a lightweight mineral oil carrier (Soltrol 170; Phillips Petroleum, Bartlesville, OK). A concentration of 14 mg urediniospores/0.7 ml oil was used, but additional spores were added if the germination rate was markedly lower than 100%. The rust spore suspension was then applied to 8 to 9-day-old plants with fully expanded primary leaves at a rate of approximately 0.09 mg/plant with an atomizer pressured at 25-30 kPa (Sun and Steffenson 2005). After inoculation, plants were kept on carts until most of the oil carrier had evaporated from the leaves. Then, they were placed in chambers misted with ultrasonic humidifiers initially for 30-40 minutes of continuous misting and thereafter for 4-8 minutes every hour for 16-18 hours in the dark. After the wetness period, light was provided by 400 W sodium vapor lamps (150-250 mmol photons $s^{-1}m^{-2}$), and the mist chamber doors were opened slightly to dissipate the heat. At this time, the humidifiers were set to run for 4-8 minutes of misting every 15 minutes for the next 2 hours. After 2 additional hours, the misters were turned off and the chamber doors opened halfway to facilitate slow drying of the plant surfaces under continuous light for the next 3 to 4 hours. Finally, when the leaf surfaces were completely dry, plants were returned to the greenhouse under the conditions previously described. Plants were fertilized with a 20:20:20 (N:P:K) water soluble fertilizer formulation (J.R. Peters, Inc., Allentown, PA) after they were returned to the BSL-3 greenhouse.

2.2.3 *Rust infection phenotyping*

Twelve to 14 days after inoculation, stem rust ITs were assessed on the first leaves of plants based on the 0 to 4 scale originally developed for wheat by Stakman et al. (1962) and modified for barley. The IT scale used for barley is based primarily on uredinial size as described by Miller and Lambert (1955). Plants with ITs ranging from 0 to 2^{3-} were classified as resistant and those from $3^{-}2$ to 3^{+} as susceptible. Individual F_3 families were grouped into three classes of homozygous resistant (HR), heterozygous or segregating (SEG), or homozygous susceptible (HS) based on the general reactions of individual plants.

2.2.4 *Statistical test*

Pearson's chi-square (χ^2) test was used to evaluate independence of segregation for genetic ratios in the F_3 generation. The chi-square statistic and associated *P*-values were calculated using the *chisq.test* function in the R statistical software program (version 2.10.1).

2.2.5 *Genotyping of parents and bulks for bulk segregant analysis*

A single locus was found to impart resistance to race TTKSK in each of the six populations (see Results section below). This simple inheritance pattern is ideal for conducting genetic analyses using bulk segregant analysis (BSA) (Michelmore et al. 1991; Quarrie et al. 1999). HR and HS F_3 families were identified after phenotyping at the seedling stage. Then, three separate sets of eight HR (24 total) and eight HS (24 total) families each were used to create bulks of the six respective populations for BSA. For the Steptoe/Hv602 population, only 20 HR families were used to create the HR bulks because of the limited number of such families identified (see Table 2.2). The separate bulks were analyzed to ensure that heterozygous F_3 families were not inadvertently included in the bulks.

2.2.5.1 *Sample preparation for DNA extraction*

One arbitrarily selected seed from each HR and HS family was grown in the greenhouse and the leaf tissue harvested for DNA extraction. Leaf tissue from plants representing eight F_3 families was bulked to create the independent HR and HS bulks for BSA. Additionally, five seeds each of the resistant parents and Steptoe also were grown and leaf tissue harvested for DNA extraction. Leaf tissue was collected two weeks after sowing when the plants were at the three to four leaf stage. Segments of tissue (~5 cm long) were cut from the second leaf of each plant, folded twice and carefully placed in a 2.0 mL microtube. The microtubes were kept on ice during sample collection. Then, the tubes were frozen in liquid nitrogen and stored at −80°C until the samples were freeze-dried or lyophilized. For freeze drying, the samples were removed from the −80°C freezer, the lids of tubes opened, and then the entire rack of tubes covered with miracloth (EMD Millipore Corporation, Billerica, MA) using tape. Samples were freeze-dried using a general purpose freeze dryer (Model 24DX48; Virtis Company, Gardiner, NY) according to the specifications of the manufacturer. After freeze drying, the samples were stored at −80°C until the day of DNA extraction.

2.2.5.2 *DNA extraction and genotyping*

The freeze-dried tissue was used for total genomic DNA extraction using the DNAeasy Plant Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. DNA sample quality was checked by separating and visualizing on a 1% agarose gel. Two µL of stock DNA, 3 µL of double-distilled water, and 5 µL of 1X BlueJuice™ Gel Loading Buffer (Life Technologies, Grand Island, NY) were loaded onto the gel along with a 1 to 3 µL ladder (size marker DNA solution) and subjected to electrophoresis. After electrophoresis, the gel was stained by rinsing it in an appropriate volume of 0.5 µg/ml ethidium bromide stain for 20 minutes and then destaining for 15 minutes in distilled water. The gel was then briefly rinsed with distilled water to remove any residual staining solution, placed on a UV-transparent plastic tray and subjected to UV transillumination for nucleic acid visualization. The amount of DNA in each sample was then quantified by measuring absorbance at 260 nm (A_{260}) with a spectrophotometer (Labomed, Inc., Culver City, CA). The DNA concentration of each sample was then

normalized to 200 ng/µL, and 5 µL of each normalized sample was submitted for genotyping.

The barley samples were genotyped with 1,536 single nucleotide polymorphism (SNP) markers of the Barley Oligonucleotide Pooled Assay 1 (BOPA1) (Close et al. 2009; Rostocks et al. 2006). The 1,536 SNP markers were tested on the resistant parents, Steptoe, and the three individual HR and HS bulks per population. Illumina Bead Array Technology was used for genotyping with the GoldenGate assay (Fan et al. 2003, 2006) at the BioMedical Genomics Center (BMGC) of the University of Minnesota. The BOPA1 SNP markers were previously mapped onto the integrated molecular genetic linkage map of barley (Close et al. 2009; Muñoz-Amatriaín et al. 2011).

2.2.5.3 *Genotype data analysis*

Data generated by the GoldenGate assay were visualized and analyzed with the Genotyping Module of the GenomeStudio data analysis software (Illumina, San Diego, CA) GSGT version 1.8.4. After visual inspection for quality control, the SNP clusters were manually adjusted to improve the overall quality of the data. Samples with a 95% or greater call rate were retained. The final SNP data for each locus were generated and exported into a Microsoft Excel spreadsheet using the automatic allele calling platform integrated into the GenomeStudio software. SNPs polymorphic between the respective resistant parents and Steptoe were identified. All of the polymorphic SNP call data were manually checked, and positive hits for BSA were noted when alleles of at least two of the resistant and/or susceptible bulks clustered close with alleles of the resistant parent or Steptoe, respectively, in the GenCall output.

2.2.6 *Allelism tests*

To determine the allelic relationships among stem rust resistance genes in the different resistant accessions and also Q21861, a half diallel was attempted. Successful crosses were obtained for most, but not all combinations due mostly to flowering time differences and poor pollen production. F_1 plants were grown in the greenhouse. F_2 seeds

were sown, and plants were inoculated with race TTKSK according to the protocol described above.

2.2.7 *Resistance spectrum of parents to other stem rust races*

To determine the resistance spectrum of the TTKSK-resistant accessions and also help resolve whether they may contain the same resistance gene complex of *rpg4*/*Rpg5*, additional races/isolates of *Puccinia graminis* were used. Race QCCJ of *Pgt* was used because the resistance gene *rpg4* (and other genes in concert [Wang et al. 2013]) is specifically effective against it at low incubation temperatures (18-20°C) (Jin et al. 1994). Another hallmark of *rpg4* is its temperature sensitivity. Thus, assays also were made with race QCCJ at 27-28°C, where the gene is rendered completely ineffective (Jin et al. 1994a). To assay the possible presence of the closely linked gene *Rpg5*, isolate 92-MN-90 of *Pgs* was used. In previous studies, *Rpg5* was found to confer a clear low reaction type to this pathogen isolate (Steffenson et al. 2009; Sun et al. 1996). Finally, race HKHJ of *Pgt* was used because it is capable of identifying *Rpg1* in the presence of other resistance genes (Sun and Steffenson 2005). Two replications of the parental accessions and control lines were evaluated in experiments that were repeated twice in time against each race or isolate. The conditions for plant growth and procedures for inoculation and IT assessment were made according to the methods described previously. The only exception was for the inoculation with race HKHJ where a concentration of 35 mg urediniospores/0.7 ml oil was used due to a lower than normal germination rate.

2.2.8 *Molecular characterization of the Rpg5 region*

Two pairs of primer sequences of sequence tag site (STS) markers, designed for *rpg4*/*Rpg5* marker development utilizing sequence data generated from the cv. Morex bacterial artificial chromosome (BAC) contig spanning the *rpg4*/*Rpg5* region (Brueggeman et. al. 2008; Wang et al. 2013), were used to genotype the resistant parents, the susceptible parent Steptoe, and line Q21861, the original source of the gene complex (Druka et al. 2000; Jin et al. 1994a; Sun et al. 1996). The markers were used to characterize the presence of *Rpg5* by the polymerase chain reaction (PCR). The PCR

reaction was set up in a 20 µL total volume comprising 2 µL of 100 ng gDNA, 12.7 µL of ddH_2O, 1 µL of 10 µM each of forward and reverse primer, 2 µL of 10x Red Taq PCR Reaction Buffer, 1 µL of Red Taq DNA Polymerase (Sigma-Aldrich, St. Louis, MO), and 0.3 µL of 10 µM dNTPs. Primer sequences for the STS markers are given in Table 2.3. The PCR cycling conditions were as follows: denaturation at 94°C for 5 minutes, 35 cycles of initial cycle at 94°C for 30 seconds; 35 cycles of annealing at 62°C for 1 minute; 35 cycles of extension at 72°C for 1 minute, and a final extension at 72°C for 7 minutes. The expected size of the PCR product is given in Table 2.3. Five µL of PCR product was electrophoresed on a 1.0% TBE 1× agarose gel stained with 3 µL of the fluorescent nucleic acid gel stain GelRed™ (Phenix Research Products, Candler, NC), followed by visualization on a UV light transilluminator. Q21861 has a 1 kb band with the *Rpg5* STS1 primer pairs, and Steptoe has a 800 bp band with the PP2C STS1 primer pairs. The *Rpg5* gene has three main domains (see Figure 2.1; Brueggeman et al. 2008). The *Rpg5* STS1 marker targets the NBS and the LRR regions and is specific to resistant lines. The PP2C STS1 marker amplifies the LRR to the PP2C region and is specific to susceptible lines. Sequencing of a small region of *Rpg5* also was conducted to determine whether the functional units of the gene [serine/threonine protein kinase (STPK) domain and the 5' end of the gene] are intact.

2.3 Results

2.3.1 *Genetics of resistance to race TTKSK of Puccinia graminis f. sp. tritici*

2.3.1.1 *Phenotyping of parents*

The landrace and wild barley accessions exhibited highly resistant ITs (modes of 0; to 0;1) in response to race TTKSK at the seedling stage. In contrast, Steptoe was susceptible, exhibiting an IT mode of 3 (occasionally 3$^-$2). The resistant accessions also showed much lower rust severities and infection responses than Steptoe in the limited Kenyan field trials where they were included (Table 2.1).

2.3.1.2 *Genetics of resistance as determined from phenotyping of F_3 families*

Most of the resistant F_3 plants exhibited ITs ranging from 0; to 12 (rarely 21; & 23^-) and could be easily differentiated from susceptible plants giving ITs of 3^-2 to 3^+. Thus, individual families of all populations could be confidentally grouped into HR, SEG, or HS (Figure 2.2).

Segregation data for F_3 families of all populations closely fit a 1:2:1 ratio for HR:SEG:HS (χ^2 ranging from 0.50 to 5.34 with *P*-values ranging from 0.07 to 0.52) (Table 2.2). These data clearly indicate that a single gene confers stem rust resistance in Hv501, Hv545, Hv602, Hv612, WBDC213 and WBDC345. To determine the gene action of the genes in the resistance sources, several different aspects were investigated. First, a limited number of F_1 plants were phenotyped. The IT mode of F_1 plants from the Steptoe/Hv545 population was similar to that of the susceptible parent Steptoe (3^-2 vs. 33^+) (Figure 2.2), suggesting a recessive or at least a partially recessive gene. Unfortunately, no other F_1 seeds were available from the other crosses. Second, the composition of individual plant reactions within each segregating F_3 family was tallied to assess possible gene action (Appendix Table 2.2). IT ranges of 0; to 23^- and 3^-2 to 3^+ were assigned for resistant and susceptible plants, respectively. At least half of the segregating families evaluated of each population (except WBDC213/Steptoe) fit a single gene ratio (Appendix Table 2.2). Some of the segregating F_3 families in the six populations did not follow a clear Mendelian ratio for an expected single recessive gene as found for the Steptoe/Hv545 F_1 plant. For the Steptoe/Hv501, Steptoe/Hv602 and WBDC345/Steptoe populations, 56% (27/48), 62% (33/53), and 48% (29/60) of the segregating F_3 families, respectively, fit a 1:3 ratio for resistant to susceptible plants, suggesting recessive gene action in the respective resistant parents (Appendix Table 2.2). Unexpectedly, however, 23% (11/48), 22% (13/60) and 28% (15/53) of the segregating F_3 families of these three respective populations showed dominant gene action, i.e. 3:1 ratio of resistant to susceptible plants (Appendix Table 2.2). None or just one segregating F_3 family of the Steptoe/Hv545, Steptoe/Hv612 and WBDC213/Steptoe populations followed a recessive gene action ratio of 1:3 (Appendix Table 2.2). The corresponding number of segregating F_3 families following a 3:1 ratio for resistant:susceptible plants for these populations was 54% (13/24), 50% (15/30), and 45% (22/49), respectively

(Appendix Table 2.2). For the Steptoe/Hv545 population, the dominant gene action discerned from the plants within the segregating families contradicts the putative partially recessive resistance gene action in Hv545 deduced from the IT of the single Steptoe/Hv545 F_1 plant.

2.3.2 *Genotyping of parents and bulks, and bulk segregant analysis*

In order to determine the chromosomal location of the resistance genes, genetic analysis was conducted using BSA. Each bulk contained eight different HR or HS F_3 families with the exception of the Steptoe/Hv602 population where each HR bulk contained seven, seven, and six different HR F_3 families. A total of 525, 552, 564, 588, 568 and 561 polymorphic SNPs were identified between the respective resistant parents (Hv501, Hv545, Hv602, Hv612, WBDC213 and WBDC345) and susceptible parent Steptoe after screening with 1,536 BOPA1 SNPs. SNP markers with possible linkage to the resistance locus were determined after establishing two broad criteria. First, those polymorphic SNPs differentiating at least two of the HR bulks (plus the resistant parent) and two of the HS bulks (plus Steptoe) were considered as putatively linked SNP markers to the resistance loci in the TTKSK-resistant barley accessions (Table 2.4). Accordingly, alleles of 18 SNPs in total (one in Steptoe/Hv501, three in Steptoe/Hv545, four in Steptoe/Hv602, and five each in Steptoe/Hv612 and WBDC345/Steptoe F_3 families) differed between two or more of the HR and HS bulks (Table 2.4). Of these, one SNP each in all of the three resistant and susceptible bulks of Steptoe/Hv545 and Steptoe/Hv612, and two SNPs in all of the three resistant and susceptible bulks of WBDC345/Steptoe clustered together with the respective resistant parent (Table 2.4 SNPs in bold).

Second, in cases where alleles of all the HR bulks were similar to the allele clusters of the resistant parent and alleles of the susceptible bulks had a different cluster from the susceptible parent, the SNP also was considered as a putative “candidate” SNP linked to the identified resistance gene in the populations (Table 2.4 SNPs in italic). This is because a genetic change or some other genetic factor might contribute to the shift of

the allele cluster of the susceptible bulks away from the respective parent. Based on these criteria, alleles of 12 SNP markers in total (two each in Steptoe/Hv501 and Steptoe/Hv545, three in Steptoe/Hv602, and five in WBDC213/Steptoe F_3 families) clustered with the resistant parent in all three resistant bulks (Table 2.4 SNPs in italic).

Most of the 1,536 SNP markers were previously mapped onto the integrated consensus map of barley (Close et al. 2009; Muñoz-Amatriaín et al. 2011). Almost all SNPs that had alleles clustered with the respective parent in all three resistant and susceptible bulks, and in all three resistant bulks (positive SNPs)--irrespective of their pattern in the HS bulks of all six populations--were located within a 10 cM region of the long arm of chromosome 5H between SNP markers 11_0582 and 11_1053 (Table 2.4; Figure 2.3; Figure 2.4). Likewise, most SNPs that clustered with the respective parent in all three resistant bulks or in all three resistant and susceptible bulks (positive SNPs) of all six populations were located within the same region of chromosome 5H. In addition, other SNPs in which two of the resistant bulks clustered with the resistant parent or in which two or more of the susceptible bulks clustered with the susceptible parent were also located in the same interval of chromosome 5H in all populations (Table 2.4; Figure 2.3; Figure 2.4). This result suggests that all six barley accessions contain a resistance gene mapping to the same region of chromosome 5H. The *rpg4/Rpg5* complex locus in Q21861 also maps to this same region of chromosome 5H (Steffenson et al. 2009). One exception to note is that in the Steptoe/Hv602 population, a SNP (11_0987) that maps at 99.39 cM on the same arm of chromosome 5H had all the three resistant bulks clustered with the resistant parent (Table 2.4; Figure 2.4). This region is interesting because Zhou (2011) identified a novel adult plant resistance locus against race TTKSK in the 69.3-103.9 cM interval of 5HL.

2.3.3 *Allelism tests*

Relationships among the gene(s) conferring resistance to race TTKSK identified in this study and *rpg4/Rpg5*

Crosses for the half diallel among the resistance sources and also Q21861 were obtained for a select number of the 21 possible combinations: Hv545/Hv602 (300 progeny), Hv545/Hv612 (140 progeny), Hv602/Hv612 (280 progeny), Q21861/Hv501 (680 progeny), Q21861/Hv545 (500 progeny), and Q21861/Hv612 (760 progeny). F_2 progeny derived from the crosses of Hv545/Hv602, Hv545/Hv612, and Hv602/Hv612 did not segregate for reaction to race TTKSK (Table 2.5). All progeny exhibited resistant ITs, indicating that the same allele imparts resistance in each of the three landraces. Likewise, F_2 progeny derived from the crosses Q21861/Hv501, Q21861/Hv545, and Q21861/Hv612 also did not segregate for reaction to race TTKSK, demonstrating that these three landraces likely carry a resistance gene allelic to the *rpg4/Rpg5* complex in Q21861. Three F_2 plants derived from Q21861/Hv612 and one F_2 plant derived from Q21861/Hv501 showed an intermediate infection type, but these were retested and later confirmed to be resistant to race TTKSK. Attempts at other crosses for the half diallel were not successful; however, some deductions may be made based on the current results. Although Hv602 was not crossed to Q21861, it likely carries a resistance gene allelic to the *rpg4/Rpg5* complex based on the allelism of Hv602 to Hv612 and Hv612 to Q21861.

2.3.4 *Resistance spectrum of parents to other stem rust races*

Landrace and wild barley accessions were inoculated with three additional races/isolates of *Puccinia graminis* to help resolve whether they contain the same resistance gene complex of *rpg4*/*Rpg5* and also to profile their resistance spectrum. The accessions exhibited similar low ITs as Q21861 (with *rpg4*/*Rpg5* and *Rpg1*) and QSM20 (with *rpg4*/*Rpg5*) to race QCCJ at low temperature. In contrast, all accessions exhibited similar high ITs as Q21861 to the race at high temperature, a condition under which the gene complex is rendered ineffective. Steptoe, the susceptible control, gave high ITs to race QCCJ at both incubation temperatures (Table 2.6). The TTKSK-resistant accessions also were evaluated against *Pgs* isolate 92-MN-90 to determine if they carry *Rpg5*. The low ITs exhibited by the six accessions were similar to those exhibited by Q21861 and QSM20, suggesting they also carry *Rpg5*. Steptoe gave a high IT to isolate 92-MN-90

showing that it lacks *Rpg5* (Table 2.6). Finally, the six TTKSK-resistant accessions were evaluated to race HKHJ to assess whether they might carry *Rpg1*. The accessions Hv501, Hv612, WBDC213 and WBDC345 exhibited similar high ITs as QSM20 (with *rpg4/Rpg5*), SM41 (with *Rpg5* only) and other controls without *Rpg1* (Steptoe and Hiproly) to race HKHJ, suggesting they lack *Rpg1*. The Swiss accessions Hv545 and Hv602 gave low to intermediate ITs: 210; and 213^{-}, respectively. These accessions exhibited similar results under repeated evaluations and may possess a different resistance spectrum. Q21861 gave a low IT to race HKHJ verifying that it has *Rpg1* (Table 2.6).

2.3.5 *Molecular characterization of the Rpg5 region*

Recent high-resolution recombinant analysis by Wang et al. (2013) indicated that the *rpg4/Rpg5* region spans a ~290 kbp physical region and contains several candidate genes. However, the smallest region required for race TTKSK and QCCJ resistance corresponds to a ~70 kbp genetic interval (Wang et al. 2013). The parental lines were genotyped at the *rpg4*/*Rpg5* region using sequence tagged site (STS) markers (Brueggeman et. al., 2008; Wang et al. 2013; GenBank accession number EU812563) to assay the presence of *Rpg5*. Genotyping through PCR indicated that all parents contain the nucleotide binding site (NBS) of the *Rpg5* gene (Table 2.7). The STS markers also were used for haplotype analysis of the parental genotypes in the functional regions of *Rpg5* through sequencing and revealed that all resistant parents contain an intact STPK (serine/threonine protein kinase) domain at the 3' end of the *Rpg5* gene. This further indicated that *Rpg5* is functional in all resistant accessions (Table 2.7). Steptoe contained the non-functional allele of *Rpg5* as it lacks the STPK domain.

2.4 Discussion

Race TTKSK is a serious threat to wheat and barley production worldwide because of its virulence on multiple resistance genes of agricultural importance. Steffenson et al. (2009) previously reported that the stem rust resistance locus *rpg4/Rpg5* in line Q21861 was the only locus described in barley that confers resistance against race

TTKSK. From a large screening effort of *Hordeum* germplasm at the seedling stage, the Swiss landraces Hv501, Hv545, Hv602 and Hv612 as well as wild barley accessions WBDC213 and WBDC345 were found to be among the most resistant accessions identified to race TTKSK (B. Steffenson, unpublished). To fully characterize the genetics of resistance in these accessions and therefore enable more efficient use in breeding, the following studies were conducted: 1) bi-parental populations were developed to determine the inheritance of resistance and define the genome position of the resistance locus using BSA; 2) allelism tests were made to resolve the relationships of genes among some of the resistance sources; 3) accessions were tested to other stem rust races to characterize the resistance spectrum of the gene(s) and postulate their possible identity; and 4) specific primers were used for detecting *Rpg5*, a gene implicated with *rpg4* in conferring resistance to race TTKSK.

Genetic analysis of the segregating populations clearly indicated that a single gene confers seedling resistance to race TTKSK in the six barley accessions (Table 2.3). Monogenic inheritance for resistance to different races/isolates of the wheat and rye stem rust pathogens have been reported in a number of barley accessions in previous studies (see Appendix Table 2.1). Steffenson et al. (2009) reported that resistance to race TTKSK in line Q21861 segregates as a single gene that lies at the *rpg4*/*Rpg5* region. It is likely that this same gene complex confers resistance in the accessions characterized in this study (see further evidence below). The population Steffenson et al. (2009) evaluated had only 129 progeny and therefore segregation of the two closely linked genes was unlikely. The recessive gene *rpg4* controls resistance to race QCCJ in Q21861 at low incubation temperatures (18−21°C) (Jin et al. 1994a). This gene was also thought to confer resistance to rye stem rust in a partially dominant fashion (Sun et al. 1996) until Brueggeman et al. (2008) identified recombinants exhibiting resistance to *Pgs* isolate 92-MN-90 and not race QCCJ from large segregating populations. The gene conferring resistance to rye stem rust is partially dominant, lies only a few recombination units from *rpg4*, and was designated as *Rpg5* (Brueggeman et al. 2008).

Efforts to characterize the gene action (recessive vs. dominant) of TTKSK resistance in the resistance sources did not yield clear-cut results. The IT mode of an F_1 plant from the Steptoe/Hv545 population and the ratio of plant reaction types within 48 to 62% of segregating F_3 families of the Steptoe/Hv501, Steptoe/Hv602 and WBDC345/Steptoe populations were suggestive of a recessive or at least partially recessive acting resistance gene. On the contrary, the ratio of plant reaction types in other segregating F_3 families was suggestive of a dominant gene. One explanation for the lack of conclusive results concerning gene action based on segregating F_3 families is that multiple genes are likely involved in the phenotype as recently found by Wang et al. (2013), and therefore some F_3 plants might represent various recombinations within the *rpg4/Rpg5* complex of genes. This contention was supported by the IT data observed in the Steptoe/Hv501 population, where some resistant families exhibited intermediate ITs instead of the typical 0; to 0;1 types shown by the resistant parent. In addition, some plants in the susceptible families of the Steptoe/Hv602 population had quite variable infection types, ranging from $3^{-}2$ to 3^{+}. This suggests again that there might be complementary resistance genes involved in the population(s). Indeed, the recessive nature of *rpg4* is determined at the *Rpg5* locus by the HvPP2C dominant susceptibility factor (R. Brueggeman, unpublished), suggesting that other genes in the vicinity may influence the nature of gene action in the resistance sources. Another mitigating factor is that barley often exhibits mesothetic reactions (a mixture of different ITs on the same plant) in response to stem rust infection, and this can confound the classification of resistant and susceptible plants within segregating families (Steffenson 1992).

The *rpg4* locus (now the *rpg4/Rpg5* complex locus) was mapped to the long arm of barley chromosome 5H using molecular markers (Borovkova et al. 1995; Druka et al. 2000). *Rpg5* was later isolated through positional cloning (Brueggeman et al. 2008). Wang et al. (2013) have recently implicated *Rpg5*, along with other tightly linked genes in the region, in *rpg4*-mediated resistance against TTKSK and QCCJ. The multiple genes in the *rpg4/Rpg5* region required for resistance to TTKSK and QCCJ often segregate as a single locus because they are very closely linked, i.e. within a ~70 kbp physical region. Research is underway to identify additional informative recombinants in the region to

resolve which gene(s) are essential for conferring TTKSK resistance (Wang et al. 2013). This information will be useful for identifying the genes needed to confer TTKSK resistance in the landrace and wild barley accessions.

After demonstrating a monogenic inheritance pattern for the resistant accessions, BSA was used to define the chromosomal locations of the resistance loci for TTKSK resistance. The linked SNP markers identified via BSA in the six populations all map to the subtelomeric region of the long arm of chromosome 5H in the 158 to 165 cM interval between SNP markers 11_0374 and 11_1053 (Table 2.4; Figure 2.4).

The SNPs detected by BSA lie in a region coincident with *rpg4/Rpg5*. A previous but lower resolution mapping study of the resistance gene *rpg4* in the Q21861/SM89010 (Q/SM) population using restriction fragment length polymorphism (RFLP) markers identified MWG740 and ABG390 as linked markers (Borovkova et al. 1995). That study mapped *rpg4* 5.7 cM distal from the RFLP marker ABG390. On recent consensus maps, ABG390 lies in the same genomic region with the SNP markers detecting the TTKSK resistance locus in the current study (Close et al. 2009; http://wheat.pw.usda.gov/GG2/index.shtml). After discovery that the *rpg4* locus consisted of more than a single gene, the *rpg4/Rpg5* complex locus in Q21861 was found to impart TTKSK resistance (Moscou et al. 2011; Steffenson et al., 2009). Steffenson et al. (2009) mapped the gene(s) conferring TTKSK resistance in line Q21861 to the *rpg4/Rpg5* complex locus based on the cosegregation of this resistance with the previously mapped *rpg4* locus conferring resistance to race QCCJ and also resistance to *Pgs* isolate 92-MN-90 (Sun et al. 1996). Moscou et al. (2011) mapped qualitative TTKSK seedling stage resistance in the Q/SM population to 146.78 cM on chromosome 5H using mRNA transcript abundance based on the Barley1 Affymetric array. Based on the chromosomal position in consensus maps of RFLP markers linked to the *rpg4* locus, specifically ABG390, the TTKSK resistance locus detected by BSA analysis in this study lies very near the *rpg4/Rpg5* complex locus. In BSA, SNP markers that were positive in each of the three resistant and susceptible bulks and also detected in more than one population were positioned at a more proximal location on chromosome 5H, closer to the

putative location of *rpg4/Rpg5* (see SNPs in bold in Figure 2.4). Other markers, not positive in all three resistant and susceptible bulks and detected in only one of the populations, were positioned at more distal locations on the chromosome. The other interesting region to note is the 69.3-103.9 cM interval of 5HL where a novel adult plant TTKSK resistance locus was identified through association mapping in barley breeding germplasm (Zhou 2011). BSA in the Steptoe/Hv602 population identified a marker (SNP 11_0987) that maps at 99.39 cM on the same arm of chromosome 5H.

Steffenson et al. (2007) identified DArT markers significantly associated with wheat stem rust (race MCCF) resistance in the *rpg4/Rpg5* gene complex region of chromosome 5H through association mapping in the WBDC. A bi-parental mapping study with one of the resistant wild barley accessions (WBDC348 also known as 'Damon') identified a single major gene conferring resistance to stem rust races MCCF and QCCJ in the same bin as *rpg4*/*Rpg5* (Alsop et al. 2007). Research is underway to continue high resolution recombinant analysis in the *rpg4/Rpg5* region to precisely map these resistant loci (Wang et al. 2013).

The BSA approach has some drawbacks. First, it does not provide a well-defined chromosomal position. However, coupled with the GoldenGate assay, it was recently employed to precisely locate a locus in soybean that confers resistance to soybean rust (Hyten et al. 2009). The authors used only three HS bulks for the procedure in one mapping population and identified one candidate region that contained the resistance gene. In the current study, we used three HR and three HS bulks for BSA in six different mapping populations and also identified only one candidate region for resistance—a chromosomal position previously found to contain a TTKSK-resistance locus (Steffenson et al. 2009). Second, the consensus map of SNP markers used for BSA was developed based on other mapping populations, whereas *rpg4* (now *rpg4/Rpg5* locus) was originally mapped in the Q/SM population with low marker resolution (Borovkova et al. 1995). In other words, disparate bi-parental mapping studies and consensus mapping studies could partly confound the interpretation of BSA results. Third, the Q/SM map initially used for mapping the *rpg4/Rpg5* locus had a very low marker density. Taken together, it is

possible that the *rpg4/Rpg5* map position identified using the Q/SM population may not exactly align with the position of the TTKSK resistance gene(s) found in this study. Nonetheless, since the ABG390 marker linked to the *rpg4/Rpg5* locus and the SNP markers identified through BSA lie in the same genomic interval, the BSA result should indicate a precise location for the TTKSK locus.

To provide additional data regarding the relationships among the resistance genes identified in the six accessions and also the *rpg4/Rpg5* complex in line Q21861, tests of allelism were made. No segregation was observed in crosses between the Swiss landraces (Hv501, Hv545 and Hv612) with unknown genes and Q21861 with the *rpg4/Rpg5* complex (Table 2.5). This indicates that the gene(s) conferring TTKSK resistance in the landraces are either allelic with those at the *rpg4/Rpg5* locus or are closely linked to it. Further confirmation of this finding was obtained from the allelism tests among selected Swiss landraces (Table 2.5). No segregation was observed in any of these populations, demonstrating that Hv501, Hv545, Hv612 and Hv602 all carry the same allele for resistance to race TTKSK and that it resides at *rpg4/Rpg5* locus.

Extra precautions were taken to make the allelism tests robust since the presence of even a single susceptible plant can change the conclusions. First, low infection or some inherent issues of the inoculation and incubation conditions might alter the expression of the true phenotype in F_2 plants. To avoid such problems, the experiment was replicated at least twice in time, and extra care was taken to maintain rigorous standard experimental conditions. Second, to reduce the possibility of mis-scoring the phenotype of progeny, each and every F_2 plant was carefully assessed for its rust reaction in comparison with numerous controls planted throughout the experiment. Mis-classifying plants was a concern because barley often exhibits mesothetic reactions in response to stem rust infection (Steffenson 1992). For the very few F_2 progeny that showed possible high infection types, the plants were grown up to maturity and F_3 families phenotyped for stem rust reaction. In each case, F_3 families derived from the suspicious F_2 plants were found to be all resistant. Conducting allelism tests with small F_2 populations may not reveal a susceptible F_2 plant if the resistance loci in question are closely linked. In this study, we

included at least 150 F_2 plants for the allelism tests among landraces, which should yield, with a probability of 99%, at least one susceptible plant if the two genes are linked within 1 cM of each other (Hanson 1958). Since the TTKSK resistance gene(s) in the four landrace accessions were suspected to be either allelic with those at the *rpg4/Rpg5* locus or closely linked to them, large populations of more than 500 F_2 plants were screened in the crosses with Q21861. No segregation was found among the F_2 progenies. Not all crosses were successful between the six resistant accessions and Q21861, even after several attempts. In particular, the resistance genes in the wild barley accessions WBDC213 and WBDC345 were not tested for allelism with the *rpg4/Rpg5* locus in Q21861 or those present in the Swiss landraces. These crosses will be made in the future to determine the relationships among the genes. Nevertheless, the allelism tests from crosses that were made with the Swiss landraces and also Q21861 showed no segregation in F_2 progeny. These data strongly suggest that the TTKSK resistance genes in the Swiss landraces are likely allelic with those at the *rpg4/Rpg5* gene complex.

To obtain more data as to whether the resistant accessions contain the same resistance gene complex of *rpg4*/*Rpg5*, additional phenotype evaluations were made with *Pgt* races QCCJ and HKHJ and *Pgs* isolate 92-MN-90. These tests were critical because the genes have unique hallmarks: *rpg4* is temperature sensitive (Jin et al. 1994), and *Rpg5* specifically confers resistance to rye stem rust without the need for other genes (Sun et al. 1996). The Swiss landraces and wild barleys exhibited resistant ITs against QCCJ at low temperature and susceptible ITs at high temperature, similar to those exhibited by Q21861 with the *rpg4/Rpg5* complex (Steffenson et al. 2009; Brueggeman et al. 2009). The resistant accessions also gave low ITs against *Pgs* isolate 92-MN-90, similar to those exhibited by Q21861, suggesting they also carry *Rpg5* (Table 2.6). These results strongly support our hypothesis that the six resistance sources contain the *rpg4/Rpg5* locus. Tests with race HKHJ indicated that Hv501, Hv612, WBDC213 and WBDC345 likely lack *Rpg1*. This is in agreement with molecular analyses indicating the absence of this gene in the Swiss landraces (B. Steffenson and R. Brueggeman, personal communication). Two accessions (Hv545 and Hv602) exhibited unexpected ITs for HKHJ under multiple evaluations. Known controls with *Rpg1* gave classical ITs of 0; to

10; when tested with HKHJ. In contrast, Hv545 and Hv602 exhibited low to intermediate ITs of 210; and 213⁻, respectively, to the race. From molecular analysis, these two landraces lack *Rpg1* (B. Steffenson and R. Brueggeman, personal communication). However, they may carry a partially effective gene against HKHJ. This result should be verified with further, including testing the two accessions with stem rust races under different temperature conditions.

Molecular haplotyping provided another strong piece of evidence concerning the presence of a functional *Rpg5* gene being present in the six resistant accessions. *Rpg5* encodes a protein with nucleotide binding-site, leucine-rich, and protein kinase domains (Brueggeman et al., 2008). Molecular characterization of the *Rpg5* region with STS markers indicated that all six resistant accessions contain a functional *Rpg5* gene (Table 2.7). A sequenced portion of the allele also revealed that the serine/threonine protein kinase (STPK) domain at the C-terminus end of the *Rpg5* gene is intact. The STPK domain is a functionally crucial unit of *Rpg5* for conferring resistance. These data demonstrate that TTKSK resistance in landrace and wild barley accessions likely involves *Rpg5*. By lieu of its close linkage to other genes, the presence of *Rpg5* in these sources also strongly suggests the presence of *rpg4* and other nearby genes needed for conferring TTKSK resistance. The latest research on stem rust resistance mediated by the *rpg4/Rpg5* region suggests that *Rpg5* is the R-gene that is responsible for the gene-for-gene interaction determining *rpg4*-mediated resistance and is the only reliable gene with polymorphism that can be used to determine the presence of *rpg4*-mediated resistance (R. Brueggeman, unpublished). In the future, any newly discovered barley accessions with TTKSK resistance should be initially screened for the presence of the functional *Rpg5* gene to determine whether the resistance might be novel or not. This test will likely serve to identify the other genes at the locus since *rpg4* and *Rpg5* are likely conserved as revealed by their discovery both in landrace and also wild barley accessions.

In summary, segregation data from F_3 families developed from crosses of landrace and wild barley accessions with the susceptible cultivar Steptoe clearly indicated that a single locus confers resistance to race TTKSK. Molecular genetic mapping by

BSA, together with molecular haplotyping for a functional *Rpg5* gene and screening with rye stem rust demonstrate that the TTKSK resistance gene in the landrace and wild barley accessions map to the *rpg4/Rpg5* region. The body of data taken together strongly suggest that the TTKSK resistance genes in the barley accessions are simply alleles of genes already implicated to impart resistance against this race—specifically the *rpg4/Rpg5* gene complex. Of the stem rust resistance genes identified in barley thus far, only the *rpg4/Rpg5* locus is effective against TTKSK, one of the most virulent races of stem rust in eastern Africa.

Wheat stem rust race TTKSK (isolate Ug99) remains a potential threat to wheat and barley production (Singh et al. 2011; Hodson et al. 2012; Mwando et al. 2012). More than 97% of barley accessions are susceptible to race TTKSK worldwide (Steffenson et al. 2011; Steffenson et al. 2012). Currently, only one described gene complex has been found to confer resistance to this race in barley. This gene is present in the rare landrace and wild barley accessions found resistant to race TTKSK, highlighting the extreme vulnerability of the *Hordeum* gene pool to the "Ug99 group" of stem rust races worldwide. Q21861 is the original source of the *rpg4/Rpg5* gene complex and is one of the best known accessions possessing a high level of adult plant resistance against race TTKSK. Several barley breeding programs in North America are introgressing *rpg4/Rpg5* into elite breeding lines for resistance to race TTKSK. However, future work should be done to identify additional sources of resistance so that barley cultivars can be developed with a broad spectrum of resistance to *Pgt* races, including race TTKSK and its variants. Gene pyramiding is a possible strategy to reduce the threat posed by the "Ug99 race group" in barley; however, care must be taken not to unduly expose the only effective gene complex over large "hotspot" regions of cultivation.

Table 2.1. Reaction of Swiss barley landraces, wild barleys and susceptible control Steptoe to stem rust race *Pgt*-TTKSK at the seedling and adult plant stages

Accession[a]	Origin	Location	Seedling reaction		Kenya 2008	Kenya 2009	Kenya 2010
			IT mode[b]	IT range[c]	Sev % / IR[d]	Sev % / IR	Sev % / IR
Hv501	Switzerland	Near Bonaduz	0;1	0; to 0;1	2 MR	--	0 R
Hv545	Switzerland	Near Disentis	0;1	0; to 0;1	5 MS	--	0.5 R/5MS
Hv602	Switzerland	Unknown	0;	0; to 10;	1 R	--	0 R
Hv612	Switzerland	Near Laret	0;	0; to 0;1	20 MS-MR	--	0 R
WBDC213	Uzbekistan	Samarkand	0;	0; to 0;1	--[e]	10 MR/20 MS-S	--
WBDC345	Uzbekistan	Kashkadarya	0;1	0; to 210;	--	Trace MS/Trace R	--
Steptoe	USA	Washington	3^+	3^-2 to 3^+	40 S	--	20 S-MS

[a] Hv (*Hordeum vulgare*) number assigned by the Station federale de recherches en production vegetale de Changins in Nyon, Switzerland; WBDC: Wild Barley (*H. vulgare ssp. spontaneum*) Diversity Collection accession described by Steffenson et al. (2007).
[b] IT mode represents the one or two most commonly observed ITs on plants, listed in order of their relative prevalence.
[c] Infection type (IT) range represents the lowest and highest ITs observed on plants. Plants were evaluated for their ITs based on the 0 to 4 scale originally developed for wheat (Stakman et al. 1962) and modified for barley (Steffenson et al. 1993). ITs 0, 0;, 1, 2, and 23^- are considered indicative of host resistance, and types 3^-, 3, and 3^+ are indicative of susceptibility.
[d] Terminal rust severity (0-100%) at the adult plant stage was based on the modified Cobb scale (Peterson et al. 1948; Stubbs et al. 1986). The infection responses (IR) were based on the size and type of uredinia observed where R: resistant; MR: moderately resistant; MS: moderately susceptible, and S: susceptible (McIntosh et al. 1995; Roelfs et al. 1992). Severity readings of "Trace" denote very low rust infection (<0.5%) in the field. The slash symbol (/) indicates the variation in stem rust severity at different times of disease rating during the growing season.
[e] Not Tested.

Table 2.2. Segregation for resistance in F_3 families from crosses of landrace and wild barley accessions with Steptoe to race TTKSK of *Puccinia graminis* f. sp. *tritici* at the seedling stage

	Number of F_3 families					
Cross	**Homozygous resistant**	**Segregating**	**Homozygous susceptible**	**Expected ratio**	**χ^2 value (2 df)**	**Probability (>χ^2)[a]**
Steptoe/Hv501	32	48	40	1:2:1	3.20	0.20 ns
Steptoe/Hv545	48	98	35	1:2:1	0.50	0.47 ns
Steptoe/Hv602	20	47	43	1:2:1	5.34	0.07 ns
Steptoe/Hv612	39	88	60	1:2:1	2.59	0.28 ns
WBDC213/Steptoe	30	52	36	1:2:1	1.32	0.52 ns
WBDC345/Steptoe	32	76	52	1:2:1	2.60	0.27 ns

[a] ns = not significant at 0.05.

Table 2.3. Primer sequences for polymerase chain reaction (PCR)-based markers in the *rpg4/Rpg5* region of barley chromosome 5H

			PCR products (bp)[c]	
Markers[a]	**Primers**	**Sequence 5′–3′[b]**	**Resistant line**	**Susceptible line**
Rpg5 STS1	LRK-F1	GGTGGATCGAAGAGAATGGAACTGC	1,046	None
	LRK-R1	GCAACCTTCATTCTGACAGACCATG		
PP2C STS1	RpgQ-F6	AGATGCACCTATCTGCATCGAGGAC	None	841
	PP2C-R2	CCCGCGGTTTGCCGATGAAGAGAGTC		

[a] Markers are designated as sequence tag sites (STS).

[b] Primers are listed with 5′–3′ sequences.

[c] Amplicon sizes for genomic DNA are shown for each marker. The Rpg5 STS1 marker is a dominant PCR marker only amplifying a product from the resistant parents, e.g. line Q21861. The PP2C STS1 marker is a dominant PCR marker only amplifying a product from susceptible lines such as Steptoe (Wang et al. 2013).

Table 2.4. Chromosomal positions of putative SNPs linked to a locus conferring seedling resistance to stem rust race TTKSK in landrace and wild barley accessions identified through the genotyping of TTKSK resistant and susceptible bulks of F_3 families via bulked segregant analysis

Chrom.	Arm	Position along chrom. (cM)[a]	SNP designation[b]	Mapping pop. in which associations were identified	No. HR bulks[c]	No. HS bulks[d]
3H	L	66.62	11_1341	Steptoe/Hv612	2	3
5H	L	51.51	11_0480	Steptoe/Hv602	1	3
		99.39	*11_0987*	*Steptoe/Hv602*	*3*	*1*
		152.93	*11_1121*	*WBDC213/Steptoe*	*3*	*1*
		155.45	*11_0582*	*WBDC213/Steptoe*	*3*	*1*
		155.66	*11_0960*	*WBDC213/Steptoe*	*3*	*0*
		157.61	11_0374	Steptoe/Hv545	3	2
		157.61	11_0374	Steptoe/Hv602	2	2
		157.61	11_0374	Steptoe/Hv612	3	2
		157.61	11_0374	WBDC345/Steptoe	2	3
		157.61	*11_0612*	*Steptoe/Hv545*	*3*	*1*
		157.61	11_0612	Steptoe/Hv602	2	2
		157.61	11_0612	Steptoe/Hv612	2	2
		157.61	**11_0612**	**WBDC345/Steptoe**	**3**	**3**
		157.61	*11_0959*	*Steptoe/Hv545*	*3*	*0*
		158.28	11_1499	WBDC345/Steptoe	2	3
		162.98	**11_1389**	**Steptoe/Hv545**	**3**	**3**
		162.98	11_1389	Steptoe/Hv612	3	2
		162.98	*11_1389*	*WBDC213/Steptoe*	*3*	*1*
		162.98	**11_1389**	**WBDC345/Steptoe**	**3**	**3**
		163.72	11_0514	Steptoe/Hv501	2	2

Table 2.4. See next page.

Table 2.4. Continued.

Chrom.	Arm	Position along chrom. (cM)[a]	SNP designation[b]	Mapping pop. in which associations were identified	No. HR bulks[c]	No. HS bulks[d]
5H	L	163.72	11_0514	Steptoe/Hv602	2	2
		163.72	11_0514	Steptoe/Hv612	2	3
		163.72	11_0655	WBDC345/Steptoe	2	3
		164.15	*11_0610*	*Steptoe/Hv602*	*3*	*1*
		164.15	*11_0610*	*WBDC213/Steptoe*	*3*	*1*
		165.28	*11_1053*	*Steptoe/Hv501*	*3*	*1*
		165.28	11_1053	Steptoe/Hv545	2	2
		165.28	11_1053	Steptoe/Hv602	2	2
		165.28	**11_1053**	**Steptoe/Hv612**	**3**	**3**
		168.44	*11_0508*	*Steptoe/Hv501*	*3*	*0*
		168.44	*11_0508*	*Steptoe/Hv602*	*3*	*0*

[a] SNP marker position according to Muñoz-Amatriaín et al. (2011).
[b] SNP marker nomenclature according to Close et al. (2009); SNP markers that were positive in each of the three resistant and susceptible bulks are indicated in bold; SNP markers that were positive in each of the three resistant bulks and in none or one of the susceptible bulks were considered as putative "candidate" SNPs. Such SNPs are indicated in italics.
[c] Number of HR bulks (out of three) which have alleles identical to the respective resistant parent.
[d] Number of HS bulks (out of three) which have alleles identical to the respective susceptible parent.

Table 2.5. Crosses made for allelism tests among race TTKSK resistant Swiss landraces and line Q21861 and resulting phenotypes observed in the F_2 populations

Female parent		Male parent		F_2 plants			
					Phenotypes		
Line name	**Gene**	**Line name**	**Gene**	**No.**	**Resistant**	**Susceptible**	**Ratio**
Q21861	*Rpg1, rpg4/Rpg5*	Hv501	Unknown	680	680	0	1:0
Hv545	Unknown	Hv602	Unknown	300	300	0	1:0
Hv545	Unknown	Hv612	Unknown	140	140	0	1:0
Q21861	*Rpg1, rpg4/Rpg5*	Hv545	Unknown	500	500	0	1:0
Hv602	Unknown	Hv612	Unknown	280	280	0	1:0
Q21861	*Rpg1, rpg4/Rpg5*	Hv612	Unknown	760	760	0	1:0

[a] Q21861 carries *rpg4/Rpg5*, a pair of closely linked resistance genes that are effective against race TTKSK. Q21861 also carries *Rpg1*, which is effective against other wheat stem rust races, but not race TTKSK.

Table 2.6. Seedling infection types (IT) of parental lines and controls in response to wheat stem rust races *Pgt*-QCCJ and *Pgt*-HKHJ and rye stem rust isolate *Pgs*-92-MN-90

	Infection type[b]			
	***Pgt*-QCCJ**		***Pgt*-HKHJ**	***Pgs*-92-MN-90**
Accession[a]	**21°C**	**28°C**	**21°C**	**21°C**
Hv501	0;1	3⁻2	3⁻2	0;
Hv545	0;1	3⁻2	210;	0;
Hv602	0;1	3⁻2	213⁻	0;1
Hv612	0;1	3⁻2	3⁻2	0;
WBDC213	0;	3⁻2	3⁻3	0;
WBDC345	0;1	3⁻2	3⁻2	0;1
Q21861	0;1	3⁻2	0;	0;
QSM20	0;1	3⁻2	33+	0;
Steptoe	3⁻2	33⁺	3⁻3	3⁻2

[a] See Table 2.1 for information on the accessions.
[b] See Table 2.1 for information on IT classification. The IT mode is reported here.

Table 2.7. Genotyping and sequencing of barley accessions for *Rpg5*

Accession	*Rpg5*[a]	Kinase[b]	5' end insertion[c]	Sequence[d]	*Rpg5* polymorphism[e]	Remark
Hv501	+	+	-	Unknown	Unknown	
Hv545	+	+	-	Unknown	Unknown	
Hv602	+	+	-	+	Unknown	
Hv612	+	+	-	Unknown	Unknown	
WBDC213	+	+	-	Unknown	Unknown	
WBDC345	+	Unknown	Unknown	Unknown	Unknown	
Q21861	+	+	-	+, D	Q21861	Reference
Steptoe	+	-	Unknown	-, D	No kinase	Susceptible parent

[a] *Rpg5* NBS (nucleotide binding site-leucine rich repeat) region was present.
[b] The C-terminal protein kinase is intact (+ for resistant genotype) or replaced by the PP2C gene (- for susceptible genotypes).
[c] The 5' end of the allele does not contain an insertion that results in a frame shift and truncated *Rpg5* protein (-).
[d] Resistant genotype (+) or susceptible genotype (-) based on the sequenced portions of the allele; the entire allele has been sequenced (D) (Wang et al. 2013).
[e] Indicates presence of polymorphism compared to Q21861 allele in sequences determining the non-functional alleles.

Figure 2.1. Domains of the stem rust resistance gene *Rpg5* based on the predicted protein structure from barley accession Q21861. NBS: nucleotide binding site; LRR: leucine-rich repeat; STPK: serine/threonine protein kinase domain also known as the protein kinase (pKinase) domain. The amino acid positions are shown above the domains in base pairs. Protein structure and amino acid position data are based on Brueggeman et al. (2008). Figure is modified from Wang et al. (2013).

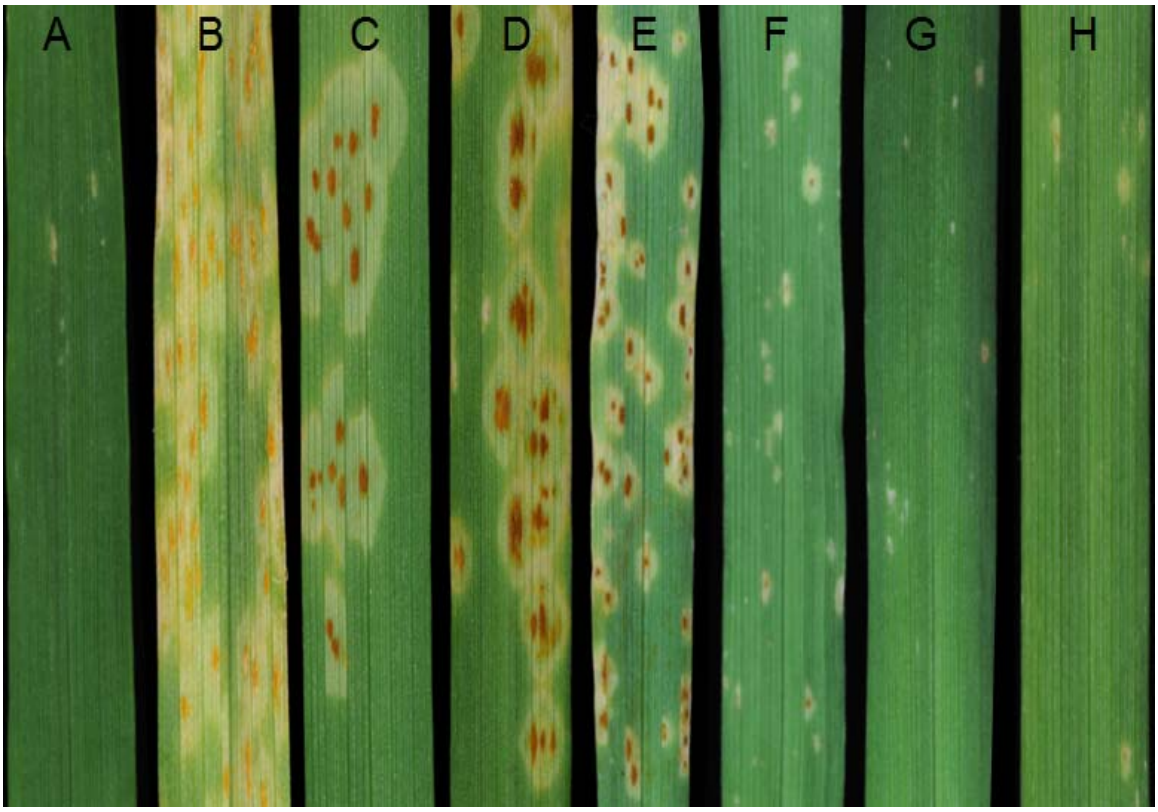

Figure 2.2. Disease phenotypes of barley landraces in response to wheat stem rust race TTKSK (**A**) highly resistant reaction of Hv545, (**B**) susceptible reaction of F_1 plant from the Steptoe/Hv545 cross, (**C**) susceptible reaction of Steptoe parent, (**D**) susceptible plant from a homozygous susceptible F_3 family of the Steptoe/Hv612 cross, (**E**) moderately susceptible plant from a segregating F_3 family of the Steptoe/Hv612 cross, (**F**) resistant plant from a segregating F_3 family of the Steptoe/Hv612 cross, (**G**) resistant plant from a homozygous resistant F_3 family of the Steptoe/Hv612 cross, and (**H**) highly resistant reaction of Hv612.

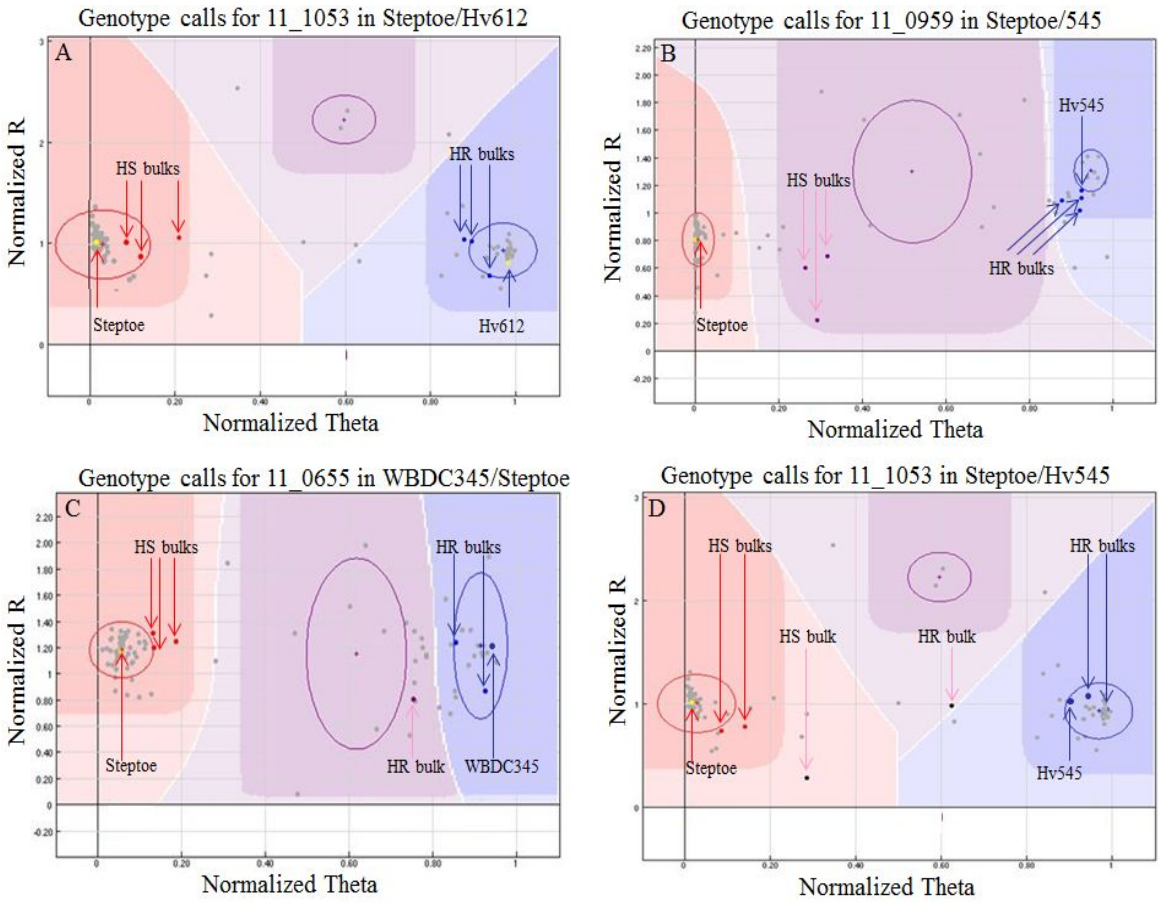

Figure 2.3. The clustering of sample GoldenGate assay results from GenomeStudio that were considered a positive hit for bulked segregant analysis where (**A**) the three susceptible bulks clustered with the susceptible genotype Steptoe and the three resistant bulks clustered with the resistant genotype Hv612, (**B**) the three resistant bulks clustered with the resistant genotype Hv545 but the three susceptible bulks did not cluster with Steptoe, (**C**) the three susceptible bulks clustered with Steptoe and two of the three resistant bulks clustered with the resistant genotype WBDC345, and (**D**) two of the susceptible bulks clustered with Steptoe and the two of the resistant bulks clustered with Hv545. The cluster position of the bulks (resistant vs. susceptible) and the parents (resistant vs. Steptoe) are indicated with arrows (HR: homozygous resistant; HS: homozygous susceptible). The *X* axis is normalized theta. A normalized theta value nearest 0 is homozygous for allele A, and a theta value nearest 1 is homozygous for allele B. The *Y* axis is normalized R (Fan et al. 2006).

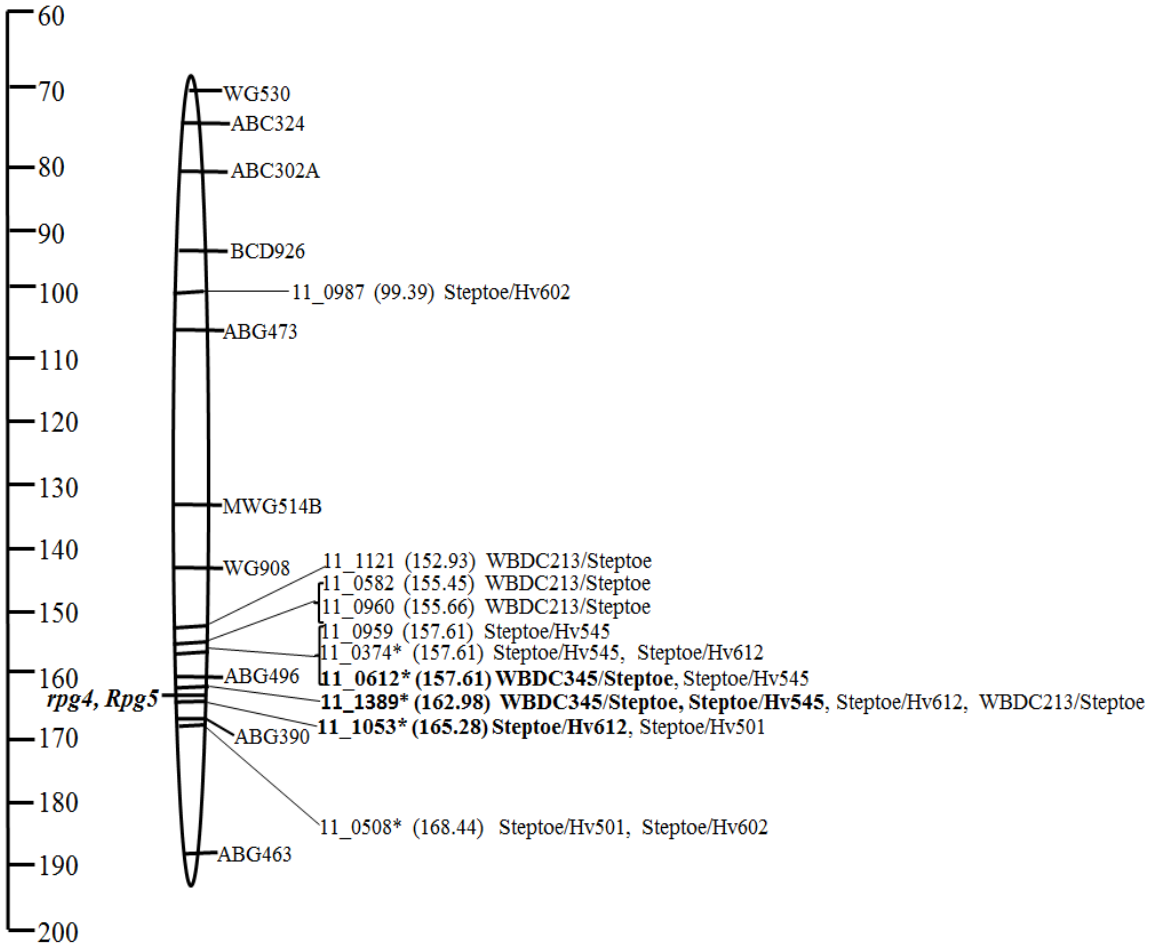

Figure 2.4. Consensus genetic map of the long arm of chromosome 5H of barley from the Steptoe/Morex (SM) doubled haploid line population as reported by Kleinhofs and Graner (2002) and modified from a presentation by Brueggeman (2011). SNP markers detected in the subtelomeric region of the long arm of chromosome 5H through bulked segregant analysis are indicated with the thinner and longer line. SNP marker names begin with numbers according to the nomenclature used in Close et al. (2009) followed by the name of the cross in which they were detected. Cumulative cM distances are in parenthesis next to the marker name as given by Muñoz-Amatriaín et al. (2011). The stem rust resistance gene complex *rpg4/Rpg5*, given in bold italics on the left-hand side of the subtelomeric region of the hypothetical chromosome arm, was previously mapped to this region (Borovkova et al. 1995; Steffenson et al. 2009). The cM position of the *rpg4/Rpg5* locus and the linked RFLP marker ABG390 is approximate based on the map position of the latter in recent barley consensus maps (http://wheat.pw.usda.gov/GG2/index.shtml). SNP markers that were positive in each of the three resistant and susceptible bulks are indicated in bold. SNPs detected in more than one population are followed by an asterisk. The line scale on the left-hand side of the figure gives approximate map distances in Kosambi cM at 10 cM interval.

Chapter 3

Genome-wide Association Mapping of Stem Rust and Spot Blotch Resistance in Barley Landraces from Ethiopia and Eritrea

3.1 Introduction

Barley (*Hordeum vulgare* ssp. *vulgare* L.) was one of the first domesticated cereals in the Fertile Crescent. Since its first domestication about 10,000 years ago, the crop has become one of the major cereals under cultivation throughout the world. However, the role the crop plays in the economy of nations and livelihood of their populations varies from country to country. In Ethiopia, barley is one of the five major cereals (along with teff [*Eragrostis tef* (Zucc.) Trotter], wheat [*Triticum aestivum* L.], maize [*Zea mays* L.] and sorghum [*Sorghum bicolor* L.]) at the core of the country's agricultural and food economy (Taffesse et al. 2011). Ethiopia is one of the largest barley-producing countries in the world with an estimated production of >1.3 million metric tons (http://faostat.fao.org/). The crop is cultivated on over 1 million hectares in the country, accounting for close to 10% of the cultivated cereal land and ~7.5% of total cereal production (averaged over 2004-2008) (Taffesse et al. 2011). Barley is one of the principal dietary staples in Ethiopia, particularly in the highlands, and contributes ~5.6-12.3% of per capita calorie consumption in the country (Berhane et al. 2011; Marwat et al. 2012).

In Ethiopia, barley is utilized for many different purposes, including food, home-made drinks, animal feed, and as a cash crop. The grain is used as food in many different forms ranging from bread making to traditional dishes (e.g. *injera, kita, dabo and kolo;* Shewayrga and Sopade 2011). In addition to its use in industrial beverages like beer, barley is used in the production of home-made drinks including tella, a locally produced beer and areki, a kind of local schnapps similar to Italian Sambuca. The straw of the barley crop is used for livestock feed, construction/thatching of houses, bedding for animals, and residue cover on farmlands.

In Ethiopia, landraces of barley with desirable agronomic traits have been utilized and preserved through generations. Such landraces have been under cultivation in Ethiopia since 3000 B.C. (Zemede 1996). Today, landraces are still the main seed sources used by subsistence farmers in Ethiopia and Eritrea (Grando and Macpherson 2005).

Modern breeding is at its infancy in Ethiopia, and most farmers still use "unimproved" landraces. Landraces represent more than 90% of the barley cultivated in Ethiopia (Hadado et al. 2010). They are cultivated in almost all regions of the country, ranging from the cool, moist to the warmer, arid agro-ecological zones (Getaneh 2007). Barley is cultivated from 1,400 meters above sea level (masl) to over 4,000 masl (Asfaw 2000), with most production occurring between 2,000 and 3,500 masl. Barley is cultivated by resource-poor farmers as a reliable crop for the highland areas of Ethiopia—perhaps as insurance for the uncertainty of very low yield or failure of other crops (Akar et al. 2004) due to low rainfall. Farmers use indigenous technologies for producing barley with knowledge passed on from generation to generation. These technologies include selection of promising "farmer's varieties" as well as practices to manage soil erosion, lodging and other agronomic husbandries.

Since its domestication, barley has emerged as one of the crop species with a narrow genetic base due to its inbreeding nature. This is in spite of the existence of thousands of diverse accessions housed in different gene banks around the world. Ethiopia is one of the richest plant genetic resource centers in the world. The East African region comprising Ethiopia and Eritrea is one of the few places in the world where very diverse barley landraces have been preserved for centuries and are still grown by farmers. Ethiopia is considered as one of the centers of diversity for barley. In fact, some early scientists (e.g. Vavilov 1951) believed that barley evolved independently in the East African Plateau (i.e. Ethiopia and Eritrea).

Various scholars have documented the existence of a rich genetic diversity in Ethiopian barley and have postulated a center of origin and/or diversification in the Horn of Africa (Negassa 1985; Orabi et al. 2007; Vavilov 1951). Today, Ethiopia and Eritrea are at least considered as centers of barley diversification (Eticha et al. 2010). Resistance against some diseases is rare in the cultivated barley gene pool commonly used for breeding. Breeding for disease resistance and other traits in barley is a work in progress, and there is a continual need for diverse genetic materials, including Ethiopian landraces. The genetic diversity of *in situ* (on-site conservation) and *ex situ* (facilities that store

accessions) germplasm collections should be systematically analyzed to identify candidate accessions that might contain unique alleles never used in cultivated breeding materials.

To characterize the extent of genetic diversity and discover valuable genes underlying agronomic traits, association genetics is being extensively used in barley and other plant species (Waugh et al. 2009). Genome-wide association study (GWAS) is a powerful approach for mapping economically and biologically valuable traits in collections of germplasm (Thornsberry et al. 2001). GWAS is based on linkage disequilibrium (LD), which refers to the non-independence of alleles in haplotypes in a population (Gaut and Long, 2003). GWAS is a widely used alternative approach to bi-parental mapping for identifying genes responsible for natural variation in phenotypic traits, including disease resistance (Comadran et al. 2009; Roy et al. 2010; Thornsberry et al. 2001). Through the technique of GWAS, it is possible to map the chromosomal location of resistance genes at a much greater resolution than is possible through bi-parental mapping (Aranzana et al. 2005).

Several GWAS efforts have been conducted for cultivated barley in Europe and the Mediterranean region (Cockram et al. 2007; Comadran et al. 2009; Kraakman et al. 2004; Rostoks et al. 2005). Recently, a large, multi-institutional research effort called the Barley Coordinated Agricultural Project (BCAP) completed characterization of the extent of genetic diversity and also the mapping of agronomic traits in US barley breeding germplasm. Analyses of the extent of LD and GWAS of disease resistance traits also has been conducted in wild barley (Morrell et al. 2005; Roy et al. 2010; Steffenson et al. 2007), as well as landraces from the Mediterranean basin (Comadran et al. 2009).

Wheat stem rust, caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & Henn. (*Pgt*), attacks both wheat and barley in many regions of the world. The disease affects the productivity of these cereal crops through interruption of nutrient and water flow to the developing kernels, resulting in shriveled grain, lower grain yields, and reduced grain quality (Stokstad 2007). The emergence in Africa of the new *Pgt* race

known as TTKSK (aka isolate Ug99) is significant because it is virulent for the most widely used *Pgt* resistance genes in wheat (Jin and Singh 2006) and barley (Steffenson et al. 2013). Spot blotch, caused by *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem. [teleomorph: *Cochliobolus sativus* (Ito and Kurib.) Drechsl. ex Dastur], is also a common foliar disease of both crops worldwide (Bockus et al. 2010; Clark 1979). Both stem rust and spot blotch can be controlled by fungicides and use of resistant cultivars, with the latter being the most cost effective and environmentally safe alternative. Most of the stem rust resistance genes identified thus far in barley are qualitative in nature (Fetch et al. 2009; Fox et al. 1995; Jedel 1990; Jin et al. 1994a; Patterson et al. 1957; Steffenson 1992; Steffenson et al. 1984; Sun et al. 1996), though some quantitative resistance was recently described to race TTKSK (Moscou et al. 2011; Zhou 2011). Likewise, both qualitative and quantitative resistances against spot blotch have been reported in barley (Arny 1951; Bilgic et al. 2005; Bilgic et al. 2006; Gonzalez Ceniceros 1990; Griffee 1925; Steffenson et al. 1996; Zhou and Steffenson 2013a).

The rich diversity of Ethiopian barley landraces has been recognized, particularly with reference to the frequency of resistance genes to various diseases. Indeed, this germplasm has provided unique resistance genes against diseases such as Barley Yellow Dwarf, powdery mildew, net blotch, scald, and loose smut (Jørgensen 1992; Metcalfe et al. 1978; Qualset 1975; Wiberg 1974; Yitbarek et al. 1998; Zhang et al. 1987). Ethiopian barley landraces preserved in genebanks could be a valuable resource for identifying new alleles controlling important agronomic traits, especially disease resistance. This germplasm has not been systematically evaluated for its reaction to stem rust and spot blotch. Given the new threats posed by stem rust race TTKSK and spot blotch isolate ND4008 (Gyawali 2010), Ethiopian barley landraces may carry important untapped genetic variation for disease resistance that can be used in breeding programs.

Diverse barley landraces, originally collected from both Ethiopia and Eritrea, have been assembled from various international genebanks. The germplasm set has been assayed for multiple phenotypes (agronomic traits, disease resistance and micronutrient concentration) and genotyped with the barley iSelect SNP (single nucleotide

polymorphism) chip that contains a total of 7,842 SNP markers. Phenotypically, the materials are very different in terms of spike morphology, kernel color, spike length, plant height, days to heading and several more traits. The long-term goal of this research is to introgress economically important genes identified from barley landraces into elite breeding lines or commercial barley cultivars. The specific objectives of this research were to: (1) characterize population structure and phenotypic variation in reaction to stem rust and spot blotch in Ethiopian/Eritrean barley landraces and (2) identify loci conferring stem rust and spot blotch resistance in the germplasm using a GWAS approach.

3.2 Materials and methods

3.2.1 *Plant materials*

Two-hundred and ninety-eight Ethiopian and Eritrean barley landraces were used in this study. To incorporate as much genetic diversity as possible in the GWAS panel, we used the following criteria to select the landraces. More than 3,000 Ethiopian and Eritrean landraces are held by the US Department of Agriculture-Agricultural Research Service (USDA-ARS), National Small Grains Collection (NSGC) in Aberdeen, ID (USDA-ARS, NGRP 2009). Of the accessions in this list, a total of 87 Ethiopian and Eritrean landraces were already included in the barley core collection developed by the NSGC (USDA-ARS, NGRP 2000) and were therefore also included in our GWAS panel. The barley core collection was assembled to represent the genetic diversity found in the cultivated portion of the barley germplasm maintained by the NSGC in such a way that the final set consisted of 10% of the total collection. The logarithm of the number of accessions per country in the entire collection was used to determine the number included in the core collection on a per country basis (H. Bockelman, personal communication). Accessions for the core collection were then selected at random within each country.

To further enhance the panel diversity, additional accessions were included with special emphasis on landraces from geographically diverse regions with historically intensive barley cultivation. The number of accessions representing each region was selected based on the area of barley cultivation within the respective areas. When

multiple accessions from a single site were available, the final accession(s) for the panel were arbitrarily selected. In the end, 132 additional accessions were selected from the NSGC along with the 87 from the core collection. These Ethiopian and Eritrean barley landraces were collected during various expeditions to the countries between the 1920s and 1970s (Qualset 1975). For example, Harry Harlan made one of the earliest collection trips to the country in 1923 (Harlan 1957). Seed of the NSGC landraces were provided courtesy of Harold E. Bockelman, the NSGC curator. Some of these sampled landraces also were included in previous studies on disease and insect resistance, feed quality, and endosperm texture variation (e.g., Bowman et al. 2001; Schaller et al. 1963; Turaspekov et al. 2008).

In addition to the NSGC holdings, additional accessions were arbitrarily selected from different genebanks whose expeditions collected germplasm in different regions and eras than those conducted by the USDA. In this regard, 63 and 17 additional Ethiopian/Eritrean landraces were obtained from the N. I. Vavilov Research Institute of Plant Industry (VIR) and the International Center for Agricultural Research in the Dry Areas (ICARDA), respectively, and included in the panel. Passport data (where available) and other details about the accessions are given in Appendix Table 3.1.

To obtain genetically pure seed stocks of the landraces, single plant selections were made from each accession and selfed twice in the greenhouse. Then, a large greenhouse increase was made to provide sufficient seed for all subsequent experiments. For the greenhouse increases, seven seeds were planted in plastic pots (13.3 cm × 13.3 cm × 10.2 cm, l × w × h) filled with a 50:50 mix of steam-sterilized native soil and Metro-Mix® 200 (Sun Gro Horticulture, Quincy, MI), a growing media containing vermiculite, sphagnum peat moss, perlite, dolomitic limestone, and a wetting agent. After planting, all pots were watered and fertilized with Osmocote® controlled-release 14-14-14 (N:P:K) (Scott's Company, Marysville, OH) (1.4 g/pot) and Peters Dark Weather 15-0-15 fertilizer (Scott's Company) (ca. 40 g/liter at 1/16 dilution). Thereafter, plants were fertilized with a 20:20:20 water-soluble fertilizer formulation (J.R. Peters, Inc., Allentown, PA) biweekly at the recommended rate. All plant grow-outs were done in a

greenhouse within the Plant Growth Facility on the St. Paul campus of the University of Minnesota in 2010 and 2011. Plants were grown at 19-23°C with a 16 hr photoperiod supplemented by 400 W high-pressure sodium halide lamps emitting a minimum of 300 μmol photons $s^{-1}m^{-2}$. When plants reached physiological maturity, seeds were harvested, dried at 35°C in a forced air dryer for two days, threshed and cleaned.

3.2.2 *Sample preparation and DNA extraction*

Seeds from the third selfed generation (S_3) of each landrace were sown and grown in the greenhouse as described above. Two weeks after planting, leaf tissue was collected from the plants for subsequent DNA extraction. For this procedure, segments of tissue (~5 to 6.5 cm long) were cut from the second leaves of plants, folded twice, and carefully placed in a 1.0 ml polypropylene microtube (BioExpress, Kaysville, UT). The microtubes were kept on ice during sample collection. Then, the microtubes were frozen in liquid nitrogen and stored at −20°C until the samples were freeze-dried or lyophilized. For freeze-drying, the samples were removed from the freezer, the lids of the microtubes opened, and the tube rack securely covered with miracloth (EMD Millipore Corporation, Temecula, CA). Samples were kept on ice until they were freeze-dried in a general purpose freeze dryer (Model 24DX48, Virtis Company, Gardiner, NY) according to the specifications of the manufacturer.

The lyophilized tissue was subjected to tissue lysis for total genomic DNA isolation. One 3 mm tungsten-carbide bead was added to microtubes containing the tissue samples and homogenized using a TissueLyser (Qiagen, Valencia, CA) for 90 seconds at 30 Hz. Then, the sample was centrifuged at 4000 rpm for 3 minutes followed by the addition of 300 μl of Buffer RLT (Qiagen). The microtubes were tightly sealed with new caps, and manually shaken back and forth in an upright position 20 times. Then, the microtubes were vortexed upside down at full speed for 20 seconds and centrifuged at 4000 rpm for 5 minutes at room temperature. DNA extraction was completed using the high-throughput BioSprint 96 DNA-liquid handling workstation and the BioSprint 96 DNA Plant Kit (Qiagen) according to the manufacturer's instructions.

The BioSprint system uses magnetic particle technology for DNA purification. Purified DNA was eluted in 200 µl 0.1X TE Buffer at pH 8.0 (Ambion, Austin, Texas). As both DNA quality and quantity are critical for genotyping, both parameters were carefully assayed. DNA yield and purity were determined by measuring absorbance at 260 nm (A_{260}) and the ratio of absorbance at 260 nm and 280 nm (A_{260}/A_{280}), respectively. DNA concentration of each sample was then adjusted to 50 ng/µl with 0.1X TE Buffer. The quality of DNA was confirmed by running 20 µl of the normalized DNA on a 1% agarose gel. Twenty microliters of normalized DNA was shipped to the USDA-ARS Biosciences Research Laboratory (Fargo, ND) in PCR plates on ice for genotyping. Five microliters of the 50 ng/µl DNA stock sample was used to run the genotyping assay.

3.2.3 *SNP genotyping and genotype data analysis*

The Ethiopian/Eritrean landrace samples were genotyped with the barley iSelect SNP chip of the expanded barley SNP marker platform using the Illumina Infinium II assay first described by Steemers et al. (2006). This assay is a whole-genome genotyping protocol with a single-base extension. Custom iSelect BeadChips can be multiplexed to assay from 3,072 to $1x10^6$ SNPs per sample. Each locus is assayed with an average 12-18x redundancy for each sample, enabling highly accurate genotype calls. The barley iSelect SNP chip contains a total of 7,842 SNPs that comprise 2,832 of the existing barley oligonucleotide pooled assay (BOPA1 and BOPA2) SNPs discovered and mapped previously (Close et al. 2009; Muñoz-Amatriaín et al. 2011), plus 5,010 new SNPs developed from next generation sequencing data (Comadran et al. 2012). The mapping information for the barley iSelect SNPs is available from the Germinate 2.7.2 Teleport iSelect Database at the James Hutton Institute (JHI) (Dundee, Scotland) (http://bioinf.scri.ac.uk). The Illumina Infinium genotyping assay was conducted under the direction of Shiaoman Chao.

The data generated by the Infinium assay were visualized and analyzed with the genotyping module of GenomeStudio data analysis software GSGT, version 1.8.4 (Illumina, San Diego, CA). The software parameters of GenCall score and no-call

threshold were optimized. GenCall score is a quality metric calculated for each genotype and ranges from 0 to 1. The GenCall scores generally decrease in value the further a sample is from the center of the cluster to which the data point is associated. The no-call threshold is the lower boundary for calling genotypes relative to its associated cluster. To obtain the highest genotyping accuracy possible, a GenCall score cutoff (no-call threshold) of 0.15 was used. This means that genotypes with a GenCall score less than 0.15 were not assigned genotypes because they were considered to be too far from the cluster centroid to make reliable genotype calls. The analysis was started with a preliminary sample quality evaluation to determine which samples may require reprocessing or removal. Each SNP was analyzed independently to identify genotypes for the entire set of samples. Visual inspections for quality control were made, and the clusters manually adjusted for SNPs with well-separated clusters. SNPs not clustering as expected and/or SNPs with low call rates were systematically excluded from the dataset prior to calling genotypes again. To improve call rate and accuracy, custom cluster profiles were generated for SNPs that did not fit well into the standard cluster positions. SNPs with overlapping clusters were manually zeroed. Those that could be scored for the majority of samples and with a 95% call frequency were retained. After all the quality evaluations, genotype calls were generated using the GenCall algorithm (version 6.3.0) implemented in the GenomeStudio software.

3.2.4 *Phenotyping landraces to wheat stem rust races TTKSK and MCCFC at the seedling stage*

Barley landraces were evaluated at the seedling stage for reaction to wheat stem rust races *Pgt*-TTKSK and MCCFC. Seedling evaluations for *Pgt*-TTKSK were conducted within the Biosafety Level-3 (BSL-3) Containment Facility on the St. Paul campus of the University of Minnesota during the winter months of 2011 and 2012. Race *Pgt*-MCCFC evaluations were conducted in a non-containment greenhouse during the winter months of 2013. For each race, five seeds of each accession were sown in plastic pots (7.6 cm × 7.6 cm × 10.8 cm, l × w × h) filled with a 50:50 mix of sterilized native soil and Metro-Mix® 200. Multiple replicates of the following classes of controls were

included in all evaluations to monitor the infection level and virulence of the two races: (1) a barley line with no known resistance genes (Hiproly [PI 60693]); (2) a barley line with *Rpg1*, a gene effective against many races including *Pgt*-MCCFC, but not TTKSK (Morex [CI 15773]); (3) barleys with the gene complex *rpg4/Rpg5*, which is effective against *Pgt*-TTKSK and MCCFC (line QSM20 and Q21861 [PI 584766] with the additional gene of *Rpg1*); and (4) a susceptible wheat control of Line E (PI 357308). After planting, all pots were watered and fertilized with Osmocote® controlled-release 14-14-14 (Scott's Company, Marysville, OH) (1.4 g/pot) and Peters Dark Weather 15-0-15 fertilizer (Scott's Company) (ca. 40 g/liter at 1/16 dilution). For the *Pgt*-TTKSK evaluations, the pots were then moved into the BSL-3 facility for the remainder of the experiment. Plants were grown in the BSL-3 greenhouse at 20-22°C and 75% RH with a 14 hr photoperiod, supplemented by 400 W high-pressure sodium lamps emitting a minimum of 300 μmol photons $s^{-1}m^{-2}$. For evaluations against *Pgt*-MCCFC, plants were grown under similar conditions, except that light was supplemented by lamps emitting 230-270 μmol photons $s^{-1}m^{-2}$ in the greenhouse. Stocks of rust isolate 04KEN156/04 of race *Pgt*-TTKSK and isolate A-5 of race MCCFC were utilized in this experiment. Rust isolates were initially increased on a susceptible wheat host (McNair701 [CItr 15288]), collected, desiccated in a 20% RH chamber, and stored in gel capsules within cryovials at −80°C until needed. On the day of inoculation, the tubes of stored urediniospores were heat shocked in a water bath at 45°C for 10 min and rehydrated in an 80% RH chamber for one hour. A small sample of urediniospores was then sprayed onto a water agar plate to assess the germination rate. After two hours of incubation in the dark at room temperature, the germination rate was checked by scanning fields on the plate using a compound microscope under low magnification. Urediniospores were weighed and placed in gelatin capsules containing a lightweight mineral oil carrier (Soltrol 170; Phillips Petroleum, Bartlesville, OK). A concentration of 10-14 mg urediniospores/0.7 ml oil was used, but additional spores were added if the germination rate was lower than 95%. The rust spore suspension was then applied to 8 to 9-day-old plants with fully expanded primary leaves at a rate of 0.10-0.14 mg/plant with an atomizer pressured at 25-30 kPa (Sun and Steffenson 2005).

After inoculation, plants were kept on carts until most of the oil carrier had evaporated from the leaves. Then, they were placed in chambers misted with ultrasonic humidifiers initially for 30-40 minutes of continuous misting and thereafter for 4-8 minutes every hour for 16-18 hours in the dark. After the 16-18 hour wetness period in the dark, light was provided by 400 W sodium vapor lamps (150-250 μmol photons $s^{-1}m^{-2}$), and the mist chamber doors were opened slightly to dissipate the heat. At this time, the humidifiers were set to run for 2-8 minutes of misting every 15 minutes for the next 2 hours. After 2 hours, the misters were turned off and the chamber doors opened halfway to facilitate slow drying of the plant surfaces under continuous light for the next 3 to 4 hours. Finally, when the leaf surfaces were completely dry, plants were returned to the greenhouses under the conditions previously described. Plants were fertilized with a 20:20:20 water-soluble formulation (J.R. Peters, Inc., Allentown, PA) after they were returned to the greenhouses.

Twelve to 14 days after inoculation, stem rust ITs were assessed on the first leaves of plants based on the 0 to 4 scale originally developed for wheat by Stakman et al. (1962) and modified for barley. The IT scale used for barley is based primarily on uredinial size as described by Miller and Lambert (1955). Two or more ITs were frequently observed on individual plants of the landraces (i.e. a mesothetic reaction) infected by stem rust. To accurately capture the stem rust phenotypes of landraces, all observed ITs on individual plants were recorded in order of their prevalence. The experiments were conducted sequentially over time using a completely randomized design and included two replicates in total. For association mapping analyses, these categorical IT data were transformed into numerical values as described by Zhou (2011). Briefly, IT 0 was coded as 0.0; IT; as 0.5, IT 1 as 2.0, IT 2 as 3.0, IT 3^- as 3.5, IT 3 as 4.0, IT 3^+ as 4.5, and IT 4 as 5.0. The numeric values were assigned to ITs in such a way to reflect the biological and epidemiological differences among the ITs in the stem rust-barley host interaction (Zhou 2011). The final numeric stem rust phenotype score for each accession was calculated from the transformed values by applying a multiplier. The multipliers applied were based on generalized IT proportions assessed from many infected leaf samples and order of their prevalence. For example, the final numeric

disease score for an accession that had only one IT was the code of the one IT observed (0.0 to 5.0) multiplied by 1.0. If a line exhibited two different ITs, the final numeric score was: (code of the first IT x 0.75) + (code of the second IT x 0.25). If three ITs were observed on an accession, the final numeric score of that accession was: (code of the first IT x 0.60) + (code of the second IT x 0.30) + (code of the third IT x 0.10). The mean of the final numeric scores of the two replications was utilized in GWAS analysis.

3.2.5 *Phenotyping landraces to African stem rust and race MCCFC at the adult plant stage*

Barley landraces also were evaluated at the adult plant stage against African stem rust and race MCCFC. Adult plant evaluations against African stem rust were conducted at Debre Zeit Agricultural Research Center in Debre Zeit, Ethiopia (June to October 2012), and those against race MCCFC were conducted at the Minnesota Agricultural Experiment Station in St. Paul during the summers of 2010, 2011 and 2012. The African stem rust nursery was established without replication owing to a lack of sufficient plot space. Experiments for race MCCFC were established in a randomized complete block design with two replicates in all three years of the study. The nursery in Ethiopia was hand-planted in single 1 m row (30-40 seeds/row) plots. Continuous rows of stem rust spreader plants (mixture of susceptible wheat cultivars) were planted parallel to the test entries to facilitate inoculum build-up and uniform distribution. Nurseries for race MCCFC were planted using a four-row planter with the center two rows being the test entries sown in short ~30 cm long rows (20-30 seeds/row) spaced ~30 cm apart and the outside two rows being susceptible spreader plants (a mixture of 80-TT-30 [CIho 16130] and Steptoe [CIho 15229]) sown in continuous rows to enhance disease development. Multiple replicates of the following classes of controls were included in the race MCCFC experiments to monitor infection levels and pathogen virulence: (1) barleys with no known resistance genes (Steptoe and Hiproly) and (2) barleys with *Rpg1*, a gene effective against many races including race MCCFC [Chevron (PI 38061)]. Hiproly, Chevron, and Q21861 were included as controls for the African stem rust nursery in Ethiopia. This nursery was artificially inoculated with a bulk of Ethiopian stem rust isolates, later

confirmed to contain races *Pgt*-TTKSK and JRCQC (P. Olivera, personal communication).

In both rust nurseries, the spreader rows were needle-inoculated at the early jointing stage with a water suspension of urediniospores (1.0 g urediniospores/900.0 mL of sterile distilled water plus 2 drops of a surfactant). The surfactant (Tween-20: polyoxyethylene-20-sorbitan monolaurate, ICI Americas, Wilmington, DE) was added to facilitate the even distribution of urediniospores within the distilled water for inoculation. Briefly, 0.5 ml of the inoculum suspension was injected into elongating stems (Welty and Barker, 1992) using a syringe and hypodermic BD Cornwall needle (Becton, Dickinson and Company, Franklin Lakes, NJ). Two tillers were inoculated about every ~60.0 cm across the spreader rows. Infection phenotypes (disease severity and infection types) were scored when the landraces were at the mid- to hard-dough stages of development. Rust severity was recorded as the visual percentage of stem tissue covered by uredinia (0-100%) based on the modified Cobb scale (Peterson et al. 1948; Stubbs et al. 1986). Infection type responses were scored based on the size and type (i.e. presence of necrosis and/or chlorosis) of uredinia observed as described by Roelfs et al. (1992) where R: resistant; MR: moderately resistant; MS: moderately susceptible, and S: susceptible. Disease severity scoring was done several times during the season, but only terminal severity data were used for GWAS.

3.2.6 *Phenotyping landraces to spot blotch isolate ND85F at the seedling stage*

Plants were sown and grown according to the same methods described for the stem rust phenotyping experiments. Multiple replicates of the following three barley genotypes were included as controls in the evaluations to monitor disease levels and the virulence level of the pathogen: (1) a barley line highly susceptible to pathotype 1 (isolate ND85F) of *C. sativus* (ND5883 [PI 643237]), (2) a barley cultivar moderately resistant to isolate ND85F (Bowman [PI 483237]), and (3) a barley line highly resistant to isolate ND85F as well as other common isolates (NDB112 [CIho 11531]). Fertilizer

treatment and growth conditions were as described above for the stem rust seedling experiments.

Inoculations with the spot blotch pathogen were done according to the methods of Fetch et al. (2008). Briefly, two-week old seedlings with fully expanded second leaves were inoculated with a conidial suspension of isolate ND85F (4,000 conidia/ml) at a rate of about 0.5 ml per plant using an atomizer (Paasche Airbrush Company, Harwood Heights, IL) pressured by CO_2 at 20 kPa. After inoculation, plants were allowed to dry slightly for a few minutes and then placed in chambers misted with ultrasonic humidifiers for 30 minutes of continuous misting. Thereafter, the humidifiers were set to run for 2 minutes every hour for 16 hours in the dark. After the 16-hour wetness period in the dark, the misters were turned off and the chamber doors opened halfway to facilitate slow drying of the plant surfaces without light for the next 4 hours. Finally, when the leaf surfaces were completely dry, plants were returned to the greenhouse under the conditions previously described. Plants were fertilized with a 20:20:20 water-soluble formulation (J.R. Peters, Inc., Altentown, PA) after they were returned to the greenhouse. Nine to 11 days after inoculation, spot blotch infection responses (IR) were assessed on the second leaves of all plants based on the 1 to 9 rating scale of Fetch and Steffenson et al. (1999). The experiment was conducted using a completely randomized design with one replicate and was repeated over time. The mean IR was utilized in GWAS.

3.2.7 *Phenotyping landraces to spot blotch isolate ND85F at the adult plant stage*

Adult plant evaluations to spot blotch were conducted at the Minnesota Agricultural Experiment Station in St. Paul during the summers of 2010, 2011, and 2012. The experiment was conducted in a randomized complete block design with two replicates in all three years of the study. The nursery was planted using a four-row planter with the center two rows being the test entries sown in short ~30 cm long rows (20-30 seeds/row) spaced ~30 cm apart and the outside two rows being susceptible spreader plants (line ND5883) sown in continuous rows to enhance disease development. Multiple replicates of the same three controls used in the seedling test were included in the field

experiment to monitor disease levels and virulence spectrum of the pathogen. Spot blotch infection was initiated in the field as described by Fetch et al. (2008). Briefly, when most of the plants were at the mid-tillering stage of development, the susceptible spreader rows were inoculated with isolate ND85F-infected barley straw (about 50 g straw/m of row). The infected barley straw was harvested from the previous season's spot blotch nursery, but was initially produced by inoculating barley line ND5883 with isolate ND85F as described by Bilgic et al. (2005). Disease severity (percentage of leaf area infected from 0 to 100%) was assessed on the top three leaves of plants (flag, flag-1, and flag-2) according to standard disease area diagrams (James 1971). Disease assessments were made twice during the growing season and three times for late maturing accessions. Terminal disease severity data were recorded for landraces at the mid-dough stage of development and then used in GWAS. All marker and phenotypic data used in these studies are available at http://triticeaetoolbox.org/barley/.

3.2.8 *Statistical analysis of phenotypic traits*

Unbiased mean estimates for each trait and an analysis of variance were generated through the restricted maximum likelihood (REML) method in SAS (SAS Institute 2008). REML is commonly used to generate estimates of variance and covariance parameters for fitting linear mixed models (Meyer and Kirkpatrick, 2005), a model used in GWAS. Heritability was estimated based on entry means using the formula: $h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_e^2/r)$, or $h^2 = \sigma_g^2 / [(\sigma_g^2) + (\sigma_y^2/y) + (\sigma_e^2/ry)]$, where σ_g^2 and σ_e^2 are the genotypic and error variance, respectively, r is the number of replications, and y is the number of years the trial was conducted. Pearson correlation coefficients were calculated to assess phenotypic correlation among traits.

3.2.9 *Population structure*

Population structure can be present in germplasm panels assembled from diverse geographic regions and during different eras. To account for this effect and decrease the number of false-positives in GWAS, the most likely number of subpopulations in the panel was investigated. The clustering program STRUCTURE (version 2.3.4) (Falush et

al. 2003; Pritchard et al. 2000a; Pritchard et al. 2000b) applies a parametric genetic mixture analysis and was used to estimate the membership probability of each barley landrace to a number of hypothetical subpopulations (*K*). STRUCTURE software employs a Bayesian model-based clustering method that infers genetic clusters (subpopulations) of individuals on the basis of their genotypes at multiple loci using a Markov Chain Monte Carlo (MCMC) maximum-likelihood approach that compares posterior probabilities of different numbers of possible population configurations. To avoid overestimation of subpopulation divergence caused by tightly linked SNP markers (Falush et al. 2003), a subset of markers with approximately 10 cM spacing was selected for the analysis. This SNP subset was used to calculate the subpopulation membership matrix (Q), which is the fractional subpopulation membership for each landrace based on its inferred ancestry. There were no *a priori* population designations assumed for the landraces. The program was run by setting most parameters to default values according to the instructions in the user's manual (Pritchard et al. 2010).

Subpopulation numbers ranging from 1 to 20 were tested using the admixture model and considering allele frequencies of SNPs correlated among populations. The correlated allele frequency setting is considered favorable when there is subtle population structure (Falush et al. 2003). The program was first run using a burn-in period of 10,000 cycles followed by 50,000 MCMC iterations with 20 independent runs at each of K=1 to 20 (Falush et al. 2003; Pritchard et al. 2000a). Posterior probability Pr(X|K) of each K was generated by the program and lnPr(X|K) plotted against each value of K. The optimal K was estimated based on the value and variation of Pr(X|K) (Rosenberg et al. 2001) as well as the rate of lnPr(X|K) change from K-1 to K (ΔK). The ad hoc quantity (ΔK) was calculated based on the second order rate of change of the likelihood function with respect to K (Evanno et al. 2005). ΔK usually shows a peak at the true value of K. The program was then run using a burn-in period of 100,000 cycles followed by 200,000 MCMC iterations with 20 independent runs at each of K=3 to 10. The optimal K value was identified based on the value and variation of Pr(X|K) and ΔK. Then, value tables for matrix Q, allele-frequency divergence among presumed subpopulations, and average

distances (expected heterozygosity) between individuals in the same cluster were generated using the determined optimum *K* value.

Principal component analysis (PCA), a multivariate analysis method that transforms a set of correlated variables (genetic markers in this case) into a smaller number of uncorrelated variables, also was used to infer population structure (Zhao et al. 2007). PCA analysis was conducted using the software program TASSEL (version 4.0) (http://www.maizegenetics.net; Bradbury et al. 2007). To satisfy the PCA requirement that all variables should have variation and no missing values, SNP markers with minor allele frequency (MAF) $\leq$0.05 were eliminated and missing values were imputed. A matrix with principal components (PCs) that represent population structure and eigenvalues (the variance explained by each of the PCs) were generated. Population stratification was visualized by plotting the first three PCs.

3.2.10 *Linkage disequilibrium*

LD between markers was measured using Haploview v4.2 (Barrett et al. 2005). Using a large number of markers affects association mapping results due to the correction for multiple tests. Utilizing fewer markers in the analysis may enable better detection of statistically significant markers associated with a trait. Therefore, to reduce the number of markers in the analysis, we selected the ones considered independent of each other, i.e. r^2 <0.8. The SNP tagger in Haploview was used to test for independence of markers to select those with an r^2 <0.8. In cases where multiple SNP markers mapping to the same location or region were found significantly associated with a trait, LD, as measured by r^2 among the neighboring markers, was calculated and presented as a "heat" map in some cases.

3.2.11 *Association mapping*

A mixed linear model (MLM), with 'efficient mixed model association' (EMMA) method (Kang et al. 2008) implemented in the GWAS package *rrBLUP* (version 4.1) in software R (version 2.15.2), was used for association analysis between SNP markers and

QTL for stem rust and spot blotch resistance in Ethiopian and Eritrean barley landrace germplasm. The mixed model equation used by the *rrBLUP* package is based on the mixed model (Yu et al. 2005): $y = X\beta+u+e$, where y is a vector of the phenotypic observations; X is a vector of the SNP marker genotypes; β is a coefficient of the SNP marker effect being estimated; v is a coefficient of population structure effect; u is a vector of random (polygene background) effects (effect of individual relatedness estimated as pairwise kinship coefficients); and e is a vector of residual effects. In the MLM, marker effect (Xβ) represents fixed effect, and relative kinship and residuals represent random effects. The variances of the random effects are given as: $Var(u) = 2KV_g$ and $Var(e) = V_R$, where *K* is an *n* x *n* matrix of kinship inferred from genotypes based on the proportion of shared allele values between a pair of individuals (*n*: number of individuals in the GWAS panel); V_g is the genetic variance; and V_R is the residual variance. The *rrBLUP* package in R for GWAS incorporates methods for obtaining best linear unbiased estimates (BLUE) of β (fixed effect) and best linear unbiased predictions (BLUP) of u (random effect) as originally developed (Kang et al. 2008; Yu et al. 2005). The EMMA association analysis method then fitted each SNP into the MLM individually and generated marker scores [$-\log_{10}$(*P*-values)].

The *rrBLUP* GWAS function incorporates a package known as QVALUE (Storey and Tibshirani 2003) for multiple testing using the Benjamini-Hochberg False Discovery Rate (BH-FDR) (Benjamini and Hochberg 1995) and handles permutations of *q*-value (an FDR adjusted *P*-value) for each test internally. At the end of the analysis, the package draws a "bar graph" as a GWAS result with the $-\log_{10}$(*P*-values) and a threshold line (FDR=*q*-value=0.05) delineating significant markers. Then, the FDR adjusted marker scores were outputted from which *P*-values were calculated.

To identify QTL conferring resistance to stem rust race MCCFC and spot blotch isolate ND85F at the adult plant stage, GWAS was first conducted on the phenotype data from individual years, designated as Ethiopian and Eritrean Barley Collection EEBC-10 (for 2010), EEBC-11 (for 2011), and EEBC-12 (for 2012). Then, the combined overall entry means of three years designated as EEBC-C (for 2010-2012) was analyzed. Next,

GWAS for adult plant spot blotch resistance was performed on phenotype data for the two-rowed (110 landraces) and six-rowed germplasm (180 landraces) separately for each year and then the mean of the three years combined. Separate analyses of row type germplasm were done because previous studies had demonstrated differences in spot blotch resistance (Bilgic et al. 2005; Steffenson et al. 1996). Two-rowed cultivars generally exhibit more variable reactions to spot blotch, and the resistance bred into them has not been broadly effective (Alsop 2009; Roy et al. 2010; Zhou and Steffenson 2013a). Thus, this analysis contributes to our understanding of the genetics of spot blotch resistance in two- and six-rowed barley germplasm.

3.2.12 *Candidate gene scan using Basic Local Alignment Search Tool (BLAST)*

Source sequences of SNP markers significantly associated with the disease resistance traits were used to identify putative candidate genes in the rice (http://rice.plantbiology.msu.edu/) and *Brachypodium* (http://www.phytozome.net/) genomes and also the NCBI database (http://blast.ncbi.nlm.nih.gov) using BLAST. In the rice and *Brachypodium* genomes, a 150 kilo base pair (kbp) window was scanned in each direction of regions homologous to the sequence of the significant SNP. In the NCBI database, a BLASTX (searching protein database using a translated nucleotide query) search was implemented to determine if sequences of significant SNP markers correspond to a known protein in barley, wheat, maize, sorghum or *Arabidopsis*.

3.3 Results

3.3.1 *Phenotypic variation for race TTKSK resistance at the seedling and adult plant stages*

The susceptible and resistant controls exhibited the expected range of ITs based on previous studies. Mostly moderately high IT modes of $3^{-}2$ (range of $3^{-}2$ to 3^{+}) (i.e. numeric score mode of 3.38 with range of 2.45 to 4.13) were observed on the susceptible barley controls. In particular, the susceptible controls of Hiproly and Morex both had an IT mode of $3^{-}2$ with range of $3^{-}2$ to $3^{-}3$ (i.e. numeric score mean of 3.55±0.16 and

3.33±0.22 and medians of 3.63 and 3.38, respectively) (Note: the lowest and highest, upper and lower quartiles and medians are given for numeric score data in Figure 3.1A as well as 3.1B, C, and D). The highly susceptible wheat control Line E exhibited a high IT mode of 4 with range of 3 to 4 (i.e. numeric score mean of 4.95±0.09 and median of 5.0) (Figure 3.1A). Low IT modes of 0; (range of 0; to 10;) (i.e. numeric score mode of 0.35 with range of 0.13 to 1.25) were observed on the resistant controls. Specifically, the resistant controls of QSM20 and Q21861 had IT modes of 0;1 and 0; (with range of 0; to 10;), respectively (i.e. numeric score mean of 0.66±0.46 and 0.21±0.11 and medians of 0.35 and 0.13, respectively) (Figure 3.1A). The EEBC panel (n=298) exhibited a wide range of ITs with IT mode of 3⁻2 and range of 1 to 3 (i.e. a numeric score mode of 3.38 with range of 2.00 to 4.00 and mean and median of 3.33±0.23 and 3.38, respectively) (Figure 3.1A). The high mean numeric IT value for the landraces indicates that *Pgt*-TTKSK resistance in the EEBC germplasm is rare. Indeed, only 15 (5.0%) lines exhibited a resistant to moderately resistant reaction (i.e. IT mode of 1 to 2). Heritability, defined here as the reproducibility of the observed phenotype on the germplasm across experiments, was estimated to be 1.00 based on entry means (Table 3.1).

In the African stem rust nursery, the susceptible controls of Hiproly and Chevron exhibited mean disease severities of 27.14±9.94 and 17.86±5.89% and medians of 30 and 15%, respectively, whereas the resistant control Q21861 exhibited a mean disease severity of 3.29±1.98% and median of 5% (Figure 3.1B). The EEBC germplasm exhibited a wide range of disease severities ranging from 10 to 50% with a mean of 24.07±10.04% and median of 20% (Figure 3.1B).

Correlation analysis was conducted to determine the degree of association between seedling phenotypes to race *Pgt*-TTKSK and adult plant phenotypes against a composite of African stem rust isolates. The correlation coefficient between the seedling numeric IT scores and adult plant severity was positive, but non-significant (r=0.03).

3.3.2 *Phenotypic variation for race MCCFC resistance at the seedling and adult plant stages*

At the seedling stage, mostly high ITs were observed on the susceptible controls as expected based on previous studies. Hiproly, the susceptible barley control, gave a high IT mode of 3^{-} with range of $3^{-}2$ to 3 (i.e. numeric score mean of 3.35±0.08 and median of 3.38). Similarly, the susceptible wheat control Line E also exhibited a high IT mode of 4 with range of 3 to 4 (i.e. numeric score mean of 4.97± 0.08 and median of 5.00) (Figure 3.1C). In contrast, the resistant controls exhibited low ITs. Morex had an IT mode of 0; with range of 0; to 10; (i.e. numeric score mean of 0.36±0.36 and median of 0.35). The other resistant controls of QSM20 and Q21861 also exhibited a low IT mode of 0; with range of 0; to 0;1 (i.e. mean numeric scores of 0.35±0.01 and 0.24±0.11 and medians of 0.35 and 0.13, respectively) (Figure 3.1C). EEBC germplasm exhibited a wide range of ITs with IT mode of $3^{-}2$ and range of 2 to 33^{+} (i.e. a numeric score mode of 3.26 with range of 1.63 to 4.26 and mean and median of 3.26±0.36 and 3.26, respectively) (Figure 3.1C). Only 2 (0.67%) lines had raw ITs that were regarded as resistant. Fifty-five (18.46%) other lines exhibited raw ITs between 1 and 2, which are regarded as moderately resistant reactions.

In the adult plant evaluations, the susceptible controls of Hiproly and Steptoe had mean (and median) disease severities of 29.17±6.07 (30.00) and 25.83±6.07 (25.00); 36.00±6.63 (35.00) and 19.17±6.07 (20.00); and 22.00±13.27 (20.00) and 16.00±3.74% (15.00), respectively, for the years of 2010, 2011, and 2012 (Figure 3.1D). Mean (and median) rust severities on the resistant control Chevron were 4.25±1.30 (5.00); 0.5±0.00 (0.50); and 3.75±2.17% (5.00) for the respective years (Figure 3.1D). The EEBC germplasm exhibited a mean (and median) rust severity of 32.31±10.27 (32.50) in 2010, 14.39±11.47 (10.00) in 2011, and 21.66±9.89 (20.00) in 2012. Disease was relatively low in 2011 as the weather was dry and unsuitable for rust development (Figure 3.1D). One (0.34), 71 (23.83), and six (2.01%) landraces exhibited mean disease severities comparable to the resistant control of Chevron, respectively, in 2010, 2011, and 2012. Considering the aggregate means across the three years of the study, only 12 (4.03%) landraces had mean disease severities comparable to Chevron.

Correlation analysis was conducted to determine the degree of association between seedling and adult plant *Pgt*-MCCFC phenotypes. The seedling infection response and adult plant severity phenotypes were positively correlated in each of the three respective years (r=0.18, 0.12, and 0.04, respectively), but the values of r were very low and only significant in 2010. The correlation coefficients for adult severity phenotypes across years were significant and positive (r=0.41 between 2010 and 2011, r=0.42 between 2010 and 2012 and r=0.13 between 2011 and 2012). Heritability/repeatability estimated based on entry means for *Pgt*-MCCFC infection response at the seedling stage and severity at the adult plant was 1.00 and 0.63, respectively (Table 3.1).

3.3.3 *Phenotypic variation for spot blotch isolate ND85F resistance at the seedling and adult plant stages*

At the seedling stage, the controls reacted as expected based on many previous experiments. High ITs (mean of 6.50±1.00 and median of 6.50; mode of 6.00 and range of 5.50 to 8.50) were observed on the susceptible control ND5883 (Note: the lowest and highest, upper and lower quartiles and medians are given for data in Figure 3.1E as well as 3.1FFigure 3.1E), moderately low IRs (mean of 3.25±0.69 and median of 3.50; mode of 3.50 and range of 1.50 to 4.50) were observed on cv. Bowman and low IRs (mean of 1.88±0.46 and median of 1.50; mode of 1.50 and range of 1.50 to 2.50) were observed on NDB112. EEBC germplasm exhibited a wide range of IRs (mean of 5.71±0.94 and median of 5.75; mode of 5.00 and range of 2.50 to 8.00). None of the landraces exhibited a resistance level comparable to NDB112. However, 4 (1.34%) and 95 (31.88%) of the accessions had a mean IR lower than Bowman and <5.00, respectively, and are therefore moderately resistant.

In the field tests, spot blotch infections were uniform and high across all years, thus facilitating the differentiation of lines with different levels of resistance (Figure 3.1F). The lowest mean spot blotch severity on EEBC germplasm was recorded in 2010 due to a confounding effect of powdery mildew infection. The three classes of controls

exhibited expected levels of disease development: moderate to high mean (and median) disease severity was observed for the susceptible control ND5883 for the respective years (55.83±14.98 (50.00), 82.08±12.66 (87.50), and 38.33±12.30% (37.50), moderately low mean (and median) severities were observed for the moderately resistant cv. Bowman (23.33±10.67 (20.00), 40.83±8.62 (40.00), and 18.75±5.45% (20.00), and very low mean (and median) severities were observed for the highly resistant control NDB112 (5.75±1.11 (3.00), 3.83±1.48 (3.00), and 7.67±0.99% (5.00) (Figure 3.1F). The EEBC germplasm exhibited mean (and median) disease severities of 21.73±12.66 (range of 2.00 to 80.00) (20.00), 41.52±13.41 (12.50 to 90.00) (40.00), and 24.60±7.67% (from 7.50 to 60.00) (22.50) for the three respective years of the study (Figure 3.1F). As was found in the seedling stage test, no line exhibited a resistance level comparable to NDB112. Twenty-three (7.72%) landraces had mean disease severities comparable to the NDB112 in 2010, a year of low disease. All landraces exhibited higher mean disease levels than NDB112 in 2011 and 2012. One hundred and eighty-four (61.74), 166 (55.71), and 58 (19.46%) landraces had comparable mean disease severities to Bowman in 2010, 2011, and 2012, respectively. Considering the aggregate means across the three years, 145 (48.66%) landraces exhibited mean disease severities comparable to Bowman and are therefore moderately resistant.

From the correlation analyses, seedling IRs were found significantly and positively correlated with adult plant disease severities in 2011 (r=0.12) and 2012 (0.15). The correlation between seedling IR and 2010 disease severity was positive, but low and non-significant (r=0.09). The disease severities on EEBC landraces across the three years were significantly positively correlated. The correlation coefficients (r) were 0.24 between 2010 and 2011, 0.18 between 2010 and 2012, and 0.33 between 2011 and 2012. Heritability/repeatability estimated based on entry means for spot blotch infection response at the seedling stage and severity at the adult plant was 1.00 and 0.79, respectively (Table 3.1).

3.3.4 *Marker data*

The platform used for genotyping the germplasm panel consisted of 7,842 SNPs of the barley iSelect SNP chip. Of this number, 2,832 SNPs were previously mapped (Close et al. 2009; Muñoz-Amatriaín et al. 2011). The remaining 5,010 SNPs were newly developed (Comadran et al. 2012). After quality evaluations during genotype calling, 85% (6,702/7,842) of the SNPs generated reliable allele data with a 95% call rate for all accessions. Nearly 99% percent of these SNPs (6,615/6,702) had >95% call frequency. Filtering based on minor allele-frequency (MAF) resulted in 5,269 SNP markers with MAF >5%. These were subsequently used for association analyses. Of this final group of SNPs, 72.46% (3,818/5,269) have known map positions on the seven barley chromosomes and span 1,112.71 cM of the genome, whereas the remaining 1,451 SNP markers have yet to be mapped. The consensus map of SNPs used was generated by merging the map from the James Hutton Institute (JHI) (Dundee, Scotland) with the 11 linkage maps from the Barley Coordinated Agricultural Project (Barley CAP) (J. Endelman, personal communication). The vast majority of markers with unknown map positions were among the newly developed markers from the JHI and have SCRI (Scottish Crop Research Institute, the former name of the JHI) designations. Both mapped and unmapped markers were used for running GWAS. Of the mapped SNPs, 38.58% (1,473/3,818) had unique map positions, and the rest had redundant map locations. The average density of SNP markers on the genome using all mapped and unique SNPs was 3.43 and 1.32 SNP markers per cM, respectively. Gaps between markers ranged from 0.01 to 9.32 cM with a mean of 0.77 cM. Only eight gaps with >5 cM were identified: two each on chromosomes 1H and 7H, and one each on 2H, 3H, 5H and 6H.

3.3.5 *Population structure*

To understand the level of genetic variation in the landrace accessions, population structure was characterized using both maximum likelihood estimation and distance-based methods. Randomly selected SNP markers (371) distributed throughout the genome with approximately 10 cM spacing were used to characterize genetic variation using the model-based program STRUCTURE 2.3.4 (Pritchard et al. 2000a). Plotting of

the likelihood values and variance of the probability samples against subpopulations (*K*) (Rosenberg et al. 2001) indicated six to seven clusters (Appendix Figure 3.1A). The likelihood reaches a maximum around *K*=6–7, and variance across runs shows a sharp rise for *K* >7. Estimation of the true value of *K* using the rate of change of the likelihood values from *K*-1 to *K* (*ΔK*) method (Evanno et al. 2005) also confirmed this result. The *ΔK* value reached a peak at *K*=7 (Figure 3.2A; Appendix Figure 3.1B), leading to the conclusion that the most probable number of subpopulations within the germplasm is 6–7. The average distance (expected heterozygosity) between individuals in the same cluster also was calculated. Close examination of the number and genetic composition of each presumed group indicated that one of the groups had only five individuals with the highest average genetic distance (Table 3.2). Three of these accessions were obtained from VIR and the other two were from ICARDA. Four accessions within this group were six-rowed, and the remaining one was segregating for row-type. Two (both from VIR) of the five had winter/facultative growth habit, while the remaining three were spring types. All five individuals in this group appeared to be admixtures, sharing similar genotypes with individuals in other groups. Thus, the likely number of subpopulations within the GWAS panel was considered to be six. The number of individuals in each group varied from 12 (12/298=4.03%) for group 5 to 110 (110/298=36.91%) for group 6 (Table 3.2). Individuals in subpopulation 3 had the highest average expected heterozygosity and individuals in subpopulations 2 and 6 were the least heterozygous (Table 3.2).

Characteristics of each subpopulation were carefully analyzed in order to make important observations of common behaviors shared by germplasm included in the respective groups. Lines in the assumed six subpopulations were not differentiated based on the well-established traits in barley: row-type (two vs. six) or growth habits (spring vs. winter). However, the detailed characteristics of each hypothetical subpopulation are presented for general information. There were no obvious patterns for subpopulation 1 as it included both two- and six-rowed materials from most regions of Ethiopia and also the two genebanks of NSGC and VIR. Thus, this group was designated as “Two-rowed/six-rowed (NSGC/VIR)” (Table 3.2). Subpopulation 2 was dominated by two-rowed germplasm (28/32) from northern Ethiopia (Tigray region) and Eritrea and obtained

solely from the NSGC. Thus, this group was designated as “Two-rowed Tigray/Eritrea (NSGC).” Landraces in subpopulation 3 were mostly six-rowed types (29/38) obtained from VIR and ICARDA (27/38) and of Ethiopian origin only (33/38). This group had several winter/facultative types and was designated as “Six-rowed VIR/ICARDA.” Subpopulation 4 was dominated by two-rowed germplasm (24/26) obtained from VIR and ICARDA (21/26) and of Ethiopian origin only (24/26). This subpopulation was designated as “Two-rowed VIR/ICARDA.” Two-rowed NSGC landraces (8/12) of Ethiopian origin (10/12) were the most common members of subpopulation 5; thus, this group was designated as “Two-rowed NSGC.” Finally, subpopulation 6 was dominated by six-rowed landraces (91/110) obtained from the NSGC and VIR (9/110) and were mostly of Ethiopian (100/110) origin. This group contained two landraces with facultative/spring growth habit in addition to spring types. This group was labeled as “Six-rowed NSGC/(VIR).”

In order to measure distances between the subpopulations and to further evaluate population structure, net nucleotide distances between pairs of subpopulations were computed (Table 3.3). The lowest differentiation between pairs of subpopulations (0.02) was found for subpopulations 1 and 6 (Table 3.3). On the other hand, subpopulation 4 had the largest differentiation (0.30 each) from subpopulations 2, 5 and 6. STRUCTURE analysis resulted in the clustering pattern presented in Figure 3.2B. Inferred ancestry of each individual landrace based on the estimated membership coefficient is shown in Appendix Table 3.2. Bar-plot of the genetic composition of individual accessions in multiple columns is presented in Appendix Figure 3.2.

Structure also was analyzed using PCA. The result of the PCA analysis was not consistent with the analysis done by STRUCTURE. However, the PCA analysis revealed that the first principal component (PC) accounted for more than 26% of the total variance. The first four PCs all together explained more than 40% of the variation among the accessions (Appendix Figure 3.3). Plots of both PC1 vs. PC2 and PC2 vs. PC3 generated one clearly observable cluster (Appendix Figure 3.3). The rest of the clustering was scattered without a distinguishable pattern. More than 90% of the accessions that

grouped together in both PC1 vs. PC2 and PC2 vs. PC3 clustering were landraces that were obtained from the NSGC. Accessions that did not fall into a clear pattern were obtained from the VIR and ICARDA genebanks. Again, the clustering of the lines in the PCA analysis did not reveal any differentiation based on row-type or other well-known traits in barley.

3.3.6 *Genome-wide association mapping of seedling and adult plant resistance*

GWAS was conducted for stem rust (races TTKSK and MCCFC) and spot blotch (isolate ND85F) at both the seedling and adult plant stages. GWAS for race *Pgt*-MCCFC and spot blotch resistance at the adult plant stage was conducted with phenotype data of individual years separately and also the overall mean across all three years. In addition, since the genetic architecture of row-type may affect the expression of spot blotch resistance, GWAS was run separately for two-rowed and six-rowed germplasm in each year and also for the entire germplasm set across all three years. Significant associations were automatically declared by the *rrBLUP* GWAS package used. After correction for multiple testing, markers with a *q*-value (an FDR adjusted *P*-value) <0.05 were considered as truly significant. To provide detailed information, significantly associated markers and markers with scores close to the FDR threshold in different sets of the mapping panel were reported.

3.3.6.1 *Genome-wide association mapping of barley spike morphology*

As a validation of the GWAS model for reliability of localizing true marker-trait associations and evaluating mapping resolution within the germplasm panel, GWAS was conducted on the spike morphology trait of row type. Barley is classified into two-rowed and six-rowed types, which is a result of having either two or six rows of florets being fertile. This trait is mainly controlled by the well-characterized Mendelian gene *Vrs1*, located on chromosome 2H, as well as other genes. Alleles at the *Vrs1* locus and some of the other spike morphology genes have been cloned (Komatsuda et al. 2007; Pourkheirandish and Komatsuda 2007; Ramsay et al. 2011; Waugh et al. 2009). The GWAS model identified 14 SNP markers significantly associated with the row-type

locus: eight on the long arm of chromosome 2H, one each on chromosomes 4H and 5H, and four unmapped markers (Table 3.4; Figure 3.3A). BLAST search of the NCBI database with the significant SNPs on chromosome 2H identified the coding sequences of the *H. vulgare* homeodomain-leucine zipper protein (*Vrs1*) gene with ≥99% identity, indicating that the SNP markers are likely located within the coding region of the gene. The significant SNPs on chromosome 2HL were mapped within a short distance (4.03 cM) of each other (Table 3.4) and are most likely detecting the same *Vrs1* locus. LD as measured by r^2 ranged from 0.004 (between SCRI_RS_171032 and 12_30897) to 0.99 (between 12_30897 and SCRI_RS_196853) (Figure 3.3B). LD between the most significant marker (12_30896) and the rest of the markers ranged from 0.10 (with SCRI_RS_221886) to 0.88 (with 11_20340), indicating that some of the markers are in high LD with the underlying gene controlling spike morphology.

The significant marker on 4H maps to the reported position of INTERMEDIUM-C (*Int-c*) *gene* (Cuesta-Marcos et al. 2010; Lundqvist and Franckowiack 1997; Komatsuda and Mano 2002) that influences both the fertility of the lateral spikelets on the inflorescence and the amount of the basal branching (tillering) in barley (Ramsay et al. 2011). The one significant marker on chromosome 5H is most likely detecting the six-rowed spike 2 (*vrs2*) or the intermedium spike-b (*int-b*) locus (Lundqvist and Franckowiack 1997). Some or all of the unmapped SNPs are likely located near or at *vrs1*, *vrs2*, *int-b*, *Int-c* or some other spike morphology genes described on chromosome 1H (Cuesta-Marcos et al. 2010). These results are in agreement with previous studies (Figure 3.3A) and validate the power of GWAS, the appropriate model for accounting for pairwise relatedness, and a sufficiently high density of markers for high-resolution mapping. Accordingly, the GWAS model was used for marker-trait associations of stem rust and spot blotch phenotypes.

3.3.6.2 *Genome-wide association mapping of Pgt-TTKSK resistance*

GWAS of *Pgt*-TTKSK resistance was conducted with three sets of SNP markers. First, all informative SNP markers were used to run GWAS. Several SNP markers with

low *P*-values were identified on chromosomes 5H and 4H, but none of them surpassed the FDR threshold (Appendix Figure 3.4A). Among other factors, correction for multiple hypothesis testing might have affected significant marker detection due to the large number of markers used. Thus, GWAS was conducted using 2,430 markers considered to be independent based on linkage disequilibrium (with an $r^2 < 0.8$) (Massman et al. 2011). However, no marker that surpassed the FDR threshold was detected using this marker set (data not shown). Finally, GWAS was conducted only with markers mapping to unique chromosomal locations to further reduce the number of markers. Retaining only one marker per unique location and omitting the redundant ones resulted in a final set of 1,533 SNPs that were well distributed throughout the genome. Using this reduced SNP set, five genomic locations represented by six markers were found significantly associated with *Pgt*-TTKSK resistance at the seedling stage (Table 3.4; Figure 3.4A). Two of the markers were located on chromosome 2H (both short and long arms), one each on the long arms of chromosomes 3H and 4H, and two on the short arm of chromosome 5H (Table 3.4). The significant marker identified on chromosome 2HS was SCRI_RS_115905 (at 41.67 cM), on chromosome 2HL SCRI_RS_109266 (172.15 cM), on chromosome 3HL SCRI_RS_180847 (152.69 cM), and on chromosome 4HL 12_30995 (54.95 cM). The two significant markers on chromosome 5HS (SCRI_RS_10929 and 11_20206) were located within a very short genetic distance (3.93 cM) of each other and were perhaps detecting the same QTL. Indeed, the LD between the markers (SCRI_RS_10929 and 11_20206) was moderate (r^2=0.26), further suggesting that they are both associated with the same QTL. The most significant marker identified was SCRI_RS_10929 (Table 3.4; Figure 3.4A). QTL associated with SNP markers were named according to the following convention: *Rpg*-, indicating the pathogen abbreviation of Reaction to *Puccinia graminis*; *qtl*-, indicating the quantitative nature of the trait; *5H*- indicating the chromosome upon which the marker lies; and SCRI_RS_10929, indicating the name of the most significant marker associated with the trait. Thus, since SCRI_RS_10929 was the most significant marker found for TTKSK resistance, the QTL designation of *Rpg-qtl-5H-SCRI_RS_10929* was applied. No significant marker

associations were found for African stem rust resistance at the adult plant stage (Appendix Figure 3.4B).

3.3.6.3 *Genome-wide association mapping of Pgt-MCCFC resistance*

GWAS of *Pgt*-MCCFC resistance was conducted with both the seedling infection response and adult plant terminal severity data. No significant associations were detected for seedling resistance (Appendix Figure 3.4C) as none of the markers had scores close to the FDR threshold. Thus, multiple testing was likely not a factor for the lack of significant associations. With respect to adult plant resistance, GWAS was conducted with data from individual years and the three combined years. A significant association was found with marker 12_10674 on the long arm of chromosome 5H from the 2012 data alone and also the 2010 and 2012 data combined (Table 3.4; Figures 3.4B and C). This QTL was designated as *Rpg-qtl-5H-12_10674* (68.83 cM). No significant QTL were detected with the 2010 and 2011 data sets individually or the combined three-year dataset (Appendix Figure 3.4D, E and F). Marker 12_10674 did have the lowest FDR adjusted *P*-value in 2010 and also in the three year combined dataset, albeit at a level just below the discovery threshold (Appendix Figure 3.4D and F).

3.3.6.4 *Genome-wide association mapping of spot blotch isolate ND85F resistance*

GWAS of spot blotch resistance was conducted with both seedling infection response and adult plant terminal severity datasets. No significant associations were detected at the seedling stage (Appendix Figure 3.5A). One marker (SCRI_RS_153785) on the short arm of chromosome 1H (43.41 cM) had an FDR adjusted *P*-value close to the FDR threshold (Appendix Figure 3.5A), but was not statistically significant. To determine the effect of marker number on the discovery of significant marker associations, GWAS also was conducted on the reduced marker set of 1,535 SNPs; however, this did not result in any marked changes in the FDR adjusted *P*-values. The SCRI_RS_153785 marker gave the highest signal just as in the complete SNP dataset (data not shown). With respect to the adult plant terminal severity data, GWAS was conducted with data from individual years and the three combined years. As in the

seedling dataset, no significant marker-trait associations were detected with data from individual years or the three years combined (Appendix Figure 3.5B, C, D, E and F for sample plots).

Basic Local Alignment Search Tool (BLAST) analysis with the source sequences of SNP markers with the lowest FDR adjusted *P*-values in GWAS with the adult plant spot blotch severity data sets of 2010 alone, 2011 alone, and 2011 and 2012 combined revealed sequences with putative functions in plant-microbe interactions (see section 3.3.7.6 below). Using the 2010 dataset, SCRI_RS_133602 was the marker (on the long arm of chromosome 5H at 119.72 cM) that had the lowest FDR adjusted *P*-value of 1.31×10^{-4}. In 2011 dataset, the marker SCRI_RS_210928 had the lowest *P*-value of 1.31×10^{-4} and was about 10 cM distal on the same arm of chromosome 5H (131.43 cM). In the combined datasets of 2011 and 2012, marker 11_21226 (on the short arm of 1H at 4.27 cM) had the lowest FDR adjusted *P*-value at 5.35×10^{-5}.

As the genetic architecture of spot blotch resistance may differ in two- and six-rowed germplasm (Bilgic et al. 2005; Steffenson et al. 1996), GWAS of spot blotch resistance also was conducted in the two germplasm sets separately. In the two-rowed set, one marker on the short arm of chromosome 4H (SCRI_RS_12719: 25.34 cM) and another unmapped marker (SCRI_RS_120247) were detected as significantly associated with spot blotch resistance based on the combined three-year mean severity data (Table 3.4; Figure 3.4D). The spot blotch resistance QTL at the SCRI_RS_12719 locus was designated as *Rcs-qtl-2H-SCRI_RS_12719*. No significant association was detected with other datasets in the two-rowed accessions (data not shown). Moreover, no significant associations were detected with any of the datasets of the six-rowed germplasm (data not shown). However, marker SCRI_RS_153785 on chromosome 1H, which gave an FDR adjusted *P*-value close to the threshold at the seedling stage in the complete GWAS panel (Appendix Figure 3.5A), also exhibited the lowest FDR adjusted *P*-value of 2.47×10^{-5} in the seedling data of six-rowed accessions alone (data not shown).

3.3.6.5 *Allele effect of significant markers on traits*

The allele frequency and allelic effect of each significant marker in reducing disease levels of stem rust and spot blotch at both the seedling and adult plant stages was estimated (Table 3.5). The beneficial (resistance) alleles of all but one of the six markers significantly associated with TTKSK resistance were minor in frequency, with allele frequencies ranging from 7 to 27% (Table 3.5). The beneficial alleles of these markers provided a 1.80 (*Rpg-qtl-5H-SCRI_RS_10929*) to 8.14% (*Rpg-qtl-2H_SCRI_RS_109266*) decrease in TTKSK numeric score over the other allele with a mean numeric score of 3.25 to 3.30 (Table 3.5). The SNP marker *SCRI_RS_109266* on chromosome 2HL had resistance allele "A" with a frequency of 70% and resulted in a mean reduction of race TTKSK numeric score by 8.14% compared to the other allele, giving a mean numeric score of 3.16 (Table 3.5). The beneficial allele "B" of *Rpg-qtl-5H-12_10674* associated with race MCCFC resistance provided a 29.81% decrease in disease severity over the other allele, resulting in a reduced mean disease severity of 21.61% (Table 3.5). The beneficial allele "B" of the QTL/marker associated with adult spot blotch resistance provided a 19.31 (*Rcs-qtl-2H-SCRI_RS_12719*) to 91.41% (*SCRI_RS_120247*) decrease in mean disease severity over the other allele (Table 3.5).

3.3.6.6 *Candidate gene scan using BLAST*

Source sequences of markers significantly associated with resistance against stem rust and spot blotch were examined by BLAST in the NCBI database. With respect to race TTKSK resistance, marker SCRI_RS_115905 on 2HS was a predicted protein in *H. vulgare* ssp. *vulgare*, but no significant similarity was detected in rice or *Brachypodium*. However, the sequence coded for a predicted mitochondrial iron transporter in other species. Mitochondrial iron transport is essential for plant growth including rice (Bashir et al. 2011). Similarly, marker SCRI_RS_109266 on chromosome 2HL was a predicted protein in *H. vulgare* ssp. *vulgare*, but again no significant homology was found in rice or *Brachypodium*. In other species, however, a predicted protein phosphatase-1 (PP1) was detected. Protein phosphatase-1 is a protein serine/threonine phosphatase that regulates a variety of cellular processes through the dephosphorylation of substrates, including recovery from stress, but also promotes apoptosis when cells are damaged beyond repair

(Ceulemans et al. 2004). Marker SCRI_RS_180847 on chromosome 3HL was a predicted protein in *H. vulgare* ssp. *vulgare* and a predicted uncharacterized protein in *B. distachyon* with no clear function in other species. Likewise, marker 12_30995 on chromosome 4HL was a predicted protein in *H. vulgare* ssp. *vulgare*, but no homologs were detected in rice, *Brachypodium* or other species. Marker SCRI_RS_10929 on the telomeric region of chromosome 5HS is a putative chalcone synthase family member in *H. vulgare* ssp. *vulgare* and several related species including *B. distachyon*, *Zea mays* and *Triticum*. Chalcone synthase is a key enzyme of the flavonoid/isoflavonoid biosynthesis pathway and plays a role in some plant-microbe interactions (Dao et al. 2011). Marker 11_20206, which is a few cM distal of SCRI_RS_10929 on chromosome 5HS, is a *H. vulgare* methyljasmonate-inducible lipoxygenase 2. It was uncharacterized in the rice library, not detected in *Brachypodium*, and had no clear function in other species. Methyljasmonate-inducible lipoxygenase 2 is involved in jasmonic acid biosynthesis and was shown to be localized within the chloroplasts of barley leaves (Vörös et al. 1998). Jasmonic acid is a phytohormone that plays a recognized role in induced defense against both biotrophic and necrotrophic fungal pathogens (Antico et al. 2012). The only marker significantly associated with race MCCFC resistance at the adult plant stage (12_10674) was a predicted uncharacterized protein in *B. distachyon*, rice and other related species.

The three markers with the lowest *P*-values in the GWAS of adult plant spot blotch resistance based on the whole germplasm panel revealed an interesting feature after BLAST analysis. The marker 11_21226 on chromosome 1HS is a predicted protein in barley, an ABC transporter D family member 1-like protein in *B. distachyon*, and peroxisomal membrane protein ABC transporter in *Oryza sativa*, *Populus trichocarpa* and *Arabidopsis lyrata*. Plant ABC transporters are involved in the growth and response to abiotic and biotic stresses, including resistance against pathogens (Kang et al. 2011). The marker on chromosome 5HL (SCRI_RS_133602) was a predicted protein in barley and a predicted serine/threonine-protein kinase tousled-like in *B. distachyon*. It also was a predicted tousled-like protein kinase in *Triticum* and *Z. mays*. Serine/threonine kinase genes encode one of the important proteins for defense signal transduction and confer disease resistance in plants (Song et al. 2005). One example of this class of genes is the

stem rust resistance gene *Rpg1* in barley (Brueggeman et al. 2006). SCRI_RS_210928 is another marker (~12 cM away on chromosome 5HL) with a low *P*-value, and its sequence encodes a predicted protein in barley, a predicted probable carboxylesterase 7-like protein in *B. distachyon*, and a gibberellin receptor GID1L2 in *Z. mays*. Carboxylesterases are hydrolyzing enzymes widely distributed in plants, where they have been implicated in roles that include disease resistance (Cunnac et al. 2007). GIBBERELLIN-INSENSITIVE DWARF1 (GID1) mediates gibberellin signaling in *O. sativa*) and *Arabidopsis thaliana* (Ueguchi-Tanaka et al. 2007). Gibberellins (GAs) are plant hormones that play diverse biological roles in growth and also defense to biotic or abiotic stress in crosstalk with jasmonate signaling (Hou et al. 2013).

Two markers were significantly associated with spot blotch resistance at the adult plant stage in the two-rowed germplasm sets. Marker SCRI_RS_12719, lies on chromosome 4HS and was detected in the three year mean severity data of the two-rowed accessions. It is a predicted protein in barley, a shikimate kinase-like protein in *Triticum aestivum*, and an uncharacterized protein in *B. distachyon* and *O. sativa*. Shikimate kinase (EC 2.7.1.71) catalyzes the direction of carbon from the central metabolism pool to a broad range of secondary metabolites involved in plant development, growth, and stress responses in the fifth reaction of the shikimate pathway (Fucile et al. 2008). The unmapped marker SCRI_RS_120247 associated with spot blotch resistance in the three year combined severity data of two-rowed germplasm is a predicted protein in barley and and related plant species.

3.4 Discussion

Two-hundred and ninety-eight (298) landraces from Ethiopia and Eritrea were assembled for this study. Population structure is an important factor that affects GWAS results. STRUCTURE, a model-based clustering method, and PCA were implemented for population structure analysis of the EEBC landraces. Six subpopulations were obtained in the STRUCTURE analysis (Table 3.2; Figure 3.2). However, no clear clustering of two- vs. six-rowed groups was found. Historically, in Ethiopia, farmers use a mixture of barley

landraces for cultivation that may include different row-types. Thus, outcrossing among accessions with different row-types and other characters possibly occurred, thereby contributing to this result. The lack of differentiation of two- vs. six-rowed types in the EEBC landraces is in contrast to breeding germplasm from North America and Europe that showed strong differentiation for row type (Malysheva-Otto et al. 2006; Zhou et al. 2012). The entire germplasm set was originally collected from Ethiopia with a few (29) from Eritrea. At the time of germplasm collection, there was no political separation between Ethiopia and Eritrea and germplasm exchange probably occurred between farmers in what is now two separate countries. Thus, differentiation based on country with geographical delineation was not expected. This was confirmed by the grouping of two-rowed landraces from northern Ethiopia (Tigray) and Eritrea together in subpopulation 2 (Figure 3.2). The results of the genetic composition study of individual lines (Figure 3.2B and Appendix Figure 3.2) demonstrate that many of the accessions are admixtures, as they shared genetic composition with individuals in multiple subpopulations. Based on the PCA analysis, there was no apparent population structure differentiating the landraces. The only apparent clustering in the PCA plots was for landraces obtained from the NSGC. This was mainly because PCA performs poorly for an admixed population, i.e. to have individuals with ancestry from more than one subpopulation (Boyko et al. 2009; Patterson et al. 2006). Individuals in admixed populations have expected allele frequencies that are a linear combination of the frequencies in their ancestors. As all individuals in the admixed population will have the same ancestry proportion, the PCA method fails to describe structure (Patterson et al. 2006). The quantitative STRUCTURE result of the proportion of the genome of each individual originating from different inferred populations indicates the existence of admixture (Figure 3.2B). Thus, we conclude that there is a lack of true and distict population structure in the association mapping panel. The possible presence of any sample structure was corrected for using the pair-wise relatedness between individuals through application of the kinship (K) model.

The landraces were evaluated against two important foliar diseases barley: stem rust and spot blotch. Among the landraces evaluated against stem rust race TTKSK at the

seedling stage, none exhibited level of resistance comparable to the resistant controls, indicating a rarity of this resistance. However, ~15 (5.03%) of the accessions exhibited a moderate level of resistance and are potential candidates for advanced testing under field conditions. These findings are not surprising given that previous works have identified a low frequency of resistance from extensive evaluations of *Hordeum* germplasm. Steffenson et al. (2013) evaluated 1,902 cultivated and 935 wild barley accessions and identified only 2.3 and 1.7% of the accessions with resistance, respectively. Likewise, Zhou (2011) found only 115 (0.30%) of 3,840 US barley breeding lines with TTKSK resistance from screening tests done at the seedling stage. Evaluation of the 298 landraces against race MCCFC at the seedling and adult plant stages revealed only 2 (0.67%) and 12 (4.03%) accessions, respectively, with a level of resistance similar to the resistant controls. At the seedling stage, 55 (18.46%) landraces were moderately resistant to race MCCFC. This 0.67% frequency of highly resistant lines to race MCCFC at the seedling stage was lower, but comparable to the 1.30% of highly resistant accessions reported in wild barley (Steffenson et al. 2007), suggesting that MCCFC resistance is also rare. Overall, stem rust resistance in *Hordeum* appears very rare. Jin et al. (1994) evaluated over 18,000 accessions of barley for their reaction to race QCC at the seedling and adult plant stages and found only a few that were resistant.

When screened against spot blotch isolate ND85F at the seedling and adult plant stages, no EEBC landrace exhibited a resistance level comparable to the six-rowed highly resistant control NDB112 at either developmental stage. However, 95 (31.88%) and 145 (48.66%) accessions exhibited a resistance level comparable to the moderately resistant control of Bowman at the seedling and adult plant stage, respectively. This percentage of spot blotch resistance at the seedling stage is relatively lower than that reported in other studies using the same *C. sativus pathotype.* Fetch et al. (2003) and Roy et al. (2010) detected a ~50 and 95% resistance percentage in wild barley accessions at the seedling stage, respectively. In contrast, evaluation of diverse cultivated barley germplasm identified a low proportion of accessions with spot blotch resistance at the adult plant stage (Fetch et al. 2008).

It appears that the frequency of resistance to spot blotch isolate ND85F is lower in the Ethiopian/Eritrean landrace germplasm than in wild barley accessions. Inoculations of Bowman and NDB112 with recently isolated pathotypes of *C. sativus* indicated the emergence of types with virulence for both accessions (Ghazvini and Tekauz 2007; Valjavec-Gratian and Steffenson 1997; Zhong and Steffenson 2001). This warrants the search for additional resistance genes in various *Hordeum* gene pools. The ultimate goal of the current work is to identify and utilize germplasm with promising resistance in breeding programs so that resistant cultivars can be developed. However, wild and landrace germplasm are not ideal for immediate use in breeding as they may contain exotic genomic regions coding for undesirable phenotypes, i.e. linkage drag. Marker assisted or genomic selection would be a preferable means to transfer stem rust or spot blotch resistance from such accessions into adapted breeding lines.

In this study, GWAS identified six SNP markers (SCRI_RS_115905, SCRI_RS_109266, SCRI_RS_180847, 12_30995, SCRI_RS_10929, 11_20206) on chromosomes 2H, 3H, 4H and 5H associated with seedling resistance to race TTKSK, one marker (12_10674) on 5H associated with adult plant resistance to race MCCFC, and two markers (SCRI_RS_12719 and SCRI_RS_120247) on chromosome 4H and unknown position associated with adult plant spot blotch resistance (Table 3.5). The markers associated with TTKSK seedling resistance are significant, but additional validation is needed to determine if the resistance is also effective at the adult plant stage, the time when stem rust usually infects the crop. The germplasm was evaluated at the adult plant stage for one season in Ethiopia, and no marker was significantly associated with resistance to African stem rust. Additional studies are in progress to obtain more rigorous phenotype data of adult plant resistance to African stem rust. In addition to the *rpg4/Rpg5* locus on chromosome 5HL conferring TTKSK resistance (Steffenson et al. 2009), QTL for adult plant resistance have been reported on all but chromosome 4H (Moscou et al. 2011; Zhou 2011). Among the QTL detected in the current study, only the *Rpg-qtl-2H_SCRI_RS_115905* (41.67 cM) region was reported to be associated with TTKSK resistance at the adult plant stage in the Q21861/SM89010 (Q/SM) population (Moscou et al. 2011). The other markers (SCRI_RS_109266, SCRI_RS_180847,

12_30995, SCRI_RS_10929, 11_20206) may be detecting unique TTKSK resistance loci (Table 3.4). Genomic regions for TTKSK seedling and adult plant resistance identified through GWAS in barley breeding germplasm by Zhou (2011) did not overlap with any of the QTL detected in this study. In a GWAS of wild barley, Steffenson et al. (2007) found Diversity Array Technology (DArT) markers significantly associated with stem rust resistance to race QCCJ at the seedling stage in the genomic region of *Rpg-qtl-3H_ SCRI_RS_180847* (152.69 cM). It is not possible to make conclusive statements as to whether the loci are the same or different due to the differences in consensus maps. Steffenson et al. (2007) also detected DArT markers associated with seedling resistance to race MCCFC on the short arm of chromosome 5H, where the QTL *Rpg-qtl-5H-SCRI_RS_10929* (2.62 cM) lies. It is possible that this region of chromosome 5H contains loci conferring resistance to both races TTKSK and MCCFC. The other remaining QTL conferring TTKSK resistance reside on chromosomes 2HL and 5HL (*Rpg-qtl-2H_SCRI_RS_109266* and *Rpg-qtl-4H_12_30995*) and probably represent unmapped or unique stem rust resistance genes/QTL.

One marker (12_10674) on the long arm of chromosome 5H was found associated with adult plant resistance to race MCCFC (Table 3.4), whereas none was found for seedling resistance. A possible factor for the lack of significant associations is the small size of the GWAS panel (n=298), the low frequency of resistance (4.03 to 18.46%), or contribution of both LD and map resolution. The *Rpg-qtl-5H-12_10674* QTL is robust as it was detected in two separate data sets of high (2010) and intermediate (2012) disease pressure (Table 3.4; Figure 3.1D). The marker was not detected in 2011, possibly due to very low disease development (Figure 3.1D). SNP markers with the same position as 12_10674 on chromosome 5HL were found associated with TTKSK resistance (both at the seedling and adult plant stages) in US barley breeding germplasm through GWAS (Zhou 2011). This chromosome 5H region also was associated with race TTKSK resistance at the adult plant stage in the Q/SM population (Moscou et al. 2011). Taken together, these results strongly indicate that the *Rpg-qtl-5H-12_10674* genomic region is important for conferring resistance against both stem rust races TTKSK and MCCFC.

Given the implication of this finding, the synteny of barley with the *B. distachyon* and rice genomes was characterized with BLAST searches with the EST sequence of barley from which the 12_10674 marker was developed. The BLAST searches provided a predicted uncharacterized protein in both *Brachypodium* and rice. However, the exploration of neighboring genomic regions revealed a putative pentatricopeptide (PPR) repeat domain containing protein (LOC_Os09g29790) just a gene upstream of the uncharacterized protein. PPR proteins (PPRPs) were recently found to function in defense against necrotrophic fungi and abiotic stress tolerance in *Arabidopsis* (Laluk et al. 2011). Zhou (2011) also indicated that a gene (RING-H2 finger gene) related to plant-microbe interaction exists in the sytenic region of the rice genome. The author postulated RING-H2 finger as a putative candidate gene conferring adult plant resistance against stem rust race TTKSK. Pending future validation studies, it appears that a major effect gene in the region confers resistance to stem rust races MCCFC and TTKSK. This is an important finding for several reasons. First, the previous studies that identified this region of chromosome 5H as associated with stem rust resistance used race TTKSK for phenotyping at the seedling and/or adult plant stages (Zhou 2011; Moscou et al. 2011). Race MCCFC is a North American stem rust race that caused severe epidemics during the 1930s. In contrast, TTKSK is a new race detected in Uganda in 1998-99 and is currently threatening cereal crops in Eastern Africa and the Middle East (Yemen and Iran). Second, the previous studies utilized US barley breeding germplasm (Zhou 2011) or a population derived from a cross between the breeding lines Q21861 and SM89010 (Moscou et al. 2011). The current study utilized barley germplasm with a completely different genetic background—that of landraces from Ethiopia and Eritrea. The discovery of the same stem rust resistance region on chromosome 5H in barley breeding germplasm from North America and landraces from Ethiopia and Eritrea suggests that the genomic region was perhaps evolutionarily conserved; i.e. evolved in wild or landrace barleys before the beginning of selection for modern breeding.

For seedling spot blotch resistance to isolate ND85F, one marker (SCRI_RS_153785) on the short arm of chromosome 1H was found associated, but not significantly so (Appendix Figure 3.5A). In US barley breeding germplasm, Zhou and

Steffenson (2013) identified SNP markers associated with spot blotch resistance at the seedling and adult plant stages that exactly mapped at the position of SCRI_RS_153785, indicating that it is possibly detecting the same QTL. Based on the barley comparative BIN (barleygenomics.wsu.edu) and SNP marker maps, the QTL identified by Zhou and Steffenson (2013) appears to be coincident with a major effect adult plant resistance QTL on chromosome 1H that was derived from line NDB112 (Wilcoxson et al. 1990). This NDB112-derived QTL was found in bi-parental mapping studies in the Steptoe/Morex population (Bilgic et al. 2005; Steffenson et al. 1996) and was named *Rcs-qtl-1H-6-7*. DArT markers significantly associated with the *Rcs-qtl-1H-6-7* QTL region also were identified in a GWAS of seedling spot blotch resistance in wild barley (Roy et al. 2010). The consistent detection of this QTL in diverse barley accessions perhaps indicates the evolutionary importance of the QTL and/or block of linked genes. The QTL was not statistically significant, however, in this study perhaps due to the low frequency of resistance, low sample size, and mapping resolution.

At the adult plant stage, no marker was found significantly associated with spot blotch resistance using the complete germplasm panel, but three markers (11_21226, SCRI_RS_133602 and SCRI_RS_210928) on chromosomes 1H and 5H had low FDR adjusted *P*-values. These markers were probably not significant due to the low frequency of resistance, map resolution, or level of LD in the population. Marker 11_21226 was located at the telomeric region of the short arm of chromosome 1H where a single gene (designated *Rcs6*) conferring resistance to pathotype 2 of *C. sativus* was identified in a population derived from a Bowman backcross line (Bilgic et al. 2006). The authors indicated that *Rcs6* was effective at both the seedling and adult plant stages. However, marker 11_21226 was neither significant nor exceedingly low in its FDR adjusted *P*-value at the seedling stage. It would be interesting to evaluate the EEBC germplasm against pathotype 2 and other characterized pathotypes of *C. sativus*. Such analyses would provide additional insight into the genetics of resistance against pathotype 2 as well as other pathotypes in the germplasm. The markers SCRI_RS_133602 and SCRI_RS_210928, located on the long arm of chromosome 5H, appear to be coincident

with *Rcs-5H-11*, a previously identified QTL for seedling spot blotch resistance in the Shechem/Harrington population (Alsop 2009).

The inheritance of spot blotch resistance depends on the growth stage and/or genetic background (Bilgic et al. 2005; Steffenson et al. 1996). Two-rowed cultivars exhibit more variable reactions to spot blotch and the resistance bred into them has not been broadly effective (Alsop 2009; Roy et al. 2010). Understanding the genetics of spot blotch resistance in two- and six-rowed germplasm is a continuing interest for breeders. In the current study, two markers (SCRI_RS_12719 and SCRI_RS_120247) on chromosome 4H and an unknown mapping location were found significantly associated with adult plant spot blotch resistance in two-rowed accessions (Table 3.5). Marker SCRI_RS_12719 appears to be located in close vicinity with the *Rcs-qtl-4H-4-6* QTL for adult plant spot blotch resistance (Yun et al. 2006). *Rcs-qtl-4H-4-6* was mapped on the short arm of chromosome 4H in an advanced backcross population derived from the wild barley accession OUH602 and Harrington, where the former contributed the resistance allele (Yun et al. 2006). Marker SCRI_RS_12719 was perhaps detecting the *Rcs-qtl-4H-4-6* QTL or an allele. The unmapped marker SCRI_RS_120247 was perhaps detecting an already characterized spot blotch QTL or a unique locus. Additional searches will be conducted to determine whether this marker is detecting an already characterized resistance QTL or a unique locus.

In summary, evaluation of Ethiopia and Eritrean barley landraces to stem rust races TTKSK and MCCFC and spot blotch isolate ND85F revealed that resistance against these diseases is very limited in the accessions. Utilizing association mapping in this unique germplasm, six SNP markers on chromosomes 2HS, 2HL, 3HL, 4HL and 5HS associated with seedling resistance to race TTKSK, one marker on 5HL associated with adult plant resistance to race MCCFC, and one marker on chromosome 4HS and one with unknown position associated with adult plant spot blotch resistance were identified. The QTL associated with race TTKSK resistance on 2HS maps to a region where a QTL against the race was previously reported at the adult plant stage. Two QTL associated with TTKSK on 3HL and 5HS lie in genomic regions containing QTL conferring

resistance against races QCCJ and MCCFC, respectively. These QCCJ and MCCFC resistance QTL were recently identified in a GWAS of wild barley at the seedling stage. Thus, the 3HL and 5HS regions possibly harbor loci conferring resistance to both races TTKSK and QCCJ, and TTKSK and MCCFC, respectively. In essence, the aforementioned TTKSK QTL on 2HS, 3HL and 5HS may not be new stem rust resistance QTL, though they could still be unique alleles. The other QTL identified for TTKSK resistance were located on chromosomes 2HL and 4HL. These two QTL mapped to genomic regions where no gene/QTL for stem rust resistance were previously identified in other studies; thus, they probably represent novel stem rust resistance loci. Additional studies are needed to validate the markers associated with TTKSK seedling resistance at the adult plant stage, as stem rust usually infects the crop towards the heading stage. More robust germplasm evaluations against African stem rust at the adult plant stage are in progress. For stem rust race MCCFC, GWAS identified a SNP marker on chromosome 5HL associated with adult plant resistance. SNP markers with the same position on 5HL as currently detected were found associated with TTKSK resistance in barley breeding germplasm through GWAS. QTL conferring race TTKSK resistance at the adult plant stage also was recently reported in this region of 5HL, indicating its importance for resistance against both stem rust races TTKSK and MCCFC. GWAS of spot blotch resistance in the landraces identified a marker on 4HS significantly associated with adult plant resistance in two-rowed accessions. The SNP marker appears to be located in close proximity with adult plant spot blotch resistance QTL found in other bi-parental mapping studies.

Even though barley landraces from Ethiopia and/or Eritrea are known to be sources of unique disease resistance genes/loci against Barley Yellow Dwarf, powdery mildew, net blotch, scald, and loose smut (Jørgensen 1992; Metcalfe et al. 1978; Qualset 1975; Wiberg 1974; Yitbarek et al. 1998; Zhang et al. 1987), only a few QTL were detected in this study. The majority of them mapped to genomic locations previously reported to contain resistance QTL against respective diseases. This could be due to a low frequency of resistance to the respective diseases studied, low number of samples (n=298), low genetic diversity in the germplasm, and/or level of mapping resolution and

LD. Populations used in GWAS should possess high phenotypic/genetic diversity (Flint-Garcia et al. 2005). Despite the few reports that claim high genetic diversity in Eritrean barley [e.g., Backes et al. 2008], the low genetic diversity actually found in this germplasm (Arregui et al. 2013; Bjørnstad et al. 1997) might have affected the GWAS outcomes. Still, this exploratory GWAS study validates the findings of previous population-based reports that stem rust resistance is rare in the *Hordeum* gene pool. Given the threat of the stem rust race TTKSK and its variants, the identification of the TTKSK resistance QTL coincident with those conferring resistance to races QCCJ and MCCFC indicates that it is perhaps possible to introgress multiple minor genes/QTL conferring broad-spectrum resistance against diverse stem rust populations. This could be achieved by selecting a small subset of accessions with beneficial alleles at different QTL. Accessions with resistance alleles can then be used in crossing with elite breeding lines or adapted cultivars. Individual markers associated with each QTL can be used for selection of lines for the next cycle of crossing until beneficial alleles are accumulated in a single genetic background. This genomic selection assisted back-crossing scheme could also be used for pyramiding of multiple QTL conferring spot blotch resistance.

Table 3.1. Variance components and heritability of resistance to stem rust and spot blotch in Ethiopian and Eritrean barley landrace germplasm

	Variance components[a]			
Trait	**Genotype**	**Year**	**Residuals**	**Heritability**
Stem rust race TTKSK (seedling)	1.03	--[b]	-0.69	1.00
Stem rust race MCCFC (seedling)	1.98	--	-1.34	1.00
Stem rust race MCCFC (adult plant)	71.56	80.21	93.04	0.63
Spot blotch isolate ND85F (seedling)	3.14	--	-1.66	1.00
Spot blotch isolate ND85F (adult plant)	174.76	115.17	44.96	0.79

[a] Generated by "proc varcomp" procedure in SAS.
[b] --: Not applicable.

Table 3.2. Characteristics of subpopulations (sp) of Ethiopian and Eritrean barley landrace germplasm

				Row morphology, genebank and growth habit information[b]									
Sp	**Distance[a]**	**No. individuals**	**Group name**	**2-rowed**	**6-rowed**	**Irregular**	**NSGC**	**VIR**	**ICARDA**	**Spring**	**Spring/ facultative**	**Winter**	**Location**
1	0.10	75 (25.17%)	Two-rowed+six-rowed (NSGC+VIR)	27	48	0	60	15	0	72	3	0	Ethiopia
2	0.08	32 (10.74%)	Two-rowed Tigray +Eritrea (NSGC)	28	1?	3	30	0	2	31	1	0	Tigray and Eritrea
3	0.31	38 (12.75%)	Six-rowed VIR +ICARDA	9	29	0	11	18	9	32	0	6	Ethiopia (33/38)
4	0.20	26 (8.72%)	Two-rowed VIR+ICARDA	24	2	0	5	19	2	26	0	0	Ethiopia (24/26)
5	0.09	12 (4.03%	Two-rowed NSGC	8	2	0	12	0	0	12	0	0	Ethiopia (10/12)
6	0.08	110 (36.91%)	Six-rowed NSGC+(VIR)	16	91	3	101	9	0	82	26	2	Ethiopia (100/110)

[a] Average distance (expected heterozygosity) between individuals in same cluster generated by the STRUCTURE program for subpopulations K=3 to 10 with a burn-in period of 100,000 cycles and 200,000 iterations with 20 independent runs.

[b] NSGC: National Small Grains collection; VIR: N.I. Vavilov Research Institute of Plant Industry; and ICARDA: International Center for Agricultural Research in the Dry Areas.

Table 3.3. Allele-frequency divergence (net nucleotide distance)[a] among subpopulations (sp) in Ethiopian and Eritrean barley landrace germplasm

sp[b]	1	2	3	4	5	6
1	-	-	-	-	-	-
2	0.06	-	-	-	-	-
3	0.24	0.24	-	-	-	-
4	0.29	0.30	0.21	-	-	-
5	0.12	0.14	0.24	0.30	-	-
6	0.02	0.07	0.24	0.30	0.12	-

[a] Computed by the STRUCTURE program for subpopulations *K*=3 to 10 with a burn-in period of 100,000 cycles and 200,000 iterations with 20 independent runs.

[b] The sp were named as 1: "Two-rowed/six-rowed (NSGC/VIR)"; 2: "Two-rowed Tigray/Eritrea (NSGC)"; 3: "Six-rowed VIR/ICARDA"; 4: "Two-rowed VIR/ICARDA"; 5: "Two-rowed NSGC"; and 6: "Six-rowed NSGC/(VIR)" (see Table 3.2).

Table 3.4. SNP markers significantly associated with row-type morphology (RT), resistance to stem rust races TTKSK and MCCFC, and resistance to spot blotch isolate ND85F (SB) in Ethiopian and Eritrean barley landrace germplasm

				q-value[d]			
Trait	**SNP marker**	**Chrom.[a]**	**Position[b]**	**EEBC**	**EEBC10+12**	**EEBC-12**	**EEBC-2-R-C**
RT	11_20781	2H	88.04	$6.79x10^{-6c}$			
RT	SCRI_RS_171032	2H	90.64	$1.63x10^{-11}$			
RT	11_20340	2H	90.99	$9.30x10^{-53}$			
RT	12_30901	2H	90.99	$3.78x10^{-20}$			
RT	12_30896	2H	91.09	$1.52x10^{-55}$			
RT	SCRI_RS_196853	2H	91.09	$2.30x10^{-8}$			
RT	12_30897	2H	91.09	$4.89x10^{-8}$			
RT	SCRI_RS_221886	2H	92.07	$7.66x10^{-5}$			
RT	11_20606	4H	31.14	$6.52x10^{-5}$			
RT	11_21001	5H	55.83	$6.04x10^{-5}$			
RT	SCRI_RS_165473	Unknown	Unknown	$1.97x10^{-21}$			
RT	SCRI_RS_72983	Unknown	Unknown	$8.15x10^{-20}$			
RT	SCRI_RS_181051	Unknown	Unknown	$7.91x10^{-5}$			
RT	SCRI_RS_237894	Unknown	Unknown	$1.09x10^{-4}$			
TTKSK	SCRI_RS_115905	2H	41.67	$1.40x10^{-4}$			
TTKSK	SCRI_RS_109266	2H	172.15	$1.90x10^{-4}$			
TTKSK	SCRI_RS_180847	3H	152.69	$9.10\ x10^{-5}$			
TTKSK	12_30995	4H	54.95	$9.20x10^{-5}$			
TTKSK	SCRI_RS_10929	5H	2.62	$2.80x10^{-5}$			
TTKSK	11_20206	5H	6.55	$1.80x10^{-4}$			
MCCFC	12_10674	5H	68.83		$6.93x10^{-9}$	$7.42x10^{-6}$	
SB	SCRI_RS_12719	4H	25.34				$3.33x10^{-6}$
SB	SCRI_RS_120247	Unknown	Unknown				$7.22x10^{-7}$

[a] Chromosome. Note: some markers have not been mapped.

[b] Genetic position (in cM) of marker on chromosome. Note: some markers have not been mapped to a chromosomal position.

[c] Multiple testing corrected *P*-value.

[d] EEBC: Includes the entire Ethiopian and Eritrean barley landrace collection evaluated over three years (2010-2012); EEBC-10+12: Includes the entire Ethiopian and Eritrean barley landrace collection evaluated in 2010 and 2012; EEBC-12: Includes the entire Ethiopian and Eritrean barley landrace collection evaluated in 2012; EEBC-2-rowed-C: Includes only two-rowed accessions from the Ethiopian and Eritrean barley landrace collection evaluated over three years (2010-2012).

Table 3.5. Trait means for each allele at chromosome positions significantly associated with resistance to stem rust races TTKSK and MCCFC and spot blotch isolate ND85F (SB) identified in Ethiopian and Eritrean barley landrace germplasm

Germ-plasm	Trait	QTL/marker	Position (cM)	Allele	Trait mean (0-4.5 or %)[d]	Allele frequency [e]
Whole set	TTKSK[a]	*Rpg-qtl-2H_SCRI_RS_115905*	2H 41.67	A	3.30	0.07
Whole set	TTKSK	*Rpg-qtl-2H_SCRI_RS_115905*	2H 41.67	B	3.54	0.93
Whole set	TTKSK	*Rpg-qtl-2H_SCRI_RS_109266*	2H 172.15	A	3.16	0.69
Whole set	TTKSK	*Rpg-qtl-2H_SCRI_RS_109266*	2H 172.15	B	3.44	0.31
Whole set	TTKSK	*Rpg-qtl-3H_SCRI_RS_180847*	3H 152.69	A	3.25	0.09
Whole set	TTKSK	*Rpg-qtl-3H_SCRI_RS_180847*	3H 152.69	B	3.33	0.91
Whole set	TTKSK	*Rpg-qtl-4H_12_30995*	4H 54.95	A	3.33	0.92
Whole set	TTKSK	*Rpg-qtl-4H_12_30995*	4H 54.95	B	3.25	0.08
Whole set	TTKSK	*Rpg-qtl-5H-SCRI_RS_10929*	5H 2.62-6.55	A	3.27	0.09
Whole set	TTKSK	*Rpg-qtl-5H-SCRI_RS_10929*	5H 2.62-6.55	B	3.33	0.91
Whole set	TTKSK	*11_20206*	5H 6.55	A	3.33	0.73
Whole set	TTKSK	*11_20206*	5H 6.55	B	3.30	0.27
Whole set	MCCFC	*Rpg-qtl-5H-12_10674*[b]	5H 68.83	A	30.79	0.58
Whole set	MCCFC	*Rpg-qtl-5H-12_10674*	5H 68.83	B	21.61	0.42
Two-rowed	SB[C]	*Rcs-qtl-2H-SCRI_RS_12719*	4H 25.34	A	47.81	0.10
Two-rowed	SB	*Rcs-qtl-2H-SCRI_RS_12719*	4H 25.34	B	27.63	0.90
Six-rowed	SB	*Rcs-qtl-2H-SCRI_RS_12719*	4H 25.34	A	34.29	0.05
Six-rowed	SB	*Rcs-qtl-2H-SCRI_RS_12719*	4H 25.34	B	28.74	0.95
Whole set	SB	*Rcs-qtl-2H-SCRI_RS_12719*	4H 25.34	A	41.80	0.07
Whole set	SB	*Rcs-qtl-2H-SCRI_RS_12719*	4H 25.34	B	28.15	0.94
Two-rowed	SB	*SCRI_RS_120247*	Unknown	A	50.80	0.15
Two-rowed	SB	*SCRI_RS_120247*	Unknown	B	26.54	0.85
Six-rowed	SB	*SCRI_RS_120247*	Unknown	A	30.00	0.03
Six-rowed	SB	*SCRI_RS_120247*	Unknown	B	28.78	0.97
Whole set	SB	*SCRI_RS_120247*	Unknown	A	48.72	0.08
Whole set	SB	*SCRI_RS_120247*	Unknown	B	27.91	0.92

[a] All markers were identified in genome-wide association mapping of reaction to stem rust race TTKSK at seedling stage.

[b] Identified in EEBC-10+12 and EEBC-12; see Table 3.4 for full explanation of these datasets.

[c] Identified in EEBC-2-rowed-C; see Table 3.4 for full explanation of these datasets.

[d] Trait mean for reaction to stem rust race TTKSK was a numeric mean (0–4.5 scale) of seedling infection response; means for reaction to stem rust race MCCFC and spot blotch were mean disease severity (%) at the adult plant stage; --: only one accession had the A allele.

[e] Allele frequency within analysis set; calculated for each significant marker.

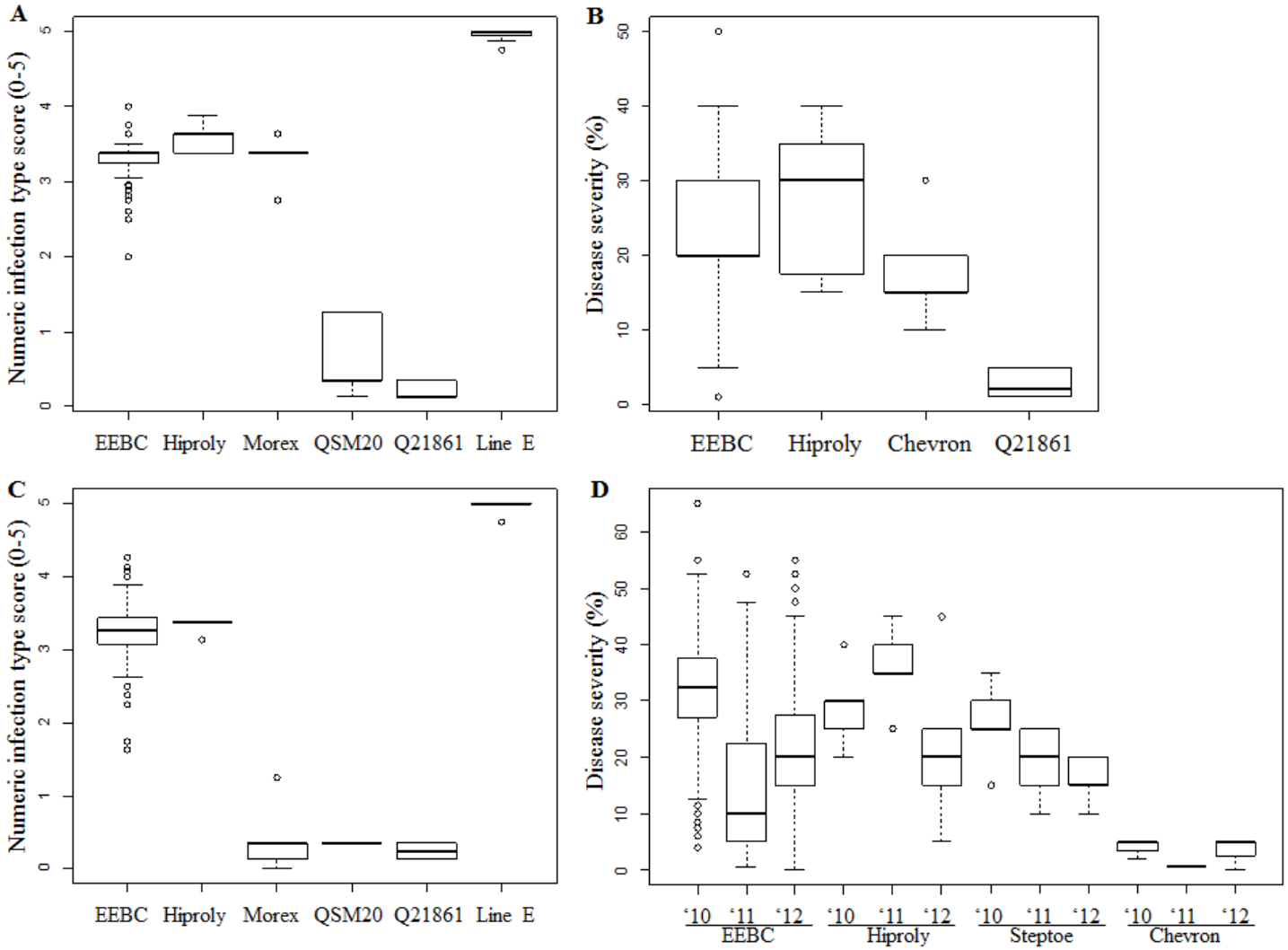

Figure 3.1. See next page.

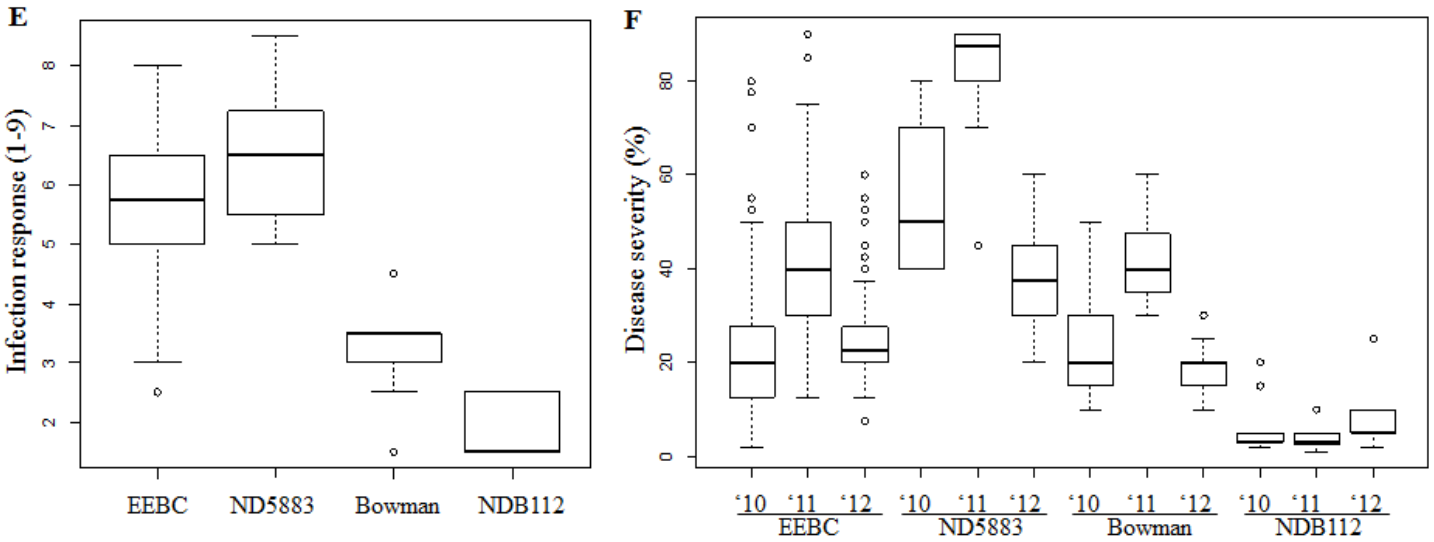

Figure 3.1. Boxplots of reaction data to stem rust (races TTKSK and MCCFC), and spot blotch (isolate ND85F) at the seedling and adult plant stages of Ethiopian and Eritrean barley landrace germplasm (EEBC) (n=298) and controls. For stem rust race *Pgt*-TTKSK, Hiproly, Morex and Chevron are susceptible controls; line QSM20 and Q21861 are resistant controls; and Line E is a susceptible wheat control. For race *Pgt*-MCCFC, Hiproly and Steptoe are susceptible controls; Morex, Chevron, line QSM20 and Q21861 are resistant controls; and Line E is a susceptible wheat control. For spot blotch, line ND5883, cv. Bowman and line NDB112 are susceptible, moderately resistant and resistant controls, respectively. (**A**) Seedling infection numeric score data to race *Pgt*-TTKSK on scale of 0 (resistant) to 5 (susceptible), (**B**) Adult plant severity (%) to domestic African stem rust races, (**C**) Seedling infection numeric score data to race *Pgt*-MCCFC on scale of 0 (resistant) to 5 (susceptible), (**D**) Adult plant severity (%) to *Pgt*-MCCFC in years 2010 ('10), 2011 ('11) and 2012 ('12); (**E**) Seedling infection response (IR) to spot blotch on scale of 1 (resistant) to 9 (susceptible), and (**F**) Adult plant severity (%) to spot blotch in years 2010 ('10), 2011 ('11) and 2012 ('12). Five statistics (bars) are represented in each boxplot from bottom to top: the smallest observation, lower quartile, median, upper quartile, and largest observation, respectively. Data points positioned outside this range are depicted as circles and are outliers.

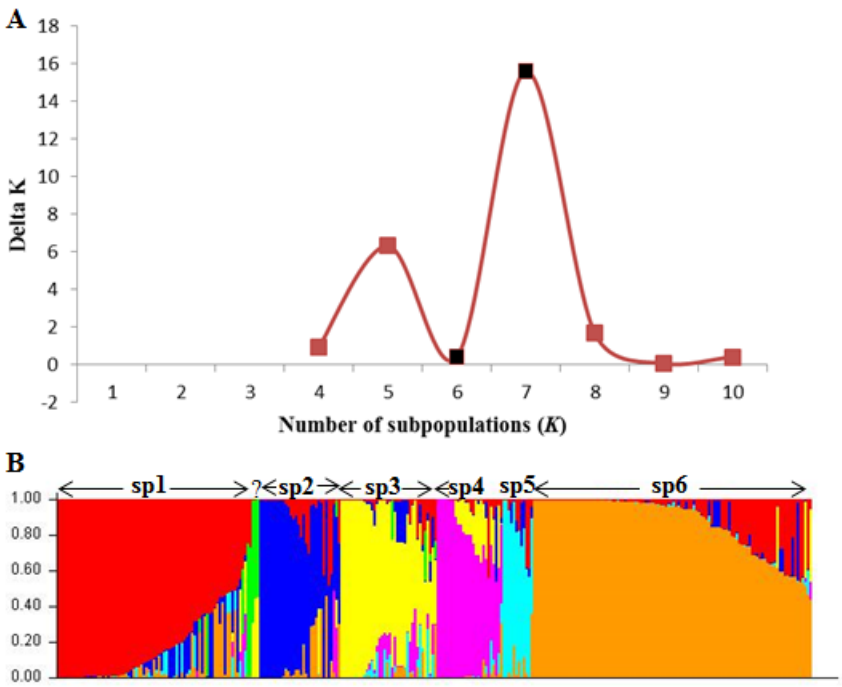

Figure 3.2. Probability of subpopulations within Ethiopian and Eritrean barley landrace germplasm based on STRUCTURE analysis. (**A**) Inference of true value of subpopulations (*K*) using the rate of lnPr(X|*K*) change from *K*-1 to *K* (*ΔK*) method for *K*=3 to 10 with a burn-in period of 100,000 cycles and 200,000 iterations with 20 independent runs. Inferred subpopulations of 6 to 7 are indicated with black square, and (**B**) Bar-plot of the individual accessions based on SNP markers generated by STRUCTURE 2.3.4 using the admixture model. Individuals were assigned into clusters based on the subpopulation membership matrix (Q) scores. Q scores represent the probability that a particular portion of an individual's genome came from population *K*. Each individual is represented by a vertical (100%) stacked column of genetic components with proportions shown in color for *K*=7. Groups for each panel are represented by colors. Each column (298 columns in total) represents a landrace's genotype and is partitioned into segments, the length of which represents the estimated genetic fraction of every accession from each of the seven inferred subpopulations. The assumed subpopulations (sp) of 1 to 6 are shown above the bar-plot. The "?" mark between sp1 and sp2 denotes a few individuals which could possibly be admixtures; and therefore not considered as a separate subpopulation on their own. The sp were named as sp1: "Two-rowed/six-rowed (NSGC/VIR)"; sp2: "Two-rowed Tigray/Eritrea (NSGC)"; sp3: "Six-rowed VIR/ICARDA"; sp4: "Two-rowed VIR/ICARDA"; sp5: "Two-rowed NSGC"; and sp6: "Six-rowed NSGC/(VIR)".

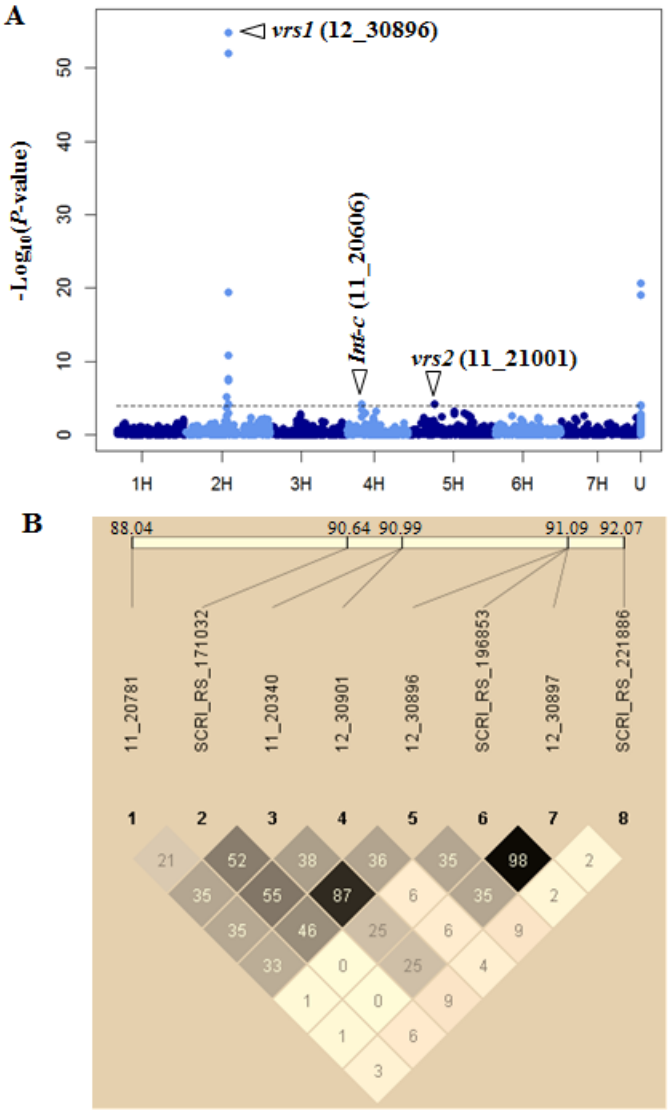

Figure 3.3. (**A**) Genome-wide association scan for row-type spike morphology of Ethiopian and Eritrean barley landrace germplasm. Vertical axis represents -$\log_{10}$(*P*-values) of the marker-trait associations after correction for multiple testing, and the horizontal axis represents the relative chromosomal position of the SNP markers across the genome. The black horizontal dotted line shows the *P*-value corresponding to a false discovery rate (FDR) of 0.05. SNP markers with peaks above this threshold level were considered as significantly associated with row-type spike morphology. Significantly associated SNPs at or in the vicinity of the genes *vrs1* (six-rowed spike 1), *Int-c* (INTERMEDIUM-C) and *vrs2* (six-rowed spike 2) are marked with arrows. (**B**) Heat map of linkage disequilibrium (LD) for eight SNP markers on chromosome 2H associated with barley spike morphology around the *Vrs1* locus. Numbers above the map are the position of the markers from the concensus map in cM. LD is displayed in the squares below the map as r^2, expressed as a percentage, between all pair-wise combinations of the eight markers.

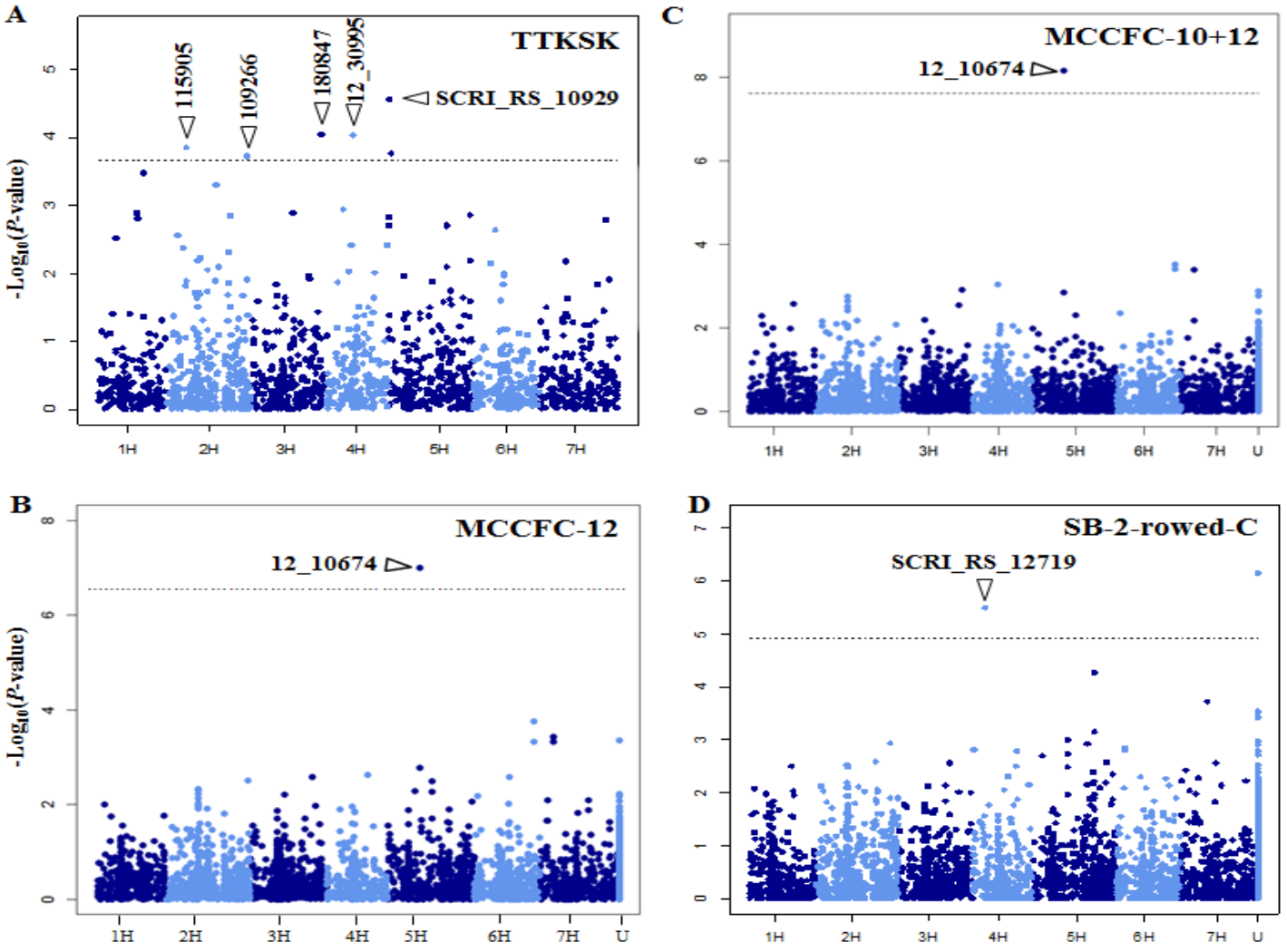

Figure 3.4. See next page.

Figure 3.4. Genome-wide association scans for marker associations to stem rust and spot blotch resistance in Ethiopian and Eritrean barley landrace germplasm (EEBC). Scans are shown for (**A**) Infection numeric score to stem rust race TTKSK at the seedling stage with 1,533 SNP markers (TTKSK). Full names of SNPs indicated with arrows on 2HS, 2HL, and 3HL are SCRI_RS_115905, SCRI_RS_109266, and SCRI_RS_180847, respectively. (**B**) Adult plant severity to stem rust race MCCFC of EEBC in 2012 (MCCFC-12), (**C**) Adult plant severity to stem rust race MCCFC of EEBC in 2010 and 2012 combined (MCCFC-10+12), and (**D**) Adult plant severity to spot blotch isolate ND85F of two-rowed EEBC germplasm with combined data of three years (SB-2-rowed-C). The genome-wide association mapping was run with 5,269 SNP markers unless stated otherwise. Vertical axis represents $-\log_{10}$(*P*-values) of the marker-trait associations after correction for multiple testing. The black horizontal dotted lines show the *P*-value corresponding to a false discovery rate (FDR) of 0.05 and the horizontal axis represents the relative chromosomal position across the barley genome. SNP markers with peaks above this threshold level were considered as significantly associated. The most significant marker for each trait or analysis panel is marked with an arrow. In (D), a non-significant marker on chromosome 4H (12_10562) was indicated with an arrow as the same marker was significant in (E).

Chapter 4

Genome-wide Association Mapping of Fusarium Head Blight Resistance and Agro-morphological Traits in Barley Landraces from Ethiopia and Eritrea

4.1 Introduction

Fusarium head blight (FHB) or scab, caused primarily by *Fusarium graminearum* Schwabe [teleomorph: *Gibberella zeae* (Schw.) Petch.], is a significant disease of wheat, barley and other small grain cereals in many countries with temperate and semi-tropical climates (Goswami and Kistler 2004; Ma et al. 2000; Parry et al. 1995). FHB was first reported in England in 1884 and was considered a major threat to wheat and barley production following the years of its discovery (Goswami and Kistler 2004; Stack 2003). In the Upper Midwest region of the United States and the Prairie provinces of Canada, epidemics of FHB occurred sporadically during the last century (McMullen et al. 1997a; Tekauz et al. 2000). However, starting in 1993, a series of widespread and severe epidemics struck wheat and barley crops, resulting in losses of more than $3 billion during the 1990s (Ward et al. 2008; Windels 2000). FHB also has been a problem in Asia, Europe, and South America in recent years, suggesting that the disease is establishing itself as perennial economically important problem (Parry et al. 1995).

Most species of *Fusarium* (including *F. graminearum*) are spread by dispersal of conidia that travel via air currents to cause new infections. The sexual stage of *F. graminearum* (*Gibberella zeae*) that develops regularly in abundance provides the pathogen with an added advantage. Ascospores produced by this phase can also serve as an inoculum source for subsequent infection and dispersal (http://www.apsnet.org). *Fusarium* infection is favored by higher levels of precipitation, which facilitates subsequent disease spread (McMullen et al. 1997b; Prom et al. 1999; Tekauz et al. 2000). FHB adversely affects yield and grain quality in several ways. Kernel infection may completely destroy kernels or make them smaller, lighter and shriveled. The most severely affected kernels are lost during harvesting and processing (McMullen and Stack 1999). Kernels also can be discolored by infection, thereby further affecting the quality and market value of the grain. In addition, most *Fusarium* species causing FHB produce mycotoxin(s), e.g. deoxynivalenol (DON), in infected grain (Prom et al. 1999). DON is a trichothecene produced by *F. graminearum* and is toxic to humans and other animals

(Scott 1990). Barley grain with DON concentrations greater than 0.5 ppm is not accepted by the malting and brewing industry (Steffenson 1998).

An integrated management approach that combines multiple control strategies is needed to reduce FHB. FHB infection can be reduced through agronomic and chemical control methods. However, the effectiveness and sustainability of these methods is debatable. Cultural practices such as crop rotation may not be very effective as *Fusarium* can overwinter as spores or mycelium in infected crop residues and survive for several years. Fungicides are expensive and provide only partial control of FHB and associated mycotoxin contamination (http://www.apsnet.org/). Host resistance alone is not effective either; however, when coupled with crop rotation, residue management, and fungicides, the severity of FHB and its detrimental effects can be markedly reduced (Steffenson 1998).

In barley, the genetic basis of FHB resistance has been intensively studied through Quantitative Trait Loci (QTL) mapping. The genetic studies indicated that FHB resistance is a complex quantitative trait with low heritability in elite germplasm and highly influenced by the environment (Urrea et al. 2002). FHB resistance also has a low correlation with DON accumulation, adding an additional challenge to FHB resistance breeding (Lamb et al. 2009). So far, no major resistance gene against FHB has been reported. Numerous bi-parental mapping studies identified minor effect QTL that act additively to confer resistance to FHB (Canci et al. 2004; Dahleen et al. 2003; Dahleen et al. 2012; de la Pena et al. 1999; Hori et al. 2005; Hori et al. 2006; Horsley et al. 2006; Lamb et al. 2009; Ma et al. 2000; Mesfin et al. 2003; Sato et al. 2008; Yu et al. 2010; Zhu et al. 1999). These studies have described QTL for FHB resistance, DON accumulation and kernel discoloration across all seven barley chromosomes (Kolb et al. 2001; Massman et al. 2011); however, the most important regions for FHB resistance lie in chromosomes 2H, 4H, and 6H.

Several of the FHB resistance QTL identified in bi-parental studies coincide with QTL for low DON concentration (e.g. Dahleen et al. 2003; Mesfin et al. 2003).

Moreover, QTL for low FHB severity and reduced DON concentration are often associated with gene(s) or QTL for various agro-morphological traits, including the spike type locus *Vrs1*, heading date, and plant height (Dahleen et al. 2003; Dahleen et al. 2012; de la Pena et al. 1999; Ma et al. 2000; Mesfin et al. 2003; Hori et al. 2005; Horsley et al. 2006; Lamb et al. 2009; Zhu et al. 1999). The only resistance QTL reported to be independent of these agronomic traits is the one on the distal portion of chromosome 2(2HL) in PI 643302 (Yu et al. 2010). The resistance locus at the centromeric region of chromosome 2H was frequently detected in most of the bi-parental mapping studies (de la Pena et al. 1999; Hori et al. 2006; Lamb et al. 2009; Ma et al. 2000; Sato et al. 2008). But this QTL is also associated with late heading (e.g. de la Pena et al. 1999). The bin 10 region of chromosome 2H was found to contain QTL that conferred reduced FHB severity and DON concentration in mapping populations with Zhedar 2, CIho 4196, and Chevron as the resistant parents (Dahleen et al. 2003; Horsley et al. 2006; Ma et al. 2000).

More recently, extensive germplasm screening of 23,255 wild (*Hordeum vulgare* ssp. *spontaneum*) and cultivated (*H. vulgare* ssp. *vulgare*) accessions has identified only a few sources of partial resistance to the disease, mostly in two-rowed barley (Huang et al. 2013). The authors collated a group of 78 accessions that possessed partial FHB resistance and haplotyped the group along with susceptible controls and other accessions. They reported that resistant QTL haplotypes on chromosomes 2H and 6H are rare in susceptible cultivars and accessions grown in the Upper Midwest region of the United States. Given the economic importance of FHB in barley, additional resistant haplotypes should be identified and incorporated into breeding programs. Currently, genome-wide association study (GWAS) is becoming a preferred statistical genetic tool to identify QTL underlying important agronomic traits, including disease resistance. The GWAS approach was recently employed in contemporary US barley breeding germplasm and identified QTL associated with FHB severity and DON concentration on all chromosomes except 7H (Massman et al. 2011).

Previously, Ethiopian barley accessions with a modest level of FHB resistance were identified (Dahl et al. 2009). With respect to the current research, diverse barley landraces, originally collected from both Ethiopia and Eritrea, were assembled from various international genebanks. The germplasm set was evaluated for FHB, DON accumulation, and various agro-morphological traits. Additionally, the germplasm was genotyped with the barley iSelect SNP (single nucleotide polymorphism) chip that contains a total of 7,842 SNP markers. The specific objectives of the study were to: (1) assess phenotypic variation for FHB resistance and DON accumulation in the barley landraces; (2) identify loci conferring FHB resistance and low DON concentration in the germplasm using a GWAS approach; and (3) examine the association of QTL for FHB resistance and DON accumulation with various agro-morphological traits.

4.2 Materials and Methods

4.2.1 *Plant materials*

Two-hundred and ninety-eight Ethiopian and Eritrean barley landraces were used in this study. To incorporate as much genetic diversity as possible in the GWAS panel, we used the following criteria to select the landraces. More than 3,000 Ethiopian and Eritrean landraces are held by the US Department of Agriculture-Agricultural Research Service (USDA-ARS), National Small Grains Collection (NSGC) in Aberdeen, ID (USDA-ARS, NGRP 2009). Of the accessions in this list, a total of 87 landraces were already included in the barley core collection developed by the NSGC (USDA-ARS, NGRP 2000) and were therefore also included in our GWAS panel. The barley core collection was assembled to represent the genetic diversity found in the cultivated portion of the barley germplasm maintained by the NSGC in such a way that the final set consisted of 10% of the total collection. The logarithm of the number of accessions per country in the entire collection was used to determine the number included in the core collection on a per country basis (H. Bockelman, personal communication). Accessions for the core collection were then selected at random within each country.

To further enhance the panel diversity, additional accessions were included with special emphasis on landraces from geographically diverse regions with historically intensive barley cultivation. The number of accessions representing each region was selected based on the area of barley cultivation within the respective areas. When multiple accessions from a single site were available, the final accession(s) for the panel were arbitrarily selected. In the end, 132 additional accessions were selected from the NSGC along with the 87 from the core collection. These Ethiopian and Eritrean barley landraces were collected during various expeditions to the countries between the 1920s and 1970s (Qualset 1975). For example, Harry Harlan made one of the earliest collection trips to the country in 1923 (Harlan 1957). Seed of the NSGC landraces were provided courtesy of Harold E. Bockelman, the NSGC curator. Some of these sampled landraces also were included in previous studies on disease and insect resistance, feed quality, and endosperm texture variation (e.g., Bowman et al. 2001; Schaller et al. 1963; Turaspekov et al. 2008).

In addition to the NSGC holdings, additional accessions were arbitrarily selected from different genebanks whose expeditions collected germplasm in different regions and eras than those conducted by the USDA. In this regard, 63 and 17 additional Ethiopian/Eritrean landraces were obtained from the N. I. Vavilov Research Institute of Plant Industry (VIR) and the International Center for Agricultural Research in the Dry Areas (ICARDA), respectively, and included in the panel. Passport data (where available) and other details about the accessions are given in Appendix Table 3.1.

To obtain genetically pure seed stocks of the landraces, single plant selections were made from each accession and selfed twice in the greenhouse. Then, a large greenhouse increase was made to provide sufficient seed for all subsequent experiments. For the greenhouse increases, seven seeds were planted in plastic pots (13.3 cm × 13.3 cm × 10.2 cm, l × w × h) filled with a 50:50 mix of steam-sterilized native soil and Metro-Mix® 200 (Sun Gro Horticulture, Quincy, MI), a growing media containing vermiculite, sphagnum peat moss, perlite, dolomitic limestone, and a wetting agent. After planting, all pots were watered and fertilized with Osmocote® controlled-release 14-14-

14 (N:P:K) (Scott's Company, Marysville, OH) (1.4 g/pot) and Peters Dark Weather 15-0-15 fertilizer (Scott's Company) (ca. 40 g/liter at 1/16 dilution). Thereafter, plants were fertilized with a 20:20:20 water-soluble formulation (J.R. Peters, Inc., Allentown, PA) biweekly at the recommended rate. All plant grow-outs were done in a greenhouse within the Plant Growth Facility on the St. Paul campus of the University of Minnesota in 2010 and 2011. Plants were grown at 19-23°C with a 16 hr photoperiod supplemented by 400 W high-pressure sodium halide lamps emitting a minimum of 300 μmol photons $s^{-1}m^{-2}$. When plants reached physiological maturity, seeds were harvested, dried at 35°C in a forced air dryer for two days, threshed and cleaned.

4.2.2 *Sample preparation and DNA extraction*

Seeds from the third selfed generation (S_3) of each landrace were sown and grown in the greenhouse as described above. Two weeks after planting, leaf tissue was collected from the plants for subsequent DNA extraction. For this procedure, segments of tissue (~5 to 6.5 cm long) were cut from the second leaves of plants, folded twice, and carefully placed in a 1.0 ml polypropylene microtube (BioExpress, Kaysville, UT). The microtubes were kept on ice during sample collection. Then, the microtubes were frozen in liquid nitrogen and stored at −20°C until the samples were freeze-dried. For freeze-drying, the samples were removed from the freezer, the lids of the microtubes opened, and the tube rack securely covered with miracloth (EMD Millipore Corporation, Temecula, CA). Samples were kept on ice until they were freeze-dried in a general purpose freeze-dryer (Model 24DX48, Virtis Company, Gardiner, NY) according to the specifications of the manufacturer.

The lyophilized tissue was subjected to tissue lysis for total genomic DNA isolation. One 3 mm tungsten-carbide bead was added to microtubes containing the tissue samples and homogenized using a TissueLyser (Qiagen, Valencia, CA) for 90 seconds at 30 Hz. Then, the sample was centrifuged at 4000 rpm for 3 minutes followed by the addition of 300 μl of Buffer RLT (Qiagen). The microtubes were tightly sealed with new caps, and manually shaken back and forth in an upright position 20 times. Then, the

microtubes were vortexed upside down at full speed for 20 seconds and centrifuged at 4000 rpm for 5 minutes at room temperature. DNA extraction was completed using the high-throughput BioSprint 96 DNA-liquid handling workstation and the BioSprint 96 DNA Plant Kit (Qiagen) according to the manufacturer's instructions.

The BioSprint system uses magnetic particle technology for DNA purification. Purified DNA was eluted in 200 µl 0.1X TE Buffer at pH 8.0 (Ambion, Austin, Texas). As both DNA quality and quantity are critical for genotyping, both parameters were carefully assayed. DNA yield and purity were determined by measuring absorbance at 260 nm (A_{260}) and the ratio of absorbance at 260 nm and 280 nm (A_{260}/A_{280}), respectively. DNA concentration of each sample was then adjusted to 50 ng/µl with 0.1X TE Buffer. The quality of DNA was confirmed by running 20 µl of the normalized DNA on a 1% agarose gel. Twenty microliters of normalized DNA was shipped to the USDA-ARS Biosciences Research Laboratory (Fargo, ND) in PCR plates on ice for genotyping. Five microliters of the 50 ng/µl DNA stock sample was used to run the genotyping assay.

4.2.3 *SNP genotyping and genotype data analysis*

The Ethiopian/Eritrean landrace samples were genotyped with the barley iSelect SNP chip of the expanded barley SNP marker platform using the Illumina Infinium II assay first described by Steemers et al. (2006). This assay is a whole-genome genotyping protocol with a single-base extension. Custom iSelect BeadChips can be multiplexed to assay from 3,072 to $1x10^6$ SNPs per sample. Each locus is assayed with an average 12-18x redundancy for each sample, enabling highly accurate genotype calls. The barley iSelect SNP chip contains a total of 7,842 SNPs that comprise the 2,832 existing barley oligonucleotide pooled assay (BOPA1 and BOPA2) SNPs discovered and mapped previously (Close et al. 2009; Muñoz-Amatriaín et al. 2011), plus 5,010 new ones developed from next generation sequencing data (Comadran et al. 2012). The mapping information for the barley iSelect SNPs is available from the Germinate 2.7.2 Teleport iSelect Database at the James Hutton Institute (JHI) (Dundee, Scotland)

(http://bioinf.scri.ac.uk). The Illumina Infinium genotyping assay was conducted under the direction of Shiaoman Chao.

The data generated by the Infinium assay were visualized and analyzed with the genotyping module of GenomeStudio data analysis software GSGT, version 1.8.4 (Illumina, San Diego, CA). The software parameters of GenCall score and no-call threshold were optimized. GenCall score is a quality metric calculated for each genotype and ranges from 0 to 1. The GenCall scores generally decrease in value the further a sample is from the center of the cluster to which the data point is associated. The no-call threshold is the lower boundary for calling genotypes relative to its associated cluster. To obtain the highest genotyping accuracy possible, a GenCall score cutoff (no-call threshold) of 0.15 was used. This means that genotypes with a GenCall score less than 0.15 were not assigned genotypes because they were considered to be too far from the cluster centroid to make reliable genotype calls. The analysis was started with a preliminary sample quality evaluation to determine which samples may require reprocessing or removal. Each SNP was analyzed independently to identify genotypes for the entire set of samples. Visual inspections for quality control were made, and the clusters manually adjusted for SNPs with well-separated clusters. SNPs not clustering as expected and/or SNPs with low call rates were systematically excluded from the dataset prior to calling genotypes again. To improve call rate and accuracy, custom cluster profiles were generated for SNPs that did not fit well into the standard cluster positions. SNPs with overlapping clusters were manually zeroed. Those that could be scored for the majority of samples and with a 95% call frequency were retained. After all the quality evaluations, genotype calls were generated using the GenCall algorithm (version 6.3.0) implemented in the GenomeStudio software.

4.2.4 *Phenotypic evaluation of landraces for FHB reaction*

FHB evaluations were conducted at the Northwest Research and Outreach Center in Crookston, MN during the summers of 2011 and 2012. The experiment was organized in a randomized complete block design with two replications in both years of the study.

The nursery was planted using a four-row planter with the test entries sown in short ~180 cm long rows (25-35 seeds/row) spaced ~30 cm apart. Accessions were planted in one row plots in 2011 and in two row plots in 2012. Multiple replicates of the following controls were included in the nursery to monitor disease development and pathogen virulence: 1) the early, highly susceptible six-rowed accession PI 383933; 2) the mid-season, six-rowed susceptible cultivar Stander (PI 564743) and mid-season, two-rowed susceptible line ICB 111809; and 3) the later maturing six-rowed partially resistant accession Chevron (PI 38061) and later maturing two-rowed partially resistant accession CIho 4196.

A mixture of 19 isolates of *F. graminearum* collected from the Red River Valley area of northwest Minnesota was used for inoculum production. Eleven of the isolates were collected in 1998, and eight were collected in 2006. Long-term storage of isolates was made on silica gel crystals stored at 4°C. The isolate mixture is routinely used to screen breeding materials for FHB resistance and represents a portion of the genetic variation existing in northwestern Minnesota (G. Thompson, personal communication).

Inoculum production and inoculations were made using the grain spawn method according to Horsley et al. (2006) with slight modifications. Whole kernel maize (*Zea mays*) seed was placed in stainless steel pans and covered with water overnight. The next morning, excess water was drained, the pans covered with aluminum foil, and the maize seed autoclaved twice for 20 min at 121°C to sterilize the grain substrate. When the maize seed cooled, usually the following morning, each pan was inoculated with pieces of Potato Dextrose Agar (PDA) colonized by one isolate of *F. graminearum*. These isolates were initially grown by placing silica gel crystals with adsorbed conidia onto PDA plates about one week before inoculation of the sterilized maize grain. The fungal isolates were allowed to colonize the grain by incubating at 25°C for 14 d in complete darkness. After this treatment, the pans of infested grain were transferred to burlap bags and dried at 32°C. When dry, the infested grain spawn inoculum was stored at room temperature until needed.

When the majority of accessions were at the five-leaf stage of development, the grain spawn was scattered over the barley plots at a rate of 5.6 g colonized grain m^{-2} (5.21 g $plot^{-1}$). The inoculum was applied with a fertilizer spreader mounted to an all-terrain vehicle. To enhance ascospore liberation and disease development, the plots were irrigated beginning the day of inoculum dispersal and continuing until the last disease evaluation dates. Irrigation was provided daily for 10 min every 70 min from 1630 to 1100 h by misting from overhead sprinklers.

Disease severity (percentage of florets infected) was assessed when the accessions were at the soft-dough stage of development. All of the spikes within a plot were considered in aggregate for the disease assays, and the percentage of florets displaying symptoms were visually estimated on a 1 (most resistant) to 9 (most susceptible) scale in comparison to the sets of standard controls. A disease rating of 1 denotes a severity ranging from about 0 to 5% with no to only a few florets infected per spike and 9 denotes a severity ranging from about 90 to 100% with nearly all or all florets infected per spike. Disease assessments were made twice during the growing season, three times for late maturing accessions. Terminal disease severity data recorded at the soft-dough stage of development were used in GWAS.

4.2.5 *Phenotypic evaluation of landraces for agro-morphological traits*

Agronomic and morphological traits may influence the amount of FHB severity observed on barley (Steffenson 1998). To determine this possible association, row-type morphology (RT), days to heading (DTH), plant height (PH), the number of nodes per cm of rachis in the spike (kernel density; KD), spike angle (SA), and exsertion length (EL) were assessed for all of the landraces. DTH was recorded as the interval after planting to the time when 50% of the plants in a row had at least 50% of the spike emerged from the boot. PH was measured as the distance from the ground to the tip of the spike, excluding awns. KD was obtained by dividing the number of nodes (attachment points of seeds) by the length of spike. Mean number of nodes was calculated by counting the number of seeds in one row of the spike and multiplying by the row type of the landrace (i.e., 2 or 6)

on three randomly selected spikes. The length of the spike was the distance from the bottom of the lowest floret to the top of highest floret of a spike, excluding the awns. Spike angle (SA) was estimated at maturity on a scale of 1 to 3 where spikes bending less than 45° from vertical were scored as 1; those bending from about 45 to 90° from vertical scored as 2; and those bending greater than 90° from vertical scored as 3. Exsertion (or peduncle) length (EL) was rated as the length from the flag leaf auricle to the bottom (i.e. start) of the spike. In instances where the spikes did not completely emerge outside the boot, EL was recorded as a negative value. When spikes emerged just above the flag leaf auricle, a value of zero was assigned for the EL.

Each accession was harvested at maturity. The samples were then dried in an oven at 35°C for two days before threshing and cleaning the seed. DON concentration of the grain samples was analyzed using gas chromatography and mass spectrometry (GC-MS) (Fuentes et al. 2005; Mirocha et al. 1998) at the Mycotoxin Diagnostic Laboratory of the University of Minnesota. DON analysis was done for only one replication in the 2011 trial and both replications in 2012 trial. All marker and phenotypic data used in these studies are available at http://triticeaetoolbox.org/barley/.

4.2.6 *Statistical analysis of phenotypic traits*

Unbiased mean estimates for each trait and an analysis of variance were generated through the restricted maximum likelihood (REML) method in SAS (SAS Institute 2008). REML is commonly used to generate estimates of variance and covariance parameters for fitting linear mixed models (Meyer and Kirkpatrick, 2005), a model used in association mapping. Heritability was estimated based on entry means using the formula $h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_e^2/r)$, or $h^2 = \sigma_g^2 / [(\sigma_g^2) + (\sigma_y^2/y) + (\sigma_e^2/ry)]$, where σ_g^2 and σ_e^2 are the genotypic and error variance, respectively, r is the number of replications, and y is the number of years the trial was conducted. Pearson correlation coefficients also were calculated to assess the phenotypic correlation among traits.

4.2.7 *Population structure*

Population structure can be present in germplasm panels assembled from diverse geographic regions and during different eras. To account for this effect and decrease the number of false-positives in association mapping analyses, the most likely number of subpopulations in the panel was investigated. The clustering program STRUCTURE (version 2.3.4) (Falush et al. 2003; Pritchard et al. 2000a; Pritchard et al. 2000b) applies a parametric genetic mixture analysis and was used to estimate the membership probability of each barley landrace to a number of hypothetical subpopulations (*K*). STRUCTURE software employs a Bayesian model-based clustering method that infers genetic clusters (subpopulations) of individuals on the basis of their genotypes at multiple loci using a Markov Chain Monte Carlo (MCMC) maximum-likelihood approach that compares posterior probabilities of different numbers of possible population configurations. To avoid overestimation of subpopulation divergence caused by tightly linked SNP markers (Falush et al. 2003), a subset of markers with approximately 10 cM spacing was selected for the analysis. This SNP subset was used to calculate the subpopulation membership matrix (Q), which is the fractional subpopulation membership for each landrace based on its inferred ancestry. There were no *a priori* population designations assumed for the landraces. The program was run by setting most parameters to default values according to the instructions in the user's manual (Pritchard et al. 2010).

Subpopulation numbers ranging from 1 to 20 were tested using the admixture model and considering allele frequencies of SNPs correlated among populations. The correlated allele frequency setting is considered favorable when there is subtle population structure (Falush et al. 2003). The program was first run using a burn-in period of 10,000 cycles followed by 50,000 MCMC iterations with 20 independent runs at each of K=1 to 20 (Falush et al. 2003; Pritchard et al. 2000a). Posterior probability $\Pr(X|K)$ of each K was generated by the program and $\ln\Pr(X|K)$ plotted against each value of K. The optimal K was estimated based on the value and variation of $\Pr(X|K)$ (Rosenberg et al. 2001) as well as the rate of $\ln\Pr(X|K)$ change from K-1 to K (ΔK). The ad hoc quantity (ΔK) was calculated based on the second order rate of change of the likelihood function with respect to K (Evanno et al. 2005). ΔK usually shows a peak at the true value of K. The program was then run using a burn-in period of 20,000 cycles followed by 100,000

MCMC iterations with 20 independent runs at each of K=3 to 10. The optimal K value was identified based on the value and variation of Pr(X|K) and ΔK. Then, value tables for matrix Q, allele-frequency divergence among presumed supopulations and average distances (expected heterozygosity) between individuals in the same cluster were generated using the determined optimum K value.

Principal component analysis (PCA), a multivariate analysis method that transforms a set of correlated variables (genetic markers in this case) into a smaller number of uncorrelated variables, also was used to infer population structure (Zhao et al. 2007). PCA analysis was conducted using the software program TASSEL (version 4.0) (http://www.maizegenetics.net; Bradbury et al. 2007). To satisfy the PCA requirement that all variables should have variation and no missing values, SNP markers with minor allele frequency (MAF) $\leq$0.05 were eliminated and missing values were imputed. A matrix with principal components (PCs) that represent population structure and eigenvalues (the variance explained by each of the PCs) were generated. Population stratification was visualized by plotting the first three PCs.

4.2.8 *Linkage disequilibrium*

LD between markers was measured using Haploview v4.2 (Barrett et al. 2005). Using large number of markers affects association mapping due to correcting for multiple hypothesis testing. Applying fewer markers may better enable detection of statistically significant markers associated with a trait. Thus, to reduce the number of markers by selecting independent sets of SNPs for running association mapping, markers with an r^2 $\geq$0.8 were considered to be dependent with each other. The SNP tagger in Haploview was used to test for independence of markers to select those with an r^2 <0.8. In cases where multiple SNP markers mapping to the same location or region were found significantly associated with a trait, LD, as measured by r^2 among the neighboring markers, was calculated and presented as a "heat" map.

4.2.9 *Association mapping*

A mixed linear model (MLM), with 'efficient mixed model association' (EMMA) method (Kang et al. 2008) implemented in the GWAS package *rrBLUP* (version 4.1) in software R (version 2.15.2), was used for association analysis between SNP markers and FHB severity, DON concentration, and associated agro-morphological traits in Ethiopian and Eritrean barley landraces. The mixed model equation used by the *rrBLUP* package is based on the mixed model (Yu et al. 2005): $y = X\beta+u+e$, where y is a vector of phenotypic observation; X is a vector of SNP marker genotypes; β is a coefficient of the SNP marker effect being estimated; u is a vector of random (polygene background) effects (effect of individual relatedness estimated as pairwise kinship coefficients); and e is a vector of residual effects. In the MLM, marker effect (Xβ) represents fixed effect, and relative kinship and residuals represent random effects. Variances of the random effects are given as: $Var(u) = 2KV_g$ and $Var(e) = V_R$, where *K* is an *n* x *n* matrix of kinship inferred from genotypes based on the proportion of shared allele values between a pair of individuals (*n*: number of individuals in the association mapping panel), V_g is the genetic variance, and V_R is the residual variance. The *rrBLUP* package in R for GWAS incorporates methods for obtaining best linear unbiased estimates (BLUE) of β (fixed effect) and best linear unbiased predictions (BLUP) of u (random effects) as originally developed (Kang et al. 2008; Yu et al. 2005). The EMMA association analysis method then fitted each SNP into the MLM individually and generated marker scores [$-\log_{10}$(*P*-values)].

The *rrBLUP* GWAS function incorporates a package known as QVALUE (Storey and Tibshirani 2003) for multiple testing using the Benjamini-Hochberg False Discovery Rate (BH-FDR) (Benjamini and Hochberg 1995) and handles permutations of *q*-value (an FDR adjusted *P*-value) for each test internally. At the end of the analysis, the package draws a "bar graph" as a GWAS result with the $-\log_{10}$(*P*-values) and a threshold line (FDR=*q*-value=0.05) delineating significant markers. Then, the FDR adjusted marker scores were outputted from which *P*-values were calculated.

GWAS was first conducted with the phenotype data of individual years separately, designated as Ethiopian and Eritrean Barley Collection EEBC-11 (for 2011),

EEBC-12 (for 2012), and then with the combined overall entry means of two years, designated as EEBC-C (2011 and 2012), to identify QTL for FHB severity, DON concentration and associated agro-morphological traits. Next, GWAS for FHB severity and DON concentration was performed with the phenotype data of two-rowed (2R: 110 landraces) and six-rowed germplasm (6R: 180 landraces) separately for each year (designated as EEBC-2R-11, EEBC-2R-12, EEBC-6R-11 and EEBC-6R-12), and mean of the two years combined (EEBC-2R-C and EEBC-6R-C) to reveal possible QTL confounded with row type and also to assess reproducibility of a given QTL region.

4.3 Results

4.3.1 *Phenotypic variation for FHB reaction and correlated traits*

Infection in the FHB nurseries was high and uniform in both years of the study, allowing for the easy differentiation of materials with varying levels of resistance. The infection level in 2011 was slightly higher in variation and amount than in 2012. The highly susceptible and susceptible six-rowed accessions of PI 383933 and Stander had mean FHB severities (1-9 scale) ranging from 7.67±0.47 (2012) to 8.45±0.50 (2011) and from 5.64±0.83 (2011) to 6.18±0.83 (2012), respectively (Figure 4.1A). Mean FHB severity of the two-rowed susceptible line ICB 111809 was lower than the six-rowed susceptible controls and ranged from 4.00±0.82 in 2012 to 5.18±0.72 in 2011. The partially resistant six-rowed and two-rowed barley accessions of Chevron and CIho 4196 had mean FHB severities ranging from 1.73±0.62 in 2011 to 2.00±0.43 in 2012 and from 2.09±0.51 in 2011 to 3.22±0.79 in 2012, respectively. The EEBC germplasm exhibited a higher mean and level of variation for FHB severity in 2011 (4.23±1.68) compared to 2012 (4.02±1.12), which was consistent with the disease severity of most controls (Figure 4.1A).

The controls and EEBC accessions exhibited marked differences in DON concentration between the two years of the study, with most having lower DON levels in 2012 (Figure 4.1B). The highly susceptible and susceptible six-rowed accessions of PI 383933 and Stander had a mean DON concentration ranging from 16.66±7.27 ppm

(2012) to 21.58±3.88 ppm (2011) and from 13.98±1.58 ppm (2012) to 47.23±9.46 ppm (2011), respectively (Figure 4.1B). Mean DON concentration of the two-rowed susceptible line ICB 111809 ranged from 3.84±2.35 ppm in 2012 to 29.19±6.00 ppm in 2011 (Figure 4.1B). The partially resistant six-rowed and two-rowed barley accessions of Chevron and CIho 4196 had mean DON concentrations ranging from 2.19±1.01 ppm (2012) to 9.42±6.81 ppm (2011) and from 2.37±1.11 ppm (2012) to 10.51±7.07 ppm (2011), respectively. EEBC germplasm had a mean DON concentration ranging from 11.62±8.14 ppm in 2012 to 39.20±24.42 ppm in 2011 (Figure 4.1B). In 2012, warm weather hastened senescence of the germplasm and may have contributed to the lower DON concentrations observed.

Two-rowed barleys usually sustain lower infection levels of FHB than six-rowed barleys. To assess whether this same trend occurs in the EEBC germplasm, the 110 two-rowed accessions were analyzed separately from the 180 six-rowed accessions. As expected, two-rowed accessions had lower mean FHB severities (3.40±1.16 and 3.47±0.94 for 2011 and 2012, respectively) than six-rowed accessions (4.77±10.74 and 4.37±1.07 for 2011 and 2012, respectively) in both years (Figure 4.1C). The same trend was observed for mean DON concentration with two-rowed types having 27.47±23.34 ppm and 7.47±5.47 ppm and six-rowed types having 46.59±22.32 ppm and 14.24±8.48 ppm for 2011 and 2012, respectively (Figure 4.1D). Heritability, defined here as the reproducibility of the observed phenotype in germplasm across experiments, was estimated based on entry means and was 0.99 for FHB severity and 0.29 for DON concentration (Table 4.1). Heritability of 0.99 for FHB severity is unusally high. This was perhaps because disease was scored on a scale of 1 to 9, taking an aggregate of spikes into account, instead of calculating the percentage of infected spikes that are arbitrarily selected for disease assessment—a commonly used method.

Agro-morphological traits (DTH, PH, KD, SA and EL) previously reported to possibly impact the level of FHB in barley also were investigated in the field. The traits showed a low to moderate degree of variation in the EEBC germplasm (Figure 4.1E). The germplasm had a mean DTH ranging from 49.81±6.91 days in 2012 to 58.31±7.74 days

in 2011 (Figure 4.1E). Mean PH of the test entries ranged from 82.27±11.02 cm in 2011 to 87.57±13.12 cm in 2012 (Figure 4.1E). Mean KD ranged from 5.58±2.64 nodes/cm of rachis in 2011 to 5.78±2.76 nodes per cm of rachis in 2012 (Figure 4.1E). The means for SA and EL were 1.51±0.68 and 2.25±7.11 cm, respectively (Figure 4.1E). Heritability estimates based on entry means were 0.71 for DTH, 0.93 for PH, 0.98 for KD, 1.00 for SA, and 0.67 for EL (Table 4.1).

4.3.2 *Correlation of FHB, DON and associated agro-morphological traits*

Correlation analysis was conducted to determine the degree of association between the disease and toxin phenotypes (FHB and DON) and various agro-morphological traits (RT, DTH, PH, KD, SA and EL). In 2011 (when all traits except SA were measured), FHB severity and DON concentration were significantly positively correlated (r=0.30) (Table 4.2). RT and KD also were significantly positively correlated with FHB severity (r=0.41 and 0.50, respectively), whereas DTH and PH were significantly negatively correlated with FHB severity (r=-0.41 and -0.30, respectively). RT, DTH and KD were significantly positively correlated with DON concentration (r=0.38, 0.19 and 0.36, respectively), whereas EL was significantly negatively correlated (r=-0.17) with DON. The correlation between PH and DON concentration was not significant (r=-0.01) (Table 4.2). Correlation analysis for FHB severity, DON concentration, DTH, PH and KD between the 2011 and 2012 datasets revealed a number of significantly positive correlations (Table 4.3). DTH and DON concentration were not significantly correlated in 2012 (r=0.01). Likewise, correlation between PH and DON concentration in 2012 was weak and non-significant (r=-0.15) (Table 4.3).

4.3.3 *Marker data*

The platform used for genotyping the GWAS panel consisted of 7,842 SNPs of the barley iSelect SNP chip. Of this set, 2,832 SNPs were previously mapped (Close et al. 2009; Muñoz-Amatriaín et al. 2011). The remaining 5,010 SNPs were newly developed at the James Hutton Institute (JHI) (Dundee, Scotland). After quality evaluations during genotype calling, 85% (6,702/7,842) of the SNPs generated reliable allele data with a

95% call rate for all accessions. Nearly 99% of these SNPs (6,615/6,702) had a >95% call frequency. Filtering based on minor allele-frequency (MAF) resulted in 5,269 SNPs with a MAF >5%, which were subsequently used for association analyses. Of these, 72.46% (3,818/5,269) have known map positions on the seven barley chromosomes spanning 1,112.71 cM with the remaining set (1,451 SNPs) yet to be mapped. The consensus map of the SNPs used was generated by merging the map from JHI with the 11 linkage maps from the Barley Coordinated Agricultural Project (Barley CAP) (J. Endelman, personal communication). The vast majority of markers with unknown map positions were among the newly developed markers and had SCRI (Scottish Crop Research Institute; the former name of the JHI) designations. Both mapped and unmapped markers were used for running GWAS. Of the mapped SNPs, 38.6% (1,473/3,818) had unique map positions and the rest had redundant map locations. The average density of SNP markers across the genome using all mapped SNPs and unique SNPs was 3.43 and 1.32 SNP markers per cM, respectively. Gaps between SNP markers ranged from 0 to 9.32 cM with a mean of 0.77 cM. Only eight gaps of >5 cM were identified: two each on chromosomes 1H and 7H, and one each on chromosomes 2H, 3H, 5H and 6H.

4.3.4 *Population structure*

STRUCTURE and PCA were implemented for population structure analysis. From the STRUCTURE analysis, six subpopulations were delineated. However, no clear clustering of two- vs. six-rowed groups was obtained as is often found in advanced breeding germplasm (citation). Two-rowed landraces were grouped into subpopulations 1, 2, 4 and 5, whereas six-rowed ones were grouped into subpopulations 1, 3 and 6. Moreover, no distinct clustering was found for accessions from the different countries of Ethiopia (269 accessions) and Eritrea (29 accessions). Accessions from the ICARDA genebank were mainly grouped into subpopulations 3 and 4, and those from the VIR genebank were mainly clustered in subpopulations 1, 3, 4 and 6. Subpopulations 1, 2, 5 and 6 were mainly composed of landraces from the NSGC genebank. Subpopulations 3 and 6 were the most genetically heterogeneous as revealed by the highest expected heterozygosity values between individuals in clusters (see chapter 3, Figure 3.2B). Based

on the PCA analysis, there was no readily apparent population structure differentiating the germplasm. The only apparent clustering in the PCA plots was for the landraces that were obtained from the NSGC genebank. Thus, there is a lack of apparent population structure in the association mapping panel. Therefore, the possible presence of any sample structure was corrected for using the pair-wise relatedness between individuals through application of the kinship (K) model.

4.3.5 *Genome-wide association mapping of FHB resistance, DON concentration and agro-morphological traits*

GWAS was conducted for FHB severity, DON concentration and related agro-morphological traits. To assess reproducibility of any given QTL region, GWAS for all traits was conducted with phenotype data for an individual year separately and also the overall mean of two years. In addition, GWAS for FHB severity and DON concentration was run for two-rowed and six-rowed germplasm separately for each year and the overall mean of two years. Significant associations were automatically declared by the GWAS package. After correction for multiple testing, markers with a *q*-value (an FDR adjusted *P*-value) <0.05 were considered as truly significant associations.

To further reduce possible false positives and highlight truly significant associations, only markers found significantly associated with FHB severity and/or DON concentration in two or more datasets of the mapping panel are reported. Significant associations in only two-rowed or six-rowed germplasm sets are included as supplementary information. Except for the RT and KD traits, none of the agro-morphological traits had coincident QTL with FHB severity or DON concentration. Thus, SNP markers significantly associated with DTH, PH, and EL are only presented as supplementary information in the appendix.

4.3.6.1 *Genome-wide association mapping of barley spike morphology*

As a validation of the GWAS model for reliability of localizing true marker-trait associations and evaluating mapping resolution within the germplasm panel, GWAS was

conducted on the spike morphology trait of row type. Barley is classified into two-rowed and six-rowed types, which is a result of having either two or six rows of florets being fertile. This trait is mainly controlled by the well-characterized Mendelian gene *Vrs1*, located on chromosome 2H, as well as other genes. Alleles at the *Vrs1* locus and some of the other spike morphology genes have been cloned (Komatsuda et al. 2007; Pourkheirandish and Komatsuda 2007; Ramsay et al. 2011; Waugh et al. 2009). The GWAS model identified 14 SNP markers significantly associated with the row-type locus: eight on the long arm of chromosome 2H, one each on chromosomes 4H and 5H, and four unmapped markers (Table 4.4; Figure 4.2A). BLAST search of the NCBI database with the significant SNPs on chromosome 2H identified the coding sequences of the *H. vulgare* homeodomain-leucine zipper protein (*Vrs1*) gene with ≥99% identity, indicating that the SNP markers are likely located within the coding region of the gene. The significant SNPs on chromosome 2HL were mapped within a short distance (4.03 cM) of each other (Table 4.4) and are most likely detecting the same *Vrs1* locus. LD as measured by r^2 ranged from 0.004 (between SCRI_RS_171032 and 12_30897) to 0.99 (between 12_30897 and SCRI_RS_196853 (Figure 4.2B). LD between the most significant marker (12_30896) and the rest of the markers ranged from 0.10 (with SCRI_RS_221886) to 0.88 (with 11_20340), indicating that some of the markers are in high LD with the underlying gene controlling spike morphology.

The significant marker on 4H maps to the reported position of INTERMEDIUM-C (*Int-c*) gene (Cuesta-Marcos et al. 2010; Lundqvist and Franckowiack 1997; Komatsuda and Mano 2002) that influences both the fertility of the lateral spikelets on the inflorescence and the amount of the basal branching (tillering) in barley (Ramsay et al. 2011). The one significant marker on chromosome 5H is most likely detecting the six-rowed spike 2 (*vrs2*) or the intermedium spike-b (*int-b*) locus (Lundqvist and Franckowiack 1997). Some or all of the unmapped SNPs are likely located near or at *vrs1*, *vrs2*, *int-b*, *Int-c* or some other spike morphology genes described on chromosome 1H (Cuesta-Marcos et al. 2010). Our results are in close agreement with previous studies (Figure 4.2B). This GWAS analysis indicates the strong power of the association mapping model to account for pairwise relatedness and achieve good mapping resolution

within the germplasm panel. Accordingly, the association mapping model was used for marker-trait associations of other phenotypes.

4.3.6.2 *Genome-wide association mapping of Fusarium head blight severity*

Using the combined overall entry means of two years (EEBC-C), two SNP markers were found significantly associated with FHB severity (Table 4.4; Figure 4.3A; Appendix Table 4.1). The markers (11_20340 and 12_30896) were located on the long arm of chromosome 2H within a very narrow interval of 0.1 cM and were likely detecting the same QTL. Indeed, LD between the markers was very high (r^2=0.88) indicating that they were associated with the same QTL. The most significant marker was 11_20340 (Table 4.4; Figure 4.3A). QTL associated with SNP markers were named according to the following convention: *Rfg*-, indicating the pathogen abbreviation of Reaction to *Fusarium graminearum*; *qtl*-, indicating the quantitative nature of the trait; *2H*-, indicating the chromosome upon which the marker lies; and 11_20340, indicating the name of the most significant marker associated with the trait. Thus, the resistance QTL on chromosome 2HL was designated *Rfg-qtl-2H_11_20340* (90.99 cM). The two markers identified in the combined dataset also were significant in individual years (Table 4.4). An unmapped marker (SCRI_RS_72983) was also significant in the 2012 dataset (Table 4.4). The "bar graphs" of GWAS scans for marker associations across datasets for individual years are presented in Appendix Figure 4.1A and B.

4.3.6.3 *Genome-wide association mapping of deoxynivalenol concentration*

In the combined dataset (EEBC-C), six SNP markers were found significantly associated with DON concentration (Table 4.4; Figure 4.3B; Appendix Table 4.1). Three of the markers (SCRI_RS_171032, 12_30901 and 11_20340) were located on the long arm of chromosome 2H, one (SCRI_RS_148392) on the long arm of chromosome 4H, and the other two (SCRI_RS_165473 and SCRI_RS_72983) were unmapped (Table 4.4). The three markers on chromosome 2HL were located within a narrow genetic distance of 0.35 cM and were likely detecting the same QTL. With respect to LD, marker SCRI_RS_171032 was in high LD with the other two associated markers 12_30901 and

11_20340 (r^2=0.55 and 0.53, respectively). The other two significantly associated markers, 12_30901 and 11_20340, had the same exact map location, but the LD between them was realtively lower (r^2=0.39). The most significant SNP identified was SCRI_RS_171032, and thus the QTL was designated *DON-qtl-2H_SCRI_RS_171032* (90.64 cM). This QTL coincides with the *Rfg-qtl-2H_11_20340* described above for FHB severity and also the row-type locus *Vrs1*. The *DON-qtl-SCRI_RS_171032* QTL also was associated with DON concentration within the 2012 DON dataset (EEBC-12) as the same marker (11_20340) was significant in both the EEBC-C and EEBC-12 datasets (Table 4.4; Figure 4.3C). Another marker (12_30896) mapping 0.10 cM proximal to 11_20340 was also significant in the EEBC-12 dataset (Table 4.4) and was probably detecting the same QTL. Marker 12_30896 was in high LD with marker 11_20340 and 12_30901 (r^2=0.88 and 0.37, respectively). On the long arm of chromosome 4H, one marker (SCRI_RS_148392) was found significantly associated with DON concentration in the EEBC-C dataset (Table 4.4; Figure 4.3B). This marker, plus another one (SCRI_RS_157650) with the exact map location, also were found significant in the EEBC-12 dataset (Table 4.4; Figure 4.3B and C). LD between these markers was high (r^2=0.84), indicating that both were likely detecting the same DON QTL. Since SCRI_RS_148392 was the most significant marker identified, the QTL was designated as *DON-qtl-4H- SCRI_RS_148392* (65.52 cM). On the short arm of chromosome 4H, one marker (12_11485) was significantly associated with DON concentration in the EEBC-12 DON dataset (Table 4.4; Figure 4.3C), and the QTL associated with it was designated as *DON-qtl-4H-12_11485* (12.91 cM). Two other significantly associated markers (SCRI_RS_165473 and SCRI_RS_72983) in the EEBC-C dataset have not been assigned map locations (Table 4.4; Figure 4.3B). SCRI_RS_165473 was also significant in the 2011 DON dataset (EEBC-11) (Table 4.4). No other significant associations were detected in the EEBC-11 dataset (Appendix Figure 4.2A).

Row-type morphology is a prominent trait delineating barley accessions in population structure-based analyses. It also may influence the level of FHB—due either to the underlying gene(s) having a pleiotropic effect or close linkage to gene(s) controlling the reaction to the disease. Not surprisingly, the two markers on chromosome

2HL significantly associated with FHB resistance and DON accumulation also were the most significant markers associated with the row-type locus *Vrs1*. The SNP markers associated with *Rfg-qtl-2H_11_20340*/*DON-qtl-SCRI_RS_171032* (90.64-90.99 cM) was one of the most significant marker associated with the row-type trait (Table 4.4). The consistent significance of these markers strongly suggests that the QTL for FHB resistance and DON accumulation coincides with the spike-type locus *Vrs1* as reported in other studies. To further investigate the possible relationship of row-type with FHB severity and DON concentration, GWAS for FHB resistance and DON concentration were conducted separately for the two- and six-rowed germplasm datasets.

For FHB severity, three markers one each on chromosomes 1H, 4H and 7H, and three unmapped markers were detected significantly associated in the two-rowed germplasm datasets of the combined two-year data or the 2012 set (Appendix Table 4.1; Appendix Figure 4.1C and D). No significant markers were identified in the separate two-rowed lines of the 2011 FHB severity dataset or any datasets of the six-rowed lines (Appendix Table 4.1; Appendix Figure 4.1E to H). The 2HL markers (11_20340 and 12_30896) significantly associated with the row-type *Vrs1* locus and the unmapped marker (SCRI_RS_72983) significantly associated with FHB severity in the whole-germplasm set were not significant in the separate two- or six-rowed germplasm datasets (Table 4.4; Appendix Table 4.1). For DON concentration, the four SNP markers (SCRI_RS_171032 and 12_30901 on chromosome 2HL and the unmapped markers SCRI_RS_165473 and SCRI_RS_72983) found significantly associated with DON concentration in the EEBC-C DON dataset were significant in the separate two-rowed germplasm datasets (Table 4.4; Appendix Table 4.1). These markers were not significant in any dataset of the separate six-rowed lines (Table 4.4). On the other hand, marker 12_11485 on 4HS, identified initially in the EEBC-12 DON dataset, also was significant in the separate six-rowed germplasm DON datasets (Table 4.4; Figure 4.3D and E; Appendix Table 4.1). Similarly, the SNP marker on chromosome 4HL (SCRI_RS_148392) found significantly associated with DON concentration in the EEBC-C and EEBC-12 DON datasets was significant in the separate six-rowed germplasm datasets (Table 4.4; Figure 4.3D and E; Appendix Table 4.1). In addition, 43

markers belonging to fifteen different genomic locations and ten other unmapped markers were found significantly associated with DON concentration exclusively in the separate two- and/or six-rowed germplasm sets (Appendix Table 4.1). The description of results for these significant associations in the individual two-rowed or six-rowed germplasm sets are presented as supplementary information (Appendix Text 4.1).

4.3.6.4 *Genome-wide association mapping of correlated agro-morphological traits*

In addition to spike-type, FHB severity and DON concentration also may be influenced by other agro-morphological traits. Thus, GWAS was conducted on several additional agro-morphological traits to investigate whether QTL underlying correlated agro-morphological traits map at a coincident location with QTL for resistance to FHB and DON accumulation.

4.3.6.4.1 *Days to heading*

Three markers on chromosome 2HS and six markers of unknown map location were significantly associated with DTH in the EEBC-C and EEBC-11 datasets (Appendix Table 4.2; Appendix Figure 4.3A). None of these markers lie at a coincident location with QTL conferring lower FHB severity or DON concentration. The description of results for the SNP markers significantly associated with DTH is presented as supplementary information (Appendix Text 4.2).

4.3.6.4.2 *Plant height*

No significant marker associations were detected for PH in any of the datasets from the Crookston location (Appendix Table 4.2; Appendix Figure 4.4C). However, six markers were found significantly associated with PH at St. Paul: four on chromosome 1H, one on chromosome 5H, and one unmapped (Appendix Table 4.2; Appendix Figure 4.3B). None of these markers were associated with QTL identified for low FHB severity or DON concentration. The description of results for the SNP markers significantly associated with PH is presented as supplementary information (Appendix Text 4.2).

4.3.6.4.3 *Kernel density*

A dense arrangement of nodes (seeds) along the rachis may facilitate the spread of FHB in spikes, leading to higher FHB severities. To investigate whether QTL underlying KD coincide with QTL for FHB severity and DON concentration, GWAS was conducted for the trait. Based on the EEBC-C dataset, ten markers were found significantly associated with KD: eight (11_20781, SCRI_RS_171032, 11_20340, 12_30901, 12_30896, 12_30897, SCRI_RS_196853 and SCRI_RS_221886) on chromosome 2HL and two (SCRI_RS_165473 and SCRI_RS_72983) that were unmapped (Table 4.4; Figure 4.3H; Appendix Table 4.2; Appendix Figure 4.3C). Of the eight significant markers on chromosome 2HL, 12_30896 had the highest significance level; thus, the QTL was designated as *KD-qtl-2H-12_30896* (91.09 cM). Seven of the markers mapped within a 1.43 cM genetic interval and one (SCRI_RS_221886) 3.10 cM away; thus, it is likely that all eight markers are detecting the same QTL. In fact, the same eight markers were significantly associated with the spike morphology trait of *Vrs1* (for details, see section 4.3.6.1 above). This QTL (*KD-qtl-2H-12_30896*) maps to a coincident location with one(s) associated with row-type morphology, FHB severity and DON concentration (Table 4.4; Figure 4.2; Figure 4.3A, B and H). In fact, these four traits share four significantly associated markers (12_30901, 11_20340, SCRI_RS_165473 and SCRI_RS_72983) in common (Table 4.4). DON concentration, KD and row-type morphology shared an additional marker (SCRI_RS_171032) in common (Table 4.4).

4.3.6.4.4 *Spike angle*

Upright spikes may tend to hold water longer (especially in crevices) than those with a nodding profile, possibly leading to greater FHB infection. To assess whether any significant effect could be detected for this trait and if such identified QTL were coincident for those controlling FHB severity and DON, GWAS was conducted for SA. No significantly associated markers were detected in any dataset for SA (data not shown).

4.3.6.4.5 *Exsertion length*

The extent to which the spike extends above the flag leaf sheath may influence FHB development as the leaf sheath retains moisture around the non-emerged portions of the spike, thereby facilitating greater infection. Thus, GWAS was conducted for EL. A marker of unknown map position was significantly associated with EL (Appendix Table 4.2). This marker was not significantly associated with any of the other traits.

4.3.6.5 *Allele effect of significant markers on trait*

The allele frequency and allelic effect (trait means) of each QTL identified in the combined dataset or two different sub-sets of data in reducing the levels of FHB severity and DON accumulation were estimated (Table 4.5). The positive allele "A" of the FHB QTL *Rfg-qtl-2H_11_20340* associated with both disease severity and DON concentration gave a mean disease severity of 2.21 in two-rowed landraces. The corresponding "B" allele gave a mean disease severity of 4.76 in the six-rowed germplasm set. However, the most common allele "B" of this QTL resulted in a mean DON concentration of 16.93 ppm and 28.70 ppm in two-rowed and six-rowed accessions, respectively (Table 4.5). In addition to 11_20340, another SNP marker (12_30901) with the same map position was significantly associated with DON detecting the QTL *Rfg-qtl-2H_11_20340*. The "A" allele of 12_30901 is associated with low disease severity and DON concentration unlike the "A" allele of 11_20340, which was associated with higher values of these traits (Table 4.5). Ninety-seven percent (56/58) of accessions with allele "A", and 23% (53/232) of accessions with allele "B" for 12_30901 were two-rowed. Seventy-seven percent (179/232) of accessions with allele "B" at this marker were six-rowed, i.e., 99% (179/180) of the six-rowed accessions had allele "B" and had higher FHB severity and DON concentration (Table 4.5). This suggests that SNP markers with the same map position that were significantly associated with DON may have alleles with negative correlation as to their effect on the expression of the trait. In addition to the major QTL on chromosome 2HL, QTL for DON concentration also were detected on the short arm of chromosome 2H (*DON-qtl-2H-12_10777*), short (*DON-qtl-4H-12_11485*) and long arms of chromosome 4H (*DON-qtl-4H-SCRI_RS_148392*), and the long arms of chromosomes 5H (*DON-qtl-5H-11_21001*) and 7H (*DON-qtl-7H-11_20824*) (Table 4.5). These QTL

have alleles with variable effects on FHB severity and DON concentration (Table 4.5). Of these, *DON-qtl-4H- SCRI_RS_148392* was the only QTL identified in the entire germplasm set. Two-rowed accessions with the more frequent positive allele "B" at *DON-qtl-4H-SCRI_RS_148392* exhibited the lowest mean disease severity and DON concentration of 3.27 and 16.14 ppm, respectively (Table 4.5).

4.4 Discussion

FHB is a devastating disease of barley causing significant yield and quality losses in many parts of the world. In this study, a diverse panel of Ethiopian and Eritrean barley landrace germplasm was used for whole-genome association mapping to identify QTL associated with FHB resistance, DON accumulation and related agro-morphological traits. Key elements for successful GWAS include high diversity in the association mapping panel, robust quality phenotypic data, and full accounting for population structure (Myles et al. 2009). Except for DON, the traits had heritability values of 0.67 and higher. The germplasm exhibited a higher level of variation for FHB severity and DON concentration, more so for the latter, in 2011 than in 2012 (Figure 4.1A and B). The explanation for these differences lie in the very nature of the FHB disease itself. The environment (mainly moisture and temperature) and associated developmental conditions such as heading time can markedly influence FHB development. In 2011, rainfall was about 15% higher than in 2012 and therefore likely favored additional infection periods for disease development (Tekauz et al. 2000). The two seasons had very similar mean, minimum and maximum temperatures during the critical growth stages (i.e. heading to late milk) of the germplasm, suggesting that temperature was not a major factor contributing to the differences in disease levels. The time of heading can influence the level of FHB and consequently DON concentration in barley (Urrea et al. 2002). DTH was about seven days earlier in 2012 than in 2011 (Figure 4.1E).

The two-rowed and six-rowed EEBC germplasm sets exhibited differences in FHB severity and DON accumulation in both years of the study with the former having lower FHB severity and DON concentration than the latter (Figure 4.1C, D). The

differences were very obvious in 2012 for disease severity and in both years for DON. Spike density may have influenced initial infection and subsequent DON accumulation in this study. Two-rowed EEBC genotypes had longer, more lax spikes. This architecture might alter the spike micro-environment making it less favorable for for fungal infection and growth and/or reduce the chances for lateral kernel to kernel spread. Alternatively, two-rowed germplasm may have a level of basal FHB resistance not present in six-rowed germplasm. This is in accordance with previous studies showing that lower FHB levels are usually found in two-rowed accessions (Choo et al. 2004; Steffenson et al. 1996; Zhou 1991).

Using GWAS with a widely used statistical model that successfully accounts for population structure (Kang et al. 2008), a QTL on the long arm of chromosome 2H (*Rfg-qtl-2H_11_20340*) was identified for FHB severity and DON accumulation in Ethiopian and Eritrean landrace germplasm (Table 4.5). This QTL likely coincides with a common FHB resistance QTL detected in bi-parental mapping studies (de la Pena et al. 1999; Hori et al. 2006; Lamb et al. 2009; Ma et al. 2000; Sato et al. 2008), which lies in the centromeric region of chromosome 2H (bins 8-10) based on the comparative Steptoe/Morex bin map (http://barleygenomics.wsu.edu) and the consensus map of SNP markers used in this study (J. Endelman, unpublished). In the EEBC, the *Rfg-qtl-2H_11_20340* QTL was detected consistently across years for FHB severity and in the overall mean of two years for DON, indicating that it has a sound genetic basis and is less affected by the environment. This QTL also was significantly associated with row-type, detecting the *Vrs1* allele controlling spike row-type. The two-rowed spike type governed by the *Vrs1* allele is generally less conducive to disease development and likely contributed to the association of FHB resistance in this region of chromosome 2H (Huang et al. 2013).

In the current study, the *Rfg-qtl-2H_11_20340* QTL shared the same common SNP markers detecting the *Vrs1* locus, suggesting the possibility of linkage or pleiotropy. Several mapping studies found that the two-rowed (i.e. *Vrs1*) trait coincided with QTL conferring FHB resistance and DON concentration (de La Pena et al. 1999; Mesfin et al.

2003; Zhu et al. 1999). It remains to be resolved whether this co-localization of the *Vrs1* locus and QTL for FHB and DON levels is due to linkage or perhaps pleiotropy of the *Vrs1* locus. Some bi-parental studies have suggested close linkage between *Vrs1* and FHB resistance QTL on chromosome 2H bin10 (Horsley et al. 2006; Mesfin et al. 2003). On the contrary, other studies have suggested a possible pleiotropic effect of *Vrs1* on FHB resistance QTL in this region as detected in two-rowed/six-rowed crosses (Sato et al. 2008). Given the presumably high resolution of GWAS, it is possible that the common FHB and DON QTL detected in this region in the EEBC germplasm is a pleiotropic effect of the *Vrs1* locus. The fact that sequences of some of the SNP markers significantly associated with FHB and DON concentration were identified from within the coding region of the *Vrs1* locus with high sequence identity asserts this hypothesis.

The *Rfg-qtl-2H_11_20340* QTL detected in EEBC germplasm may still represent a unique allele for FHB resistance. In previous studies, FHB resistance QTL at the centromeric region of chromosome 2HL were identified using bi-parental mapping of barley accessions from other countries. To resolve whether the identified QTL are novel or not, additional genetic tests should be conducted. In a recent study (Huang et al. 2013), the haplotype diversity of multiple barley accessions with partial resistance was studied and distinct haplotypes at the FHB resistance QTL on chromosome 2H bin 8 and 10, were reported. However, only two barley accessions from Ethiopia (not among the EEBC accessions used in this study) were included in the analysis. Similar analyses should be conducted in the future with some of the germplasm used in this study to identify the haplotype structure of this candidate region in Ethiopian and Eritrean barley landraces. This may provide valuable information as to whether such germplasm contains FHB resistance haplotypes different from other resistance sources.

The FHB QTL *Rfg-qtl-2H_11_20340* was detected by two SNP markers (11_20340 and 12_30896) in the combined dataset, but not in the separate two- or six-rowed datasets. However, the AA-AA "haplotype" for the two markers was predominately found in six-rowed accessions. Ninety-three percent (168/180) of six-rowed accessions had the AA-AA haplotype at these markers. On the other hand, 94%

(103/110) of the two-rowed accessions had the BB-BB haplotype. The ‘‘A’’ allele was associated with generally higher levels of FHB and DON, whereas the “B” allele was associated with generally lower levels (Table 4.5). In addition to 11_20340, another SNP marker (12_30901) with the same map position was significantly associated with DON concentration detecting the QTL *Rfg-qtl-2H_11_20340*. The positive allele “A” of the 12_30901 marker had a low frequency and provided a mean reduction in disease severity and DON concentration of 21.14 and 46.80%, respectively, compared to accessions without the allele in the entire germplasm panel (Table 4.5).

In addition to the most common QTL on chromosome 2HL, QTL for DON concentration also were detected on the short arm of chromosome 2H (*DON-qtl-2H-12_10777*), short (*DON-qtl-4H-12_11485*) and long arms of chromosome 4H (*DON-qtl-4H-SCRI_RS_148392*), and the long arms of chromosomes 5H (*DON-qtl-5H-11_21001*) and 7H (*DON-qtl-7H-11_20824*) (Table 4.5). These QTL have alleles with variable effects on FHB severity and DON concentration (Table 4.5). Except for *DON-qtl-4H-SCRI_RS_148392*, the remaining QTL were identified in a limited germplasm set, and no QTL for FHB severity were identified in these regions. This indicates that these QTL may have a minor effect in DON accumulation and are probably affected by the environment.

The *DON-qtl-4H-SCRI_RS_148392* QTL detected on chromosome 4HL mapped approximately to bin 7 where FHB resistance QTL were previously identified (Hori et al. 2006; Sato et al. 2008). This suggests that this QTL is possibly the same or an allele of these QTL. *DON-qtl-4H-SCRI_RS_148392* also was detected by another marker (SCRI_RS_157650) sharing the same exact position. Allele “A” of the SCRI_RS_157650 marker contributed to lower FHB severity and DON concentration unlike the allele “A” of SCRI_RS_148392 that contributed to an increase in disease severity and DON accumulation (Table 4.5). The SCRI_RS_157650 marker was discovered in the 2012 data set where DON concentration was relatively low and appeared to have a negative correlation with SCRI_RS_148392 in allele effect.

In addition to row-type, heading date and other agro-morphological traits may affect the level of FHB severity (de La Pena et al. 1999). In most of the bi-parental mapping studies cited above, the centromeric region of chromosome 2H (especially bin 8) was consistently associated with FHB resistance, DON accumulation and heading date. However, no significant marker associated with DTH was detected in this region in the current study. Likewise, no FHB severity or DON concentration QTL coincident with PH were detected in this study. Among the other agro-morphologic traits correlated with disease, a QTL for KD mapped to the same chromosome 2HL location as *Rfg-qtl-2H_11_20340*, sharing identical significant markers. This was likely due to the confounding effect of the row-type trait as the same QTL also coincided with the *Vrs1* locus. In addition to the markers on chromosome 2HL, two unmapped markers (SCRI_RS_165473 and SCRI_RS_72983) were significantly associated with FHB severity, DON concentration, RT and KD. These unmapped markers are probably associated with one of the same described QTL or perhaps other one(s) at a unique genomic location. Future research will be done to map such SNP markers significantly associated with the important phenotypic traits considered in this investigation.

In summary, one common QTL conferring low FHB severity and DON concentration and a separate QTL conferring low DON were identified through whole-genome association mapping in barley landraces from Ethiopia and Eritrea. The common QTL identified for FHB and DON on chromosome 2HL appeared to be linked with or a pleotropic effect of the row-type locus *Vrs1*. The other QTL identified for DON in the combined dataset and again in a separate dataset was located on chromosome 4HL. These two QTL (the 2HL and 4HL) mapped to genomic regions where QTL for FHB resistance were identified in other studies. Thus, they most likely are not unique QTL. Interestingly, both of these QTL were not associated with heading date or plant height unlike other studies. Thus, resistant germplasm with this QTL may be used in breeding programs without the issue of confounding effects from heading date or plant height. Importantly, these QTL could well be new alleles preserved in the unique germplasm used in this study. Barley accessions used in previous mapping studies were either breeding lines, unimproved varieties, or raw germplasm selections from other countries. To gain a better

understanding of the genetics of FHB resistance in the Ethiopian and Eritrean barley germplasm, more tests should be done to validate the consistency of the QTL detected in this study, primarily those on chromosomes 2HL and 4HL. This could be done through other studies including bi-parental mapping or allele-sequencing of putative QTL regions. It is also a common practice to evaluate materials for three to four years to identify reliable sources of FHB resistance, given the inherent genotype-by-environment interactions of the pathosystem. Additional barley landraces from Ethiopia should be tested over several years and at multiple locations to obtain more rigorous phenotype data for association genetics.

Table 4.1. Variance components and heritability of resistance to Fusarium head blight, deoxynivalenol (DON) concentration, and various agro-morphological traits (days to heading, plant height, kernel density, spike angle and spike exsertion length) in Ethiopian and Eritrean barley landrace germplasm

	Variance components[a]			
Trait	**Genotype**	**Year[b]**	**Residuals**	**Heritability**
FHB	2.83	0.03	0.02	0.99
DON	75.42	272.34	206.18	0.29
Days to heading	48.93	34.48	10.79	0.71
Plant height	170.21	16.96	19.60	0.93
Kernel density	7.02	0.0038	0.64	0.98
Spike angle	0.54	--	-0.03	1.00
Exsertion length	27.84	--	27.18	0.67

[a] Generated by "proc varcomp" procedure in SAS.
[b] --: Not applicable.

Table 4.2. Pearson correlation coefficients for Fusarium head blight severity, deoxynivalenol (DON) concentration, and various agro-morphological traits (days to heading, plant height, kernel density and spike exsertion length) in Ethiopian and Eritrean barley landrace germplasm at Crookston, MN in MN in 2011

Trait[a]	**RT**	**FHB**	**DON**	**DTH**	**PH**	**KD**	**EL**
RT	--						
FHB	0.41[b]	--					
DON	0.38[b]	0.30[b]	--				
DTH	0.30[b]	-0.41[b]	0.19[b]	--			
PH	-0.13[b]	-0.30[b]	-0.01	0.07	--		
KD	0.92[b]	0.50[b]	0.36[b]	0.22[b]	-0.19[b]	--	
EL	-0.32[b]	0.17[b]	-0.17[b]	-0.62[b]	0.41[b]	-0.30[b]	--

[a] RT: row type; FHB: Fusarium head blight severity; DON: deoxynivalenol concentration; DTH: days to heading; PH: plant height; KD: kernel density; EL: exsertion length.

[b] Significant at *P*-value of 0.01. The rest are not significantly different from zero at *P*-value 0.01.

Table 4.3. Pearson correlation coefficients for Fusarium head blight severity, deoxynivalenol concentration, days to heading (DTH), plant height (PH) and kernel density (KD) data in Ethiopian and Eritrean barley landrace germplasm at Crookston, MN in 2011 and 2012

Trait[a]	FHB 11	FHB 12	DON 11	DON 12	DTH 11	DTH 12	PH 11	PH 12	KD 11	KD 12
FHB11	--									
FHB12	0.61[b]	--								
DON11	0.29[b]	0.26[b]	--							
DON12	0.43[b]	0.46[b]	0.48[b]	--						
DTH11	-0.38[b]	-0.17[b]	0.24[b]	-0.004	--					
DHT12	-0.30[b]	-0.19[b]	0.29[b]	0.01	0.76[b]	--				
PH11	-0.33[b]	-0.33[b]	-0.03	-0.10	0.11	0.09	--			
PH12	-0.19[b]	-0.25[b]	0.001	-0.15	0.10	0.07	0.55[b]	--		
KD11	0.53[b]	0.48[b]	0.37[b]	0.44[b]	0.21[b]	0.17[b]	-0.19[b]	-0.06	--	
KD12	0.48[b]	0.47[b]	0.36[b]	0.43[b]	0.19[b]	0.18[b]	-0.14	-0.03	0.88[b]	--

[a] FHB: Fusarium head blight severity in 2011 and 2012; DON: deoxynivalenol concentration in 2011 and 2012; DTH: days to heading in 2011 and 2012; PH: plant height in 2011 and 2012; and KD: kernel density in 2011 and 2012.

[b] Significant at *P*-value of 0.01. The rest are not significantly different from zero at *P*-value of 0.01.

Table 4.4. SNP markers significantly associated with Fusarium head blight (FHB) severity, deoxynivalenol (DON) concentration, row-type morphology (RT) and kernel density (KD) in Ethiopian and Eritrean barley landrace germplasm at Crookston, MN in 2011 and 2012

			q-value for FHB[d]								
SNP marker	**Chrom.[a]**	**Position[b]**	**EEBC-C**	**EEBC-11**	**EEBC-12**	**EEBC-2R-C**	**EEBC-2R-11**	**EEBC-2R-12**	**EEBC-6R-C**	**EEBC-6R-11**	**EEBC-6R-12**
11_20340	2H	90.99	$1.44x10^{-8c}$	$2.14x10^{-8}$	$8.51x10^{-8}$	*0.07*	*0.04*	*1.00*	*1.00*	*1.00*	*1.00*
12_30896	2H	91.09	$9.88x10^{-8}$	$1.62x10^{-8}$	$1.95x10^{-6}$	*1.00*	*1.00*	*1.00*	*0.66*	*0.49*	*0.71*
SCRI_RS_72983	Unknown	Unknown	*$2.06x10^{-5}$*	*$5.02x10^{-5}$*	$1.114x10^{-5}$	*0.73*	*0.89*	*0.08*	*1.00*	*1.00*	*1.00*
			q-value for DON[d]								
SNP marker	**Chrom.[a]**	**Position[b]**	**EEBC-C**	**EEBC-11**	**EEBC-12**	**EEBC-2R-C**	**EEBC-2R-11**	**EEBC-2R-12**	**EEBC-6R-C**	**EEBC-6R-11**	**EEBC-6R-12**
SCRI_RS_171032	2H	90.64	$9.04x10^{-6c}$	*$9.54x10^{-5}$*	*$6.80x10^{-4}$*	*$3.12x10^{-4}$*	$1.74x10^{-4}$	*$1.19x10^{-3}$*	*1.00*	*1.00*	*1.00*
12_30901	2H	90.99	$3.39x10^{-6}$	*$5.69x10^{-5}$*	*$1.22x10^{-4}$*	$6.73x10^{-5}$	$2.11x10^{-4}$	*$4.61x10^{-4}$*	*1.00*	*1.00*	*1.00*
11_20340	2H	90.99	$4.21x10^{-6}$	*$8.71x10^{-4}$*	$1.85x10^{-5}$	*1.00*	*1.00*	*1.00*	*1.00*	*1.00*	*1.00*
12_30896	2H	91.09	*$4.55x10^{-5}$*	*$9.04x10^{-3}$*	$2.93x10^{-5}$	*1.00*	*1.00*	*1.00*	*0.90*	*0.89*	*0.42*
12_11485	4H	12.91	*$7.93x10^{-4}$*	*0.03*	$2.45x10^{-8}$	*0.42*	*0.88*	*0.02*	$7.50x10^{-6}$	*$1.18x10^{-3}$*	$1.47x10^{-5}$
SCRI_RS_148392	4H	65.52	$7.74x10^{-6}$	*0.01*	$3.26x10^{-8}$	*0.02*	*0.18*	*0.01*	$3.23x10^{-4}$	*0.04*	$4.86x10^{-6}$
SCRI_RS_157650	4H	65.52	*$1.77x10^{-4}$*	*0.06*	$1.22x10^{-6}$	*0.03*	*0.21*	*0.03*	*$3.05x10^{-3}$*	*0.14*	*$7.02x10^{-5}$*
SCRI_RS_165473	Unknown	Unknown	$7.75x10^{-7}$	$4.54x10^{-6}$	*$8.38x10^{-5}$*	$6.70x10^{-5}$	$2.94x10^{-4}$	*$1.67x10^{-4}$*	*1.00*	*1.00*	*1.00*
SCRI_RS_72983	Unknown	Unknown	$1.06x10^{-6}$	*$1.83x10^{-5}$*	*$7.09x10^{-5}$*	$1.51x10^{-5}$	$7.36x10^{-5}$	*$1.43x10^{-4}$*	*1.00*	*1.00*	*1.00*
			q-value for RT[e]								
SNP marker	**Chrom.[a]**	**Position[b]**	**EEBC**								
11_20781	2H	88.04	$6.79x10^{-6c}$								
SCRI_RS_171032	2H	90.64	$1.63x10^{-11}$								
11_20340	2H	90.99	$9.30x10^{-53}$								
12_30901	2H	90.99	$3.78x10^{-20}$								
12_30896	2H	91.09	$1.52x10^{-55}$								
SCRI_RS_196853	2H	91.09	$2.30x10^{-8}$								
12_30897	2H	91.09	$4.89x10^{-8}$								
SCRI_RS_221886	2H	92.07	$7.66x10^{-5}$								
SCRI_RS_165473	Unknown	Unknown	$1.97x10^{-21}$								
SCRI_RS_72983	Unknown	Unknown	$8.15x10^{-20}$								
SCRI_RS_181051	Unknown	Unknown	$7.91x10^{-5}$								
SCRI_RS_237894	Unknown	Unknown	$1.09x10^{-4}$								
			q-value for KD[f]								
SNP marker	**Chrom.[a]**	**Position[b]**	**EEBC-C**								
11_20781	2H	88.04	$2.61x10^{-6c}$								

Table 4.4. See next page.

Table 4.4. Continued.

			q-value for KD[f]
SNP marker	Chrom.[a]	Position[b]	**EEBC-C**
SCRI_RS_171032	2H	90.64	$3.56x10^{-10}$
11_20340	2H	90.99	$1.58x10^{-46}$
12_30901	2H	90.99	$1.55x10^{-16}$
12_30896	2H	91.09	$7.68x10^{-47}$
12_30897	2H	91.09	$1.40x10^{-10}$
SCRI_RS_196853	2H	91.09	$1.56x10^{-10}$
SCRI_RS_221886	2H	92.07	$2.34x10^{-5}$
SCRI_RS_165473	Unknown	Unknown	$4.20x10^{-20}$
SCRI_RS_72983	Unknown	Unknown	$1.23x10^{-19}$

[a] Chromosome. Note: some markers have not been mapped.

[b] Genetic position (in cM) of marker on chromosome. Note: some markers have not been mapped to a chromosomal position.

[c] Multiple testing corrected *P*-value. Non-significant *q*-values are presented for comparison purposes and are indicated in italics.

[d] EEBC-C: Ethiopian and Eritrean barley collection with combined data of 2011 and 2012; EEBC-11: test from 2011 that includes data from the entire collection; EEBC-12: test from 2012 that includes data from the entire collection; EEBC-2R-C: Combined two-year (2011 & 2012) data of two-rowed accessions only; EEBC-2R-11: 2011 data of two-rowed accessions only; EEBC-2R-12: 2012 data of two-rowed accessions only; EEBC-6R-C: Combined two-year data of six-rowed accessions ; EEBC-6R-11: 2011 data of six-rowed accessions only ; EEBC-6R-12: 2012 data of six-rowed accessions only;

[e] EEBC: Comprises 110 two-rowed and 180 six-rowed germplasm.

[f] EEBC-C: Combined two-year data of the entire collection.

Table 4.5. Trait means for each allele at chromosome positions significantly associated with Fusarium head blight (FHB) severity and deoxynivalenol (DON) concentration identified in the combined dataset or two different sub-sets of data in Ethiopian and Eritrean barley landrace germplasm

Germplasm	Trait	QTL/marker	Position (cM)	Allele	FHB	DON	Allele frequency[1]	Identified in
Whole set	FHB + DON	*Rfg-qtl-2H_11_20340*[a]	2H 90.99-91.09	A	4.37	28.54	0.61	
Whole set	FHB + DON	*Rfg-qtl-2H_11_20340*	2H 90.99-91.09	B	3.53	17.24	0.39	EEBC-C, EEBC-11 and EEBC-12[a]
Two-rowed	FHB + DON	*Rfg-qtl-2H_11_20340*	2H 90.99-91.09	A	2.21	25.10	0.06	
Two-rowed	FHB + DON	*Rfg-qtl-2H_11_20340*	2H 90.99-91.09	B	3.45	16.93	0.94	
Six-rowed	FHB + DON	*Rfg-qtl-2H_11_20340*	2H 90.99-91.09	A	4.48	28.70	0.95	
Six-rowed	FHB + DON	*Rfg-qtl-2H_11_20340*	2H 90.99-91.09	B	4.76	22.44	0.05	
Whole set	DON	*12_30901*[b]	2H 90.99	A	3.36	14.29	0.20	
Whole set	DON	*12_30901*	2H 90.99	B	4.26	26.86	0.80	
Two-rowed	DON	*12_30901*	2H 90.99	A	3.35	14.18	0.51	
Two-rowed	DON	*12_30901*	2H 90.99	B	3.50	21.43	0.49	
Six-rowed	DON	*12_30901*	2H 90.99	A	ND[j]	ND	0.01	
Six-rowed	DON	*12_30901*	2H 90.99	B	4.51	28.40	0.99	EEBC-C[b]
Whole set	DON	*DON-qtl-2H_SCRI_RS_171032*[b]	2H 90.64	A	4.02	24.03	0.51	
Whole set	DON	*DON-qtl-2H_SCRI_RS_171032*	2H 90.64	B	4.26	24.33	0.49	
Two-rowed	DON	*DON-qtl-2H_SCRI_RS_171032*	2H 90.64	A	3.41	16.87	0.52	
Two-rowed	DON	*DON-qtl-2H_SCRI_RS_171032*	2H 90.64	B	3.50	17.12	0.48	
Six-rowed	DON	*DON-qtl-2H_SCRI_RS_171032*	2H 90.64	A	4.42	28.38	0.50	
Six-rowed	DON	*DON-qtl-2H_SCRI_RS_171032*	2H 90.64	B	4.82	28.47	0.50	
Whole set	DON	*DON-qtl-2H-12_10777*[c]	2H 30.36	A	4.17	22.44	0.70	
Whole set	DON	*DON-qtl-2H-12_10777*	2H 30.36	B	3.82	28.32	0.30	EEBC-2R-C, EEBC-2R-11 and EEBC-2R-11[c]
Two-rowed	DON	*DON-qtl-2H-12_10777*	2H 30.36	A	3.42	14.63	0.79	
Two-rowed	DON	*DON-qtl-2H-12_10777*	2H 30.36	B	3.37	29.16	0.21	
Six-rowed	DON	*DON-qtl-2H-12_10777*	2H 30.36	A	4.78	28.58	0.64	
Six-rowed	DON	*DON-qtl-2H-12_10777*	2H 30.36	B	4.01	28.30	0.36	
Whole set	DON	*DON-qtl-4H-12_11485*[d]	4H 12.91	A	4.19	12.82	0.71	
Whole set	DON	*DON-qtl-4H-12_11485*	4H 12.91	B	4.93	18.49	0.29	EEBC-12, EEBC-6R-C and EEBC-6R-12[d]
Two-rowed	DON	*DON-qtl-4H-12_11485*	4H 12.91	A	3.22	16.53	0.65	
Two-rowed	DON	*DON-qtl-4H-12_11485*	4H 12.91	B	3.71	18.45	0.35	
Six-rowed	DON	*DON-qtl-4H-12_11485*	4H 12.91	A	4.16	26.71	0.74	
Six-rowed	DON	*DON-qtl-4H-12_11485*	4H 12.91	B	5.63	33.53	0.26	
Whole set	DON	*DON-qtl-4H- SCRI_RS_148392*[e]	4H 65.52	A	4.8	28.7	0.33	EEBC-C, EEBC-12, EEBC-6R-C and EEBC-6R-12[e]
Whole set	DON	*DON-qtl-4H- SCRI_RS_148392*	4H 65.52	B	3.7	21.88	0.67	
Two-rowed	DON	*DON-qtl-4H- SCRI_RS_148392*	4H 65.52	A	3.78	20.21	0.28	

Table 4.5. See next page.

Table 4.5. Continued.

Germplasm	Trait	QTL/marker	Position (cM)	Allele	FHB	DON	Allele frequency[J]	
Two-rowed	DON	*DON-qtl-4H- SCRI_RS_148392*	4H 65.52	B	3.27	16.14	0.72	EEBC-C, EEBC-12,
Six-rowed	DON	*DON-qtl-4H- SCRI_RS_148392*	4H 65.52	A	5.28	32.82	0.37	EEBC-6R-C and
Six-rowed	DON	*DON-qtl-4H- SCRI_RS_148392*	4H 65.52	B	4.04	25.87	0.63	EEBC-6R-12[e]
Whole set	DON	*DON-qtl-5H-11_21001*[f]	5H 55.83	A	4.12	24.52	0.75	
Whole set	DON	*DON-qtl-5H-11_21001*	5H 55.83	B	3.91	22.81	0.25	
Two-rowed	DON	*DON-qtl-5H-11_21001*	5H 55.83	A	3.31	16.31	0.75	EEBC-2R-C and
Two-rowed	DON	*DON-qtl-5H-11_21001*	5H 55.83	B	3.68	20.05	0.25	EEBC-2R-11[f]
Six-rowed	DON	*DON-qtl-5H-11_21001*	5H 55.83	A	4.63	29.49	0.75	
Six-rowed	DON	*DON-qtl-5H-11_21001*	5H 55.83	B	4.18	24.98	0.25	
Whole set	DON	*DON-qtl-7H-11_20824*[g]	7H 107.49	A	4.05	23.88	0.81	
Whole set	DON	*DON-qtl-7H-11_20824*	7H 107.49	B	4.19	25.15	0.19	
Two-rowed	DON	*DON-qtl-7H-11_20824*	7H 107.49	A	3.23	16.30	0.65	EEBC-6R-C and
Two-rowed	DON	*DON-qtl-7H-11_20824*	7H 107.49	B	3.72	19.03	0.35	EEBC-6R-12[g]
Six-rowed	DON	*DON-qtl-7H-11_20824*	7H 107.49	A	4.43	27.12	0.92	
Six-rowed	DON	*DON-qtl-7H-11_20824*	7H 107.49	B	5.52	42.68	0.08	
Whole set	DON	*SCRI_RS_165473*[h]	Unknown	A	3.34	14.25	0.21	
Whole set	DON	*SCRI_RS_165473*	Unknown	B	4.24	26.85	0.79	EEBC-C,
Two-rowed	DON	*SCRI_RS_165473*	Unknown	A	3.33	14.17	0.52	EEBC-11,
Two-rowed	DON	*SCRI_RS_165473*	Unknown	B	3.49	21.50	0.48	EEBC-2R-C
Six-rowed	DON	*SCRI_RS_165473*	Unknown	A	5.00	18.82	0.01	EEBC-2R-11[h]
Six-rowed	DON	*SCRI_RS_165473*	Unknown	B	4.49	28.35	0.99	
Whole set	FHB + DON	*SCRI_RS_72983*[i]	Unknown	A	3.26	13.89	0.10	
Whole set	FHB + DON	*SCRI_RS_72983*	Unknown	B	4.28	26.81	0.90	EEBC-12 for FHB,
Two-rowed	FHB + DON	*SCRI_RS_72983*	Unknown	A	3.27	13.87	0.25	EEBC-C,
Two-rowed	FHB + DON	*SCRI_RS_72983*	Unknown	B	3.57	21.39	0.75	EEBC-2R-C and
Six-rowed	FHB + DON	*SCRI_RS_72983*	Unknown	A	ND[k]	ND	0.00	EEBC-2R-11[i]
Six-rowed	FHB + DON	*SCRI_RS_72983*	Unknown	B	4.52	28.40	1.00	

[j] Not determined. There were no six-rowed germplasm accessions with the BB allele. One accession that had the BB allele did not yield phenotype data.

[k] Not determined. There were no six-rowed germplasm accessions with the AA allele.

[l] Resistant allele frequency within analysis set; calculated for the most significant marker where more than marker was significant.

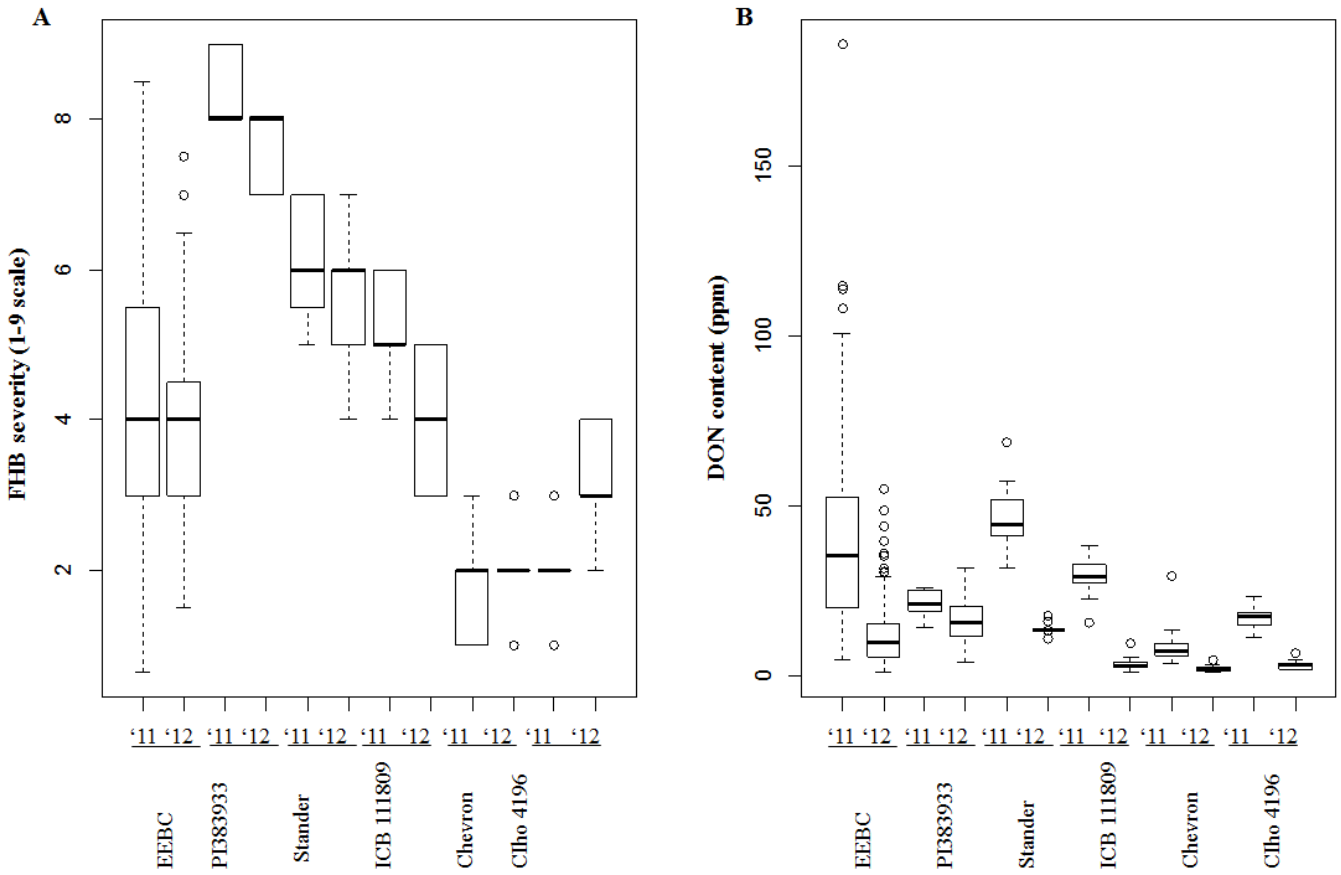

Figure 4.1. See next page.

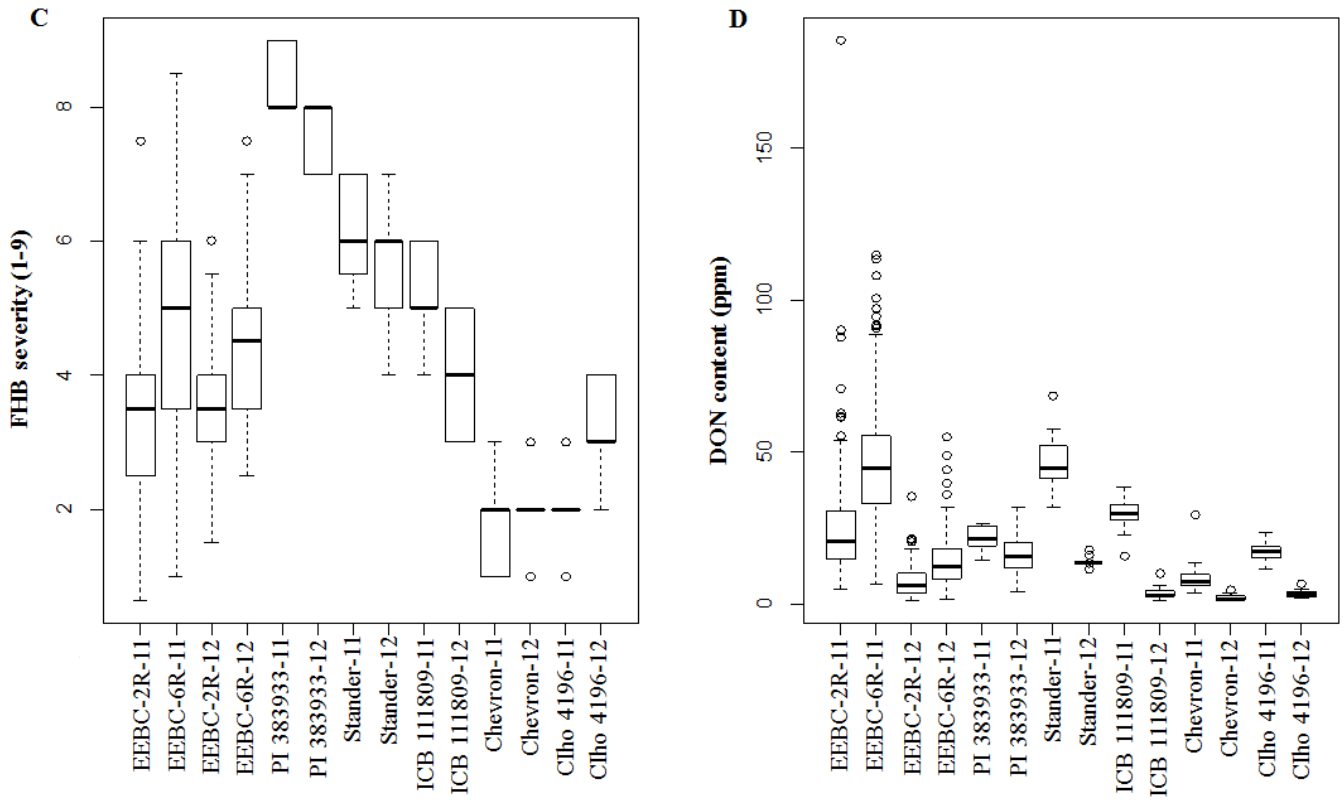

Figure 4.1. See next page.

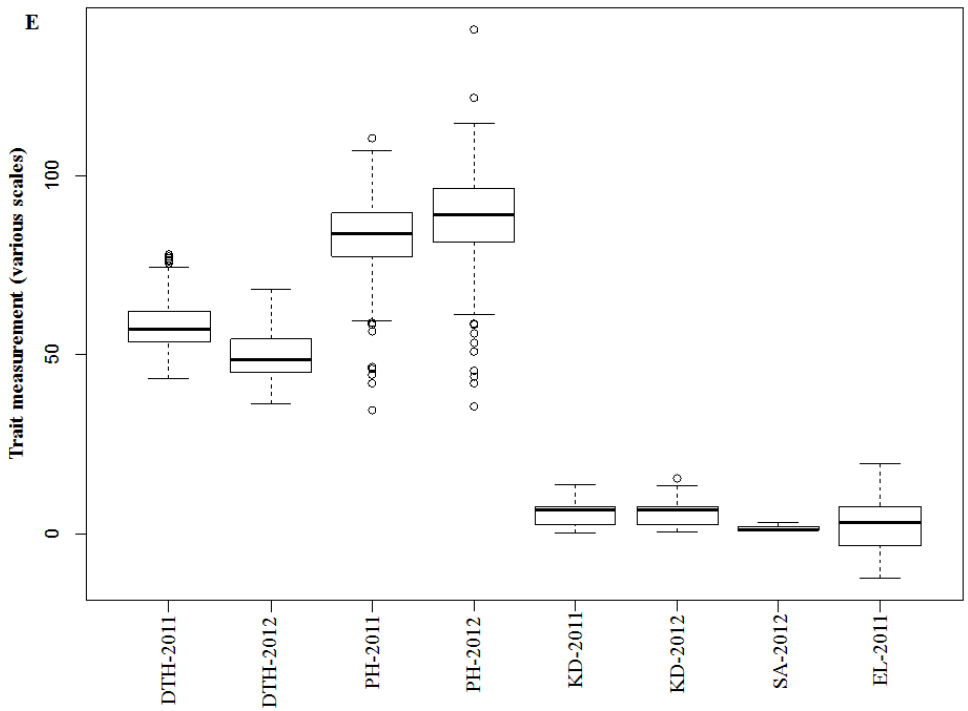

Figure 4.1. Boxplots of Fusarium head blight (FHB) severity, deoxynivalenol (DON) concentration and associated agro-morphological traits of Ethiopian and Eritrean barley landrace germplasm (EEBC) and controls at Crookston, MN. PI 383933, cv. Stander and ICB 111809 are the susceptible controls and cv. Chevron and CIho 4196 are the resistant controls. (**A**) Fusarium head blight severity data on scale of 1 (most resistant) to 9 (most susceptible) of EEBC germplasm and controls in 2011 and 2012, (**B**) DON concentration data (ppm) of EEBC germplasm and controls in 2011 and 2012, (**C**) Fusarium head blight severity data of two-rowed and six-rowed EEBC germplasm and controls in 2011 and 2012, (**D**) DON concentration data (ppm) of two-rowed and six-rowed EEBC germplasm and controls in 2011 and 2012, (**E**) Agro-morphological traits of landrace germplasm in 2011 and 2012 (DTH: days to heading in days; PH: plant height in cm; KD: kernel density in number of nodes per cm of rachis; SA: spike angle on scale of 1 to 3; and EL: exsertion length in cm). Five statistics (bars) are represented in each boxplot from bottom to top: the smallest observation, lower quartile, median, upper quartile, and largest observation, respectively. Data points positioned outside this range and depicted as circles are outliers.

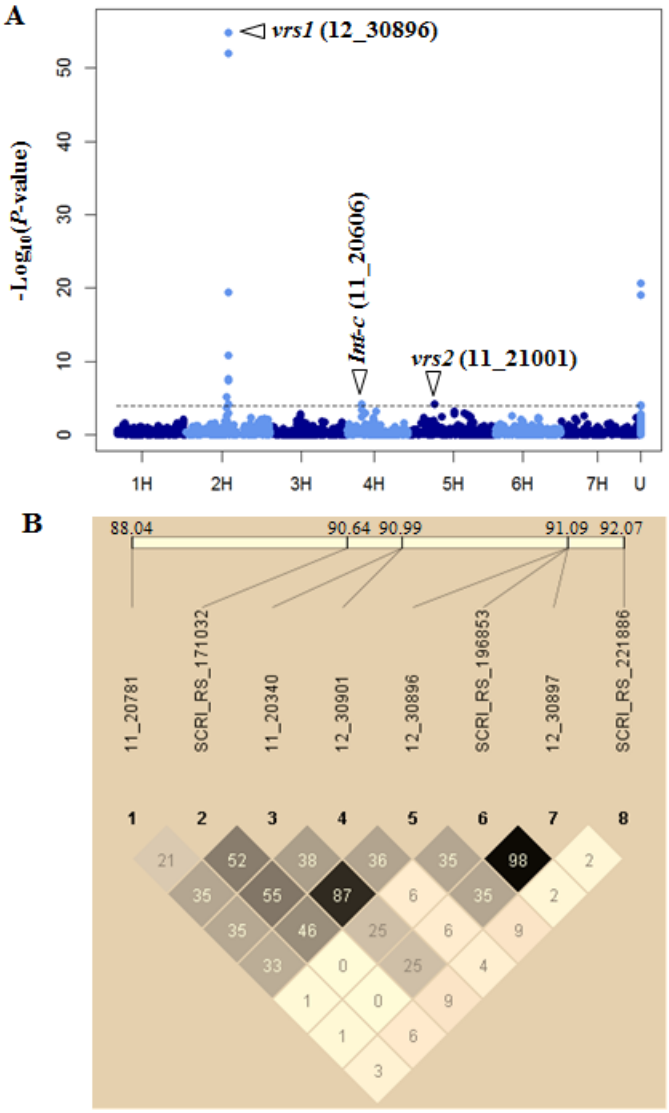

Figure 4.2. (**A**) Genome-wide association scan for row-type spike morphology of Ethiopian and Eritrean barley landrace germplasm. Vertical axis represents -$\log_{10}$(*P*-values) of the marker-trait associations after correction for multiple testing, and the horizontal axis represents the relative chromosomal position of the SNP markers across the genome. The black horizontal dotted line shows the *P*-value corresponding to a false discovery rate (FDR) of 0.05. SNP markers with peaks above this threshold level were considered as significantly associated with row-type spike morphology. Significantly associated SNPs at or in the vicinity of the genes *vrs1* (six-rowed spike 1), *Int-c* (INTERMEDIUM-C) and *vrs2* (six-rowed spike 2) are marked with arrows. (**B**) Heat map of linkage disequilibrium (LD) for eight SNP markers on chromosome 2H associated with barley spike morphology around the *Vrs1* locus. Numbers above the map are the position of the markers from the concensus map in cM. LD is displayed in the squares below the map as r^2, expressed as a percentage, between all pair-wise combinations of the eight markers.

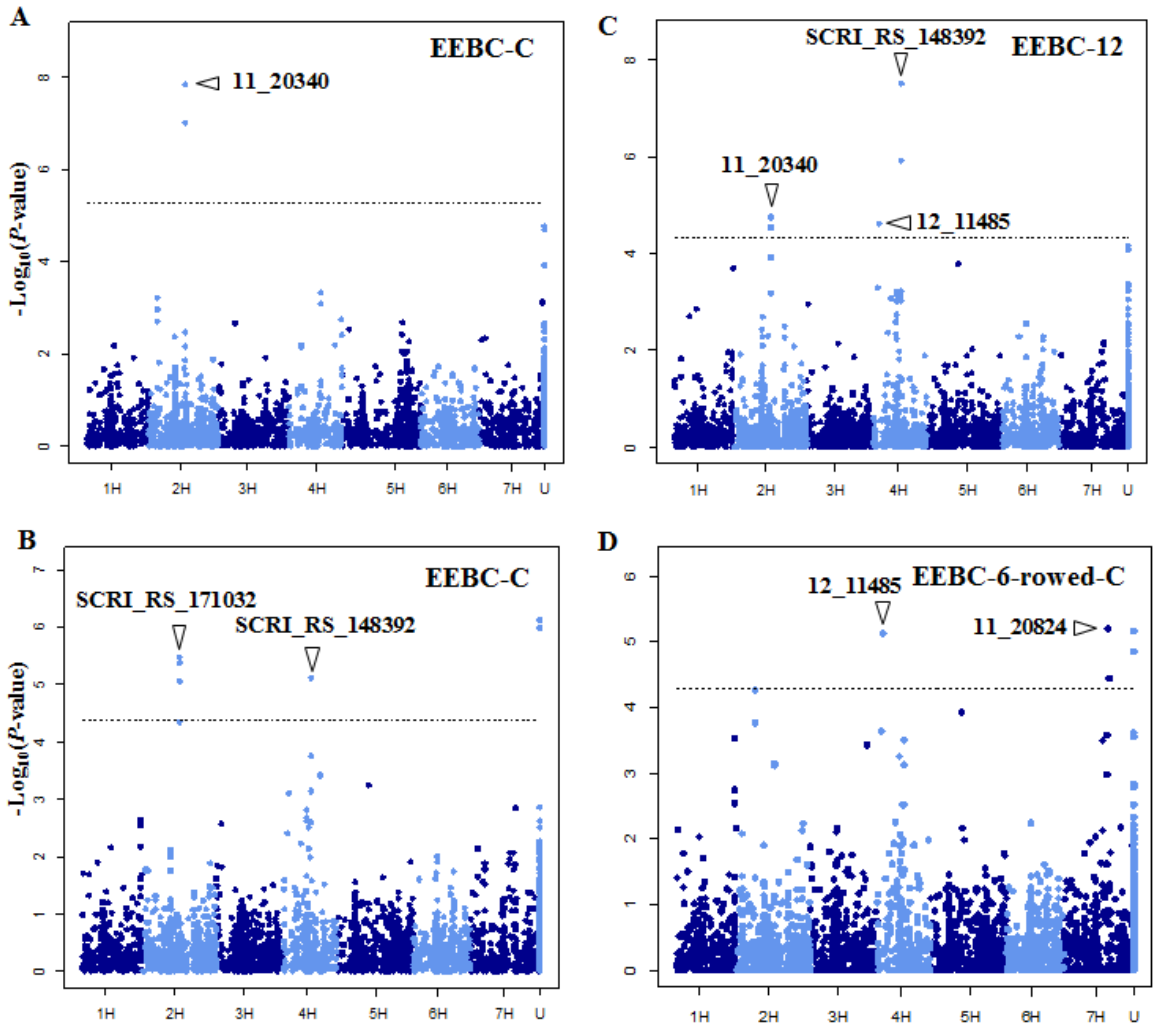

Figure 4.3. See next page.

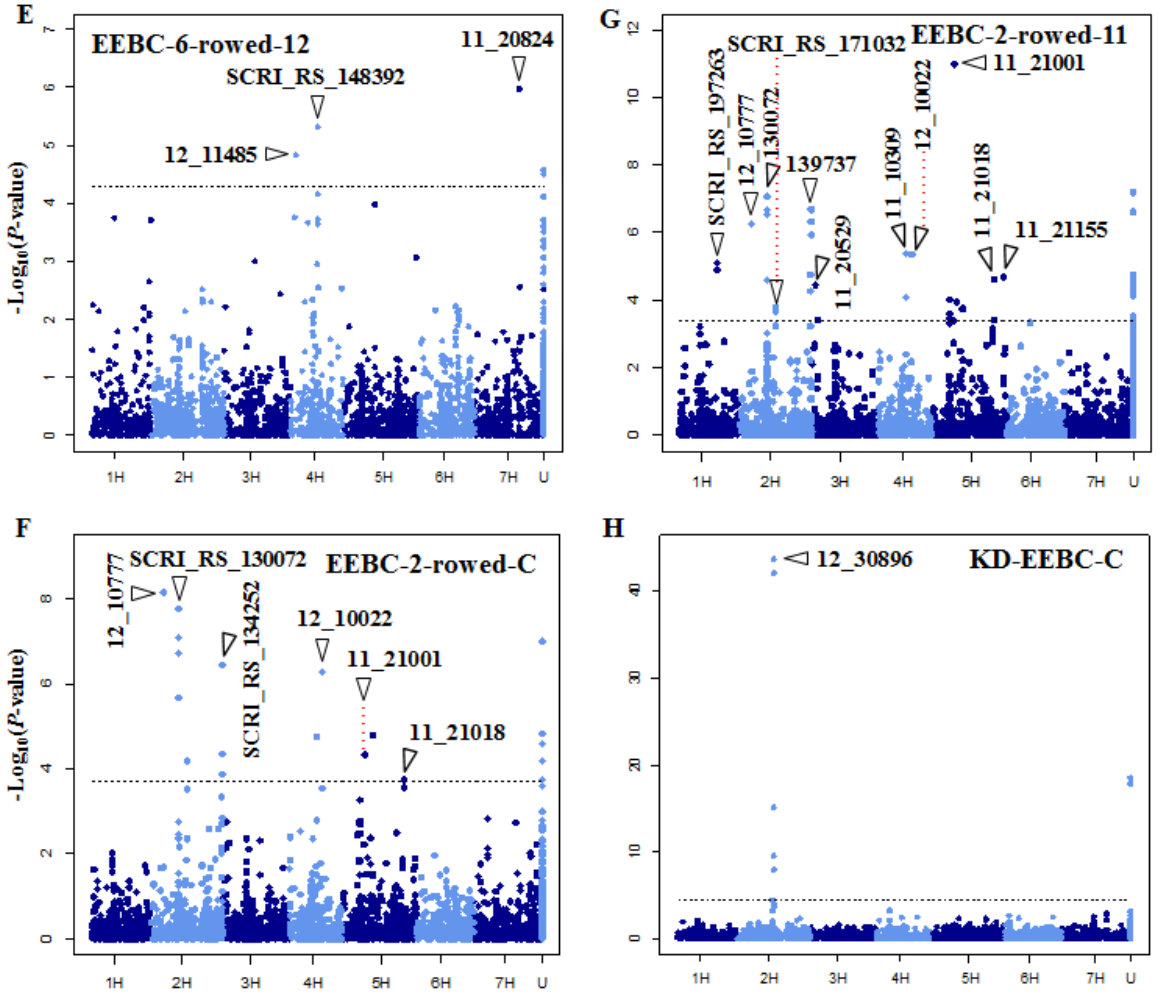

Figure 4.3. Genome-wide association scan for marker associations with Fusarium head blight (FHB) severity, deoxynivalenol (DON) concentration and kernel density (KD) in the Ethiopian and Eritrean Barley Collection (EEBC). Scans are shown for (**A**) FHB severity for the whole set of landrace germplasm combined over two years (EEBC-C), (**B**) DON concentration for the whole set of landrace germplasm combined over two years (EEBC-C), (**C**) DON concentration for the individual year of 2012 (EEBC-12), (**D**) combined DON concentration in the six-rowed germplasm set (EEBC-6-rowed-C) only, (**E**) DON concentration in the six-rowed germplasm set in 2012 (EEBC-6-rowed-12) only, (**F**) combined DON concentration in the two-rowed germplasm set (EEBC-2-rowed-C) only, (**G**) DON concentration in the two-rowed germplasm set in 2011 (EEBC-2-rowed-11) only, and (**H**) kernel density combined over two years (KD-EEBC-C). Vertical axis represents -$\log_{10}$(*P*-values) of the marker-trait associations after correction for multiple testing, and the horizontal axis represents the relative chromosomal position of the SNP markers across the genome. The black horizontal dotted lines show the *P*-value corresponding to a false discovery rate (FDR) of 0.05. SNP markers with peaks above this threshold level were considered as significantly associated. The most significant marker for each trait or analysis panel is marked with arrows. The full names of two of the most significant markers indicated in 'G' on chromsome 2HL are SCRI_RS_130072 and SCRI_RS_139737.

Chapter 5

Genome-wide Association Mapping of Zinc and Iron Concentration in Barley Landraces from Ethiopia and Eritrea

5.1 Introduction

Barley (*Hordeum vulgare* ssp. *vulgare* L.) is one of the oldest cultivated crop plants and the fourth most important cereal crop in the world after maize (*Zea mays*), rice (*Oryza sativa*) and wheat (*Triticum* spp.) (Schulte et al. 2009; http://faostat.fao.org). The crop has been part of a sustainable food source for humans since pre-historic time. Today, less than 2% of the barley grain produced is used directly for food in the US and the European Union. However, it is major part of a staple diet in Central and West Asia (China), South Asia (India), North and East Africa (Morocco & Ethiopia/Eritrea), and the mountainous regions of the Andean countries of South America (Bolivia, Ecuador, Colombia and Peru). In these countries, more than 60% of the barley produced is used directly for human consumption (Grando and Macpherson 2005; Newman and Newman 2006). In the US, barley is gaining renewed attention as a "new" food due to its health benefit effects (Baik and Ullrich 2008; http://barleygenome.org/). Barley grain contains about 65–68% starch, 10–17% protein, 4–9% β-glucan, 2–3% free lipids and 1.5–2.5% minerals (Czuchajowska et al. 1998; Izydorczyk et al. 2000; Quinde et al. 2004), depending upon the barley genotype and environment in which it is grown (for a review, see Baik and Ullrich 2008).

Other nutritional attributes of barley include many vitamins and micronutrients, including niacin (vitamin B3), thiamin (vitamin B1), calcium, iron, magnesium, phosphorous, potassium, selenium, zinc and copper (http://www.nal.usda.gov). Zinc and iron deficiencies are regarded as two of the "big five" micronutrient deficiencies in humans throughout the world, along with iodine, selenium and vitamin A deficiencies (Genc et al. 2005). Micronutrient malnutrition, also referred to as "hidden hunger", affects over two billion people throughout the world (Welch and Graham 1999; White et al. 2009). Specifically, zinc, iron, folate and vitamin A malnutrition are the most important human health challenges on a global scale (www. copenhagenconsensus.com). The problem of "hidden hunger" is more significant in resource-poor parts of the world, where alternative food sources are scarce to supplement cereal-based food materials,

which are inherently low in composition of micronutrients, especially if grown on soils deficient in such nutrition (Cakmak 2008).

Deficiencies of some of these micronutrients in the human diet could be alleviated through improving the dietary composition of locally grown staple food crops (Mayer et al. 2008). Currently, multiple options are under exploration to achieve this (Graham et al. 2001). For example, use of microorganisms such as plant-growth promoting bacteria for improving the nutritional status of wheat is under investigation (e.g. Rana et al. 2012) as is biofortification through the application of fertilizers (Cakmak et al. 2010; Zhang et al. 2010; Zhang et al. 2012). Perhaps the most promising option for increasing the level of micronutrients in cereals is through breeding (Genc et al. 2005; Welch and Graham 2004; Schachtman and Barker 1999; White et al. 2009). Micronutrient enhancement through plant breeding or via a molecular genetics approach is preferable for several reasons. First, once developed, staple crops with improved food quality traits can easily be obtained, adopted, and used by subsistence farmers in developing countries. Second, nutritionally-enriched cultivars provide a sustainable supply of micronutrients since additional inputs are not required to sustain the level of nutrients in demand. Third, use of cultivars genetically-fortified for nutritional value is environmentally safe as it does not require introduction of chemical fertilizers, micro-organisms or other inputs (White and Broadley 2011). However, an integrated approach of micronutrient enhancement that combines all feasible options may have to be used to alleviate micronutrient deficiencies over a large scale.

Zinc and iron deficiencies prominently contribute to global health problems, especially in developing countries. Along with vitamin A, iodine and selenium, deficiencies in zinc and iron account for over 65% of childhood deaths worldwide (Genc et al. 2005; Welch and Graham 2004). Iron deficiency is the most prevalent nutritional disorder in the world, affecting 66-80% of the global population. Over 30% of this total cohort show symptomatic anemia, the vast majority being from developing countries (WHO; https://apps.who.int/nut/ida.htm; Welch and Graham 2004). About 50% of the world's population is at risk for zinc deficiency (Hotz and Brown 2004). Together, zinc

and iron deficiencies are highly prominent public health problems in the world, but especially so in Ethiopia (Gebremedhin et al. 2011; Haidar et al. 2005). Over the last decade, a global effort launched under the auspices of HarvestPlus (http://www.harvestplus.org/) has made significant progress to enhance the micronutrient levels of staple cereal crops.

Genetic diversity for zinc and iron concentration has been studied in wheat, rice and maize as a prerequisite of consideration for improvement through breeding (for review, see Welch and Graham 2004). In wheat, studies are currently underway to characterize the genetic basis and molecular mechanisms affecting zinc and iron accumulation in grain (Cakmak et al. 2010). Chromosomal regions associated with grain zinc and iron concentration in wheat have been reported (Genc et al. 2009; Rawer et al. 2011; Shi et al. 2008; Tiwari et al. 2009; Xu et al. 2012). Additionally, quantitative trait loci (QTL) controlling micronutrient accumulation also were identified in rice (e.g. Neelamraju et al. 2012; Stangoulis et al. 2007) and maize (e.g Qin et al 2012; Šimić et al. 2012). Moreover, genetic diversity studies for micronutrient composition have recently been reported in sorghum (Kumar et al. 2012; Shegro et al. 2012). These studies highlight the recent research initiatives to characterize the genetic diversity and underlying QTL involved in grain micronutrient concentration and their potential application in breeding.

In barley, the effort to develop molecular markers for use in breeding for increased micronutrient accumulation (such as zinc) is just starting. Several studies have reported on QTL controlling zinc nutrition in barley. Lonergan et al. (2009) studied zinc uptake and distribution (zinc concentration in the shoot, stem and seed) in a doubled haploid mapping population of barley. They identified several QTL on chromosomes 1H, 2H and 5H associated with genetic variation for grain zinc concentration. In another separate study by the same author, three QTL for grain zinc concentration in barley also were reported on chromosomes 2HS, 2HL and 5HL, in addition to the one controlling leaf zinc concentration on chromosome 4HS (Lonergan 2001). A region on the long arm of chromosome 4H also was found associated with shoot zinc concentration at the early vegetative stage of growth (Lonergan 2002). QTL linked with barley seed zinc

accumulation were identified on the short and long arms of chromosome 2H in another doubled haploid population (Sadeghzadeh 2008; Sadeghzadeh et al. 2010; Shi et al. 2010). In a study involving greenhouse-grown plants, QTL determining grain zinc concentration were detected on chromosomes 3HL and 4HS (Sadeghzadeh 2008). Only a few published reports indicate the existence of genetic variation in grain iron concentration in barley (El-Haramein and Grando 2010; Ma et al. 2004; Yan et al. 2013); however, no known study has been reported on the genetics of iron accumulation in barley grains or other plant parts.

Barley is one of the principal dietary staples in Ethiopia and contributes ~5.6-12.3% of per capita calorie consumption in the country (Berhane et al. 2011; Marwat et al. 2012). In the highland regions of northeastern Ethiopia, barley is the number one food crop and is used in over 20 types of dishes and beverages (Shewayrga and Sopade 2011). Barley also is used as a major part of a staple diet in some other developing countries, e.g. Bolivia, China, Colombia, Eritrea, and Morocco. Its composition of medically important β-glucans (useful for reduction of blood cholesterol, control of heart disease and type-2 diabetes) has recently been recognized in the US (Baik and Ullrich 2008; http://barleygenome.org/). Consequently, increasing micronutrient composition of the crop through genetic fortification (breeding) would have a major impact in alleviating micronutrient deficiencies in developing and developed countries alike.

In addition to traits such as disease resistance (Metcalfe et al. 1978; Yitbarek et al. 1995), barley germplasm from Ethiopian has been recognized as a source of genetic variation for traits of nutritional value. Munck et al. (1970) identified a high-protein, high-lysine barley line (Hiproly), and a sister line CIho 4362 with high protein concentration by screening accessions from the World Barley Collection. Both Hiproly and CIho 4362 are lines of Ethiopian origin. Hiproly was the first and the only spontaneous mutant identified in barley for high lysine concentration (Shewry 2006) and provides more essential amino acids than any other cereal grain (Pomeranz 1973). The existence of such rare and valuable mutants in Ethiopian barley indicates the significance of this regional germplasm. Other early studies also identified Ethiopian and Eritrean

barleys with high productivity, high protein concentration, and large grain as well as those potentially valuable for brewing (Orlov 1929). Lance and Nilan (1980) also reported that Ethiopian barley landraces have promising malting and brewing quality.

To characterize the extent of genetic diversity and discover valuable genes underlying traits, association mapping is being extensively used in barley and other plant species (Waugh et al. 2009). Genome-wide association study (GWAS) is a powerful approach for mapping economically and biologically valuable traits in collections of germplasm (Thornsberry et al. 2001). GWAS is based on linkage disequilibrium (LD), which refers to the non-independence of alleles in haplotypes of a population (Gaut and Long, 2003). GWAS is a widely used alternative approach to bi-parental mapping for identifying genes responsible for natural variation in phenotypic traits, including nutritional quality (Shao et al. 2011; Wei et al. 2013; Zhao et al. 2011). Through the technique of GWAS, it is possible to map the chromosomal location of genes/QTL controlling traits of interest at a much greater resolution than is possible through bi-parental mapping (Flint-Garcia et al. 2005).

Diverse barley landraces, originally collected from both Ethiopia and Eritrea, have been assembled from various international genebanks. This germplasm has been assayed for multiple phenotypes (including nutritional traits) and genotyped with the barley iSelect SNP (single nucleotide polymorphism) chip that contains a total of 7,842 SNP markers. The long-term goal of this research is to introgress economically important genes identified from barley landraces into elite breeding lines or commercial barley cultivars. The specific objectives of the current study were to: (1) characterize the genetic variation in grain zinc and iron concentration and kernel weight in Ethiopian/Eritrean barley landraces; and (2) identify QTL associated with these traits in the germplasm using a GWAS approach.

5.2 Materials and methods

5.2.1 *Plant materials*

Two-hundred and ninety-eight Ethiopian and Eritrean barley landraces were used in this study. To incorporate as much genetic diversity as possible in the GWAS panel, we used the following criteria to select the landraces. More than 3,000 Ethiopian and Eritrean landraces are held by the US Department of Agriculture-Agricultural Research Service (USDA-ARS), National Small Grains Collection (NSGC) in Aberdeen, ID (USDA-ARS, NGRP 2009). Of the accessions in this list, a total of 87 Ethiopian and Eritrean landraces were already included in the barley core collection developed by the NSGC (USDA-ARS, NGRP 2000) and were therefore also included in our GWAS panel. The barley core collection was assembled to represent the genetic diversity found in the cultivated portion of the barley germplasm maintained by the NSGC in such a way that the final set consisted of 10% of the total collection. The logarithm of the number of accessions per country in the entire collection was used to determine the number included in the core collection on a per country basis (H. Bockelman, personal communication). Accessions for the core collection were then selected at random within each country.

To further enhance the panel diversity, additional accessions were included with special emphasis on landraces from geographically diverse regions with historically intensive barley cultivation. The number of accessions representing each region was selected based on the area of barley cultivation within the respective areas. When multiple accessions from a single site were available, the final accession(s) for the panel were arbitrarily selected. In the end, 132 additional accessions were selected from the NSGC along with the 87 from the core collection. These Ethiopian and Eritrean barley landraces were collected during various expeditions to the countries between the 1920s and 1970s (Qualset 1975). For example, Harry Harlan made one of the earliest collection trips to the country in 1923 (Harlan 1957). Seed of the NSGC landraces were provided courtesy of Harold E. Bockelman, the NSGC curator. Some of these sampled landraces also were included in previous studies on disease and insect resistance, feed quality, and

endosperm texture variation (e.g., Bowman et al. 2001; Schaller et al. 1963; Turaspekov et al. 2008).

In addition to the NSGC holdings, additional accessions were arbitrarily selected from different genebanks whose expeditions collected germplasm in different regions and eras than those conducted by the USDA. In this regard, 63 and 17 additional Ethiopian/Eritrean landraces were obtained from the N. I. Vavilov Research Institute of Plant Industry (VIR) and the International Center for Agricultural Research in the Dry Areas (ICARDA), respectively, and included in the panel. Passport data (where available) and other details about the accessions are given in Appendix Table 3.1.

To obtain genetically pure seed stocks of the landraces, single plant selections were made from each accession and selfed twice in the greenhouse. Then, a large greenhouse increase was made to provide sufficient seed for all subsequent experiments. For the greenhouse increases, seven seeds were planted in plastic pots (13.3 cm × 13.3 cm × 10.2 cm, l × w × h) filled with a 50:50 mix of steam-sterilized native soil and Metro-Mix® 200 (Sun Gro Horticulture, Quincy, MI), a growing media containing vermiculite, sphagnum peat moss, perlite, dolomitic limestone, and a wetting agent. After planting, all pots were watered and fertilized with Osmocote® controlled-release 14-14-14 (N:P:K) (Scott's Company, Marysville, OH) (1.4 g/pot) and Peters Dark Weather 15-0-15 fertilizer (Scott's Company) (ca. 40 g/liter at 1/16 dilution). Thereafter, plants were fertilized with a 20:20:20 water-soluble formulation (J.R. Peters, Inc., Allentown, PA) biweekly at the recommended rate. All plant grow-outs were done in a greenhouse within the Plant Growth Facility on the St. Paul campus of the University of Minnesota in 2010 and 2011. Plants were grown at 19-23°C with a 16 hr photoperiod supplemented by 400 W high-pressure sodium halide lamps emitting a minimum of 300 μmol photons $s^{-1}m^{-2}$. When plants reached physiological maturity, seeds were harvested, dried at 35°C in a forced air dryer for two days, threshed and cleaned.

5.2.2 *Sample preparation and DNA extraction*

Seeds from the third selfed generation (S_3) of each landrace were sown and grown in the greenhouse as described above. Two weeks after planting, leaf tissue was collected from the plants for subsequent DNA extraction. For this procedure, segments of tissue (~5 to 6.5 cm long) were cut from the second leaves of plants, folded twice, and carefully placed in a 1.0 ml polypropylene microtube (BioExpress, Kaysville, UT). The microtubes were kept on ice during sample collection. Then, the microtubes were frozen in liquid nitrogen and stored at −20°C until the samples were freeze-dried or lyophilized. For freeze-drying, the samples were removed from the freezer, the lids of the microtubes opened, and the tube rack securely covered with miracloth (EMD Millipore Corporation, Temecula, CA). Samples were kept on ice until they were freeze-dried in a general purpose freeze dryer (Model 24DX48, Virtis Company, Gardiner, NY) according to the specifications of the manufacturer.

The lyophilized tissue was subjected to tissue lysis for total genomic DNA isolation. One 3 mm tungsten-carbide bead was added to microtubes containing the tissue samples and homogenized using a TissueLyser (Qiagen, Valencia, CA) for 90 seconds at 30 Hz. Then, the sample was centrifuged at 4000 rpm for 3 minutes followed by the addition of 300 µl of Buffer RLT (Qiagen). The microtubes were tightly sealed with new caps, and manually shaken back and forth in an upright position 20 times. Then, the microtubes were vortexed upside down at full speed for 20 seconds and centrifuged at 4000 rpm for 5 minutes at room temperature. DNA extraction was completed using the high-throughput BioSprint 96 DNA-liquid handling workstation and the BioSprint 96 DNA Plant Kit (Qiagen) according to the manufacturer's instructions.

The BioSprint system uses magnetic particle technology for DNA purification. Purified DNA was eluted in 200 µl 0.1X TE Buffer at pH 8.0 (Ambion, Austin, Texas). As both DNA quality and quantity are critical for genotyping, both parameters were carefully assayed. DNA yield and purity were determined by measuring absorbance at 260 nm (A_{260}) and the ratio of absorbance at 260 nm and 280 nm (A_{260}/A_{280}), respectively. DNA concentration of each sample was then adjusted to 50 ng/µl with 0.1X TE Buffer. The quality of DNA was confirmed by running 20 µl of the normalized DNA on a 1%

agarose gel. Twenty microliters of normalized DNA was shipped to the USDA-ARS Biosciences Research Laboratory (Fargo, ND) in PCR plates on ice for genotyping. Five microliters of the 50 ng/µl DNA stock sample was used to run the genotyping assay.

5.2.3 *SNP genotyping and genotype data analysis*

The Ethiopian/Eritrean landrace samples were genotyped with the barley iSelect SNP chip of the expanded barley SNP marker platform using the Illumina Infinium II assay first described by Steemers et al. (2006). This assay is a whole-genome genotyping protocol with a single-base extension. Custom iSelect BeadChips can be multiplexed to assay from 3,072 to $1x10^6$ SNPs per sample. Each locus is assayed with an average 12-18x redundancy for each sample, enabling highly accurate genotype calls. The barley iSelect SNP chip contains a total of 7,842 SNPs that comprise the 2,832 existing barley oligonucleotide pooled assay (BOPA1 and BOPA2) SNPs discovered and mapped previously (Close et al. 2009; Muñoz-Amatriaín et al. 2011), plus 5,010 new ones developed from next generation sequencing data (Comadran et al. 2012). The mapping information for the barley iSelect SNPs is available from the Germinate 2.7.2 Teleport iSelect Database at the James Hutton Institute (JHI) (Dundee, Scotland) (http://bioinf.scri.ac.uk). The Illumina Infinium genotyping assay was conducted under the direction of Shiaoman Chao.

The data generated by the Infinium assay were visualized and analyzed with the genotyping module of GenomeStudio data analysis software GSGT, version 1.8.4 (Illumina, San Diego, CA). The software parameters of GenCall score and no-call threshold were optimized. GenCall score is a quality metric calculated for each genotype and ranges from 0 to 1. The GenCall scores generally decrease in value the further a sample is from the center of the cluster to which the data point is associated. The no-call threshold is the lower boundary for calling genotypes relative to its associated cluster. To obtain the highest genotyping accuracy possible, a GenCall score cutoff (no-call threshold) of 0.15 was used. This means that genotypes with a GenCall score less than 0.15 were not assigned genotypes because they were considered to be too far from the

cluster centroid to make reliable genotype calls. The analysis was started with a preliminary sample quality evaluation to determine which samples may require reprocessing or removal. Each SNP was analyzed independently to identify genotypes for the entire set of samples. Visual inspections for quality control were made, and the clusters manually adjusted for SNPs with well-separated clusters. SNPs not clustering as expected and/or SNPs with low call rates were systematically excluded from the dataset prior to calling genotypes again. To improve call rate and accuracy, custom cluster profiles were generated for SNPs that did not fit well into the standard cluster positions. SNPs with overlapping clusters were manually zeroed. Those that could be scored for the majority of samples and with a 95% call frequency were retained. After all the quality evaluations, genotype calls were generated using the GenCall algorithm (version 6.3.0) implemented in the GenomeStudio software.

5.2.4 *Analysis of grain samples for zinc and iron concentration and fifty seed weight*

Two sets of seed samples of the germplasm panel were used for analysis of zinc and iron concentration. The first consisted of seeds from the third selfed generation (S_3) produced in a greenhouse during the winter months of 2011. The second sample consisted of seeds grown from the S_3 generation at the Minnesota Agricultural Experiment Station in St. Paul during the summer of 2012. The experiment was conducted in a randomized complete block design with two replicates. Each accession was harvested at maturity, and the grain samples dried in an air dryer oven at 35°C for two days. When conducting studies of micronutrient concentration in grain samples, contamination from dust and possibly threshing equipment can occur. To monitor the former, concentrations of aluminum were taken in addition to zinc and iron. To eliminate the latter source of contamination, all samples were hand-threshed using non-metallic equipment. Multiple replicates of the following controls were included in the evaluations to monitor data outputs: (1) the hulless two-rowed variety Atahualpa with high zinc and iron concentration (El-Haramein and Grando 2010); (2) the hulless two-rowed Ethiopian barley cultivar with high-protein and lysine concentration [Hiproly (PI 60693)]; and (3) other standard controls (referred to as “rose leaf verification standard” plus a multi-

element certified standard) used by the analysis laboratory to confirm quantification and identification of corresponding elements in the analyses (B. Barber, personal communication). Atahualpa and Hiproly were not grown in same environment as the test lines in this study.

The micronutrient analyses were conducted at the Research Analytical Laboratory on the St. Paul campus of the University of Minnesota. The dry ashing method was used. Briefly, all samples were ashed in a muffle furnace for 12 h at 485°C. Then, the ash was dissolved in 5 mL of 20% HCl followed by a dilution with 5 mL of deionized water. Elemental determinations were performed with a simultaneous multi-element inductively coupled plasma-optical emission spectrometer (ICP-OES) (Perkin Elmer Optima 3000 ICP-OES; Perkin Elmer, Waltham, MA). The ICP-OES provides concentration assays for several elements, including zinc and iron in mg/Kg (ppm). Seed weight was determined based on 50 arbitrarily selected kernels from each landrace from the S_3 generation. All marker and phenotypic data used in these studies are available at http://triticeaetoolbox.org/barley/.

5.2.5 *Statistical analysis of phenotypic traits*

Unbiased mean estimates for zinc and iron, and an analysis of variance were generated through the restricted maximum likelihood (REML) method in SAS (SAS Institute 2008). REML is commonly used to generate estimates of variance and covariance parameters for fitting mixed linear models (Meyer and Kirkpatrick 2005), a type used in GWAS. For heritability estimation, variance components of each trait were generated by "proc varcomp" in SAS. Heritability was calculated using the formula $h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_e^2/r)$, or $h^2 = \sigma_g^2 / [(\sigma_g^2) + (\sigma_y^2/y) + (\sigma_e^2/ry)]$, where σ_g^2 and σ_e^2 are the genotypic and error variance, respectively, r is the number of replications, and y is the number of trial years. Pearson correlation coefficients were calculated to assess phenotypic correlation among traits.

5.2.6 *Population structure*

Population structure can be present in GWAS panels assembled from diverse geographic regions and during different eras. To account for this effect and decrease the number of false-positives in GWAS, the most likely number of subpopulations in the panel was investigated. The clustering program STRUCTURE (version 2.3.4) (Falush et al. 2003; Pritchard et al. 2000a; Pritchard et al. 2000b) applies a parametric genetic mixture analysis and was used to estimate the membership probability of each barley landrace to a number of hypothetical subpopulations (*K*). STRUCTURE software is a Bayesian model-based clustering method that infers genetic clusters (subpopulations) of individuals on the basis of their genotypes at multiple loci using a Markov Chain Monte Carlo (MCMC) maximum-likelihood approach that compares posterior probabilities of different numbers of possible population configurations. To avoid overestimation of subpopulation divergence caused by tightly linked SNP markers (Falush et al. 2003), a subset of markers with approximately 10 cM spacing was selected for the analysis. This SNP subset was used to calculate the subpopulation membership matrix (Q), which is the fractional subpopulation membership for each landrace based on its inferred ancestry. There were no *a priori* population designations assumed for the landraces. The program was run by setting most parameters to default values according to the instructions in the user's manual (Pritchard et al. 2010).

Subpopulation numbers ranging from 1 to 20 were tested using the admixture model and considering allele frequencies of SNPs correlated among populations. The correlated allele frequency setting is considered favorable when there is subtle population structure (Falush et al. 2003). The program was first run using a burn-in period of 10,000 cycles followed by 50,000 MCMC iterations with 20 independent runs at each of K=1 to 20 (Falush et al. 2003; Pritchard et al. 2000a). Posterior probability $\Pr(X|K)$ of each K was generated by the program and $\ln\Pr(X|K)$ plotted against each value of K. The optimal K was estimated based on the value and variation of $\Pr(X|K)$ (Rosenberg et al. 2001) as well as the rate of $\ln\Pr(X|K)$ change from K-1 to K (ΔK). The ad hoc quantity (ΔK) was calculated based on the second order rate of change of the likelihood function with respect to K (Evanno et al. 2005). ΔK usually shows a peak at the true value of K. The program was then run using a burn-in period of 100,000 cycles followed by 200,000

MCMC iterations with 20 independent runs at each of K=3 to 10. The optimal K value was identified based on the value and variation of Pr(X|K) and ΔK. Then, value tables for matrix Q, allele-frequency divergence among presumed supopulations, and average distances (expected heterozygosity) between individuals in the same cluster were generated using the determined optimum K value.

Principal component analysis (PCA), a multivariate analysis method that transforms a set of correlated variables (genetic markers in this case) into a smaller number of uncorrelated variables, also was used to infer population structure (Zhao et al. 2007). PCA analysis was conducted using the software program TASSEL (version 4.0) (http://www.maizegenetics.net; Bradbury et al. 2007). To satisfy the PCA requirement that all variables should have variation and no missing values, SNP markers with minor allele frequency (MAF) $\leq$0.05 were eliminated and missing values were imputed. A matrix with principal components (PCs) that represent population structure and eigenvalues (the variance explained by each of the PCs) were generated. Population stratification was visualized by plotting the first three PCs.

5.2.7 *Linkage disequilibrium*

LD between markers was measured using Haploview v4.2 (Barrett et al. 2005). Using large number of markers affects association mapping due to correcting for multiple hypothesis testing. Applying fewer markers may better enable detection of statistically significant markers associated with a trait. Thus, to reduce the number of markers by selecting independent sets of SNPs for running association mapping, markers with an r^2 $\geq$0.8 were considered to be dependent with each other. The SNP tagger in Haploview was used to test for independence of markers to select those with an r^2 <0.8. In cases where multiple SNP markers mapping to the same location or region were found significantly associated with a trait, LD, as measured by r^2 among the neighboring markers, was calculated and presented as a "heat" map in some cases.

5.2.8 *Association mapping*

To identify QTL for zinc and iron concentration and fifty seed weight, association analyses were conducted between SNP markers and the respective phenotypic traits. A mixed linear model (MLM), with 'efficient mixed model association' (EMMA) method (Kang et al. 2008) was implemented in the GWAS package *rrBLUP* (version 4.1) in software R (version 2.15.2). The mixed model equation used by the *rrBLUP* package is based on the mixed model (Yu et al. 2005): $y = X\beta+u+e$, where y is a vector of phenotypic observation; X is a vector of SNP marker genotypes; β is a coefficient of the SNP marker effect being estimated; P is a matrix of principal component vectors accounting for population structure (estimated using the first four top principal components as covariates); v is a coefficient of population structure effect; u is a vector of random (polygene background) effects (effect of individual relatedness estimated as pairwise kinship coefficients); and e is a vector of residual effects. In the MLM, marker effect (Xβ) represents fixed effect, and relative kinship and residuals represent random effects. The variances of the random effects are given as: $Var(u) = 2KV_g$ and $Var(e) = V_R$, where *K* is an *n* x *n* matrix of kinship inferred from genotypes based on the proportion of shared allele values between a pair of individuals (*n*: number of individuals in the GWAS panel); V_g is the genetic variance; and V_R is the residual variance. The *rrBLUP* package in R for GWAS incorporates methods for obtaining best linear unbiased estimates (BLUE) of β (fixed effect) and best linear unbiased predictions (BLUP) of u (random effect) as originally developed (Kang et al. 2008; Yu et al. 2005). The EMMA association analysis method then fitted each SNP into the MLM individually and generated marker scores [$-\log_{10}$(*P*-values)].

The *rrBLUP* GWAS function incorporates a package known as QVALUE (Storey and Tibshirani 2003) for multiple testing using the Benjamini-Hochberg False Discovery Rate (BH-FDR) (Benjamini and Hochberg 1995) and handles permutations of the *q*-value (an FDR adjusted *P*-value) for each test internally. At the end of the analysis, the package draws a "bar graph" as a GWAS result with the $-\log_{10}$(*P*-values) and a threshold line (FDR=*q*-value=0.05) delineating significant markers. Then, the FDR adjusted marker scores were outputted from which *P*-values were calculated.

5.2.9 *Candidate gene scan using Basic Local Alignment Search Tool (BLAST)*

Source sequences of SNP markers significantly associated with the traits were used to identify putative candidate genes in the rice (http://rice.plantbiology.msu.edu/) and *Brachypodium* (http://www.phytozome.net/) genomes, and in the National Center for Biotechnology Information (NCBI) database (http://blast.ncbi.nlm.nih.gov) using BLAST. In the rice and *Brachypodium* genomes, a 150 kilo base pair (kbp) window was scanned in each direction of regions homologous to the sequence of the significant SNP. In the NCBI database, a BLASTX (searching protein database using a translated nucleotide query) search was implemented to determine if sequences of significant SNP markers correspond to a known protein in barley, wheat, maize, sorghum or *Arabidopsis*.

5.3 Results

5.3.1 *Phenotypic variation for zinc concentration from greenhouse and field experiments*

Grain samples of the EEBC (Ethiopian and Eritrean Barley Collection) accessions obtained from greenhouse and field grown plants were used for assays of zinc concentration. Barley cultivar Atahualpa, included as a control in the analyses of the EEBC samples from both environments, yielded high levels of zinc: mean (and median) of 56.57±4.50 mg/Kg (57.53 mg/Kg) and 49.04±4.04 mg/Kg (47.90 mg/Kg) in the analyses of samples grown in the greenhouse and field, respectively (Figure 5.1A). For Hiproly, a cultivar from Ethiopia known for other nutritional attributes, the mean (and median) concentration of zinc in the analyses of samples from the respective environments was 71.74±3.18 mg/Kg (73.12 mg/Kg) and 60.64±5.66 mg/Kg (61.36 mg/Kg), respectively (Figure 5.1A). Overall, accessions of the EEBC sampled from the greenhouse generally had lower zinc concentrations than those from the field. Mean (and median) zinc concentrations of 30.72±8.66 mg/Kg (29.31 mg/Kg) and 48.46±11.67 mg/Kg (47.02 mg/Kg) were obtained from samples produced in the greenhouse and field, respectively (Figure 5.1A). The minimum and maximum zinc concentrations produced by EEBC accessions harvested from the respective environments were 6.23 and 71.96 mg/Kg and 19.69 and 87.42 mg/Kg, respectively (Figure 5.1A). Three EEBC accessions

(EEBC236, EEBC257 and EEBC297) had a higher zinc concentration than the mean plus one standard deviation of Atahualpa in the analysis of greenhouse samples (Figure 5.1A). In the analysis of samples from the field, 27.24% (73/268) of the accessions had a higher zinc concentration than the mean plus one standard deviation of Atahualpa (Figure 5.1A). The correlation coefficient for zinc concentration between greenhouse vs. field grown samples was low, but significant (r=0.26). The heritability/reproducibility of zinc concentration in EEBC germplasm sampled from the field was 0.65 based on entry means (Table 5.1). Heritability was not estimated from the greenhouse samples because only one replicate was analyzed for zinc concentration.

5.3.2 *Phenotypic variation for iron concentration from greenhouse and field experiments*

Grain samples obtained from both greenhouse and field grown plants also were assayed for iron concentration. Atahualpa, a control cultivar known to have a high iron concentration, exhibited a mean (and median) iron concentration of 58.40±2.54 mg/Kg (58.59 mg/Kg) and 51.16±3.83 mg/Kg (51.20 mg/Kg) from the analysis of samples from the greenhouse and field, respectively (Figure 5.1B). For Hiproly, mean (and median) iron concentrations of 48.94±2.16 mg/Kg (48.96 mg/Kg) and 45.07±1.79 mg/Kg (44.60 mg/Kg) were obtained from the analyses of samples from the greenhouse and field, respectively (Figure 5.1B). As was the case for zinc, the EEBC germplasm sampled from the greenhouse generally had lower iron concentrations than those from the field (Figure 5.1B). EEBC germplasm had a mean (and median) iron concentration of 37.66±10.09 mg/Kg (36.21 mg/Kg) and 56.07±11.32 mg/Kg (55.15 mg/Kg) from samples grown in the greenhouse and field, respectively (Figure 5.1B). The minimum and maximum iron concentrations produced by EEBC accessions harvested from the greenhouse and field were 7.73 and 74.78 mg/Kg and 27.26 and 109.60 mg/Kg, respectively (Figure 5.1B). Although Atahualpa was not grown in the same environment as the landraces, eight EEBC accessions had a higher iron concentration than the mean plus one standard deviation of Atahualpa in the analysis of greenhouse samples (Figure 5.1B). In the analysis of samples from the field, 41.49% (138/268) of the accessions had a higher iron concentration than the mean plus one standard deviation of Atahualpa (Figure 5.1B). The

correlation coefficient for iron concentration between greenhouse vs. field grown plants was positive, but very low (r=0.10) and non-significant. Heritability/reproducibility of iron concentration in EEBC germplasm sampled from the field was 0.59 based on entry means (Table 5.1). Heritability was not estimated from the greenhouse samples because only one replicate was analyzed for iron concentration. Correlation coefficients for zinc vs. iron concentration from greenhouse (r=0.52) and field (r=0.63) samples were positive and highly significant.

The mean (and median) fifty seed weight of EEBC germplasm was 2.57±0.45 g (2.63 g) (ranging from 1.38 to 3.75 g) (data not shown). Heritability was not determined as only one replicate of data was available. With respect to correlation, fifty seed weight was significantly correlated with zinc (r=0.23) and iron (r=0.20) concentration of field grown samples. Correlation coefficients between fifty seed weight and zinc and iron concentration in grain samples grown in the greenhouse were positive but very low (r=0.03 and 0.08, respectively) and non-significant.

5.3.3 *Marker data*

The platform used for genotyping the GWAS panel consisted of 7,842 SNPs of the barley iSelect SNP chip. Of these, 2,832 SNPs were previously mapped (Close et al. 2009; Muñoz-Amatriaín et al. 2011). The remaining 5,010 SNPs were newly developed from next generation sequencing data (Comadran et al. 2012). After quality evaluations during genotype calling, 85% (6,702/7,842) of the SNPs generated reliable allele data with 95% call rate for all accessions. Nearly 99% of these SNPs (6,615/6,702) had a >95% call frequency. Filtering based on minor allele-frequency (MAF) resulted in 5,269 SNPs with a MAF >5%. This final set of SNP markers was used for association analyses. Of this set, 72.5% (3,818/5,269) have known map positions on the seven barley chromosomes spanning 1,112.71 cM of the genome, whereas the remaining 1,451 SNP markers have no known chromosomal position. The consensus map of the SNPs used was generated by merging the map from the James Hutton Institute (JHI) (Dundee, Scotland) with the 11 linkage maps from the Barley Coordinated Agricultural Project (Barley CAP)

(J. Endelman, personal communication). The vast majority of markers with unknown map positions were among the newly developed markers and had SCRI (Scottish Crop Research Institute; the former name of the JHI) designations. Both mapped and unmapped markers were used for running GWAS. Of the mapped SNPs, 38.6% (1,473/3,818) had unique map positions and the rest had redundant map locations. The average density of SNP markers across the genome using all mapped SNPs and unique SNPs was 3.43 and 1.32 SNP markers per cM, respectively. Gaps between SNP markers ranged from 0 to 9.32 cM with a mean of 0.77 cM. Only eight gaps of >5 cM were identified: two each on chromosomes 1H and 7H, and one each on chromsomes 2H, 3H, 5H and 6H.

5.3.4 *Population structure*

STRUCTURE and PCA were implemented for population structure analysis. From the STRUCTURE analysis, six subpopulations were delineated. However, no clear clustering of two- vs. six-rowed groups was obtained as is often found in advanced breeding germplasm (citation). Two-rowed landraces were grouped into subpopulations 1, 2, 4 and 5, whereas six-rowed ones were grouped into subpopulations 1, 3 and 6. Moreover, no distinct clustering was found for accessions from the different countries of Ethiopia (269 accessions) and Eritrea (29 accessions). Accessions from the ICARDA genebank were mainly grouped into subpopulations 3 and 4, and those from the VIR genebank were mainly clustered in subpopulations 1, 3, 4 and 6. Subpopulations 1, 2, 5 and 6 were mainly composed of landraces from the NSGC genebank. Subpopulations 3 and 6 were the most genetically heterogeneous as revealed by the highest expected heterozygosity values between individuals in clusters.

Based on the PCA analysis, there was no readily apparent population structure differentiating the germplasm. The only apparent clustering in the PCA plots was for the landraces that were obtained from the NSGC genebank. Thus, there is a lack of apparent population structure in the association mapping panel. Therefore, the possible presence of

any sample structure was corrected for using the pair-wise relatedness between individuals through application of the kinship (K) model.

5.3.5 *Genome-wide association mapping*

To identify QTL for zinc and iron concentration in the grain of Ethiopian and Eritrean barley landraces, GWAS was conducted with trait data from the greenhouse and field. For fifty seed weight, data from greenhouse grown samples only were used for GWAS. A significant association was automatically declared by the *rrBLUP* GWAS package used for the analyses. After correction for multiple testing, markers with a *q*-value (an FDR adjusted *P*-value) <0.05 were considered as truly significant. Given that an FDR adjusted *P*-value threshold of 0.05 means that 5% of significant tests will result in false positives, number of spurious associations was greatly reduced.

5.3.5.1 *Genome-wide association mapping of barley spike morphology*

As a validation of the GWAS model for reliability of localizing true marker-trait associations and evaluating mapping resolution within the germplasm panel, GWAS was conducted on the spike morphology trait of row type. Barley is classified into two-rowed and six-rowed types, which is a result of having either two or six rows of florets being fertile. This trait is mainly controlled by the well-characterized Mendelian gene *Vrs1*, located on chromosome 2H, as well as other genes. Alleles at the *Vrs1* locus and some of the other spike morphology genes have been cloned (Komatsuda et al. 2007; Pourkheirandish and Komatsuda 2007; Ramsay et al. 2011; Waugh et al. 2009). The GWAS model identified 14 SNP markers significantly associated with the row-type locus: eight on the long arm of chromosome 2H, one each on chromosomes 4H and 5H, and four unmapped markers (Table 5.2; Figure 5.2A). BLAST search of the NCBI database with the significant SNPs on chromosome 2H identified the coding sequences of the *H. vulgare* homeodomain-leucine zipper protein (*Vrs1*) gene with ≥99% identity, indicating that the SNP markers are likely located within the coding region of the gene. The significant SNPs on chromosome 2HL were mapped within a short distance (4.03 cM) of each other (Table 5.2) and are most likely detecting the same *Vrs1* locus. LD as

measured by r^2 ranged from 0.004 (between SCRI_RS_171032 and 12_30897) to 0.99 (between 12_30897 and SCRI_RS_196853 (Figure 5.2B). LD between the most significant marker (12_30896) and the rest of the markers ranged from 0.10 (with SCRI_RS_221886) to 0.88 (with 11_20340), indicating that some of the markers are in high LD with the underlying gene controlling spike morphology.

The significant marker on 4H maps to the reported position of INTERMEDIUM-C (*Int-c*) gene (Cuesta-Marcos et al. 2010; Lundqvist and Franckowiack 1997; Komatsuda and Mano 2002) that influences both the fertility of the lateral spikelets on the inflorescence and the amount of the basal branching (tillering) in barley (Ramsay et al. 2011). The one significant marker on chromosome 5H is most likely detecting the six-rowed spike 2 (*vrs2*) or the intermedium spike-b (*int-b*) locus (Lundqvist and Franckowiack 1997). Some or all of the unmapped SNPs are likely located near or at *vrs1*, *vrs2*, *int-b*, *Int-c* or some other spike morphology genes described on chromosome 1H (Cuesta-Marcos et al. 2010). Our results are in close agreement with previous studies (Figure 5.2B). This GWAS analysis indicates the strong power of the association mapping model to account for pairwise relatedness and achieve good mapping resolution within the germplasm panel. Accordingly, the association mapping model was used for marker-trait associations of other phenotypes.

5.3.5.2 *Genome-wide association mapping of zinc and iron concentration and fifty seed weight*

GWAS of zinc and iron concentration were conducted on grain samples from greenhouse and field grown plants. For zinc concentration of grain samples produced in the greenhouse, GWAS identified four significantly associated SNP markers (SCRI_RS_10655, 12_10051, 12_30355 and SCRI_RS_234548) on chromosome 6H (Table 5.2; Figure 5.3A). The most significant marker was SCRI_RS_10655; thus, the zinc concentration QTL was designated *Zn-qtl-6H_SCRI_RS_10655* (128.72 cM) (Figure 5.3A). The QTL was named according to the following convention: *Zn-*, indicating the trait abbreviation; *qtl-*, indicating the quantitative nature of the trait; *6H-*, indicating the

chromosome upon which the marker lies; and SCRI_RS_10655, indicating the name of the most significant marker associated with the trait on chromsomes 6H. Three of the significant SNPs on chromosome 6HL had the same exact genetic position and were linked within a short distance (5.73 cM) of SCRI_RS_10655 (Table 5.2). Thus, they may be detecting the same QTL. From LD analysis, the three significant markers mapping at the same locus were in perfect LD (r^2=1) with each other and were in strong LD with marker SCRI_RS_10655 (r^2 ranging from 0.33 to 0.34) (Appendix Figure 5.1). In addition, SNP marker 12_11485 on the short arm of chromsome 4H had a low *P*-value of $1.34x10^{-4}$, but it was not significant based on the FDR threshold (Figure 5.3A). Among other factors, correction for multiple hypothesis testing might have affected detection of significantly associated markers due to the large number of markers used in the analysis. Thus, GWAS was conducted using 2,430 markers considered to be independent based on linkage disequilibrium (with an $r^2 < 0.8$) (Massman et al. 2011). However, no additional significant marker-trait associations were detected using this marker set (data not shown). Finally, GWAS was conducted only with markers mapping to unique chromosomal locations to further reduce the number of markers. Retaining only one marker per unique location and omitting the redundant ones resulted in a final set of 1,534 SNPs that were well distributed throughout the genome. Using this reduced SNP set, the 12_11485 SNP marker on chromosome 4H and the SCRI_RS_10655 on 6H were found significantly associated with zinc concentration in the greenhouse dataset (Table 5.2; Figure 5.3B). The zinc concentration QTL on the short arm of chromosome 4H was designated *Zn-qtl-4H_ 12_11485* (12.91 cM) after the significant marker 12_11485 (Table 5.2; Figure 5.3B).

No marker was significantly associated with zinc concentration from the field samples using either the full or reduced set of SNP markers (Appendix Figure 5.2A). Likewise, no significant associations were detected in the GWAS of iron concentration from either the greenhouse or field samples (Appendix Figure 5.2B and C). Since there were no SNP markers with *P*-values close to the FDR threshold (Appendix Figure 5.2A, B and C), GWAS with a reduced number of independent or unique markers did not yield

any significant associations for either zinc concentration (from field samples) nor iron concentration (from either greenhouse and field samples).

GWAS of fifty seed weight identified five (three mapped and two unmapped) significantly associated SNP markers (Table 5.2; Figure 5.3C). The three mapped markers were located within a very short genetic distance (0.10 cM) of each other on the long arm of chromosome 2H and were likely detecting the same QTL. With regard to LD, these three markers were in strong LD with each other. Marker 12_30896, mapping 0.10 cM away from the other two markers, was in high LD with markers 12_30901 and 11_20340 (r^2=0.37 and 0.88, respectively). The markers 12_30901 and 11_20340 had the same map position and were in strong LD (r^2=0.39) with each other. The most significant marker identified was 11_20340 (90.99 cM) (Table 5.2; Figure 5.3C); thus, the QTL associated with this trait was designated as *50Swt-qtl-2H-11_20340* with *50Swt-* indicating the trait abbreviation. This QTL also was associated with row-type morphology (see proceeding section). When GWAS was conducted separately in the two- and six-rowed datasets, no significant associations were identified for fifty seed weight (data not shown).

5.3.6 *Allele effect of significant markers on trait*

The allele frequency and allelic effect of each significant marker in increasing levels of grain zinc concentration and fifty seed weight were estimated (Table 5.3). The favorable allele (minor allele "B") of the significant markers associated with zinc (12_11485 and SCRI_RS_10655) provided a mean concentration of 33.09 to 36.52 mg/Kg (Table 5.3). This is an increase of 10.78 to 23.84% compared to the 29.87 and 29.49 mg/Kg mean zinc concentration in the accessions without the allele. For fifty seed weight, the accessions containing the minor allele "B" of the significant markers 11_20340 and 12_30896 and minor allele "A" of the significant markers 12_30901 and SCRI_RS_165473 had a mean fifty seed weight ranging from 2.68 to 2.96 g (Table 5.3). This is an increase of 7.20 to 19.84% compared to the mean fifty seed weight of accessions without the favorable alleles.

5.3.7 *Candidate gene scan using BLAST*

Sequences of SNPs identified as associated with zinc concentration on chromosomes 4H and 6H were used to examine the rice (http://rice.plantbiology.msu.edu/) and *Brachypodium* (http://www.phytozome.net/) genomes, and NCBI database (http://blast.ncbi.nlm.nih.gov) using BLAST search. No homology was found in the BLAST search with the 12_11485 sequence in both the rice and *Brachypodium* genome databases. In the NCBI database, the only hit was a *H. vulgare* subsp. *vulgare* cDNA clone. BLAST search with the EST sequence of SCRI_RS_10655 in the rice genome identified a zinc finger C3HC4-type family protein as one of the most significant hits. This protein family has diverse roles in plant physiologic processes, including homeostasis, development, cell division, growth, and hormone and stress responses (Smalle and Vierstra 2004). Another homologous sequence identified from the rice genome was a VQ (valine-glutamine) domain containing protein, which like the zinc finger C3HC4-type family protein, has an apparently similar role in plant growth, development, and response to environmental conditions—most likely by acting as a cofactor of transcription factors (Cheng et al. 2012). No homology was found for any of the sequences in the *Brachypodium* genome database. Using BLAST in the NCBI database, a predicted protein in *H. vulgare* subsp. *vulgare* and a pentatricopeptide repeat (PPR)-containing protein in *B. distachyon* were found as the most significant hits. PPR proteins regulate transcription in the mitochondria and chloroplasts, perhaps through binding to organellar transcripts (Lurin et al. 2004).

The sequence of the most significant SNP (11_20340) found associated with fifty seed weight on chromosome 2H was examined by BLAST search in the rice, *Brachypodium* and NCBI genome databases. The BLAST searches identified a vacuolar-processing enzyme in *Brachypodium*, *Zea mays*, and *Sorghum bicolor* and a vacuolar-processing enzyme precursor in rice. Vacuolar processing enzymes are essential for proper processing of seed storage proteins in *Arabidopsis* and rice (Shimada et al. 2003; Kumamaru et al. 2010; Wang et al. 2009).

5.4 Discussion

Zinc and iron are essential micronutrients for human growth, development, and maintenance of the immune system (Walker et al. 2005). Deficiencies in zinc and iron nutrition are widespread worldwide, especially among children and women in developing countries (Chandyo et al. 2009) and in poor families of some developed countries. Meat products and legumes are especially rich in zinc and iron (http://www.livestrong.com); however, in resource-poor parts of the world, these food sources are too expensive and therefore not available to supplement cereal-based foodstuffs, which are inherently low in micronutrient composition, especially if grown on soils deficient in such minerals (Cakmak 2008). Ethiopia is a developing country where zinc and iron deficiencies are prominent public health problems (Gebremedhin et al. 2011; Haidar et al. 2005). In addition to other agronomic and nutrient supplementation methods, the amount of these trace elements could be enhanced through improving the dietary composition of locally grown food crops (Mayer et al. 2008; White and Broadley 2011).

In the current study, two-hundred and ninety-eight (298) barley landraces collected from Ethiopia and Eritrea were sampled for their zinc and iron concentration and kernel weight to study their diversity for the traits and to possibly identify genetic loci controlling them through a GWAS approach. Among the 298 accessions grown in the field, more than one-quarter had a higher zinc concentration than Atahualpa, a barley cultivar known to have high zinc and iron levels (El-Haramein and Grando 2010). However, in greenhouse-grown grain samples, only three accessions had a higher zinc concentration than Atahualpa. Eight (2.99%) and 138 (41.49%) of the accessions had a higher iron concentration than Atahualpa in the greenhouse and field-grown grain samples, respectively. One shortcoming of these comparisons is that Atahualpa was not grown in the same environment as the EEBC accessions. Field samples of grain from the landraces had consistently higher levels than the greenhouse grown samples. This may be related to differences in the amount of zinc in the field soil and in the potting mix used to grow plants in the greenhouse.

The correlation coefficients for zinc and iron concentration between the greenhouse and field samples were low (r=0.10 to 0.26). In general, zinc and iron concentration were lower in the greenhouse samples. Coefficients of correlation between the concentration of the respective micronutrients in the greenhouse and field also were low. This was probably due to differences in crop husbandry practices and soil fertility between the greenhouse and field experiments. Zinc- and iron-deficient soils affect plant growth, and therefore result in a deficiency of micronutrients in food crops (Sillanpaa 1982).

To obtain more robust micronutrient data in future studies, all plants should be grown in a highly uniform standard plant growth mix for greenhouse experiments and in replicated multi-location sites for field experiments. This was not possible in the present study given the limited seed and resources for testing the micronutrient concentration. Rather, this was an initial exploratory study to assess the diversity of these traits in this unique cultivated germplasm.

Natural contamination of iron via dust can occur in the field (El-Haramein and Grando 2010) or during threshing. The concentration of aluminum can be used to monitor possible contamination from dust. A concentration of <3 mg/Kg is a natural background level for aluminum (El-Haramein and Grando 2010). In our analysis, the mean aluminum concentration in greenhouse and field samples were 2.54 and 3.79 mg/Kg, respectively. These aluminum levels are very similar to each other and to normal background levels; thus, background contamination of iron from dust was likely negligible. No significant contamination of zinc by dust has been reported (El-Haramein and Grando 2010).

Zinc and iron concentration were highly and positively correlated with each other both in the greenhouse (r=0.52) and field (r=0.63). These values are comparable to a correlation coefficient of 0.64 reported between iron and zinc concentration in the field in Syria (El-Haramein and Grando 2010). A significant correlation between grain zinc and iron concentration also was reported in wheat (Genc et al. 2009). This suggests that the traits might have some common genetic mechanisms underlying their uptake,

accumulation, or concentration. This is a desirable phenomenon because of the possibility to enhance the concentration of both micronutrients simultaneously. The heritability of zinc and iron concentration in the grain was 0.65 and 0.59 in the field, respectively. El-Haramein and Grando (2010) reported heritability of 0.64 and 0.61 for zinc concentration, and 0.59 and 0.56 for iron concentration in the grain of barley landraces from 34 countries grown in two separate fields in Syria. This indicates that the heritability of the traits is very much consistent across germplasm and sites of evaluation.

The concentration of zinc and iron in the Ethiopian/Eritrean landraces was higher than that reported for landraces from many different countries. Analysis of 333 barley landraces from 34 countries indicated that those from Ethiopia were a promising source of high zinc and iron levels in grain next to those from Morocco (El-Haramein and Grando 2010). In the same study, one Ethiopian landrace had a high range of zinc and iron concentrations of 32.70 to 39.20 and 28.30 to 45.80 mg/Kg, respectively. The maximum zinc and iron concentrations in this one Ethiopian landrace were, however, lower than the maximum amounts found in the current analysis by more than one-half. In the current study, 42 and 209 accessions had zinc concentrations higher than 39 mg/Kg in the greenhouse and field, respectively. Whereas, 112 and 228 accessions had iron concentrations higher than 46 mg/Kg in the greenhouse- and field-grown samples, respectively. Differences in soil fertility levels where the accessions were grown might have contributed to the different zinc and iron levels detected in the two studies. A genetic variation study in grain iron concentration was conducted in 274 “standard” Japanese varieties at Okayama University in Japan, and 274 varieties from the barley core collection of the USDA-ARS NSGC in Aberdeen, ID (Ma et al. 2004). The study revealed iron concentrations ranging from 24.6 to 63.3 mg/Kg in the “standard” varieties and 21.0 to 83.0 mg/Kg in the NSGC core set. The Ethiopian and Eritrean landraces in this study exhibited a wider range of variation (ranging from 7.73 to 109.60 mg/Kg) in iron concentration.

Yan et al. (2013) investigated the grain zinc and iron concentration of 92 wild barley accessions from Israel and 10 barley cultivars from China. The authors found zinc

and iron concentrations ranging from 66.30 to 493.90 mg/Kg (mean=173.90 mg/Kg) and 10.80 to 329.10 mg/Kg (mean=74.30mg/Kg), respectively, in the wild barley genotypes. The range of zinc and iron concentrations found in the Ethiopian and Eritrean landraces in our study are lower than reported in these wild barley accessions. Yan et al. (2013) indicated that barley cultivars from China exhibited lower values and lower diversity for grain zinc and iron concentration than the wild accessions. On the other hand, the mean iron concentrations we found (56.07 mg/Kg) in the landraces was relatively lower, but comparable to wild barley (74.30 mg/Kg). Wild barley often exhibits greater genetic variation than landrace or cultivated germplasm for many economically important traits, including those of nutritional value. However, due to the linkage of "undesirable" genes in wild relatives with agronomically or nutritionally important traits, more advanced cultivated germplasm is preferable for use in breeding. The variation in zinc and iron concentration in Ethiopian and Eritrean landraces is valuable because landraces are favored over wild barley germplasm in breeding. In addition, since landraces are the primary seed stocks used for cultivation in Ethiopia, those with enhanced micronutrient concentrations can readily be used directly for production in the country.

Thousand-kernel weight is one of several important food quality traits in barley (El-Haramein and Grando 2010). Due to limitations in seed stocks, we assessed the weight of just 50 seeds. The mean fifty seed weight of the Ethiopian/Eritrean GWAS panel was 2.57 g (range of 1.38 to 3.75 g). Extrapolation of 50-seed weights (mean and range) into 1000-kernel weights gives a mean of 51.40 g (range of 27.60 to 75.00 g) for the Ethiopian/Eritrean germplasm. These latter values are close to the 1000-kernel weight range of 19.30 to 64.50 g reported by El-Haramein and Grando (2010) for barley landraces from 34 countries. This limited comparison suggests that the estimated 1000-kernel weights of Ethiopian and Eritrean landraces is relatively higher than those reported for other landrace germplasm.

The ultimate goal of the current work was to identify and utilize germplasm with high levels of micronutrients (zinc and iron) in breeding programs so that cultivars with more balanced nutrition can be developed. In fact, landraces from Ethiopia can essentially be

used directly as they are still the majority type grown in the country. Marker assisted or genomic selection would be a preferable means to transfer genes/QTL coding for high zinc and iron concentrations from the Ethiopian landraces into adapted breeding lines in other countries.

GWAS identified two SNP markers one each on chromosomes 4H (12_11485) and 6H (SCRI_RS_10655) associated with grain zinc concentration in greenhouse produced grain, and five markers, either on 2H (11_20340, 12_30896 and 12_30901) or at unknown positions (SCRI_RS_165473 and SCRI_RS_72983), associated with kernel weight (Table 5.2). The SNP markers associated with zinc concentration from greenhouse grown plants are perhaps important, but would be particularly useful for development of zinc-rich cultivars if they were detected in the field as well. Zinc concentration was analyzed in grain produced from the field during one growing season, but no significantly associated markers were identified.

The genetics of zinc accumulation in barley grain has been documented in a few studies. Lonergan et al. (2009) identified several QTL, on chromosomes 1H, 2H and 5H, associated with grain zinc concentration in a doubled haploid mapping population of barley. The double haploid mapping population utilized by Lonergan et al. (2009) was developed from a cross between an Algerian landrace, Sahara 3771 (high zinc accumulator) and the Australian barley cv. Clipper (low zinc accumulator). In the current study, no QTL associated with zinc concentration were detected on these chromosomes. A QTL linked to grain zinc concentration was reported on chromosome 4HS in doubled haploid lines grown in a glasshouse (Sadeghzadeh 2008). The *Zn-qtl-4H_12_11485* (12.91 cM) QTL associated with grain zinc concentration on 4HS in the current study is distal to the one identified by Sadeghzadeh (2008) by about 50 cm and is therefore unique. Lonergan et al. (2001) also found a QTL on 4HL that was associated with leaf zinc concentration in barley. However, as it lies on the other arm of the chromosome, this QTL cannot be the same as *Zn-qtl-4H_12_11485.* The *Zn-qtl-6H_SCRI_RS_10655* (128.72 cM) QTL reported here is also likely unique as no other QTL controlling this micronutrient have been reported on chromosome 6H in the literature.

Zinc is a trace element vital for all life forms with its role in gene expression, cell division and replication (Tauris et al. 2009). Zinc also is an essential cofactor for over 300 enzymes that are involved in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as in the homeostasis of other micronutrients (Broadley et al. 2007). Proteins known as zinc finger C3HC4-type family protein and VQ domain-containing protein were identified in the rice genome from BLAST searches with the EST sequence of *Zn-qtl-6H_SCRI_RS_10655*. C3HC4-type zinc finger proteins play diverse roles in plant physiologic processes, including homeostasis, development, cell division, growth, and hormone and stress responses (Smalle and Vierstra 2004). VQ (valine-glutamine) domain-containing proteins apparently have similar roles in plant growth, development, and response to environmental conditions, most likely by acting as a cofactor of transcription factors (Cheng et al. 2012). BLAST search with the *Zn-qtl-6H_ SCRI_RS_10655* sequence in the NCBI database identified pentatricopeptide repeat (PPR)-containing protein. PPR proteins regulate transcription in mitochondria and chloroplasts, perhaps through binding to organellar transcripts (Lurin et al. 2004). Taken together, these results suggest that the *Zn-qtl-6H_SCRI_RS_10655* locus is biologically important for barley and could be targeted for breeding and isolation. The two QTL associated with grain zinc concentration were detected in the greenhouse samples where zinc concentrations were generally lower than those from the field. These two QTL are probably environment-specific, requiring special growing conditions for full expression and should be validated in a future study.

For kernel weight, three markers on the long arm of chromosome 2H (11_20340, 12_30896 and 12_30901) and two markers with unknown positions (SCRI_RS_165473 and SCRI_RS_72983) were found significantly associated (Table 5.2). The three markers on chromosome 2HL also were associated with the row-type morphology (Table 5.2). Spike morphology (two- vs. six-rowed) has a profound effect on kernel weight since two-rowed lines have only two rows of fertile florets and therefore the seeds will be larger due to less competition for photosynthates. GWAS confirmed this as the significant markers lie closely linked to or in the coding region of the homeodomain-leucine zipper I-class homeobox gene representing the row-type locus *Vrs1*. The two unmapped markers

associated with kernel weight may be detecting the same chromosome 2HL locus or possibly another one at a different location. QTL controlling kernel weight/size and the *Vrs1* locus were found to coincide in several bi-parental (Ayoub et al. 2002; Jensen 1996; Kjaer and Marquez-Cedillo et al. 2000; Xue et al. 2010) and some association mapping studies (Comadran et al. 2011). This region of chromosome 2HL also harbors QTL controlling grains per spike, spikes per plant, spike length and grain yield (Comadran et al. 2011; Kjaer and Jensen 1996; Xue et al. 2010; this thesis chapter 4). This indicates that these traits are under the control of tightly linked genes or perhaps the pleiotropic effect of a single gene located in the region.

The search for a candidate gene for the *50Swt-qtl-2H-11_20340* QTL associated with kernel weight identified a vacuolar-processing enzyme in *Brachypodium*, *Z. mays*, and *S. bicolor* and a vacuolar-processing enzyme precursor in rice. A vacuolar-processing enzyme converts proprotein precursors into their respective mature forms (Hara-Nishimura et al. 1991). In *Arabidopsis* and rice, vacuolar processing enzymes are essential for proper processing of seed storage proteins (Kumamaru et al. 2010; Shimada et al. 2003; Wang et al. 2009). Nakaune et al. (2005) showed that a vacuolar-processing enzyme is involved in seed coat formation at the early stage of seed development. In tomato fruits, vacuolar-processing enzymes play roles in controlling sugar accumulation (Ariizumi et al. 2011). The vacuolar-processing enzyme precursor identified from rice is presumably a seed storage protein or related compound that is a target of processing enzymes in vacuoles.

Despite the use of a dense SNP marker map, no QTL associated with grain iron concentration were detected in this study. In fact, no QTL controlling iron concentration have been reported in barley so far. On the other hand, the current study identified SNP markers from two genomic locations (one each on 4HS and 6HL) that were associated with grain zinc concentration. A known QTL region on chromosome 2HL also was found associated with kernel weight. A QTL linked to leaf zinc concentration on chromosome 4HL was previously reported by Lonergan (2001), and later Sadeghzadeh (2008) identified a grain zinc concentration QTL on chromosome 4HS. Lonergan (2001)

identified the QTL using a population developed from a cross between the landrace Sahara 3771 and cv. Clipper. Their work was later extended to an analysis of zinc accumulation in seed and mature tissue. Lonergan et al. (2009) identified QTL determining grain zinc concentration on chromosomes 1H, 2H and 5H. Though not all of the beneficial alleles came from the landrace Sahara 3771, they did obtain positive transgressive segregation for grain zinc concentration (S. Barker, personal communication), suggesting that this trait in barley could be enhanced through breeding.

In staple cereals, some progress has been made in developing micronutrient-biofortified lines through molecular and transgenic breeding approaches. Biofortified wheat produced by the Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT) has increased quantities of zinc absorbed by adult women (Rosado et al. 2009). Recently**,** a study by Tako et al. (2013) reported that a maize (*Zea mays* L.) hybrid line with high iron bioavailability provides more absorbable iron and improves the iron condition of deficient chickens. Folate, iron and vitamin A-biofortified rice lines have been developed and their health benefits for vulnerable people are under assessment (Haas et al. 2005; Steur et al. 2010; http://www.goldenrice.org/). Recently, iron-biofortified rice was produced by the introduction of multiple genes involved in iron nutrition (Masuda et al. 2012). Rawer et al. (2011) developed wheat (*Aegilops kotschyi*) addition and substitution lines with high grain iron and zinc concentration and identified chromosomes that possess gene(s) for high grain micronutrients. Studies on increasing the grain zinc and iron concentrations of sorghum through genetic means are currently in progress (Kumar et al. 2013).

The high variation in grain zinc and iron concentrations in the germplasm studied and SNP markers associated with grain zinc concentration are potentially valuable resources for breeding. However, further investigations need to be conducted to confirm the relative importance of the chromosomal regions/genes and to devise methods of selection to enhance the nutritional value of barley. Further evaluations of the germplasm may also identify additional QTL that may be expressed under different test environments. In addition to micronutrient enhancement, barley germplasm with high

concentrations of zinc and iron in the grain are likely “micronutrient-efficient” genotypes. They likely perform better than zinc and iron-inefficient genotypes in soils with poor mineral nutrition. Micronutrient-efficient genotypes may be targeted for breeding and cultivation as they can produce greater yield and accumulate more micronutrients in the seed under soil conditions with severe micronutrient deficiencies (Sadeghzadeh et al. 2010).

Table 5.1. Variance components and heritability of zinc and iron concentration in Ethiopian and Eritrean barley landrace germplasm

	Variance components[a]		
Trait	**Genotype**	**Residual**	**Heritability**
Zinc	75.66	82.26	0.65
Iron	71.35	100.51	0.59

[a] Generated by "proc varcomp" procedure in SAS.

Table 5.2. SNP markers significantly associated with row-type morphology (RT), grain zinc concentration (Zn) and fifty seed weight (50Swt) in Ethiopian and Eritrean barley landrace germplasm[a]

Trait	SNP marker	Chrom.[b]	Position[c]	*q-value*[d]
RT	11_20781	2H	88.04	$6.79x10^{-6c}$
RT	SCRI_RS_171032	2H	90.64	$1.63x10^{-11}$
RT	11_20340	2H	90.99	$9.30x10^{-53}$
RT	12_30901	2H	90.99	$3.78x10^{-20}$
RT	12_30896	2H	91.09	$1.52x10^{-55}$
RT	SCRI_RS_196853	2H	91.09	$2.30x10^{-8}$
RT	12_30897	2H	91.09	$4.89x10^{-8}$
RT	SCRI_RS_221886	2H	92.07	$7.66x10^{-5}$
RT	11_20606	4H	31.14	$6.52x10^{-5}$
RT	11_21001	5H	55.83	$6.04x10^{-5}$
RT	SCRI_RS_165473	Unknown	Unknown	$1.97x10^{-21}$
RT	SCRI_RS_72983	Unknown	Unknown	$8.15x10^{-20}$
RT	SCRI_RS_181051	Unknown	Unknown	$7.91x10^{-5}$
RT	SCRI_RS_237894	Unknown	Unknown	$1.09x10^{-4}$
Zn	12_11485	4H	12.91	$7.71x10^{-5}$
Zn	12_10051	6H	122.99	$2.84x^{-5}$
Zn	12_30355	6H	122.99	$3.03x^{-5}$
Zn	SCRI_RS_234548	6H	122.99	$3.34x^{-5}$
Zn	SCRI_RS_10655	6H	128.72	$2.91x10^{-6}$
50Swt	11_20340	2H	90.99	$4.14x10^{-8}$
50Swt	12_30901	2H	90.99	$7.37x10^{-8}$
50Swt	12_30896	2H	91.09	$5.52x10^{-8}$
50Swt	SCRI_RS_165473	Unknown	Unknown	$1.70x10^{-8}$
50Swt	SCRI_RS_72983	Unknown	Unknown	$9.76x10^{-8}$

[a] The entire Ethiopian and Eritrean barley landrace collection (298 lines) grown in the greenhouse.
[b] Chromosome; some markers were not mapped.
[c] Genetic position (in cM) of marker on chromosome; some markers have unknown position.
[d] Multiple testing corrected *P*-value.

Table 5.3. Trait means for each allele at chromosome positions significantly associated with grain zinc concentration (Zn) and fifty seed weight (50Swt) identified in Ethiopian and Eritrean barley landrace germplasm[a]

Trait	QTL/marker	Chrom.[b]	Position[c]	Allele	Trait mean[d]	Allele Frequency[e]
Zn	*Zn-qtl-4H_12_11485*	4H	12.91	A	29.87	0.71
Zn	*Zn-qtl-4H_12_11485*	4H	12.91	B	33.09	0.29
Zn	*Zn-qtl-6H_SCRI_RS_10655*	6H	128.72	A	29.49	0.82
Zn	*Zn-qtl-6H_SCRI_RS_10655*	6H	128.72	B	36.52	0.18
50Swt	*50Swt-qtl-2H-11_20340*	2H	90.99	A	2.49	0.61
50Swt	*50Swt-qtl-2H-11_20340*	2H	90.99	B	2.68	0.39
50Swt	*12_30896*	2H	91.09	A	2.50	0.59
50Swt	*12_30896*	2H	91.09	B	2.68	0.41
50Swt	*12_30901*	2H	90.99	A	2.96	0.20
50Swt	*12_30901*	2H	90.99	B	2.47	0.80
50Swt	*SCRI_RS_165473*	Unknown	Unknown	A	2.93	0.21
50Swt	*SCRI_RS_165473*	Unknown	Unknown	B	2.47	0.79
50Swt	*SCRI_RS_72983*	Unknown	Unknown	A	NA	0.10
50Swt	*SCRI_RS_72983*	Unknown	Unknown	B	2.47	0.90

[a] All markers were identified in genome-wide association mapping of respective traits in the entire germplasm set grown in the greenhouse.
[b] Chromosome; some markers were not mapped.
[c] Genetic position (in cM) of marker on chromosome; some markers have unknown position.
[d] Trait mean for zinc concentration was in mg/Kg; mean for fifty seed weight was mean weight of fifty arbitrarily selected seeds in g.
[e] Allele frequency within the whole germplasm set; calculated for each significant marker. "NA": No accession contained homozygous allele A for the marker.

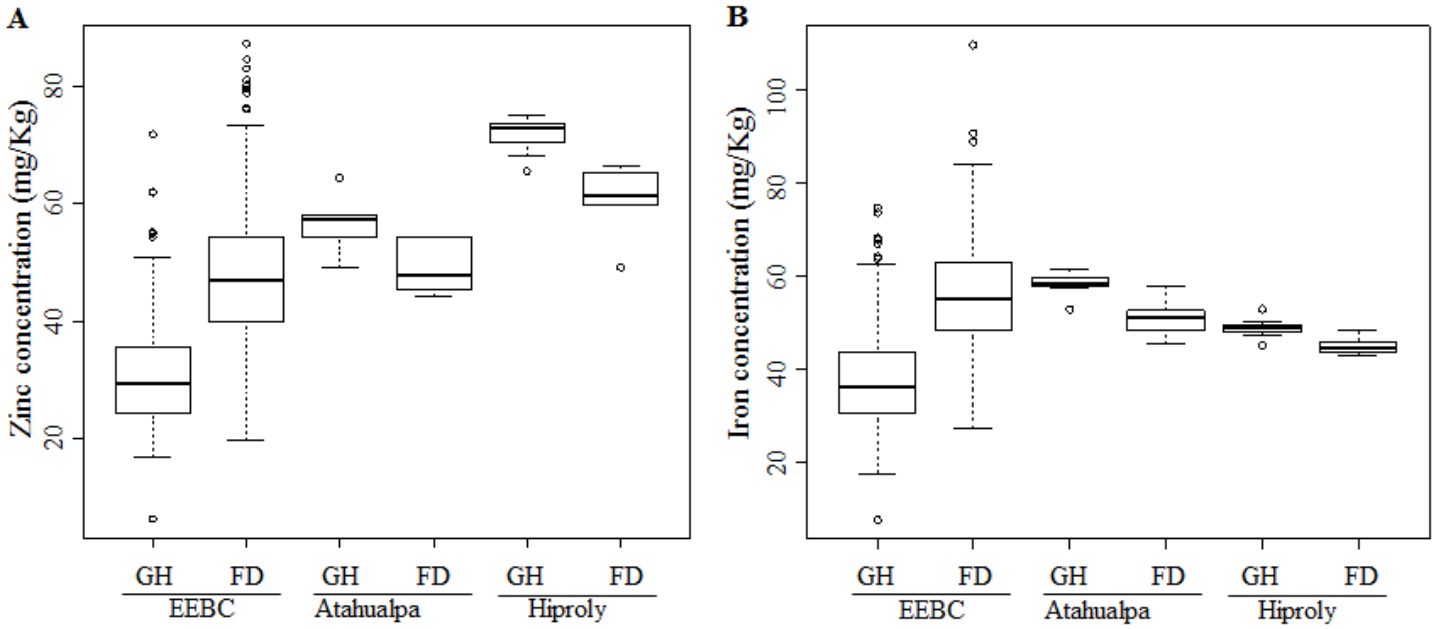

Figure 5.1. Boxplots of zinc and iron concentration in grain of Ethiopian and Eritrean barley landrace germplasm and controls grown in the greenhouse and field. Atahualpa was used as a control for its high zinc and iron concentration and Hiproly is a cultivar with high lysine and protein concentration derived from an Ethiopian barley. (**A**) Grain zinc concentration (mg/Kg) of landrace germplasm and controls produced in the greenhouse (GH) and field (FD), and (**B**) Grain iron concentration (mg/Kg) of landrace germplasm and controls produced in the greenhouse (GH) and field (FD). Five statistics (bars) are represented in each boxplot from bottom to top: the smallest observation, lower quartile, median, upper quartile, and largest observation, respectively. Data points positioned outside this range and depicted as circles are outliers.

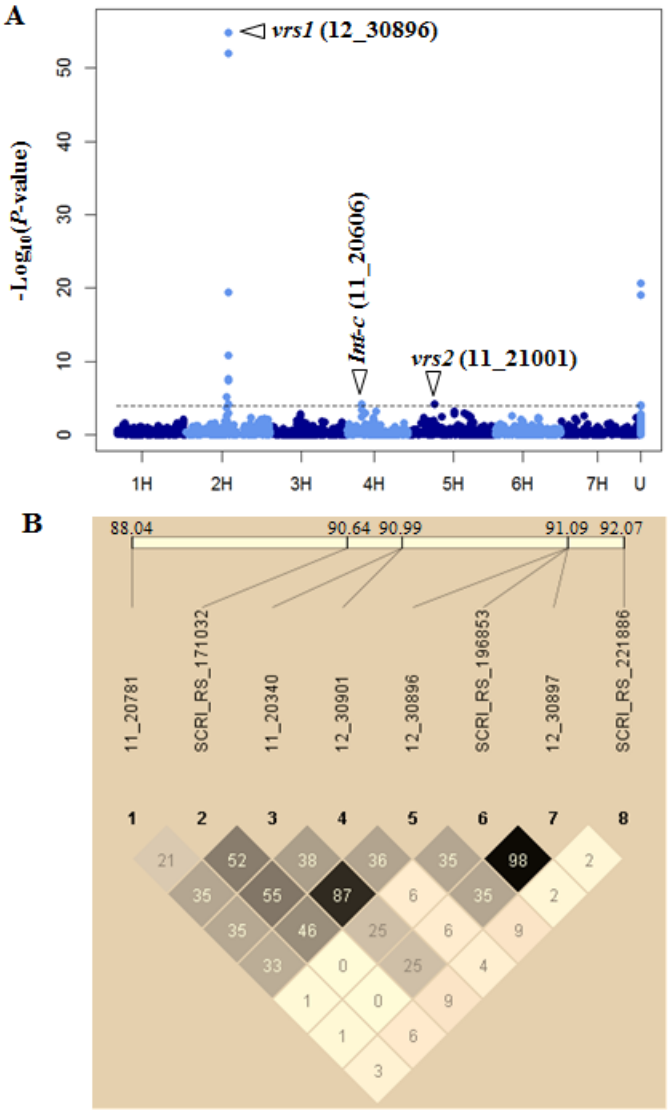

Figure 5.2. (**A**) Genome-wide association scan for row-type spike morphology of Ethiopian and Eritrean barley landrace germplasm. Vertical axis represents -$\log_{10}$(*P*-values) of the marker-trait associations after correction for multiple testing, and the horizontal axis represents the relative chromosomal position of the SNP markers across the genome. The black horizontal dotted line shows the *P*-value corresponding to a false discovery rate (FDR) of 0.05. SNP markers with peaks above this threshold level were considered as significantly associated with row-type spike morphology. Significantly associated SNPs at or in the vicinity of the genes *vrs1* (six-rowed spike 1), *Int-c* (INTERMEDIUM-C) and *vrs2* (six-rowed spike 2) are marked with arrows. (**B**) Heat map of linkage disequilibrium (LD) for eight SNP markers on chromosome 2H associated with barley spike morphology around the *Vrs1* locus. Numbers above the map are the position of the markers from the concensus map in cM. LD is displayed in the squares below the map as r^2, expressed as a percentage, between all pair-wise combinations of the eight markers.

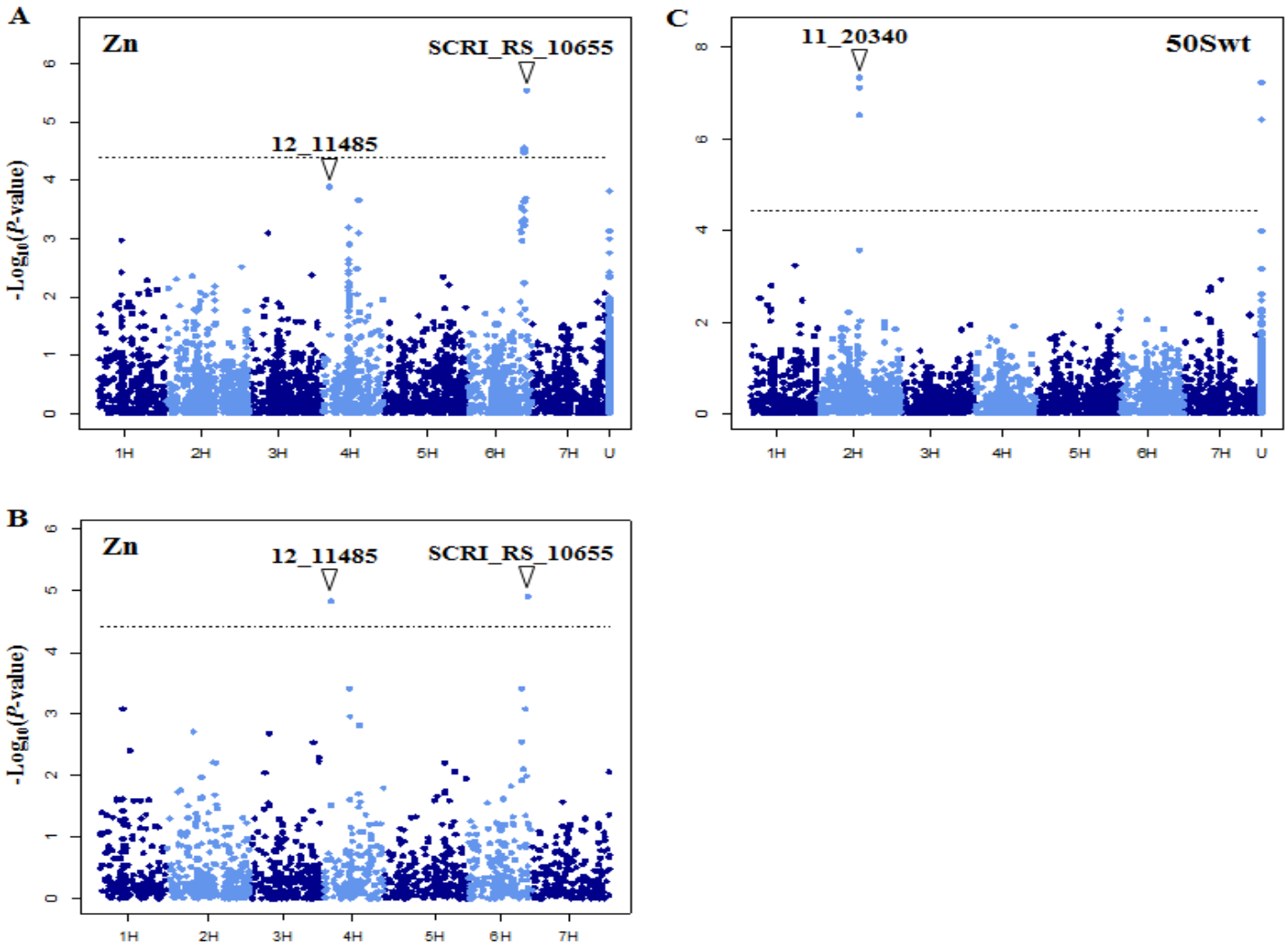

Figure 5.3. See next page.

Figure 5.3. Genome-wide association scans for marker associations with grain zinc concentration and fifty seed weight of Ethiopian and Eritrean barley landrace germplasm. Scans are shown for (**A**) grain zinc concentration (Zn) of greenhouse grown plants with 5,269 SNP markers, (**B**) grain zinc concentration of greenhouse grown plants with 1,534 SNP markers and (**C**) fifty seed weight (50Swt) of greenhouse grown plants with 5,269 SNP markers. Vertical axis represents $-\log_{10}$(*P*-values) of the marker-trait associations after correction for multiple testing, and the horizontal axis represents the relative chromosomal position of the SNP markers across the genome. The black horizontal dotted lines show the *P*-value corresponding to a false discovery rate (FDR) of 0.05. SNP markers with peaks above this threshold level were considered as significantly associated. The most significant markers are marked with arrows.

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Appendices

Appendix Table 2.1. Stem rust resistance genes in barley and their chromosomal locations

Gene	Line identified from	Year discovered	Effective against *Pgt* race	Map location	Remark[a]	References
Rpg1	Chevron (CIho 1111), Peatland (CIho 5267), Kindred (CIho 6969); carried by Morex and line Q21861	1933	All except QCCJ & TTKSK, and 92-MN-90**	Telomeric region of short arm of chromosome 7H	Provides all stage resistance; widely used	Powers and Hines 1933; Sogaard and von Wettstein-Knowles 1987; Steffenson 1992; Kilian et al. 1994; Brueggeman et al. 2002
Rpg2	Hietpas-5 (CIho 7124)	1957	HTMJ, JCMN	Not known	Low level of resistance (all stages)	Patterson et al. 1957; Jedel 1989; Jedel 1990; Sun and Steffenson 2005
Rpg3	PI 382313	1990	MCCFC, JCMN, QTH, HTC, RKQ, TPM	Not known	Resistant to moderately resistant level at all stages	Jedel 1989; Jedel 1990; Sun and Steffenson 2005
rpg4[b, c]	Q21861 (PI 584766)	1994?	QCCJ, MCCFC	Long-arm of chromosome 5H	Provides all stage resistance	Dill-Macky et al. 1992; Jin et al. 1994a; Borovkova et al. 1995; Sun et al. 1996; Druka et al. 2000
RpgU	Peatland	1995?	QCCJ	Not known	Moderate adult plant resistance	Fox et al. 1995
Rpg5[b, c]	Q21861	1996	92-MN-90[d]	Long arm of chromosome 5H	Provides all stage resistance	Sun et al. 1996, Druka et al. 2000
rpgBH	Black Hulless (CIho 666)	1984	Race HQ (isolate 76-32-1355)[d]	Not known	Low level of all stage resistance	Steffenson et al. 1984; Jedel 1989; Jedel 1990; Sun and Steffenson 2005
rpg6	*H. bulbosum* and *H. vulgare* introgression line 212Y1	2004	QCCJ, MCCFC	Short arm of chromosome 6H	Seedling stage resistance; adult plant resistance is unknown	Fetch et al. 2009

[a] It is not known whether *Rpg2*, *Rpg3* and *rpgBH* are responsible for seedling and adult plant resistance in the respective accessions.
[b] *rpg4* requires a functional *Rpg5* gene for full resistance against *Pgt* race QCCJ (Brueggeman et al. 2009).
[c] *rpg4*/*Rpg5* in combination are effective against *Pgt* races QCCJ, MCCFC, TTKSK and RCRS (Brueggeman et al. 2009).
[d] Isolate of rye stem rust *P. graminis* f. sp. *secalis* (*Pgs*).

Appendix Table 2.2. Segregation for resistance and susceptibility among segregating F_3 families from crosses of landrace and wild barley accessions with Steptoe to race TTKSK of *Puccinia graminis* f. sp. *tritici* at the seedling stage

Cross Steptoe/Hv501	3:1 resistance to susceptibility ratio				1:3 resistance to susceptibility ratio			
	Resistant plants	Susceptible plants	χ^2 value (1 df)	Probability ($>\chi^2$)[a]	Resistant plants	Susceptible plants	χ^2 value (1 df)	Probability ($>\chi^2$)[a]
Family 1	19	8	0.36	0.55 ns	19	8	30.82	2.83×10^{-8}
Family 2	9	14	14.31	1.55×10^{-4}	9	14	2.51	0.11 ns
Family 3	8	16	22.22	2.43×10^{-5}	8	16	0.89	0.35 ns
Family 4	16	13	7.13	7.59×10^{-3}	16	13	16.05	6.16×10^{-5}
Family 5	4	22	51.29	7.95×10^{-13}	4	22	1.37	0.24 ns
Family 6	16	12	5.69	0.02	16	12	17.64	2.67×10^{-5}
Family 7	7	22	47.05	6.91×10^{-12}	7	22	0.65	0.42 ns
Family 8	15	10	3.00	0.08 ns	15	10	16.33	5.31×10^{-5}
Family 9	8	17	24.65	6.86×10^{-7}	8	17	0.65	0.42 ns
Family 10	10	18	26.17	3.12×10^{-7}	10	18	2.28	0.13 ns
Family 11	8	15	20.79	5.13×10^{-6}	8	15	1.27	0.26 ns
Family 12	17	10	2.09	0.15 ns	17	10	20.75	5.22×10^{-6}
Family 13	14	10	3.44	0.06 ns	14	10	13.28	2.68×10^{-4}
Family 14	4	23	52.16	5.11×10^{-13}	4	23	1.49	0.22 ns
Family 15	16	10	2.51	0.11 ns	16	10	18.51	1.69×10^{-5}
Family 16	12	9	3.64	0.06 ns	12	9	10.36	1.29×10^{-3}
Family 17	9	17	22.62	1.98×10^{-6}	9	17	1.28	0.26 ns
Family 18	14	14	10.81	1.01×10^{-3}	14	14	10.81	$1.01 \times^{-3}$
Family 19	6	9	11.62	6.54×10^{-4}	6	9	2.38	0.12 ns
Family 20	10	16	18.51	1.69×10^{-5}	10	16	2.51	0.11 ns
Family 21	8	19	29.64	5.20×10^{-8}	8	19	0.31	0.58 ns
Family 22	14	14	10.21	1.40×10^{-3}	14	14	10.21	1.40×10^{-3}
Family 23	8	2	6.61	0.01	8	2	13.88	1.95×10^{-4}
Family 24	4	17	30.09	4.12×10^{-8}	4	17	0.97	0.32 ns
Family 25	19	8	0.31	0.58 ns	19	8	29.64	5.20×10^{-8}
Family 26	9	18	24.14	8.94x10	9	18	1.00	0.32 ns
Family 27	16	10	2.46	0.12 ns	16	10	17.86	2.37×10^{-5}
Family 28	16	10	2.51	0.11 ns	16	10	18.51	1.69×10^{-5}
Family 29	13	13	8.19	4.21×10^{-3}	13	13	8.19	4.21×10^{-3}
Family 30	15	11	4.03	0.04	15	11	13.60	2.26×10^{-4}

Cross Steptoe/Hv501	3:1 resistance to susceptibility ratio				1:3 resistance to susceptibility ratio			
	Resistant plants	Susceptible plants	χ^2 value (1 df)	Probability (>χ^2)[a]	Resistant plants	Susceptible plants	χ^2 value (1 df)	Probability (>χ^2)[a]
Family 31	8	21	36.05	1.93×10^{-9}	8	21	0.14	0.71 ns
Family 32	13	15	12.19	4.80×10^{-4}	13	15	6.86	8.83×10^{-3}
Family 33	3	22	55.17	1.11×10^{-13}	3	22	2.39	0.12 ns
Family 34	4	24	57.12	4.09×10^{-14}	4	24	1.81	0.18 ns
Family 35	14	10	3.62	0.06 ns	14	10	12.76	3.54×10^{-4}
Family 36	15	11	4.15	0.04	15	11	14.82	1.18×10^{-4}
Family 37	11	13	10.01	1.55×10^{-3}	11	13	5.27	0.02
Family 38	8	17	21.80	3.02×10^{-6}	8	17	1.11	0.29 ns
Family 39	4	23	54.21	1.81×10^{-13}	4	23	1.59	0.21 ns
Family 40	3	20	47.09	6.79×10^{-12}	3	20	1.75	0.19 ns
Family 41	14	10	3.56	0.06 ns	14	10	14.22	1.62×10^{-4}
Family 42	7	18	29.45	5.73×10^{-8}	7	18	0.12	0.73 ns
Family 43	10	20	27.78	1.36×10^{-7}	10	20	1.11	0.29 ns
Family 44	3	20	47.09	6.79×10^{-12}	3	20	1.75	0.19 ns
Family 45	7	19	32.05	1.50×10^{-8}	7	19	0.05	0.82 ns
Family 46	7	19	32.05	1.50×10^{-8}	7	19	0.05	0.82 ns
Family 47	5	21	43.13	5.13×10^{-11}	5	21	0.46	0.50 ns
Family 48	6	19	34.68	3.89×10^{-9}	6	19	0.01	0.91 ns
Steptoe/Hv545								
Family 1	26	25	15.69	7.45×10^{-7}	26	25	18.36	1.83×10^{-5}
Family 2	3	8	13.36	2.57×10^{-4}	3	8	0.03	0.862 ns
Family 3	39	17	0.86	0.35 ns	39	17	59.52	1.21×10^{-14}
Family 4	35	18	2.27	0.13 ns	35	18	47.60	5.22×10^{-12}
Family 5	16	11	3.57	0.06 ns	16	11	16.90	3.94×10^{-5}
Family 6	7	3	0.13	0.72 ns	7	3	10.80	1.02×10^{-3}
Family 7	14	7	0.78	0.38 ns	14	7	19.44	1.04×10^{-5}
Family 8	23	21	12.12	4.99×10^{-4}	23	21	17.45	0.0000294
Family 9	22	19	9.96	1.60×10^{-3}	22	19	17.96	2.26×10^{-5}
Family 10	17	13	5.38	0.02	17	13	16.04	6.19×10^{-5}
Family 11	20	20	13.33	2.61×10^{-4}	20	20	13.33	261×10^{-4}
Family 12	20	5	0.33	0.56 ns	20	5	40.33	2.14×10^{-10}
Family 13	30	1	7.84	5.11×10^{-3}	30	1	85.17	2.74×10^{20}

Cross Steptoe/Hv545	3:1 resistance to susceptibility ratio				1:3 resistance to susceptibility ratio			
	Resistant plants	Susceptible plants	χ^2 value (1 df)	Probability ($>\chi^2$)[a]	Resistant plants	Susceptible plants	χ^2 value (1 df)	Probability ($>\chi^2$)[a]
Family 14	31	9	0.13	0.72 ns	31	9	58.80	1.75×10^{-14}
Family 15	31	5	2.37	0.12 ns	31	5	71.70	2.50×10^{-17}
Family 16	28	0	9.33	2.25×10^{-3}	28	0	84.00	4.95×10^{20}
Family 17	16	1	3.31	0.07 ns	16	1	43.31	4.66×10^{11}
Family 18	13	2	1.09	0.30 ns	13	2	30.42	3.48×10^{-8}
Family 19	24	19	8.44	3.67×10^{-3}	24	19	21.78	3.07×10^{-6}
Family 20	44	2	10.46	1.22×10^{-3}	44	2	122.46	1.83×10^{-28}
Family 21	38	9	0.86	0.35 ns	38	9	78.19	9.35×10^{-19}
Family 22	43	0	14.33	1.5×10^{-4}	43	0	129.00	6.78×10^{-30}
Family 23	56	17	0.11	0.74 ns	56	17	104.11	1.91×10^{-24}
Family 24	11	5	0.33	0.56 ns	11	5	16.33	5.31×10^{-5}
Steptoe/Hv602								
Family 1	8	4	0.96	0.33 ns	8	4	9.49	2.07×10^{-3}
Family 2	16	4	0.30	0.58 ns	16	4	30.78	289×10^{-8}
Family 3	9	4	0.47	0.49 ns	9	4	12.02	5.26×10^{-4}
Family 4	14	11	5.06	0.02 ns	14	11	13.39	2.53×10^{-4}
Family 5	10	14	14.88	1.14×10^{-4}	10	14	3.75	0.05 ns
Family 6	4	17	33.52	7.07×10^{-9}	4	17	0.42	0.51 ns
Family 7	4	20	43.56	4.12×10^{-11}	4	20	0.89	0.35 ns
Family 8	9	18	26.00	3.41×10^{-7}	9	18	1.08	0.30 ns
Family 9	14	18	20.68	5.43×10^{-9}	14	18	8.04	4.58×10^{-3}
Family 10	5	11	14.23	1.62×10^{-4}	5	11	0.75	0.39 ns
Family 11	14	9	2.39	0.12 ns	14	9	15.17	9.84×10^{-5}
Family 12	6	23	45.62	1.44×10^{-11}	6	23	0.29	0.59 ns
Family 13	15	10	3.17	0.08 ns	15	10	17.06	3.63×10^{-5}
Family 14	11	9	4.54	0.03 ns	11	9	10.16	1.44×10^{-3}
Family 15	6	22	44.48	2.57×10^{-11}	6	22	0.23	0.63 ns
Family 16	9	7	3.00	0.08 ns	9	7	8.33	3.89×10^{-3}
Family 17	6	17	24.13	8.99×10^{-7}	6	17	1.64	0.20 ns
Family 18	11	6	1.07	0.30 ns	11	6	13.00	3.11×10^{-4}
Family 19	8	18	26.16	3.14×10^{-7}	8	18	0.48	0.49 ns
Family 20	4	16	28.45	9.62×10^{-8}	4	16	0.62	0.43 ns

Cross Steptoe/Hv602	3:1 resistance to susceptibility ratio				1:3 resistance to susceptibility ratio			
	Resistant plants	Susceptible plants	χ^2 value (1 df)	Probability ($>\chi^2$)[a]	Resistant plants	Susceptible plants	χ^2 value (1 df)	Probability ($>\chi^2$)[a]
Family 21	7	14	17.93	2.29×10^{-5}	7	14	0.88	0.35 ns
Family 22	6	20	37.38	9.70×10^{-10}	6	20	0.05	0.82 ns
Family 23	5	14	27.08	1.95×10^{-7}	5	14	0.25	0.61 ns
Family 24	3	10	18.69	1.54×10^{-5}	3	10	0.03	0.87 ns
Family 25	3	5	5.20	0.02	3	5	0.93	0.33 ns
Family 26	4	17	31.06	2.51×10^{-9}	4	17	0.72	0.40 ns
Family 27	3	23	52.00	5.55×10^{-13}	3	23	2.48	0.12 ns
Family 28	20	2	2.89	0.09 ns	20	2	46.89	7.51×10^{-12}
Family 29	8	11	10.96	9.29×10^{-4}	8	11	2.96	0.09 ns
Family 30	4	18	33.69	6.45×10^{-9}	4	18	0.84	0.36 ns
Family 31	2	20	42.46	7.23×10^{-11}	2	20	3.35	0.07 ns
Family 32	6	16	24.67	6.82×10^{-7}	6	16	0.22	0.64 ns
Family 33	8	12	13.07	3.01×10^{-4}	8	12	2.40	0.12 ns
Family 34	4	9	11.58	6.65×10^{-4}	4	9	0.75	0.39 ns
Family 35	4	14	25.39	4.69×10^{-7}	4	14	0.12	0.73 ns
Family 36	1	10	25.48	4.46×10^{-7}	1	10	1.48	0.22 ns
Family 37	5	15	22.33	2.29×10^{-6}	5	15	1.00	0.32 ns
Family 38	5	10	14.95	1.10×10^{-4}	5	10	0.67	0.41 ns
Family 39	1	18	46.87	7.60×10^{-12}	1	18	3.80	0.05 ns
Family 40	2	20	41.33	1.28×10^{-10}	2	20	3.62	0.06 ns
Family 41	2	12	27.52	1.55×10^{-7}	2	12	0.86	0.35 ns
Family 42	3	14	28.22	108×10^{-7}	3	14	0.52	0.47 ns
Family 43	5	1	0.33	0.56 ns	5	1	9.48	2.08×10^{-3}
Family 44	13	2	1.41	0.24 ns	13	2	25.85	3.69×10^{-7}
Family 45	8	7	3.58	0.06 ns	8	7	6.08	0.01
Family 46	4	8	11.11	8.58×10^{-4}	4	8	0.44	0.50 ns
Family 47	10	9	5.59	0.02	10	9	7.54	6.04×10^{-3}
Family 48	4	15	23.12	1.52×10^{-6}	4	15	2.48	0.12 ns
Family 49	9	1	1.62	0.20 ns	9	1	18.03	2.18×10^{-5}
Family 50	10	7	2.30	0.13 ns	10	7	9.85	1.70×10^{-3}
Family 51	17	6	0.01	0.90 ns	17	6	29.35	6.05×10^{-8}
Family 52	4	12	16.97	3.79×10^{-5}	4	12	3.85	0.05 ns

Cross Steptoe/Hv602	3:1 resistance to susceptibility ratio				1:3 resistance to susceptibility ratio			
	Resistant plants	Susceptible plants	χ^2 value (1 df)	Probability ($>\chi^2$)[a]	Resistant plants	Susceptible plants	χ^2 value (1 df)	Probability ($>\chi^2$)[a]
Family 53	14	5	1.45	0.23 ns	14	5	19.69	9.09×10^{-6}
Steptoe/Hv612								
Family 1	37	1	9.92	1.63×10^{-3}	31	76	78.65	7.43×10^{-19}
Family 2	37	0	11.45	7.15×10^{-4}	9	80	70.98	3.60×10^{-17}
Family 3	45	3	12.24	4.69×10^{-5}	14	116	83.37	6.80x10-^{20}
Family 4	46	2	11.51	6.91×10^{-4}	27	109	96.50	8.94×10^{-23}
Family 5	13	9	2.88	0.09 ns	19	46	78.45	8.21×10^{-19}
Family 6	33	5	3.27	0.07 ns	19	82	78.58	7.70×10^{-19}
Family 7	44	8	5.35	0.02	41	119	129.17	6.23×10^{-31}
Family 8	29	8	1.61	0.20 ns	29	82	96.99	6.98×10^{-24}
Family 9	32	6	1.81	0.18 ns	25	79	96.93	7.16×10^{-23}
Family 10	34	4	4.33	0.04	25	81	92.58	6.46×10^{-22}
Family 11	35	8	0.99	0.32 ns	31	88	121.87	2.46×10^{-30}
Family 12	34	3	6.05	0.01	27	82	91.03	1.42×10^{-21}
Family 13	35	6	2.39	0.12 ns	29	85	111.39	4.85×10^{-26}
Family 14	34	2	7.08	0.01	26	78	91.52	1.10×10^{-21}
Family 15	35	5	3.31	0.07 ns	33	83	125.91	3.21×10^{-29}
Family 16	27	5	1.97	0.16 ns	25	69	88.14	6.11×10^{-21}
Family 17	27	2	7.52	0.01	18	71	54.89	1.28×10^{-13}
Family 18	24	6	0.50	0.48 ns	21	62	81.29	1.95×10^{19}
Family 19	35	1	8.98	2.73×10^{-3}	26	75	98.64	3.03×10^{-24}
Family 20	39	0	12.09	5.07×10^{-4}	36	83	132.58	1.12×10^{-30}
Family 21	27	9	0.11	0.75 ns	22	74	89.09	3.78×10^{-21}
Family 22	30	3	4.53	0.03	27	71	95.61	1.40×10^{-22}
Family 23	32	3	4.88	0.03	22	73	85.93	1.86×10^{-20}
Family 24	32	1	8.04	4.59×10^{-3}	27	71	95.61	1.40×10^{-23}
Family 25	23	9	2.51	0.11 ns	21	74	67.84	1.77×10^{-17}
Family 26	18	6	1.20	0.27 ns	20	54	64.93	7.75×10^{-16}
Family 27	37	5	4.11	0.04	19	90	85.08	2.86×10^{-20}
Family 28	37	5	3.76	0.05 ns	20	86	92.48	6.78×10^{-22}
Family 29	29	10	0.22	0.64 ns	30	81	114.00	1.30×10^{-28}
Family 30	31	7	1.36	0.24 ns	30	81	108.16	2.48×10^{-26}

Cross WBDC213/Steptoe	3:1 resistance to susceptibility ratio				1:3 resistance to susceptibility ratio			
	Resistant plants	Susceptible plants	χ^2 value (1 df)	Probability ($>\chi^2$)[a]	Resistant plants	Susceptible plants	χ^2 value (1 df)	Probability ($>\chi^2$)[a]
Family 1	5	7	7.11	0.01	10	24	41.33	1.28×10^{-10}
Family 2	13	6	1.06	0.30 ns	14	38	36.80	1.31×10^{-9}
Family 3	13	6	0.44	0.51 ns	9	38	43.39	4.49×10^{-11}
Family 4	9	9	6.00	0.01	12	36	50.00	1.54×10^{-12}
Family 5	12	6	0.67	0.41 ns	8	36	40.22	2.27×10^{-10}
Family 6	8	10	8.96	2.76×10^{-3}	14	36	57.56	3.29×10^{-14}
Family 7	10	5	0.56	0.46 ns	7	30	34.07	5.33×10^{-10}
Family 8	9	8	4.41	0.04	13	34	53.43	2.68×10^{-13}
Family 9	10	3	0.03	0.87 ns	6	26	29.41	5.86×10^{-8}
Family 10	8	8	5.33	0.02	10	32	42.33	7.70×10^{-11}
Family 11	9	11	9.60	1.95×10^{-3}	12	40	51.47	7.28×10^{-13}
Family 12	13	2	1.09	0.30 ns	4	30	31.27	2.25×10^{-8}
Family 13	11	4	0.02	0.88 ns	10	30	41.67	1.08×10^{-10}
Family 14	9	14	18.60	1.61×10^{-5}	18	46	97.87	4.48×10^{-23}
Family 15	10	9	5.07	0.02	10	38	45.39	1.62×10^{-11}
Family 16	6	11	14.29	1.56×10^{-4}	13	34	53.43	2.68×10^{-13}
Family 17	15	4	0.20	0.65 ns	7	38	36.07	1.91×10^{-9}
Family 18	17	3	1.07	0.30 ns	5	40	41.67	1.08×10^{-10}
Family 19	7	8	6.42	0.01	10	30	41.67	1.08×10^{-10}
Family 20	12	8	2.40	0.12 ns	11	40	48.87	2.74×10^{-12}
Family 21	8	12	13.07	3.01×10^{-4}	15	40	61.67	4.07×10^{-14}
Family 22	10	9	5.07	0.02	10	38	45.39	1.62×10^{-11}
Family 23	5	12	18.84	1.42×10^{-5}	13	34	53.43	2.68×10^{-13}
Family 24	11	9	4.27	0.04	10	40	46.67	8.41×10^{-12}
Family 25	7	11	12.52	403×10^{-4}	13	36	53.56	2.51×10^{-13}
Family 26	6	7	5.56	0.02	9	26	16.07	6.09×10^{-5}
Family 27	6	5	2.45	0.12 ns	5	22	24.76	6.50×10^{-7}
Family 28	11	10	6.07	0.01	14	42	64.80	8.29×10^{-16}
Family 29	12	8	2.40	0.12 ns	9	40	44.87	2.11×10^{-11}
Family 30	17	0	5.67	0.02	4	34	35.43	2.64×10^{-9}
Family 31	16	5	0.07	0.80 ns	8	42	50.40	1.25×10^{-12}
Family 32	11	5	0.33	0.56 ns	6	32	34.33	4.64×10^{-9}

Cross WBDC213/Steptoe	3:1 resistance to susceptibility ratio				1:3 resistance to susceptibility ratio			
	Resistant plants	Susceptible plants	χ^2 value (1 df)	Probability ($>\chi^2$)[a]	Resistant plants	Susceptible plants	χ^2 value (1 df)	Probability ($>\chi^2$)[a]
Family 33	13	5	0.07	0.79 ns	7	36	38.89	4.49×10^{-9}
Family 34	13	2	1.20	0.27 ns	5	30	23.47	1.27×10^{-6}
Family 35	16	2	1.85	0.17 ns	2	36	38.89	4.49×10^{-10}
Family 36	7	8	6.08	0.01	11	30	39.25	3.73×10^{-10}
Family 37	11	5	0.33	0.56 ns	6	32	34.33	4.64×10^{-9}
Family 38	7	2	0.78	0.38 ns	2	18	9.33	2.25×10^{-3}
Family 39	6	3	2.00	0.16 ns	5	18	6.00	0.01
Family 40	8	12	13.81	2.03×10^{-4}	12	40	57.60	3.22×10^{-14}
Family 41	5	10	11.81	5.90×10^{-4}	10	30	23.21	1.45×10^{-6}
Family 42	11	9	4.27	0.04	12	40	51.47	7.28×10^{-13}
Family 43	9	8	4.41	0.04	10	34	43.20	4.95×10^{-11}
Family 44	12	6	0.80	0.37 ns	10	36	34.40	4.49×10^{-9}
Family 45	6	13	19.11	124×10^{-5}	14	38	57.60	3.22×10^{-14}
Family 46	10	11	8.87	2.90×10^{-3}	17	42	77.40	1.40×10^{-19}
Family 47	10	8	3.49	0.06 ns	13	36	47.53	5.43×10^{-12}
Family 48	7	10	9.27	2.33×10^{-3}	14	34	40.27	2.22×10^{-10}
Family 49	8	7	4.07	0.04	10	30	20.00	7.74×10^{-6}
WBDC345/Steptoe								
Family 1	12	10	4.91	0.03	12	10	10.24	1.37×10^{-3}
Family 2	15	12	5.29	0.02	15	12	13.00	3.11×10^{-4}
Family 3	12	9	4.67	0.03	12	9	14.00	1.83×10^{-4}
Family 4	6	24	52.00	5.55×10^{-14}	6	24	0.57	0.45 ns
Family 5	2	9	38.89	4.49×10^{-10}	2	9	4.67	0.03
Family 6	11	24	50.82	1.01×10^{-12}	11	24	4.15	0.04
Family 7	18	11	5.64	0.02	18	11	30.24	3.81×10^{-8}
Family 8	20	6	0.05	0.82 ns	20	6	37.38	9.70×10^{-10}
Family 9	15	14	8.71	3.16×10^{-3}	15	14	11.48	7.05×10^{-4}
Family 10	20	9	0.97	0.32 ns	20	9	33.69	6.46×10^{-9}
Family 11	10	14	14.88	1.14×10^{-5}	10	14	3.75	0.05 ns
Family 12	15	11	4.00	0.05 ns	15	11	13.90	1.923×10^{-4}
Family 13	20	4	1.12	0.29 ns	20	4	39.05	4.13×10^{-10}
Family 14	15	15	10.86	9.84×10^{-4}	15	15	10.86	9.84×10^{-4}

Cross WBDC345/Steptoe	3:1 resistance to susceptibility ratio				1:3 resistance to susceptibility ratio			
	Resistant plants	Susceptible plants	χ^2 value (1 df)	Probability ($>\chi^2$)[a]	Resistant plants	Susceptible plants	χ^2 value (1 df)	Probability ($>\chi^2$)[a]
Family 15	4	12	20.14	7.21×10^{-6}	4	12	0.06	0.81 ns
Family 16	4	14	28.37	1.00×10^{-6}	4	14	0.14	0.71 ns
Family 17	10	26	57.33	3.68×10^{-14}	10	26	2.48	0.12 ns
Family 18	21	8	0.14	0.71 ns	21	8	36.05	1.93×10^{-9}
Family 19	12	15	14.00	1.83×10^{-4}	12	15	5.69	0.02
Family 20	9	13	12.04	5.22×10^{-4}	9	13	3.35	0.07 ns
Family 21	7	21	38.75	4.81×10^{-10}	7	21	0.04	0.85 ns
Family 22	4	8	11.11	8.58×10^{-4}	4	8	0.44	0.50 ns
Family 23	6	7	5.43	0.02	6	7	2.95	0.09 ns
Family 24	10	18	23.94	9.95×10^{-7}	10	18	1.81	0.18 ns
Family 25	12	15	13.44	2.46×10^{-4}	12	15	5.44	0.02
Family 26	14	13	7.48	0.01	14	13	10.05	1.53×10^{-3}
Family 27	13	13	8.38	3.79×10^{-3}	13	13	8.38	3.79×10^{-3}
Family 28	22	3	2.25	0.13 ns	22	3	52.92	3.47×10^{-13}
Family 29	8	20	33.42	7.43×10^{-9}	8	20	0.23	0.63 ns
Family 30	14	10	3.45	0.06 ns	14	10	13.69	2.15×10^{-4}
Family 31	7	20	36.05	1.92×10^{-9}	7	20	0.05	0.82 ns
Family 32	14	6	1.21	0.27 ns	14	6	18.28	1.91×10^{-5}
Family 33	9	7	2.88	0.09	9	7	7.90	4.94×10^{-3}
Family 34	12	14	11.15	8.41×10^{-4}	12	14	6.01	0.01
Family 35	5	21	40.19	2.30×10^{-10}	5	21	0.57	0.45 ns
Family 36	11	16	16.90	3.94×10^{-5}	11	16	3.57	0.06 ns
Family 37	5	14	22.87	1.74×10^{-6}	5	14	0.07	0.80 ns
Family 38	6	20	37.38	9.70×10^{-10}	6	20	0.05	0.82 ns
Family 39	7	18	26.62	2.48×10^{-7}	7	18	0.43	0.51 ns
Family 40	9	19	27.43	1.63×10^{-7}	9	19	0.76	0.38 ns
Family 41	11	10	5.73	0.02	11	10	8.40	3.76×10^{-3}
Family 42	10	12	9.27	2.33×10^{-3}	10	12	4.93	0.03
Family 43	8	19	28.62	8.81×10^{-8}	8	19	0.33	0.56 ns
Family 44	6	19	34.68	3.89×10^{-9}	6	19	0.01	0.91 ns
Family 45	6	19	33.38	7.56×10^{-9}	6	19	0.05	0.82 ns
Family 46	5	22	45.94	1.22×10^{-11}	5	22	0.60	0.44 ns

Cross WBDC345/Steptoe	3:1 resistance to susceptibility ratio				1:3 resistance to susceptibility ratio			
	Resistant plants	Susceptible plants	χ^2 value (1 df)	Probability ($>\chi^2$)[a]	Resistant plants	Susceptible plants	χ^2 value (1 df)	Probability ($>\chi^2$)[a]
Family 47	11	14	12.81	3.44×10^{-4}	11	14	4.81	0.03
Family 48	3	6	8.33	3.89×10^{-3}	3	6	0.33	0.56 ns
Family 49	17	6	0.06	0.81 ns	17	6	28.17	1.11×10^{-7}
Family 50	16	8	0.89	0.35 ns	16	8	22.22	2.43×10^{-6}
Family 51	18	9	1.00	0.32 ns	18	9	25.00	5.73×10^{-7}
Family 52	10	13	12.19	4.81×10^{-4}	10	13	4.19	0.04
Family 53	12	3	0.20	0.65 ns	12	3	24.20	8.68×10^{-7}
Family 54	9	18	24.14	8.94×10^{-7}	9	18	1.00	0.32 ns
Family 55	6	16	25.61	4.18×10^{-7}	6	16	0.10	0.75 ns
Family 56	6	20	40.67	1.81×10^{-9}	6	20	0.22	0.64 ns
Family 57	10	11	7.84	0.01	10	11	5.41	0.02
Family 58	9	15	17.32	3.16×10^{-5}	9	15	1.96	0.16 ns
Family 59	4	18	39.73	2.92×10^{-10}	4	18	0.62	0.43 ns
Family 60	7	11	13.31	2.63×10^{-4}	7	11	2.02	0.16 ns

[a] ns = not significant at 0.05.

Appendix Table 3.1. Passport information for the 298 Ethiopian and Eritrean barley landraces (*Hordeum vulgare* ssp. *vulgare*) (EEBC) used in this study

				Collection site/origin						
No.	**Genotype[a]**	**Identifier[b]**	**Plant ID[c]**	**Country**	**State**	**Province**	**Latitude**	**Longitude**	**Elevation (m)**	**Year**
1	EEBC001	PI 193538	8738	Ethiopia	Harari	Harer	9.32	42.12	1935	1950
2	EEBC002	PI 193986	8894	Ethiopia	Oromia	Shewa	9.73	39.67	3274	1950
3	EEBC003	PI 195967[d]	9743	Ethiopia	Amhara	Welo	11.13	39.63	2495	1951
4	EEBC004	PI 195988	9842	Ethiopia	Amhara	Welo	11.18	40.02	1670	1951
5	EEBC005	PI 196858	10034	Ethiopia	Oromia	Welega	9.08	36.55	2107	1951
6	EEBC006	PI 277383	S-5	Ethiopia	Oromia	Kefa	6.98	35.58	1486	1961
7	EEBC007	PI 277387	S-9	Eritrea	Unknown	Seraye	14.89	38.82	1967	1961
8	EEBC008	PI 296456	H-2187	Eritrea	Unknown	Asmara	15.33	38.93	2336	1964
9	EEBC009	PI 295438	ELS 6302-70	Ethiopia	Oromia	Shewa	9.30	38.75	2400	1963
10	EEBC010	PI 298765	ELS 6402-375	Ethiopia	Oromia	Bale	7.05	39.63	3508	1964
11	EEBC011	PI 298769[e]	ELS 6402-379	Ethiopia	Oromia	Bale	7.05	39.63	3508	1964
12	EEBC012	PI 298796	ELS 6402-414	Ethiopia	Amhara	Gonder	12.12	37.78	2013	1964
13	EEBC013	PI 296454	H-2163	Eritrea	Unknown	Senhit	15.78	38.46	1553	1964
14	EEBC014	PI 298736	ELS 6402-277	Ethiopia	Oromia	Shewa	9.78	38.68	3080	1964
15	EEBC015	CIho 13745	CI 13745	Eritrea	Unknown	Asmara	15.33	38.93	2336	1967
16	EEBC016	PI 331228	R56	Ethiopia	Oromia	Ilubabor	8.45	36.35	1973	1967
17	EEBC017	CIho 13761	CI 13761	Ethiopia	Oromia	Shewa	9.68	39.53	2789	1967
18	EEBC018	CIho 14976	Yaha	Eritrea	Unknown	Asmara	15.33	38.93	2379	1964
19	EEBC019	CIho 14977	ELS 6402-564	Eritrea	Unknown	Akele Guzai	14.97	39.05	2263	1964
20	EEBC020	PI 347245	265	Ethiopia	Southern	Gemu Gefa	5.28	37.35	1810	1970
21	EEBC021	PI 382191	GAW 28-4	Ethiopia	Amhara	Gonder	12.15	37.72	2231	1970
22	EEBC022	PI 382251	GAW 60-6	Ethiopia	Tigray	Tigre	14.27	38.85	1990	1970
23	EEBC023	PI 382269	GAW 65-3	Ethiopia	Tigray	Tigre	14.28	39.15	1977	1970
24	EEBC024	PI 382313	GAW 79-3	Ethiopia	Tigray	Tigre	13.53	39.57	2440	1970
25	EEBC025	PI 382325	GAW 81-6	Ethiopia	Tigray	Tigre	12.93	39.52	2446	1970
26	EEBC026	PI 382379	GAW 106-6	Ethiopia	Oromia	Shewa	8.97	37.67	2393	1970
27	EEBC027	PI 382448	GAW 142-5	Ethiopia	Oromia	Shewa	8.28	39.50	2008	1970
28	EEBC028	PI 382695[f]	GAW 11-2	Ethiopia	Oromia	Shewa	9.68	39.63	2964	1970
29	EEBC029	PI 382743	GAW 32-4	Ethiopia	Amhara	Gonder	12.50	37.42	1968	1970
30	EEBC030	PI 382865	GAW 48-17	Ethiopia	Amhara	Gonder	12.78	37.65	2782	1970
31	EEBC031	PI 383041	GAW 110-1	Ethiopia	Oromia	Shewa	8.97	37.65	2669	1970
32	EEBC032	PI 383089	GAW 122-1	Ethiopia	Oromia	Shewa	9.27	37.18	2414	1970
33	EEBC033	PI 383108	GAW 126-3	Ethiopia	Oromia	Welega	9.13	37.28	2893	1970

No.	Genotype[a]	Identifier[b]	Plant ID[c]	Collection site/origin						
				Country	State	Province	Latitude	Longitude	Elevation (m)	Year
34	EEBC034	PI 383150	GAW 144-5	Ethiopia	Oromia	Shewa	8.08	39.55	2736	1970
35	EEBC035	PI 386392	IAR/B/136-3	Ethiopia	Oromia	Shewa	9.03	38.15	2274	1974
36	EEBC036	PI 386393	IAR/B/141	Ethiopia	Oromia	Arusi	7.07	38.78	2666	1974
37	EEBC037	PI 386458	IAR/B/411-2	Ethiopia	Oromia	Shewa	8.92	38.65	2241	1974
38	EEBC038	PI 386505	IAR/B/513	Ethiopia	Amhara	Gojam	10.35	37.73	2487	1974
39	EEBC039	PI 386655	IAR/B/246-2	Ethiopia	Amhara	Gonder	12.77	37.62	2932	1974
40	EEBC040	PI 386704	IAR/B/337-1	Ethiopia	Oromia	Shewa	9.17	37.75	1665	1974
41	EEBC041	PI 386855	IAR/B/76	Ethiopia	Tigray	Tigre	13.13	39.50	2023	1974
42	EEBC042	PI 386881	IAR/B/110	Ethiopia	Southern	Sidamo	6.37	38.62	2720	1974
43	EEBC043	PI 386920	IAR/B/159	Ethiopia	Oromia	Arusi	7.85	39.97	2480	1974
44	EEBC044	PI 386997	IAR/B/216-1	Ethiopia	Oromia	Arusi	7.58	38.90	2432	1974
45	EEBC045	PI 387016	IAR/B/244-2	Ethiopia	Amhara	Gonder	12.28	37.45	1786	1974
46	EEBC046	PI 387033	IAR/B/280-1	Ethiopia	Oromia	Kefa	7.67	36.83	1716	1974
47	EEBC047	PI 387049	IAR/B/292-1	Ethiopia	Oromia	Kefa	7.60	36.73	1809	1974
48	EEBC048	PI 387195	IAR/B/450	Ethiopia	Tigray	Tigre	12.67	40.33	338	1974
49	EEBC049	PI 387201	IAR/B/467-2	Ethiopia	Amhara	Gonder	12.63	37.48	2184	1974
50	EEBC050	PI 387231	IAR/B/514-2	Ethiopia	Harari	Harer	9.58	41.87	1234	1974
51	EEBC051*	CIho 1604[g,h]	Hillsa	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1921
52	EEBC052	CIho 2226	Steudelli	Ethiopia	Harari	Harer	9.58	41.87	1234	1920
53	EEBC053	CIho 4221	494b	Ethiopia	Amhara	Welo	11.50	40.00	3050	1924
54	EEBC054*	CIho 11709	CI 11709	Ethiopia	Oromia	Kefa	7.83	36.67	2288	1960
55	EEBC055*	CIho 11788[i,j]	Harar	Ethiopia	Harari	Harer	9.50	41.50	Unknown	1960
56	EEBC056*	CIho 11812	Abyssinian 16	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1962
57	EEBC057*	CIho 11813	Abyssinian 1118	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1962
58	EEBC058*	CIho 11849	Alemaya Mago	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1962
59	EEBC059*	CIho 13141	CI 13141	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1962
60	EEBC060*	CIho 13353	CI 13353	Ethiopia	Amhara	Gonder	12.60	37.47	2201	1964
61	EEBC061	CIho 13355	CI 13355	Ethiopia	Amhara	Gojam	10.35	37.73	2470	1964
62	EEBC062	CIho 13398	2095	Ethiopia	Harari	Harer	9.50	41.50	2339	1960
63	EEBC063*	CIho 13737	CI 13737	Ethiopia	Oromia	Shewa	9.32	42.12	1935	1967
64	EEBC064*	CIho 13740	CI 13740	Eritrea	Unknown	Asmara	15.33	38.93	2336	1967
65	EEBC065*	CIho 13741	CI 13741	Eritrea	Unknown	Asmara	15.33	38.93	2336	1967
66	EEBC066*	CIho 13742	CI 13742	Eritrea	Unknown	Asmara	15.33	38.93	2336	1967
67	EEBC067*	CIho 13743	CI 13743	Eritrea	Unknown	Asmara	15.33	38.93	2336	1967
68	EEBC068*	CIho 13746	CI 13746	Eritrea	Unknown	Asmara	15.33	38.93	2336	1967
69	EEBC069	CIho 13753	CI 13753	Ethiopia	Oromia	Arusi	7.65	39.22	2500	1967

No.	Genotype[a]	Identifier[b]	Plant ID[c]	Collection site/origin						
				Country	State	Province	Latitude	Longitude	Elevation (m)	Year
70	EEBC070	CIho 13767	CI 13767	Ethiopia	Amhara	Gonder	12.12	37.78	2022	1967
71	EEBC071	CIho 13776	CI 13776	Ethiopia	Oromia	Bale	7.00	39.40	2400	1967
72	EEBC072	CIho 13777	CI 13777	Ethiopia	Oromia	Bale	7.00	39.40	2400	1967
73	EEBC073	CIho 14092[k]	507	Ethiopia	Amhara	Welo	11.50	40.00	3355	1924
74	EEBC074*	CIho 14124[k]	473	Ethiopia	Amhara	Welo	11.13	39.63	2495	1923
75	EEBC075*	CIho 14880	ELS 6402-467	Eritrea	Unknown	Asmara	15.33	38.93	2336	1964
76	EEBC076*	CIho 14881	ELS 6402-467	Eritrea	Unknown	Asmara	15.33	38.93	2336	1964
77	EEBC077*	CIho 14882	ELS 6402-468	Eritrea	Unknown	Asmara	15.33	38.93	2336	1964
78	EEBC078*	CIho 14883	ELS 6402-469	Eritrea	Unknown	Asmara	15.33	38.93	2336	1964
79	EEBC079	CIho 14886	ELS 6402-472	Ethiopia	Harari	Harer	9.50	41.50	Unknown	1964
80	EEBC080	CIho 14926	ELS 6402-513	Ethiopia	Harari	Harer	9.50	41.50	Unknown	1964
81	EEBC081	CIho 14975	ELS 6402-562	Ethiopia	Tigray	Tigre	14.13	38.72	2257	1964
82	EEBC082*	CIho 14978	ELS 6402-565	Ethiopia	Tigray	Tigre	13.65	39.57	2495	1964
83	EEBC083	CIho 14986	ELS 6402-573	Ethiopia	Oromia	Arusi	7.95	39.13	2135	1964
84	EEBC084*	PI 60694[k]	Kober	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1924
85	EEBC085	PI 130582	462	Ethiopia	Tigray	Tigre	13.50	39.50		1937
86	EEBC086*	PI 151787[k]	Abyssinian 12	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1945
87	EEBC087*	PI 151795[l]	Abyssinian 21	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1945
88	EEBC088*	PI 186424[i]	Deficiens d'Abyssinia	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1950
89	EEBC089	PI 193531	8701	Ethiopia	Harari	Harer	9.58	41.87	1234	1950
90	EEBC090*	PI 193799	8853	Ethiopia	Oromia	Shewa	9.03	38.70	2402	1950
91	EEBC091	PI 193980[k]	8807	Ethiopia	Amhara	Gojam	10.35	37.73	2487	1950
92	EEBC092*	PI 194951	9243	Ethiopia	Amhara	Gonder	12.60	37.47	2201	1950
93	EEBC093*	PI 194952[f,k]	9244	Ethiopia	Amhara	Gonder	12.60	37.47	2201	1950
94	EEBC094	PI 194961[k]	9253	Ethiopia	Amhara	Gonder	12.60	37.47	2201	
95	EEBC095	PI 195959	9646	Ethiopia	Amhara	Welo	11.13	39.63	2495	1951
96	EEBC096	PI 197216	9946	Ethiopia	Harari	Harer	9.40	42.32	2084	1951
97	EEBC097	PI 273899	1863	Ethiopia	Harari	Harer	9.32	42.12	1935	1960
98	EEBC098*	PI 273905	1997	Ethiopia	Oromia	Kefa	7.83	36.67	2288	1960
99	EEBC099*	PI 277393[e]	S-18	Ethiopia	Oromia	Shewa	9.07	38.50	2402	1961
100	EEBC100*	PI 283441	C.P.I. 15700	Ethiopia	Unknown	Unknown	9.68	39.53	2789	1962
101	EEBC101*	PI 285118	S-36	Eritrea	Unknown	Unknown	Unknown	Unknown	Unknown	1962
102	EEBC102	PI 285120	S-38	Ethiopia	Harari	Harer	9.38	41.60	2349	1962
103	EEBC103*	PI 285124	S-42	Ethiopia	Oromia	Shewa	Unknown	Unknown	Unknown	1962
104	EEBC104*	PI 286388	CI 12089	Eritrea	Unknown	Asmara	15.33	38.93	2336	1963
105	EEBC105*	PI 286389	CI 12090	Eritrea	Unknown	Unknown	Unknown	Unknown	Unknown	1963

No.	Genotype[a]	Identifier[b]	Plant ID[c]	Collection site/origin						
				Country	State	Province	Latitude	Longitude	Elevation (m)	Year
106	EEBC106	PI 286398	121	Ethiopia	Tigray	Tigre	14.12	39.47	2359	1963
107	EEBC107*	PI 290346	Abessinische 6 Zlg	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1963
108	EEBC108*	PI 295373	ELS 6302-5	Ethiopia	Oromia	Shewa	8.75	38.95	1800	1963
109	EEBC109*	PI 295442	ELS 6302-74	Ethiopia	Oromia	Shewa	9.35	38.77	2750	1963
110	EEBC110*	PI 295581	ELS 6302-213	Ethiopia	Oromia	Shewa	7.20	38.60	1525	1963
111	EEBC111*	PI 296453	H-2170	Eritrea	Unknown	Asmara	15.33	38.93	2336	1964
112	EEBC112*	PI 296455[n,o]	H-2164	Ethiopia	Tigray	Tigre	13.50	39.47	2044	1964
113	EEBC113*	PI 296457	H-2180	Ethiopia	Tigray	Tigre	14.20	39.42	2815	1964
114	EEBC114*	PI 296459	H-2213	Eritrea	Unknown	Unknown	14.68	39.42	2403	1964
115	EEBC115*	PI 296460	H-2227	Eritrea	Unknown	Senhit	15.78	38.46	1553	1964
116	EEBC116	PI 296467	H-2171	Ethiopia	Southern	Gemu Gefa	5.78	36.57	1401	1964
117	EEBC117*	PI 296472	H-2183	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1964
118	EEBC118	PI 296479	H-2162	Ethiopia	Southern	Sidamo	6.48	38.52	2746	1964
119	EEBC119	PI 296488	H-2190	Ethiopia	Southern	Gemu Gefa	6.17	36.62	1952	1964
120	EEBC120	PI 296499	H-2185	Ethiopia	Southern	Gemu Gefa	5.78	36.57	1401	1964
121	EEBC121	PI 296511	H-2215	Ethiopia	Southern	Sidamo	6.20	38.70	2447	1964
122	EEBC122	PI 296517	H-2222	Ethiopia	Southern	Gemu Gefa	5.78	36.57	1401	1964
123	EEBC123	PI 296533	H-2204	Ethiopia	Amhara	Welo	11.13	39.63	2495	1964
124	EEBC124	PI 298309	ELS 6402-263	Ethiopia	Oromia	Bale	7.00	39.40	2400	1967
125	EEBC125	PI 298310	ELS 6302-220	Ethiopia	Oromia	Arusi	7.98	39.15	2375	1963
126	EEBC126	PI 298324	ELS 6402-261	Ethiopia	Oromia	Bale	7.00	39.98	2900	1964
127	EEBC127	PI 298325	ELS 6402-262	Ethiopia	Oromia	Bale	7.00	39.98	2900	1964
128	EEBC128	PI 298326	ELS 6402-264	Ethiopia	Oromia	Bale	7.00	39.98	2900	1964
129	EEBC129	PI 298327	ELS 6402-265	Ethiopia	Oromia	Bale	7.00	39.98	2900	1964
130	EEBC130	PI 298328	ELS 6402-266	Ethiopia	Oromia	Bale	7.00	40.02	2900	1964
131	EEBC131	PI 298329	ELS 6402-267	Ethiopia	Oromia	Bale	7.00	40.02	2900	1964
132	EEBC132	PI 298330	ELS 6402-268	Ethiopia	Oromia	Bale	7.00	40.02	2900	1964
133	EEBC133	PI 298340	ELS 6302-225	Ethiopia	Harari	Harer	9.25	41.25	2130	1963
134	EEBC134	PI 298656	ELS 6402-269	Ethiopia	Amhara	Gojam	11.27	36.83	1952	1964
135	EEBC135	PI 298679	ELS 6402-357	Ethiopia	Oromia	Arusi	7.98	39.13	2288	1964
136	EEBC136	PI 298688[e]	ELS 6402-384	Ethiopia	Oromia	Bale	7.05	39.63	3508	1964
137	EEBC137	PI 298690[e]	ELS 6402-374	Ethiopia	Oromia	Bale	7.05	39.63	3508	1964
138	EEBC138	PI 298691[e]	ELS 6402-385	Ethiopia	Oromia	Bale	7.05	39.63	3508	1964
139	EEBC139*	PI 298708[n,o]	ELS 6402-317	Ethiopia	Oromia	Kefa	7.73	36.58	2562	1964
140	EEBC140	PI 298724	ELS 6402-353	Ethiopia	Oromia	Arusi	8.53	39.27	2196	1964
141	EEBC141*	PI 298738	ELS 6402-282	Ethiopia	Oromia	Shewa	9.78	38.68	3080	1964

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142	EEBC142	PI 298766	ELS 6402-376[d]	Ethiopia	Oromia	Bale	7.05	39.63	3508	1964
143	EEBC143	PI 298770	ELS 6402-380	Ethiopia	Oromia	Bale	7.05	39.63	3508	1964
144	EEBC144*	PI 316801	HOR 2541	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1966
145	EEBC145*	PI 316835	HOR 2720	Ethiopia	Oromia	Kefa	7.67	36.83	1716	1959
146	EEBC146*	PI 316874	S 7244	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1959
147	EEBC147*	PI 328976	S 139	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1968
148	EEBC148*	PI 329000	S 3192	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1968
149	EEBC149*	PI 331217	290b	Eritrea	Unknown	Asmara	15.33	38.93	2336	1968
150	EEBC150	PI 331237	P65	Ethiopia	Tigray	Tigre	12.92	39.52	3500	1967
151	EEBC151*	PI 356264	E 81/2	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1970
152	EEBC152*	PI 356333[p]	E 1/183	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1970
153	EEBC153*	PI 356505	E 435/5	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1970
154	EEBC154*	PI 356580	E 508/3	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1970
155	EEBC155*	PI 356666	E 586/6	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1970
156	EEBC156	PI 358287	71	Ethiopia	Oromia	Ilubabor	8.30	35.58	1700	1970
157	EEBC157	PI 358599	22852	Ethiopia	Tigray	Tigre	13.87	39.60	2440	1970
158	EEBC158*	PI 382182[n,o]	GAW 3-2	Ethiopia	Oromia	Shewa	9.17	39.12	2618	1970
159	EEBC159	PI 382184	GAW 17-2	Ethiopia	Oromia	Shewa	9.50	38.87	2632	1970
160	EEBC160	PI 382223	GAW 50-12	Ethiopia	Amhara	Gonder	13.10	37.85	2721	1970
161	EEBC161	PI 382226	GAW 51-2	Ethiopia	Tigray	Tigre	14.12	38.50	2044	1970
162	EEBC162*	PI 382275	GAW 66-3	Ethiopia	Tigray	Tigre	14.32	39.23	2226	1970
163	EEBC163*	PI 382296	GAW 76-3	Ethiopia	Tigray	Tigre	13.98	39.58	2368	1970
164	EEBC164*	PI 382343	GAW 86-2	Ethiopia	Tigray	Tigre	12.52	39.53	2495	1970
165	EEBC165	PI 382373[f]	GAW 104-4	Ethiopia	Oromia	Shewa	8.57	38.00	2118	1970
166	EEBC166	PI 382434	GAW 137-3	Ethiopia	Oromia	Shewa	8.53	39.25	1633	1970
167	EEBC167*	PI 382437	GAW 138-3	Ethiopia	Oromia	Shewa	8.42	39.38	1401	1970
168	EEBC168*	PI 382506	GAW 71-16	Ethiopia	Tigray	Tigre	14.27	39.45	2491	1970
169	EEBC169	PI 382510	GAW 81-5	Ethiopia	Tigray	Tigre	12.93	39.52	2446	1970
170	EEBC170	PI 382630	GAW 153-5	Ethiopia	Amhara	Welo	11.08	39.73	1815	1970
171	EEBC171	PI 382642	GAW 27-3	Ethiopia	Amhara	Gojam	10.58	37.45	2063	1970
172	EEBC172	PI 382725	GAW 27-1	Ethiopia	Amhara	Gojam	10.58	37.45	2063	1970
173	EEBC173*	PI 382798	GAW 42-1	Ethiopia	Amhara	Gonder	12.72	37.43	2263	1970
174	EEBC174*	PI 382839	GAW 47-1	Ethiopia	Amhara	Gonder	12.77	37.62	2932	1970
175	EEBC175*	PI 382860	GAW 48-12	Ethiopia	Amhara	Gonder	12.78	37.65	2782	1970
176	EEBC176*	PI 382911	GAW 72-8	Ethiopia	Tigray	Tigre	14.27	39.47	2443	1970
177	EEBC177	PI 382952	GAW 84-5	Ethiopia	Tigray	Tigre	12.85	39.57	2871	1970

No.	Genotype[a]	Identifier[b]	Plant ID[c]	Collection site/origin						
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178	EEBC178*	PI 382982	GAW 87-1	Ethiopia	Tigray	Tigre	12.52	39.53	2495	1970
179	EEBC179*	PI 383031	GAW 99-4	Ethiopia	Oromia	Shewa	8.68	38.23	2147	1970
180	EEBC180	PI 383101	GAW 125-1	Ethiopia	Oromia	Welega	9.22	37.37	2389	1970
181	EEBC181*	PI 383136	GAW 141-2	Ethiopia	Oromia	Shewa	8.28	39.42	1703	1970
182	EEBC182	PI 386385	IAR/B/133	Ethiopia	Oromia	Welega	9.00	35.50		1974
183	EEBC183	PI 386398	IAR/B/214-2	Ethiopia	Oromia	Arusi	7.53	39.25	2817	1974
184	EEBC184*	PI 386406	IAR/B/252	Eritrea	Unknown	Unknown	15.33	38.67	1977	1974
185	EEBC185*	PI 386407	IAR/B/253	Eritrea	Unknown	Unknown	15.33	38.67	1977	1974
186	EEBC186	PI 386412	IAR/B/266	Ethiopia	Harari	Harer	9.58	41.87	1234	1974
187	EEBC187	PI 386415	IAR/B/272	Ethiopia	Oromia	Ilubabor	8.17	36.33	2258	1974
188	EEBC188	PI 386416	IAR/B/273	Ethiopia	Oromia	Ilubabor	8.17	36.33	2258	1974
189	EEBC189*	PI 386462	IAR/B/434	Ethiopia	Tigray	Tigre	14.17	38.90	1890	1974
190	EEBC190	PI 386475	IAR/B/452	Ethiopia	Tigray	Tigre	14.13	38.72	2184	1974
191	EEBC191	PI 386514	IAR/B/148-3	Ethiopia	Oromia	Arusi	7.07	38.78	2666	1974
192	EEBC192*	PI 386524	IAR/B/431	Ethiopia	Southern	Sidamo	6.48	38.52	2746	1974
193	EEBC193	PI 386525	IAR/B/432-3	Ethiopia	Southern	Sidamo	6.48	38.52	2746	1974
194	EEBC194*	PI 386526	IAR/B/527-2	Ethiopia	Oromia	Shewa	9.68	39.53	2789	1974
195	EEBC195*	PI 386559	IAR/B/37	Ethiopia	Amhara	Gonder	11.85	38.02	2692	1974
196	EEBC196	PI 386581	IAR/B/99-2	Ethiopia	Southern	Gemu Gefa	6.03	37.55	1269	1974
197	EEBC197	PI 386584	IAR/B/107	Ethiopia	Southern	Sidamo	6.57	38.50	2560	1974
198	EEBC198*	PI 386601	IAR/B/155-2	Ethiopia	Oromia	Arusi	7.53	39.25	2817	1974
199	EEBC199*	PI 386650	IAR/B/239-1	Ethiopia	Amhara	Gonder	13.15	37.90	2842	1974
200	EEBC200	PI 386661	IAR/B/257-3	Ethiopia	Amhara	Gojam	10.35	37.73	2487	1974
201	EEBC201	PI 386666	IAR/B/271	Ethiopia	Oromia	Ilubabor	7.50	35.50	2417	1974
202	EEBC202*	PI 386723	IAR/B/361-2	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1974
203	EEBC203*	PI 386759	IAR/B/400	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1974
204	EEBC204	PI 386833	IAR/B/31	Ethiopia	Amhara	Gojam	11.00	36.92	2661	1974
205	EEBC205*	PI 386838	IAR/B/38-1	Ethiopia	Amhara	Gonder	11.85	38.02	2692	1974
206	EEBC206*	PI 386844	IAR/B/57	Ethiopia	Tigray	Tigre	14.10	38.50	2286	1974
207	EEBC207	PI 386863	IAR/B/85	Ethiopia	Oromia	Welega	9.10	35.45	1524	1974
208	EEBC208	PI 386873	IAR/B/101-1	Ethiopia	Southern	Gemu Gefa	6.03	37.55	1269	1974
209	EEBC209	PI 386897	IAR/B/123	Ethiopia	Southern	Sidamo	6.42	38.32	1593	1974
210	EEBC210*	PI 386940	IAR/B/175-1	Ethiopia	Oromia	Arusi	7.07	38.78	2666	1974
211	EEBC211*	PI 386993	IAR/B/214-3	Ethiopia	Oromia	Arusi	7.53	39.25	2817	1974
212	EEBC212*	PI 387098	IAR/B/328-3	Ethiopia	Oromia	Shewa	8.92	38.65	2241	1974
213	EEBC213	PI 387184	IAR/B/427	Ethiopia	Southern	Sidamo	5.00	39.00	Unknown	1974

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				Country	State	Province	Latitude	Longitude	Elevation (m)	Year
214	EEBC214	PI 387186	IAR/B/428-2	Ethiopia	Southern	Sidamo	5.00	39.00	Unknown	1974
215	EEBC215*	PI 387202	IAR/B/473	Eritrea	Unknown	Seraye	14.89	38.82	1967	1974
216	EEBC216	PI 387226	IAR/B/506-1	Ethiopia	Tigray	Tigre	12.98	39.55	2989	1974
217	EEBC217	PI 387240	IAR/B/521-3	Ethiopia	Oromia	Shewa	9.00	38.25	2133	1974
218	EEBC218	PI 447116	ST 1256 Harar	Ethiopia	Harari	Harer	9.50	41.50	Unknown	1980
219	EEBC219*	PI 548736	HOR 2928	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1990
220	EEBC220	EN 17695	Unknown	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1949
221	EEBC221	EN 17696	Fayiks	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1949
222	EEBC222	EN 18830	Local	Ethiopia	Oromia	Shewa	Unknown	Unknown	Unknown	1959
223	EEBC223	EN 18838	Local	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1959
224	EEBC224	EN 18842	Local	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1959
225	EEBC225	EN 18843	Local	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1959
226	EEBC226	EN 18844	Local	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1959
227	EEBC227	EN 18846	Local	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1959
228	EEBC228	EN 21272	DZ02-610	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1972
229	EEBC229	EN 22900	DZ02- 622	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
230	EEBC230	EN 22968	DZ02-456	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1976
231	EEBC231	EN 25027	DZ02-228	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
232	EEBC232	EN 20054	LINIYA AHOR 2930/66	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1969
233	EEBC233	EN 21984	Athiopien-AB.9	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1917
234	EEBC234	EN 22905	DZ02-24	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1976
235	EEBC235	EN 22940	DZ02-158	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1976
236	EEBC236	EN 22951	DZ02-237	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1976
237	EEBC237	EN 23026	III-29K	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1976
238	EEBC238	EN 23027	III-39CH	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1976
239	EEBC239	EN 23028	III-39D	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1976
240	EEBC240	EN 23036	I-22B	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1976
241	EEBC241	EN 23042	I-57A	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1976
242	EEBC242	EN 23055	II-164B	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1976
243	EEBC243	EN 23060	III-36A	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1976
244	EEBC244	EN 23062	III-38	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1976
245	EEBC245	EN 23065	III-45B	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1976
246	EEBC246	EN 25016	Local	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
247	EEBC247	EN 25025	DZ02-174	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
248	EEBC248	EN 25029	DZ02-249	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
249	EEBC249	EN 25030	DZ02-269	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown

No.	Genotype[a]	Identifier[b]	Plant ID[c]	Collection site/origin						
				Country	State	Province	Latitude	Longitude	Elevation (m)	Year
250	EEBC250	EN 25036	DZ02-400	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
251	EEBC251	EN 25038	DZ02-600	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
252	EEBC252	EN 25810	Abyssinian	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1979
253	EEBC253	EN 26045	Ethiopia AB.1122	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1979
254	EEBC254	EN 26335	III-75	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1980
255	EEBC255	EN 26557	Local	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1980
256	EEBC256	EN 26605	Local	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1980
257	EEBC257	EN 26606	Local	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1980
258	EEBC258	EN 26695	Local	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1980
259	EEBC259	EN 26696	Local	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1980
260	EEBC260	EN 27093	Local	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
261	EEBC261	EN 27094	Local	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
262	EEBC262	EN 28218	Local	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1986
263	EEBC263	EN 30808	EP 73	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
264	EEBC264	EN 30809	Ab 15	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
265	EEBC265	EN 30810	H-2210	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
266	EEBC266	PI 296508	H-2211	Ethiopia	Amhara	Gonder	12.60	37.47	2201	1964
267	EEBC267	PI 296509	H-2212 Or Ab 1118	Ethiopia	Amhara	Gonder	12.97	37.77	2561	1964
268	EEBC268	EN 30814	Ab 1119	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
269	EEBC269	EN 21165	DZ02-401	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1972
270	EEBC270	EN 21223	II-95A	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1972
271	EEBC271	EN 23003	DZ02-693	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1976
272	EEBC272	EN 25550	II-23	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1978
273	EEBC273	EN 25557	II-147B	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1978
274	EEBC274	EN 26528	EH 12B/F3.Q.A.2.B	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1980
275	EEBC275	EN 26531	76-12-1	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1980
276	EEBC276	EN 26539	EH20-F3-A-A	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1980
277	EEBC277	EN 26547	EH12B/F3.M.1.A.1.A	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1980
278	EEBC278	EN 26548	EH12B/F3.M.1.A.4.A	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1980
279	EEBC279	EN 26559	Local	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1980
280	EEBC280	EN 26582	Local	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1980
281	EEBC281	EN 27090	Local	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
282	EEBC282	EN 3241	Local	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	1918
283	EEBC283	Land09 109	Unknown	Eritrea	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
284	EEBC284	Land09 110	Unknown	Eritrea	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
286	EEBC286	Land09 112	Unknown	Eritrea	Akabe Saat	Unknown	Unknown	Unknown	Unknown	Unknown

				Collection site/origin						
				Country	**State**	**Province**	**Latitude**	**Longitude**	**Elevation (m)**	**Year**
287	EEBC287	Land09 113	Unknown	Unknown	Arta	Unknown	Unknown	Unknown	Unknown	Unknown
288	EEBC288	Land09 114	Unknown	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
289	EEBC289	Land09 115	Unknown	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
290	EEBC290	Land09 116	Unknown	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
291	EEBC291	Land09 117	Unknown	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
292	EEBC292	Land09 118	Unknown	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
293	EEBC293	Land09 119	Unknown	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
294	EEBC294	Land09 120	Unknown	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
295	EEBC295	Land09 121	Unknown	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
296	EEBC296	Land09 122	Unknown	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
297	EEBC297	Land09 123	Unknown	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
298	EEBC298	Land09 124	Unknown	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
299	EEBC299	Land09 125	Unknown	Ethiopia	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown

[a] EEBC001-EEBC219: obtained from the USDA-ARS, National Small Grains Collection (NSGC), Aberdeen, Idaho, USA; * Core germplasm; EEBC220-EEBC282: obtained from N.I. Vavilov Research Institute of Plant Industry (VIR), St. Petersburg, Russia; EEBC282-EEBC299: obtained from the International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria.

[b] Given as accession prefix (PI or CIhO) followed by accession number (ACNO). EN (entry number) designations were assigned for accessions obtained from VIR; landraces obtained from ICARDA were designated by internal laboratory reference numbers preceded by the prefix "Land09" as used in a 2009 nursery in Saint Paul, MN.

[c] Plant ID number or plant name; the ID was uncertain for some accessions; some names (e.g. local) were redundant.

[d] This accession was also used in the study of Bonman et al. 2005.

[e] These six accessions were also used in the study of Qualset and Schaller (1969).

[f] These three accessions were also used in the study of Webster et al. 1980.

[g] This accession was also used in the study Larter and Enns (1962).

[h] This accession was also used in the study Bonman et al. 2005.

[i] This accession was also used in the study Metcalfe (1966).

[j] This accession was also used in the study Moseman and Metcalfe (1969).

[k] These seven accessions were also used in the study of Schaller et al. 1963.

[l] This accession was also used in the study Buchannon and McDonald (1965).

[m] This accession was also used in the study Bockelman et al. 1980.

[n] These three accessions were also used in the study of Bowman et al. 2001.

[o] These three accessions were also used in the study of Turaspekov et al. 2008.

[p] This accession was also used in the study Reid et al. 1980.

Appendix Table 3.2. Estimated subpopulation membership coefficients of 298 Ethiopian and Eritrean barley landraces using *K*=7 as inferred from STRUCTURE

				Inferred ancestry of individuals into subpopulations (sp)[a]						
No.	**Genotype**	**Identifier**	**Plant ID**	**1**	**?**	**2**	**3**	**4**	**5**	**6**
1	EEBC001	PI 193538	8738	0.011	0	0.002	0	0	0.001	0.985
2	EEBC002	PI 193986	8894	0.251	0	0.001	0	0	0.003	0.744
3	EEBC003	PI 195967	9743	0.002	0	0.002	0	0	0.001	0.995
4	EEBC004	PI 195988	9842	0.863	0	0.098	0	0	0.021	0.018
5	EEBC005	PI 196858	10034	0.002	0	0.078	0.66	0.005	0.253	0.001
6	EEBC006	PI 277383	S-5	0.765	0	0.211	0	0	0.005	0.019
7	EEBC007	PI 277387	S-9	0.001	0	0.997	0	0	0	0.001
8	EEBC008	PI 296456	H-2187	0.015	0	0.116	0	0	0.856	0.012
9	EEBC009	PI 295438	ELS 6302-70	0.433	0	0.001	0	0	0	0.565
10	EEBC010	PI 298765	ELS 6402-375	0.004	0	0.001	0	0	0	0.995
11	EEBC011	PI 298769	ELS 6402-379	0.003	0	0.001	0	0	0.001	0.995
12	EEBC012	PI 298796	ELS 6402-414	0.116	0	0.064	0	0	0.805	0.015
13	EEBC013	PI 296454	H-2163	0.042	0	0.419	0.003	0.525	0.002	0.008
14	EEBC014	PI 298736	ELS 6402-277	0.061	0	0.001	0	0	0.002	0.935
15	EEBC015	CIho 13745	CI 13745	0.004	0	0.001	0	0	0	0.994
16	EEBC016	PI 331228	R56	0.787	0	0.208	0	0	0.002	0.002
17	EEBC017	CIho 13761	CI 13761	0.308	0	0.002	0	0	0.001	0.689
18	EEBC018	CIho 14976	Yaha	0.002	0.095	0.464	0.16	0.278	0	0.001
19	EEBC019	CIho 14977	ELS 6402-564	0.044	0	0.619	0	0	0.001	0.336
20	EEBC020	PI 347245	265	0.989	0	0.005	0	0	0.001	0.005
21	EEBC021	PI 382191	GAW 28-4	0.428	0	0.565	0	0	0.001	0.006
22	EEBC022	PI 382251	GAW 60-6	0.301	0	0.696	0	0	0.001	0.002
23	EEBC023	PI 382269	GAW 65-3	0.003	0.001	0.992	0	0	0.002	0.002
24	EEBC024	PI 382313	GAW 79-3	0.027	0	0.922	0	0	0.005	0.046
25	EEBC025	PI 382325	GAW 81-6	0.001	0	0.997	0	0	0	0.001
26	EEBC026	PI 382379	GAW 106-6	0.803	0	0.192	0	0	0.001	0.004
27	EEBC027	PI 382448	GAW 142-5	0.984	0	0.001	0	0	0	0.014
28	EEBC028	PI 382695	GAW 11-2	0.049	0	0.002	0	0	0.001	0.948
29	EEBC029	PI 382743	GAW 32-4	0.303	0	0.013	0	0	0.001	0.683
30	EEBC030	PI 382865	GAW 48-17	0.003	0	0.001	0	0	0	0.995
31	EEBC031	PI 383041	GAW 110-1	0.002	0	0.001	0	0	0.001	0.996
32	EEBC032	PI 383089	GAW 122-1	0.033	0	0.002	0	0	0.004	0.961
33	EEBC033	PI 383108	GAW 126-3	0.003	0	0.001	0	0	0.001	0.995
34	EEBC034	PI 383150	GAW 144-5	0.002	0	0.001	0.001	0.002	0.001	0.994

35	EEBC035	PI 386392	IAR/B/136-3	0.67	0.067	0.003	0	0	0.001	0.259
36	EEBC036	PI 386393	IAR/B/141	0.007	0	0.085	0	0	0.031	0.876
37	EEBC037	PI 386458	IAR/B/411-2	0.005	0	0.001	0	0	0.001	0.993
38	EEBC038	PI 386505	IAR/B/513	0.955	0	0.039	0	0	0.001	0.005
39	EEBC039	PI 386655	IAR/B/246-2	0.095	0	0.001	0	0	0.001	0.903
40	EEBC040	PI 386704	IAR/B/337-1	0.84	0	0.157	0	0	0.001	0.003
41	EEBC041	PI 386855	IAR/B/76	0.025	0	0.003	0	0	0.003	0.969
42	EEBC042	PI 386881	IAR/B/110	0.344	0	0.007	0	0.001	0.024	0.624
43	EEBC043	PI 386920	IAR/B/159	0.969	0	0.023	0	0	0.001	0.006
44	EEBC044	PI 386997	IAR/B/216-1	0.002	0	0.001	0	0	0	0.996
45	EEBC045	PI 387016	IAR/B/244-2	0.307	0	0.004	0	0	0.001	0.688
46	EEBC046	PI 387033	IAR/B/280-1	0.423	0	0.007	0	0	0	0.569
47	EEBC047	PI 387049	IAR/B/292-1	0.809	0	0.002	0	0	0.17	0.019
48	EEBC048	PI 387195	IAR/B/450	0.012	0	0.002	0	0	0.001	0.985
49	EEBC049	PI 387201	IAR/B/467-2	0.005	0	0.001	0	0	0	0.993
50	EEBC050	PI 387231	IAR/B/514-2	0.603	0	0.01	0	0	0.001	0.386
51	EEBC051	CIho 1604	Hillsa	0.009	0	0.001	0	0	0.001	0.99
52	EEBC052	CIho 2226	Steudelli	0.997	0	0.001	0	0	0.001	0.002
53	EEBC053	CIho 4221	494b	0.458	0	0.003	0	0	0.001	0.537
54	EEBC054	CIho 11709	CI 11709	0.841	0	0.005	0	0	0.135	0.018
55	EEBC055	CIho 11788	Harar	0.309	0	0.038	0.291	0.206	0.147	0.009
56	EEBC056	CIho 11812	Abyssinian 16	0.007	0	0.002	0	0.001	0.031	0.958
57	EEBC057	CIho 11813	Abyssinian 1118	0.443	0.001	0.061	0.35	0.045	0.093	0.007
58	EEBC058	CIho 11849	Alemaya Mago	0.176	0.281	0.001	0.536	0.002	0.001	0.003
59	EEBC059	CIho 13141	CI 13141	0.387	0	0.043	0.002	0.001	0.564	0.003
60	EEBC060	CIho 13353	CI 13353	0.01	0	0.096	0.228	0.599	0.045	0.021
61	EEBC061	CIho 13355	CI 13355	0.004	0	0.159	0	0	0.72	0.116
62	EEBC062	CIho 13398	2095	0.424	0	0.002	0	0	0.001	0.573
63	EEBC063	CIho 13737	CI 13737	0.008	0	0.045	0	0	0	0.946
64	EEBC064	CIho 13740	CI 13740	0.005	0	0.001	0	0	0	0.993
65	EEBC065	CIho 13741	CI 13741	0.362	0	0.002	0	0	0.001	0.635
66	EEBC066	CIho 13742	CI 13742	0.356	0	0.001	0	0	0	0.641
67	EEBC067	CIho 13743	CI 13743	0.357	0	0.001	0	0	0	0.64
68	EEBC068	CIho 13746	CI 13746	0.165	0	0.005	0	0	0	0.829
69	EEBC069	CIho 13753	CI 13753	0.008	0	0.001	0	0	0	0.991
70	EEBC070	CIho 13767	CI 13767	0.243	0	0.002	0.002	0	0.001	0.751
71	EEBC071	CIho 13776	CI 13776	0.001	0	0.001	0	0	0	0.997
72	EEBC072	CIho 13777	CI 13777	0.004	0	0.013	0.001	0.001	0.001	0.981

73	EEBC073	CIho 14092	507	0.137	0	0.015	0	0	0.001	0.847
74	EEBC074	CIho 14124	473	0.003	0	0.002	0	0	0	0.994
75	EEBC075	CIho 14880	ELS 6402-467	0.036	0	0.001	0	0	0.001	0.962
76	EEBC076	CIho 14881	ELS 6402-467	0.234	0.095	0.007	0.364	0.001	0.001	0.298
77	EEBC077	CIho 14882	ELS 6402-468	0.643	0	0.333	0	0	0.001	0.023
78	EEBC078	CIho 14883	ELS 6402-469	0.256	0	0.001	0	0	0.001	0.741
79	EEBC079	CIho 14886	ELS 6402-472	0.546	0	0.032	0	0	0.005	0.416
80	EEBC080	CIho 14926	ELS 6402-513	0.048	0	0.001	0	0	0.001	0.95
81	EEBC081	CIho 14975	ELS 6402-562	0.007	0	0.533	0	0	0.001	0.46
82	EEBC082	CIho 14978	ELS 6402-565	0.213	0	0.77	0	0	0.001	0.015
83	EEBC083	CIho 14986	ELS 6402-573	0.015	0	0.001	0	0	0.001	0.983
84	EEBC084	PI 60694	Kober	0.007	0	0.004	0	0	0.001	0.988
85	EEBC085	PI 130582	462	0.25	0	0.443	0.001	0	0.001	0.305
86	EEBC086	PI 151787	Abyssinian 12	0.007	0	0.021	0	0.001	0.033	0.938
87	EEBC087	PI 151795	Abyssinian 21	0.06	0	0.086	0.805	0.026	0.013	0.01
88	EEBC088	PI 186424	Deficiens d'Abyssinia	0.002	0	0.001	0	0	0.002	0.994
89	EEBC089	PI 193531	8701	0.163	0	0.003	0	0	0.001	0.833
90	EEBC090	PI 193799	8853	0.212	0	0.001	0	0	0	0.787
91	EEBC091	PI 193980	8807	0.202	0	0.001	0	0	0	0.795
92	EEBC092	PI 194951	9243	0.002	0	0.001	0	0	0.002	0.995
93	EEBC093	PI 194952	9244	0.005	0	0.001	0	0	0	0.993
94	EEBC094	PI 194961	9253	0.004	0	0.014	0	0	0.001	0.981
95	EEBC095	PI 195959	9646	0.022	0	0.107	0.568	0.004	0.297	0.001
96	EEBC096	PI 197216	9946	0.01	0	0.002	0	0	0.001	0.986
97	EEBC097	PI 273899	1863	0.974	0	0.004	0	0	0.001	0.021
98	EEBC098	PI 273905	1997	0.737	0	0.006	0	0	0.001	0.256
99	EEBC099	PI 277393	S-18	0.017	0	0.005	0	0	0.016	0.962
100	EEBC100	PI 283441	C.P.I. 15700	0.508	0	0.236	0.05	0.085	0.12	0.002
101	EEBC101	PI 285118	S-36	0.011	0	0.005	0.179	0.794	0.002	0.009
102	EEBC102	PI 285120	S-38	0.001	0	0.001	0.092	0.904	0.001	0.001
103	EEBC103	PI 285124	S-42	0.255	0	0.003	0	0	0.001	0.741
104	EEBC104	PI 286388	CI 12089	0.002	0	0.996	0	0	0	0.001
105	EEBC105	PI 286389	CI 12090	0.04	0	0.918	0	0	0.001	0.04
106	EEBC106	PI 286398	121	0.015	0	0.004	0.176	0.794	0.001	0.01
107	EEBC107	PI 290346	Abessinische 6 Zlg	0.134	0	0.005	0.658	0.172	0.001	0.03
108	EEBC108	PI 295373	ELS 6302-5	0.994	0.001	0.001	0	0	0.001	0.003
109	EEBC109	PI 295442	ELS 6302-74	0.386	0.368	0.002	0	0.241	0.001	0.003
110	EEBC110	PI 295581	ELS 6302-213	0.996	0	0.001	0	0	0.001	0.002

111	EEBC111	PI 296453	H-2170	0.142	0	0.808	0	0	0	0.048
112	EEBC112	PI 296455	H-2164	0.024	0	0.486	0.001	0.455	0.001	0.033
113	EEBC113	PI 296457	H-2180	0.486	0	0.479	0	0	0	0.034
114	EEBC114	PI 296459	H-2213	0.004	0	0.003	0.986	0.001	0.004	0.002
115	EEBC115	PI 296460	H-2227	0.041	0	0.061	0	0	0.536	0.362
116	EEBC116	PI 296467	H-2171	0.685	0	0.073	0	0	0.181	0.061
117	EEBC117	PI 296472	H-2183	0.939	0	0.002	0	0	0.053	0.005
118	EEBC118	PI 296479	H-2162	0.992	0	0.002	0	0	0	0.006
119	EEBC119	PI 296488	H-2190	0.384	0	0.002	0	0	0.586	0.027
120	EEBC120	PI 296499	H-2185	0.078	0	0.041	0.517	0.092	0.268	0.003
121	EEBC121	PI 296511	H-2215	0.996	0	0.002	0	0	0.001	0.001
122	EEBC122	PI 296517	H-2222	0.392	0	0.005	0	0	0.132	0.471
123	EEBC123	PI 296533	H-2204	0.009	0	0.001	0	0	0.001	0.989
124	EEBC124	PI 298309	ELS 6402-263	0.989	0	0.001	0	0	0.001	0.009
125	EEBC125	PI 298310	ELS 6302-220	0.003	0	0.007	0	0	0.809	0.18
126	EEBC126	PI 298324	ELS 6402-261	0.99	0	0.001	0	0	0.001	0.007
127	EEBC127	PI 298325	ELS 6402-262	0.99	0	0.001	0	0	0.001	0.008
128	EEBC128	PI 298326	ELS 6402-264	0.986	0	0.001	0	0	0	0.012
129	EEBC129	PI 298327	ELS 6402-265	0.989	0	0.004	0	0	0.001	0.005
130	EEBC130	PI 298328	ELS 6402-266	0.989	0	0.001	0	0	0.001	0.009
131	EEBC131	PI 298329	ELS 6402-267	0.937	0	0.002	0	0	0.001	0.059
132	EEBC132	PI 298330	ELS 6402-268	0.984	0	0.001	0	0	0	0.014
133	EEBC133	PI 298340	ELS 6302-225	0.011	0	0.002	0	0	0.001	0.985
134	EEBC134	PI 298656	ELS 6402-269	0.744	0	0.21	0	0	0.021	0.024
135	EEBC135	PI 298679	ELS 6402-357	0.68	0	0.282	0.001	0.035	0.001	0.002
136	EEBC136	PI 298688	ELS 6402-384	0.011	0	0.163	0	0	0.001	0.824
137	EEBC137	PI 298690	ELS 6402-374	0.003	0	0.002	0	0	0.001	0.994
138	EEBC138	PI 298691	ELS 6402-385	0.003	0	0.002	0	0	0.001	0.994
139	EEBC139	PI 298708	ELS 6402-317	0.015	0	0.003	0	0	0.004	0.978
140	EEBC140	PI 298724	ELS 6402-353	0.909	0	0.03	0	0	0.002	0.059
141	EEBC141	PI 298738	ELS 6402-282	0.024	0	0.001	0	0	0	0.973
142	EEBC142	PI 298766	ELS 6402-376	0.004	0	0.027	0	0	0.011	0.957
143	EEBC143	PI 298770	ELS 6402-380	0.002	0	0.001	0	0	0.001	0.996
144	EEBC144	PI 316801	HOR 2541	0.901	0	0.052	0.005	0.007	0.032	0.003
145	EEBC145	PI 316835	HOR 2720	0.06	0	0.004	0	0	0.906	0.029
146	EEBC146	PI 316874	S 7244	0.173	0	0.001	0	0	0.733	0.092
147	EEBC147	PI 328976	S 139	0.003	0	0.002	0.606	0.384	0.004	0.001
148	EEBC148	PI 329000	S 3192	0.997	0	0.001	0	0	0	0.002

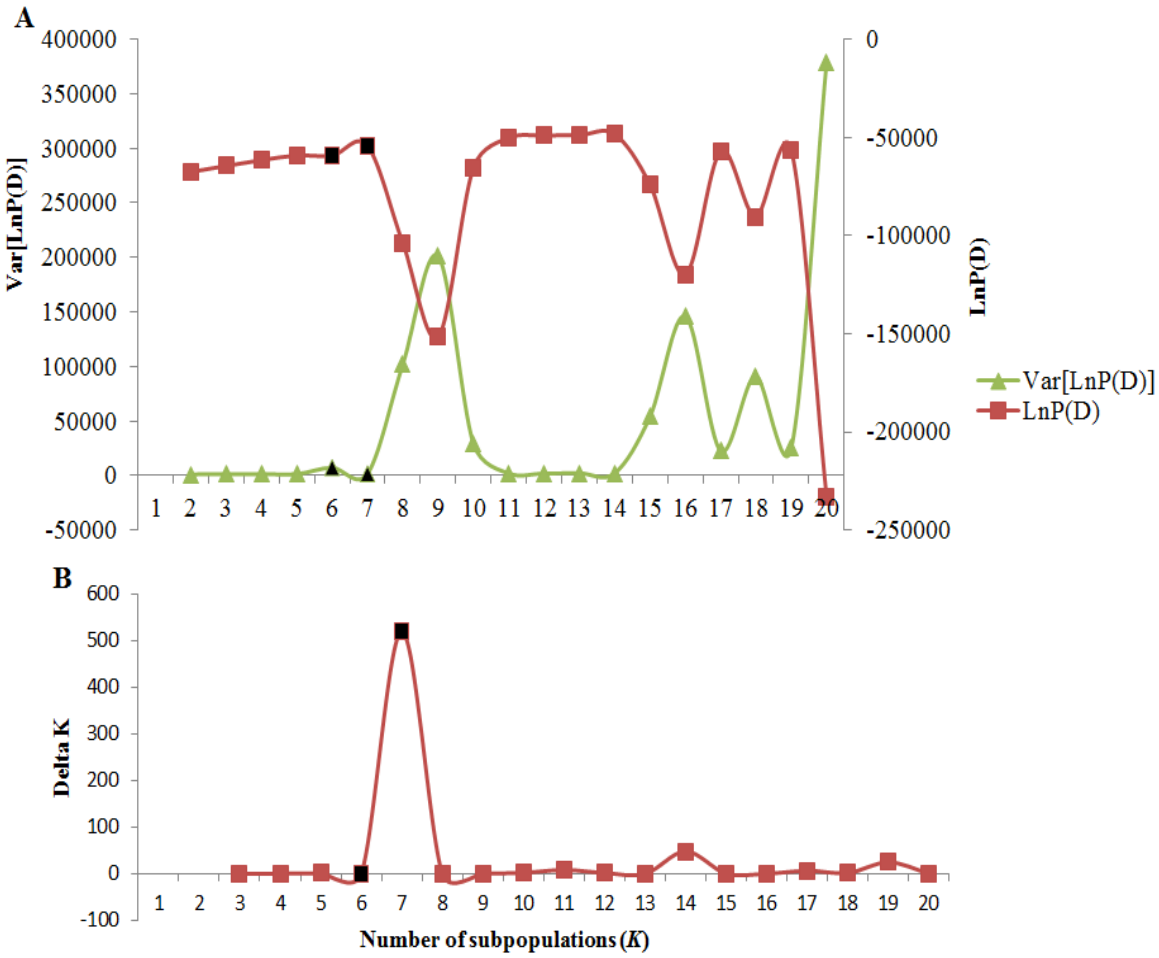
149	EEBC149	PI 331217	290b	0.005	0	0.981	0	0.001	0.001	0.012
150	EEBC150	PI 331237	P65	0.004	0	0.993	0	0	0.001	0.002
151	EEBC151	PI 356264	E 81/2	0.051	0	0.002	0	0	0.004	0.942
152	EEBC152	PI 356333	E 1/183	0.006	0	0.148	0	0	0.001	0.845
153	EEBC153	PI 356505	E 435/5	0.002	0	0.001	0	0	0	0.996
154	EEBC154	PI 356580	E 508/3	0.42	0	0.487	0	0	0.001	0.092
155	EEBC155	PI 356666	E 586/6	0.085	0	0.001	0	0	0.001	0.913
156	EEBC156	PI 358287	71	0.988	0	0.002	0	0	0.008	0.002
157	EEBC157	PI 358599	22852	0.015	0	0.248	0	0.001	0.733	0.003
158	EEBC158	PI 382182	GAW 3-2	0.992	0	0.002	0	0	0	0.005
159	EEBC159	PI 382184	GAW 17-2	0.992	0	0.002	0	0	0	0.005
160	EEBC160	PI 382223	GAW 50-12	0.209	0	0.221	0	0	0.001	0.569
161	EEBC161	PI 382226	GAW 51-2	0.08	0	0.734	0	0	0.001	0.185
162	EEBC162	PI 382275	GAW 66-3	0.025	0	0.679	0	0	0.001	0.295
163	EEBC163	PI 382296	GAW 76-3	0.089	0	0.866	0	0	0.001	0.044
164	EEBC164	PI 382343	GAW 86-2	0.005	0	0.992	0	0	0.001	0.002
165	EEBC165	PI 382373	GAW 104-4	0.2	0	0.063	0	0	0.001	0.736
166	EEBC166	PI 382434	GAW 137-3	0.636	0	0.361	0	0	0.001	0.002
167	EEBC167	PI 382437	GAW 138-3	0.635	0	0.362	0	0	0.001	0.002
168	EEBC168	PI 382506	GAW 71-16	0.541	0	0.443	0	0	0.001	0.014
169	EEBC169	PI 382510	GAW 81-5	0.109	0	0.874	0	0	0.001	0.016
170	EEBC170	PI 382630	GAW 153-5	0.483	0	0.512	0	0	0	0.003
171	EEBC171	PI 382642	GAW 27-3	0.324	0	0.008	0	0	0.001	0.667
172	EEBC172	PI 382725	GAW 27-1	0.005	0	0.148	0	0	0.001	0.847
173	EEBC173	PI 382798	GAW 42-1	0.017	0	0.001	0	0	0.001	0.981
174	EEBC174	PI 382839	GAW 47-1	0.003	0	0.001	0	0	0	0.995
175	EEBC175	PI 382860	GAW 48-12	0.434	0	0.006	0.003	0	0.027	0.529
176	EEBC176	PI 382911	GAW 72-8	0.019	0	0.594	0	0.001	0.002	0.383
177	EEBC177	PI 382952	GAW 84-5	0.334	0	0.001	0	0	0.001	0.664
178	EEBC178	PI 382982	GAW 87-1	0.185	0	0.001	0.001	0	0.038	0.775
179	EEBC179	PI 383031	GAW 99-4	0.002	0	0.001	0	0	0	0.996
180	EEBC180	PI 383101	GAW 125-1	0.034	0	0.001	0	0	0.002	0.963
181	EEBC181	PI 383136	GAW 141-2	0.001	0	0	0	0	0	0.997
182	EEBC182	PI 386385	IAR/B/133	0.987	0	0.002	0	0	0.008	0.002
183	EEBC183	PI 386398	IAR/B/214-2	0.01	0	0.001	0	0	0.001	0.989
184	EEBC184	PI 386406	IAR/B/252	0.001	0	0.997	0	0	0	0.001
185	EEBC185	PI 386407	IAR/B/253	0.001	0	0.997	0	0	0	0.001
186	EEBC186	PI 386412	IAR/B/266	0.549	0	0.148	0	0	0.001	0.301

187	EEBC187	PI 386415	IAR/B/272	0.893	0	0.096	0	0	0.007	0.004
188	EEBC188	PI 386416	IAR/B/273	0.835	0	0.161	0	0	0.001	0.003
189	EEBC189	PI 386462	IAR/B/434	0.247	0	0.726	0	0	0.001	0.026
190	EEBC190	PI 386475	IAR/B/452	0.001	0	0.997	0	0	0	0.001
191	EEBC191	PI 386514	IAR/B/148-3	0.001	0	0	0	0	0.998	0.001
192	EEBC192	PI 386524	IAR/B/431	0.274	0	0.13	0	0	0.001	0.594
193	EEBC193	PI 386525	IAR/B/432-3	0.273	0	0.13	0	0	0.001	0.595
194	EEBC194	PI 386526	IAR/B/527-2	0.001	0	0	0	0	0.998	0.001
195	EEBC195	PI 386559	IAR/B/37	0.001	0	0	0.997	0	0	0.001
196	EEBC196	PI 386581	IAR/B/99-2	0.917	0	0.062	0	0	0.007	0.013
197	EEBC197	PI 386584	IAR/B/107	0.642	0	0.009	0	0	0.002	0.346
198	EEBC198	PI 386601	IAR/B/155-2	0.001	0	0.001	0	0	0	0.997
199	EEBC199	PI 386650	IAR/B/239-1	0.16	0	0.001	0	0	0.001	0.837
200	EEBC200	PI 386661	IAR/B/257-3	0.006	0	0.148	0	0	0.001	0.845
201	EEBC201	PI 386666	IAR/B/271	0.827	0	0.161	0	0	0.006	0.006
202	EEBC202	PI 386723	IAR/B/361-2	0.015	0	0.001	0	0	0.001	0.983
203	EEBC203	PI 386759	IAR/B/400	0.002	0	0.001	0	0	0.001	0.995
204	EEBC204	PI 386833	IAR/B/31	0.03	0	0.002	0	0	0.001	0.967
205	EEBC205	PI 386838	IAR/B/38-1	0.003	0	0.004	0	0	0.001	0.992
206	EEBC206	PI 386844	IAR/B/57	0.033	0	0.68	0	0	0.001	0.286
207	EEBC207	PI 386863	IAR/B/85	0.002	0	0.002	0	0	0.001	0.994
208	EEBC208	PI 386873	IAR/B/101-1	0.522	0	0.003	0	0	0.108	0.366
209	EEBC209	PI 386897	IAR/B/123	0.927	0	0.001	0	0	0.002	0.069
210	EEBC210	PI 386940	IAR/B/175-1	0.007	0	0	0	0	0.002	0.99
211	EEBC211	PI 386993	IAR/B/214-3	0.109	0	0.001	0	0	0.001	0.888
212	EEBC212	PI 387098	IAR/B/328-3	0.002	0	0.001	0	0	0	0.996
213	EEBC213	PI 387184	IAR/B/427	0.01	0	0.018	0.792	0.036	0.14	0.003
214	EEBC214	PI 387186	IAR/B/428-2	0.018	0	0.434	0.003	0.002	0.001	0.541
215	EEBC215	PI 387202	IAR/B/473	0.01	0	0.001	0	0	0.001	0.986
216	EEBC216	PI 387226	IAR/B/506-1	0.054	0	0.001	0	0.001	0.001	0.943
217	EEBC217	PI 387240	IAR/B/521-3	0.992	0	0.001	0	0	0.004	0.002
218	EEBC218	PI 447116	ST 1256 Harar	0.802	0	0.002	0	0	0.167	0.028
219	EEBC219	PI 548736	HOR 2928	0.573	0	0.007	0	0	0	0.42
220	EEBC220	EN 17695		0.001	0.221	0.002	0.713	0.058	0.005	0.001
221	EEBC221	EN 17696	FAYIKS	0.002	0	0.002	0.768	0.225	0.001	0.001
222	EEBC222	EN 18830	LOCAL	0.508	0	0.003	0	0	0.108	0.381
223	EEBC223	EN 18838	LOCAL	0.001	0	0	0.998	0.001	0	0
224	EEBC224	EN 18842	LOCAL	0.064	0	0.003	0	0	0	0.932

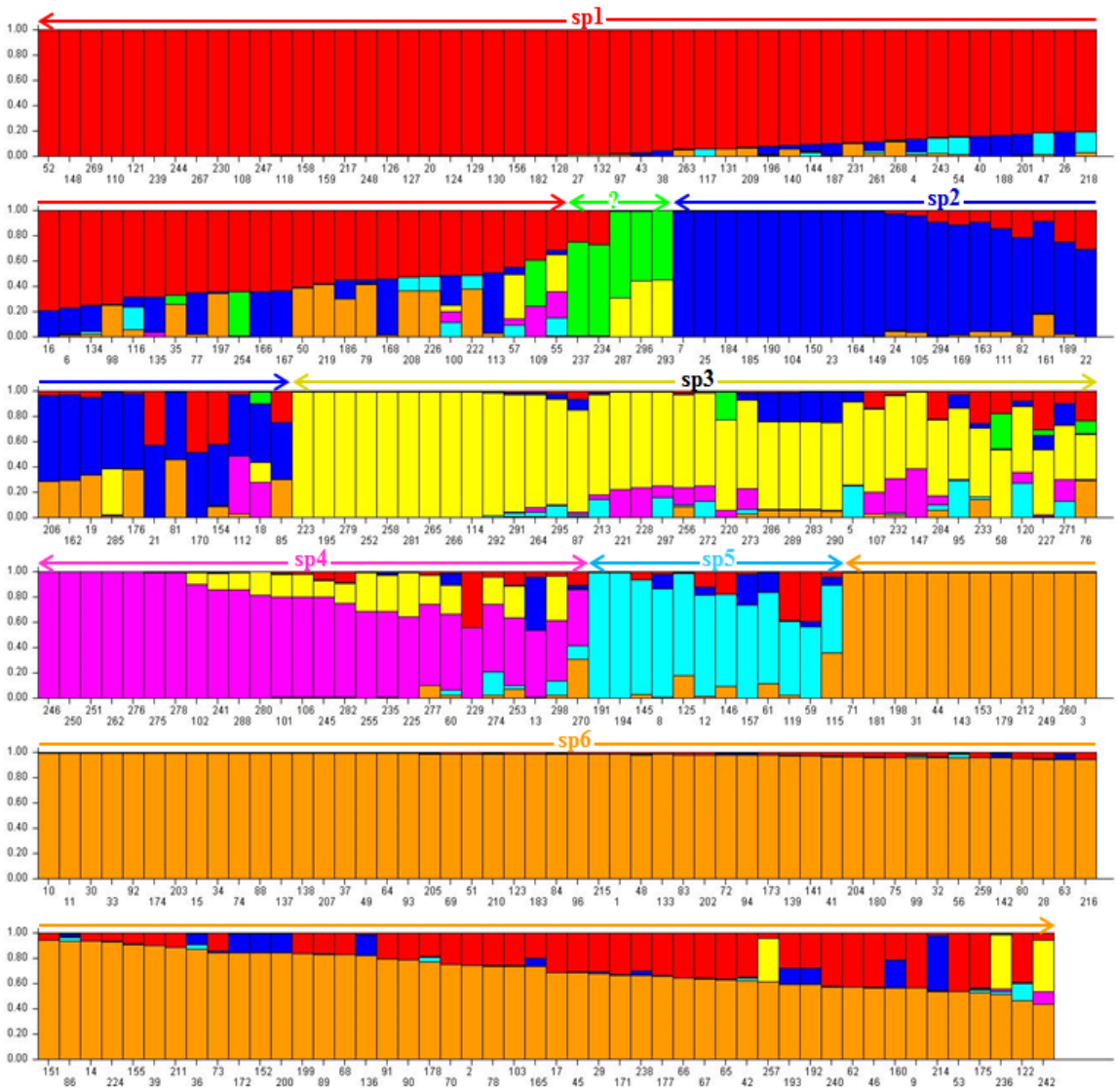
225	EEBC225	EN 18843	LOCAL	0.001	0	0	0.351	0.644	0.002	0.001
226	EEBC226	EN 18844	LOCAL	0.518	0	0.003	0	0	0.108	0.371
227	EEBC227	EN 18846	LOCAL	0.303	0.047	0.113	0.512	0.015	0.003	0.008
228	EEBC228	EN 21272	DZ02-610	0.001	0	0	0.761	0.237	0	0
229	EEBC229	EN 22900	DZ02- 622	0.437	0	0.002	0.001	0.557	0.001	0.002
230	EEBC230	EN 22968	DZ02-456	0.995	0	0.001	0	0	0.001	0.003
231	EEBC231	EN 25027	DZ02-228	0.892	0	0.001	0	0	0	0.106
232	EEBC232	EN 20054	LINIYA AHOR 2930/66	0.027	0	0.002	0.657	0.276	0.012	0.026
233	EEBC233	EN 21984	Athiopien-AB.9	0.256	0	0.033	0.54	0.001	0.023	0.146
234	EEBC234	EN 22905	DZ02-24	0.267	0.72	0.001	0	0	0.001	0.011
235	EEBC235	EN 22940	DZ02-158	0.006	0	0.016	0.286	0.682	0.004	0.006
236	EEBC236	EN 22951	DZ02-237	0.006	0	0.008	0.427	0.018	0.022	0.519
237	EEBC237	EN 23026	III-29K	0.245	0.745	0.001	0	0	0	0.008
238	EEBC238	EN 23027	III-39CH	0.297	0.002	0.032	0	0	0.001	0.667
239	EEBC239	EN 23028	III-39D	0.996	0	0.001	0	0	0	0.002
240	EEBC240	EN 23036	I-22B	0.42	0	0.003	0	0	0	0.576
241	EEBC241	EN 23042	I-57A	0.004	0	0.004	0.132	0.854	0.005	0.001
242	EEBC242	EN 23055	II-164B	0.054	0	0.003	0.402	0.099	0.001	0.442
243	EEBC243	EN 23060	III-36A	0.849	0	0.004	0	0	0.125	0.02
244	EEBC244	EN 23062	III-38	0.996	0	0.001	0	0	0	0.003
245	EEBC245	EN 23065	III-45B	0.064	0	0.007	0.126	0.793	0.002	0.008
246	EEBC246	EN 25016	LOCAL	0	0	0	0	0.999	0	0
247	EEBC247	EN 25025	DZ02-174	0.994	0	0.001	0	0	0.001	0.003
248	EEBC248	EN 25029	DZ02-249	0.992	0	0.001	0	0	0.003	0.003
249	EEBC249	EN 25030	DZ02-269	0.003	0	0.001	0	0	0	0.996
250	EEBC250	EN 25036	DZ02-400	0	0	0	0	0.999	0	0
251	EEBC251	EN 25038	DZ02-600	0	0	0	0	0.999	0	0
252	EEBC252	EN 25810	ABYSSINIAN	0.001	0	0	0.996	0.003	0	0
253	EEBC253	EN 26045	ETHIORIA AB.1122	0.105	0	0.009	0.244	0.537	0.029	0.075
254	EEBC254	EN 26335	III-75	0.637	0.353	0.006	0	0.001	0.001	0.002
255	EEBC255	EN 26557	LOCAL	0.001	0	0	0.31	0.687	0.001	0.001
256	EEBC256	EN 26605	LOCAL	0.015	0	0.011	0.737	0.136	0.014	0.087
257	EEBC257	EN 26606	LOCAL	0.041	0	0.001	0.337	0.001	0.003	0.617
258	EEBC258	EN 26695	LOCAL	0.001	0	0	0.996	0.003	0	0
259	EEBC259	EN 26696	LOCAL	0.04	0	0.001	0	0	0.001	0.958
260	EEBC260	EN 27093	LOCAL	0.002	0	0.001	0	0	0.001	0.996
261	EEBC261	EN 27094	LOCAL	0.881	0	0.069	0	0	0.027	0.023
262	EEBC262	EN 28218	LOCAL	0	0	0	0	0.999	0	0

263	EEBC263	EN 30808	EP 73	0.942	0	0.004	0	0	0	0.053
264	EEBC264	EN 30809	Ab 15	0.01	0	0.018	0.891	0.038	0.031	0.012
265	EEBC265	EN 30810	H-2210	0.002	0	0.001	0.994	0	0.002	0.001
266	EEBC266	EN 30812	H-2212	0.002	0	0.001	0.994	0	0.002	0.001
267	EEBC267	EN 30813	Ab 1118	0.996	0	0.001	0	0	0	0.002
268	EEBC268	EN 30814	Ab 1119	0.866	0	0.012	0	0	0.003	0.117
269	EEBC269	EN 21165	DZ02-401	0.997	0	0.001	0	0	0	0.001
270	EEBC270	EN 21223	II-95A	0.104	0	0.03	0.003	0.448	0.106	0.309
271	EEBC271	EN 23003	DZ02-693	0.093	0	0.172	0.435	0.168	0.129	0.003
272	EEBC272	EN 25550	II-23	0.002	0.001	0.011	0.732	0.125	0.128	0.001
273	EEBC273	EN 25557	II-147B	0.011	0	0.06	0.7	0.164	0.036	0.03
274	EEBC274	EN 26528	EH 12B/F3.Q.A.2.B	0.036	0	0.003	0.214	0.539	0.181	0.028
275	EEBC275	EN 26531	76-12-1	0.001	0	0	0.001	0.998	0	0
276	EEBC276	EN 26539	EH20-F3-A-A	0	0	0	0	0.999	0	0
277	EEBC277	EN 26547	EH12B/F3.M.1.A.1.A	0.026	0	0.001	0.225	0.642	0.002	0.104
278	EEBC278	EN 26548	EH12B/F3.M.1.A.4.A	0.001	0	0	0.001	0.997	0	0
279	EEBC279	EN 26559	LOCAL	0.001	0	0	0.997	0	0.001	0.001
280	EEBC280	EN 26582	LOCAL	0	0	0	0.18	0.818	0.001	0
281	EEBC281	EN 27090	LOCAL	0.001	0	0	0.996	0.003	0	0
282	EEBC282	EN 3241	LOCAL	0.085	0	0.002	0.163	0.741	0.002	0.007
283	EEBC283	LAND09 109	ETH IRA/B/251	0.007	0	0.235	0.693	0.002	0.004	0.059
284	EEBC284	LAND09 110	ETH IRA/B/252	0.218	0	0.008	0.598	0.069	0.049	0.058
285	EEBC286	LAND09 112	ETH IRA/B/57	0.004	0	0.604	0.366	0	0.007	0.018
286	EEBC287	LAND09 113	-	0.008	0	0.232	0.694	0.002	0.005	0.059
287	EEBC288	LAND09 114	-	0.001	0.689	0.001	0.308	0	0.001	0.001
288	EEBC289	LAND09 115	-	0.003	0	0.002	0.137	0.854	0.002	0.002
289	EEBC290	LAND09 116	-	0.008	0	0.232	0.694	0.002	0.004	0.06
290	EEBC291	LAND09 117	-	0.007	0	0.237	0.693	0.002	0.005	0.055
291	EEBC292	LAND09 118	-	0.01	0	0.014	0.933	0.001	0.033	0.009
292	EEBC293	LAND09 119	-	0.006	0.001	0.001	0.972	0	0.015	0.005
293	EEBC294	LAND09 120	-	0	0.546	0	0.451	0.002	0	0
294	EEBC295	LAND09 121	-	0.092	0	0.897	0	0	0.002	0.008
295	EEBC296	LAND09 122	-	0.015	0	0.047	0.836	0.008	0.088	0.006
296	EEBC297	LAND09 123	-	0.001	0.553	0	0.444	0	0.001	0
297	EEBC298	LAND09 124	-	0.002	0	0.001	0.741	0.094	0.161	0.002
298	EEBC299	LAND09 125	-	0.029	0	0.005	0.35	0.479	0.114	0.022

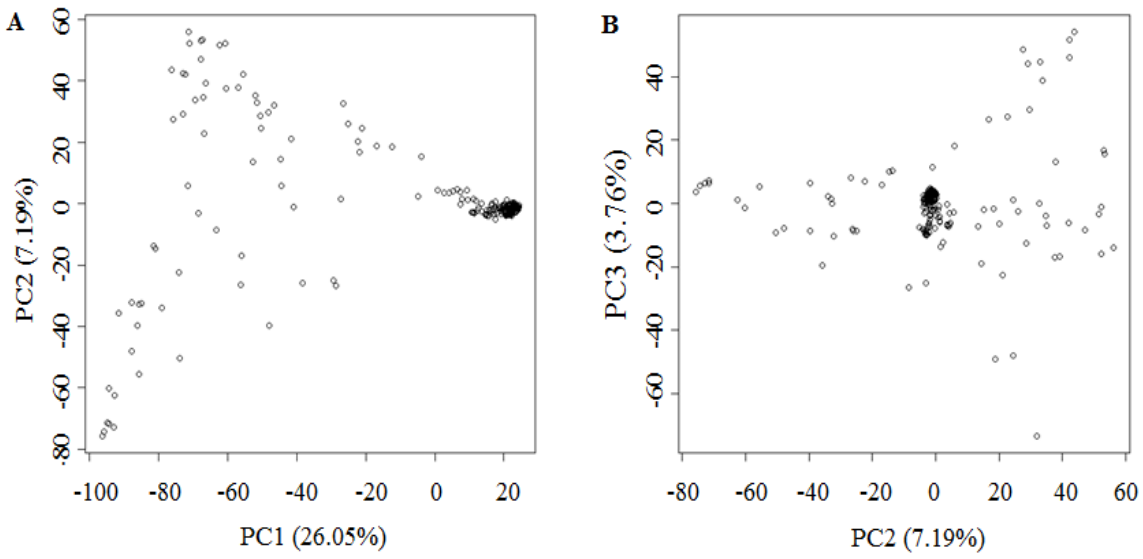
[a] The sp were named as sp1: “Two-rowed/six-rowed (NSGC/VIR)”; sp2: “Two-rowed Tigray/Eritrea (NSGC)”; sp3: “Six-rowed VIR/ICARDA”; sp4: “Two-rowed VIR/ICARDA”; sp5: “Two-rowed NSGC”; and sp6: “Six-rowed NSGC/(VIR)”. “?” denotes a cluster not considered as a separate subpopulation.



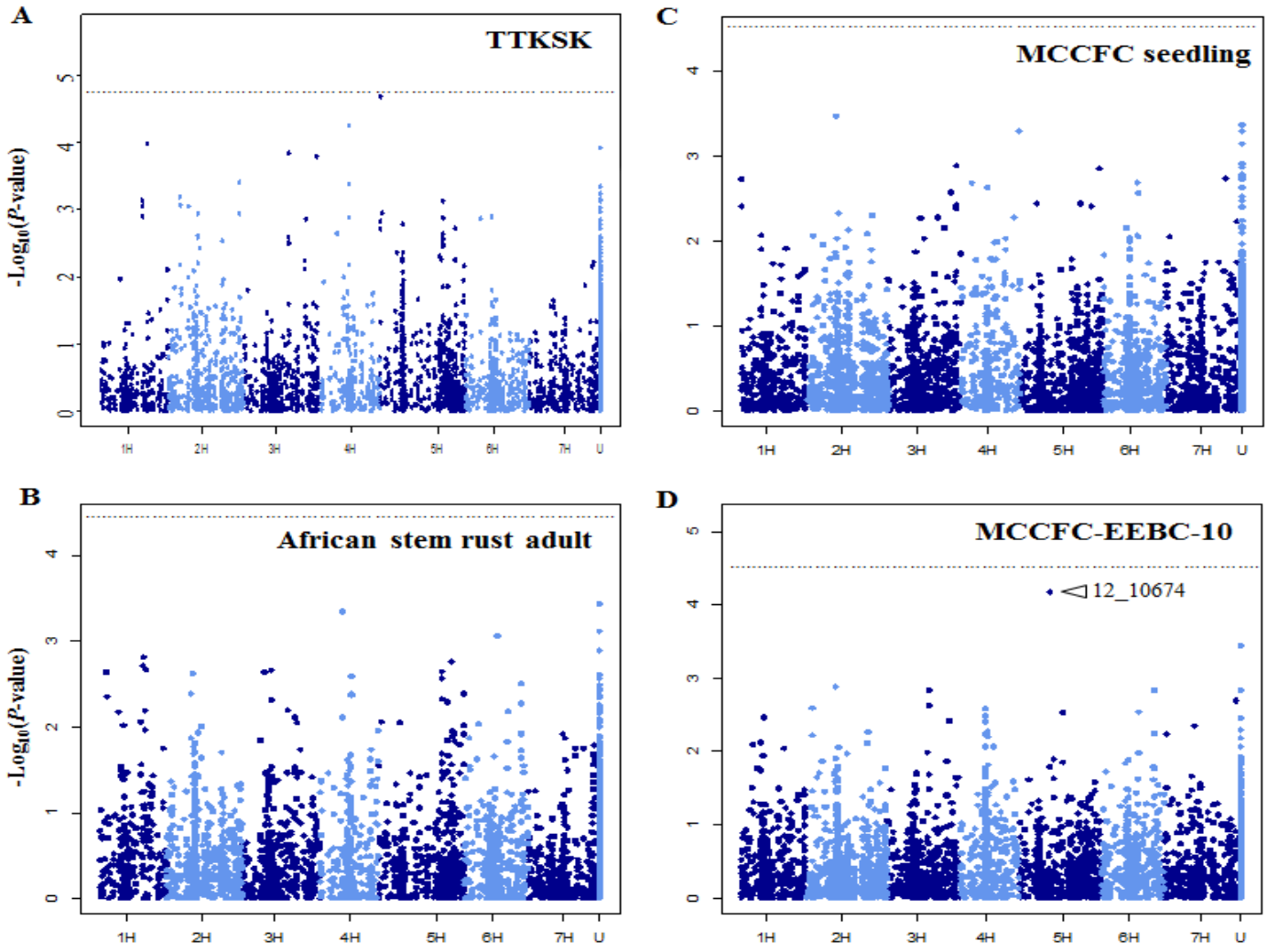
Apppendix Figure 3.1. Probability of subpopulations within Ethiopian and Eritrean barley landrace germplasm based on STRUCTURE analysis. (**A**) Plot of the average logarithm of the probability of data likelihood [LnP(D)] and its variance {var[LnP(D)]} as a function of the number of assumed subpopulations (*K*); with *K* allowed to range from 2 to 20. The likelihood data for each value of *K* was generated from 20 independent runs with a burn-in period of 10,000 cycles and 50,000 iterations. The optimal *K* was estimated based on the value and variation of Pr(X|*K*). Labels for [LnP(D)] and var[LnP(D)] are shown as legends on the right of the plot. Inferred subpopulations of 6-7 with [LnP(D)] and var[LnP(D)] methods are indicated with black rectangle and black triangle, respectively. (**B**) Inference of true value of *K* using the rate of lnPr(X|*K*) change from *K*-1 to *K* (*ΔK*) method for *K*=2 to 20. Run parameters were similar to (A). Inferred subpopulations of 6-7 are indicated with black rectangle.



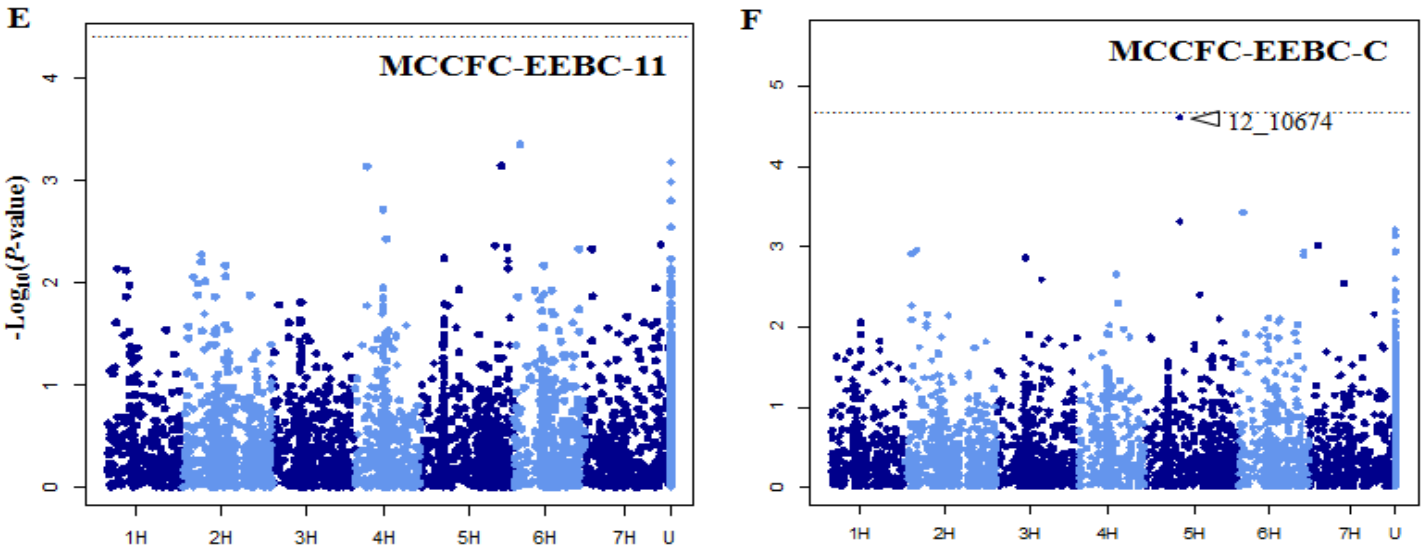
Appendix Figure 3.2. Bar-plot of individual Ethiopian and Eritrean barley landrace germplasm in multiple lines based on SNP markers generated by STRUCTURE 2.3.4 using the admixture model. Individuals were assigned into clusters based on the subpopulation membership matrix (Q) scores. Q coefficients represent the fractional subpopulation membership for each landrace based on its inferred ancestry. The likelihood data for each value of *K* was generated for *K*=3 to 10 from 20 independent runs with a burn-in period of 100,000 cycles and 200,000 iterations. Each individual is represented by a vertical (100%) line of genetic components proportions shown in color for *K*=7. Groups for each panel are represented by colors as indicated on the top. Each column (298 columns in total) represents a landrace's genotype, the length of which represents the estimated genetic fraction of every accession from each of the seven inferred subpopulations. The assumed subpopulations (sp) of 1 to 6 are shown above the bar-plot. The '?' mark between sp1 and sp2 denotes few individuals which could possibly be admixtures; and therefore not considered as a subpopulation on their own. The sp were named as sp1: "Two-rowed/six-rowed (NSGC/VIR)"; sp2: "Two-rowed Tigray/Eritrea (NSGC)"; sp3: "Six-rowed VIR/ICARDA"; sp4: "Two-rowed VIR/ICARDA"; sp5: "Two-rowed NSGC"; and sp6: "Six-rowed NSGC/(VIR)".



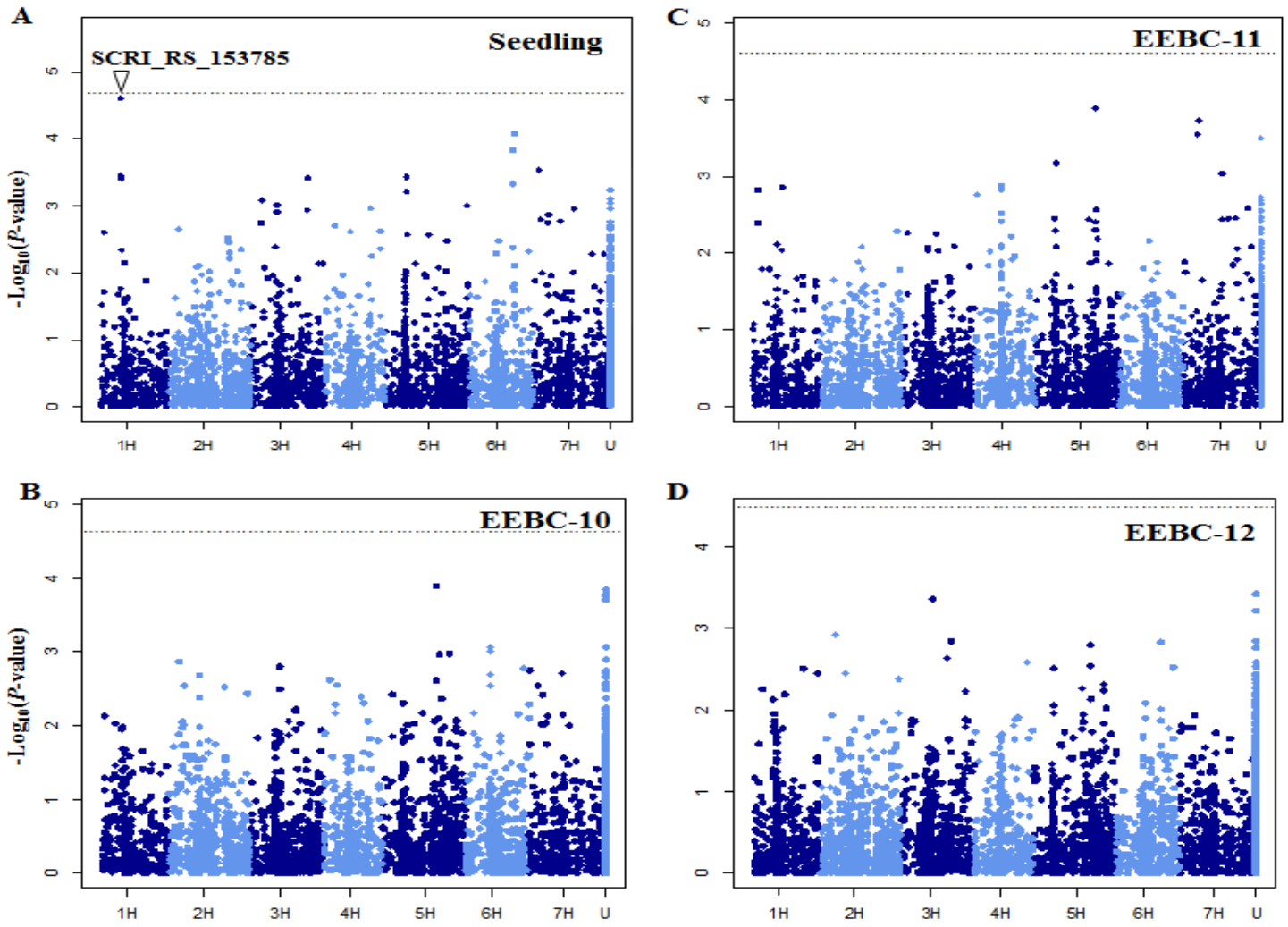
Appendix Figure 3.3. Principal component analysis of Ethiopian and Eritrean barley landrace germplasm. *Scatter plots* represent the grouping of the individuals according to (**A**) the first principal component (PC) versus the second one and (**B**) the second component versus the third one. The three principal coordinates all together explain 37% of the variation among the accessions.



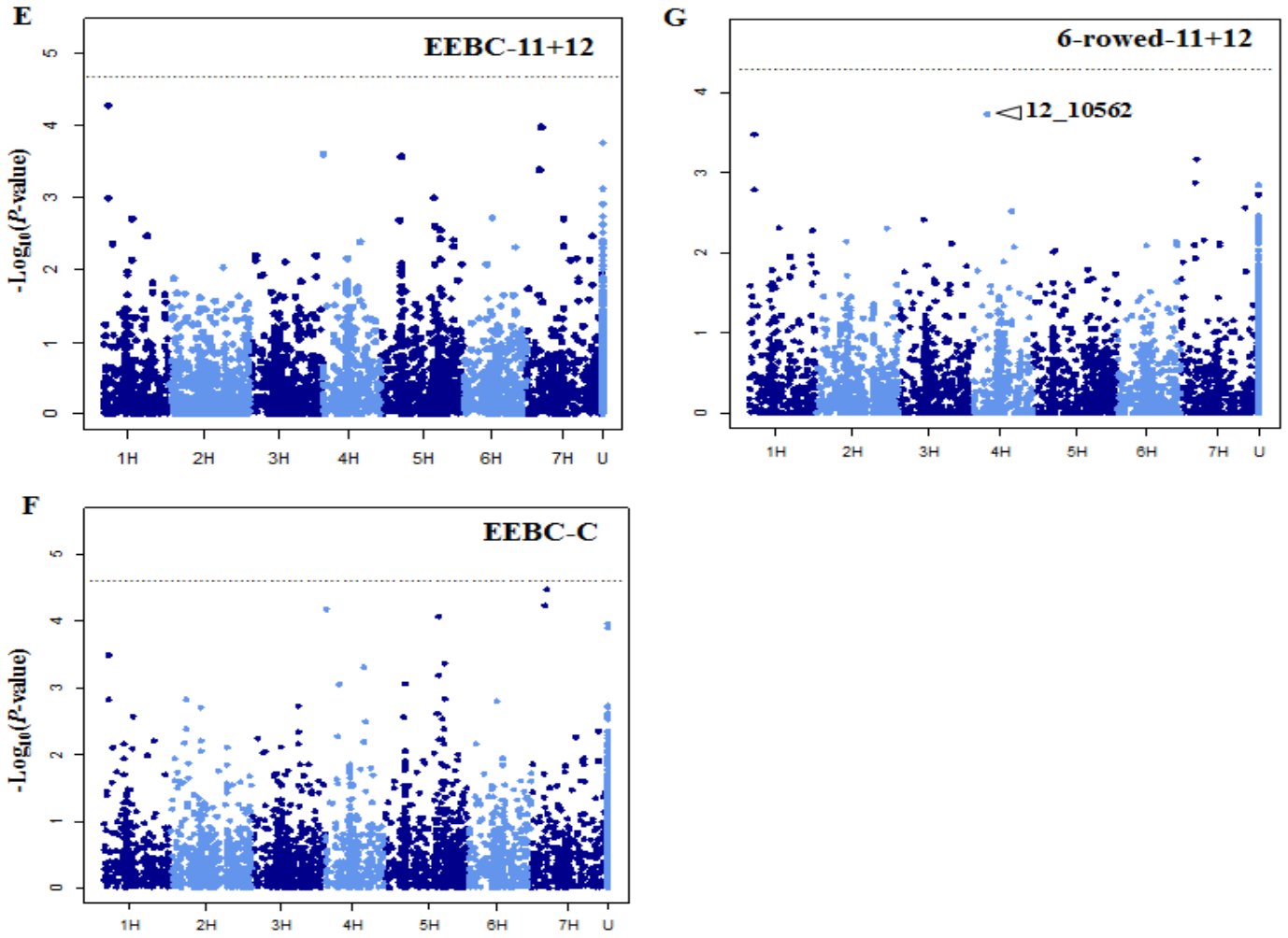
Appendix Figure 3.4. See next page.



Appendix Figure 3.4. Genome-wide association scans for marker associations to stem rust and spot blotch resistance in Eritrean barley landrace germplasm (EEBC). Scans are shown for (**A**) Infection response to stem rust race TTKSK at the seedling stage (TTKSK), (**B**) Adult plant severity to African stem rust at adult plant stage (African stem rust adult), (**C**) Infection response to stem rust race MCCFC at the seedling stage (MCCFC seedling), (**D**) Adult plant severity to race MCCFC at adult plant stage of EEBC in 2010 (MCCFC-EEBC-10), (**E**) Adult plant severity to race MCCFC at adult plant stage of EEBC in 2011 (MCCFC-EEBC-11), and (**F**) Adult plant severity to race MCCFC at adult plant stage of three years combined (MCCFC-EEBC-C). The genome-wide association mapping was run with 5,269 SNP markers. Vertical axis represents $-\log_{10}$(*P*-values) of the marker-trait associations after correction for multiple testing, and the horizontal axis represents the relative chromosomal position of the SNP markers across the genome. The black horizontal dotted lines show the *P*-value corresponding to a false discovery rate (FDR) of 0.05. SNP markers with peaks above this threshold level were considered as significantly associated. Marker with the highest peak here and has been significant in other analysis panel but not with the current data set is marked with arrows for reference.



Appendix Figure 3.5. See next page.



Appendix Figure 3.5. See next page.

Appendix Figure 3.5. Genome-wide association scans for marker associations spot blotch resistance in Ethiopian and Eritrean barley landrace germplasm (EEBC). Scans are shown for (**A**) Infection response at the seedling stage, (**B**) Adult plant severity at adult plant stage of EEBC in 2010 (EEBC-10), (**C**) Adult plant severity at adult plant stage of EEBC in 2011 (EEBC-11), (**D**) Adult plant severity at adult plant stage of EEBC in 2012 (EEBC-12), (**E**) Adult plant severity at adult plant stage of EEBC in 2010 and 2012 combined (EEBC-10+12), (**F**) Adult plant severity at adult plant stage of three years combined (EEBC-C), and (**G**) Adult plant mean severity at adult plant stage of 2011 and 2012 of the six-rowed germplasm set (6-rowed-11+12). The genome-wide association mapping was run with 5,269 SNP markers. Vertical axis represents $-\log_{10}$(*P*-values) of the marker-trait associations after correction for multiple testing, and the horizontal axis represents the relative chromosomal position of the SNP markers across the genome. The black horizontal dotted lines show the *P*-value corresponding to a false discovery rate (FDR) of 0.05. SNP markers with peaks above this threshold level were considered as significantly associated. Though not significant, a marker with a score near the FDR threshold on the short arm of chromosome 1H was indicated with an arrow on the plot in (A). In (H), a non-significant marker on the short arm of chromosome 4H was labeled for reference as it had the highest peak and was significant in in other analysis panel.

Appendix Text 4.1. Genome-wide association mapping of deoxynivalenol (DON) concentration in separate two- or six-rowed germplasm sets

To investigate the possible relationship of spike-type with DON concentration, GWAS for DON concentration was conducted separately for the two- and six-rowed germplasm datasets. The 12_11485 SNP marker on chromosome 4HS (12.91 cM) identified in the EEBC-12 DON dataset also was significant in the six-rowed combined dataset (EEBC-6R-C) and the 2012 six-rowed dataset (EEBC-6R-12) (Appendix Table 4.1; Figure 4.3D and E). In addition, 43 markers belonging to fifteen genomic locations and ten other unmapped markers were found significantly associated with DON concentration exclusively in the separate two- and/or six-rowed germplasm sets (Appendix Table 4.1). In the EEBC-6R-C and EEBC-6R-12 datasets, a second significant marker (11_20824) was detected on the long arm of chromosome 7H (Appendix Table 4.1; Figure 4.3D and E). Thus, this QTL was designated as *DON-qtl-7H-11_20824* (107.49 cM). The QTL *DON-qtl-7H-11_20824* on chromosome 7HL appeared to be located in bin 10-11 where no FHB QTL were previously reported. This may represent a unique resistance QTL that could be useful in breeding. Ninety-two percent (165/180) of the six-rowed accessions had the "A" allele at 11_20824. These accessions had a lower mean FHB severity and DON concentration than the remaining six-rowed accessions (15) that had the "B" allele (Table 4.5) for the marker. In addition, two unmapped markers (SCRI_RS_189331 and SCRI_RS_119390) were significantly associated in the EEBC-6R-12 and/or EEBC-6R-C DON datasets (Appendix Table 4.1). Another marker (11_20092) located 2.91 cM distal was also significantly associated with DON concentration in the EEBC-6R-C DON datasets (Appendix Table 4.1), and was probably detecting the same QTL as 11_20824. In addition, two unmapped markers (SCRI_RS_119390 and SCRI_RS_189331) were sifnificantly associated with DON concentration in the EEBC-6R-C and EEBC-6R-12 DON datasets (Appendix Table 4.1).

In the two-rowed germplasm set with the combined mean DON data (EEBC-2R-C), nine genomic regions were found to contain significant markers. On the short arm of chromosome 2H, marker 12_10777 was found significantly associated with DON concentration (Appendix Table 4.1; Figure 4.3F and G; Appendix Figure 4.2B). The QTL for DON concentration in this region was designated as *DON-qtl-2H-12_10777* (30.36 cM). Six markers on the long arm of chromsome 2H were significantly associated with DON concentration in the EEBC-2R-C dataset (Appendix Table 4.1; Figure 4.3F). Most of these markers were also significant in the EEBC-2R-11 (Appendix Table 4.1; Figure 4.3G). Some of these markers and several others mapping to the same location were also significant in the EEBC-2R-12 (Appendix Table 4.1; Appendix Figure 4.2B). Since these markers are mapping to the same position or 0.55 cm away, they are likely detecting the same QTL. The most significant marker was SCRI_RS_130072; thus, the QTL was designated as *DON-qtl-2H-SCRI_RS_130072* (69.00 cM). On the long arm of chromsome 2H, marker SCRI_RS_202469 was significant in the EEBC-2R-12 (Appendix Table 4.1; Appendix Figure 4.2B). Towards the telomeric end of 2HL, three markers were significantly associated with DON concentration in the EEBC-2R-C dataset (Appendix Table 4.1; Figure 4.3F). The most significant marker was SCRI_RS_134252; thus, the

QTL was designated as *DON-qtl-2H-SCRI_RS_134252* (177.58). Two of the markers and three more were also significant in the EEBC-2R-11 DON dataset (Appendix Table 4.1; Figure 4.3G). The markers were mapping within a very short genetic interval (1.7 cM) and were perhaps associated with the same QTL.

On the long arm of chromsome 4H, a marker (11_10309) was significantly associated with DON concentration in the EEBC-2R-C dataset (Appendix Table 4.1; Figure 4.3G). The QTL identified by this marker was designated as *DON-qtl-4H-11_10309* (67.91 cM). Another marker (12_10022: 80.62 cM) was also identified on 4HL in the same DON dataset (Appendix Table 4.1; Figure 4.3G). On the long arm of chromosome 5H, a second significant marker (11_21001) was identified in this dataset; thus, this QTL was designated as *DON-qtl-5H-11_21001* (55.83 cM) (Appendix Table 4.1; Figure 4.3F). Marker 11_21001 also was significantly associated with row-type morphology. Both *DON-qtl-2H-12_10777* (30.36 cM) and *DON-qtl-5H-11_21001* (55.83 cM) also were significant in the two-rowed germplasm set in 2011 (EEBC-2R-11) (Appendix Table 4.1; Figure 4.3G). Another SNP marker (11_20246) 5 cM distal to *DON-qtl-5H-11_21001* and likely detecting the same QTL also was identified on chromosome 5HL in the EEBC-2R-11 dataset. The *DON-qtl-5H-11_21001* QTL on chromosome 5HL approximately mapped to bin 5 and appears to be unique given that no other known FHB QTL were reported previously at this locus. This putative DON QTL on chromosome 5HL was detected by the same marker that was significantly associated with the row-type trait. This marker was most likely detecting the six-rowed spike 2 (*vrs2*) or the intermedium spike-b (*int-b*) locus located on chromosome 5HL (Lundqvist and Franckowiack 1997).

In addition, other unique markers on chromosomes 1H, 2H, 3H, and 5H were found to be significantly associated with DON concentration in the EEBC-2R-11 data set (Appendix Table 4.1; Figure 4.3G). On the long arm of chromosome 1H, two markers (SCRI_RS_197263 and 12_31163) were discovered as significantly associated with DON concentration in the EEBC-2R-11 data set (Appendix Table 4.1). The most significant one was SCRI_RS_197263; thus, the QTL was designated as *DON-qtl-1H-SCRI_RS_197263* (92.50 cM). These two markers lie within a very short interval (0.10 cM) and are probably detecting the same QTL. On chromosome 2H, in addition to 12_10777 described above, several additional markers were significantly associated with DON concentration in the EEBC-2R-11 dataset (Appendix Table 4.1). On the long arm of chromosome 2H (ca. 69.00 cM), seven markers were found significantly associated with DON concentration (Appendix Table 4.1; Figure 4.3G). The most significant one SCRI_RS_130072; thus, the QTL was designated as *DON-qtl-2H- SCRI_RS_130072* (69.00 cM). All but one (SCRI_RS_237688 at 69.55 cM) of the seven associated markers had the exact same genetic position. These seven markers are probably detecting the same QTL for DON concentration. On the same arm of chromosome 2H (ca. 179.00 cM), two markers mapped within 1.00 cM of each other and perhaps were associated with the same QTL (Appendix Table 4.1; Figure 4.3G). The most significant marker was SCRI_RS_139737; thus, this QTL was designated as *DON-qtl-2H-SCRI_RS_139737* (178.08 cM). On the short arm of chromosome 3H, one marker (11_20529) was

significantly associated with DON concentration in the EEBC-2R-11 dataset (Appendix Table 4.1; Figure 4.3G). The QTL detected by this marker was designated as *DON-qtl-3H-11_20529* (8.33 cM). Another marker (SCRI_RS_130264) mapping 5.85 cM away and likely detecting the same QTL was detected in the EEBC-2R-11 dataset (Appendix Table 4.1).

On the long arm of chromosome 5H, in addition to 11_21001 and 11_20246 described above, several markers mapping to two unique genomic regions were significantly associated with DON concentration in the EEBC-2R-11 dataset (Appendix Table 4.1; Figure 4.3G). Two markers (11_21018 and 12_31375) on chromosome 5HL with the exact map location were significantly associated with DON concentration in the EEBC-2R-11 dataset (Appendix Table 4.1). The most significant marker was 11_21018, and therefore the QTL was designated as *DON-qtl-5H-11_21018* (153.47 cM). Since the markers lie at the same location, it is possible that they are detecting the same QTL. The marker 11_21018 was also significant in the EEBC-2R-C DON dataset (Appendix Table 4.1). Located 23 cM distal on the same arm of chromosome 5H, another significantly associated marker (11_21155) was detected (Appendix Table 4.1; Figure 4.3G). This marker is most likely detecting another QTL, and was thus designated as *DON-qtl-5H-11_21155* (176.52 cM). In addition, six unmapped markers (SCRI_RS_114308, 11_10750, SCRI_RS_171, 12_20632, SCRI_RS_196483 and SCRI_RS_239653) were significantly associated with DON concentration specifically in the EEBC-2R-11 dataset (Appendix Table 4.1). Two unmapped significant marker (11_10750 and SCRI_RS_220205) in the EEBC-2R-11 DON dataset also was significant in the EEBC-2R-C dataset (Appendix Table 4.1). No significant marker-trait associations were detected in the EEBC-6R-11 datasets (Appendix Figure 4.2C).

Appendix Text 4.2 *Genome-wide association mapping of days to heading and plant height*

Days to heading (DTH)

Three markers on the short arm of chromosome 2H and six markers of unknown map location were significantly associated with DTH in the EEBC-C and EEBC-11 datasets (Appendix Table 4.2; Appendix Figure 4.3A). The most significant marker identified on chromosome 2HS was 12_30872; thus, the QTL was designated as *DTH-qtl-2H-12_30872* (38.60 cM). The two other markers (SCRI_RS_195810, 12_30871) in this region lie at or within a short genetic interval (5.86 cM) of 12_30872 and may be detecting the same QTL. With respect to LD, marker 12_30872 was in high LD with the other two markers SCRI_RS_195810 and 12_30871 (r^2=0.40 and 0.98, respectively) strongly suggesting that the markers were associated with the same locus. The same three markers also were found significantly associated with DTH in the EEBC-11 dataset (Appendix Table 4.2; Appendix Figure 4.4A). This region of 2HS was previously identified to contain one of the two main photoperiod response genes in barley, *Ppd-H1*. Thus, the SNP markers on 2HS are likely detecting this gene. The *Ppd-H1* gene regulates flowering time under long days (Laurie et al. 1995; Faure et al. 2007). The *DTH-qtl-2H-12_30872* QTL was identified in the 2011 dataset, a year in which the mean DTH was relatively longer, and also the mean of the combined two-year dataset. In the 2012 dataset, though not significant, strong signals appeared on the long arm of chromosome 1H, likely detecting the other photoperiod response gene *Ppd-H2* (Appendix Figure 4.4B). *PPDH2* function is assumed to be limited to promoting flowering under a short photoperiod (Laurie et al. 1995). Though it is not possible to make a biological interpretation and its significance on gene expression, the mean DTH was noticeably shorter in 2012. In the region of the major QTL associated with DTH on 2HS, a significant marker (12_10777) representing a putative DON QTL was detected in the GWAS of the two-rowed germplasm set. Interestingly, five two-rowed accessions that had a winter or facultative growth habit and thus did not head in the field had failed genotype data at this locus. The six unmapped markers significantly associated with DTH (Appendix Table 4.2) may be detecting *Ppd-H1* and/or some other gene(s) underlying photoperiod response in barley. In the EEBC-12 dataset, no significant associations were detected for DTH (Appendix Figure 4.4B).

Plant height (PH)

No significant marker associations were detected for PH in any of the datasets from the Crookston location (Appendix Table 4.2; Appendix Figure 4.4C. However, six markers were found significantly associated with PH at St. Paul: four on chromosome 1H, one on chromosome 5H, and one unmapped (Appendix Table 4.2; Appendix Figure 4.3B). The most significant marker identified on chromosome 1H was 12_30350; thus, the QTL was designated as *PH-qtl-1H-12_30350* (50.10 cM). Three of the four markers

mapped at 50.20 cM on the short arm of chromosome 2H and one just 0.1 cM away (Appendix Table 4.2). It is likely that all four markers are detecting the same QTL. There was strong LD among the markers (r^2 values ranging from 0.95 to 0.99 between markers 12_30350 and SCRI_RS_221609, and SCRI_RS_221609 and SCRI_RS_152642, respectively). On the long arm of chromosome 5H, one marker (11_10127) was significantly associated with PH, so this QTL was named *PH-qtl-5H-11_10127* (59.03 cM). The unmapped significant marker was possibly detecting one of these same QTL or perhaps another PH QTL.

Appendix Table 4.1. SNP markers significantly associated with Fusarium head blight (FHB) severity and deoxynivalenol (DON) concentration in Ethiopian and Eritrean barley landrace germplasm

			q-value for FHB[d]								
SNP marker	**Chrom.[a]**	**Position[b]**	**EEBC-C**	**EEBC-11**	**EEBC-12**	**EEBC-2R-C**	**EEBC-2R-11**	**EEBC-2R-12**	**EEBC-6R-C**	**EEBC-6R-11**	**EEBC-6R-12**
11_20383	1H	136.65	--	--	--	$8.99x10^{-6}$	*$3.88x10^{-4}$*	*$4.72x10^{-3}$*	*0.37*	*0.85*	*0.47*
11_20340	2H	90.99	$1.44x10^{-8c}$	$2.14x10^{-8}$	$8.51x10^{-8}$	*0.07*	*0.04*	*1.00*	*1.00*	*1.00*	*1.00*
12_30896	2H	91.09	$9.88x10^{-8}$	$1.62x10^{-8}$	$1.95x10^{-6}$	*1.00*	*1.00*	*1.00*	*0.66*	*0.49*	*0.71*
11_11180	4H	50.70	--	--	--	--	--	$2.43x10^{-9}$	--	--	--
SCRI_RS_152228	7H	19.32	--	--	--	--	--	$4.70x10^{-6}$	--	--	--
SCRI_RS_72983	Unknown	Unknown	*$2.06x10^{-5}$*	*$5.02x10^{-5}$*	$1.114x10^{-5}$	*0.73*	*0.89*	*0.08*	*1.00*	*1.00*	*1.00*
SCRI_RS_196677	Unknown	Unknown	--	--	--	--	--	$3.86x10^{-6}$	--	--	--
SCRI_RS_236528	Unknown	Unknown	--	--	--	--	--	$3.86x10^{-6}$	--	--	--
SCRI_RS_148653	Unknown	Unknown	--	--	--	--	--	$4.39x10^{-6}$	--	--	--
			q-value for DON[d]								
SNP marker	**Chrom.[a]**	**Position[b]**	**EEBC-C**	**EEBC-11**	**EEBC-12**	**EEBC-2R-C**	**EEBC-2R-11**	**EEBC-2R-12**	**EEBC-6R-C**	**EEBC-6R-11**	**EEBC-6R-12**
SCRI_RS_197263	1H	92.5	--	--	--	--	$8.13x10^{-6}$	--	--	--	--
12_31163	1H	92.6	--	--	--	--	$1.26x10^{-5}$	--	--	--	--
12_10777	2H	30.36	--	--	--	$7.59x10^{-9}$	$5.74x10^{-7}$	$3.26x10^{-7}$	--	--	--
11_20032	2H	69.00	--	--	--	$1.98x10^{-7}$	$2.94x10^{-7}$	--	--	--	--
12_31175	2H	69.00	--	--	--	$2.15x10^{-6}$	--	$2.12x10^{-6}$	--	--	--
SCRI_RS_130072	2H	69.00	--	--	--	$1.86x10^{-8}$	$8.55x10^{-8}$	$1.09x10^{-4}$	--	--	--
SCRI_RS_197997	2H	69.00	--	--	--	$1.98x10^{-7}$	$2.94x10^{-7}$		--	--	--
SCRI_RS_230984	2H	69.00	--	--	--	$1.98x10^{-7}$	$2.94x10^{-7}$		--	--	--
12_11288	2H	69.00	--	--	--	--	$2.57x10^{-5}$	--	--	--	--
12_30068	2H	69.00	--	--	--	--	$2.57x10^{-5}$	--	--	--	--
SCRI_RS_237688	2H	69.55	--	--	--	$8.71x10^{-8}$	$2.26x10^{-7}$	$1.21x10^{-4}$	--	--	--
11_10436	2H	69.55	--	--	--	--	--	$1.84x10^{-6}$	--	--	--
11_10632	2H	69.55	--	--	--	--	--	$4.94x10^{-7}$	--	--	--
11_20374	2H	69.55	--	--	--	--	--	$4.94x10^{-7}$	--	--	--
11_20442	2H	69.55	--	--	--	--	--	$2.84x10^{-7}$	--	--	--
12_31256	2H	69.55	--	--	--	--	--	$3.86x10^{-5}$	--	--	--
SCRI_RS_188255	2H	69.55	--	--	--	--	--	$4.94x10^{-7}$	--	--	--
SCRI_RS_171032	2H	90.64	$9.04x10^{-6c}$	*$9.54x10^{-5}$*	*$6.80x10^{-4}$*	*$3.12x10^{-4}$*	$1.74x10^{-4}$	*$1.19x10^{-3}$*	*1.00*	*1.00*	*1.00*
12_30901	2H	90.99	$3.39x10^{-6}$	*$5.69x10^{-5}$*	*$1.22x10^{-4}$*	$6.73x10^{-5}$	$2.11x10^{-4}$	*$4.61x10^{-4}$*	*1.00*	*1.00*	*1.00*
11_20340	2H	90.99	$4.21x10^{-6}$	*$8.71x10^{-4}$*	$1.85x10^{-5}$	*1.00*	*1.00*	*1.00*	*1.00*	*1.00*	*1.00*
12_30896	2H	91.09	*$4.55x10^{-5}$*	*$9.04x10^{-3}$*	$2.93x10^{-5}$	*1.00*	*1.00*	*1.00*	*0.90*	*0.89*	*0.42*

Appendix Table 4.1. See next page.

Appendix Table 4.1. Continued.

			q-value for DON[d]								
SNP marker	**Chrom.[a]**	**Position[b]**	**EEBC-C**	**EEBC-11**	**EEBC-12**	**EEBC-2R-C**	**EEBC-2R-11**	**EEBC-2R-12**	**EEBC-6R-C**	**EEBC-6R-11**	**EEBC-6R-12**
SCRI_RS_202469	2H	149.00	--	--	--	--	--	$2.32x10^{-5}$	--	--	--
11_20681	2H	177.38	--	--	--	--	$1.77x10^{-5}$	--	--	--	--
SCRI_RS_223897	2H	177.38	--	--	--	--	$5.29x10^{-5}$	--	--	--	--
SCRI_RS_134252	2H	177.58	--	--	--	$3.75x10^{-7}$	--	--	--	--	--
12_30378	2H	178.08	--	--	--	$4.57x10^{-5}$	$4.82x10^{-7}$	--	--	--	--
SCRI_RS_139737	2H	178.08	--	--	--	$1.37x10^{-4}$	$2.16x10^{-7}$	--	--	--	--
SCRI_RS_151056	2H	179.08	--	--	--	--	$1.18x10^{-6}$	--	--	--	--
11_20529	3H	8.33	--	--	--	--	$3.57x10^{-5}$	--	--	--	--
SCRI_RS_130264	3H	14.18	--	--	--	--	$3.93x10^{-4}$	--	--	--	--
12_11485	4H	12.91	*$7.93x10^{-4}$*	*0.03*	$2.45x10^{-8}$	*0.42*	*0.88*	*0.02*	$7.50x10^{-6}$	*$1.18x10^{-3}$*	$1.47x10^{-5}$
SCRI_RS_148392	4H	65.52	$7.74x10^{-6}$	*0.01*	$3.26x10^{-8}$	*0.02*	*0.18*	*0.01*	$3.23x10^{-4}$	*0.04*	$4.86x10^{-6}$
SCRI_RS_157650	4H	65.52	*$1.77x10^{-4}$*	*0.06*	$1.22x10^{-6}$	*0.03*	*0.21*	*0.03*	*$3.05x10^{-3}$*	*0.14*	*$7.02x10^{-5}$*
11_10309	4H	67.91	--	--	--	$1.84x10^{-5}$	$4.27x10^{-6}$	--	--	--	--
11_11319	4H	67.91	--	--	--	--	$8.50x10^{-5}$	--	--	--	--
11_20178	4H	80.52	--	--	--	--	$4.43x10^{-6}$	--	--	--	--
12_10022	4H	80.62	--	--	--	$5.29x10^{-7}$	$4.40x10^{-6}$	--	--	--	--
11_20737	5H	43.92	--	--	--	--	$2.50x10^{-5}$	--	--	--	--
11_21318	5H	43.92	--	--	--	--	$1.01x10^{-4}$	--	--	--	--
12_30717	5H	43.92	--	--	--	--	$3.61x10^{-4}$	--	--	--	--
12_10953	5H	54.46	--	--	--	--	$4.14x10^{-4}$	--	--	--	--
11_21001	5H	55.83	--	--	--	$4.84x10^{-5}$	$1.12x10^{-11}$	--	--	--	--
11_20246	5H	60.99	--	--	--	--	$1.21x10^{-4}$	--	--	--	--
SCRI_RS_232871	5H	76.14	--	--	--	$1.65x10^{-5}$	$1.83x10^{-4}$	--	--	--	--
11_21018	5H	153.47	--	--	--	$1.84x10^{-4}$	$2.49x10^{-5}$	--	--	--	--
12_31375	5H	153.47	--	--	--	--	$3.96x10^{-4}$	--	--	--	--
11_21155	5H	176.52	--	--	--	--	$2.08x10^{-5}$	--	--	--	--
12_30358	6H	42.43	--	--	--	--	--	$4.80x10^{-8}$	--	--	--
11_20824	7H	107.49	--	--	--	--	--	--	$6.25x10^{-6}$	--	$1.08x10^{-6}$
11_20092	7H	110.40	--	--	--	--	--	--	$3.59x10^{-5}$	--	--
SCRI_RS_165473	Unknown	Unknown	$7.75x10^{-7}$	$4.54x10^{-6}$	*$8.38x10^{-5}$*	$6.70x10^{-5}$	$2.94x10^{-4}$	*$1.67x10^{-4}$*	*1.00*	*1.00*	*1.00*
SCRI_RS_72983	Unknown	Unknown	$1.06x10^{-6}$	*$1.83x10^{-5}$*	*$7.09x10^{-5}$*	$1.52x10^{-5}$	$7.36x10^{-5}$	*$1.43x10^{-4}$*	*1.00*	*1.00*	*1.00*
11_10750	Unknown	Unknown	--	--	--	$1.03x10^{-7}$	$2.53x10^{-7}$	--	--	--	--
SCRI_RS_220205	Unknown	Unknown	--	--	--	$2.67x10^{-5}$	$3.05x10^{-5}$	--	--	--	--

Appendix Table 4.1. See next page.

Appendix Table 4.1. Continued.

			q-value for DON[d]								
SNP marker	**Chrom.[a]**	**Position[b]**	**EEBC-C**	**EEBC-11**	**EEBC-12**	**EEBC-2R-C**	**EEBC-2R-11**	**EEBC-2R-12**	**EEBC-6R-C**	**EEBC-6R-11**	**EEBC-6R-12**
12_20632	Unknown	Unknown	--	--	--	--	$6.11x10^{-5}$	--	--	--	--
SCRI_RS_114308	Unknown	Unknown	--	--	--	--	$6.62x10^{-8}$	--	--	--	--
SCRI_RS_171	Unknown	Unknown	--	--	--	--	$1.80x10^{-5}$	--	--	--	--
SCRI_RS_185563	Unknown	Unknown	--	--	--	--	$4.21x10^{-5}$	--	--	--	--
SCRI_RS_196483	Unknown	Unknown	--	--	--	--	$2.49x10^{-5}$	--	--	--	--
SCRI_RS_197405	Unknown	Unknown	--	--	--	--	$3.62x10^{-4}$	--	--	--	--
SCRI_RS_119390	Unknown	Unknown	--	--	--	--	--	--	$1.43x10^{-5}$	--	$2.69x10^{-5}$
SCRI_RS_189331	Unknown	Unknown	--	--	--	--	--	--	$6.78x10^{-6}$	--	$3.20x10^{-5}$

[a] Chromosome. Note: some markers have not been mapped

[b] Genetic position (in cM) of marker on chromosome. Note: some markers have not been mapped to a chromosomal position.

[c] *q*-value (multiple testing corrected *P*-value). For markers significant in the combined dataset, non-significant *q*-values are presented for comparison purposes and are indicated in italic.

[d] EEBC-C: Ethiopian and Eritrean barley collection with combined data of 2011 and 2012; EEBC-11: test from 2011 that includes data from the entire collection; EEBC-12: test from 2012 that includes data from the entire collection; EEBC-2R-C: Combined two year (2011 & 2012) data of two-rowed accessions only; EEBC-2R-11: 2011 data of two-rowed accessions only; EEBC-2R-12: 2012 data of two-rowed accessions only; EEBC-6R-C: Combined two year data of six-rowed accessions only; EEBC-6R-11: 2011 data of six-rowed accessions only; EEBC-6R-12: 2012 data of six-rowed accessions only. '--': No QTL detected.

Appendix Table 4.2. SNP markers significantly associated with row-type morphology (RT), days to heading (DTH), plant height (PH), kernel density (KD), spike angle (SA) and spike exsertion length (EL) in Ethiopian and Eritrean barley landrace germplasm

				q-value for agro-morphlogical data[d]						
Trait	**SNP marker**	**Chrom.[a]**	**Position[b]**	EEBC	EEBC-C	EEBC-11	EEBC-12	EEBC-StP10	EEBC-2R-C	EEBC-6R-C
RT	11_20781	2H	88.04	$6.79x10^{-6c}$						
RT	SCRI_RS_171032	2H	90.64	$1.63x10^{-11}$						
RT	11_20340	2H	90.99	$9.30x10^{-53}$						
RT	12_30901	2H	90.99	$3.78x10^{-20}$						
RT	12_30896	2H	91.09	$1.52x10^{-55}$						
RT	SCRI_RS_196853	2H	91.09	$2.30x10^{-8}$						
RT	12_30897	2H	91.09	$4.89x10^{-8}$						
RT	SCRI_RS_221886	2H	92.07	$7.66x10^{-5}$						
RT	11_20606	4H	31.14	$6.52x10^{-5}$						
RT	11_21001	5H	55.83	$6.04x10^{-5}$						
RT	SCRI_RS_165473	Unknown	Unknown	$1.97x10^{-21}$						
RT	SCRI_RS_72983	Unknown	Unknown	$8.15x10^{-20}$						
RT	SCRI_RS_181051	Unknown	Unknown	$7.91x10^{-5}$						
RT	SCRI_RS_237894	Unknown	Unknown	$1.09x10^{-4}$						
DTH	SCRI_RS_195810	2H	32.74		$2.29x10^{-6}$	$2.22x10^{-8}$	--			
DTH	12_30872	2H	38.60		$7.52x10^{-7}$	$4.66x10^{-9}$	--			
DTH	12_30871	2H	38.60		$1.87x10^{-6}$	$1.48x10^{-8}$	--			
DTH	BK_14	Unknown	Unknown		$4.41x10^{-7}$	$2.87x10^{-9}$	--			
DTH	BK_12	Unknown	Unknown		$4.52x10^{-7}$	$3.50x10^{-9}$	--			
DTH	BK_16	Unknown	Unknown		$6.56x10^{-7}$	$5.31x10^{-9}$	--			
DTH	BK_13	Unknown	Unknown		$7.52x10^{-7}$	$1.44x10^{-8}$	--			
DTH	BK_15	Unknown	Unknown		$1.00x10^{-6}$	$6.78x10^{-9}$	--			
DTH	SCRI_RS_150519	Unknown	Unknown		$3.01x10^{-5}$	$5.98x10^{-5}$	--			
PH	12_30350	1H	50.10		--	--	--	$9.45x10^{-7}$		
PH	SCRI_RS_204810	1H	50.20		--	--	--	$2.47x10^{-6}$		
PH	SCRI_RS_221609	1H	50.20		--	--	--	$2.81x10^{-6}$		
PH	SCRI_RS_152642	1H	50.20		--	--	--	$4.44x10^{-6}$		
PH	11_10127	5H	59.03		--	--	--	$3.46x10^{-5}$		
PH	SCRI_RS_150519	Unknown	Unknown		--	--	--	$5.77x10^{-7}$		
KD	11_20781	2H	88.04		$2.61x10^{-6c}$					
KD	SCRI_RS_171032	2H	90.64		$3.56x10^{-10}$					
KD	11_20340	2H	90.99		$1.58x10^{-46}$					

Appendix Table 4.2. See next page.

Appendix Table 4.2. Continued.

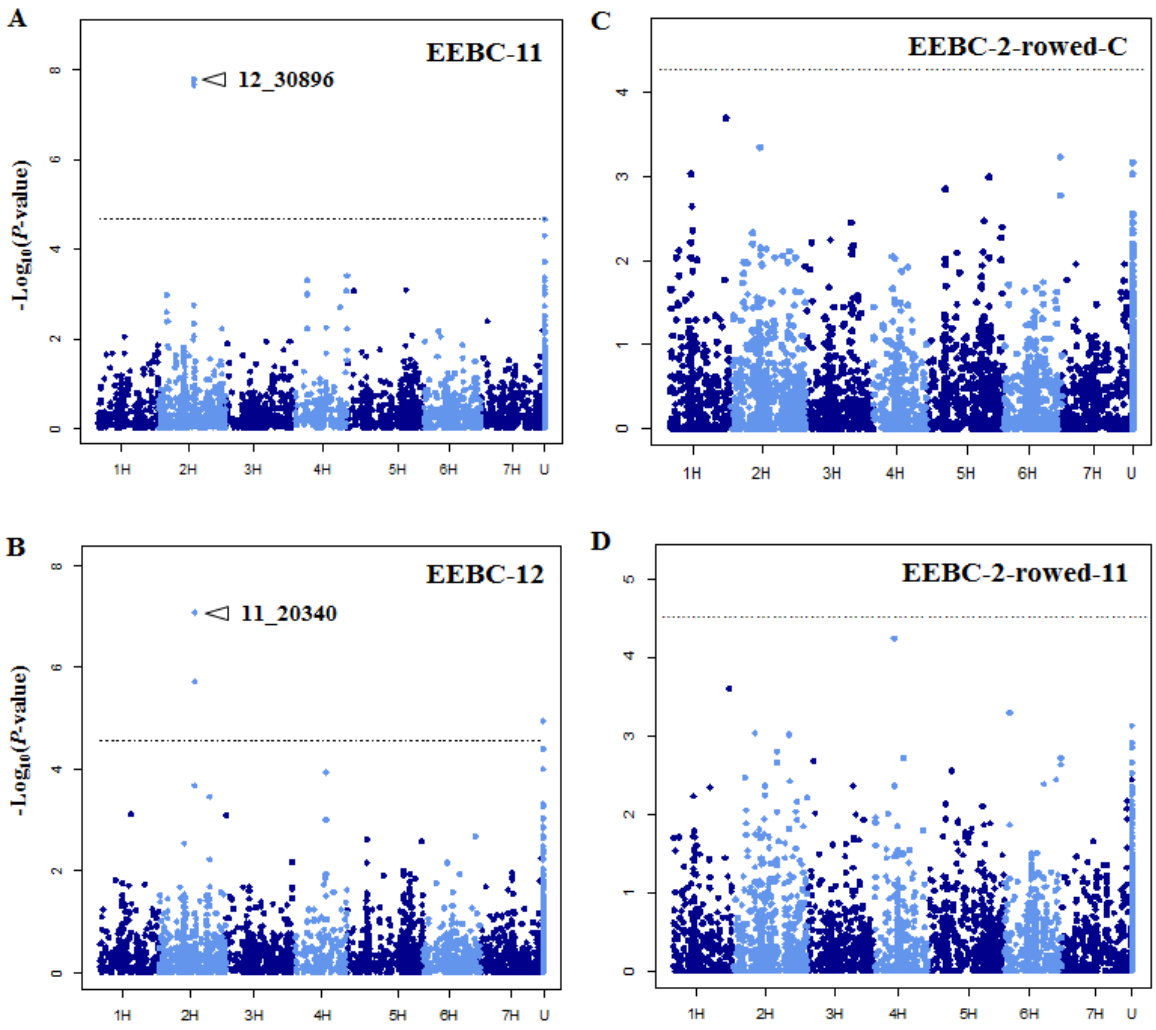
				q-value for agro-morphlogical data[d]						
Trait	**SNP marker**	**Chrom.[a]**	**Position[b]**	EEBC	EEBC-C	EEBC-11	EEBC-12	EEBC-StP10	EEBC-2R-C	EEBC-6R-C
KD	12_30901	2H	90.99		$8.55x10^{-16}$					
KD	12_30896	2H	91.09		$1.55x10^{-16}$					
KD	12_30897	2H	91.09		$7.68x10^{-47}$					
KD	SCRI_RS_196853	2H	91.09		$1.40x10^{-10}$					
KD	SCRI_RS_221886	2H	92.07		$2.34x10^{-5}$					
KD	SCRI_RS_165473	Unknown	Unknown		$4.20x10^{-20}$					
KD	SCRI_RS_72983	Unknown	Unknown		$1.23x10^{-19}$					
SA	--	--	--		NA	NA	--			
EL	SCRI_RS_171997	Unknown	Unknown		NA	$6.11X10^{-6}$	NA			

[a] Chromosome. Note: some markers have not been mapped

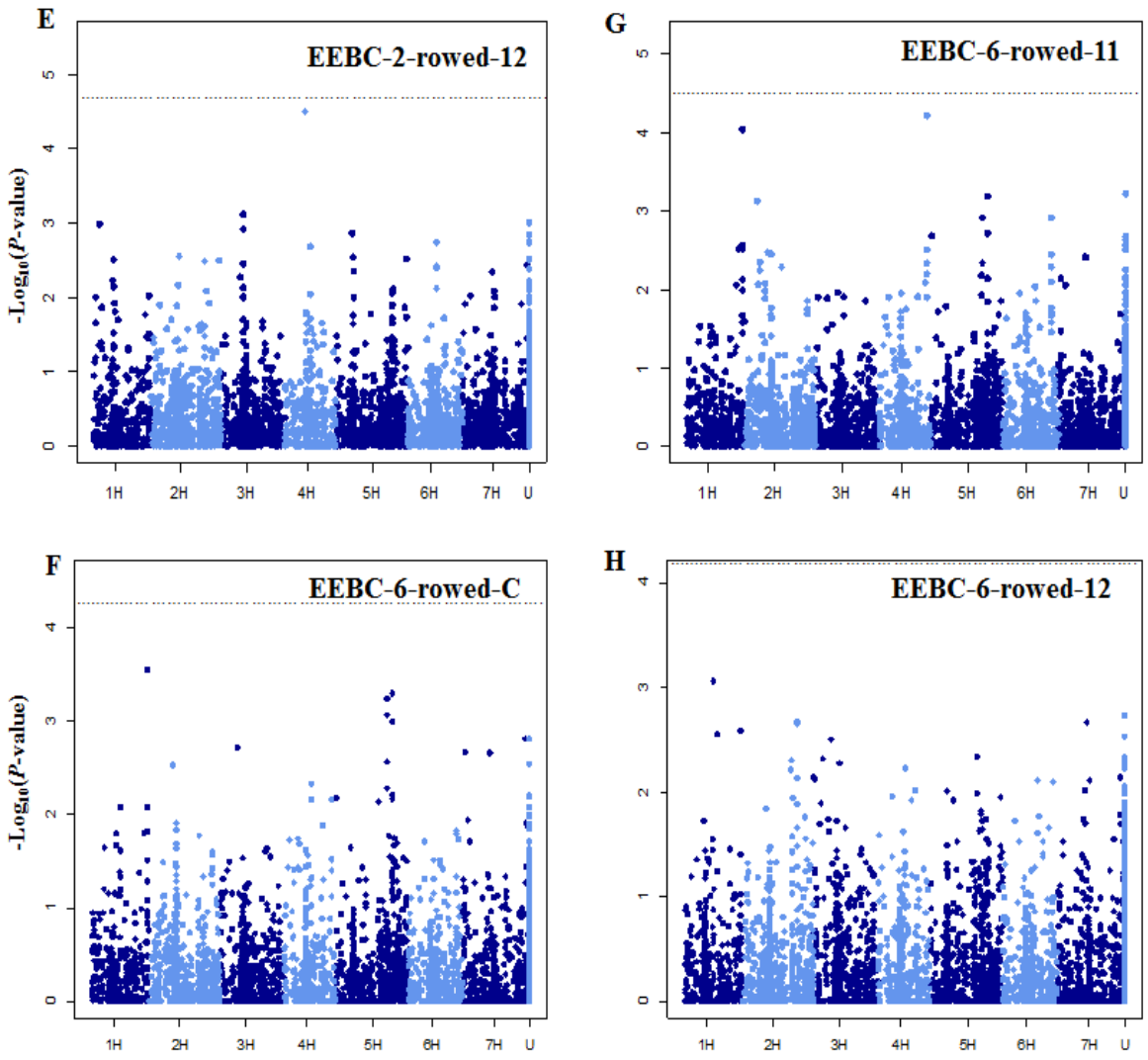
[b] Genetic position (in cM) of marker on chromosome. Note: some markers have not been mapped to a chromosomal position.

[c] Multiple testing corrected *P*-value.

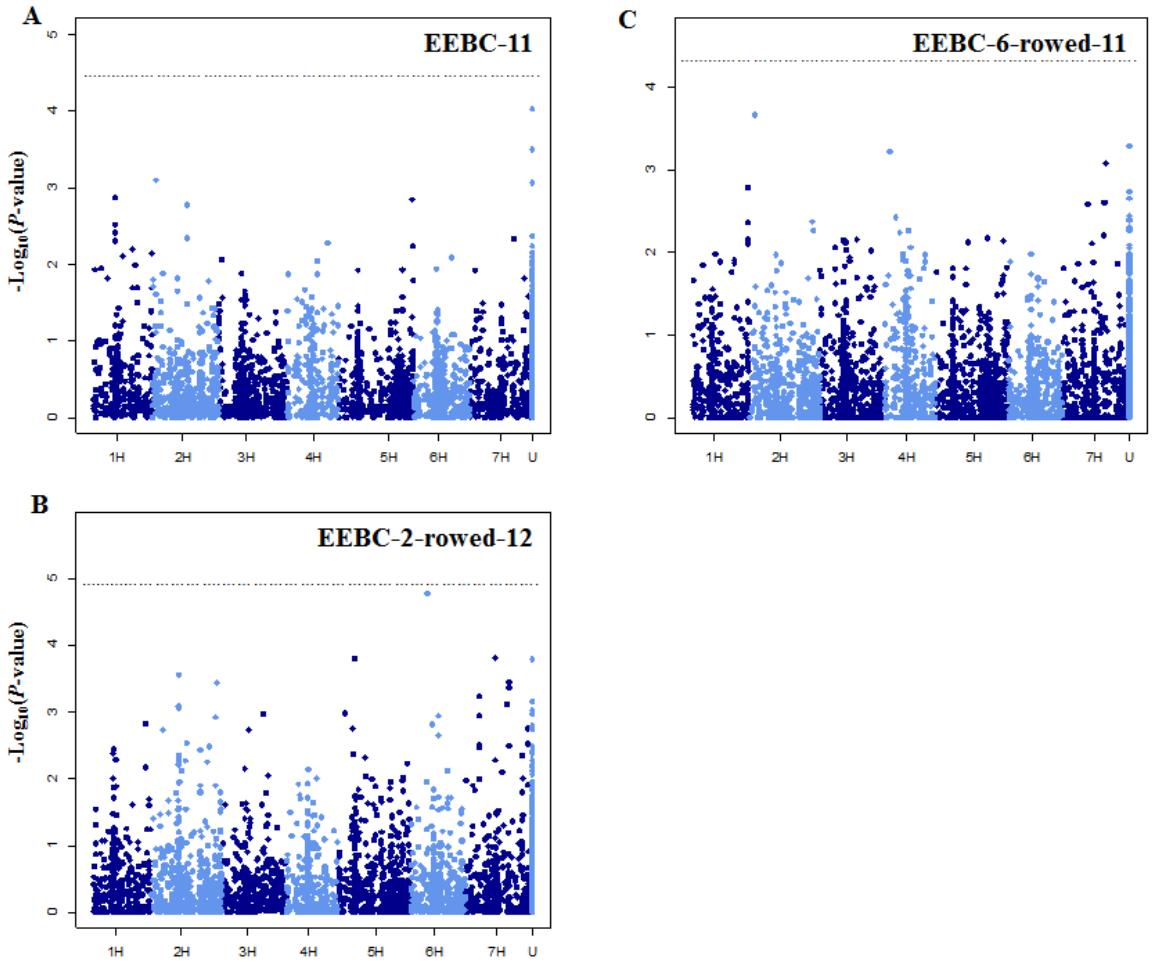
[d] EEBC: Ethiopian and Eritrean barley collection comprises 110 two-rowed and 180 six-rowed germplasm; EEBC-C: Combined data of 2011 and 2012 of the entire collection; EEBC-11: test from 2011 that includes data from the entire collection; EEBC-12: test from 2012 that includes data from the entire collection; EEBC- StP10: test from 2010 that includes data from the entire collection at Saint Paul, MN; EEBC-2R-C: Combined two year (2011 & 2012) data of two-rowed accessions only; EEBC-6R-C: Combined two year data of six-rowed accessions only; '--': No QTL detected; NA: analysis not applicable/data not available.



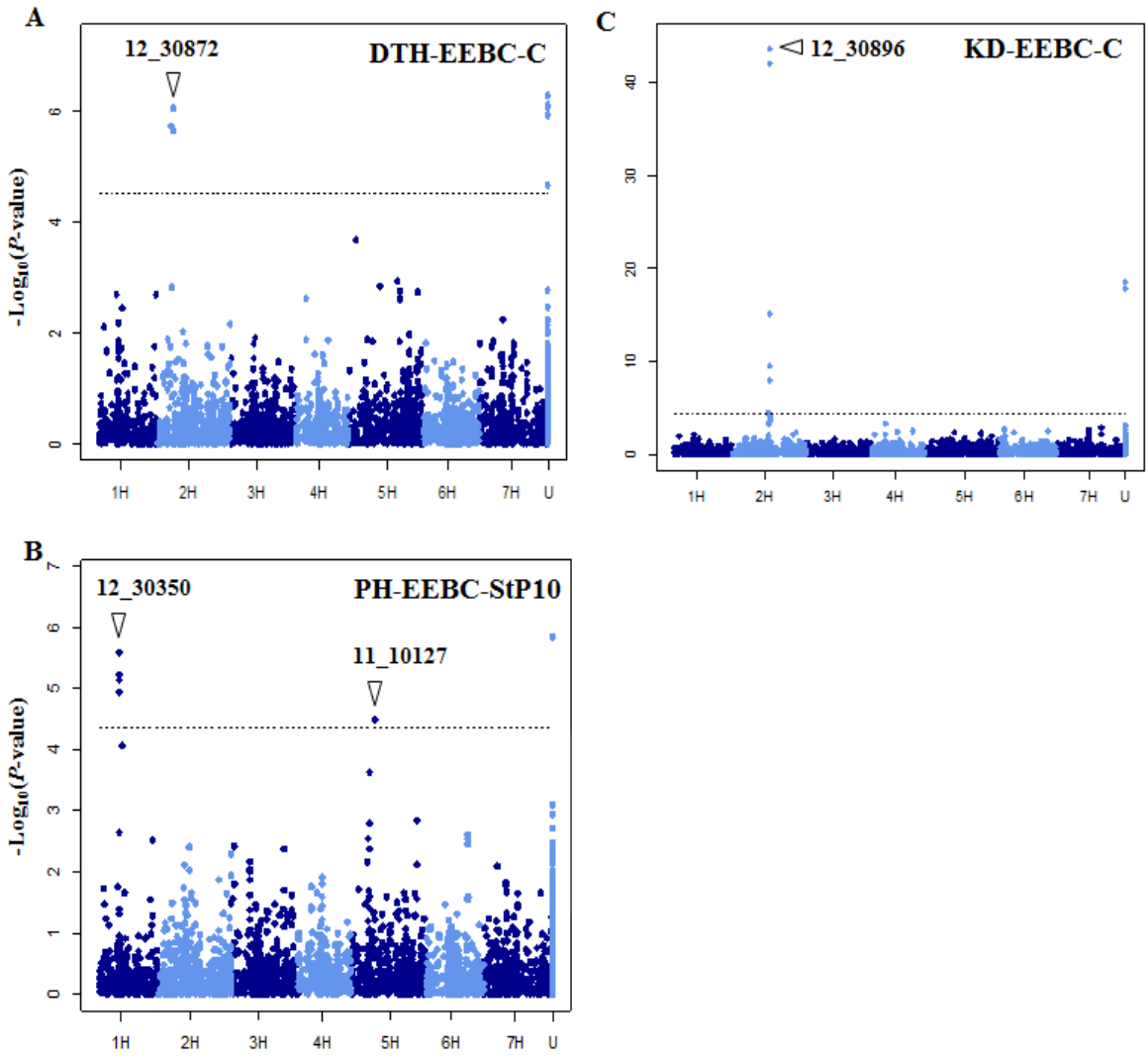
Appendix Figure 4.1. See next page.



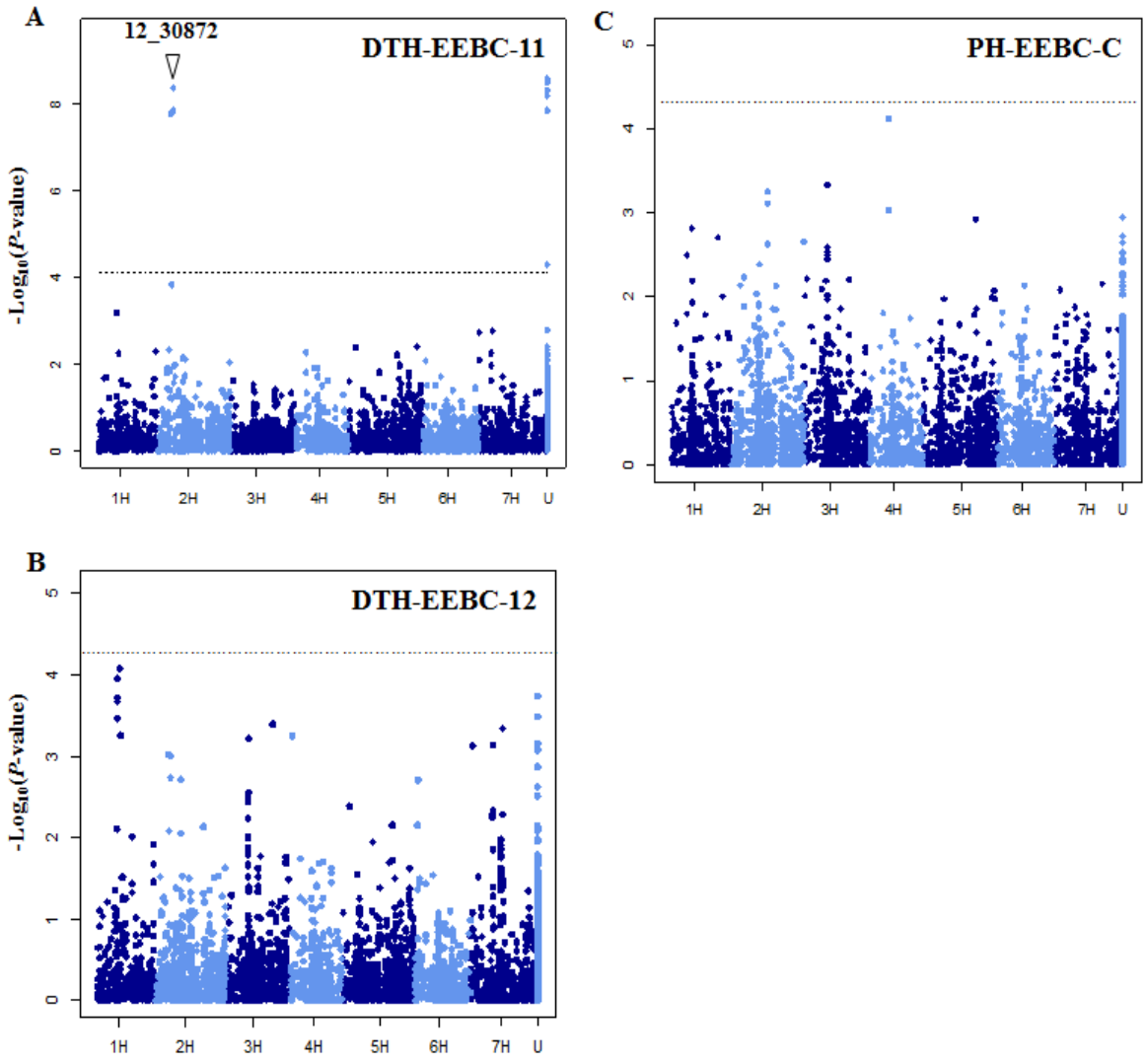
Appendix Figure 4.1. Genome-wide association scan for marker associations with Fusarium head blight (FHB) severity of Ethiopian and Eritrean barley collection (EEBC) evaluated in 2011 and 2012. Scans are shown for the whole set of landrace germplasm (**A**) FHB severity in individual year of 2011 (EEBC-11), and (**B**) FHB severity in 2012 (EEBC-12). Scans are also shown for the two- and six-rowed subsets (**C**) FHB severity combined years for two-rowed set (EEBC-2-rowed-C), (**D**) FHB severity for two-rowed set in 2011 (EEBC-2-rowed-11), (**E**) FHB severity for two-rowed set in 2012 (EEBC-2-rowed-12), (**F**) FHB severity combined years for six-rowed set (EEBC-6-rowed-C), (**G**) FHB severity for six-rowed set in 2011 (EEBC-6-rowed-11), (**H**) FHB severity for six-rowed set in 2012 (EEBC-6-rowed-12). Vertical axis represents -$\log_{10}$(*P*-values) of the marker-trait associations after correction for multiple testing, and the horizontal axis represents the relative chromosomal position of the SNP markers across the genome. The black horizontal dotted lines show the *P*-value corresponding to a false discovery rate (FDR) of 0.05. SNP markers with peaks above this threshold level were considered as significantly associated. The most significant marker for each trait or analysis panel is marked with arrows.



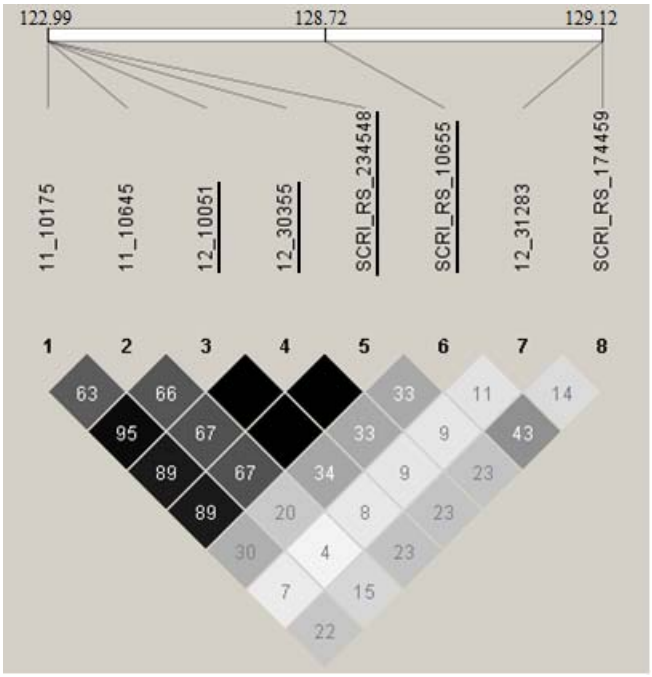
Appendix Figure 4.2. Genome-wide association scan for marker associations with deoxynivalenol (DON) concentration of Ethiopian and Eritrean barley collection (EEBC) evaluated in 2011 and 2012. Scans are shown for (**A**) DON concentration in individual year of 2012 for the whole set of landrace germplasm (EEBC-12), (**B**) DON concentration for the two-row subset in 2012 (EEBC-2-rowed-12), (**C**) DON concentration for the six-row subset in 2011 (EEBC-6-rowed-11). Vertical axis represents -$\log_{10}$(*P*-values) of the marker-trait associations after correction for multiple testing, and the horizontal axis represents the relative chromosomal position of the SNP markers across the genome. The black horizontal dotted lines show the *P*-value corresponding to a false discovery rate (FDR) of 0.05. SNP markers with peaks above this threshold level were considered as significantly associated.



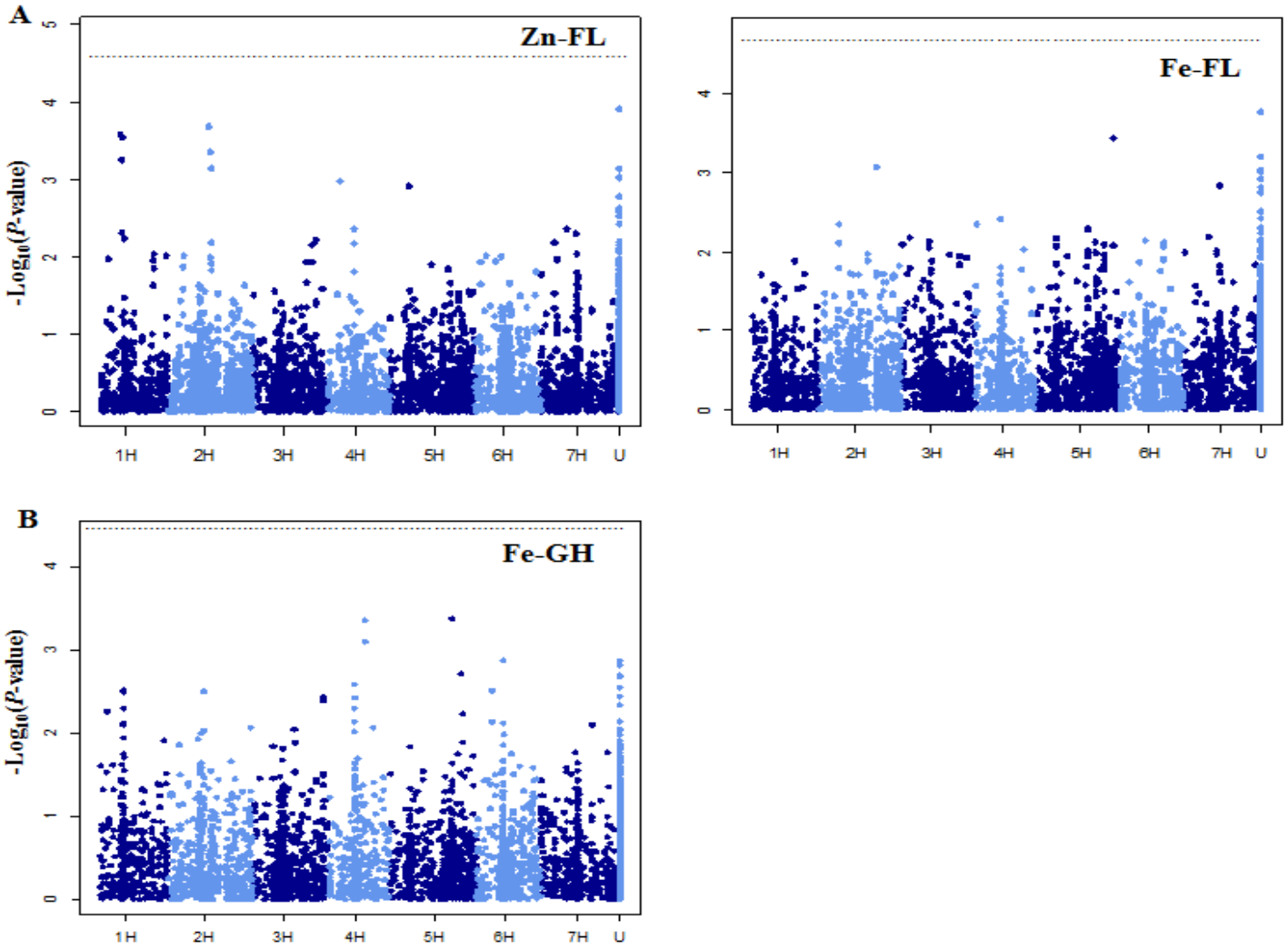
Appendix Figure 4.3. Genome-wide association scan for marker associations with agro-morphological traits in Ethiopian and Eritrean barley collection (EEBC). Scans are shown for (**A**) days to heading combined over two years (DTH-EEBC-C), (**B**) plant height for individual year of 2010 from Saint Paul (PH-EEBC-StP10), and (**C**) kernel density combined over two years (KD-EEBC-C). Vertical axis represents -$\log_{10}$(*P*-values) of the marker-trait associations after correction for multiple testing, and the horizontal axis represents the relative chromosomal position of the SNP marker across the genome. The black horizontal dotted lines show the *P*-value corresponding to a false discovery rate (FDR) of 0.05 and the horizontal axis represents the relative chromosomal position of the SNP markers across the genome. SNP markers with peaks above this threshold level were considered as significantly associated. The most significant marker for each trait or analysis panel is marked with arrows.



Appendix Figure 4.4. Genome-wide association scan for marker associations with agro-morphological traits in Ethiopian and Eritrean barley collection (EEBC). Scans are shown for (**A)** days to heading in individual year of 2011 (DTH-EEBC-11), (**B**) days to heading in 2012 (DTH-EEBC-12), and (**C**) plant height combined over two years (PH-EEBC-C). Vertical axis represents $-\log_{10}$(*P*-values) of the marker-trait associations after correction for multiple testing, and the horizontal axis represents the relative chromosomal position of the SNP markers across the genome. The black horizontal dotted lines show the *P*-value corresponding to a false discovery rate (FDR) of 0.05. SNP markers with peaks above this threshold level were considered as significantly associated. The most significant marker for days to heading is marked with an arrow.



Appendix Figure 5.1. Heat map of linkage disequilibrium (LD) for a genetic region significantly associated with grain zinc concentration on the long arm of chromosome 6H. Four SNP markers significantly associated with the trait are underlined. Numbers above the map are the position of the markers from the concensus map in cM. LD is displayed in the squares below the map as r^2, expressed as a percentage, between all pair-wise combinations of the eight markers.



Appendix Figure 5.2. See next page.

Appendix Figure 5.2. Genome-wide association scans for marker associations with grain zinc and iron concentration in Ethiopian and Eritrean barley landrace germplasm. Scans are shown for (**A**) Zinc concentration of field grown plants (Zn-FL), (**B**) Iron concentration of greenhouse grown plants (Fe-GH), and (**C**) Iron concentration of field grown plants (Fe-FL). The genome-wide association mapping was run with 5,269 SNP markers. Vertical axis represents $-\log_{10}$(*P*-values) of the marker-trait associations after correction for multiple testing, and the horizontal axis represents the relative chromosomal position of the SNP markers across the genome. The black horizontal dotted lines show the *P*-value corresponding to a false discovery rate (FDR) of 0.05. SNP markers with peaks above this threshold level were considered as significantly associated. In (A), a non-significant marker each on chromosomes 4H and 6H was indicated with an arrow for reference as the same marker was significant in other analysis set (See Figure 5.3A).