Association Mapping for Net Blotch Resistance in Barley and a Study of Barley/Cereal Yellow Dwarf Virus in Minnesota

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Dedication

This thesis is dedicated to my parents Dr. Bishnu Bilas Adhikari and Bimala Adhikari for making all the sacrifices to provide me a quality education and to my loving wife Pratibha Acharya for all the encouragement, valuable suggestions and constant support.

Abstract

A number of diseases affect the quality and yield of barley (*Hordeum vulgare* L.). Net form of net blotch caused by *Pyrenophora teres* f. *teres* (*Ptt*) is an economically important disease of barley. The best management strategy for net blotch is breeding for resistance. Resistance genes for net blotch are isolate specific and thus continued efforts are required to identifying resistance genes or quantitative trait loci (QTL) that are effective against an evolving pathogen. Disease resistance can often be sourced from wild germplasm and from landraces where genetic diversity is higher than within a breeding program. Genome wide association studies (GWAS) provide an effective approach to identifying resistance loci in a large population of unrelated individuals. GWAS were conducted to identify resistance loci in a collection of barley landraces from Ethiopia and Eritrea (EEBC) and large populations of elite barley breeding germplasm submitted to Barley Coordinated Agricultural Project (Barley CAP) from ten United States barley breeding programs. Two loci, located in chromosomes 5H and 6H, were identified in the EEBC. The Barley CAP germplasm was divided into smaller association mapping panels based on breeding program origin, and row type and separate analyses were conducted on each of these panels. QTL were identified in chromosome 3H, 4H, 6H and at unmapped locations in the combined Barley CAP germplasm. Several unique loci were identified in individual breeding program panels and in both the two-row and six-row panels. Differences in the location of QTL providing resistance to net blotch in the two-row and six-row panels were observed suggesting that the loci were distinct. Resistance QTL in the Barley CAP were concentrated mostly in chromosomes 4H and 6H in the two-rowed panel, and in chromosomes 3H and 6H in six-rowed panel. Another important disease of barley is Barley yellow dwarf (BYD) caused by *Barley/cereal yellow dwarf virus* (B/CYDV) of Luteovirus family. The distribution pattern and population structure of B/CYDV in Minnesota was studied using 243 samples of largely symptomatic cereals and grasses collected in 2013, 2014 and 2015. Analysis by reverse transcription polymerase chain reaction using virus strain specific primers revealed that BYDV-PAV is presently the most prevalent strain of B/CYDV of cereals in Minnesota.

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Appendix Fig. 4.10 Manhattan plot for markers used in a genome-wide association mapping study examining resistance to *Pyrenophora teres* f. *teres* in the MN panel with all barley breeding lines submitted to the Barley Coordinated Agricultural Project by the University of Minnesota from 2006 to 2009. The x-axis shows the chromosomal positions of the markers and the y-axis the -log(p) values of the markers. UNK indicates markers with unknown map positions. The dotted line represents the significance threshold for a false discovery rate of 0.05 198

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Appendix Fig. 4.15 Manhattan plot for markers used in a genome-wide association mapping study examining resistance to *Pyrenophora teres* f. *teres* in the USU panel with all barley breeding lines submitted to the Barley Coordinated

Agricultural Project by Utah State University from 2006 to 2009. The x-axis shows the chromosomal positions of the markers and the y-axis the -log(p) values of the markers. UNK indicates markers with unknown map positions. The dotted line represents the significance threshold for a false discovery rate of 0.05 203

Appendix Fig. 4.16 Manhattan plot for markers used in a genome-wide association mapping study examining resistance to *Pyrenophora teres* f. *teres* in the VA panel with all barley breeding lines submitted to the Barley Coordinated Agricultural Project by Virginia Polytechnic Institute and State University from 2006 to 2009. The x-axis shows the chromosomal positions of the markers and the y-axis the -log(p) values of the markers. UNK indicates markers with unknown map positions 204

Appendix Fig. 4.17 Manhattan plot for markers used in a genome-wide association mapping study examining resistance to *Pyrenophora teres* f. *teres* in the WSU panel with all barley breeding lines submitted to the Barley Coordinated Agricultural Project by Washington State University from 2006 to 2009. The x-axis shows the chromosomal positions of the markers and the y-axis the -log(p) values of the markers. UNK indicates markers with unknown map positions. The dotted line represents the significance threshold for a false discovery rate of 0.05 205

Chapter 1

General Background: Barley, Net Blotch, Genome-Wide Association Mapping and Barley/Cereal Yellow Dwarf

1.1 Barley

Barley (*Hordeum vulgare* L.) is one of the oldest cultivated crops and ranks fourth among the cereals in worldwide production (143 million tons) behind rice, wheat and maize. Barley is estimated to be produced annually on 49 million hectares of land worldwide (http://faostat.fao.org). Russia, France, Germany, Australia and Ukraine are the top five barley producing countries, while the United States (US) was ranked as the 11th largest producer of barley in 2014 (FAOSTAT). The US produces about 4.68 million tons on 1.3 million hectares of land annually (http://faostat.fao.org). Twenty-seven US states, mainly in the Northern Great Plains, Intermountain West, and Pacific Northwest produce barley, with North Dakota having the largest production in the country in 2015 (1.61 million tons from 425,250 hectares). The state of Minnesota produced 201,100 tons of barley from 40,468 hectares in 2015 (http://www.nass.usda.gov).

Barley was one of the first agricultural crops to be domesticated, along with wheat, peas and lentils, with domestication centering in the Fertile Crescent about 10,000 years ago (Badr et al. 2000). Tibet is also considered one of the centers of domestication of barley, although the Fertile Crescent is recognized as the primary center of origin, domestication and diversity (Dai et al. 2012). Following domestication, barley was primarily used as food, but as wheat and rice consumption surpassed barley, the end uses of barley were diversified to include feed and malt production (Baik and Ullrich 2008). Today, barley is mostly used as animal feed or for malt production, with its use for human food being quite limited. Barley as a food crop is most important in Asia and northern Africa, particularly in extreme climates (Baik and Ullrich 2008). Barley straw

may be used for animal feed and bedding or as a roofing material. Barley grown for human consumption is often used in soups or as an extender for vegetable proteins. Barley flour is used for making baby food in the US and for making flat breads in Africa and Asia (Taylor 2012). Barley used for feed and malt have different quality characteristics. High grain protein and high test weight are desirable for feed barley, whereas barley for malt production needs to have high starch, high α-amylase, less protein and the kernels need to be uniformly of a plumpness suitable for malting. There are different varieties suited for malting or feed purposes. Historically, barley was first used for animal feed in the US, but the use of barley for feed has been constantly declining, while the use of barley for malt and food has been increasing. Most of the barley acreage in the US today is planted to malt quality varieties because of the premium price it fetches. Currently, about 66% of the barley in US is used domestically for malting and processed food production, 12% is exported, and 22% is used for animal feed (http://www.nationalbarley.com).

Barley has a broad ecological adaptation, and it is usually grown in areas of the world unsuitable for production of other cereals, including maize and rice. Barley grows well in dry environments due to its drought tolerant properties and because it matures earlier than other cereals. Optimum production of barley is reportedly attained in cool, dry weather conditions, in well drained fertile loam soil or light clay with a pH range of 6.0 to 8.5 and a balanced application of nitrogen and phosphorus (Rasmusson 1985). Barley is, however, grown across a wide range of climates, and production stretches from the extremely cold highlands of Tibet and Ethiopia, to the hot dry lowlands of North Africa and the moderate climate of California. Similarly, barley cultivation spans a wide

range of latitudes with commercial production ranging from 70°N in Norway to 10°N in Ethiopia. Barley grown in tropical and sub-tropical climates is generally grown as a winter crop, as in Australia, or in the cool temperatures of high altitudes, as in Ethiopia. Although barley grows well in temperate climates where seasons are cool and dry, it may also be productive in hot and dry climates or cool and humid climates. Barley generally performs most poorly in hot and humid climates, principally due to the pressure of diseases.

Barley belongs to the grass family *Poaceae* and the tribe *Triticeae*. Barley in its cultivated form has either a winter or spring growth habit. Winter barley requires vernalization for flowering, and thus it performs better in longer day length conditions. Modern spring barley cultivars do not have a vernalization requirement and will either perform well in longer day environments, or have no response to day length. *Hordeum spontaneum* L., the wild progenitor of barley, has a winter growth type, which suggests that winter growth habit is the ancestral trait of barley (Turner et al. 2005), and modern spring types have evolved from winter types. The spring barleys grown in North America and Western Europe generally show a reduced response to photoperiod, which allows them to extend their period of vegetative growth and yield well (Turner et al. 2005). The growth habit of barley is determined by allelic variation at the *VRN-1* locus and/or *VRN-2* locus. Deletions at the *VRN-1* result in a spring growth habit (Fu et al. 2005). In the US, most of the barley grown in Northern Great Plains is of spring habit, whereas the barley grown in the Pacific Northwest is generally of winter habit. Some southern states, including Texas and Oklahoma, also grow significant amounts of winter barley.

1.2 Net Blotch

1.2.1 Introduction

Net blotch, caused by *Pyrenophora teres* (Drechs.) (anamorph *Drechslera teres*), is one of the most common and economically important foliar diseases of barley (Jordan 1981; Khan 1987; Martens et al. 1988; Steffenson 1997). Net blotch is caused by two different forms of *P. teres* and they produce two different types of symptoms. The net form of net blotch (NFNB), characterized by net-like lesions, is caused by *P. teres* f. *teres* (Smedegaard) (*Ptt*) and the spot form of net blotch (SFNB), characterized by circular to elliptical lesions, is caused by *P. teres* f. *maculata* (Smedegaard-Peterson) (*Ptm*) (Serenius et al. 2005). Net blotch infection can cause marked decreases in the yield and quality of barley in North America and other barley growing regions of the world, including Western Australia (Khan 1987) and Western Europe (Jordan 1981). NFNB has been historically more prevalent and economically important disease in North America but SFNB epidemics have also been frequent in recent years (Burlakoti et al. 2017; Liu et al. 2011). Generally, yield losses from NFNB range from 10 to 40% in areas where favorable environmental conditions, especially high humidity and cool temperatures, result in frequent net blotch epidemics (Liu et al. 2011; Ma et al. 2004). Yield losses from the NFNB in susceptible cultivars have been reported to be as high as 20% in Denmark (Jordan 1981) and 26% in Western Australia (Khan 1989). In addition to yield losses, NFNB infections reduce the malting and feed quality of barley by reducing kernel weight, plumpness, and bulk density (Grewal et al. 2008).

1.2.2 Symptoms

The symptoms of net blotch are seen as lesions on the leaf and leaf sheaths during the growing season. The lesions of net blotch initially appear as small circular to elliptical spots and soon enlarge, developing narrow, dark brown striations (Steffenson 1997). The net-like symptoms of NFNB are characterized by narrow, dark-brown, longitudinal transverse stripes, forming a net-like pattern on infected leaves, whereas SFNB symptoms consist of dark-brown, circular to elliptical lesions, surrounded by chlorotic or necrotic halos (Liu et al. 2011). The lesions of both forms of net blotch are usually restricted in width by leaf veins in adult plants, but they can extend up to 25 mm in length. Severely infected leaves can turn completely necrotic and die as a result of development of either form of net blotch, however the type of lesion that develops is dependent upon the form of the pathogen. The SFNB symptoms in barley seedlings are distinct from spot blotch caused by *Cochliobolus sativus*, whereas they look similar in mature plants (Steffenson 1997). Early lesions in spot blotch infection start as black to chocolate brown lesions without chlorotic margin; chlorosis is developed in the later stages of infection whereas the early symptoms in SFNB appear as similar lesions to spot blotch but with a chlorotic margin.

1.2.3 Host Range

Barley (*Hordeum vulgare* (L.)) and its wild progenitor (*Hordeum spontaneum L*., are the primary hosts of *P. teres*, although the pathogen may also infect a few wild relatives of barley including (but not limited to): *Hordeum marinum* (Hudson), *Hordeum murinum* (L.), *Hordeum brachyantherum* (Nevskii), and *Hordeum distichon* (L.). *P. teres*

can also attack other more distantly related species, including *Avena sativa* (L.) (oats), *Avena fatua* (L.) (common wild oat) and *Triticum aestivum* (L.) (bread wheat) (Liu et al. 2011).

1.2.4 The Pathogen

Pyrenophora teres is an ascomycete fungus from the subphylum *Pezizomycotina*; Class *Dothideomycete*; Order *Pleosporales* and Family *Pleosporaceae*. The anamorph stage is *Drechslera teres* (Sacc.) (syn. *Helminthosporium teres* Sacc.). The teleomorphic stage of the fungus is characterized by the dark globose shaped pseudothecia, 1-2 mm in diameter, that are produced towards the end of growing season on crop residue and also in culture. *P. teres* is heterothallic, and the fusion of thalli from two different compatible mating types is required to produce fertile pseudothecia. The asci, measuring 30–61 µm × 180–274 µm, are club shaped, and arise from a short stalk within the pseudothecia. Ascospores, borne in the asci, measure 18–28 µm × 43–61 µm, are light brown and ellipsoidal, and often have three to four transverse septa and one or two longitudinal septa in the median cells. They are rounded at both ends with constrictions evident at the septa.

The anamorph, *D. teres,* produces conidia on conidiophores. Conidiophores usually arise singly, or in groups of two or three, and are slightly swollen at the base. Conidia measure 30–174 µm ×15–23 µm and are smooth, cylindrical and straight with rounded ends. The conidia are subhyaline to yellowish-brown colored, and often with four to six pseudosepta (Liu et al. 2011; Martens et al. 1988; Steffenson 1997). *Dreschlera teres* produces globose to pear shaped pycnidia in host tissue and culture. The

pycnia give rise to pycnidiospores that are hyaline, non-septate and spherical or ellipsoidal (Martens et al. 1988; Steffenson 1997).

1.2.5 Disease Cycle

P. teres can persist as mycelia in seed, rendering it seed-borne, but it may also survive in crop debris between growing seasons (Ma et al. 2004; Steffenson 1997). Seed-borne inoculum serves to introduce net blotch to new fields, whereas conidia and ascospores formed in fields with a history of net blotch epidemics are considered to be the most important sources of primary inoculum (Steffenson 1997). Disease development in barley seedlings by seed-borne mycelium occurs best at temperatures of around 10-15 ˚C. Inoculum in crop debris generally survives as pseudothecia on the surface of infected barley stubble from one season to another. When conditions become favorable, cool and moist for a sustained period of time, ascospores are produced from the pseudothecia. As many as 400 ascospores may be produced per square centimeter of surface area of stubble (McLean et al. 2009). Infection of barley by ascospores requires free surface moisture or high (95-100%) relative humidity. Each ascospore will germinate to form an appressorium and an infection peg, which subsequently penetrates the epidermis of the host. Differences in growth patterns of *Ptt* and *Ptm* have been reported inside the host. *Ptm* is reported to grow biotrophically, forming a vesicle intracellularly in epidermal cells before switching to intercellular necrotrophic growth, whereas, *Ptt* does not have the initial biotrophic growth phase (Hysing and Wiik 2013; Lightfoot and Able 2010).

Ascospores are considered to be the primary inoculum driving net blotch epidemics, and they may be aerially or splash dispersed to initiate infection (Mathre 1982). Conidia, which also produced on infected tissue in stubble, may also serve as primary inoculum (Mathre 1982), although they usually serve as a source of secondary inoculum when they are produced on mature and senescent leaves in the later part of the growing season (Jordan 1981). Sporulation occurs on conidiophores formed on the surface of the primary lesions when the relative humidity is near 100% (Mathre 1982). Conidial sporulation is diurnal, with light promoting sporulation. An eighteen hour light period is generally enough to stimulate spore production from the conidiophores at temperatures between 15 °C and 25 °C (Mathre 1982).

1.2.6 Disease Management

Net blotch management may include chemical control, cultural practices and host resistance. Chemical control to manage net blotch has generally focused both on the application of foliar fungicides, and seed treatments (Hysing and Wiik 2013). Foliar application of fungicides to the upper leaves during grain filling provides effective chemical control. Whilst applications at early growth stages are generally not economically justifiable (Liu et al. 2011). Seed treatments have been effective in reducing net blotch incidence, and their efficacy was demonstrated when the disease was reported to have reemerged in the barley growing areas of New Zealand and Australia in the late 1970s following the withdrawal of mercury-based seed treatments (Hampton 1980). However, seed treatments alone are not considered reliable for the management of net blotch (Hysing and Wiik 2013). Cultural practices including crop rotation away from

barley and tillage, have been promoted to reduce the amount of primary inoculum (McLean et al. 2009). The use of resistant cultivars, coupled with cultural practices to reduce disease risk, is considered the most reliable way to effectively manage net blotch (Martens et al. 1988; Mathre 1982; Steffenson 1997).

1.2.7 Genetics of Host Resistance

The genetics of resistance to net blotch in barley is complicated, with resistance appearing to be both quantitative and qualitative in nature (Ma et al. 2004; Steffenson and Webster 1992; Steffenson et al. 1996). Studies have characterized resistance as qualitative, or governed by two or three genes in specific populations (Afanasenko et al. 2007; Bockelman et al. 1977; Khan and Boyd 1969; Manninen et al. 2006; Mode and Schaller 1958; O'Boyle et al. 2011; Schaller 1955). More recent studies mapping resistance loci for *P. teres* in barley consider net blotch resistance to be quantitative (Cakir et al. 2003; Grewal et al. 2008; Lehmensiek et al. 2008; Raman et al. 2003). Some studies have tried to map resistance loci both quantitatively and qualitatively and reported that the inheritance fits either model perfectly (Friesen et al. 2006; Ma et al. 2004; Steffenson et al. 1996).

The first reported major gene for net blotch resistance was *Pt1* detected in the cultivar Tifang (CI 4407-1), that reportedly conferred resistance at the seedling stage (Schaller 1955). A second gene major gene (*Pt2*), linked to *Pt1*, was subsequently identified in the cultivars Ming (CI 4797), Harbin (CI 4929) and Manchuria (CI 2335). Two other unlinked *P. teres* resistance genes, were reported in two accessions, CI 4922 and CI 2750 (Mode and Schaller 1958). Later, another resistance gene, *Pta* (also

designated as *Pt,,d*) was identified in Tifang, Ming and CI 9819 (Khan and Boyd 1969). Bockelman et al. (1977) conducted a trisomic analysis using Tifang, Clho 7584 and Clho 9819 and mapped the resistance gene *Rpt1a* from Tifang to chromosome 3H, *Rpt2,* from Cl 9819, to chromosome 5H and *Rpt3d* from Cl 7584 to chromosome 2H. Bockelman et al. (1977) also mapped two other genes, named *Pt1b* and *Pt2c,* located on chromosomes 1H and 5H, respectively, in the line CI 9819. The results from these studies were difficult to compare and interpret as different sources of inoculum, and partially divergent sets of germplasm, were used (Graner et al. 1996). Much later, Manninen et al. (2000) mapped a single major resistance gene in a population developed by crossing CI 9819 (resistant parent) and Rolfi (susceptible parent) to chromosome 6H. This resistance gene was validated in the same population using a different set of *P. teres* isolates, and was again mapped to chromosome 6H and designated as *Rpt5* (Manninen et al. 2006).

Afanasenko et al. (2007) studied the genetics of host-pathogen interactions in twelve net blotch resistant barley accessions (CI 4922, CI 5401, CI 9819, CI 9825, Diamond, Harbin, c-8721, c-8755, c-15811, c-19979, c-21849 and c-23874) using various isolates obtained from Europe, the US, Canada and Australia and concluded that resistance in barley to *Ptt* is mostly isolate specific and controlled by one or two genes. Similarly, O'Boyle et al. (2011) crossed five net blotch resistant parents (Clho 5098, Clho 2291, Nomini, NDB112 and ND5883) with a susceptible parent, Hector, to generate set of populations each segregating for resistance genes effective against ND89-19, the most virulent isolate of *Ptt* identified to date in the Upper Midwest of the US. Their results indicated that the resistant spring barley lines, Clho 2291 and NDB112, share a single dominant resistant gene and that Clho 5098 and the winter barley

cultivar Nomini share a single dominant gene which is different from the gene, shared by Clho 2291 and NDB112.

Several recent studies have mapped loci governing resistance to net blotch in barley and consider the resistance to be quantitative (Cakir et al. 2003; Ma et al. 2004; Manninen et al. 2006; Pierre et al. 2010; Steffenson et al. 1996). A double haploid population from a Steptoe/Morex cross (Steptoe is a net blotch resistant cultivar while Morex is net blotch susceptible and a quality standard for malting barley in the Upper Midwest) was evaluated to identify the loci involved in resistance to *Ptt* at both the seedling and adult plant stages by Steffenson et al. (1996). In this study, two to three loci were found to confer resistance to net blotch isolates ND89-19 and ND85F at the seedling stage, whereas, seven loci were found to contribute resistance in adult plants. The quantitative trait loci (QTL) conferring resistance at the seedling stage were mapped to chromosomes 4H and 6H, while the QTL conferring resistance at the adult plant stage were mapped to chromosomes 1H, 2H, 3H, 4H, 6H and 7H, with two loci identified on 6H. The seven QTL accounted for 67.6% of total phenotypic variance (Steffenson et al. 1996). In the same study, Steffenson et al. (1996) tried to fit Mendelian segregation ratios both for two and three resistance gene models. The segregation ratios fitted perfectly for both the two gene and three gene models, depending on how the phenotypic scores were characterized, indicating that resistance was governed by 2-3 genes in the Steptoe/Morex population. Several other researchers (Cakir et al. 2003; Friesen et al. 2006; Grewal et al. 2008; Ma et al. 2004; Manninen et al. 2006; Steffenson et al. 1996) have reported major effect QTL for seedling resistance to *P. teres* in chromosome 6H in various resistance sources using bi-parental mapping populations.

Friesen et al. (2006) mapped a major QTL that explained more than 89% of phenotypic variance, to chromosome 6H, in a double haploid population developed by crossing Q21861 and SM89010. The same region was also identified by Cakir et al. (2003) in two Australian bi-parental mapping populations tested using Australian *Ptt* isolates. In another study conducted using the Canadian bi-parental population CDC Dolly (net blotch susceptible) x TR251 (net blotch resistant) and Canadian isolates of *P. teres*, a major QTL (*QRpt6*), conferring resistance to net blotch at the seedling stage, was mapped to chromosome 6H (Grewal et al. 2008). The same QTL was also found to be effective at the adult plant stage to some isolates of *P. teres* in that same study (Grewal et al. 2008).

Most of the studies examining resistance to *P. teres* have reported resistance genes, or major effect QTL effective at the seedling stage, in chromosome 6H (Ma et al. 2004). Mapping net blotch resistance at the adult plant stage appears to be more complex. In a study done by Lehmensiek et al. (2008) to map adult plant resistance using Australian bi-parental mapping populations and Australian net blotch isolates, QTL governing resistance were found on all barley chromosomes. Similarly, Steffenson et al. (1996) reported detecting resistance loci on all chromosomes except 5H.

Although both quantitative and qualitative resistance genes have been identified in several sources, the nature of resistance appears to be dependent on the source of resistance, plant developmental stage (seedling and adult) and the pathogen isolate used for screening. Integration of quantitative resistance into barley germplasm, as an alternative strategy to the use of single genes for resistance, appears to be prudent considering that the pathogen can mutate to overcome single major effect resistance

genes (Ma et al. 2004; O'Boyle et al. 2011). Despite the efforts made to characterize and map genes conferring resistance to *P. teres* in barley, relatively few net blotch resistance sources appear to have been effectively utilized in breeding programs (O'Boyle et al. 2011).

1.3 Genome-Wide Association Mapping

1.3.1 Association Analysis and Linkage Disequlibrium

Association mapping or genome-wide association studies (GWAS), also known as linkage disequilibrium (LD) mapping, is the statistical association of genotypes or single nucleotide polymorphisms (SNPs)/SNP haplotypes in a group of individuals (populations) to their phenotypes (Ersoz et al. 2007; Rafalski 2010).

Linkage disequilibrium is a measure of the degree of non-random association of alleles at different loci in a natural population caused by linkage, selection and admixture (Flint-Garcia et al. 2003; Yu and Buckler 2006). In a random mating population with an absence of selection or mutation, polymorphic loci will segregate independently and be in linkage equilibrium (Flint-Garcia et al. 2003). In contrast, natural populations always have linkage, selection and admixture, which cause the polymorphic alleles at different loci to have non-random association or LD. Linkage disequilibrium can be used to map polymorphic alleles, genes or QTLs having an effect on phenotype (Gupta et al. 2005; Rafalski 2010). Linkage disequilibrium can also be measured as the degree of non-random association of markers, which in a statistical sense, is the covariance of polymorphism for two molecular markers (Gupta et al. 2005).

Association mapping has been used extensively and successfully in dissecting complex human diseases (Ersoz et al. 2007). With the development of third generation sequencing technology, providing high throughput, reduced cost and higher marker densities, association analysis is feasible in plants. It has been successfully employed in dissecting complex quantitative traits, including numerous traits in the model plant species *Arabidopsis thaliana* (Atwell et al. 2010), agronomic traits in rice (Huang et al. 2010), disease resistance and kernel size in maize (Kump et al. 2011; Poland et al. 2011; Tian et al. 2011), disease resistance and agronomic traits in barley (Massman et al. 2011; Pasam et al. 2012) and milling quality in wheat (Breseghello and Sorrells 2006).

The use of LD in bi-parental QTL mapping, generally starts with the making of desired crosses using parents with known pedigrees to create a population segregating for a trait of interest and ends with mapping genomic regions co-segregating with linked polymorphic markers (Flint-Garcia et al. 2003; Rafalski 2010). Linkage based analysis using bi-parental mapping populations has two significant drawbacks: the first being that it can only take into account the limited number of recombination events in bi-parental crosses which causes the resolution of mapping to be very low, often in the range of several centimorgans. The second drawback of linkage based analysis using bi-parental crosses is that it only takes into account the two alleles, one derived from each parent, at a locus, and is thus missing the immense allelic diversity at a given locus in a natural population (Ersoz et al. 2007; Flint-Garcia et al. 2003; Zhu et al. 2008).

Association mapping, by contrast to bi-parental mapping is done with a population of unrelated individuals taking into account historical and evolutionary recombination events. Thus, it offers higher resolution mapping and allows multiple

alleles to be analyzed at a locus by making use of the genetic diversity present in a population. This approach also reduces the time and resources needed to identify resistance genes as there is no need to develop specific mapping populations (Rafalski 2010; Yu and Buckler 2006; Zhu et al. 2008). Results from association analyses are applicable to a wider base of germplasm than the results of a bi-parental mapping which are generally only pertinent to the same or genetically related populations (Zhu et al. 2008).

Depending on the objectives and the scale of study, association mapping can be done in two ways; candidate-gene association mapping, or whole genome scan or genome wide association study (GWAS). Candidate-gene association mapping aims to find associations between DNA polymorphisms of selected candidate genes and phenotypic variation for certain traits (Zhu et al. 2008). To undertake candidate-gene association mapping, one needs to have a detailed knowledge of the biochemical pathway related to the trait of interest and knowledge of other associated regulatory genes in the pathway. The major drawback of candidate-gene association mapping is that additional genes associated with phenotypic variance of a given trait might escape identification (Rafalski 2010). The whole genome scan approach, or GWAS, tries to test the hypothesis that "one (or more) of the genetic loci being considered is either causal for the trait or in linkage disequilibrium with the causal locus" (Rafalski 2010). The use of GWAS depends on scanning the whole genome for genetic variation with a large number of markers and trying to associate the genetic variation to complex phenotypic traits (Zhu et al. 2008).

The key to any association study is detecting the LD between functional loci and markers linked physically to those loci. The density of markers needed for association analysis is determined from the decay of LD over the physical distance in the population under consideration. The rapid decay of LD calls for the need of a higher density of markers to capture those located close enough to the functional loci, whereas, in cases where there is a slow decay of LD, a lower density of markers may be used in a GWAS (Yu and Buckler 2006). A low density of markers does, however, result in a decrease in the resolution of the map that would be generated.

The genetic resolution of any association analysis will be dependent on the amount of recombination available in the population. As compared to a bi-parental mapping population, the populations used in association analyses have a much greater amount of recombination within them. The individuals of the population used in association mapping are distantly related and a high degree of recombination will have occurred in them since their divergence from their last common ancestor. In a bi-parental population, a degree of recombination can be achieved with additional rounds of intercrossing and using large progeny sets, but it is generally not feasible to do this in terms of resources and time. The amount of recombination available in an association mapping population may be increased by using a larger population size and this will ultimately increase the resolution of the mapping. To get better resolution with a smaller population size, a higher marker density, providing better genome coverage, is required (Rafalski 2010).

1.3.2 Population Structure

Population structure is the division of the population under consideration for association analysis into distinct sub-populations or groups based on kinship (Rafalski 2010). In a more simplistic way, population structure identifies the individuals of the population that are more related than average (Sneller et al. 2009). Population structure is more pronounced in those cultivated species that have had been subjected to a severe bottleneck during domestication. Factors including geographical origin, breeding pattern and selection for favored traits, can give rise to significant population structure (Atwell et al. 2010). Population structure creates LD between unlinked loci and subsequently results in false positives in the downstream statistical analysis in GWAS. When the allele frequencies between sub-populations in an association mapping panel are significantly different, genetic loci without any effect on the phenotype may also appear to be co-segregating with the trait of interest and thus spurious associations may appear (Ersoz et al. 2007).

Many statistical models or methods have been developed to account for population structure within an association mapping panel. These models include the genomic control model (GC model) (Devlin et al. 2001), the structured association method (SA method) (Pritchard et al. 2000), the quantitative trait association study model (Q-model) (Thornsberry et al. 2001), the unified mixed linear model (Q+K model) (Yu et al. 2005), the step wise regression analysis (SGR method) (Setakis et al. 2006) and principle component analysis (PCA method) (Price et al. 2006).

Wang et al. (2012) compared six statistical models (GC model, SA method, Q-model, Q+K model, SGR method and PCA method) to account for population structure in barley. They conducted association mapping for 32 morphological and ten agronomic traits in a highly structured population of 615 commercial barley cultivars, originating from the United Kingdom (UK). The objective of the study was to compare the effectiveness of the models in reducing the false positive rates, but still maintaining the statistical power to identify associations. They found that the unified mixed linear model (Q+K) gave the best results.

1.3.3 Population Structure and Linkage Disequilibrium in Barley

There is generally a high degree of population structure corresponding to geographical origin, head morphology (row type), end use quality (e.g. traits for malt and feed), breeding history (or breeding programs) and growth habit (winter vs. spring) in cultivated barley germplasm. It has been evident from large scale studies of population structure and LD in US barley germplasm (Hamblin et al. 2010; Muñoz-Amatriaín et al. 2014) and worldwide spring barley germplasm (Pasam et al. 2012) that LD in barley varies in in relation to population structure.

Morrell et al. (2005) studied the population structure and LD of wild barley germplasm collected in Southwest Asia and reported LD decay below basal levels, at about 7 cM, but indicated that the extent of LD in cultivated germplasm was considerably higher than that for wild germplasm. Hamblin et al. (2010) studied the population structure and LD in 1,816 barley lines originating from ten US breeding programs and found that the germplasm separated into distinct sub-populations based on growth habit,

head type (two vs. six row), end use quality and breeding history, depending on the statistical model used to fit the data. Using the no-admixture model, they were able to differentiate the population into ten different sub-populations corresponding to the ten breeding programs whose materials were used for the study. When the admixture model was used, the population stratified into seven sub-populations corresponding to growth habit and row type combinations from different breeding programs. Although the decay of average LD was different for different sub-populations, the average decay of LD for the US barley population examined was 20-30 cM, and thus much higher than for wild germplasm.

Pasam et al. (2012) studied LD and population structure in a collection of 224 spring barley lines thought to be representative of the worldwide diversity in barley. They were able to assign individuals to six sub-populations based on spike morphology and geographical origin using various methods. They reported that the average LD decay of the population was 5-10 cM for individual chromosomes and 7 cM for the whole genome. They also reported that LD decayed slower in two-rowed cultivars compared to six-rowed cultivars, and that LD decays slower in sub-populations than in the whole population. Similarly, Muñoz-Amatriaín et al. (2014) studied LD and population structure in the US barley core collection and found that the 2,417 barley accessions could be differentiated into five sub-populations based on their geographical locations and row type morphology. They also reported different levels of LD decay within the barley genome, with LD being higher in the regions harboring traits involved in domestication and breeding selection.

1.4 Barley Yellow Dwarf

1.4.1 Introduction

Barley yellow dwarf (BYD) is the most economically important and widespread viral disease of cereals. This disease affects more than 150 species of the *Poaceae* family including barley, wheat, oats, sorghum, rye, triticale, maize, rice and many wild grasses (Plumb 1983). BYD can cause significant yield losses in wheat, barley and oats by reducing the number of grain produced per plant, especially if infection occurs at early growth stages (Gildow and Rochow 1983). Barley yellow dwarf is caused by *Barley yellow dwarf virus* (BYDV) and *Cereal yellow dwarf virus* (CYDV) in the *Luteovirideae* group. BYDV and CYDV are transmitted by aphids in a persistent, circulative non-propagative manner. The virus particles are phloem restricted and can also be spread mechanically, though the primarily spread of the virus is by means of aphid vectors (Plumb 1983). Rochow (1969) first characterized differences among BYDV isolates. He identified five strains from New York that were transmitted preferentially by different aphid vectors. The International Committee on the Taxonomy of Viruses (ICTV) has recently reclassified the strains into two different genera within the same family of *Luteoviridae* according to their phylogenetic relationship, epidemiology, host range and genome structure. The RPV strain, or *Cereal yellow dwarf virus,* is designated as the genus *Polerovirus*, whereas *Barley yellow dwarf virus,* i.e., PAV, MAV, SGV and RMV strains, are designated as the genus *Luteovirus* (Table 1.1). The ICTV also has recently added two more strains, RPS and PAS, to the genera *Polerovirus* and *Luteovirus*, respectively. The RPS strain is a variant of the Mexican strains of RPV that was defined

as a new strain based on amino acid sequence identity. Similarly, the new PAS strain is a variant of the PAV strain. The isolates that have more than 90% amino acid sequence similarity with the PAV-NY strain (originally identified by Rochow), remain designated as strain PAV, whereas the group that shared less than 90% of their amino acid sequence similarity with PAV-NY was designated PAS (http://ictvonline.org/virustaxonomy.asp). Other strains such as BYDV-GPV, vectored by *Schizaphis graminum* and *Rhopaloshiphum padi* (Wang et al. 1998), and BYDV-GAV, vectored by *S. graminum* and *Sitobion avenae* (Wang et al. 2000), have been reported in China. In addition to these strains, two additional strains, BYDV-kerII and BYDV-kerIII, which appear to be derived from the PAV strain, were detected by Svanella-Dumas et al. (2013) in *Poa cookii,* from the Kerguelen islands of Antarctica, and are reported to be vectored by *R. padi*. BYDV-kerII and BYDV-kerIII were added to genus *Luteovirus* within family *Luteoviridiae* in 2014 by the ICTV (Table 1.1). The discovery and differentiation of many strains of B/CYDV has led to the introduction of the term "BYDV complex" by Svanella-Dumas et al. (2013).

R. padi is the most efficient vector of the BYDV strains RPV and PAV. This aphid accounts for about 90% of BYDV infections in Europe (Fabre et al. 2003). In a study done in a controlled environment in New York using PAV strains and fourth instar aphids of *R. padi* and *S. avenae*, Chay et al. (1996) observed no significant difference in the efficiency of transmission between the two aphid species. The efficiency of transmission appears to be different for different BYDV strains and aphid species combinations under different environmental conditions. Environmental factors including temperature and wind affect the vector population, their migration speed, the time they

spend to acquire or transmit viruses and thus the pattern, timing and spread of viruses. For example 5-15 °C is considered the temperature range for *R. padi* to spread the virus and increase viral load (Fabre et al. 2003).

Once the virus is acquired by the aphid vector, the aphid becomes infective throughout its life. BYDV transmission is ineffective if feeding periods on host plants are less than 24 hours. Thus, migratory aphids, that only probe briefly on the plants during their transit, are not very effective in transmitting BYD (Irwin and Thresh 1990).

1.4.2 Distribution and Economic Losses

BYD has been reported in most of the cereal growing areas of the world (Singh et al. 1993). Losses due to BYD generally range from 1-3% annually, but losses as high as 20-30% have been reported by CIMMYT (Pike 1990; Singh et al. 1993). Average yield losses in Australia and the UK are reported to be around 2% (Schaller 1984). Higher yield losses have been reported in spring wheat in Manitoba, Canada (7%) (Gill 1980) and in spring oats in Pennsylvania (87%) (Gildow and Frank 1988). The highest losses to BYD tend to occur when conditions are conducive for large aphid populations.

BYD infection in cereals results in yield losses due to a decrease in the number of plants per row, number of tillers per plant, number of florets per head and seed weight (Hoffman and Kolb 1998; Jensen et al. 1971). If the BYD infection occurs at an early stage of crop growth (4-6 weeks after emergence), plants may compensate for yield loss by increasing the number of seeds per head or increasing seed weight, which can be dependent on varieties (Gildow and Frank 1988). If the BYD infection occurs late in the

season or at later growth stages, the window of compensation for yield loss is smaller and compensation may not be apparent (McKirdy et al. 2002).

Calculating yield losses due to B/CYDV is difficult because the symptoms caused by the virus, like yellowing and reddening of leaves, can also be caused by many abiotic stresses and thus symptoms can be easily confused in the field. To accurately assess yield losses due to virus infection controlled inoculation studies need to be conducted. McKirdy et al. (2002) studied losses due to BYD using yield gaps in wheat and oat. Yield gaps are the difference between the maximum attainable yield in a particular plot over years in the absence of virus infection and maximum attained yield in the presence of viral infection. McKirdy et al. (2002) reported yield gaps of 48-80%.

Yield losses to BYD vary significantly under natural and inoculated conditions. When susceptible cultivars are inoculated by viruliferous aphids at seedling growth stages, yield losses as high as 20% and 38% have been reported in barley under field conditions (Edwards et al. 2001). The major economic loss in barley to BYD, is not directly due to yield loss, but due to a decrease in plumpness or starch content of grain that ultimately decreases malting quality. Reduction in malting quality to BYD decreases the value of the crop significantly (Edwards et al. 2001).

1.4.3 Symptoms

The symptoms of BYD infection differ according to crop, cultivar, growth stage of the plant at the time of infection, strain of virus, aphid vector population and environmental conditions. Generally, visible symptoms include severe stunting of the plant, if the plant was infected in early stages of growth, reduction in root growth and

development, delay and prevention of heading, and changes in the color of leaves (Schaller 1984). Color changes in the vegetative part of the plant following BYD infection differ by crop species. Yellowing of leaves associated with BYD is generally present in all grass species, especially barley where a bright yellow discoloration starts from the tip and margins of the leaf and rapidly progresses throughout the whole leaf. Oats leaves turn reddish to purple and, in addition the leaves of the infected plant stiffens (Schaller 1984). In wheat, the leaf margins occasionally might be serrated and the heads may stay erect at ripening. In Maize, leaves frequently turn purple following infection with BYD, whereas in rice leaves might turn orange. Generally, the discoloration starts to appear 7-20 days after infection in all cereals.

Chay et al. (1996) inoculated Coast Black, a BYD susceptible oat cultivar, with two different virulent field isolates of BYDV- PAV strain to observe the symptoms using fourth instar aphids of two different species, *R. padi* and *S. avenae*, in controlled conditions. They observed that symptoms were visible as progressive yellowing of leaves from tip to basal portions and spread of yellowing from the younger leaves to older leaves. The symptoms varied between the two isolates tested, although stunting and yellowing were observed for both strains examined. In infection by some isolates, the emergence of the third leaf was delayed and this leaf was abnormal looking with notched leaf margins and/or the blade twisted in a cork-screw fashion. Timing of appearance of stunting and yellowing symptoms, and in the spread of symptoms in the plant, varied according to different field isolates due to difference in virulence among the isolates.

1.4.4 Epidemiology and Vector Dynamics

The duration of the access period and the virus titer in the source plant are the two most important factors in the acquisition and transmission of B/CYDV by aphid vectors. For acquisition of *Luteoviruses*, the aphid's stylets must come in contact with the phloem. The time aphids need to come in contact with the phloem and the time virus particles remain in the hindgut of the aphid play an important role in determining transmission efficiency. The acquisition period is more important in virus transmission than virus titer in the source plant. Virus titer has been reported to be higher in younger plants than older plants and even higher on younger leaves of the same plant (Gray et al. 1991). Virus titer and temperature have a direct relationship on transmission with higher temperature increasing the virus replication in the infected plant leading to higher efficiency of transmission. Other factors, like the developmental stage of the plant, virus source and temperature also play an important role in efficiency and specificity of transmission.

The acquisition period and transmission efficiency differ considerably among different virus-vector combinations. *R. padi* has been reported to acquire virus particles of RPV or PAV isolates in 15 minutes although the highest efficiency is reported to be at 12-24 hours. *S. avenae* was unable to acquire MAV or PAV isolates within a 15 minute feeding period, but it was able to pick up those isolates within a 4-6 hour feeding period. The highest transmission efficiency for both strains was reached after 72 hours of aphid feeding on an infected host (Gray et al. 1991).

1.4.5 Virus Genome Organization

The BYDV virus particle has a single-stranded, positive sense RNA genome of 6 kb in length and has no poly-A tail (Rochow 1969). The BYDV genome has six open reading frames (ORFs). ORF 2 encodes an RNA dependent RNA polymerase (RdRp), which when expressed is fused with a protein encoded by ORF 1. ORF 3 encodes for the coat protein. The ORF 3 also forms a minor coat protein by read through (i.e. the translation doesn't stop after the ORF ends, but goes on to another contiguous ORF) into ORF 5. ORF 4 encodes for a protein that facilitates the virus to infect phloem tissues. The function of ORF 6 is not yet known (Hull 2009). The *Poleroviruses* or CYDV have an extra ORF at the 5' end called ORF 0, which is absent in BYDV. ORF 1 in CYDV is also not homologous to ORF 1 in BYDV. ORFs 3, 4 and 5 are homologous in BYDV and CYDV, and are referred to as the "*Luteoviridae*" block (Miller et al, 2002). Polymerase chain reaction (PCR) primers for B/CYDV detection have been designed from the coat protein region or ORF 3 (Robertson et al. 1991) and for strain differentiation from ORF 2, ORF 3, and ORF 4 (Malmstrom and Shu 2004).

1.4.6 Diagnostic Tests for Virus Infection

Enzyme-linked immunosorbent assay (ELISA) is an important and widely used method for plant virus detection (Voller et al. 1976) which involves using antisera against the coat protein of a particular strain of a virus (Balaji et al. 2003). Although many variations of ELISA are used for the detection of viruses (Makkouk and Comeau 1994; Van Regenmortel 2012), polymerase chain reaction (PCR) and quantitative real-time reverse transcription polymerase reaction (Q-RT-PCR), are also used for the detection

and differentiation of virus strains in the host cells (Balaji et al. 2003). ELISA has been a cost efficient means of detecting B/CYDV as compared to PCR, but it has the limitation of being less specific and less sensitive. Moreover, multiple strain specific antisera are required to differentiate between the B/CYDV strains (Deb and Anderson 2008).

Even though ELISA is considered a more cost effective tool for the detection of viruses, a need for greater specificity and sensitivity has led to the increased use of PCR and its diverse forms, in laboratories worldwide. Efforts to cut down the cost of PCR runs led to the development of multiplex PCR assays. Multiplexed RT-PCR, which combines multiple primer sets detecting different viruses into a single multiplexed amplification step have been developed. Multiplexed PCR is rapid, and can be a sensitive, highly specific and efficient technique to detect multiple strains or viruses in a single assay (Deb and Anderson 2008). Malmstrom and Shu (2004) developed the first multiplexed PCR assay to detect and differentiate B/CYDV strains, which was later modified by Deb and Anderson (2008) to detect eight important wheat viruses: *Barley yellow dwarf virus* (strains PAV, MAV, SGV and RMV), *Cereal yellow dwarf virus* (strain RPV), *Wheat spindle streak mosaic virus* (WSSMV), *Wheat streak mosaic virus* (WSMV) and *Soil-borne wheat mosaic virus* (SBWMV).

Previously, primer pairs designed from the coat protein coding genes were used to detect and quantify B/CYDV (Table 1.2) (Balaji et al. 2003; Vincent et al. 1990), but three strains of BYDV (PAV, MAV and SGV) have a very high level of sequence identity in the ORF 3 region and the primer pairs designed from coat protein coding genes of these strains resulted in misidentification in RT-PCR assays. To overcome this problem, primer pairs have since been designed from unique regions of the viral genome

(including ORF 3), with the strain unique regions having been identified by aligning two to six sequences for different strains (Table 1.2) (Deb and Anderson 2008; Malmstrom and Shu 2004).

1.4.7 Genetics of Tolerance and Resistance

The reaction of plants to viruses was classified as resistance and tolerance by Cooper and Jones (1983). Resistance is when a plant reduces virus multiplication and spread along with a reduction of symptom development and yield losses; tolerance is when the plant does not restrict virus multiplication, but still manages to reduce symptom development and yield losses (Cooper and Jones 1983). Although resistance to BYD has been widely reported in the past from various sources (Sharma et al. 1984; Singh et al. 1993), it was actually tolerance that was identified according to the definition of Cooper and Jones (1983). The widespread presence of tolerance was only discovered after the introduction of ELISA since virus titer was high in lines previously thought to be resistant. The study of tolerance to BYD is complex, as the tolerance results from complex interactions of the plant, vector, virus, virus serotypes, environmental conditions and the physiological state of the plant at the time of inoculation (Ayala et al. 2002).

A single dominant gene '*Bdv1*' was reported to be responsible for tolerance against the MAV strain of BYDV when Anza, a North American spring wheat, and nine other CIMMYT advanced breeding lines were intercrossed with the BYDV susceptible wheats Bobwhite and/or Bagula in various CIMMYT locations (Singh et al. 1993). Resistance to BYDV has also been detected in various *Agropyron* species (Sharma et al.

1984) and *Thinopyrum, Elymus*, *Leymus*, *Roegneria*, and *Psathyrostachy* species which have been used as sources of resistance genes such as *Bdv2* and *Bdv3* (Zhang et al. 2009).

A number of major resistance genes for BYDV have been reported in barley; *Ryd1* (Rasmusson and Schaller 1959), *Ryd2* (Schaller 1984), *Ryd3* (Niks et al. 2004) and most recently *Ryd4* (Scholz et al. 2009). *Ryd1* is reportedly not very efficient in controlling the disease and thus it has not used in barley breeding programs (Ordon et al. 2009). In contrast, *Ryd2,* a semi-dominant gene identified from extensive screening of Ethiopian barley landraces, and has been extensively incorporated in various spring and winter barley cultivars (Delogu et al. 1995; Šíp et al. 2006). The expression of the resistance phenotype of lines carrying Ryd2 differs according to the genetic background. *Ryd2* also has some negative traits associated with it, such as increased plant height, poor yield and lower grain quality. Ryd2, however has been extensively used in winter barley breeding programs, and to a lesser extent in spring barley breeding programs worldwide (Delogu et al. 1995). The *Ryd2* gene has been mapped close to the centromere of chromosome 3H (Collins et al. 1996). *Ryd3* was identified in a cross between the Ethiopian barley line L94 and Vada. *Ryd3* maps to chromosome 6H and this gene provides a high degree of resistance to BYDV-MAV and BYDV-PAV (Niks et al. 2004). The recently identified, *Ryd4,* is an effective major gene for resistance which was initially identified in the tetraploid wild barley species *H. bulbosum* and later transferred to *H. vulgare.* The gene maps to chromosome 3H and provides complete resistance, or immunity, to strain BYDV-PAV (Scholz et al. 2009).

A number of quantitative trait loci (QTL) for resistance to BYDV have been reported in oats (Barbosa-Neto et al. 2000). Some QTLs associated with tolerance to

BYD in oats have also been reported (Jin et al. 1998; Zhu et al. 2003). Zhu et al. (2003) identified four QTLs; BYDq1, BYDq2, BYDq3 and BYDq4 associated with tolerance to BYDV-PAV strains. These QTL explained up to 58% of the observed phenotypic variance, including the epistatic effect between the four QTLs.

1.4.8 Disease Management

Altering the planting date in order to avoid aphid flights was found to be an efficient control strategy of BYD in winter wheat in a study conducted during the growing seasons of 1976, 1978 and 1979 in Indiana (Carrigan et al. 1981). The same strategy might not be useful in other geographical locations where there are adverse effects from altering the planting time on crop growth and yield (Gray et al. 1996). Perennial grasses have always been considered as a reservoir of BYDV/CYDV inoculum for spring sown cereals, although the strain composition of viruses in perennial grasslands and managed agro-ecosystems are generally different to those in cereal crops (Irwin and Thresh 1990, Seabloom et al. 2009). The strains causing epidemics in small grains fields, like RPV, are reported to be less prevalent in grasslands in the Upper Midwest (Irwin and Thresh 1990). The recent increase in the prevalence of winter wheat production in the Upper Midwest may also provide hosts for the survival of B/CYDV between seasons. These “green bridges” make the management of BYD increasingly difficult in the Upper Midwest, as it has in Europe.

Another potential method of BYD management is the use of insecticidal sprays to control aphid populations in cereal crops (Araya and Cambron 1992). Insecticidal sprays do not generally control primary infections of the plants with BYD since the viruliferous

aphids will already have probed a number of plants and introduced BYD before the insects are detected and symptoms become apparent alerting growers to the need for insecticide applications. Insecticides may however reduce the secondary spread of BYD, although this management strategy is generally considered to be not very effective. The high cost of application, poor effectiveness when applied after the symptoms are evident, and the environmental impacts of pesticides, reduce the likelihood that insect control will be undertaken to control BYD.

Prophylactic spraying of foliar insecticides may also be practiced for the control of aphid vectors. However, prophylactic spraying in the mid- to late-season often results in poor penetration of product in the then well developed, dense leaf canopies, within which aphids tend to hide on the underside of leaves and in leaf whorls. The prophylactic sprays of any contact insecticide are therfore not considered beneficial in the control of B/CYDV (Gray et al. 1996). Imidacloprid (Trade names: Admire, Conguard, Premise, Temprid, Winner etc.)
(1-[6-chloro-3-pyridinyl) methyl]-N-nitro-2-imidazolidinimine) and synthetic pyrethroids have been used as seed treatments providing the alternative to foliar applied insecticides. The contact and systemic properties of Imidacloprid provide protection from the time of seedling emergence well into the growing season, reducing the aphid population and fecundity (Gray et al. 1996; McKirdy and Jones 1996). The economic return from the use of Imidacloprid is however not always consistent. Although Royer et al (2005) indicated that Imidacloprid has been shown to increase the yield of the crop to which it is applied they did not suggest what caused the observed yield increase.

Breeding for resistance is therefore considered the best strategy for management of BYD in any cereal crop, and it has been very successful in the case of barley through the introgression of the *Ryd2* resistance gene (Delogu et al. 1995).

Table 1.1 Barley/Cereal yellow dwarf virus strains and their most efficient aphid vectors

Virus	Family	Genus	Strains	Most efficient aphid vectors	Reference
CYDV[a]	*Luteoviridae*	*Polerovirus*	RPV	*Rhopalosiphum padi*	Rochow 1969
CYDV	*Luteoviridae*	*Polerovirus*	RPS	*Rhopalosiphum padi*	ICTV[b] 2014
BYDV[c]	*Luteoviridae*	*Luteovirus*	PAS	*Rhopalosiphum padi, Sitobion avenae*	ICTV 2014
BYDV	*Luteoviridae*	*Polerovirus*	RMV	*Rhopalosiphum maidis*	Rochow 1969
BYDV	*Luteoviridae*	*Luteovirus*	MAV	*Sitobion avenae*	Rochow 1969
BYDV	*Luteoviridae*	*Luteovirus*	PAV	*Rhopalosiphum padi, Sitobion avenae,* and others	Rochow 1969
BYDV	*Luteoviridae*	Unassigned[d]	SGV	*Schizaphis graminum*	Rochow 1969
BYDV	*Luteoviridae*	Unassigned	GPV	*Schizaphis graminum, Rhopalosiphum padi*	ICTV 2014
BYDV	*Luteoviridae*	*Luteovirus*	kerII	*Rhopalosiphum padi*	ICTV 2014
BYDV	*Luteoviridae*	*Luteovirus*	kerIII	*Rhopalosiphum padi*	ICTV 2014

[a] CYDV = *Cereal yellow dwarf virus.*
[b] ICTV = International Committee for the Taxonomy of Viruses.
[c] BYDV = *Barley yellow dwarf virus.*
[d] Viral strains which have yet to be assigned to a genus by the ICTV.

Table 1.2 Name and sequence of forward and reverse PCR primers designed for detection of BYDV and CYDV used in this study

Target virus and strain	Primer name	Direction	Primer Sequence (5'-3')	PCR Product size (bp)	NCBI Accession no.	Reference
BYDV-PAV	PAV-coat protein	F[a]	AATGCCCAGCGCTTTCAG	91	X17261	Vincent et al. 1990
	PAV-coat protein	R[b]	GCGGACGCGTGTGACTTAA			
CYDV-RPV	RPV-coat protein	F	ACGAGTTGGACCCCCATTG	101	X17259	Vincent et al. 1990
	RPV-coat protein	R	GATCATCTTCGCTGGGAAGCT			
BYDV-PAV	PAVL	F	AGAGGAGGGGCAAATCCTGT	295	D11032	Deb and Anderson 2008
	PAVR	R	ATTGTGAAGGAATTAATGTA			
BYDV-MAV	MAVL1	F	CAACGCTTAACGCAGATGAA	175	D11028	Deb and Anderson 2008
	MAVR1	R	AGGACTCTGCAGCACCATCT			
BYDV-SGV	SGVL1	F	ACCAGATCTTAGCCGGGTTT	237	AY541039.1	Deb and Anderson 2008
	SGVR2	R	CTGGACGTCGACCATTTCTT			
BYDV-RMV	RMVL1	F	GACGAGGACGACGACCAAGTGGA	365	L12757.1	Deb and Anderson 2008
	RMVR	R	GCCATACTCCACCTCCGATT			
CYDV-RPV	RPVL	F	ATGTTGTACCGCTTGATCCAC	400	AF235168.2	Deb and Anderson 2008
	RPVR	R	GCGAACCATTGCCATTG			

[a] F= Forward primer.
[b] R= Reverse primer.

Chapter 2

Study of Barley/Cereal Yellow Dwarf Virus in Minnesota

2.1 Introduction

Barley yellow dwarf (BYD), caused by *Barley yellow dwarf virus* (BYDV) and *Cereal yellow dwarf virus* (CYDV), is the most economically important and widespread viral disease of cereals. This disease affects more than 150 species of the *Poaceae* family including barley, wheat, oats, rye, triticale, sorghum, maize, rice and many wild grasses (Plumb 1983). BYD is also an important viral disease in natural ecosystems and has been credited for causing significant changes in the species diversity within grasslands by facilitating the invasion and colonization of exotic annual grasses at the expense of native perennials (Malmstrom et al. 2006). Native perennial grasses have a reduced fecundity and increased mortality in the presence of B/CYDV, and are consequently less competitive than exotic annuals in interspecific competition (Malmstrom et al. 2006). BYD can cause significant yield losses in barley, wheat and oats. Yield losses are greatest when infection occurs early and is especially devastating when plants are infected at the seedling stage (Gildow and Rochow 1983). BYD has been reported to cause yield losses up to 38% in barley, 46% in wheat, and 15% in oats (Edwards et al. 2001; Larkin et al. 2002; Ordon et al. 2009). Significant yield losses and reductions in malt quality in Upper Midwestern spring barley cultivars have also been reported (Edwards et al. 2001).

BYD is transmitted by aphids in a persistent, circulative, non-propagative manner. The virus particles are phloem restricted and although they can be spread mechanically, BYD is primarily spread by means of aphid vectors (Plumb 1983). The disease is vectored by at least 25 aphid species, in six different genera, with varying levels of efficiency (Halbert and Voegtlin 1995). Bird cherry-oat aphid (*Rhopalosiphum padi*) and

English grain aphid (*Sitobion avenae*) are the two most important vectors of B/CYDV in North America and Europe (Fabre et al. 2003). The efficiency of transmission is variable for different B/CYDV strains, aphid and plant species combinations. The symptoms of BYD include severe stunting of plants infected in the early stages of growth, while infections at all stages may cause reductions in root growth and development; delays in, or the prevention of, heading; reduced grain number; along with changes in the color of leaves (Schaller 1984). Color changes in the vegetative parts of the plant differ by crop species. In barley symptomatic leaves are bright yellow, while in wheat, oats, rye and triticale some reddening is generally present in addition to the yellowing observed in barley (Schaller 1984). In maize, leaves infected by B/CYDV frequently turn purple, while in rice infected leaves are reported to turn orange (Schaller 1984). The discoloration of leaves generally first appears at the tip and margins of the leaf but will then spread rapidly throughout the whole leaf.

BYDV was first classified into five different strains by Rochow (1969) based on vector specificity. The International Committee on the Taxonomy of Viruses (ICTV) recently reclassified these five strains into two different genera within the family Luteoviridae according to their phylogenetic relationship, epidemiology, host range and genome structure. The strain RPV was assigned to the genus *Polerovirus* (*Cereal yellow dwarf virus*) whereas the strains PAV, MAV, SGV and RMV were assigned to the genus *Luteovirus* (*Barley yellow dwarf virus*). The ICTV added strain RPS to the genus *Polerovirus* and strains PAS, kerII and kerIII (Svanella-Dumas et al. 2013) to the genus *Luteovirus* in 2013. Strain RPS was initially distinguished out of a group of Mexican strains previously recognized as RPV (Fauquet et al. 2005). Similarly, the strains PAS,

kerII and kerIII were identified among strains previously considered to be PAV (Fauquet et al. 2005). The division of these newer strains (RPS, PAS, kerII and ker III) from RPV and PAV was made based on amino acid sequence divergence in the viral gene products. Isolates having less than 90% amino acid sequence similarity across the viral gene products were designated as new strains. In addition to the strains of BYDV already listed, there are three additional strains, GPV, GAV and GAS, reported to be specific to China (Wang et al. 1998; Wang et al. 2000, Zhang et al. 2004).

Breeding for resistance and/or tolerance in cereals is the most important strategy for management of B/CYDV. A number of major genes for resistance or tolerance to BYDV have been reported in barley including; *Ryd1* (Rasmusson and Schaller 1959), *Ryd2* (Schaller 1984), *Ryd3* (Niks et al. 2004) and *Ryd4* (Scholz et al. 2009). Of these genes, *Ryd3* and *Ryd4* have been used extensively in breeding programs. *Ryd3* provides tolerance to MAV and PAV strains, whereas *Ryd4* provides tolerance only to strain PAV. Resistance quantitative trait loci (QTL) have also been reported in oats, though these QTL are only effective against PAV strains (Barbosa-Neto et al. 2000; Zhang et al. 2009). Similarly in wheat, resistance/tolerance genes *Bdv1*, *Bdv2* and *Bdv3* are not effective against all strains. *Bdv1* is only effective against strain MAV (Singh et al. 1993), *Bdv2* is effective against strains PAV, GPV and GAV (Zhang et al. 2004) and *Bdv3*, derived from intermediate wheat grass, is highly effective against strain RPV (CYDV) while only moderately effective against the BYDV strains MAV and PAV (Anderson et al. 1998; Sharma et al. 1984).

BYD used to be a sporadic disease in the northern United States (US). However, the incidence of BYD has been on the rise in recent years, attributed to an increase in the

cultivation of winter cereals, and shorter and warmer winters that has facilitated the increased planting of cover crops including rye, which may harbor B/CYDV over the winter. Recent epidemics of BYD in the US have been reported in Minnesota and North Dakota in 1999 (Edwards et al. 2001), 2012 and 2015 (M. J. Smith, personal communication) and in Idaho in 2014 and 2015 (Marshall and Rashed 2014).

The incorporation of resistance/tolerance genes into the germplasm in US cereal breeding programs has been neglected for many years. As the resistances are known to be effective against specific strains of B/CYDV, identification of the prevalent strains in the region is critical to the selection of effective resistance/tolerance genes and/or QTL. The objective of this study was to study the strain composition of B/CYDV present in cereals of Minnesota.

2.2 Materials and Methods

2.2.1 Disease Sample Collection

Leaf samples symptomatic for BYD were collected from cereals planted on the Minnesota Agricultural Experiment Stations (MAES) in 2013, 2014 and 2015. In addition to the MAES locations, samples were also collected from commercial fields of wheat and barley. In all 65, 46 and 132 samples were collected in 2013, 2014 and 2015, respectively (Table 2.1, Table 2.2, Appendix 2.1). Samples were collected from spring wheat, spring barley, spring oats, intermediate wheat grass (*Thinopyrum intermedium*) (IWG) and other wild grasses in the family *Poaceae,* which were growing close to symptomatic plants. The wild grass species were mostly asymptomatic.

Samples were generally collected based on visible symptoms, including yellowing or reddening of leaf tips and margins, stunting and the inability of heads to emerge from the boot due to the twisting of flag leaves or growing tips. Leaf tissue (1-2 leaves) of symptomatic plants were harvested, transferred to plastic bags which were labeled and immediately placed on ice to reduce the activity of any RNA degrading enzymes on the viral RNA. Samples were transported to the lab and stored at -80 ^{o}C until the RNA extractions were undertaken.

2.2.2 RNA Extraction

For each sample, a 1-2 cm long section of tissue was cut from the leaf, with a scalpel, and transferred into a labeled 2 ml screw cap plastic microcentrifuge tube. Two sterilized glass beads (0.5 mm diameter) were then added to the tube. The tube contents were flash frozen by submerging in liquid nitrogen for 3-5 mins and the leaf tissue homogenized using a VWR Hard Tissue Homogenizer (VWR International, Radnor, PA) for 2-3 mins. RNA was extracted from the homogenized leaf tissue using a Qiagen RNAeasy Plant Minikit (Qiagen, Valencia, CA) following the manufacturer's instructions. Briefly, the homogenized tissue was mixed with RLT buffer for cell lysis and the mixture was centrifuged. The supernatant was used for subsequent steps while the plant debris was discarded. Ethanol (96-100%) was added to facilitate precipitation of nucleic acids present in the supernatant. The precipitated RNA was bound to a column and the column was washed with buffer (RW1) to remove any proteins, carbohydrates and fatty acids in the precipitate. A second buffer (RPE) was used to remove any residual salts from the column after washing with the RW1 buffer. Finally, RNA was eluted from

the column with 40 μl of RNAse free water. The RNA concentration was quantified and the quality of the RNA was assessed, using the 260:280 nm and the 260:230 nm absorbance ratio measurements, using a spectrophotometer (Nanodrop 2000c, Thermo Scientific, USA). The RNA was stored at -80 ^{o}C until used.

2.2.3 Reverse Transcription Polymerase Chain Reaction (RT-PCR)

The primers used for the reverse transcription polymerase chain reaction (RT-PCR) were those designed by Malmstrom and Shu (2004) for their two-step multiplex PCR protocol. The protocol consists of two multiplex PCRs , referred to as a two- step multiplex PCR. The primers used for specific tests, along with their sequence information, are provided in Table 2.3.

The first step of the multiplex PCR was designed to differentiate B/CYDV isolates into two subgroups. Subgroup I consisted of strains PAV, MAV and SGV, while subgroup II consisted of strains RPV, RMV and GPV. The second step of the two-step PCR was designed to distinguish strains within subgroup I. The cDNA synthesis was conducted using the Yan-R primer (Table 2.3). In the first step of this process cDNA was synthesized using 2 μl (200-500 ng) of the sample RNA, incubated at 45 ^{o}C for 60 min using Qiagen Reverse Transcription kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. The cDNA was then utilized for the multiplex PCR. Primers Shu-F, S2a-F and S2b-F were used as forward primers, and Yan-R was used as the universal reverse primer. Primers Shu-F and Yan-R allowed the detection of subgroup I strains, producing a PCR product of 830 base pairs (bp). Primers S2aF and S2bF in combination with Yan-R, allowed the detection of sub-group II strains by producing PCR

products of 372 bp. The total reaction volume of the first-step of the multiplex PCR was 20 μl, consisting of 2 μl of cDNA (100-400 ng), 1.5 mM $MgCl_2$, 200 mM of each dNTP, 1X PCR buffer and 1 unit of GoTaq DNA Polymerase (Promega Co. Madison, WI, USA). The primers used in the multiplex reaction were 0.2 μM of Shu-F, 0.3 μM of Yan-R and 0.035 μM of both S2a-F and S2b-F. The PCR conditions were as follows: polymerase activation (95 ^oC, 2 min) and 40 cycles of: denaturation (95 ^oC, 30 s); annealing (60 ^oC, 30 s) and extension (72 ^oC, 30 s), followed by a final extension (72 ^oC, 7 min). The PCR was conducted in a Peltier Thermal Cycler (PTC-200, MJ Research, Watertown, MA). PCR products were analyzed by gel electrophoresis in a 1.3% TBE agarose gel and visualized under UV illumination with a Bio-Rad gel Doc system (Bio-Rad Laboratories Inc. Hercules, CA, USA). The bands were stained with ethidium bromide (EtBr) (0.33 mg EtBr/ml of TBE agarose gel) mixed into the agarose solution before casting. Fragment sizes were compared with a 100 bp DNA ladder (New England Biolabs, Ipswich, MA).

To reduce non-specific binding, the GoTaq DNA Polymerase was replaced with Platinum Taq DNA Polymerase (Promega Co. Madison, WI) and PCR was conducted for all samples using the same protocol. To remove potential PCR artifacts as a result of using a multiplex protocol, strain identification was conducted using individual primer pairs in single reactions. RT-PCR beads (GE Healthcare, Little Chalfont, UK) were used to minimize pipetting errors in the PCR reaction. The RT-PCR beads were reconstituted by addition of 38 μl of deionized distilled water and 4 μl of a mixture of primer pairs (0.4 mM each) for each PCR reaction. The reconstituted RT-PCR reaction mix was divided into four micro-centrifuge tubes, and 2 μl of the template RNA was added to the tubes for

each reaction. Reactions were conducted in a total volume of 12.5 μl. The RT-PCR reconstituted mix contained 200 μM of each dNTP, 10 mM Tris-HCl (pH 9.0), 60 mM KCl and 1.5 mM $MgCl_2$. Single primer pairs for subgroup I (Shu-F and Yan-R, 0.4 mM each) and subgroup II (S2a-F and Yan-R, 0.4 mM each) were used in separate reactions. The PCR conditions were: reverse transcription (42 ^oC, 45 min); DNA polymerase activation and reverse transcriptase deactivation (95 ^oC for 5 min), followed by 40 cycles of denaturation (95 ^oC, 30 s); annealing (60 ^oC, 45 s); extension (72 ^oC, 30 s) and a final extension step (72 ^oC, 7 min). PCR was conducted on an MJ-Mini Thermal Cycler (Bio-Rad Laboratories Inc. Hercules, CA, USA). The PCR products were analyzed by gel electrophoresis on a 1.5% agarose gel and visualized under UV illumination with a Bio-Rad gel documentation system (Bio-Rad Laboratories Inc. Hercules, CA, USA). The PCR products were stained post-run using 20 mg/ml EtBr in 100 ml of 1X TBE buffer, followed by three step destaining by washing the gel with 100 ml 1X TBE buffer for 10 min in each step. Fragment sizes were compared with a 100 bp DNA ladder (New England Biolabs, Ipswich, MA).

2.2.4 PCR Protocol Design

In the first multiplex PCR assay, conducted using GoTaq DNA polymerase, when the PCR products were visualized in an agarose gel, non-specific bands and smearing were visible, in addition to the bands representing the expected fragment sizes. The non-specific binding was successfully removed by using a hot start DNA polymerase (Platinum Taq from Invitrogen), but the results obtained from this assay did not match the initial results obtained using the GoTaq DNA polymerase. The results using hot start

polymerase produced false negatives when compared to the results obtained from GoTaq polymerase.

To determine if the non-specific binding was an artifact of the multiplex PCR process, an assay was designed for the detection of either subgroup I or II strains using single primer pairs in separate reactions. Primer pair Shu-F and Yan-R were used to identify BYDV samples positive for subgroup I in the first PCR. In the second PCR, primer pairs S2a-F and Yan-R were used to identify samples positive for subgroup II. In this assay, a premixed RT-PCR kit (GE Healthcare, Piscataway, NJ) was used. For strain identification within subgroup I, separate PCR reactions were conducted for PAV, MAV and SGV strains using the appropriate primer pairs (Table 2.3).

2.3 Results

2.3.1 PCR for B/CYDV Subgroup Identification

A total of 65, 46 and 132 samples were collected in 2013, 2014 and 2015, respectively (Table 2.2). These samples were tested for the presence of B/CYDV subgroup I strains using the primer pair Shu-F and Yan-R. The samples testing positive for subgroup I produced the expected fragment size of 830 bp (Fig. 2.1). To test for the presence of subgroup II strains, RT-PCR was conducted using the primer pair S2a-F and Yan-R (Table 2.3). Samples testing positive for the presence of subgroup II produced the expected fragment size of 372 bp (Fig. 2.2).

Of the 65 samples from 2013, eight tested positive for subgroup I and produced the expected fragment size of 590 bp (Fig. 2.3.). Four of these positive samples were hard red spring wheat (HRSW) samples collected in Fergus Falls, MN and the remaining four

were spring barley samples collected from Kimball, MN (Appendix 2.2). In addition, one spring barley sample from Kimball in 2013 tested positive for the presence of subgroup II. None of the samples collected from Crookston (27 HRSW and 20 spring barley) tested positive (Table 2.4).

Nine of the 45 samples from 2014 tested positive for subgroup I and all nine of these samples were from HRSW. The positive samples included four out of the four samples collected from Sabin, three of the 18 samples from Le Center and one each collected from Perley (out of five samples collected) and Fergus Falls (out of nine samples collected). In addition three samples from Le Center and one sample from Sabin tested positive for subgroup II (Table 2.4). Sample number 97 (ex Sabin) and sample number 9 (ex Le Center) (Appendix 2.2) had mixed infections, testing positive for both subgroups I and II. None of the spring barley (all samples were collected from Oklee (6) or Strathcona (2)) tested positive for either of the subgroups.

One hundred and thirty-two samples were collected from various locations in 2015. Sampling in 2015 was conducted from additional hosts including spring oats, intermediate wheat grass (IWG) and other grasses (including winter wheat) (Table 2.2). Of the 48 HRSW samples collected, 24 samples tested positive; of which 21 tested positive for subgroup I, two sample tested positive for subgroup II and one sample recorded a mixed infection (Table 2.5, Appendix 2.2). Of the 24 HRSW samples that tested positive, 13 were from St. Paul, four from Perley and seven were from Oklee (Table 2.4). The sample recording a mixed infection was from Oklee and of the two samples that tested positive for subgroup II, there was one each from Perley and St. Paul. Only nine of 39 spring barley samples tested positive for BYDV, of which seven tested

positive for subgroup I and two for subgroup II, with no mixed infections detected (Table 2.5). Of the 20 spring oats samples tested, four samples tested positive for subgroup I and three samples tested positive for subgroup II with no mixed infections detected (Table 2.5). Of the 18 IWG samples collected from St. Paul, only one sample tested positive and that was positive for subgroup II. Six wild grasses and one winter wheat were sampled in 2015, of which only two tested positive, one for subgroup I and one for subgroup II, with no mixed infections detected (Table 2.5). In summary, more than 50% of the HRSW samples collected from the most intensely sampled site (St. Paul) in 2015 tested positive, whereas only two of 19 spring barley samples from Crookston tested positive (Table 2.4). None of the seven spring oats samples from Stephen tested positive, although it should be noted that they exhibited characteristic BYDV symptoms, including stunting and reddening of leaves (Table 2.4).

2.3.2 Strain Identification within Subgroup I

Of the 51 samples that tested positive for subgroup I (including three mixed infections), strains could only be identified for 42. All 42 samples that tested positive for subgroup I produced an amplicon of 590 bp which identified them as strain PAV (Fig. 2.2; Table 2.5). The six remaining samples, did not amplify a product with the primers for stains PAV, MAV or SGV and thus it was not possible to determine their strain identity within subgroup I (Table 2.5). Of the three samples that yielded a mixed infection, two tested positive for strain PAV and for the third sample the strain in subgroup I could not be determined, though all three did test positive for subgroup II as was expected.

The PCR protocol for MAV and SGV strain identification was repeated using known MAV and SGV strains provided by Dr. Eric Seabloom at University of Minnesota (these were originally obtained from Dr. Stewart M. Gray, Cornell University) as positive controls, in separate PCRs. The positive controls produced faint bands, but none of the samples we sampled that reported mixed infections amplified a B/CYDV specific product.

2.4 Discussion

B/CYDV used to be sporadic problem in the northern US but the frequency of BYD epidemics is on the rise in recent years, largely due to climate change and increased winter cereals production facilitating the survival of the virus over winter. Generally, B/CYDV are introduced into spring-grown cereals in the Upper Midwest by viruliferous aphids travelling north from cultivated grass species in the southern plains. There are several strains of B/CYDV and all strains are transmitted by aphids, albeit with variable levels of efficiency. The resistance/tolerance genes deployed in breeding are strain-specific. Hence, it is imperative that we know what strains are prevalent in a region before we decide which genes to deploy via the breeding programs or make other disease management decisions. This study was aimed at assessing the strain composition of B/CYDV in Minnesota, so that gene deployment strategies can be re-assessed.

Our results indicate that B/CYDV occurs in Minnesota, although the incidence fluctuates between years. From the samples we examined, BYDV-PAV appears to be the predominant strain in Minnesota as 70% of the 62 samples that tested positive were

positive for this strain (Table 2.5). PAV and PAS strains have been reported to be the predominant strains in natural ecosystems of Alaska and eastern Europe (Robertson and French 2007a) and agricultural ecosystems of Kansas (Rotenberg et al. 2016) and eastern Europe (Jarošová et al. 2013). We were unable to differentiate strains within subgroup I for six samples that tested positive for subgroup I. Our inability to identify the strains within subgroup I may have been because the primers used in this study were designed from limited sequence information present in the Genbank sequence database (Malmstrom and Shu 2004), meaning that sequence information may not adequately represent the current diversity of B/CYDV. Only seven complete sequences for PAV accessions (AF218798, AF235167, D11032/D01214, NC_002160, NC_004750, X07653, D85783), four complete sequences for MAV accessions (AY220739, D11028/D01213, NC_003680, NC_004666), and partial sequences for SGV accessions (U06866, U06865, AY541039) have been previously reported (Malmstrom and Shu 2004). Field isolates of B/CYDV might have immense sequence diversity since the virus is prevalent in very high incidence and across diverse hosts found in both agricultural and natural landscapes. The divergence of strains identified by Rochow (1969) into new strains in recent years is evidence that field derived B/CYDV isolates are highly diverse. It is possible that primers designed from the currently limited sequence information available in Genbank may not amplify all isolates. Moreover, these primers were mostly designed using the sequence from ORF-3 of the genome, which encodes for the coat protein of the virus and is a very highly conserved region of the genome of viruses, in general (Malmstrom and Shu 2004). While primers derived from coat protein serve as an excellent diagnostic tool to detect presence or absence of a virus, they may

not be sufficient to distinguish variations within a same species because the region being highly conserved. In this protocol, the primers were able to detect the presence or absence of virus and classify them into broad categories, as the subgroups, but they were not specific enough to classify all the field isolates to individual strain level. This may be one of the reasons why, the subgroup identification was successful, but strain differentiation was unsuccessful for a limited number of the samples in this study.

Differences in the incidence of B/CYDV strains were observed across years, locations and hosts (Table 2.4). The incidence of BYD appeared to be higher in 2015 compared to 2014 and 2013. Most (86%) of the samples from 2013 showed no amplification of B/CYDV when tested by RT-PCR (Table 2.2). Similarly, all of plants sampled from Crookston in 2013 were negative and in 2015 most (92%) were negative (Table 2.4). As a large proportion of the samples collected in 2013 (72%) were from Crookston, the pattern of sampling may explain the low frequency of BYD detected in 2013 compared to the other years where the samples were collected over a larger geographic area. Only one of the eighteen IWG samples tested positive for B/CYDV, although all of the samples collected were symptomatic, exhibiting yellowing and reddening of leaves. The incidence of B/CYDV may have been lower in the IWG population given that it is known to have some resistance/tolerance to BYDV. Sharma et al. (1984) reported that IWG was resistant to strains BYDV-PAV and CYDV-RMV after screening a collection of IWG and other wheat relatives against these strains. IWG was later identified as the donor of the resistance gene Bdv3, that confers resistance to BYDV in a line of wheat (P29) that was developed by chromosome (7D) substitution using IWG (Anderson et al. 1998, Balaji et al. 2003, Zhang et al. 2004). Sampling based on visual

symptoms may also be inaccurate in IWG and other hosts as similar visual symptoms may result from numerous diseases and physiological disorders, including nutrient stress, water logging, drought, cold weather and phytotoxicity to herbicides. The symptoms of BYD can readily be confused with Aster yellows, a disease that has previously been reported by Hollingsworth et al. (2008) in northwestern Minnesota where most of the samples for this study were collected. Another possible explanation of multiple samples testing negative for B/CYDV by RT-PCR despite having visual symptoms indicative of BYD, may be that the primers used in this protocol could not amplify field isolates. This is a possibility given that six samples were identified as being positive for BYDV in subgroup I, but then could not be further differentiated.

There are several protocols available for detecting B/CYDV, but the RT-PCR protocol used in this study was based on the protocol designed by Malmstrom and Shu (2004) and is the protocol most widely used by others (Deb and Anderson 2008; Li et al. 2008; Robertson and French 2007b). The results of our study indicated that non-specific amplification can occur both in the multiplex PCR which aims to identify the subgroups and in the enhanced multiplex PCR which identifies the strains within subgroup I. This issue was first reported by Malmstrom and Shu (2004), and later by Deb and Anderson (2008) using the same set of primers. As a result, single sets of primer pairs were used in our study for diagnosis and strain differentiation within the subgroup I. In addition, instead of manually mixing the components of RT-PCR and conducting the cDNA synthesis separately, RT-PCR beads with pre-mixed RT-PCR components, were used. This eliminated the need to conduct cDNA synthesis as a separate step and increased the effectiveness of the assay, since a larger volume of reverse transcription product was

being used for PCR, and pipetting errors were minimized. The changes we implemented to the protocol developed by Malmstrom and Shu (2004) eliminated the appearance of excess bands in the gel, presumably by reducing non-specific binding. The drawback of performing separate tests is that the tests ultimately require more reagents and take longer than the multiplexed PCR protocol, thus the modifications we utilized make it less suitable for processing a large number of samples.

The PAV strain of the BYDV complex appears to be the most dominant strain causing BYD in Minnesota. PAV is also reported to be the dominant strain in both agricultural and natural ecosystems in the southern Great Plains (Rotenberg et al. 2016). Most of the epidemics of BYD in the northern Great Plains are initiated by viruliferous aphids, migrating from winter wheat fields in the southern US. Based on the results of this study, it can be concluded that efforts in Minnesota should be directed towards breeding cereals for tolerance or resistance to the PAV strain of BYDV. Despite PAV being the most common strain of BYDV in the Great Plains (Rotenburg et al. 2016) and Minnesota (Hollingsworth et al. 2008), there remains the possibility for development of new strains from existing B/CYDV strains, as was reported recently (Robertson and French 2007a; Svanella-Dumas et al. 2013). It would be prudent to conduct routine scouting for and testing of B/CYDV isolates to monitor the population for changes in strain composition as an understanding of the virus diversity is needed to respond effectively with an appropriate management strategies, including host resistance. Future research should also be directed toward designing and developing better diagnostic protocols capable of reliably detecting strains of B/CYDV. Presumably effective diagnostic would rely on PCR protocols informed by accurate sequence information.

Table 2.1 Summary of leaf samples (total sample number and number of samples that tested positive for B/CYDV) collected from cereals within Minnesota in 2013, 2014 and 2015 (data combined for years) categorized by host and location

Host / Location	Hard red spring wheat		Spring barley		Spring oats		Intermediate wheat grass		Other grasses	
	SN[a]	+ve[b]	SN	+ve	SN	+ve	SN	+ve	SN	+ve
St. Paul	25	13	9	3	4	2	18	1	7	2
Crookston	27	0	39	2	-	-	-	-	-	-
Fergus Falls	19	5	-	-	-	-	-	-	-	-
Kimball	-	-	5	5	-	-	-	-	-	-
Le Center	18	4	-	-	-	-	-	-	-	-
Oklee	12	7	9	1	-	-	-	-	-	-
Perley[**]	15	5	3	1	6	5	-	-	-	-
Sabin	4	4			-	-	-	-	-	-
Stephen	2	0	5	2	7	0	-	-	-	-
Strathcona	1	0	2	0	-	-	-	-	-	-
Canningham[*]	-	-	-	-	2	0	-	-	-	-
Polk County[*]	1	0	1	0	-	-	-	-	-	-
Marshall County*	1	0	-	-	-	-	-	-	-	-
Total	125	38	75	14	20	7	18	1	7	2

[a] SN = number of samples collected from each host in 2013, 2014 and 2015, combined.
[b] +ve = number of samples that tested positive for B/CYDV.
[c] "-" indicates that no samples were collected.
[*] Denotes locations where samples were collected from grower fields by disease scouts.
[**] In Perley in 2015, only the spring oat samples were collected by disease scouts.

Table 2.2 Summary of leaf samples (total sample number and number of samples that tested positive for B/CYDV) collected from small grains (hard red spring wheat, spring barley, spring oats, intermediate wheat grass) and grasses in the family *Poaceae* in Minnesota in the years 2013, 2014 and 2015

Host / Year	Hard red spring wheat		Spring barley		Spring oats		Intermediate wheat grass		Other grasses		Total	
	SN[a]	+ve[b]	SN	+ve	SN	+ve	SN	+ve	SN	+ve	SN	+ve
2013	39	3	26	5	-[c]	-	-	-	-	-	65	8
2014	37	10	8	0	-	-	-	-	-	-	45	10
2015	48	24	39	9	20	7	18	1	7	2	132	43

[a] SN = number of samples collected across all locations combined, for each year of the study.
[b] +ve = Number of samples that tested positive for B/CYDV.
[c] "-" indicates that no samples were collected.

Table 2.3 Primers used in reverse transcription polymerase chain reactions (RT-PCR) for the classification (subgroup and strain) of B/CYDV isolates obtained from the leaf tissue of small grains and grasses collected in Minnesota from 2013-2015 using a two-step multiplex polymerase chain reaction

Target subgroup and strain(s) for identification	Primer	Primer sequence (5′ →3′)	Expected PCR product size (bp)
First Step Primers			
Subgroup I (strains PAV, MAV and SGV)	Shu-F Yan-R	TACGGTAAGTGCCCAACTCC TGTTGAGGAGTCTACCTATTTG	830
Subgroup II (strains RPV and RMV)	S2a-F Yan-R	TCACCTTCGGGCCGTCTCTATCAG TGTTGAGGAGTCTACCTATTTG	372
Second Step Primers			
Subgroup I strain PAV	PAV-F Yan-R	ACCTAGACGCGCAAATCAAA TGTTGAGGAGTCTACCTATTTG	590
Subgroup I strain MAV	MAV2-F Yan-R	AATAACCGCAGGAGAAATGG TGTTGAGGAGTCTACCTATTTG	590
Subgroup I strain SGV	Shu-F SGV-R1[a] SGV-R2[a]	TACGGTAAGTGCCCAACTCC ACATTTCTTCGTGTGTTGCG ACATTTTTGCGTGCGTTGCG	254

[a] SVG-R1 and SGV-R2 were used in a mix (1:1) as reverse primers, as per the enhanced multiplex PCR protocol of Malstrom and Shu (2004).

Table 2.4 Total and B/CYDV positive leaf samples listed by year and host. Samples were collected from hard red spring wheat (HRSW), spring barley (SB), spring oats (SO), intermediate wheat grass (IWG) and other grasses (including one winter wheat sample) from various locations in Minnesota

Year	Location	HRSW		SB		SO		IWG		Grasses	
2013		**Total**[a]	**+ve**[b]	**Total**	**+ve**	**Total**	**+ve**	**Total**	**+ve**	**Total**	**+ve**
	Fergus Falls	10	4	-	-	-	-	-	-	-	-
	Kimball	-	-	5	5	-	-	-	-	-	-
	Crookston	27	0	20	0	-	-	-	-	-	-
	Polk CO*	1	0	1	0	-	-	-	-	-	-
	Marshall CO*	1	0	-	-	-	-	-	-	-	-
	Sub-Total	**39**	**4**	**26**	**5**	**-**	**-**	**-**	**-**	**-**	**-**
2014											
	Fergus Falls	9	1	-	-	-	-	-	-	-	-
	Le Center	18	4	-	-	-	-	-	-	-	-
	Perley	5	1	-	-	-	-	-	-	-	-
	Oklee	-	-	6	0	-	-	-	-	-	-
	Sabin	4	4	-	-	-	-	-	-	-	-
	Strathcona	1	0	2	0	-	-	-	-	-	-
	Sub-Total	**37**	**10**	**8**	**0**	**-**	**-**	**-**	**-**	**-**	**-**
2015											
	Stephen	2	0	5	2	7	0	-	-	-	-
	St. Paul	25	13	9	3	4	2	18	1	2	2
	Perley	10	4	3	1	6*	5	-	-	-	-
	Oklee	11	7	3	1	-	-	-	-	-	-
	Crookston	-	-	19	2	1	0	-	-	5	0
	Canningham	-	-	-	-	2*	0	-	-	-	-
	Sub-Total[c]	**48**	**24**	**39**	**9**	**20**	**7**	**18**	**1**	**7**	**2**
	TOTAL[d]	**124**	**38**	**73**	**14**	**20**	**7**	**18**	**1**	**7**	**2**

[a] Total number of samples collected for a given location and year.
[b] Number of samples that tested positive for B/CYDV.
[c] Number of samples collected that tested positive for B/CYDV in a given year of the study.
[d] Number of samples collected and those that tested positive for a host, across all three years of the study.
* Denotes locations where samples collected from grower fields by disease scouts.

Table 2.5 Number of leaf samples (collected and tested positive for B/CYDV), classified by subgroup and B/CYDV strains. The samples, collected from various locations in Minnesota from 2013 to 2015, are presented by year and host. The number of mixed infections (samples positive for subgroups I and II), the number of negative samples, and the total number of samples examined in the study are also provided

Year	Host[a]	Subgroup I				Subgroup II	Mixed Infection	Negative	Total
		PAV	*MAV*	*SGV*	UnID[b]				
2013	HRSW	4	-[c]	-	-	-	-	35	39
	SB	4	-	-	-	1	-	25	30
2014	HRSW	7	-	-	-	1	2	28	38
2015	HRSW	17	-	-	4	2	1	24	48
	SB	6	-	-	1	2	-	30	39
	SO	3	-	-	1	3	-	13	20
	IWG		-	-	-	1	-	17	18
	WW*	1	-	-	-	-	-	-	1
	Other grasses	-	-	-	-	1	-	5	6
	Total	42	-	-	6	11	3	177	239

[a] Hosts are hard red spring wheat (HRSW), spring barley (SB), spring oats (SO), intermediate wheat grass (IWG) and winter wheat (WW). The one winter wheat sample included here is in the "other grasses" category elsewhere in the manuscript.

[b] UnID denotes samples that could not be identified as belonging to strain PAV MAV or SGV of Subgroup I (i.e. no PCR product was detected).

[c] "-" denotes that no sample tested positive for the corresponding B/CYDV subgroup or strain.

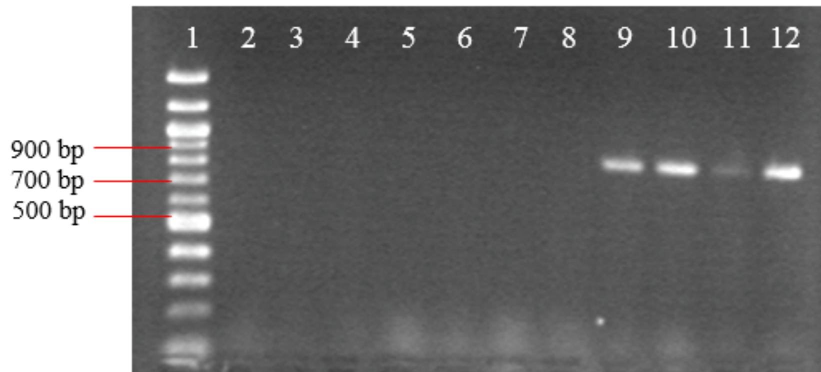

Fig. 2.1 Agarose gel of RT-PCR assay for identification of samples positive for subgroup I. Numbers 1-12 indicate the 12 lanes in the agarose gel. Lane 1: New England Bio labs 100 bp DNA ladder (2 ng), lane 2: BY175 (Sample IDs), lane 3: BY176, lane 4: BY177, lane 5: BY178, lane 6: BY179, lane 7: BY180, lane 8: BY181, lane 9: BY182, lane 10: BY183, lane 11: BY184 and lane 12: BY185. Lane 9 (BY182), lane 10 (BY183), lane 11 (BY184) and lane 12 (BY185) have the 830 bp Shu-F fragment. Full accession information for these samples is provided in Appendix Table 2.1.

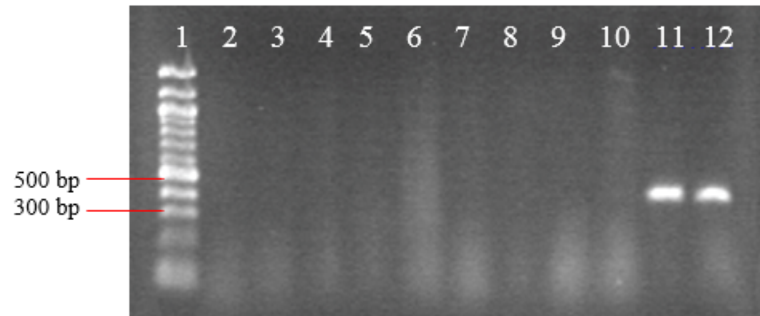

Fig. 2.2 Agarose gel of RT-PCR assay for identification of samples positive for subgroup II. Numbers 1-12 indicate lanes in the agarose gel. Lane 1: New England Bio labs 100 bp DNA ladder (2 ng), lane 2: BY197 (Sample ID), lane 3: BY198, lane 4: BY199, lane 5: BY200, lane 6: BY201, lane 7: BY202, lane 8: BY203, lane 9: BY204, lane 10: BY205, lane 11: BY206 and lane 12: BY207. Lane 11 (BY206) and lane 12 (BY207) have the 372 bp S2-F fragment. Full accession information for these samples is provided in Appendix Table 2.1.

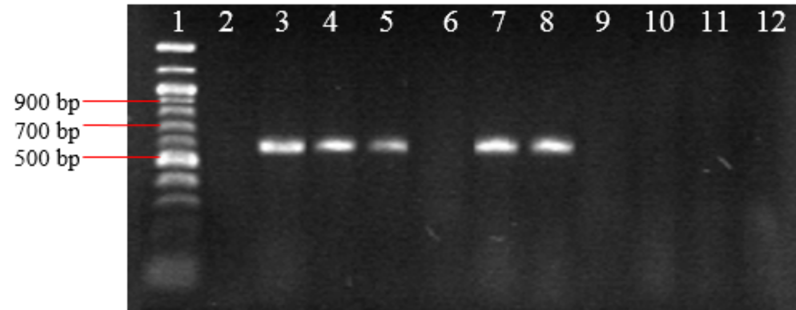

Fig. 2.3 Agarose gel of RT-PCR assay for identification of samples positive for strain PAV. Numbers 1-12 indicate lanes in the agarose gel. Lane 1: New England Bio labs 100 bp DNA ladder (2 ng), lane 2: BY191 (Sample ID), lane 3: BY192, lane 4: BY195, lane 5: BY196, lane 6: BY201, lane 7: BY202, lane 8: BY232, lane 9: BY117, lane 10: BY124, lane 11: BY157 and lane 12: BY183. Lane 3 (BY192), lane 4 (BY195), lane 5 (BY196), lane 7 (BY202) and lane 8 (BY232) have the 590 bp PAV-F fragment diagnostic for PAV. Full accession information for these samples is provided in Appendix Table 2.1.

Chapter 3

Genome-Wide Association Mapping for Net blotch Resistance in an Ethiopian and Eritrean Barley Collection

3.1 Introduction

Net blotch, caused by *Pyrenophora teres* (Drechs.), is one of the most common and economically important foliar diseases of barley (Jordan 1981; Khan 1987; Martens et al. 1988; Steffenson 1997). Net blotch causes losses both in the yield and quality of barley in North America and other barley growing regions of the world, including Australia (Khan 1987) and western Europe (Jordan 1981). Yield losses may range from 10 to 40% in areas where favorable environmental conditions, especially high humidity and cool temperatures, result in frequent net blotch epidemics (Liu et al. 2011; Ma et al. 2004). Yield losses of 20% were reported in susceptible cultivars in both Western Australia (Khan 1989) and Denmark (Jordan 1981). Net blotch infections reduce the malting and feed quality of barley by reducing kernel weight and plumpness (Grewal et al. 2008).

Two different forms of *P. teres* are recognized, and these forms result in different symptoms. In the net form of net blotch (NFNB), caused by *P. teres* f. *teres* (Smedegaard), net-like symptoms develop that are characterized by narrow, dark brown, longitudinal transverse stripes forming net-like pattern on infected leaves (Serenius et al. 2005). The symptoms of the spot form of net blotch (SFNB), caused by *P. teres* f. *maculata* (Smedegaard-Petersen), by contrast, consist of dark brown, circular to elliptical lesions surrounded by chlorotic or necrotic halo (Liu et al. 2011).

P. teres infects barley, wild relatives of barley and other related species from the genera of *Bromus, Avena* and *Triticum* (Liu et al. 2011). *P. teres* is an ascomycete fungus with the anamorph stage *Drechslera teres.* The pathogen can be seedborne, persisting as

mycelia, and it can also survive in the crop debris (Ma et al. 2004; Steffenson 1997). Infection of barley seedlings by seed borne mycelium occurs best at temperatures of 10-15 ^{o}C. Seed borne inoculum also serves to introduce *P. teres* to new fields, whereas conidia and ascospores are the most important sources of primary inoculum in fields where infections have occurred previously (Steffenson 1997). Inoculum may survive on infected stubble from one season to another, with pseudothecia developing and releasing ascospores in favorable conditions. Ascospores are either aerially or splash dispersed. Conidia, also produced on infected residues and may serve as primary inoculum, although ascospores are considered the principal inoculum. Conidia more commonly serve as a source of secondary inoculum, facilitating disease spread from mature and senescent leaves to younger tissues (Jordan 1981).

Finding host resistance to net blotch and incorporating resistance into adapted cultivars has been one of the major objectives of barley breeding programs. Multiple studies mapping sources of net blotch resistance have been conducted (Cakir et al. 2003; Friesen et al. 2006; Grewal et al. 2008; Lehmensiek et al. 2008; Ma et al. 2004; Manninen et al. 2006; Pierre et al. 2010; Steffenson et al. 1996). In these QTL mapping studies, several minor effect QTL for resistance to net blotch, expressed both at seedling and adult plant stages, have been reported. These QTL have been located on chromosomes 1H, 3H, 4H, 5H and 7H. Most of the studies have also reported major effect QTL on chromosome 6H, however, the region detected is large, spanning several centimorgans (cM). Most of these QTL studies have been done in bi-parental mapping populations, using a single source of net blotch resistance and utilizing simple sequence repeats (SSR) markers with low resolution. Recent advances in next generation

sequencing have enabled plant scientists to generate thousands of single nucleotide polymorphism (SNP) markers and develop genetic maps with much higher resolution, enabling genome wide association studies. Using genome wide association studies for QTL mapping of agronomic traits and disease resistance increases the power and statistical rigor of the genetic analysis. Association mapping is done with a population of unrelated individuals, where historical and evolutionary recombination events are represented in the population, facilitating higher resolution in mapping and the identification of more alleles at a locus by making use of the genetic diversity present in the population. This approach reduces the time and resources, since there is no need for developing specific mapping populations (Rafalski 2010; Yu and Buckler 2006; Zhu et al. 2008).

Barley originated and was domesticated in the Fertile Crescent about 8000 years ago (Badr et al. 2000; Dai et al. 2012). Ethiopia and Eritrea, which lie in the vicinity of the Fertile Crescent, are considered to be one of the centers of diversity of barley, along with the Himalayan region (Dai et al. 2012). Barley has been cultivated in Ethiopia and Eritrea since 3000 BC in the form of landraces, and it is still cultivated across a wide range of altitudes, climates and soil conditions (Lakew et al. 1997; Mamo 2013). The diversity of environments in which barley is cultivated has likely had a pronounced effect on the evolution of landraces for adaptation to a range of environmental factors. Barley landraces from east Africa are also thought to have unique genes and allelic combinations that may provide resistance to pathogens that could be utilized in modern barley breeding programs. Novel genes for resistance to diseases such as barley yellow dwarf (Delogu et al. 1995), powdery mildew (Jørgensen 1992), scald (Grønnerød et al. 2002) and loose

smut (Thomas and Metcalfe 1984) have previously been identified from Ethiopian landraces. Ethiopian lines have been reported as sources of net blotch resistance in numerous studies (Bockelman et al. 1977; Manninen et al. 2006; O'Boyle et al. 2011). The specific objectives of this study were to (1) assess phenotypic variation for net blotch resistance in a collection of Ethiopian and Eritrean barley landraces, and (2) to use an association mapping approach to identify novel loci conferring resistance to the net form of net blotch.

3.2 Materials and Methods

3.2.1 Plant Materials

A collection of barley landraces, referred to as the Ethiopian and Eritrean Barley Collection (EEBC), was used in this study (Table 3.1). The EEBC is a collection of 298 landraces of Ethiopian and Eritrean origin and various growth habits, selected to represent the genetic diversity of barley in this region of east Africa and for use in developing a diversity panel to be used in a genome-wide association mapping study (Mamo 2013). The collection includes 219 lines from the USDA-ARS National Small Grains Collection (NSGC), including 87 lines from the barley core collection. The barley core collection is a subset of the NSGC, including about 10% of the total lines in the NSGC and selected to represent the overall genetic diversity present in the NSGC. In addition to the lines from the core collection of the NSGC, the EEBC includes 63 and 17 landraces of Ethiopian/Eritrean origin 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 (Mamo 2013). The number of lines used in this study varied from 262 to 273 among experiments, with use based on seed availability.
The lines tested were of either of spring or facultative growth habit (Table 3.1). Spring types are defined as lines with a spring growth habit and have no vernalization requirement. Facultative types are defined as plants that have little or no vernalization requirement and that would flower earlier than the winter types, that require a cold period to initiate flowering. Essentially, facultative types are intermediate between the spring and winter types.

In addition to these lines, a susceptible check (Stander) and a resistant check (CIho9819), selected from the differential set used to characterize *P. teres* f. *teres* were included in each experiment.

3.2.2 *Pyrenophora teres* f. *teres* (*Ptt*) Isolate Culture, Testing and Selection

Isolates of *P. teres* f. *teres* (*Ptt*) from the University of Minnesota's Small Grains Pathology (SGP) collection were used for field and greenhouse inoculations (Table 3.2). The six isolates tested were 30107003, 30107004, 30107005, 30112001, 30112002, and 30199012. Single conidial cultures had previously been derived and the pure cultures were stored on silica gel at -20 ^{o}C.

Greenhouse experiments for isolate characterization

The characterization of the *Ptt* isolates on the differential set was done in the Plant Growth Facility West complex on the St. Paul campus of the University of Minnesota in 2013 and 2014. Isolates were characterized on a differential set (Table 3.3) consisting of 30 spring barley lines (Steffenson and Webster 1992). A differential set is a panel of lines

possessing different genes conferring resistance to a particular pathogen that can be used for characterizing pathogen isolates. The differential set for net blotch consists of lines with specific reported resistance genes, lines with uncharacterized resistances that are different from previously reported genes, and lines previously used for studying variance in virulence of *Ptt* (Steffenson and Webster 1992). The six isolates were inoculated onto seedlings of each of the lines in the differential set in a series of experiments, each experiment was set up as a randomized complete block design with three replicates. A separate experiment was conducted for each *Ptt* isolate.

Greenhouse growth conditions

Plants were grown in Conetainers (International Greenhouse Company, Danville, IL; 3.81 cm diameter, 20.92 cm height) using Metro-Mix® 300 (Sun Gro Horticulture, Quincy, MI) soilless media containing vermiculite, sphagnum peat moss, perlite, dolomitic limestone and a wetting agent. Three to five seeds of each barley line were planted per Conetainer at a depth of 1.5-2.0 cm. The Conetainers were set in Conetainer-racks that held 96 pots per rack. Plants were grown in 18-22 ^{o}C and with 18 hours of supplemental lighting provided by high-pressure sodium lamps (400 W, emitting a minimum of 300 μmol photons s^{-1} m^{-2}). The plants were fertilized one week after planting with 200 ppm concentration of a N:P:K (20:20:20) water-soluble fertilizer (Peters Professional General Purpose Fertilizer; J.R. Peters, Inc., Allentown, PA) made up to a concentration of 1.2 g/l applied at 20-25 ml per pot. The plants were watered every other day throughout the experiment.

Culture of Ptt isolates

Isolates were recovered from storage on silica gel by transferring a small number of silica gel particles to each of three to five Petri-plates containing V8 agar media, which served as starter cultures. These starter cultures were incubated under a combination of cool-white and UV light, (36 μ mol $m^{-2} s^{-1}$) with alternating 12 h light and dark periods at room temperature (20-22 ^{o}C). After five days, a 5-8 mm plug, taken from the outer circumference of the starter culture, was transferred to each of 12-15 V8 agar media plates for multiplication. The inoculum multiplication plates were incubated under the same conditions as the starter plates, except they were incubated for 10-14 days.

Inoculum preparation

Conidia were washed from the 10-14 day-old cultures by flooding the plates with 15-20 ml of sterile distilled water and rubbing the mycelial mat with a bent glass rod to release the conidia. The suspension was filtered through cheesecloth and the concentration of the resulting conidial suspension was determined using a hemocytometer and adjusted to 25,000 spores/ml by diluting the suspension with sterile distilled water. Polyoxyethylene-20-sorbitan monolaurate, ($C_{58}H_{114}O_{26}$; ICI Americas, Wilmington, DE) was added to the conidial suspension (one drop per 100 ml) as a surfactant, to reduce the surface tension of the inoculum on the leaf surface.

Inoculation

The 10-14 day old seedlings were inoculated until runoff using a 1-liter hand pump sprayer (Solo 415 Handheld Sprayer; Solo Inc., Newport News, VA). The inoculated seedlings were then placed in a dew chamber (100% relative humidity and fluorescent light at 6 μmol $m^{-2}s^{-1}$) for 24 hours. Following the infection period, the

seedlings were returned to the greenhouse bench and were grown under the previous conditions for seven days before assessment.

Assessment of net blotch

The plants were assessed for net blotch infection seven days after inoculation. The scoring was done according to Tekauz (1985) using a 1-10 numerical scale where one was the most resistant and ten was the most susceptible. The reaction class for each line was determined from the arithmetic mean of the values for the three replicates. The numerical scale ratings (infection response), taken using the Tekauz scale, were then used to classify reactions into four broader reaction classes; resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S) that corresponded to the Tekauz infection responses of 1-3, 4-5, 6-7 and 8-10, respectively.

Data summary and isolate selection

The mean infection response of each of the differential lines was calculated for each isolate tested (data not shown). The selection of the isolates to be used in future studies was based on the infection response and the origin of the isolates tested. Isolate 30107005 was the least aggressive isolate tested across all the lines in the differential set, recording low mean infection responses (Table 3.4). The mean numerical rating of isolate 30107005 on the susceptible check 'Stander' was 5.7 (with a range from 5.0 to 6.0), which was considered a moderately susceptible reaction class. Other isolates resulted in mean infection response up to 8.7 (e.g. 30112002 on 'Stander'). Because isolate 30107005 lacked virulence it was not examined further. Isolate 30107003 was more variable compared to other isolates on the differential set and was selected for additional studies. Isolates 30102002 and 30112001 produced similar response patterns on the

differential set and although isolate 30112001 appeared to be more virulent on some of the differentials than isolate 30102002, isolate 30102002 was selected for further studies because it was collected from Minnesota and could be used in field experiments. After isolates 30107005 and 30112001 were eliminated from the study, the remaining four isolates (30107003, 30107004, 30112002, and 30199012) were selected for the greenhouse screening of the EEBC lines. Isolates 30115001 and 30115002 also appeared to be good candidates for the EEBC screening, but they were received too late to be included in further experiments. Although isolates 30115001 and 30115002 were characterized on the differential set (Table 3.4), there was not enough seed of the EEBC lines to conduct a greenhouse screening for these isolates as part of this study.

3.2.3 Phenotyping the EEBC for Net Blotch Reaction in the Greenhouse

Spring and facultative barley landraces from the EEBC were used in this study. Four separate experiments were conducted using 269, 267, 264 and 262 EEBC lines with *Ptt* isolates 30107003, 30107004, 30112002 and 30199012, respectively. In addition to the EEBC lines, a susceptible check (Stander) and a resistant check (CIho9819) were included in each experiment.

Experimental lines were arranged in a randomized complete block design with three replicates. The checks were not included in the randomization but rather one Conetainer of each check line was added to each Conetainer rack, with the checks placed at an arbitrary position within the Conetainer rack. Plant growth conditions, inoculation methods and disease scoring were as described previously (See section 3.2.2).

3.2.4 Genotyping of the EEBC Landraces

The EEBC landraces were genotyped with the barley iSelect SNP chip of the expanded SNP marker platform using the Illumina Infinium II assay in 2013 at the USDA-ARS Biosciences Research Laboratory in Fargo, ND (Mamo 2013). The barley iSelect SNP chip contains 7,842 SNPs consisting of 2,832 SNPs from the barley oligonucleotide pooled assays (BOPA1 and BOPA2) and an additional 5,010 SNPs developed by Comadran et al. (2012) using next generation sequencing data. The BOPA1 and BOPA2 SNPs were developed by Close et al. (2009) and mapped by Muñoz-Amatriaín et al. (2011). The SNP data and consensus map used for this study was downloaded from The Triticeae Toolbox (https://triticeaetoolbox.org) public repository.

3.2.5 Genome-Wide Association Mapping

Genome-wide association mapping was conducted using marker dataset (Barley iSelect SNP chip) and individual phenotypic datasets from the four experiments conducted with *Ptt* isolates 30107003, 30107004, 30112002 and 30199012 and on a dataset formed by combining the datasets from each of the individual isolates. The three different marker datasets used were the full marker dataset (5,269 markers), the mapped marker dataset (3,818 markers) and markers with unique map positions (1,473 markers). The mapped marker dataset had markers with known map positions in the consensus map (Muñoz-Amatriaín et al. 2011), and in the marker dataset with unique map positions, markers identified as having duplicate map positions were removed.

Mixed linear model (MLM) with the ‘efficient mixed model association’ (EMMA) method (Kang et al. 2008) was used for conducting association mapping. The

analysis was conducted in package rrBLUP version 4.4 (Endelman 2011) in software R (version 3.2.3) using the GWAS function. The rrBLUP package uses the mixed model $y = X\beta + u + e$, where y is a vector of phenotypic values; X is a vector of SNP marker genotypes; β is the coefficient of the SNP marker being estimated; u is a vector of polygenic background of lines (effect of individual relatedness estimated as a pairwise kinship coefficients) and e is a vector of residual effects (Yu et al. 2005). In the equation, $X\beta$ represents fixed effect and e represents random effects. For the combined analysis using phenotypes from all four experiments, the environment mean was modeled using the fixed option in the GWAS function. A separate column 'Env' was passed on along with the phenotypic values, which consisted of a fixed variable for each environment or dataset, for example '2' for phenotypic values from the 30112002 dataset and '3' for phenotypic values from the 30107003 dataset. All the analyses were done in a P + K model taking the first two principal components as co-variates. The model then incorporated environmental effects as a fixed effect in the kinship matrix. The marker score –log(p) values were generated after the EMMA method fitted each marker individually in the MLM. The marker scores -log(p) were plotted against marker positions in the map to generate a Manhattan plot. The significance threshold for marker trait association is calculated by calculating a q-value using the qvalue package (Storey and Tibshirani 2003). The p-value, corresponding to a q-value of 0.05, was determined by interpolation. A false discovery rate (FDR) of 0.05 was used as a threshold for significance in all of the analyses as implemented in rrBLUP package (Endelman 2011).

3.3 Results

3.3.1 Phenotypic Variation for Net Blotch in the EEBC Germplasm

The EEBC germplasm, tested against four isolates of *Ptt* (30107003, 30107004 30112002, and 30199012), displayed a wide spectrum of responses from susceptible to resistant (Fig. 3.1). Of the disease infection response scores taken across all four experiments, 2.6% of the tested lines were categorized into the resistant reaction class, while 29.5% were in the moderately resistant reaction class, 62.2% were in the moderately susceptible reaction class and 5.7% were in the susceptible reaction class. Only one line, EEBC 123, was resistant against all four *Ptt* isolates it was tested against. Line EEBC 170 was resistant against two isolates (30107003 and 30199012) and moderately resistant to a third (30112002). Line EEBC 219 was resistant to isolates 30199012 and 30112002. The remaining lines that exhibited a resistant response were only resistant to one of the four isolates they were tested against.

Across all experiments, the mean disease infection response for the EEBC lines was 6.0, the median was 6.0 and the range was from 2.3 to 8.7 (Fig. 3.2). The mean infection response for the resistant check, Clho9819, was 3.1, the median infection response was 3.0 and the range was from 2.0 to 4.0. The mean infection response for the susceptible check, Stander, was 8.1, while the median infection response was 8.0 and the range was from 6.0 to 9.0.

3.3.2 Marker Data

The marker data used for the association mapping study was downloaded from The Triticeae Toolbox (T3). The SNP marker data had been generated by genotyping the

lines with iSelect SNP chip of the expanded barley SNP marker platform using the Illumina Infinium assay (Mamo, 2013). The barley iSelect SNP chip contains 7,842 markers including 2,832 markers from the barley oligonucleotide pooled assays (BOPA1 and BOPA2) (Close et al. 2009; Muñoz-Amatriaín et al. 2011) and 5,010 new SNPs developed from next generation sequencing data (Comadran et al. 2012). The consensus map prepared by Muñoz-Amatriaín et al. (2011) was used for the analysis. Quality control during genotype calling removed 1,140 markers, leaving 6,702 (85.5%) remaining markers. Additional filtering based on various metrics, including a call frequency of >95% and a minor allele-frequency of >5%, removed an additional 1,433 markers. Of the final group of 5,269 markers, 3,818 (72.5%) had known marker positions spanning 1,112.71 cM of the genome and only 1,473 of the 3,818 markers had unique map positions. The average marker density on the map was 3.43 SNPs per cM, when using all mapped markers, whereas when using the markers with unique map positions, the marker density was only 1.32 SNP per cM. Eight gaps greater than 5 cM were identified in the map; two each on chromosomes 1H and 7H and one each on chromosomes 2H, 3H, 5H and 6H (Mamo, 2013).

3.3.3 Population Structure

Principal component analysis (PCA) is a multivariate analysis that transforms and compresses the dimensionality of data to make patterns evident, thus revealing both similarities and differences among the observations within the data. PCA transforms the set of observations of putatively correlated variables, to a set of values of linearly uncorrelated variables called principal components (PCs). In this analysis, the first PC

had the largest possible variance and each succeeding PC has the highest possible variance provided they are orthogonal to the first PC.

PCA is widely used to infer the population structure in populations using genotypic information. The population structure of the EEBC population was inferred by conducting PCA in rrBLUP. The requirements of the data set used in a PCA are that data need to be variable and not have missing values. In order to undertake PCA on this data set, missing marker values were imputed using the population mean of the markers. The results of the PCA indicated that the first two PCs (clusters or subgroups based on genotypes) explained about 24% and 6% of variance in the population, respectively (Fig. 3.3). These two PCs were considered to have contributed to the population structure of the population based on the marker data and were then incorporated in the P+K model of EMMA to account for population structure (Fig. 3.4).

3.3.4 Genome-Wide Association Mapping for Net Blotch Resistance in EEBC

Genome-wide association mapping for resistance to isolate 30107003

In the association mapping for resistance to isolate 30107003 using the complete set of markers, no significant associations were found (Fig. 3.5). Marker 11_10405, located on chromosome 5H at 171.16 cM, with a -log(p) value of 3.15, had the highest value of all the markers included in the study (Fig. 3.5). When the analyses were done using the set of mapped markers and the set of markers with unique map positions, no significant marker-trait associations were evident although Marker 11_10405 had the

highest -log(p) values in the analyses using both the mapped markers (Fig. 3.6) and markers with unique map positions (Fig. 3.7).

Genome-wide association mapping for resistance to isolate 30107004

No significant marker-trait associations were detected in the three analyses, conducted using the complete set of markers, the set of mapped markers or the set of markers with unique map positions, for resistance to *Ptt* isolate 30107004. Marker 11_10405, on chromosome 5H at 171.16 cM, had the highest -log(p) values, recording values of 4.88, 4.67 and 4.27 for the complete marker set analysis (Fig. 3.8), mapped marker analysis (Fig. 3.9) and the analysis using the markers having unique map positions (Fig. 3.10), respectively.

Genome-wide association mapping for resistance to isolate 30112002

Three different association mapping analyses were done for resistance to isolate 30112002 using the complete set of markers, the set of mapped markers and markers having unique map positions, respectively. When the analysis was done using the complete marker set of 5,269 markers, no significant associations were observed. In this analysis, marker SCRI_RS_221843, located on chromosome 2H at 62.90 cM with a -log(p) value of 3.45, had the lowest p-value (Fig. 3.11). That no markers surpassed the significance threshold may have been the result of corrections made for multiple testing since a large number of markers were being tested. To see if this might be the case, two other analyses were done using those markers that were mapped (3,818) and those markers having unique map positions (1,473). In the analysis using the mapped markers,

no significant associations were observed and the marker with the highest –log(p) value in the analysis was SCRI_RS_199178, located on chromosome 1H at 45.23 cM with a -log(p) value of 3.27. In this analysis, marker SCRI_RS_221843, which had the lowest p-value in the analysis utilizing the complete marker set, had the second largest p-value with a -log(p) value of 3.26 (Fig. 3.12). When the analysis was done using only those markers with unique map positions, the same two markers (SCRI_RS_199178 and SCRI_RS_221843) had the highest -log(p) values, although neither surpassed the significance threshold (Fig. 3.13).

Genome-wide association mapping for resistance to isolate 30199012

No significant marker-trait associations were detected when the full marker set was used for the analysis or when the set of markers representing unique map positions was used. However, when the set of mapped markers was used for the analysis, one significant SNP marker, SCRI_RS_142541, located at 59.00 cM in chromosome 6H with a -log(p) value of 4.94 (Fig. 3.14), was detected. Marker SCRI_RS_142541 also had the highest –log(p) values in the other two analyses. Using the complete marker set, marker SCRI_RS_142541 had a -log(p) value of 4.76 (Fig. 3.15) and for the marker set with unique map positions the -log(p) value of this marker was 4.25 (Fig. 3.16).

Genome-wide association mapping for resistance to net blotch in the combined analysis

To combine the four experiments and conduct association mapping, the environment was modeled in the mixed linear model as a fixed effect. The same approach for the combined analysis was implemented for markers using the three datasets, where

the complete set of markers, the set of mapped markers and the set of markers having unique map positions were used in separate analyses. In the analysis using the complete set of markers, a significant marker-trait association was observed for marker SCRI_RS_194337, located in chromosome 5H at 176.52 cM, with the marker having a -log(p) value of 5.07 (Fig. 3.17). This same marker was also significant when the set of mapped markers were used to conduct the analysis (-log(p) value of 4.91; Fig. 3.18) and when the set of markers having unique map positions were used (-log(p) value of 4.24; Fig. 3.19). Marker SCRI_RS_221843, which had the highest -log(p) value in the analysis of the data set from isolate 30102002, had the second highest -log(p) values in the complete marker set analysis and in the mapped marker set analysis for the combined data. Marker SCRI_RS_142541, which was significant in the dataset for isolate 30199012, and marker 11_1045, which had the highest -log(p) values in the datasets for isolates 30107003 and 30107004, were among the top five markers in the combined analysis based on -log(p) values, although neither was significant. The linkage disequilibrium (LD) between markers SCRI_RS_194337 and 11_1045 in chromosome 5H was calculated to be $R^2 = 0.035$ which indicates that they reside in separate haplotypes.

Markers in the 0.1 percentile of p-value distribution

The Bonferroni method and the FDR method for calling the significance thresholds for markers are considered to be stringent tests of marker significance in association mapping studies. A less stringent method to establish the significance of markers has been used in previous studies (Chan et al. 2010; Pasam et al. 2012) and is

based on calling markers, having p-values in the lowest 0.1 percentile of the p-value distribution, significant. The markers in the lowest 0.1 percentile of p-values that were common to each of the three datasets (complete set of markers, mapped markers and markers with unique map positions) are presented in Table 3.5. There were a few common markers across datasets significant at the 0.1 percentile threshold from chromosome 2H and chromosome 5H. Marker SCRI_RS_10670 from chromosome 2H at 92.8 cM was common across three datasets (30107003, 30199012 and combined). SCRI_RS_8366 and SCRI_RS_8366 at 62.9 cM of chromosome 2H were significant for datasets of 30199012 and all isolates combined. Similarly, marker SCRI_RS_194337, at 176.5 cM of chromosome 5H, was common across two datasets (30102002 and combined) is was significant at the FDR threshold of 0.05. In the datasets for isolates 30107003 and 30107004 marker 11_10405, located at 171.2 cM of chromosome 5H, was significant at the 0.1 percentile threshold.

3.4 Discussion

Between 262 and 268 lines from the EEBC were screened for resistance to each of four isolates of *P. teres* f. *teres* included in this study. The proportion of resistant lines in the EEBC lines screened to each isolate ranged from 0.4% to 5.3%. The majority of the lines were moderately susceptible (44.7% - 73.7%), lines that were moderately resistant were the next largest category (12% - 47.7%), while the proportion of lines categorized as susceptible (2.2% - 13.9%) was low, but still generally larger than the proportion of plants that were resistant. Often in studies screening a large number of landraces and wild germplasm only a small proportion of lines are identified as resistant

to a given disease (Fetch et al. 2008; Mamo 2013). In a study examining the spot blotch and net blotch resistance of more than 5,000 barley lines from the NSGC, Fetch et al. (2008) reported that 3.4% of the lines tested were resistant to net blotch. Mamo (2013) conducted studies screening for resistance to stem rust and spot blotch using the same EEBC germplasm used in this study. In this work, Mamo (2013) reported that 5.0% of the lines were resistant to moderately resistant against *Puccinia graminis* f. sp. *tritici* race TTKSK, whereas only 0.7% lines were found to be resistant against race MCCFC. When the same population was screened for response to *Cochliobolus sativus* (spot blotch), only 1.3% of the lines exhibited resistance.

The reaction of EEBC landraces to *P. teres* f. *teres* were statistically different for the four isolates tested. The isolates used in this study are generally representative of the virulence of isolates available in a collection of isolates obtained from the northern Great Plains, with the four isolates selected following characterization on a differential set. A few lines in the EEBC demonstrated effective resistance to more than one isolate. For example, line EEBC 123 was resistant to all four isolates tested and isolates EEBC 170 and EEBC 219 which were each resistant to two isolates. The lines identified as resistant to one or more *Ptt* isolates need to be examined further to understand the resistance and determine if they are suitable candidates to serve as sources of resistance to *Ptt* in US barley breeding programs.

Association mapping was conducted using a mixed linear model with EMMA (Kang et al. 2008) on the complete set of markers. The analysis did not yield any significant associations for any of the four data sets.

Adjustment of the p-value is done in GWAS using various methods such as Bonferroni and FDR to control the Type-I error, and is often called ‘correction for multiple testing’. One of the disadvantages of p-value adjustment, in large scale GWAS studies involving many markers, is that the threshold for significance level is reduced to such a degree that it increases the probability of having type-II errors, or false negatives. Since, p-value adjustment is dependent on the number of tests being carried out, or the number of markers being tested, analyses using a reduced number of markers was done to see if the original results obtained were influenced by the correction for multiple testing. Two additional marker sets, a set of markers with known map positions and another set of markers with unique map positions, were used for this purpose. Despite reducing the number of markers in the data set, the analyses of the data did not yield any additional significant associations for the three datasets (datasets for isolates 30107003, 30107004 and 30112002). In the two analyses using the smaller number of markers for isolate 30199012, a significant SNP was observed on chromosome 6H. Specifically, marker SCRI_RS_142541 at 59.10 cM of chromosome 6H was significant when only the mapped markers were used; however, in the analysis with the smallest set of markers, those with unique map positions, this SNP was not significant at a FDR of 0.05.

Multiple testing correction was not apparent for analyses conducted on datasets for isolates 30107003, 30107004 and 30112002 since decreasing the number of markers (i.e. the number of tests) did not yield any significant markers. The -log(p) value, or probability for individual markers, decreased with a reduction of the number of markers included in a test in most of the cases (Table 3.5). The dataset for isolate 30199012 was an exception, where the -log(p) values increased when the set of mapped markers was

used, and marker SCRI_RS_14254 surpassed the significance threshold. When the number of tests was further reduced in the analysis, as was done using the marker set including only unique map positions, marker SCRI_RS_14254 was no longer significant. A penalty for multiple testing appears to be evident in this case, although too few tests may reduce the statistical power, limiting the identification of significant markers.

In the analysis using the combined phenotypic data from all four experiments, a significant marker SCRI_RS_194337 was observed in chromosome 5H at 176.52 cM. This marker was not significant in any other analyses. Marker SCRI_RS_194337 was in the lowest 0.1 percentile rank of p-values in the dataset for isolate 30112002. Marker 11_10405, from a region near to SCRI_RS_194337 on chromosome 5H (at 171.16 cM), was in the lowest 0.1 percentile across all three analyses for the datasets for isolates 30107003 and 30107004. The LD calculated between SCRI_RS_194337 and 11_10405 was, however, not significant. This result is in agreement with previous studies, where QTLs, and genes on chromosome 5H, have been identified as providing resistance to net blotch. Bockelman et al. (1977) reported that the resistance gene *Rpt2c* in line CI 7584 was on chromosome 5H. Similarly, Grewal et al. (2008) reported a minor effect QTL (5H-*QRptts5*) on chromosome 5H that explained 7% of the variation for resistance at the seedling stage in a double haploid population derived from the net blotch susceptible cultivar CDC Dolly and the net blotch resistant line TR251. A minor effect QTL was also detected by Lehmensiek et al. (2008), in several Australian double haploid bi-parental populations, that conferred resistance to the net form of net blotch. The location of this QTL was reported to be at 109.00 cM of 5H in the map developed using DArT and SSR markers where the closest marker to the QTL was bPb-8462. In other studies, Manninen

et al. (2006) reported a minor effect QTL in chromosome 5H identified using a double haploid population derived from Rolfi x CI 9819, where CI 9819 was the resistant parent. Interestingly, line CI 9819 originated from Ethiopia and was included as a resistant check in the Manninen et al. (2006) study. Line EEBC 123, which was resistant to all four isolates tested in our experiments, performed at least as well as the resistant check CI 9819. Unfortunately, the results of our study cannot be directly compared with their results as there are no common markers to facilitate a comparison. Considering however that all of the previous studies were of bi-parental populations, while this study involved an association mapping study utilizing a diverse panel of germplasm, it is probable that the QTL detected in this study are novel, although there is no conclusive evidence to demonstrate this.

Multiple studies (Cakir et al. 2003; Friesen et al. 2006; Grewal et al. 2008; Ma et al. 2004; Manninen et al. 2006; Steffenson et al. 1996) have reported a major effect QTL on chromosome 6H for seedling resistance to NFNB using various resistance sources in bi-parental populations. Some of the studies (Ma et al. 2004; Manninen et al. 2006; Steffenson et al. 1996) have identified the same region on the long arm of chromosome 6H with common markers conferring resistance to NFNB. Similarly, Cakir et al. (2003) and Friesen et al. (2006) identified a region on chromosome 6H close to the SSR marker Bmag0173 that conferred net blotch resistance. Steffenson et al. (1996) also reported a minor QTL on the short arm of chromosome 6H. In the study presented here, a significant marker trait association was also detected on chromosome 6H at 59.01 cM (marker SCRI_RS_142541; *Ptt* isolate 30199012), which is consistent with the results of other studies that report a major QTL for reaction to net blotch in long arm of 6H.

Making inferences from the wealth of mapping studies reported previously, it is apparent that these studies, cumulatively, are identifying QTLs in a large section of chromosome 6H spanning from the short arm to the long arm. This suggests that this chromosome contains multiple resistance genes, perhaps with several allelic forms, conferring net blotch resistance. It seems likely that the marker identified in this study is detecting a locus in the same large region identified in previous studies. The lack of a common genetic map and common markers, means that the results of this study cannot be directly compared with previous studies. As SNP markers provide higher resolution, it is reasonable to assume that the location of QTL identified in our study are more precise than those reported previously. Fine mapping of the region identified by these SNP markers would be necessary to derive additional information about the genes or QTLs in this region of the barley genome that are conferring net blotch resistance.

Table 3.1 Line information of Ethiopian and Eritrean barley landraces* used in the study

Number	Genotype[a]	Identifier[b]	Plant ID[c]	Row Type	Growth Habit[d]	Origin[e]	Source[f]
1	EEBC 001	CIho 9603	8738	6	S	ETH	NSGC
2	EEBC 002	CIho 9655	8894	6	S	ETH	NSGC
3	EEBC 003	CIho 9801	9743	6	S	ETH	NSGC
4	EEBC 004	CIho 9822	9842	2	S	ETH	NSGC
5	EEBC 005	CIho 9941	10034	6	W	ETH	NSGC
6	EEBC 006	CIho 11727	S-5	2	S	ETH	NSGC
7	EEBC 007	CIho 11731	S-9	2	S	ERI	NSGC
8	EEBC 008	CIho 12179	H-2187	2	S	ERI	NSGC
9	EEBC 009	CIho 12572	ELS6302-70	6	S	ETH	NSGC
10	EEBC 010	CIho 12917	ELS6402-375	6	F	ETH	NSGC
11	EEBC 011	CIho 12921	ELS6402-379	6	S	ETH	NSGC
12	EEBC 012	CIho 12948	ELS6402-414	6	S	ETH	NSGC
13	EEBC 013	CIho 13063	H-2163	2	S	ERI	NSGC
14	EEBC 014	CIho 13094	ELS6402-277	6	S	ETH	NSGC
15	EEBC 015	CIho 13745	CI13745	6	S	ERI	NSGC
16	EEBC 016	CIho 13759	R56	2	S	ETH	NSGC
17	EEBC 017	CIho 13761	CI13761	6	S	ETH	NSGC
18	EEBC 018	CIho 14976	YAHA	2	S	ERI	NSGC
19	EEBC 019	CIho 14977	ELS6402-564	2	S	ERI	NSGC
20	EEBC 020	PI 347245	PI347245	2	S	ETH	NSGC
21	EEBC 021	PI 382191	GAW28-4	2	S	ETH	NSGC
22	EEBC 022	PI 382251	GAW60-6	2	S	ETH	NSGC
23	EEBC 023	PI 382269	GAW65-3	2	S	ETH	NSGC
24	EEBC 024	PI 382313	GAW79-3	2	S	ETH	NSGC
25	EEBC 025	PI 382325	GAW81-6	2	S	ETH	NSGC
26	EEBC 026	PI 382379	GAW106-6	2	S	ETH	NSGC
27	EEBC 027	PI 382448	GAW142-5	2	S	ETH	NSGC
28	EEBC 028	PI 382695	GAW11-2	6	F	ETH	NSGC

Table 3.1 continued

Number	Genotype	Identifier	Plant ID	Row Type	Growth Habit	Origin	Source
29	EEBC 029	PI 382743	GAW32-4	6	S	ETH	NSGC
30	EEBC 030	PI 382865	GAW48-17	6	S	ETH	NSGC
31	EEBC 031	PI 383041	GAW110-1	6	S	ETH	NSGC
32	EEBC 032	PI 383089	GAW122-1	6	S	ETH	NSGC
33	EEBC 033	PI 383108	GAW126-3	6	S	ETH	NSGC
34	EEBC 034	PI 383150	GAW144-5	6	S	ETH	NSGC
35	EEBC 035	PI 386392	PI386392	6	S	ETH	NSGC
36	EEBC 036	PI 386393	IAR/B/141	2	S	ETH	NSGC
37	EEBC 037	PI 386458	IAR/B/411-2	6	S	ETH	NSGC
38	EEBC 038	PI 386505	IAR/B/513	2	S	ETH	NSGC
39	EEBC 039	PI 386655	IAR/B/246-2	6	S	ETH	NSGC
40	EEBC 040	PI 386704	IAR/B/337-1	6	S	ETH	NSGC
41	EEBC 041	PI 386855	IAR/B/76	6	S	ETH	NSGC
42	EEBC 042	PI 386881	IAR/B/110	6	S	ETH	NSGC
43	EEBC 043	PI 386920	IAR/B/159	6	S	ETH	NSGC
44	EEBC 044	PI 386997	IAR/B/216-1	6	F	ETH	NSGC
45	EEBC 045	PI 387016	IAR/B/244-2	6	S	ETH	NSGC
46	EEBC 046	PI 387033	IAR/B/280-1	6	S	ETH	NSGC
47	EEBC 047	PI 387049	IAR/B/292-1	6	S	ETH	NSGC
48	EEBC 048	PI 387195	IAR/B/450	6	S	ETH	NSGC
49	EEBC 049	PI 387201	IAR/B/467-2	6	S	ETH	NSGC
50	EEBC 050	PI 387231	IAR/B/514-2	6	S	ETH	NSGC
51	EEBC 051	CIho 1604	HILLSA	2	S	ETH	NSGC
52	EEBC 052	CIho 2226	STEUDELLI	2	S	ETH	NSGC
53	EEBC 053	CIho 4221	494B	6	S	ETH	NSGC
54	EEBC 054	CIho 11709	CI11709	2	S	ETH	NSGC
55	EEBC 055	CIho 11788	HARAR	2	S	ETH	NSGC
56	EEBC 056	CIho 11812	ABYSSINIAN16	6	S	ETH	NSGC

Table 3.1 continued

Number	Genotype	Identifier	Plant ID	Row Type	Growth Habit	Origin	Source
57	EEBC 057	CIho 11813	ABYSSINIAN1118	6	S	ETH	NSGC
58	EEBC 058	CIho 11849	ALEMAYA_MAGO	6	S	ETH	NSGC
59	EEBC 059	CIho 13141	CI13141	2	S	ETH	NSGC
60	EEBC 060	CIho 13353	CI13353	2	S	ETH	NSGC
61	EEBC 061	CIho 13355	CI13355	6	S	ETH	NSGC
62	EEBC 062	CIho 13398	2095	6	S	ETH	NSGC
63	EEBC 063	CIho 13737	CI13737	6	S	ETH	NSGC
64	EEBC 064	CIho 13740	CI13740	6	S	ERI	NSGC
65	EEBC 065	CIho 13741	CI13741	6	F	ERI	NSGC
66	EEBC 066	CIho 13742	CI13742	6	F	ERI	NSGC
67	EEBC 067	CIho 13743	CI13743	6	F	ERI	NSGC
68	EEBC 068	CIho 13746	CI13746	6	F	ERI	NSGC
69	EEBC 069	CIho 13753	CI13753	6	S	ETH	NSGC
70	EEBC 070	CIho 13767	CI13767	6	S	ETH	NSGC
71	EEBC 071	CIho 13776	CI13776	6	S	ETH	NSGC
72	EEBC 072	CIho 13777	CI13777	6	S	ETH	NSGC
73	EEBC 073	CIho 14092	CIHO14092	6	S	ETH	NSGC
74	EEBC 074	CIho 14124	CIHO14124	6	S	ETH	NSGC
75	EEBC 075	CIho 14880	ELS6402-467|CIHO14880	6	S	ERI	NSGC
76	EEBC 076	CIho 14881	ELS6402-467|CIHO14881	6	S	ERI	NSGC
77	EEBC 077	CIho 14882	ELS6402-468	6	S	ERI	NSGC
78	EEBC 078	CIho 14883	ELS6402-469	6	S	ERI	NSGC
79	EEBC 079	CIho 14886	ELS6402-472	6	S	ETH	NSGC
80	EEBC 080	CIho 14926	ELS6402-513	6	S	ETH	NSGC
81	EEBC 081	CIho 14975	ELS6402-562	2	S	ETH	NSGC
82	EEBC 082	CIho 14978	ELS6402-565	2	S	ETH	NSGC
83	EEBC 083	CIho 14986	ELS6402-573	6	S	ETH	NSGC
84	EEBC 084	PI 60694	KOBER	2	F	ETH	NSGC

Table 3.1 continued

Number	Genotype	Identifier	Plant ID	Row Type	Growth Habit	Origin	Source
85	EEBC 085	PI 130582	PI130582	6	S	ETH	NSGC
86	EEBC 086	PI 151787	ABYSSINIAN12	2	S	ETH	NSGC
87	EEBC 087	PI 151795	ABYSSINIAN21	6	S	ETH	NSGC
88	EEBC 088	PI 186424	DEFICIENS_DABYSSINIA	6	S	ETH	NSGC
89	EEBC 089	PI 193531	8701	6	S	ETH	NSGC
90	EEBC 090	PI 193799	8853	6	S	ETH	NSGC
91	EEBC 091	PI 193980	8807	6	S	ETH	NSGC
92	EEBC 092	PI 194951	9243	6	S	ETH	NSGC
93	EEBC 093	PI 194952	9244	6	S	ETH	NSGC
94	EEBC 094	PI 194961	9253	6	S	ETH	NSGC
95	EEBC 095	PI 195959	9646	6	S	ETH	NSGC
96	EEBC 096	PI 197216	9946	6	S	ETH	NSGC
97	EEBC 097	PI 273899	1863	6	S	ETH	NSGC
98	EEBC 098	PI 273905	1997	6	S	ETH	NSGC
99	EEBC 099	PI 277393	S-18	6	S	ETH	NSGC
100	EEBC 100	PI 283441	C.P.I.15700	2	S	ETH	NSGC
101	EEBC 101	PI 285118	S-36	2	S	ERI	NSGC
102	EEBC 102	PI 285120	S-38	2	S	ETH	NSGC
103	EEBC 103	PI 285124	S-42	6	S	ETH	NSGC
104	EEBC 104	PI 286388	CI12089	2	S	ERI	NSGC
105	EEBC 105	PI 286389	CI12090	2	S	ERI	NSGC
106	EEBC 106	PI 286398	PI286398	2	S	ETH	NSGC
107	EEBC 107	PI 290346	ABESSINISCHE6ZLG	6	S	ETH	NSGC
108	EEBC 108	PI 295373	ELS6302-5	6	S	ETH	NSGC
109	EEBC 109	PI 295442	ELS6302-74	2	S	ETH	NSGC
110	EEBC 110	PI 295581	ELS6302-213	6	S	ETH	NSGC
111	EEBC 111	PI 296453	H-2170	2	S	ERI	NSGC
112	EEBC 112	PI 296455	H-2164	2	S	ETH	NSGC

Table 3.1 continued

Number	Genotype	Identifier	Plant ID	Row Type	Growth Habit	Origin	Source
113	EEBC 113	PI 296457	H-2180	6	F	ETH	NSGC
114	EEBC 114	PI 296459	H-2213	6	S	ERI	NSGC
115	EEBC 115	PI 296460	H-2227	6	S	ERI	NSGC
116	EEBC 116	PI 296467	H-2171	2	S	ETH	NSGC
117	EEBC 117	PI 296472	H-2183	2	S	ETH	NSGC
118	EEBC 118	PI 296479	H-2162	2	S	ETH	NSGC
119	EEBC 119	PI 296488	H-2190	2	S	ETH	NSGC
120	EEBC 120	PI 296499	H-2185	2	S	ETH	NSGC
121	EEBC 121	PI 296511	H-2215	6	S	ETH	NSGC
122	EEBC 122	PI 296517	H-2222	6	S	ETH	NSGC
123	EEBC 123	PI 296533	H-2204	6	S	ETH	NSGC
124	EEBC 124	PI 298309	ELS6402-263	6	S	ETH	NSGC
125	EEBC 125	PI 298310	ELS6302-220	2	S	ETH	NSGC
126	EEBC 126	PI 298324	ELS6402-261	6	S	ETH	NSGC
127	EEBC 127	PI 298325	ELS6402-262	6	S	ETH	NSGC
128	EEBC 128	PI 298326	ELS6402-264	6	S	ETH	NSGC
129	EEBC 129	PI 298327	ELS6402-265	6	S	ETH	NSGC
130	EEBC 130	PI 298328	ELS6402-266	6	S	ETH	NSGC
131	EEBC 131	PI 298329	ELS6402-267	6	S	ETH	NSGC
132	EEBC 132	PI 298330	ELS6402-268	6	S	ETH	NSGC
133	EEBC 133	PI 298340	ELS6302-225	6	S	ETH	NSGC
134	EEBC 134	PI 298656	ELS6402-269	2	S	ETH	NSGC
135	EEBC 135	PI 298679	ELS6402-357	2	S	ETH	NSGC
136	EEBC 136	PI 298688	ELS6402-384	2	S	ETH	NSGC
137	EEBC 137	PI 298690	ELS6402-374	2	S	ETH	NSGC
138	EEBC 138	PI 298691	ELS6402-385	2	S	ETH	NSGC
139	EEBC 139	PI 298708	ELS6402-317	2	S	ETH	NSGC
140	EEBC 140	PI 298724	ELS6402-353	6	S	ETH	NSGC

Table 3.1 continued

Number	Genotype	Identifier	Plant ID	Row Type	Growth Habit	Origin	Source
141	EEBC 141	PI 298738	ELS6402-282	6	S	ETH	NSGC
142	EEBC 142	PI 298766	ELS6402-376D	6	S	ETH	NSGC
143	EEBC 143	PI 298770	ELS6402-380	6	F	ETH	NSGC
144	EEBC 144	PI 316801	HOR2541	2	S	ETH	NSGC
145	EEBC 145	PI 316835	HOR2720	2	S	ETH	NSGC
146	EEBC 146	PI 316874	S7244	6	S	ETH	NSGC
147	EEBC 147	PI 328976	S139	6	S	ETH	NSGC
148	EEBC 148	PI 329000	S3192	6	S	ETH	NSGC
149	EEBC 149	PI 331217	290B	2	S	ERI	NSGC
150	EEBC 150	PI 331237	P65	2	S	ETH	NSGC
151	EEBC 151	PI 356264	E81/2	6	S	ETH	NSGC
152	EEBC 152	PI 356333	E1/183	6	S	ETH	NSGC
153	EEBC 153	PI 356505	E435/5	6	S	ETH	NSGC
154	EEBC 154	PI 356580	E508/3	6	F	ETH	NSGC
155	EEBC 155	PI 356666	E586/6	6	F	ETH	NSGC
156	EEBC 156	PI 358287	PI358287	6	S	ETH	NSGC
157	EEBC 157	PI 358599	22852	2	S	ETH	NSGC
158	EEBC 158	PI 382182	GAW3-2	2	S	ETH	NSGC
159	EEBC 159	PI 382184	GAW17-2	2	S	ETH	NSGC
160	EEBC 160	PI 382223	GAW50-12	2	S	ETH	NSGC
161	EEBC 161	PI 382226	GAW51-2	2	S	ETH	NSGC
162	EEBC 162	PI 382275	GAW66-3	2	S	ETH	NSGC
163	EEBC 163	PI 382296	GAW76-3	2	S	ETH	NSGC
164	EEBC 164	PI 382343	GAW86-2	2	S	ETH	NSGC
165	EEBC 165	PI 382373	GAW104-4	2	S	ETH	NSGC
166	EEBC 166	PI 382434	GAW137-3	2	S	ETH	NSGC
167	EEBC 167	PI 382437	GAW138-3	2	S	ETH	NSGC
168	EEBC 168	PI 382506	GAW71-16	2	F	ETH	NSGC

Table 3.1 continued

Number	Genotype	Identifier	Plant ID	Row Type	Growth Habit	Origin	Source
169	EEBC 169	PI 382510	GAW81-5	6	S	ETH	NSGC
170	EEBC 170	PI 382630	GAW153-5	2	S	ETH	NSGC
171	EEBC 171	PI 382642	GAW27-3	2	F	ETH	NSGC
172	EEBC 172	PI 382725	GAW27-1	6	F	ETH	NSGC
173	EEBC 173	PI 382798	GAW42-1	6	W	ETH	NSGC
174	EEBC 174	PI 382839	GAW47-1	2	F	ETH	NSGC
175	EEBC 175	PI 382860	GAW48-12	6	S	ETH	NSGC
176	EEBC 176	PI 382911	GAW72-8	6	S	ETH	NSGC
177	EEBC 177	PI 382952	GAW84-5	6	S	ETH	NSGC
178	EEBC 178	PI 382982	GAW87-1	6	S	ETH	NSGC
179	EEBC 179	PI 383031	GAW99-4	6	S	ETH	NSGC
180	EEBC 180	PI 383101	GAW125-1	6	S	ETH	NSGC
181	EEBC 181	PI 383136	GAW141-2	2	F	ETH	NSGC
182	EEBC 182	PI 386385	IAR/B/133	6	F	ETH	NSGC
183	EEBC 183	PI 386398	IAR/B/214-2	6	F	ETH	NSGC
184	EEBC 184	PI 386406	IAR/B/252	2	S	ERI	NSGC
185	EEBC 185	PI 386407	IAR/B/253	2	S	ERI	NSGC
186	EEBC 186	PI 386412	IAR/B/266	2	S	ETH	NSGC
187	EEBC 187	PI 386415	IAR/B/272	2	S	ETH	NSGC
188	EEBC 188	PI 386416	IAR/B/273	2	S	ETH	NSGC
189	EEBC 189	PI 386462	IAR/B/434	2	S	ETH	NSGC
190	EEBC 190	PI 386475	IAR/B/452	2	S	ETH	NSGC
191	EEBC 191	PI 386514	IAR/B/148-3	2	S	ETH	NSGC
192	EEBC 192	PI 386524	IAR/B/431	2	S	ETH	NSGC
193	EEBC 193	PI 386525	IAR/B/432-3	2	S	ETH	NSGC
194	EEBC 194	PI 386526	IAR/B/527-2	2	S	ETH	NSGC
195	EEBC 195	PI 386559	IAR/B/37	6	S	ETH	NSGC
196	EEBC 196	PI 386581	IAR/B/99-2	6	S	ETH	NSGC

Table 3.1 continued

Number	Genotype	Identifier	Plant ID	Row Type	Growth Habit	Origin	Source
197	EEBC 197	PI 386584	IAR/B/107	6	S	ETH	NSGC
198	EEBC 198	PI 386601	IAR/B/155-2	6	F	ETH	NSGC
199	EEBC 199	PI 386650	IAR/B/239-1	6	F	ETH	NSGC
200	EEBC 200	PI 386661	IAR/B/257-3	6	F	ETH	NSGC
201	EEBC 201	PI 386666	IAR/B/271	6	S	ETH	NSGC
202	EEBC 202	PI 386723	IAR/B/361-2	6	S	ETH	NSGC
203	EEBC 203	PI 386759	IAR/B/400	2	F	ETH	NSGC
204	EEBC 204	PI 386833	IAR/B/31	6	S	ETH	NSGC
205	EEBC 205	PI 386838	IAR/B/38-1	6	F	ETH	NSGC
206	EEBC 206	PI 386844	IAR/B/57	2	S	ETH	NSGC
207	EEBC 207	PI 386863	IAR/B/85	6	F	ETH	NSGC
208	EEBC 208	PI 386873	IAR/B/101-1	6	S	ETH	NSGC
209	EEBC 209	PI 386897	IAR/B/123	6	S	ETH	NSGC
210	EEBC 210	PI 386940	IAR/B/175-1	6	S	ETH	NSGC
211	EEBC 211	PI 386993	IAR/B/214-3	6	S	ETH	NSGC
212	EEBC 212	PI 387098	IAR/B/328-3	6	S	ETH	NSGC
213	EEBC 213	PI 387184	IAR/B/427	2	S	ETH	NSGC
214	EEBC 214	PI 387186	IAR/B/428-2	6	W	ETH	NSGC
215	EEBC 215	PI 387202	IAR/B/473	6	F	ERI	NSGC
216	EEBC 216	PI 387226	IAR/B/506-1	6	S	ETH	NSGC
217	EEBC 217	PI 387240	IAR/B/521-3	6	S	ETH	NSGC
218	EEBC 218	PI 447116	ST1256HARAR	6	S	ETH	NSGC
219	EEBC 219	PI 548736	HOR2928	6	S	ETH	NSGC
220	EEBC 220	EN 17695	EEBC220	6	S	ETH	VIR
221	EEBC 221	EN 17696	FAYIKS	6	S	ETH	VIR
222	EEBC 222	EN 18830	EEBC222	6	S	ETH	VIR
223	EEBC 223	EN 18838	EEBC223	6	S	ETH	VIR
224	EEBC 224	EN 18842	EEBC224	6	S	ETH	VIR

Table 3.1 continued

Number	Genotype	Identifier	Plant ID	Row Type	Growth Habit	Origin	Source
225	EEBC 225	EN 18843	EEBC225	2	S	ETH	VIR
226	EEBC 226	EN 18844	EEBC226	6	S	ETH	VIR
227	EEBC 227	EN 18846	EEBC227	6	S	ETH	VIR
228	EEBC 228	EN 21272	DZ02-610	6	S	ETH	VIR
229	EEBC 229	EN 22900	DZ02-622	2	S	ETH	VIR
230	EEBC 230	EN 22968	DZ02-456	2	S	ETH	VIR
231	EEBC 231	EN 25027	DZ02-228	6	S	ETH	VIR
232	EEBC 232	EN 20054	LINIYA_AHOR2930/66	2	S	ETH	VIR
233	EEBC 233	EN 21984	ATHIOPIEN-AB.9	6	S	ETH	VIR
234	EEBC 234	EN 22905	DZ02-24	6	S	ETH	VIR
235	EEBC 235	EN 22940	DZ02-158	2	S	ETH	VIR
236	EEBC 236	EN 22951	DZ02-237	6	S	ETH	VIR
237	EEBC 237	EN 23026	III-29K	6	S	ETH	VIR
238	EEBC 238	EN 23027	III-39CH	6	S	ETH	VIR
239	EEBC 239	EN 23028	III-39D	6	S	ETH	VIR
240	EEBC 240	EN 23036	I-22B	6	S	ETH	VIR
241	EEBC 241	EN 23042	I-57A	2	S	ETH	VIR
242	EEBC 242	EN 23055	II-164B	6	S	ETH	VIR
243	EEBC 243	EN 23060	III-36A	6	S	ETH	VIR
244	EEBC 244	EN 23062	III-38	6	S	ETH	VIR
245	EEBC 245	EN 23065	III-45B	6	S	ETH	VIR
246	EEBC 246	EN 25016	EEBC246	2	S	ETH	VIR
247	EEBC 247	EN 25025	DZ02-174	6	S	ETH	VIR
248	EEBC 248	EN 25029	DZ02-249	6	S	ETH	VIR
249	EEBC 249	EN 25030	DZ02-269	6	S	ETH	VIR
250	EEBC 250	EN 25036	DZ02-400	2	S	ETH	VIR
251	EEBC 251	EN 25038	DZ02-600	2	S	ETH	VIR
252	EEBC 252	EN 25810	ABYSSINIAN	6	S	ETH	VIR

Table 3.1 continued

Number	Genotype	Identifier	Plant ID	Row Type	Growth Habit	Origin	Source
253	EEBC 253	EN 26045	ETHIOPIA_AB.1122	2	S	ETH	VIR
254	EEBC 254	EN 26335	III-75	2	S	ETH	VIR
255	EEBC 255	EN 26557	EEBC255	2	S	ETH	VIR
256	EEBC 256	EN 26605	EEBC256	6	S	ETH	VIR
257	EEBC 257	EN 26606	EEBC257	6	S	ETH	VIR
258	EEBC 258	EN 26695	EEBC258	6	S	ETH	VIR
259	EEBC 259	EN 26696	EEBC259	6	S	ETH	VIR
260	EEBC 260	EN 27093	EEBC260	6	S	ETH	VIR
261	EEBC 261	EN 27094	EEBC261	6	S	ETH	VIR
262	EEBC 262	EN 28218	EEBC262	2	S	ETH	VIR
263	EEBC 263	EN 30808	EP73	6	S	ETH	VIR
264	EEBC 264	EN 30809	AB15	6	S	ETH	VIR
265	EEBC 265	EN 30810	H-2210	6	S	ETH	VIR
266	EEBC 266	EN 30812	H-2211	6	S	ETH	VIR
267	EEBC 267	EN 30813	EEBC267	6	S	ETH	VIR
268	EEBC 268	EN 30814	AB1119	6	S	ETH	VIR
269	EEBC 269	EN 21165	DZ02-401	6	S	ETH	VIR
270	EEBC 270	EN 21223	II-95A	6	S	ETH	VIR
271	EEBC 271	EN 23003	DZ02-693	6	S	ETH	VIR
272	EEBC 272	EN 25550	II-23	6	S	ETH	VIR
273	EEBC 273	EN 25557	II-147B	6	S	ETH	VIR
274	EEBC 274	EN 26528	EH12B/F3.Q.A.2.B	2	S	ETH	VIR
275	EEBC 275	EN 26531	76-12-1	2	S	ETH	VIR
276	EEBC 276	EN 26539	EH20-F3-A-A	2	S	ETH	VIR
277	EEBC 277	EN 26547	EH12B/F3.M.1.A.1.A	2	S	ETH	VIR
278	EEBC 278	EN 26548	EH12B/F3.M.1.A.4.A	2	S	ETH	VIR
279	EEBC 279	EN 26559	EEBC279	6	S	ETH	VIR
280	EEBC 280	EN 26582	EEBC280	2	S	ETH	VIR

Table 3.1 continued

Number	Genotype	Identifier	Plant ID	Row Type	Growth Habit	Origin	Source
281	EEBC 281	EN 27090	EEBC281	6	S	ETH	VIR
282	EEBC 282	EN 3241	EEBC282	2	S	ETH	ICARDA
283	EEBC 283	LAND09 109	EEBC283	6	S	ERI	ICARDA
284	EEBC 284	LAND09 110	EEBC284	6	S	ERI	ICARDA
285	EEBC 286	LAND09 112	EEBC286	6	S	ERI	ICARDA
286	EEBC 287	LAND09 113	EEBC287	6	S	ERI	ICARDA
287	EEBC 288	LAND09 114	EEBC288	6	S	ETH	ICARDA
288	EEBC 289	LAND09 115	EEBC289	6	S	ETH	ICARDA
289	EEBC 290	LAND09 116	EEBC290	6	S	ETH	ICARDA
290	EEBC 291	LAND09 117	EEBC291	6	S	ETH	ICARDA
291	EEBC 292	LAND09 118	EEBC292	6	S	ETH	ICARDA
292	EEBC 293	LAND09 119	EEBC293	6	S	ETH	ICARDA
293	EEBC 294	LAND09 120	EEBC294	6	S	ETH	ICARDA
294	EEBC 295	LAND09 121	EEBC295	6	S	ETH	ICARDA
295	EEBC 296	LAND09 122	EEBC296	6	S	ETH	ICARDA
296	EEBC 297	LAND09 123	EEBC297	6	S	ETH	ICARDA
297	EEBC 298	LAND09 124	EEBC298	6	S	ETH	ICARDA
298	EEBC 299	LAND09 125	EEBC299	6	S	ETH	ICARDA

[a] Genotype = EEBC number assigned by Mamo (2013) for lines in the Ethiopian and Eritrean barley collection (EEBC).
[b] Identifier; accession numbers with the prefix PI or CIho are designations assigned by NSGC, accession numbers with the prefix EN designated accessions from the VIR; landraces obtained from ICARDA were designated by internal laboratory reference numbers preceded by the prefix "LAND09"following their inclusion in a 2009 nursery in Saint Paul, MN.
[c] Plant ID = number or plant name; some accession ID's were uncertain and the EEBC number was assigned here also.
[d] Growth Habit; S - spring, W - winter, F - facultative.
[e] Origin - country of origin; ETH - Ethiopia, ERI - Eritrea.
[f] Source; NSGC - National small grains collection, VIR - N.I. Vavilov Institute of Plant Genetic Resources, ICARDA - International Center for Agricultural Research in the Dry Areas, Beirut, Lebanon.
*Additional information on these lines can be found in Mamo 2013 (See, Appendix Table 3.1 in Mamo 2013)

Table 3.2 *Pyrenophora teres* f. *teres* isolates used in this study

SGP ID[a]	Other ID[b]	Year of collection	State of Collection
30107003	LDNHO5 pt-5	-	North Dakota
30107004	LDNHO4 pt-15	-	North Dakota
30107005	NDB 89-19-1	-	North Dakota
30112001	ND99-16	1999	North Dakota
30112002	MN01-10	2001	Minnesota
30115001	MN-12-06	2006	Minnesota
30115002	MN-14-02	2002	Minnesota
30199012	-	1999	North Dakota

[a] SGP ID = Identification code given to *Pyrenophora teres* f. *teres* isolates in Dr. Ruth Dill-Macky's Small Grains Pathology laboratory at the University of Minnesota.
[b] Other ID = Identification codes given by Dr. Brian Steffenson's group at the University of Minnesota and used in publications from this research group.

Table 3.3 Differential lines used for characterizing the *Pyrenophora teres* f. *teres*

Entry No.	Cultivar[a]
1	Tifang
2	Lake Shore
3	Atlas
4	Rojo
5	Coast
6	Manchurian
7	Ming
8	CIho9819
9	Algerian
10	Kombar
11	CI 11458
12	CI 5791
13	Harbin
14	CIho 7584
15	Prato
16	Manchuria
17	CIho5822
18	CI 4922
19	Hazera
20	Cape
21	Beecher
22	Rika
23	NDB112
24	FR 926-77
25	Hector
26	SM89010
27	Heartland
28	CI 9214
29	Stander
30	BT-462

[a] Name of cultivar or line from the differential set (Afanasenko et al. 1995; Afanasenko et al. 2009; Steffenson and Webster 1992).

Table 3.4 Heat map showing the reaction of the net blotch differential lines to eight *Pyrenophora teres* f. *teres* isolates (30107003, 30107004, 30107005, 30112001, 30112002, 30115001, 30115002 and 30199012), tested in a randomized complete block design experiment with three replicates

Differential Line[a]	30107003			30107004			30107005			30112001			30112002			30115001			30115002			30199012		
	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
Tifang	4[b]	2	3	5	6	6	6	2	7	8	6	7	7	7	6	7	6	7	7	8	8	3	5	6
Lake Shore	5	3	5	7	6	6	3	2	4	6	7	7	8	7	7	8	5	5	9	8	7	3	6	4
Atlas	5	6	5	4	6	7	5	4	5	8	7	8	6	7	7	9	7	6	5	6	6	9	8	8
Rojo	5	4	5	2	1	2	1	3	2	5	4	6	7	6	7	3	4	4	3	3	3	3	3	4
Coast	5	3	4	4	4	3	2	7	3	4	4	5	5	3	3	8	4	3	3	2	4	4	3	3
Manchurian	5	7	7	7	7	5	1	2	4	8	8	8	8	6	7	7	7	9	9	10	9	7	7	7
Ming	3	2	3	6	7	7	7	2	3	6	8	6	4	7	7	7	6	7	9	9	10	4	5	4
CIho9819	3	3	3	2	1	2	1	1	1	5	3	4	4	3	3	4	3	3	3	2	1	3	3	3
Algerian	5	6	5	4	4	3	1	2	2	5	6	6	6	6	7	6	3	4	4	3	4	3	3	5
Kombar	8	7	8	8	7	7	6	5	2	9	7	8	8	6	6	6	6	6	9	7	9	7	6	7
CI 11458	5	2	6	4	4	5	3	5	3	4	4	4	6	5	6	7	8	7	7	5	5	5	5	6
CI 5791	3	3	3	2	2	2	2	1	1	5	5	3	6	3	2	4	3	2	4	2	3	2	3	3
Harbin	4	5	6	3	2	4	2	3	5	6	4	5	6	3	6	6	3	3	3	8	5	4	3	6
CIho 7584	3	4	4	1	1	3	1	2	2	5	3	3	3	3	3	6	3	4	5	2	5	3	3	5
Prato	6	6	4	4	5	7	5	3	6	8	7	8	6	7	7	7	8	7	9	8	8	6	6	6
Manchuria	7	6	7	6	5	7	5	4	5	7	6	9	8	6	6	7	9	7	8	9	7	9	7	7
CIho5822	4	4	4	2	5	7	2	4	5	5	5	3	7	6	4	5	7	7	3	4	7	3	5	6
CI 4922	2	3	3	5	9	7	3	6	1	8	8	7	7	5	7	4	4	3	4	7	4	7	5	6
Hazera	3	4	5	5	5	5	2	5	5	7	6	6	6	4	7	5	7	7	6	4	4	6	6	6
Cape	7	7	6	5	7	8	3	7	4	7	8	7	6	6	6	6	7	7	9	9	7	7	6	7
Beecher	6	4	4	4	4	6	6	6	5	7	7	7	4	5	3	8	8	7	6	5	6	7	7	6
Rika	6	8	7	4	4	5	3	3	4	7	6	5	7	8	7	7	7	5	7	7	6	4	5	6
NDB112	3	2	3	6	4	4	3	5	3	3	3	4	6	4	4	3	2	5	3	7	6	3	3	2
FR 926-77	3	5	6	4	3	5	3	1	2	4	3	4	4	4	6	3	2	2	5	3	4	4	3	3
Hector	7	9	9	8	7	8	7	6	6	7	9	9	8	7	9	8	9	9	8	9	10	9	7	8
SM89010	8	7	8	2	7	2	2	nd	2	7	5	7	5	3	3	7	5	5	5	nd	9	2	2	5
Heartland	3	3	2	4	3	5	5	3	2	6	5	6	5	4	4	2	3	3	4	5	3	5	3	4
CI 9214	5	4	6	2	2	nd[c]	1	1	2	7	5	4	4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Stander	6	7	7	8	7	8	5	6	6	9	8	9	10	8	8	9	9	9	10	10	10	7	9	9
BT-462	4	4	5	4	5	6	5	3	2	6	6	6	5	5	6	7	6	7	6	5	5	5	6	6

[a] Cultivar or line in the differential set.

[b] Values provided are infection response (1-10 scale) according Tekauz (1985). In the conditional color formatting used, green indicates the resistant end of the infection type scale (1) while red indicates the susceptible end of the infection type scale (10).

[c] nd = Missing data.

Table 3.5 Markers in the lowest 0.1 percentile of -log(p) value distribution in the genome-wide association mapping (AM) analyses conducted using phenotypic datasets from net blotch screening experiments done in controlled environment using individual isolates of *Pyrenophora teres* f. *teres* and single nucleotide polymorphism (SNP) markers

Dataset[a]	SNP[b]	CHR[c]	cM[c]	Complete set[d]	Mapped markers[e]	Unique map positions[f]
30112002	SCRI_RS_221843	2H	62910	3.44	3.27	
	SCRI_RS_8366	2H	62910	3.34	3.06	
	SCRI_RS_199178	1H	45210	3.21	3.28	3.67
	SCRI_RS_238412	UNK[g]	13180	2.92	X[f]	
	11_10165	6H	17580	2.77	2.83	
	SCRI_RS_194337	5H	176520	2.65		
30107003	11_10405	5H	171160	3.16	3.33	3.37
	12_30691	2H	64700	3.02	2.85	
	11_20674	2H	65000	2.98	2.91	
	SCRI_RS_5194	3H	106960	2.95	3.09	
	SCRI_RS_10670	2H	92800	2.85		
30107004	11_10405	5H	171160	4.88	4.67	4.27
	SCRI_RS_605	UNK	13610	3.56	X	
	SCRI_RS_157480	2H	16170	3.26	3.18	
	12_30155	2H	16170	3.07	2.99	
	11_20252	3H	6460	3.05	2.92	
30199012	SCRI_RS_142541	6H	59010	4.76	4.95*	4.26
	SCRI_RS_154288	UNK	5500	3.52	X	
	SCRI_RS_7392	UNK	13870	3.35	X	
	SCRI_RS_138463	2H	93930	3.28	3.40	
	SCRI_RS_10670	2H	92800	3.17	3.36	
	SCRI_RS_128449	2H	92800	X	3.13	
Combined	SCRI_RS_194337	5H	176520	5.08*	4.92*	4.24*
	SCRI_RS_221843	2H	62910	4.05	4.05	
	SCRI_RS_8366	2H	62910	3.81	3.66	
	SCRI_RS_10670	2H	92800	3.55	3.43	
	SCRI_RS_605	UNK	13610	3.52	X	

[a] Phenotypic datasets used for individual AM analyses.
[b] SNPs that were in the 0.1 percentile of –log(p) value distribution for each analysis.
[c] Chromosomes on which the SNPs are mapped.
[d] -log(p)value of corresponding SNPs when the complete marker set was used for AM.
[e] -log(p) value of corresponding SNPs when only mapped markers were used for AM.
[f] -log(p) value of corresponding SNPs when only markers with unique map positions were used for AM.
[g] UNK refers to markers with unknown map positions.
[f] X denotes no position (markers with unknown map positions).
* -log(P) value of markers that were significant at the false discovery rate of 0.05.

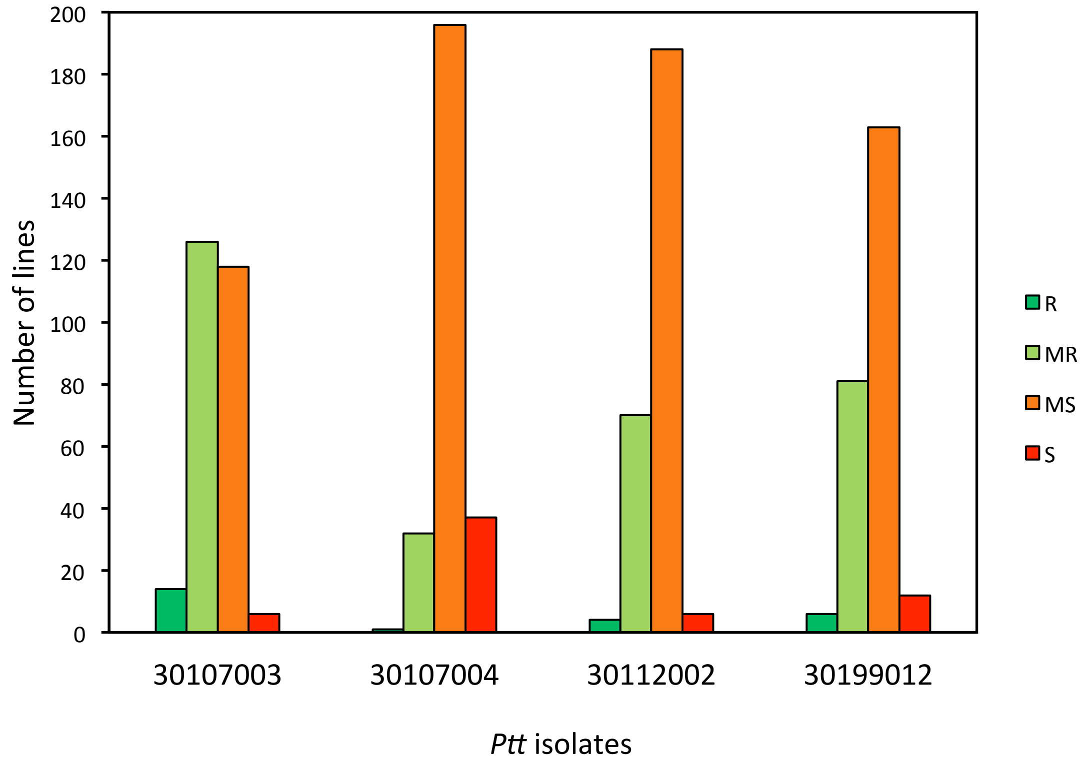

Figure 3.1 Frequency distribution for the reaction of a collection of 298 Ethiopian and Eritrean barley lines to four *Pyrenophora teres* f. *teres* (*Ptt*) isolates (30107003, 30107004, 30112002 and 30199012). The infection type categories used are R, resistant; MR, moderately resistant; MS, moderately susceptible and S, susceptible and were defined by the Tekauz (1985) net blotch infection response numerical scale using ranges of 1-3, 4-5, 6-7 and 8-10, respectively.

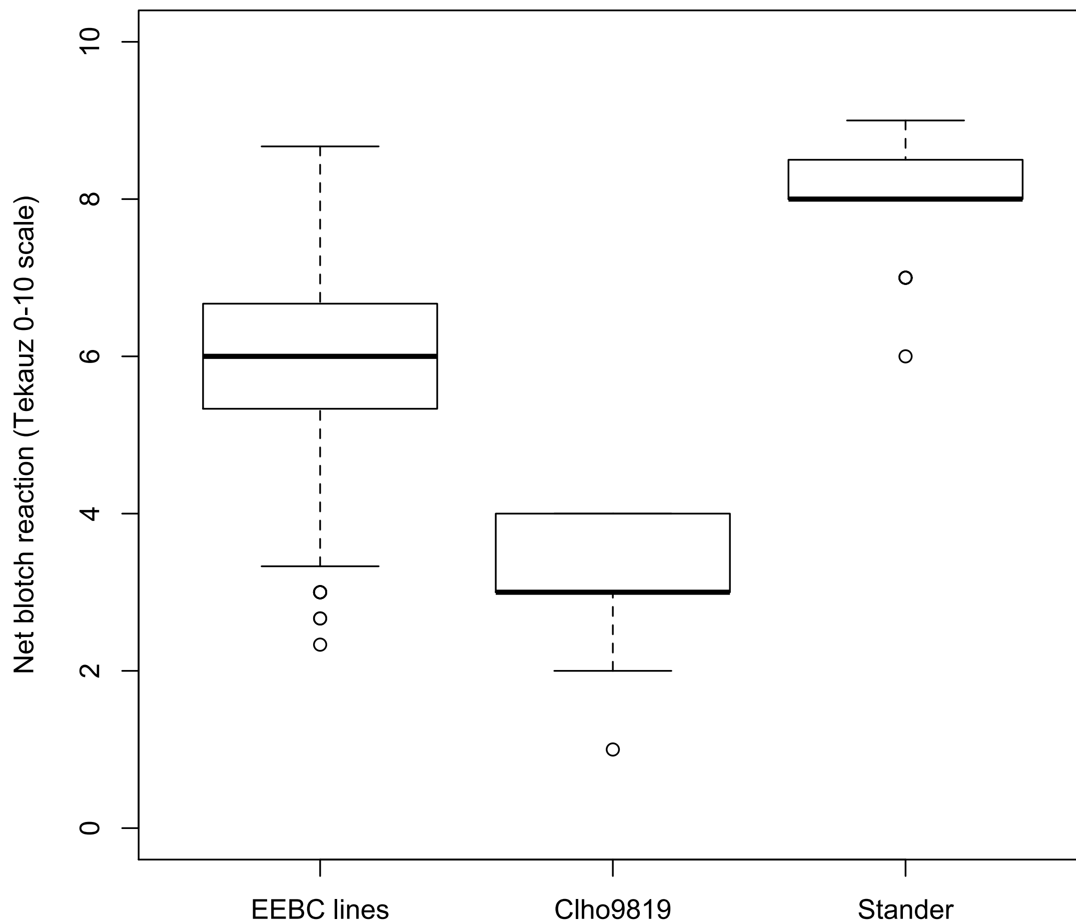

Figure 3.2 Reaction of a collection of 298 Eritrean and Ethiopian barley lines (EEBC) to *Pyrenophora teres* f. *teres* isolates compared to Clho9819, the resistant check, and Stander, the susceptible check. The five statistics (bars) represented in each boxplot are, from bottom to top: the lowest observation, lower quartile, median, upper quartile, and highest observation, respectively. Data points positioned outside this range are depicted as circles and were considered to be outliers. Net blotch infection types were assigned according to the 0-10 scale of Tekauz (1985).

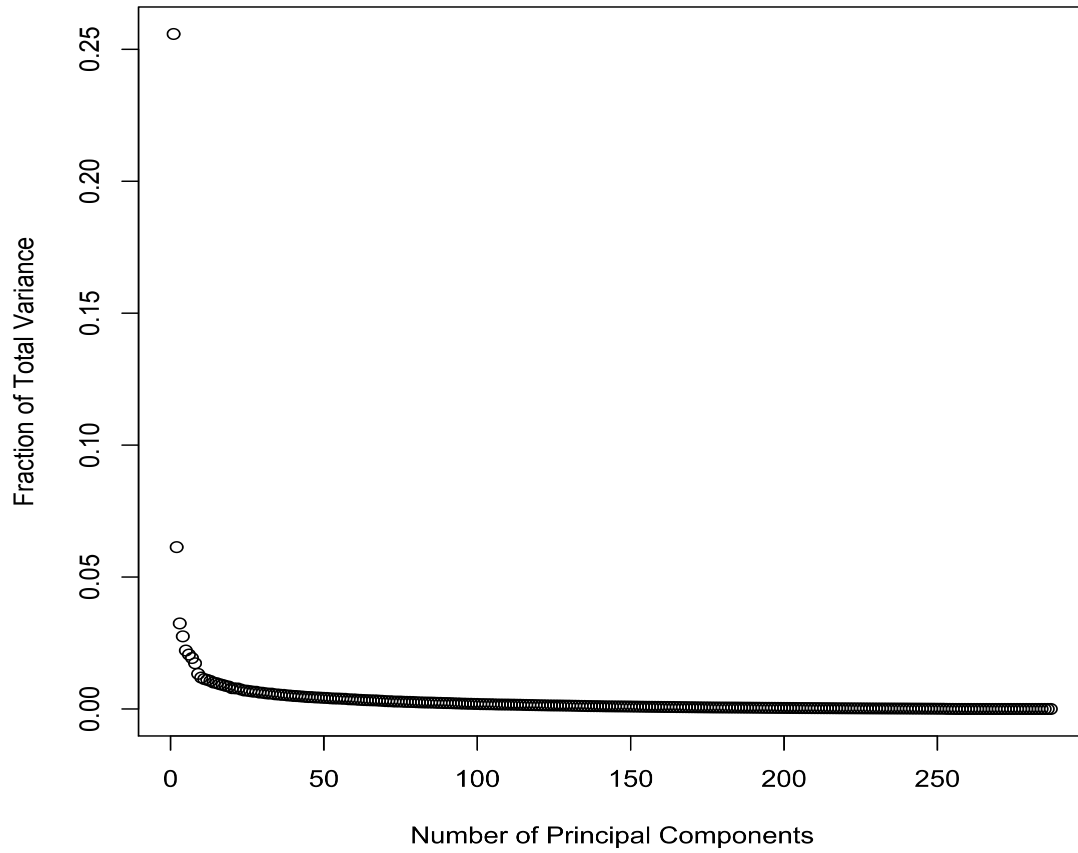

Figure 3.3 Fraction of total variance explained by principal components (PC) in the principal component analysis. The number of principal components is represented by the x-axis and the fraction of total variance explained by each PC is represented by the y-axis.

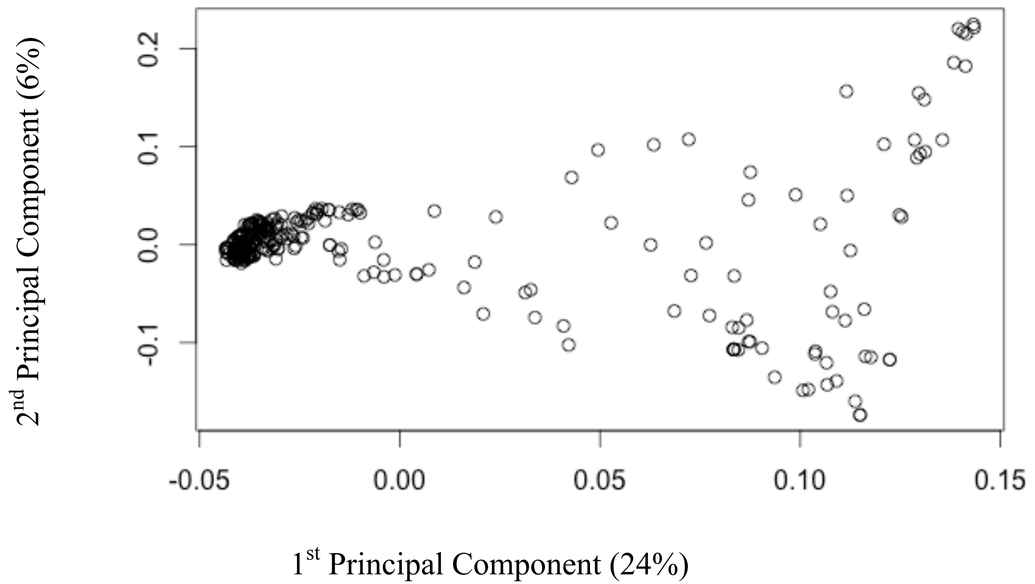

Figure 3.4 Principal component analysis of a collection of 273 Ethiopian and Eritrean barley lines. Percentages given in parentheses indicate the proportion of total variation explained by each principal component.

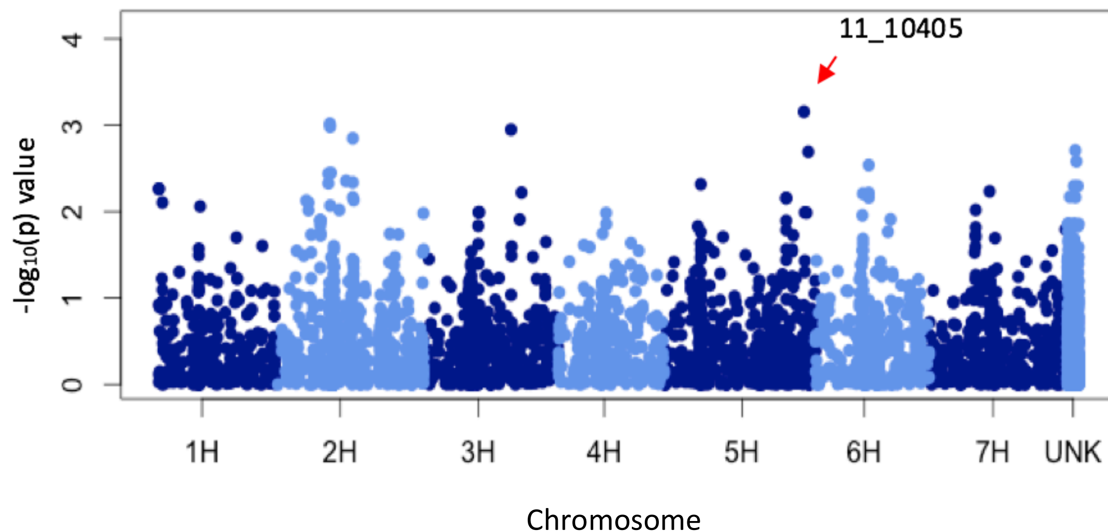

Figure 3.5 Genome-wide association scan of Ethiopian and Eritrean barley landraces germplasm for markers associated with resistance to *Pyrenophora teres* f. *teres* isolate 30107003 using complete set of markers. Vertical axis represents $-\log_{10}(p)$ values of the marker-trait association after correction for multiple testing and the horizontal axis represents the relative chromosomal position of the markers across the genome. Marker 11_10405 at 171.16 cM of chromosome 5H had the highest $-\log_{10}(p)$ value.

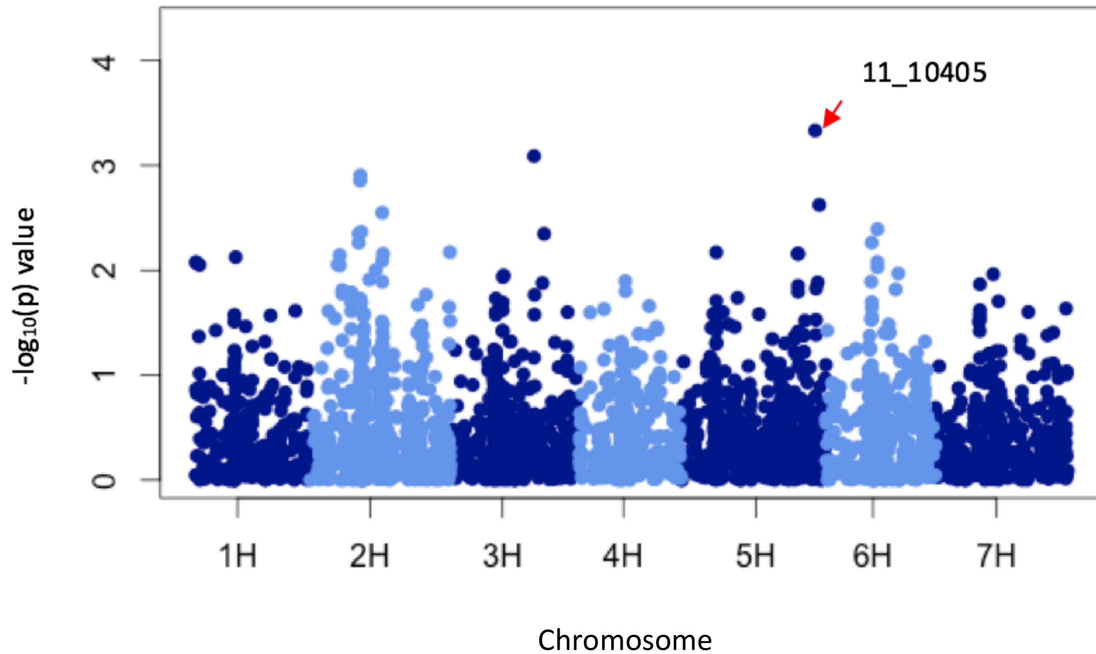

Figure 3.6 Genome-wide association scan of Ethiopian and Eritrean barley landrace germplasm for markers associated with resistance to *Pyrenophora teres* f. *teres* isolate 30107003 using mapped markers only. Vertical axis represents $-\log_{10}(p)$ values of the marker-trait association after correction for multiple testing and the horizontal axis represents the relative chromosomal position of the markers across the genome. Marker 11_10405 at 171.16 cM of chromosome 5H had the highest $-\log_{10}(p)$ value.

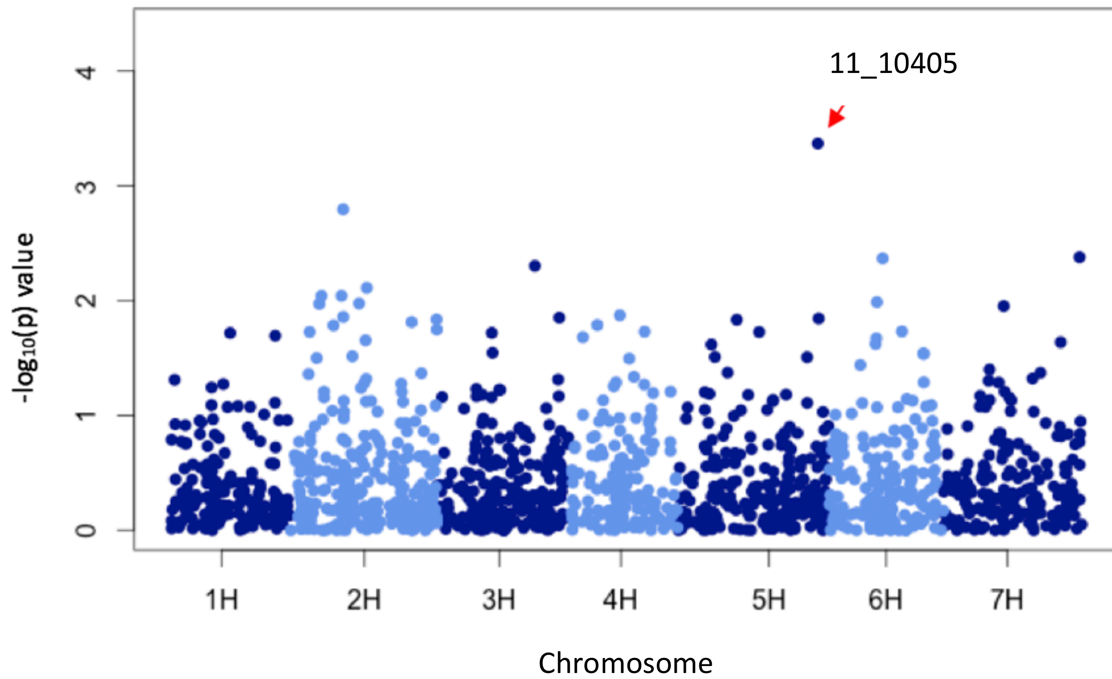

Figure 3.7 Genome-wide association scan of Ethiopian and Eritrean barley landrace germplasm for markers associated with resistance to *Pyrenophora teres* f. *teres* isolate 30107003 using markers with unique map positions only. Vertical axis represents $-\log_{10}(p)$ values of the marker-trait association after correction for multiple testing and the horizontal axis represents the relative chromosomal position of the markers across the genome. Marker 11_10405 at 171.16 cM of chromosome 5H had the highest $-\log_{10}(p)$ value.

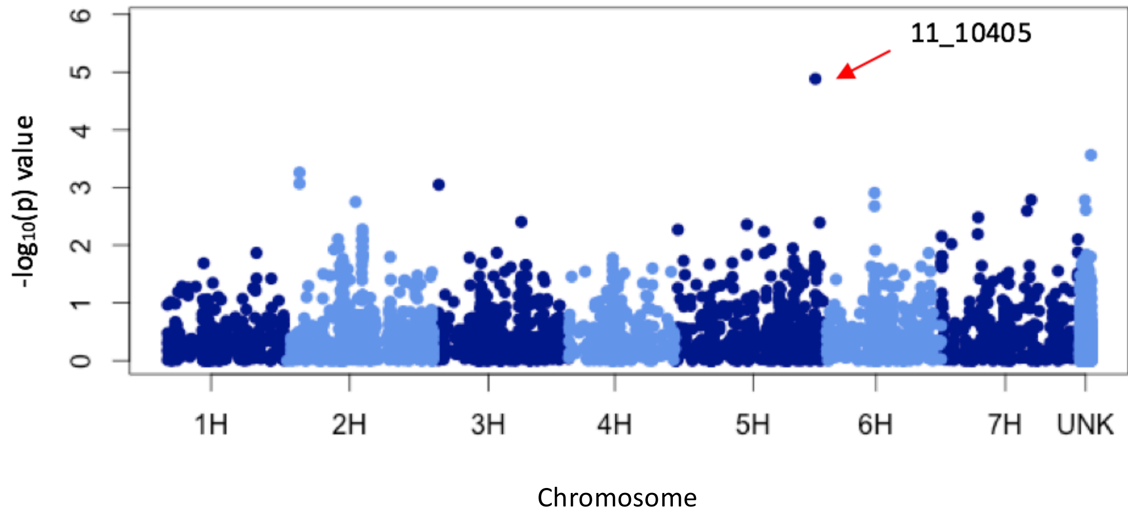

Figure 3.8 Genome-wide association scan of Ethiopian and Eritrean barley landrace germplasm for markers associated with resistance to *Pyrenophora teres* f. *teres* isolate 30107004 using complete set of markers. Vertical axis represents $-\log_{10}(p)$ values of the marker-trait association after correction for multiple testing and the horizontal axis represents the relative chromosomal position of the markers across the genome. Marker 11_10405 at 171.16 cM of chromosome 5H had the highest $-\log_{10}(p)$ value.

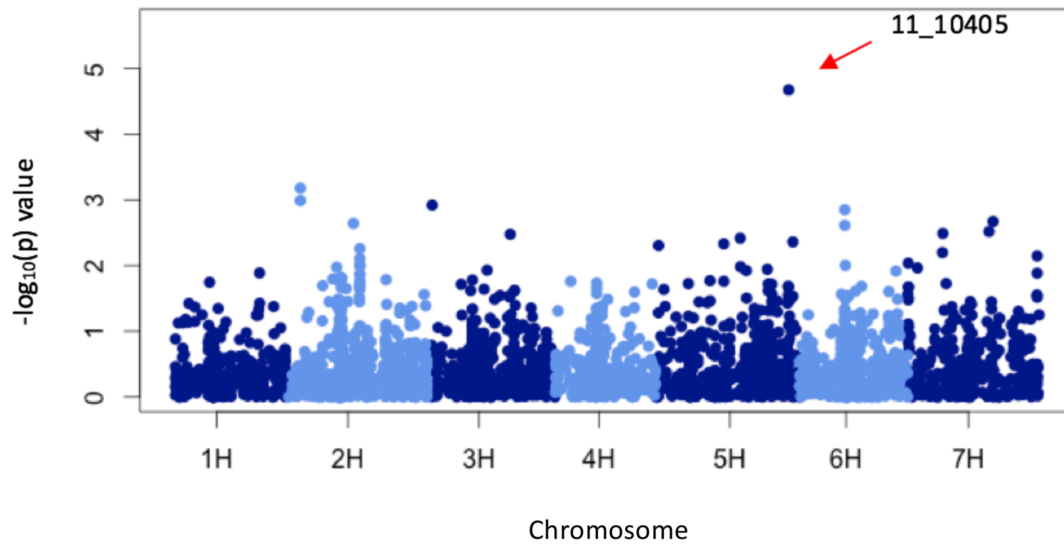

Figure 3.9 Genome-wide association scan of Ethiopian and Eritrean barley landrace germplasm for markers associated with resistance to *Pyrenophora teres* f. *teres* isolate 30107004 using mapped markers only. Vertical axis represents -$\log_{10}$(p) values of the marker-trait association after correction for multiple testing and the horizontal axis represents the relative chromosomal position of the markers across the genome. Marker 11_10405 at 171.16 cM of chromosome 5H had the highest -log10(p) value.

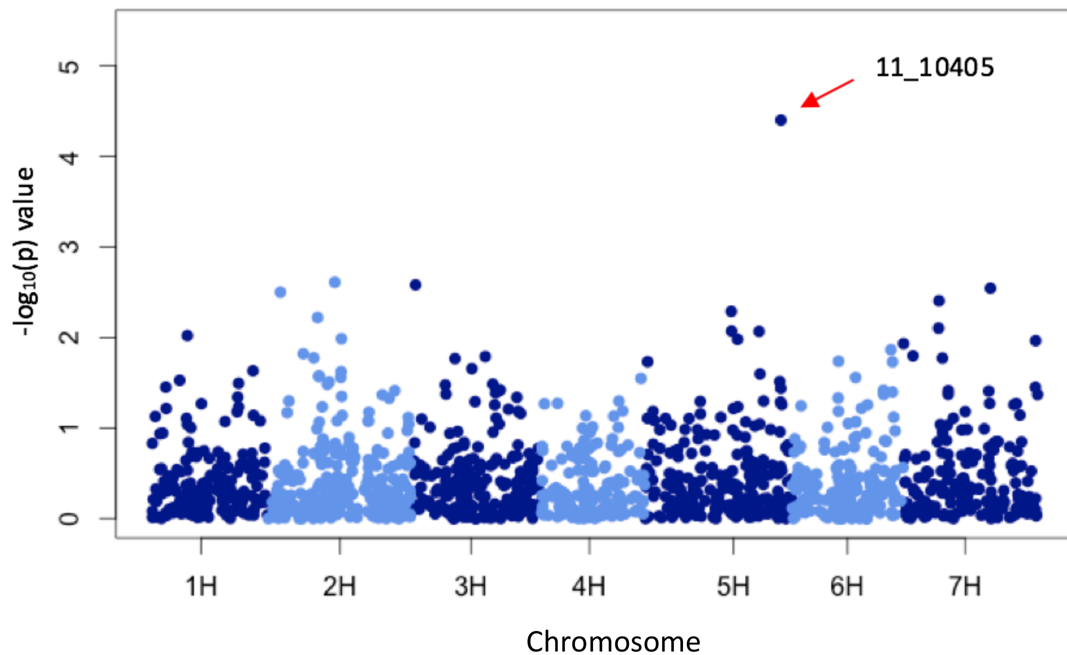

Figure 3.10 Genome-wide association scan of Ethiopian and Eritrean barley landrace germplasm for markers associated with resistance to *Pyrenophora teres* f. *teres* isolate 30107004 using markers with unique map positions. Vertical axis represents $-\log_{10}(p)$ values of the marker-trait association after correction for multiple testing and the horizontal axis represents the relative chromosomal position of the markers across the genome. Marker 11_10405 at 171.16 cM of chromosome 5H had the highest $-\log_{10}(p)$ value.

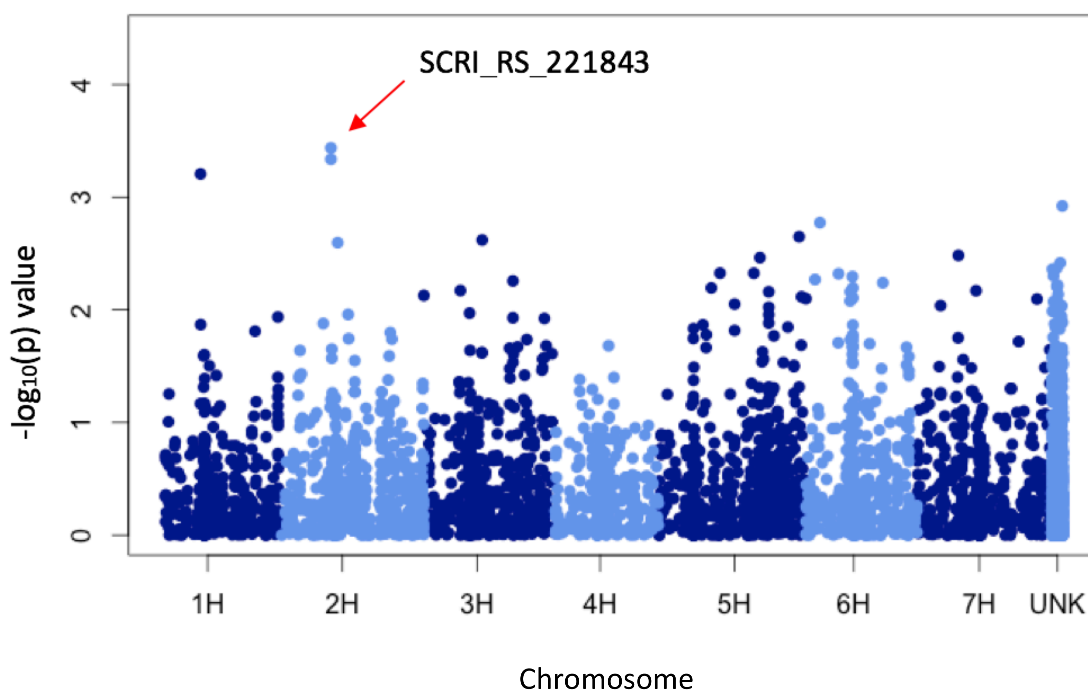

Figure 3.11 Genome-wide association scan of Ethiopian and Eritrean barley landrace germplasm for markers associated with resistance to *Pyrenophora teres* f. *teres* isolate 30112002 using the complete set of SNP markers. Vertical axis represents the $-\log_{10}(p)$ values of the marker-trait association after correction for multiple testing and the horizontal axis represents the relative chromosomal position of the markers across the genome. Marker SCRI_RS_221843 at 62.91 cM of chromosome 2H had the highest $-\log_{10}(p)$ value.

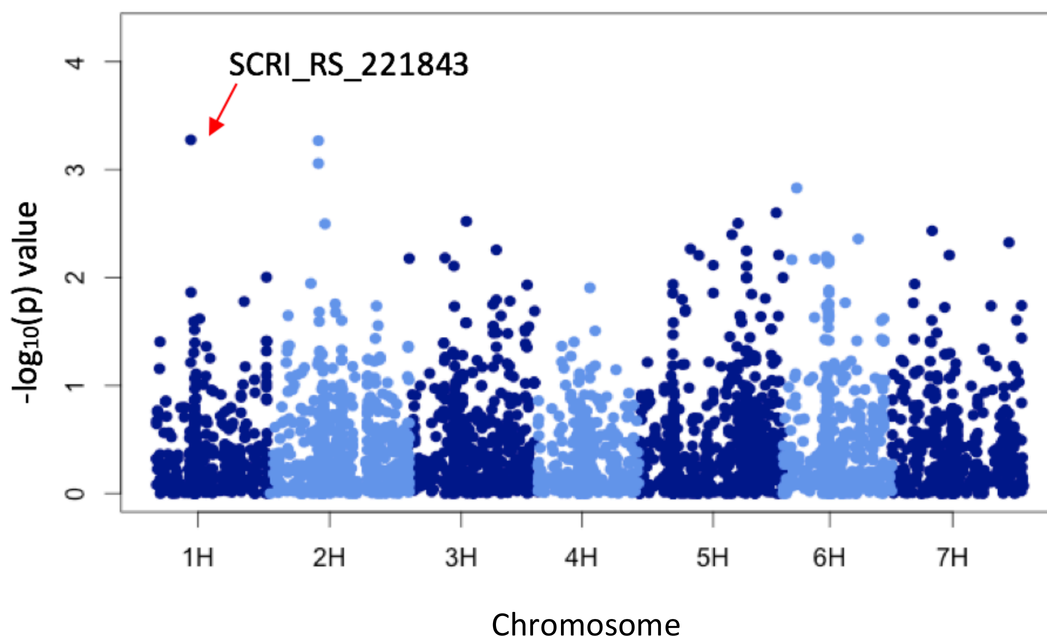

Figure 3.12 Genome-wide association scan of Ethiopian and Eritrean barley landrace germplasm for markers associated with resistance to *Pyrenophora teres* f. *teres* isolate 30112002 using mapped markers only. Vertical axis represents $-\log_{10}(p)$ values of the marker-trait association after correction for multiple testing and the horizontal axis represents the relative chromosomal position of the markers across the genome. Marker SCRI_RS_221843 at 62.91 cM of chromosome 2H had the highest $-\log_{10}(p)$ value.

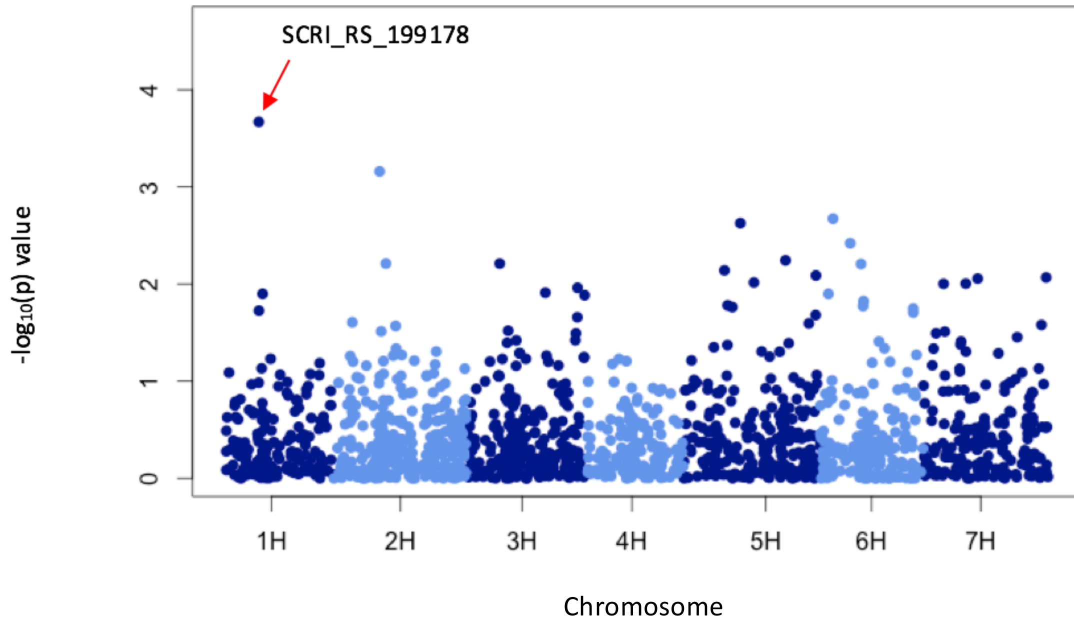

Figure 3.13 Genome-wide association scan of Ethiopian and Eritrean barley landrace germplasm for markers associated with resistance to *Pyrenophora teres* f. *teres* isolate 30112002 using markers with unique map positions only. Vertical axis represents $-\log_{10}(p)$ values of the marker-trait association after correction for multiple testing and the horizontal axis represents the relative chromosomal position of the markers across the genome. Marker SCRI_RS_199178 at 45.2 cM of chromosome 1H had the highest $-\log_{10}(p)$ value.

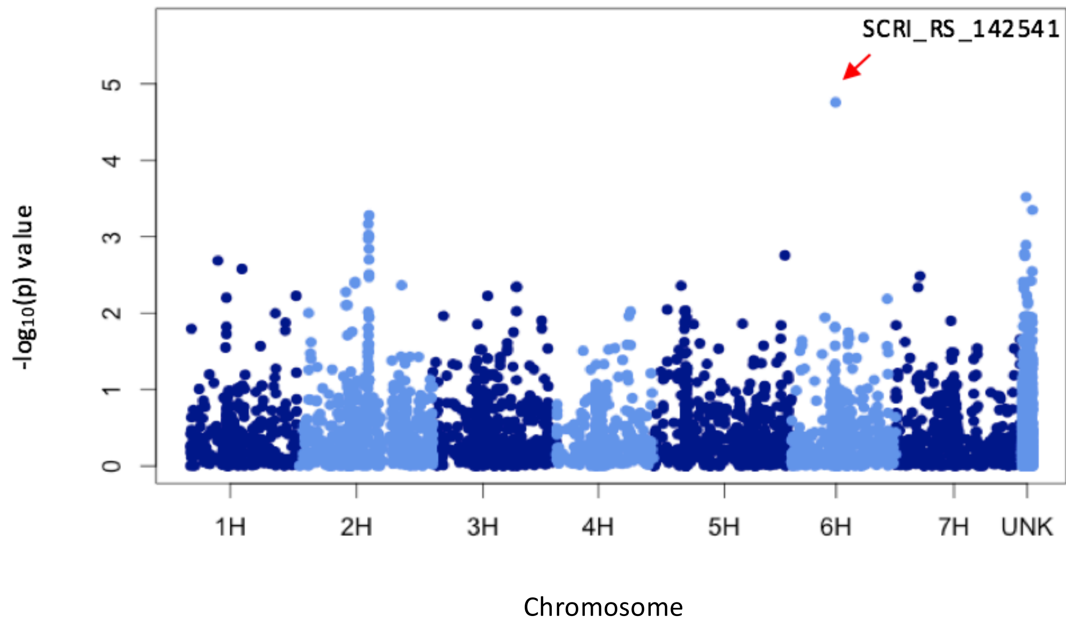

Figure 3.14 Genome-wide association scan of Ethiopian and Eritrean barley landrace germplasm for markers associated with resistance to *Pyrenophora teres* f. *teres* isolate 30199012 using complete set of markers. Vertical axis represents $-\log_{10}(p)$ values of the marker-trait association after correction for multiple testing and the horizontal axis represents the relative chromosomal position of the markers across the genome. Marker SCRI_RS_142541 at 59.01 cM of chromosome 6H had the highest $-\log_{10}(p)$ value.

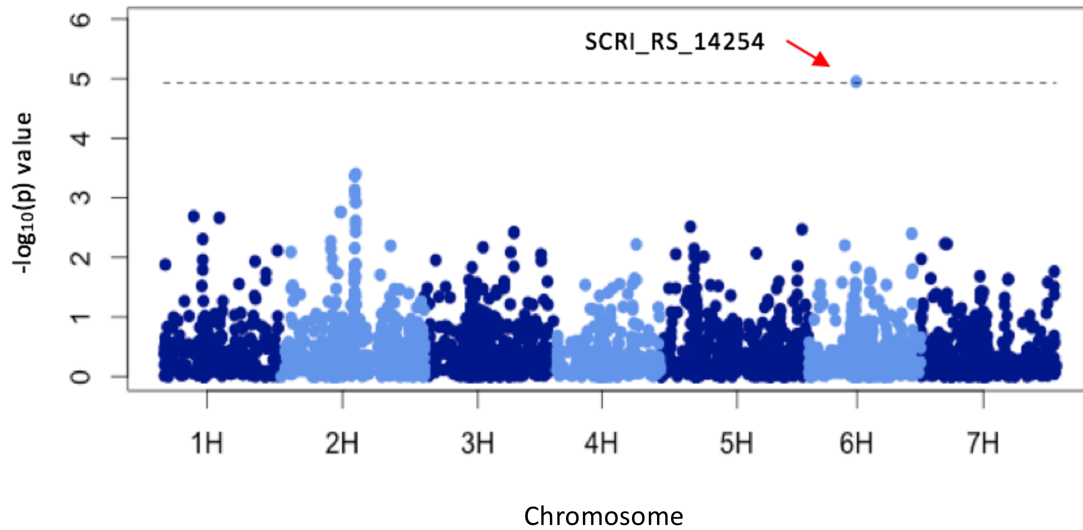

Figure 3.15 Genome-wide association scan of Ethiopian and Eritrean barley landrace germplasm for markers associated with resistance to *Pyrenophora teres* f. *teres* (*Ptt*) isolate 30199012 using mapped markers only. Vertical axis represents $-\log_{10}(p)$ values of the marker-trait association after correction for multiple testing and the horizontal axis represents the relative chromosomal position of the markers across the genome. The horizontal dotted line shows the p-value corresponding to a false discovery rate of 0.05. SNP markers above this threshold were considered as significantly associated with *Ptt* resistance. Marker SCRI_RS_142541 at 59.01 cM of chromosome 6H had a $-\log_{10}(p)$ value higher than the significance threshold.

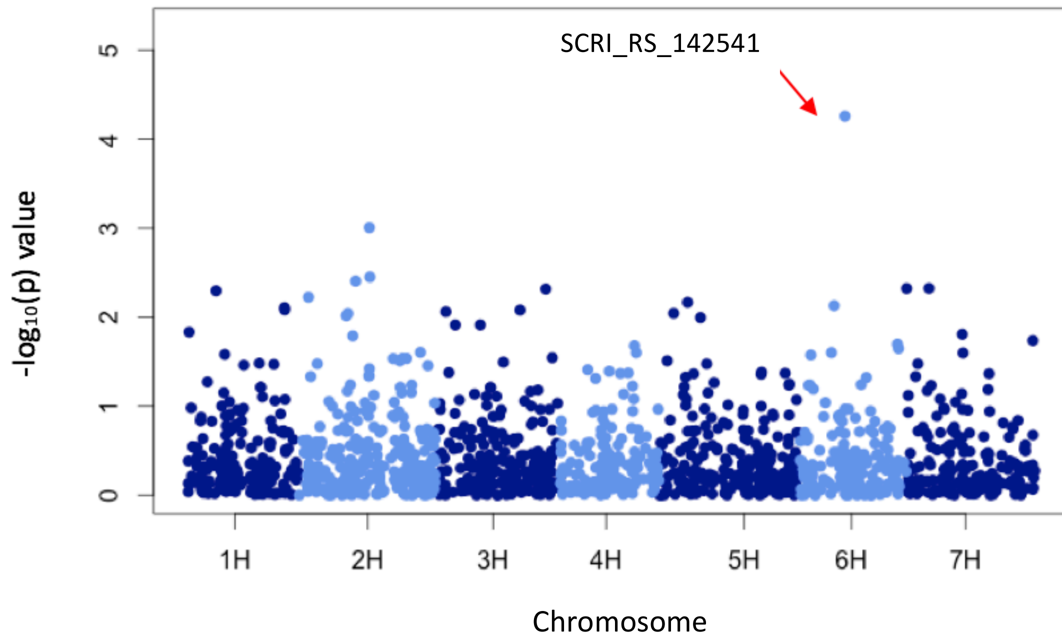

Figure 3.16 Genome-wide association scan of Ethiopian and Eritrean barley landrace germplasm for markers associated with resistance to *Pyrenophora teres* f. *teres* isolate 30199012 using markers with unique map positions. Vertical axis represents $-\log_{10}(p)$ values of the marker-trait association after correction for multiple testing and the horizontal axis represents the relative chromosomal position of the markers across the genome. Marker SCRI_RS_142541 at 59.01 cM of chromosome 6H had the highest $-\log_{10}(p)$ value.

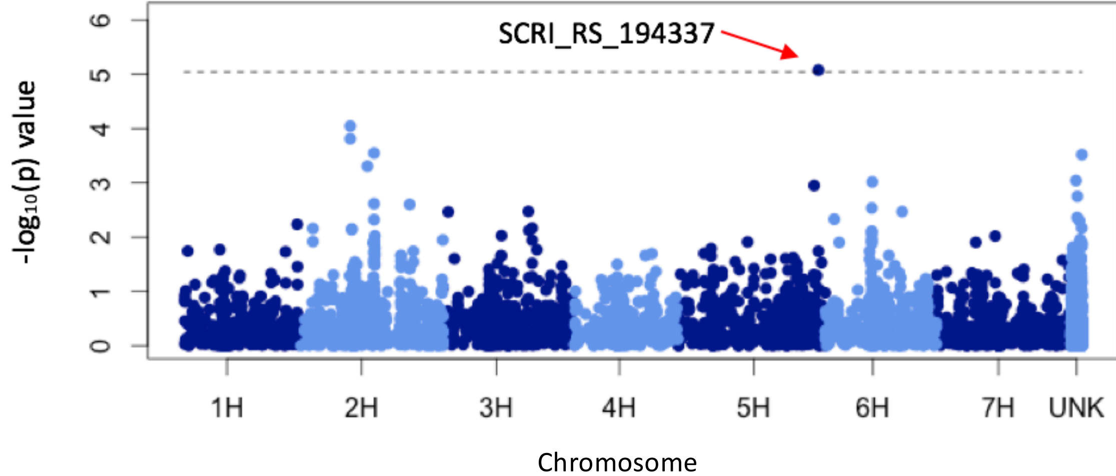

Figure 3.17 Genome-wide association scan of Ethiopian and Eritrean barley landrace germplasm for markers associated with resistance to *Pyrenophora teres* f. *teres* (*Ptt*) isolates (30112002, 30107003, 30107004 and 30199012) using markers with mapped markers. Vertical axis represents $-\log_{10}(p)$ values of the marker-trait association after correction for multiple testing and the horizontal axis represents the relative chromosomal position of the markers across the genome. The horizontal dotted line shows the p-value corresponding to a false discovery rate of 0.05. Markers above this threshold were considered as significantly associated with *Ptt* resistance. Marker SCRI_RS_194337 at 176.52 cM of chromosome 5H had a $-\log_{10}(p)$ value higher than the significance threshold.

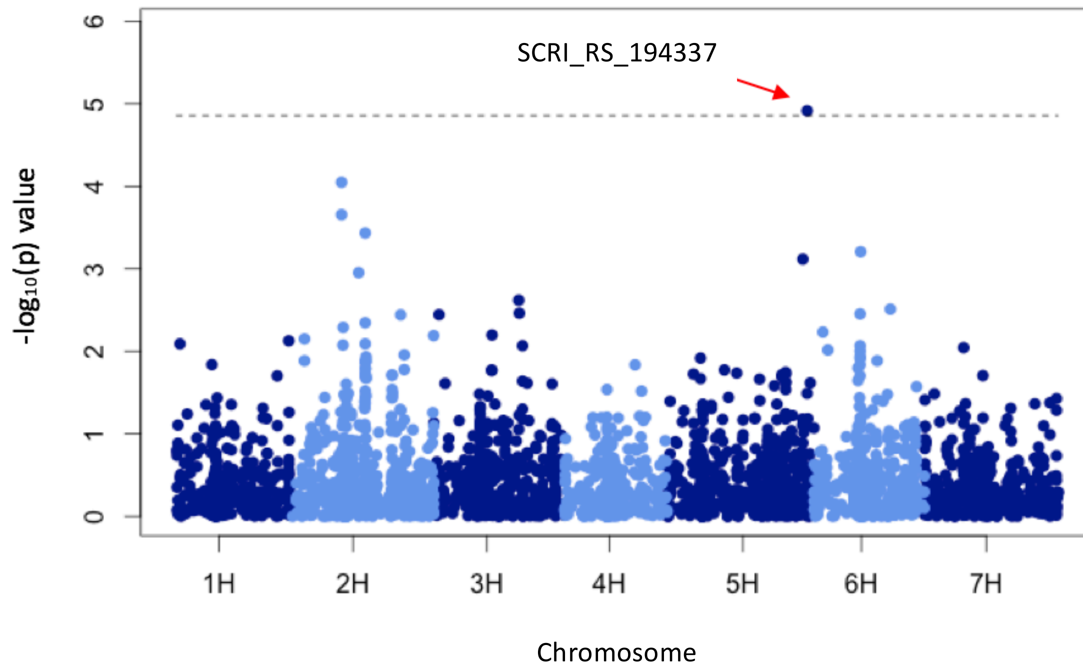

Figure 3.18 Genome-wide association scan of Ethiopian and Eritrean barley landrace germplasm for markers associated with resistance to *Pyrenophora teres* f. *teres* (*Ptt*) isolates (30112002, 30107003, 30107004 and 30199012) using mapped markers. Vertical axis represents -$\log_{10}$(p) values of the marker-trait association after correction for multiple testing and the horizontal axis represents the relative chromosomal position of the markers across the genome. The horizontal dotted line shows the p-value corresponding to a false discovery rate of 0.05. Markers above this threshold were considered as significantly associated with *Ptt* resistance. Marker SCRI_RS_194337 at 176.52 cM of chromosome 5H had a -log10(p) value higher than the significance threshold.

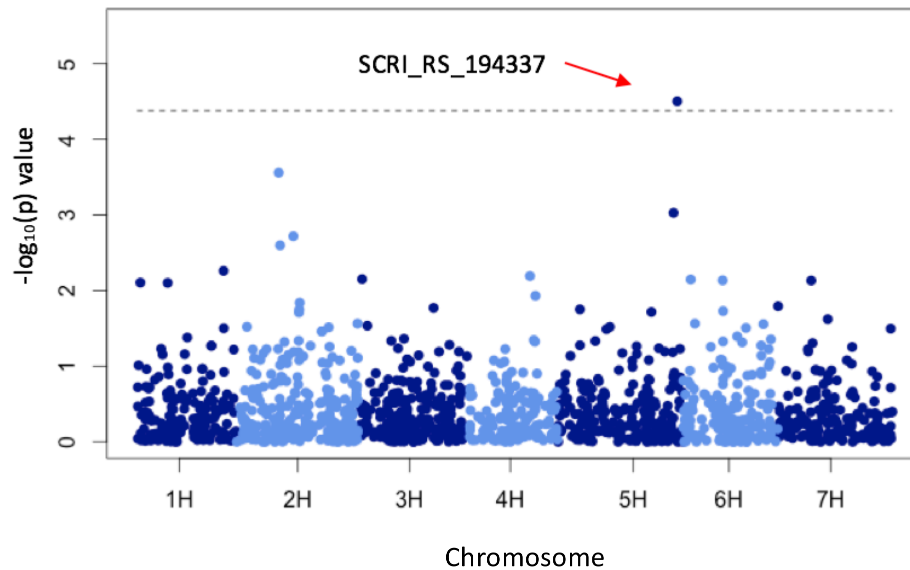

Figure 3.19 Genome-wide association scan of Ethiopian and Eritrean barley landrace germplasm for markers associated with resistance to *Pyrenophora teres* f. *teres* (*Ptt*) isolates (30112002, 30107003, 30107004 and 30199012) using markers with unique map positions. Vertical axis represents $-\log_{10}(p)$ values of the marker-trait association after correction for multiple testing and the horizontal axis represents the relative chromosomal position of the markers across the genome. The horizontal dotted line shows the p-value corresponding to a false discovery rate of 0.05. Markers above this threshold were considered as significantly associated with *Ptt* resistance. Marker SCRI_RS_194337 at 176.52 cM of chromosome 5H had a -log10(p) value higher than the significance threshold.

Chapter 4

Genome-Wide Association Mapping for Net Blotch Resistance in Contemporary Barley Breeding Germplasm from the United States Barley Coordinated Agricultural Project

4.1 Introduction

Net blotch, caused by *Pyrenophora teres* (Drechsler), is one of the most common and economically important foliar diseases of barley (Jordan 1981; Khan 1987; Martens et al. 1988; Steffenson 1997). Net blotch causes losses in the yield and quality of barley worldwide. Yield losses generally range from 10 to 40% in areas where environmental conditions, especially high humidity and cool temperatures, result in frequent net blotch epidemics (Liu et al. 2011; Ma et al. 2004). Net blotch infections reduce both the malting and feed quality of barley by reducing kernel weight (Grewal et al. 2008).

Pyrenophora teres infects barely, wild relatives of barley and other related species from the genera *Bromus, Avena* and *Triticum* (Liu et al. 2011). *Pyrenophora teres* is an ascomycete fungus with the anamorphic stage *Drechslera teres* (Sacc.) (syn. *Helminthosporium teres* Sacc.). *Pyrenophora teres* is further classified into two forms: *P. teres* f. *teres* (Smedegaard) (*Ptt*) and *P. teres* f. *maculata* (Smedegaard-Petersen) (*Ptm*), being the net form of net blotch (NFNB) and spot form of net blotch (SFNB), respectively. In the case of NFNB, the net-like symptoms that develop are characterized by narrow, dark-brown, longitudinal transverse stripes forming net-like pattern on infected leaves (Serenius et al. 2005). The symptoms of SFNB by contrast, consist of dark-brown, circular to elliptical lesions that are surrounded by chlorotic or necrotic halos (Liu et al. 2011).

Pyrenophora teres survives between growing seasons in crop debris, though it can be seedborne (Ma et al. 2004; Steffenson 1997). Ascospores and conidia from crop residues are reported to be the most important sources of primary inoculum formed on fields where infections have occurred previously (Steffenson 1997). Ascospores are

released from pseudothecia that develop on infected stubble and are either aerially or splash dispersed. Conidia, also produced on infected residues, may serve as primary inoculum, although ascospores are generally considered the principal primary inoculum (Steffenson 1997). Conidia may serve as a source of secondary inoculum, facilitating disease spread within the canopy (Jordan 1981). Seedborne mycelium facilitate the dispersal of the pathogen to new fields.

Finding and incorporating host resistance to net blotch into adapted cultivars has been one of the principal objectives of barley breeding programs in the United States (Steffenson and Smith 2006). Multiple studies mapping sources of net blotch resistance have been conducted in barley (Cakir et al. 2003; Friesen et al. 2006; Grewal et al. 2008; Lehmensiek et al. 2008; Pierre et al. 2010; Ma et al. 2004;; Manninen et al. 2006; Steffenson et al. 1996). In these studies, multiple minor effect quantitative trait loci (QTL) for resistance to net blotch, effective at the seedling and/or adult plant stages, have been reported. These QTL have been located on chromosomes 1H, 3H, 4H, 5H and 7H. Most of the studies have also reported major effect QTL on chromosome 6H, with the region detected being very large, spanning several centimorgans (cM). Most of the QTL studies conducted previously have been done utilizing bi-parental mapping populations developed with a single source of net blotch resistance and utilizing older marker technologies that have low resolution. Bi-parental mapping is suitable for mapping qualitative traits but it is not ideal for complex quantitative traits like net blotch resistance. QTL identified by bi-parental mapping cannot be easily validated or readily used for breeding because of their variable expression and effectiveness (Sneller et al. 2009). Another challenge in utilizing QTL identified from bi-parental mapping in

breeding is that most QTL are identified in wide-cross bi-parental mapping populations and are specific to that population and thus may not be segregating in other breeding populations (Sneller et al. 2009).

Recent advances in next generation sequencing have enabled plant scientists to generate thousands of single nucleotide polymorphism (SNP) markers and develop genetic maps with higher resolution than was previously possible. The development of affordable sequencing platforms has enabled scientists to undertake genome wide association studies to map QTL for agronomic traits and disease resistance with increased power and statistical rigor. Association mapping is generally done with a population of unrelated individuals, where the diversity represented in the population facilitates higher resolution mapping and the identification of multiple alleles at a given locus. Association mapping can also reduce the time and resources needed to identify QTL since there is no need of developing specific mapping populations (Rafalski 2010; Yu and Buckler 2006; Zhu et al. 2008).

The Triticeae Coordinated Agricultural Project (TCAP; http://www.triticeaecap.org) is a coordinated project of ten barley and 36 wheat breeding programs in the United States organized to facilitate the sharing of high throughput marker data and trait data from elite breeding germplasm. The Barley Coordinated Agricultural Project (Barley CAP), which predated but is now part of the TCAP, similarly involved ten barley breeding programs from across the United States. The Barley CAP germplasm included elite germplasm (96 entries from each of ten breeding programs per year) submitted annually for each of four years (2006-2009). The four sets of 960 lines were evaluated for various agronomic traits (Mohammadi et al. 2015;

Mohammadi et al. 2014; Pauli et al. 2014) and for their reaction to diseases including; stem rust (Zhou et al. 2014), spot blotch (Zhou and Steffenson 2013a) and Septoria speckled leaf blotch (Zhou and Steffenson 2013b). The major objective of this study was to conduct a genome wide association mapping study of the Barley CAP germplasm using the marker data, along with phenotypic data for net blotch reaction, to identify favorable alleles for net blotch resistance in this core set of United States barley germplasm.

4.2 Materials and Methods

4.2.1 Plant Materials

The association mapping panel for this study consisted of 3,490 elite barley breeding lines from the ten barley breeding programs participating in the Barley CAP (Table 4.1). The lines were selected and provided by the breeding programs of Busch Agricultural Resources, Inc. (BARI), Montana State University (MSU), North Dakota State University (N2 and N6), Oregon State University (OSU), University of Minnesota (MN), United States Department of Agriculture, Agriculture Research Service in Aberdeen, Idaho (USDA), Utah State University (USU), Virginia Polytechnic Institute and State University (VA), and Washington State University (WSU). All the lines were spring types except those from OSU and VA. Lines from MSU, N2 and WSU were two-row types and lines from MN, N6, OSU, USU and VA were six-row types. Entries from BARI and USDA included both two-row and six-row lines. In addition to the panels derived from individual breeding programs, combined panels were developed for each of

the 960 lines submitted to the Barley CAP in 2006, 2007, 2008 and 2009, designated as CAP-I, CAP-II, CAP-III and CAP-IV, respectively; across years and breeding programs for all 2-rowed and 6-rowed lines, designated as the 2-row and 6-row panels, respectively. The final panel used in this association mapping study was consisted of all the lines submitted to Barley CAP, combined across years and breeding programs.

4.2.2 Phenotyping for Net Blotch

Experimental design

The ninety-six lines from each program were evaluated for reaction to net blotch in the Plant Growth Facility West complex at the University of Minnesota St. Paul campus between 2008 and 2010. Each breeding program's material was evaluated in a separate experiment. Individual experiments consisted of three replicates arranged in a randomized complete block design. In addition to the 96 program lines submitted by a breeding program, a resistant and susceptible check was included in each experiment. Stander, a six row malting variety from the University of Minnesota, served as the susceptible check, while BT-462, a line identified as resistant and included in the net blotch differential set (Steffenson and Webster 1992), was included as the resistant check. The lines were grown in 'Conetainers' (International Greenhouse Company, Danville, IL; 3.81 cm diameter, 20.92 cm height) using Metro-Mix 200 (Scotts Company LLC, Marysville, OH) as the growing media. The Conetainers were arranged in a 98-cell rack (7 x 14 configuration), where Conetainers were placed only in every other row to provide adequate space for plant growth. The plants were grown in a greenhouse maintained at 18-22 ^{o}C and were provided 18 hours of supplemental light with 400 W high-pressure

sodium lamps emitting a minimum of 300 µmol photons s^{-1} m^{-2}. Entries were planted at the rate of 3-5 seeds per Conetainer at a depth of 1.5-2.0 cm. The plants were fertilized one week after planting with a water-soluble fertilizer (20-20-20, N-P-K; Peters Professional General Purpose Fertilizer; J.R. Peters, Inc., Allentown, PA) at a concentration of 1.2 $g.l^{-1}$, applied at a rate of 20-25 ml per pot. The plants were watered every other day throughout the experiment.

Production of P. teres f. *teres inoculum and inoculation protocol*

Four *Ptt* isolates (30199002-1, 30199003-1, 30100003 and 30100004) were used for inoculation. Isolates 30199002-1 and 30199003-1 were collected in 1999 and isolates 30100003 and 30100004 were collected in 2000, all four were from commercial barley production fields in the Red River Valley on either the Minnesota or North Dakota side of the state border. Isolates were cultured on V8 agar media for 10-14 days under a combination of cool white and UV light at room temperature (20-25 ^{o}C). Conidia were harvested from 10-14 day old cultures by flooding each plate with 15-20 ml of sterile distilled water, and rubbing the mycelial mat with a bent glass rod to release the conidia. The resulting spore suspension was filtered through cheesecloth to remove mycelium. The conidial concentration was determined using a hemocytometer and adjusted to 25,000 spores/ml by diluting the suspension as needed with sterile distilled water. Polyoxyethylene (20) sorbitan monolaurate, ($C_{58}H_{114}O_{26}$, ICI Americas, Wilmington, DE) was added to the conidial suspension (one drop per 100 ml) as a surfactant to facilitate even distribution of spores in the suspension and to reduce surface tension on the plant surface during inoculation.

The 10-14-day-old seedlings [growth stage 13-14 on the Zadoks scale (Zadoks et al. 1974)] were inoculated to runoff using a hand pump sprayer (Solo 415 Handheld Sprayer; Solo Inc. Newport News, VA). The inoculated seedlings were then placed in a dew chamber (100% relative humidity; cool white fluorescent light at 6 µmol $m^{-2}.s^{-1}$) for 24 hours. Following the infection period, the seedlings were returned to the greenhouse bench and grown under the previous conditions until disease assessment.

Assessment of net blotch severity

The plants were assessed for net blotch infection seven days after inoculation. The scoring was done according to Tekauz (1985) using a 1-10 numerical scale where one was the most resistant and ten was the most susceptible. The reaction class for each line was determined from the arithmetic mean of the values for the three replicates. The numerical scale ratings (infection response), taken using the Tekauz scale, were then used to classify reactions into four broader reaction classes; resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S) that corresponded to the Tekauz infection responses of 1-3, 4-5, 6-7 and 8-10, respectively.

4.2.3 Genotypic Data

The barley lines from Barley CAP were genotyped with two Illumina GoldenGate assays, called Barley Oligonucleotide Pooled Assays One and Two (abbreviated to BOPA1 and BOPA2, respectively), each with 1,536 SNP markers (Close et al. 2009), at the USDA-ARS genotyping facility in Fargo, ND. The Illumina GoldenGate assay is a large scale genotyping assay which can analyze 384-3072 different loci in up to 96

individuals using an allele specific oligo hybridization along with fluorescently labeled universal amplification primers to differentiate genotypes, in a reliable and cost effective manner (Tindall et al. 2010). The consensus map developed by Muñoz-Amatriaín et al. (2011) was used for this study. The SNP marker data and consensus map was downloaded from the Triticeae Toolbox public repository (https://triticeaetoolbox.org/barley). SNP markers with minor allele frequencies of less than 5% and with 50% missing marker data were removed from the analysis. Similarly, lines with 10% or more missing data were also removed.

4.2.4 Association Mapping

A ‘mixed linear model’ (MLM) with ‘efficient mixed model association’ (EMMA) method (Kang et al. 2008) was used for the association mapping analysis. The analysis was conducted using package rrBLUP version 4.4 (Endelman 2011) in the software R (version 3.2.3) using the GWAS function. The rrBLUP package uses the mixed model $y = X\beta + u + e$, where y is the vector of phenotypic values; X is the vector of SNP marker genotypes; β is the coefficient of the SNP marker being estimated; u is the vector of polygenic background of lines (effect of individual relatedness estimated as a pairwise kinship coefficients) and e is the vector of residual effects (Yu et al. 2005). In the equation, $X\beta$ represents fixed effect and e represents random effects. To incorporate the environmental effects arising from different experiments, the environmental mean was modeled using the fixed option in the GWAS function.

In addition to the combined analysis, using all the 3,490 lines from Barley CAP germplasm, separate association mapping analyses for the panels derived from each

breeding program were also conducted. Lines were also grouped by year of submission to the Barley CAP (CAP-I, CAP-II, CAP-III and CAP-IV) and by row type (2-row and 6-row) to create an additional six panels, and an association mapping analysis was done for each of these panels and a panel developed from all entries in the study (Barley CAP). The number of lines and markers used in each panel are presented in Table 4.1.

For panels containing only 2-rowed or 6-rowed lines, a K only model was used in the analysis. For the panels comprised of 2-rowed and 6-rowed barley types together, the analysis was done using a P+K model, using the first two principal components as covariates. The combined panel (Barley CAP) and the CAP-I, CAP-II, CAP-III, CAP-IV, USDA and BARI panels were analyzed with a P+K model, taking into account the first two principal components. The marker score -log(p) values were generated after the EMMA method fitted each marker individually in the mixed linear model. The marker scores [-log(p)] were plotted against marker positions in the map to generate "Manhattan" plots (Appendices 4.1-4.17). The significance threshold for a marker trait association (MTA) was derived by calculating a q-value using the qvalue software package (Storey and Tibshirani 2003). The p-value, corresponding to a q-value of 0.05, was determined by interpolation in the package. The additive genetic effect of each marker on the phenotype was extracted using the 'mixed.solve' function from rrBLUP package.

Once the significant markers associated with net blotch resistance were obtained, linkage disequilibrium (LD) between all the markers within a 50 cM interval was calculated. If the LD between any two markers was high ($R^2 \geq 0.8$), they were considered to be identifying the same QTL. If the LD was low ($R^2 < 0.8$) then they were considered to be identifying different QTL.

4.3 Results

4.3.1 Phenotyping for Net Blotch

The full spectrum of disease infection responses, from 1-10 on the Tekauz reaction scale, was observed in the germplasm. The average disease infection response for each line was used to determine both the reaction class [resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S)] and the frequency distributions of lines across disease reaction classes for each of the ten breeding programs, the two- and six-rowed barley panels and for all lines included in each of the four years of the Barley CAP (Fig. 4.1). The frequency distribution of all datasets examined generally followed a normal distribution with highest proportion of lines generally being moderately susceptible (infection responses 6-7) and the lowest proportion of lines being either susceptible or resistant. The combined dataset including all the panels exhibited a normal distribution with highest proportion of lines being MS (45.2%) followed by MR (25.5%), R (16.8%) and S (12.5%) (Fig. 4.1).

The mean disease infection response for the susceptible check, Stander, ranged from 7.0 to 9.7 with a median infection response of 8.0, whereas the resistant check, BT-462, ranged from 1.7 to 5.0 in individual experiments with a median infection response of 2.8. The disease infection response of the checks included in each experiment, are provided in Table 4.2.

The data for the N2 and BARI panels did not follow a normal distribution and in both these panels there were more lines in the resistant reaction class than in any of the other three classes (Fig. 4.1).

4.3.2 Marker Data

The number of markers used in the study ranged from 919 to 2,380 with an average of 1,961 markers (Table 4.1). The genetic map of Muñoz-Amatriaín et al. (2011) used in this study is 1,127 cM, and the average marker density on the map ranged from 0.8 to 2.1 per cM across the different panels.

Linkage disequilibrium (LD) analysis from previous studies conducted using the same germplasm set, reported levels of LD extending up to 10 cM (Hamblin et al. 2010; Massman et al. 2011; Mohammadi et al. 2015). In this study, an average of 1,961 markers was used, which should have provided sufficient coverage across the genome for conducting an association mapping study as the LD and genome length would indicate that as few as 113 markers would be sufficient for association mapping.

4.3.3 Association Mapping

Association mapping for individual breeding programs

No significant marker-trait associations were observed in either the OSU or VA panel which consisted of winter barley lines. Three significant SNP markers were observed in the USU panel on chromosome 3H. SNP markers 12_31409 and 11_20252 identified the first QTL (LD = 0.92) in the region spanning 72.60 to 64.60 cM and marker 11_20252 identified a second QTL located at 59.1 cM (Table 4.3).

Three SNP markers, 11_10964, 11_20266 and 11_21339 from a single location of 65.08 cM in chromosome 6H, were significant in the WSU panel. The three SNP markers were in high LD (0.9-0.96); thus, they were considered to be identifying a single QTL.

In the MSU panel, 15 significant SNP markers were identified in chromosome 6H (Table 4.3). The SNP markers spanned a region of 58.91 to 65.68 cM. Nine SNP markers (11_10270, 11_21339, 11_20266, 11_10964, 11_10635, 11_20058, 11_21310, 11_10189, 12_30346) spanning a region of 64.29-65.68 cM had high LD between them (R^2 = 0.8-1.0) and were considered to be identifying a single QTL. Similarly, four SNP markers (11_20835, 11_10227, 12_31178, 11_10377) spanning a region of 58.91-59.33 cM were in high LD (R^2 = 0.82-0.96) and were similarly considered to be identifying a single QTL. The remaining two SNP markers, 12_30144 and 11_11067 at 60.21 and 62.91 cM, respectively, each identified single QTL.

In the panel from USDA, five significant markers (12_30144, 12_30857, 11_20835, 11_10227, 12_31178) were identified in chromosome 6H, spanning the region from 58.91 to 60.21 cM, in addition to a marker (12_31230) from unknown location (Table 4.3). The pairwise LD between the five SNP markers in chromosome 6H was very high (R^2 = 0.9-1.0) and this region has significant overlap with the region identified with significant SNP markers in chromosome 6H in the MSU panel (Fig. 4.2).

Three significant SNPs (11_10377, 11_21281, 11_10244) were identified in chromosome 6H and one SNP in 3H (11_10343, 164.42 cM) in the MN panel. SNP marker 11_10377 had a map location of 59.33 that overlaps with the region (58-60 cM) detected in the MSU and USDA panels (Fig. 4.2). SNP markers 11_21281 and 11_10244 were within the region from 51.94 to 52.20 cM and demonstrated high LD (R^2 = 0.93).

Three QTL were detected in the N6 panel, with two detected in chromosome 3H and one in 6H. The first QTL detected in chromosome 3H was at 5.91 cM, identified by SNP marker 11_20159, and the second one was at 6.46-6.68 cM identified by two SNP

markers, 12_20252 and 12_10173, which were in high LD ($R^2 = 0.89$). Another QTL was detected spanning the region from 58.34 to 59.33 cM in chromosome 6H identified by two SNP markers, 12_30569 and 11_10513, in high LD ($R^2 = 0.94$). The location of this QTL in 6H overlaps with the QTL identified in the MSU, USDA and MN panels.

Six QTLs were identified in the BARI panel. Three of these QTLs were identified in chromosome 3H by three separate SNP markers (11_20159, 11_20252, 12_10173) located at 5.91 cM, 6.46 cM and 6.68 cM, respectively. Two additional QTLs were identified in chromosome 6H, the first one at 58.91 cM identified by two SNPs (12_30681 and 11_10749) with high LD ($R^2 = 0.95$), and the second one was identified by a single SNP marker 12_30144 at 60.21 cM. The sixth QTL identified in the BARI panel was associated with SNP marker 12_31230, at an unknown location. Although the SNP location is unknown, the marker had the very high $-\log_{10}(p)$ value of 10.15.

Association mapping for combined panels

Fifteen marker-trait associations with significant SNP markers were observed in the Barley CAP combined panel (all panels) (Table 4.4). Five markers were on chromosome 3H, one on chromosome 4H, eight on chromosome 6H and one was from an unknown location. Pairwise LD was calculated for significant SNPs within a region of 5 cM. Two SNPs (11_10227 and 11_20835) at 58.91 cM were in high LD ($R^2 = 0.99$) with each other and were thus considered to be identifying same QTL.

In the 2-row panel, sixteen marker-trait associations, or significant SNP markers, were observed. Thirteen SNP markers were significant in chromosome 6H, spanning the region from 57.64 to 64.29 cM. Individual SNP markers were also identified in

chromosomes 1H and 4H and at an unknown location (Table 4.4). Seven SNP markers, 11_10227, 11_20835, 11_20329, 12_31178, 11_10377, 12_30857 and 12_30144, with map positions ranging from 58.91-60.21 cM, identified a single QTL whereas SNP markers 11_10270, 11_10189, 11_20058 and 11_21310, from 64.29-65.38 cM, identified a second QTL.

In the 6-row panel, nine marker trait associations were observed. Four significant SNP markers (11_20159, 11_20252, 12_10173 and 11_20976) were identified in the region from 5.91 to 11.94 cM in chromosome 3H, but the LD between them was low (R^2 = 0.09-0.47) so it appeared that all of them were identifying individual loci. Three SNP markers (11_11153, 12_30305, 12_21482) at 58.91 cM were significant in chromosome 6H, two of which (11_11153 and 12_30305) were in high LD (R^2 = 1.0), thus these two were considered to be identifying a single QTL. The other two SNP markers that were significant in the 6-row panel were identified in chromosome 1H at 107.18 cM (12_30191) and at an unknown location (12_31230).

In the CAP-I panel, eight SNP markers were significant. Seven SNP markers were identified in chromosome 6H spanning a region from 58.34 to 65.38 cM and one SNP marker was significant in chromosome 3H, at 6.68 cM. Three SNP markers (11_21310, 11_20058 and 11_10189) from the same map position at 65.38 cM in chromosome 6H were in high LD (R^2 = 0.9-1.0) with each other and thus identified a single QTL.

In the CAP-II panel, nine significant marker-trait associations were observed, of which six were in chromosome 6H, spanning a region from 58.91 to 60.21 cM and three were from 3H spanning a region from 5.91 to 6.68 cM. Two SNP markers (11_10227 and

11_20835) at 58.91 cM in chromosome 6H were in high LD (R^2 = 0.89) and they identified a single QTL, while the remaining seven SNPs identified individual QTL.

Fourteen significant SNP markers were identified in the CAP-III panel, of which nine were in chromosome 6H, spanning a region from 58.34 to 60.21 cM. The remaining five SNP markers included two each in chromosomes 4H and 2H and one in chromosome 3H. Two SNPs, 11_20835 and 11_10227, from 58.91 cM of 6H were in high LD (R^2 = 0.92) and thus identified a single QTL. The two SNPs on chromosome 4H (11_10480 and 12_30450), located at 53.87 cM, were also in high LD (R^2 = 1.0) and identified a single QTL.

Six significant marker-trait associations were observed in the CAP-IV panel, of which five were in chromosome 6H between 58.91 and 64.29 cM, and one was from an unknown location. Of the five significant SNP markers in chromosome 6H, two SNP markers (11_20835 and 11_10227 from 58.91 cM) were in high LD (R^2 = 1.0) and thus they identified a single QTL.

Twenty-two SNP markers were detected in more than one association mapping panel (Table 4.6). Marker 11_20159 at 5.91 cM and marker 12_10173 at 6.68 cM in chromosome 3H were detected in seven panels, which were mostly combined panels. Similarly, markers 11_20835 and 11_10227 at 58.91 cM, 12_31178 at 59.21 cM and 11_10377 at 59.33 cM in chromosome 6H were significant in eight panels (MSU, N2, USDA, CAP-II, CAP-III, CAP-IV, 2-row and the Barley CAP combined). One SNP (12_31230), from an unknown location, was significant in all the combined panels except the BARI, N6, USDA, 2-row and CAP-IV panels. Similarly, markers from the region spanning 58-60 cM in chromosome 6H (11_10227 (58.91 cM), 11_20835 (58.91 cM),

11_10749 (58.91 cM), 12_30681 (58.91 cM), 11_10377 (59.33 cM), 12_30857 (59.33 cM), 12_30144 (60.21 cM)) (Fig. 4.2), were found to be common across several panels.

4.3.4 Marker Effects and Allele Frequencies

The additive genetic effects of individual markers in all the association mapping panels was calculated (Table 4.3 and Table 4.4). In addition to the marker effects, allele frequencies of all the SNP markers significant in individual breeding programs, were calculated to provide insight into the distribution of favorable alleles in the individual breeding programs. The favorable allele, or the allele contributing to resistance, was identified for the significant SNP markers across all analyses by looking at the alleles of lines with resistant phenotype. For example, for SNP marker 11_10635 in chromosome 6H with allelic forms T and C; T was determined to be the favorable (resistant) allele and C was determined to be the unfavorable allele, since all of the lines which had resistant phenotype had the T allele for SNP 11_10635 (Table 4.5).

Examining the allele frequencies of SNPs across different breeding program panels revealed segregation in all the breeding programs for resistance to *Ptt* as none of the SNPs demonstrated fixed allelic frequencies. The majority of SNPs observed had their allelic frequency skewed towards the unfavorable allele. Of the 56 MTAs with known map positions identified across the ten breeding program panels examined in this study, 28 had an allelic frequency skewed towards the unfavorable allele. In some panels with better net blotch resistance, the allelic frequencies were skewed towards the favorable allele. For example, five out of the six MTAs in the BARI panel, two out of three MTAs in the USU panel, and three out of five MTAs in the N6 panel had their

allele frequencies skewed toward the favorable allele. Although, the majority of the identified SNPs (across all panels) had allele frequencies skewed towards the unfavorable alleles, six out of 15 SNPs from the MSU and N2 panels had their allelic distribution skewed towards the favorable allele. In some panels, the allele frequency of all the significant SNPs appeared to be skewed towards the unfavorable allele. For example, in the WSU panel, all the significant SNP markers for resistance (11_10964, 11_20266 and 11_21339) have their allelic frequency skewed towards the unfavorable allele.

4.4 Discussion

Lines in all of the association mapping panels followed the expected, or normal, distribution pattern of disease response with higher numbers of individuals in the MR and MS categories for net blotch than in the R and S reaction classes, except in the N2 and BARI panels. In the N2 and BARI panels, over 60% of the lines evaluated fell into the resistant class. The germplasm in BARI and N2 breeding programs have had more selection for net blotch resistance as net blotch is an important constraint for barley production in the northern Great Plains and lines from the North Dakota 2-rowed breeding program have been used as sources of net blotch resistance in Australia (Jerome D. Franckowaik, personal communication).

Significant marker trait associations were not observed in the VA and OSU panels in this study. However, in a study done by Berger et al. (2013) using the same germplasm from the Virginia Polytechnic Institute and State University barley breeding program included in the Barley CAP using BOPA markers, two significant SNP markers, located at 39 and 60 cM in chromosome 5H were reported. No significant marker trait

associations were observed in the VA panel in this study and the SNPs with the highest -log_{10}(p) were from chromosome 1H. The difference in these findings, may be due to the use of different isolates of *Ptt* in the two studies.

Thirty-eight markers were found to be associated with net blotch resistance in the panels examined. Of the 38 significant markers, 24 markers were from a region spanning 51.94 – 65.38 cM in chromosome 6H. An additional eight markers were identified on chromosome 4H and seven markers were identified in chromosome 3H. This indicates that chromosomes 6H, 4H and 3H are the principal locations for net blotch resistance loci within the barley genome. Studies using bi-parental populations have also mapped net blotch resistance to regions in chromosomes 6H and 4H (McLean et al. 2009). Steffenson et al. (1996) mapped major QTLs conferring seedling resistance to net blotch in chromosomes 4H and 6H in a study of the 'Steptoe/Morex' doubled haploid population. The major QTL identified in this 6-row x 6-row population by was in the long arm of chromosome 6H. In another study, Ma et al. (2004) detected a major QTL in the short arm of chromosome 6H and a minor QTL in the short arm of chromosome 2H using a 6-rowed bi-parental population generated from a 'Chevron/Stander' cross. A major resistance gene was also mapped to the centromeric region of chromosome 6H in a population derived from a cross between 'Rolfi 'and 'CI 9819'and minor effect QTL were detected in chromosomes 1H, 3H, 4H, 5H and 7H using Finnish isolates of *P. teres* (Manninen et al. 2000; Manninen et al. 2006). Similarly, Grewal et al. (2008) also mapped a major QTL, *QRpt6,* in chromosome 6H, which was effective at both the seedling and adult plant stages, in a 'CDC Dolly/TR251' population. Some minor effect QTLs also were reported in chromosomes 4H and 5H in that study. Major effect QTLs

have also been identified in chromosome 6H by other groups (Cakir et al. 2003; Friesen et al. 2006) using both different sources of resistance and different *Ptt* isolates. The SNPs identified in chromosome 6H in the combined and individual association mapping panels of this study reside in the region spanning from the short arm to long arm (51.94 to 65.38 cM) in chromosome 6H. This region likely includes the major genes and QTLs identified previously. Our results cannot be directly compared with previous studies as different genetic maps were used and there are no common markers between these maps for this region of the genome. The previous studies were conducted using bi-parental mapping populations and older marker technologies. In our association mapping study, the large set of elite germplasm and new high throughput markers provided the capacity for higher resolution mapping. Thus, the results of our study yield better resolution of QTLs and enable the selection of resistance sources in individual breeding programs for the purpose of integrating net blotch resistance.

Previous studies, conducted to map resistance loci for net blotch (Bockelman et al. 1977; Grewal et al. 2008; Lehmensiek et al. 2008; Manninen et al. 2006), have reported both minor effect QTLs and major genes (Bockelman et al. 1977) in chromosome 5H, although no significant marker-trait associations were observed in chromosome 5H in any of the panels examined in this study. Previous studies also reported minor effect QTL in chromosome 3H (Manninen et al. 2000; Manninen et al. 2006). In our study, two SNP markers (11_20159 and 11_20252) located at 5.91 and 6.46 cM in chromosome 3H, respectively, were detected both in the combined 6-row panel and in the N6 panel, with -log(p) values of 10.74 and 10.49, respectively. These two SNP markers were also significant in the CAP-II panel and appear to identify a region of the

genome associated with resistance to *Ptt* in 6-rowed germplasm.

Marker trait associations for net blotch resistance in chromosome 4H was only observed in the N2 panel of this study, although this chromosome is reported to be associated with net blotch resistance by others (Grewal et al. 2008; Manninen et al. 2006; McLean et al. 2009; Steffenson et al. 1996). Three SNPs, located at 53.87 cM in chromosome 4H in the N2 panel, were significant and the allelic frequency for two of them (11_10577 and 11_21073) is skewed towards the favorable allele. A few other SNPs from the same location 53.87 cM of chromosome 4H were also significant in other panels (11_20269 in combined 2-row and Barley CAP panel, 11_10480 and 12_30450 in CAP-III panel) in this study.

More MTAs were detected in the 2-rowed panels (MSU and N2) compared to 6-rowed panels (N6 and MN). Similarly, a higher number of MTAs were detected in the BARI and USDA panels which were a mix of both 6-rowed and 2-rowed lines, compared to the panels comprised only of 6-rowed lines. The same phenomenon was evident in the combined 2-row and 6-row panels where more MTAs were detected in the 2-row panel than in the 6-row panel despite the 6-row panel having nearly equal number of lines and similar distributions of resistant and susceptible types.

Marker trait associations in the 2-rowed panels were detected most often in chromosome 6H, while MTAs in the 6-rowed panels were detected most often in chromosome 3H. MTAs in chromosomes 6H and 3H were evident in the MSU and N2 panels, which were exclusively 2-rowed panels, while in the MN and N6 panels, which are exclusively 6-rowed panels, and the BARI panel, which is a mix of 2-rowed and 6-

rowed lines, MTAs were identified in chromosomes 6H and 3H (Table 4.2). Of the MTAs identified in chromosome 6H, none of the MTAs significant in the 2-rowed and 6-rowed panels were in common. It appears therefore that resistance to the net form of net blotch is conferred by different loci in these two categories of germplasm. This suggests that different loci should be adopted in 6-row and 2-row barley breeding programs to integrate resistance.

Despite a proportion of lines in the OSU and VA panels appearing to be resistant to net blotch in the phenotypic evaluation, no marker trait associations for resistance were detected in these winter barley lines. The lack of MTAs detected could be because the frequency of resistance is very low and thus these rare variants fall below the threshold during the analysis.

The MSU and N2 panels had the most MTAs detected amongst the individual breeding programs examined. The MSU panel had 15 significant SNP markers, all mapping to chromosome 6H and spanning a region from 58.91-65.68 cM. The strongest associations were detected at 65.68 cM, which is different from the results of the other breeding program panels examined, with the exception of the WSU panel. The WSU panel had three SNPs significant at 65.68 cM, these being the same SNP markers that had the highest $-\log_{10}(p)$ values in the MSU panel. The significance of these SNP markers in these two panels may reflect common traits derived through germplasm exchange between the two programs, both being public 2-rowed spring barley breeding programs serving similar geographical regions. Despite the commonalities some differences between the significant SNPs in these two panels were also evident. In the N2 panel, 15 significant SNP markers were identified, six of the significant SNP markers were located

in chromosome 4H, while the remaining nine were in chromosome 6H. The strongest association was observed at 58.91 cM, in chromosome 6H in the N2 panel, which is at a different position from the strongest association detected in the MSU panel. The region strongly associated with net blotch resistance in the N2 panel spanned from 57.64 to 60.21 cM, peaking at 58.91 cM, which is in contrast with the MSU panel, although two SNP markers (11_20835 and 11_10227) were common at 58.91 cM. The BARI and USDA panels had a similar pattern of distribution of significant SNP markers to the N2 panel. The BARI and USDA panels have either the same two SNP markers (11_20835 and 11_10227) or SNP markers from the same 58-60 cM region of chromosome 6H significantly associated with net blotch resistance. In the case of the N6 and MN panels, which are both 6-rowed panels, none of the SNP markers in chromosomes 3H or 6H appear to be common. The SNP markers significant in the MN and N6 panels are from different positions in the same chromosome. The University of Minnesota barley breeding program and North Dakota State University's 6-rowed barley breeding program serve the barley industry in the same geographical region, and they have exchanged germplasm frequently for the last two decades (Kevin P. Smith and Richard D. Horsley, personal communication). Despite the commonalities in the germplasm pool, the alleles conferring net blotch resistance appear to be segregating at different loci. Thus, it appears that genomic regions associated with net blotch resistance are generally unique for these breeding programs, even though the MTAs were detected within the same chromosomes.

The analysis of United States barley breeding germplasm using high density markers provides insights into net blotch resistance. The elite germplasm from ten individual breeding programs were analyzed separately in this study and the significant

SNP markers detected identify loci that are important from a breeding perspective. To provide additional information to breeders, the frequency of alleles for significant markers in individual breeding programs was calculated (Table 4.5). Based on the allelic frequency of markers, breeders can make an informed decision as to direct resources to intensify selection within the program to skew the population towards the favorable alleles or to seek sources of resistance from outside of a given breeding program.

Net blotch is an economically important disease on barley in the United States and incorporating net blotch resistance is a priority for many of the breeding programs included in this study (Steffenson and Smith 2006). Resistance to net blotch is a complex trait and previous studies have identified genetic resistance conferred by QTL located within a large region of chromosome 6H. This study, using genome wide association mapping and conducted on elite barley breeding germplasm, provides valuable insights about the nature and distribution of net blotch resistance in barley. Our results confirm previous studies and have contributed a higher level of confidence in MTAs given the broader scale of germplasm we evaluated and the higher density of markers used in our association mapping study. This study also provides some insight for barley breeders about the nature of the genetic resistance to *Ptt* in their germplasm and thus may help them design strategies for effectively integrating resistance more effectively.

Table 4.1 Number of barley lines, number of single nucleotide polymorphism (SNP) markers and marker density across the genome for 17 different association mapping panels used to identify QTL associated with resistance to *Pyrenophora teres* f. *teres*

Panels	Number of Lines	Number of SNP markers	Marker density (SNPs/cM)	Panel Description
BARI	378	2022	1.8	Busch Agricultural Resources Inc.
MN	384	919	0.8	University of Minnesota
MSU	383	1631	1.4	Montana State University
N2	361	1742	1.5	North Dakota State University (two-row)
N6	379	1010	0.9	North Dakota State University (six-row)
OSU	279	2267	2.0	Oregon State University
USDA	381	2144	1.9	USDA Aberdeen
USU	320	2144	1.9	Utah State University
VA	245	1596	1.4	Virginia Polytechnic Institute and State University
WSU	380	1977	1.8	Washington State University
2-row	1692	2017	1.8	Combined panel with 2-rowed lines from all years and breeding programs
6-row	1798	2137	1.9	Combined panel with 6-rowed lines from all years and breeding programs
CAP-I	869	2336	2.1	Combined panel with lines from all breeding programs submitted to Barley Coordinated Agricultural Project (Barley CAP) in 2006
CAP-II	917	2380	2.1	Combined panel with lines from all breeding programs submitted to Barley CAP in 2007
CAP-III	852	2354	2.1	Combined panel with lines from all breeding programs submitted to Barley CAP in 2008
CAP-IV	852	2322	2.1	Combined panel with lines from all breeding programs submitted to Barley CAP in 2009
Barley CAP	3490	2342	2.1	Combined panel with lines from all years and breeding programs

Table 4.2 Mean infection response of Stander (susceptible) and BT-462 (resistant) included as checks in the screening of lines for response to *Pyrenophora teres* f. *teres* from ten breeding programs as part of the Barley Coordinated Agricultural Project in 2006, 2007, 2008 and 2009, referred to as Barley CAP-I, Barley CAP-II, Barley CAP-III and Barley CAP-IV, respectively

Breeding Program[a]	**Barley CAP-I (2006 entries)**		**Barley CAP-II (2007 entries)**		**Barley CAP-III (2008 entries)**		**Barley CAP-IV (2009 entries)**	
Check	Stander	BT-462	Stander	BT-462	Stander	BT-462	Stander	BT-462
BARI	8.0[b]	2.3	8.7	3.0	7.0	2.0	6.0	2.3
MN	8.3	2.7	8.7	3.3	8.0	2.3	7.7	2.7
MSU	8.0	2.7	7.7	2.3	6.0	3.0	7.0	3.0
N2	8.0	2.7	8.7	2.7	9.0	3.3	6.0	1.3
N6	9.0	2.3	8.3	3.3	9.0	2.7	7.3	3.0
OSU	9.0	3.7	8.7	4.3	7.3	3.7	7.0	3.7
USDA	8.7	3.3	8.0	2.7	7.7	1.7	8.0	2.3
USU	8.0	3.0	8.7	3.0	8.0	4.0	6.7	2.3
VA	9.7	5.0	9.3	4.7	8.0	2.0	8.3	4.0
WSU	7.3	2.7	8.0	2.3	8.0	3.7	7.7	3.0

[a] Breeding programs participating in the Barley CAP included; Busch Agricultural Resources Inc. (BARI), University of Minnesota (MN), Montana State University (MSU), North Dakota State University two-row (N2), North Dakota State University six-row (N6), Oregon State University (OSU), University of Minnesota (MN), USDA-ARS, Idaho (USDA), Utah State University (USU), and Washington State University (WSU).

[b] Responses were scored on the Tekauz (1985) numerical infection response scale.

Table 4.3 Significant marker-trait associations for net blotch (*Pyrenophora teres* f. *teres*) resistance identified in eight breeding program panels from lines submitted to the Barley Coordinated Agricultural Project (Barley CAP) from 2006 to 2009. Significant single nucleotide polymorphism (SNP) markers identified are given with their location (chromosome and map position), corresponding -log(p) value and marker effect. The association mapping panels used were; Busch Agricultural Resources Inc. (BARI), University of Minnesota (MN), Montana State University (MSU), North Dakota State University 2-row (N2), North Dakota State University 6-row (N6), USDA-ARS, Idaho (USDA), Utah State University (USU), and Washington State University (WSU)

Panel	SNP	Chr[a]	Map Position[b] (cM)	-log(p) Value[c]	Marker Effect
BARI	11_20159	3H	5.91	3.917	-0.050
	11_20252	3H	6.46	3.887	-0.053
	12_10173	3H	6.68	8.155	0.064
	12_30681	6H	58.91	5.428	0.033
	11_10749	6H	58.91	4.482	-0.026
	12_30144	6H	60.21	7.227	0.068
	12_31230	UNK[d]	0.88	10.146	0.090
MN	11_10343	3H	164.42	3.408	0.044
	11_21281	6H	51.94	3.416	0.029
	11_10244	6H	52.2	3.416	-0.029
	11_10377	6H	59.33	3.617	0.045
MSU	11_20835	6H	58.91	4.486	0.048
	11_10227	6H	58.91	4.153	0.045
	12_31178	6H	59.21	6.223	-0.058
	11_10377	6H	59.33	3.757	0.058
	12_30144	6H	60.21	5.321	0.052
	11_11067	6H	62.91	5.558	-0.054
	11_10270	6H	64.29	9.568	0.067
	11_21339	6H	65.08	8.667	0.062
	11_20266	6H	65.08	8.131	0.062
	11_10964	6H	65.08	4.375	0.045
	11_10635	6H	65.38	10.393	-0.050
	11_20058	6H	65.38	10.393	-0.057
	11_21310	6H	65.38	10.221	0.058
	11_10189	6H	65.38	9.579	-0.060
	12_30346	6H	65.68	10.221	-0.059
N2	11_10577	4H	53.87	4.849	-0.060
	11_10756	4H	53.87	3.898	0.051
	11_21073	4H	53.87	4.849	-0.060
	11_20269	4H	53.87	5.297	0.067

Table 4.3 continued

Panel	SNP	Chr[a]	Map Position[b] (cM)	-log(p) Value[3]	Marker Effect
N2	11_10942	4H	53.87	4.186	0.062
	12_30620	4H	59.32	3.771	-0.047
	12_30473	6H	57.64	4.122	-0.060
	12_30569	6H	58.34	4.592	0.067
	11_20835	6H	58.91	8.824	0.096
	11_10227	6H	58.91	8.510	0.093
	12_31178	6H	59.21	8.213	-0.098
	11_10377	6H	59.33	7.497	0.083
	11_10270	6H	64.29	6.475	0.080
	12_30857	6H	59.71	5.728	0.066
	12_30144	6H	60.21	5.728	0.066
N6	12_31230	UNK	0.45	17.739	0.349
	11_20159	3H	5.91	6.418	-0.200
	11_20252	3H	6.46	9.718	0.250
	12_10173	3H	6.68	8.347	0.200
	12_30569	6H	58.34	6.529	0.110
	11_10513	6H	59.33	5.444	-0.090
USDA	12_31230	UNK	1.07	4.056	0.046
	11_20835	6H	58.91	4.217	0.038
	11_10227	6H	58.91	4.188	0.038
	12_31178	6H	59.21	4.155	-0.038
	12_30857	6H	59.71	4.969	0.044
	12_30144	6H	60.21	5.987	0.044
USU	11_20252	3H	6.46	4.247	0.033
	12_31409	3H	7.26	4.837	-0.039
	11_20159	3H	5.91	4.862	-0.046
WSU	11_10964	6H	65.08	4.868	0.009
	11_20266	6H	65.08	4.868	0.009
	11_21339	6H	65.08	4.868	0.009

[a] Chr = chromosome.
[b] Map position of the SNP is based on map published by Muñoz-Amatriaín et al. (2011).
[c] -log(p) value of the SNP from corresponding association mapping panel.
[d] UNK denotes that the chromosomal location of the SNP is unknown.

Table 4.4 Significant marker-trait associations for net blotch (*Pyrenophora teres* f. *teres*) resistance identified in seven combined panels comprised of lines submitted to the Barley Coordinated Agricultural Project (Barley CAP) from 2006 to 2009. Significant single nucleotide polymorphism (SNP) markers identified are given with their location (chromosome and map position), corresponding -log(p) value and marker effect. The association mapping panels used were; the combined panel with all lines from all years and breeding programs (Barley CAP), the combined panel with all 2-rowed lines from all years and breeding programs (2-row), the combined panel with all 6-rowed lines from all years and breeding programs (6-row), and four combined panels with all lines from all breeding programs submitted to the Barley CAP in 2006, 2007, 2008 and 2009, referred to as CAP-I, CAP-II, CAP-III and CAP IV, respectively

Panel	SNP	Chr[a]	Map Position[b]	-log(p)[c]	Marker Effect
Barley CAP	11_20159	3H	5.91	3.489	0.012
	11_20252	3H	6.46	4.832	-0.015
	12_10173	3H	6.68	6.860	0.018
	12_31409	3H	7.26	8.152	0.009
	11_21398	3H	8.86	4.540	0.001
	11_20269	4H	53.87	3.316	0.008
	12_30473	6H	57.64	5.497	-0.011
	12_30569	6H	58.34	4.858	0.009
	11_10227	6H	58.91	5.504	0.007
	11_20835	6H	58.91	5.248	0.004
	12_30681	6H	58.91	3.448	0.002
	12_31178	6H	59.21	10.120	-0.004
	11_10377	6H	59.33	10.610	0.016
	12_30144	6H	60.21	8.259	0.004
	12_31230	UNK[d]	1.33	15.363	-0.010
6-row	12_31230	UNK	1.13	24.738	-0.009
	12_30191	1H	107.18	4.463	0.018
	11_20159	3H	5.91	10.740	0.009
	11_20252	3H	6.46	10.492	0.011
	12_10173	3H	6.68	5.512	-0.012
	11_20976	3H	11.94	4.814	0.001
	11_11153	6H	58.91	4.179	0.011
	12_30305	6H	58.91	4.060	0.010
	12_21482	6H	58.91	3.943	0.002
2-row	12_30672	1H	50.99	3.858	0.002
	11_20269	4H	53.87	4.068	-0.007
	12_30473	6H	57.64	4.174	0.016
	12_30569	6H	58.34	4.653	-0.013
	11_10227	6H	58.91	12.466	-0.001
	11_20835	6H	58.91	11.904	-0.002
	11_20329	6H	58.91	3.617	-0.002
	12_31178	6H	59.21	14.394	0.001
	11_10377	6H	59.33	11.880	0.001
	12_30857	6H	59.71	6.853	0.002
	12_30144	6H	60.21	10.563	-0.002
	11_10270	6H	64.29	5.719	0.001

Table 4.4 continued

Panel	SNP	Chr[a]	Map Position[b]	-log(p)[c]	Marker Effect
2-row (cont.)	11_20058	6H	65.38	4.510	0.001
	11_21310	6H	65.38	4.857	-0.002
	12_30848	UNK	0.81	4.298	0.002
CAP-I	12_10173	3H	6.68	4.455	0.073
	12_30473	6H	57.64	4.340	-0.050
	12_30569	6H	58.34	6.234	0.083
	11_10377	6H	59.33	4.991	0.078
	12_30144	6H	60.21	8.660	0.102
	11_21310	6H	65.38	5.071	0.058
	11_20058	6H	65.38	4.013	-0.056
	11_10189	6H	65.38	3.877	-0.047
	12_31230	UNK	1.33	5.389	0.097
CAP-II	11_20159	3H	5.91	5.351	-0.099
	11_20252	3H	6.46	6.666	-0.102
	12_10173	3H	6.68	8.320	0.106
	11_10227	6H	58.91	5.705	0.063
	11_20835	6H	58.91	5.705	0.063
	12_31178	6H	59.21	7.790	-0.085
	11_10377	6H	59.33	8.182	0.086
	12_30857	6H	59.71	4.785	0.066
	12_30144	6H	60.21	6.640	0.085
	12_31230	UNK	1.42	15.201	0.150
CAP-III	11_10651	2H	74.49	4.614	0.081
	11_20159	3H	5.91	3.595	-0.074
	12_10173	3H	6.68	7.753	0.095
	11_10480	4H	53.87	3.864	-0.047
	12_30450	4H	53.87	3.781	-0.044
	12_30473	6H	57.64	5.101	-0.066
	12_30569	6H	58.34	3.577	0.062
	11_20835	6H	58.91	4.937	0.062
	11_10227	6H	58.91	4.641	0.059
	12_30681	6H	58.91	3.630	0.036
	12_31178	6H	59.21	6.403	-0.077
	11_10377	6H	59.33	6.892	0.080
	12_30857	6H	59.71	4.908	0.069
	12_30144	6H	60.21	7.412	0.094
	12_31230	UNK	1.37	6.316	0.088
CAP-IV	11_10227	6H	58.91	7.449	0.040
	11_20835	6H	58.91	6.795	0.038
	12_31178	6H	59.21	8.017	-0.045
	11_10377	6H	59.33	6.670	0.031
	11_10270	6H	64.29	4.541	0.028
	12_10393	UNK	0.26	4.739	0.027
	11_10189	6H	65.38	5.223	0.000

[a] Chr = Chromosome.
[b] Map position (cM)of the SNP is based on the map published by Muñoz-Amatriaín et al. (2011).
[c] -log(p) value of the SNP from corresponding association mapping panel.
[d] UNK denotes that the chromosomal location of the SNP is unknown.

Table 4.5 Distribution of individuals with homozygous and heterozygous alleles for single nucleotide polymorphism (SNP) markers with significant marker-trait association for net blotch (*Pyrenophora teres* f. *teres*) resistance identified in association mapping panels from lines submitted by eight breeding programs to the Barley Coordinated Agricultural Project (Barley CAP) from 2006 to 2009

SNP	Chr[b]	Map Pos[c]	Panels[d]	Number of lines with corresponding alleles[e]			
11_20159	3H	5.91		A	C	A/C	N[f]
			BARI	127	247	127	124
			N6	124	246	5	4
			USU	104	211	2	3
11_20252	3H	6.46		T	C	T/C	N
			BARI	136	237	1	
			N6	99	273	6	1
			USU	73	239	3	4
12_10173	3H	6.68		T	C	T/C	
			BARI	80	294		
			N6	229	143	6	
12_31409	3H	7.26		T	C	T/C	
			USU	241	77	1	
11_10343	3H	164.42		T	C	T/C	
			MN	357	25	2	
11_10577	4H	53.87		T	C	T/C	
			N2	132	224	5	
11_10756	4H	53.87		T	G	T/G	
			N2	223	133	5	
11_10942	4H	53.87		T	C	T/C	
			N2	24	335	2	
11_20269	4H	53.87		T	C	T/C	
			N2	237	118	5	
11_21073	4H	53.87		A	G	A/G	
			N2	132	224	5	
12_30620	4H	59.32		A	G	A/G	
			N2	337	19	5	
11_21281	6H	51.94		A	G	A/G	
			MN	363	19	2	
11_10244	6H	52.2		A	T	A/T	
			MN	19	363	2	
12_30473	6H	57.64		A	G	A/G	
			N2	121	234	6	
12_30569	6H	58.34		T	G	T/G	
			N2	235	121	5	
			N6	343	35	1	
11_10227	6H	58.91		T	G	T/G	
			MSU	258	125		
			N2	256	104	1	
			USDA	243	136	2	
11_20835	6H	58.91		A	G	A/G	
			MSU	257	116	1	
			N2	252	104	5	
			USDA	133	243	5	
11_10749	6H	58.91		T	C		
			BARI	327	46		

Table 4.5 continued

SNP	Chr	Map Pos	Panels	Number of lines with corresponding alleles[e]			
12_30681	6H	58.91		T	C		
			BARI	51	327		
12_31178	6H	59.21		G	C	G/C	N
			MSU	144	237	2	
			N2	229	122	9	
			USDA	138	225	6	12
11_10377	6H	59.33		A	G	A/G	
			MSU	242	140	1	
			N2	130	224	7	
			MN	331	52	1	
11_10513	6H	59.33		T	C	T/C	
			N6	35	342	2	
12_30857	6H	59.71		T	C	T/C	
			N2	254	103	3	
			USDA	231	145	5	
12_30144	6H	60.21		A	G	A/G	
			MSU	300	82	1	
			N2	254	103	4	
			USDA	212	167	2	
			BARI	148	224	2	
11_11067	6H	62.91		T	C	T/C	
			MSU	104	278	1	
11_10270	6H	64.29		A	G	A/G	
			MSU	330	50	3	
			N2	125	231	4	
11_10964	6H	65.08		A	G	A/G	
			WSU	324	55	1	
			MSU	304	78	3	
11_20266	6H	65.08		T	C	T/C	
			WSU	327	55	1	
			MSU	331	53	1	
11_21339	6H	65.08		C	G	C/G	
			WSU	327	55	1	
			MSU	329	54		
11_10189	6H	65.38		A	G		
			MSU	33	350		
11_10635	6H	65.38		T	C		
			MSU	33	350		
11_20058	6H	65.38		T	C		
			MSU	33	350		
11_21310	6H	65.38		T	C	T/C	
			MSU	350	32	1	
12_30346	6H	65.68		G	C	G/C	
			MSU	32	350	1	

[a] Chr = Chromosomal location of the SNP.
[b] Map Pos = Map position (cM) of the SNP based on the map developed by Muñoz-Amatriaín et al. (2011).
[c] The eight panels examined were; Busch Agricultural Resources Inc. (BARI), University of Minnesota (MN), Montana State University (MSU), North Dakota State University 2-row (N2), North Dakota State University 6-row (N6), USDA-ARS, Idaho (USDA), Utah State University (USU), and Washington State University (WSU).
[d] Number of lines with the corresponding A, T, G or C allele for the particular marker.
[e] N = number of lines with missing data for a particular SNP.

Table 4.6 Significant marker trait associations for net blotch (*Pyrenophora teres* f. *teres*) resistance detected in more than one association mapping panel from lines submitted by ten breeding programs to the Barley Coordinated Agricultural Project (Barley CAP) from 2006 to 2009. Significant SNP markers are given with their location, (chromosome and map position) and the panels in which they were detected. Association mapping panels used were; Busch Agricultural Resources Inc. (BARI), University of Minnesota (MN), Montana State University (MSU), North Dakota 2-row (N2), North Dakota 6-row (N6), Utah State University (USU), USDA, Idaho (USDA), Washington State University (WSU), combined panel with all 2-rowed lines from all years and breeding programs (2-row), combined panel with all 6-rowed lines from all years and breeding programs (6-row), four combined panels with all lines from all breeding programs submitted to the Barley CAP in 2006, 2007, 2008 and 2009, referred to as CAP-I, CAP-II, CAP-III and CAP IV, respectively, and the combined panel with all lines from all years and breeding programs (Barley CAP)

SNP marker[a]	Chr[b]	Map Position[c] (cM)	Panels in which the SNP was detected
11_20159	3H	5.91	BARI, N6, USU, 6-row, CAP-II, CAP-III, Barley CAP
11_20252	3H	6.46	BARI, N6, USU, 6-row, CAP-II, Barley CAP
12_10173	3H	6.68	BARI, N6, 6-row, CAP-I, CAP-II, CAP-III, Barley CAP
12_31409	3H	7.26	USU, Barley CAP
11_20269	4H	53.87	N2, 2-row, Barley CAP
12_30473	6H	57.64	N2, 2-row, Barley CAP
12_30569	6H	58.34	N2, N6, 2-row, CAP-I, CAP-III, Barley CAP
11_10227	6H	58.91	MSU, N2, USDA, 2-row, CAP-II, CAP-III, CAP-IV, Barley CAP
11_20835	6H	58.91	MSU, N2, USDA, 2-row, CAP-II, CAP-III, CAP-IV, Barley CAP
12_30681	6H	58.91	BARI, CAP-III, Barley CAP
12_31178	6H	59.21	MSU, N2, USDA, 2-row, CAP-II, CAP-III, CAP-IV, Barley CAP
11_10377	6H	59.33	MN, MSU, 2-row, CAP-I, CAP-II, CAP-III, CAP-IV, Barley CAP
12_30857	6H	59.71	N2, USDA, 2-row, CAP-II, CAP-III
12_30144	6H	60.21	MSU, N2, USDA, 2-row, CAP-I, CAP-II, CAP-IV
11_10270	6H	64.29	MSU, N2, 2-row, CAP-IV
11_10964	6H	65.08	MSU, WSU
11_20266	6H	65.08	MSU, WSU
11_21339	6H	65.08	MSU, WSU
11_10189	6H	65.38	MSU, 2-row, CAP-I
11_20058	6H	65.38	MSU, 2-row, CAP-I
11_21310	6H	65.38	MSU, 2-row, CAP-I
12_31230	UNK[d]	0.45	BARI, N6, USDA, 6-row, CAP-I, CAP-II, CAP-III, Barley CAP

[a] SNP = Single nucleotide polymorphism marker.
[b] Chr = Chromosome.
[c] Map position of the SNP on the chromosome is based on map from Muñoz-Amatriaín et al. (2011).
[d] UNK denotes that the chromosomal location of the SNP is unknown.

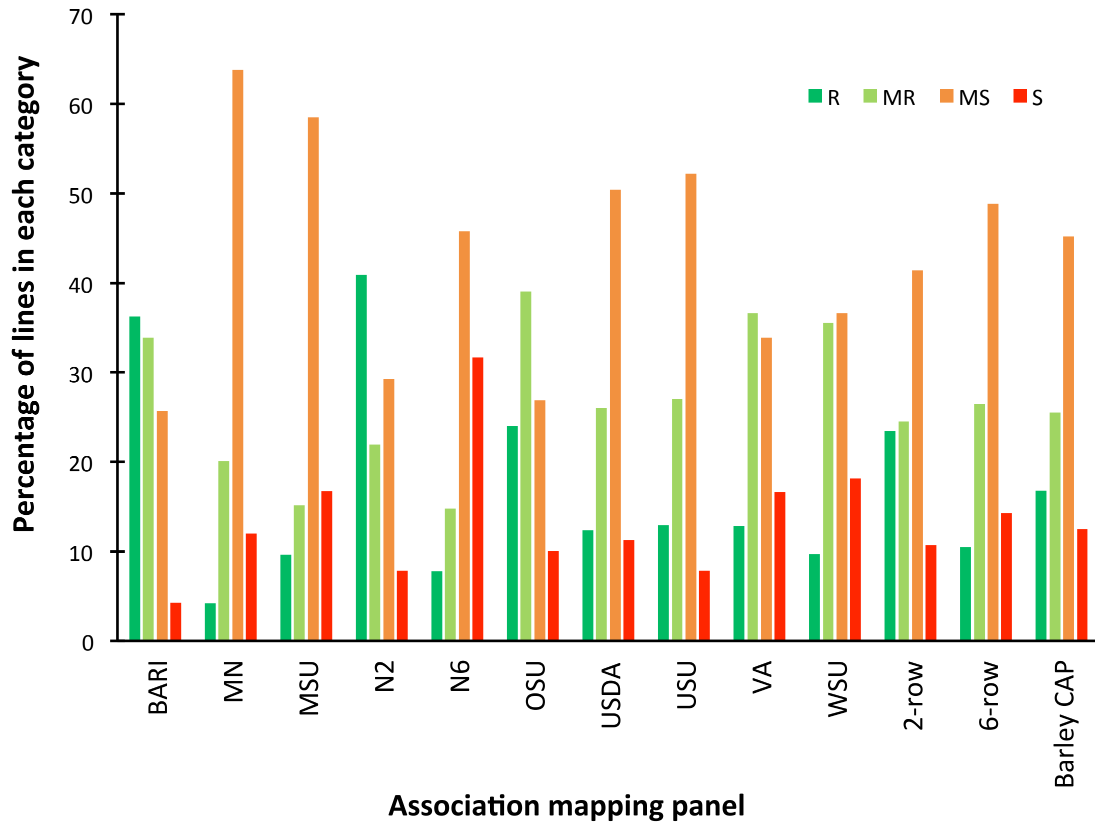

Figure 4.1 Frequency distribution of barley lines for reaction to *Pyrenophora teres* f. *teres* (*Ptt*) in 13 panels used for association mapping. The disease reaction classes used; R, resistant; MR, moderately resistant; MS, moderately susceptible; and S, susceptible, corresponded to the Tekauz (1985) infection responses of 1-3, 4-5, 6-7 and 8-10, respectively. The 13 association mapping panels examined were; Busch Agricultural Resources Inc. (BARI), University of Minnesota (MN), Montana State University (MSU), North Dakota State University 2-row (N2), North Dakota State University 6-row (N6), Oregon State University (OSU), USDA-ARS, Idaho (USDA), Utah State University (USU), Virginia Polytechnic Institute and State University (VA), Washington State University (WSU), all 2-rowed lines from all breeding programs submitted to Barley CAP from 2006 to 2009 (2-row), all 6-rowed lines from all breeding programs submitted to the Barley CAP from 2006 to 2009 (6-row) and all lines submitted to the Barley CAP in the years from 2006 to 2009 combined (Barley CAP).

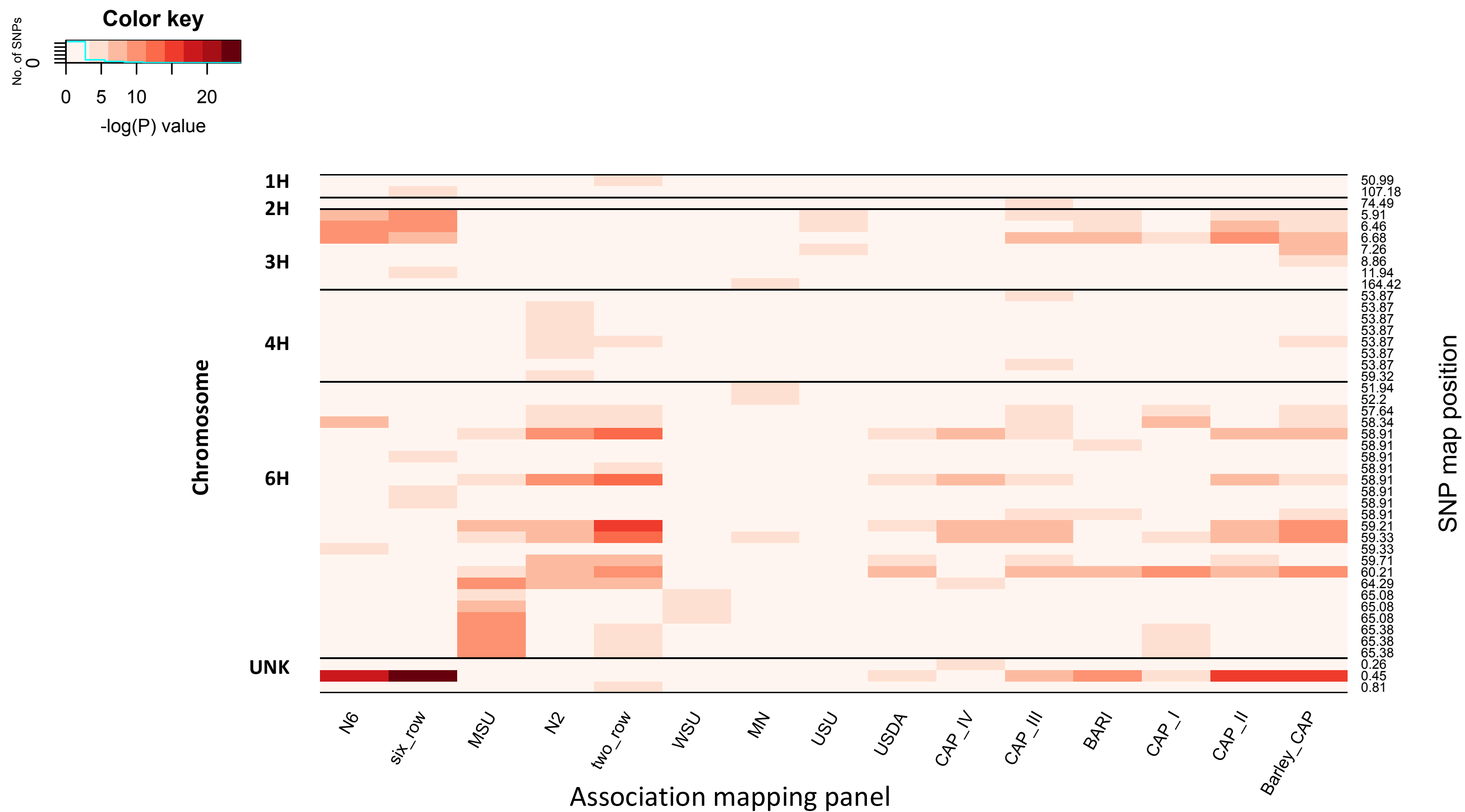

Figure 4.2 Heat map of -log(p) values of single nucleotide polymorphism (SNP) markers with significant marker trait associations for resistance to *Pyrenophora teres* f. *teres* for 13 association mapping panels. There were no significant markers identified on chromosome 5H. The association mapping panels used were; Busch Agricultural Resources Inc. (BARI), University of Minnesota (MN), Montana State University (MSU), North Dakota State University 2-row (N2), North Dakota State University 6-row (N6), USDA-ARS, Idaho (USDA), Utah State University (USU), Washington State University (WSU), all 2-rowed lines from all breeding programs submitted to Barley CAP from 2006 to 2009 (2-row), all 6-rowed lines from all breeding programs submitted to the Barley CAP from 2006 to 2009 (6-row), and all the lines submitted to the Barley CAP in the years from 2006 to 2009 combined (Barley CAP). UNK denotes SNP markers with unknown map positions.

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Appendix Table 2.1 Accession information for samples of hard red spring wheat, spring barley, spring oats, intermediate wheat grass, corn and other grasses collected in Minnesota from 2013 to 2015

Sample ID	Date Collected	Location	Crop[a]	Variety/Line
BY1	7/3/14	Fergus Falls	HRSW	MN13423
BY2	7/3/14	Fergus Falls	HRSW	Vantage
BY3	7/3/14	Fergus Falls	HRSW	MN13468
BY4	7/3/14	Fergus Falls	HRSW	MN13353
BY5	7/3/14	Fergus Falls	HRSW	MN13407
BY6	7/3/14	Fergus Falls	HRSW	MN13407
BY7	7/3/14	Fergus Falls	HRSW	MN13341
BY8	7/3/14	Le Center	HRSW	SD4299
BY9	7/3/14	Le Center	HRSW	MN06075-4
BY10	7/3/14	Le Center	HRSW	LNR10125
BY11	7/3/14	Le Center	HRSW	ND817
BY12	7/3/14	Le Center	HRSW	MN11079-7
BY13	7/3/14	Le Center	HRSW	Elgin-ND
BY14	7/3/14	Le Center	HRSW	HRS3378
BY15	7/3/14	Le Center	HRSW	Norden
BY16	7/3/14	Le Center	HRSW	HRS3419
BY17	7/3/14	Le Center	HRSW	Glenn
BY18	7/3/14	Le Center	HRSW	SY Rowyn
BY19	7/3/14	Le Center	HRSW	WB Mayville
BY20	7/14/14	Perley	HRSW	LCS Powerplay
BY21	7/14/14	Perley	HRSW	Marshall
BY22	7/14/14	Perley	HRSW	Prosper
BY23	7/14/14	Perley	HRSW	LCS Albany
BY24	7/14/14	Fergus Falls	HRSW	SY Soren
BY25	7/14/14	Perley	HRSW	LCS Iguaca
BY26	7/3/14	Le Center	HRSW	MN07098-6-Lr34

Appendix Table 2.1 continued

Sample ID	Date Collected	Location	Crop	Variety/Line
BY27	7/3/14	Le Center	HRSW	MN11452-3
BY28	7/3/14	Le Center	HRSW	RB07
BY29	7/3/14	Le Center	HRSW	Faller
BY30	7/3/14	Le Center	HRSW	MN08165-8
BY31	7/26/13	Crookston	SB	M150 (FEG215-05)
BY32	7/26/13	Crookston	SB	M156 (FEG234-18)
BY33	7/26/13	Crookston	SB	MS10S4115-03
BY34	7/26/13	Crookston	SB	Innovation
BY35	7/26/13	Crookston	SB	Rawson
BY36	7/26/13	Crookston	SB	MS10S4005-005
BY37	7/26/13	Crookston	SB	M157 (FEG260-09)
BY38	7/26/13	Crookston	SB	ND22421
BY39	7/26/13	Crookston	SB	07-N6-88-H-929H
BY40	7/26/13	Crookston	SB	MS10S4115-03
BY41	7/26/13	Crookston	SB	ND22421
BY42	7/26/13	Crookston	SB	G10W016-04
BY43	7/26/13	Crookston	SB	MS10S4013-023
BY44	7/26/13	Crookston	SB	Celebration
BY45	7/26/13	Crookston	SB	G10W016-04
BY46	7/26/13	Crookston	HRSW	MN10201-4
BY47	7/26/13	Crookston	HRSW	MN06075-4
BY48	8/1/13	Crookston	HRSW	ND817
BY49	8/1/13	Crookston	HRSW	Breaker
BY50	7/26/13	Crookston	SB	FEG250-16
BY51	7/26/13	Crookston	SB	Pinnacle
BY52	7/26/13	Crookston	SB	M159 (MS10S4013-019)

Appendix Table 2.1 continued

Sample ID	Date Collected	Location	Crop	Variety/Line
BY53	7/26/13	Crookston	HRSW	Select
BY54	7/26/13	Crookston	SB	Rasmusson
BY55	7/26/13	Crookston	HRSW	Forefront
BY56	7/26/13	Crookston	HRSW	ND817
BY57	7/26/13	Crookston	HRSW	MN10204-6
BY58	7/26/13	Crookston	HRSW	Barlow
BY59	8/1/13	Crookston	HRSW	MN10030-4
BY60	8/1/13	Crookston	HRSW	Propser
BY61	8/1/13	Crookston	HRSW	Samson
BY62	8/1/13	Crookston	HRSW	MN10261-1
BY63	8/1/13	Crookston	HRSW	Barlow
BY64	8/1/13	Crookston	HRSW	Advance
BY65	7/26/13	Crookston	SB	G10W016-04
BY66	7/22/13	Polk County	SB	unknown[e]
BY67	8/1/13	Crookston	HRSW	Elgin ND
BY68	7/22/13	Marshall County	HRSW	unknown
BY69	7/22/13	Polk County	HRSW	unknown
BY70	8/1/13	Crookston	HRSW	MN10292-8
BY71	7/9/13	Kimball	SB	unknown
BY72	7/9/13	Kimball	SB	unknown
BY73	7/9/13	Kimball	SB	unknown
BY74	7/9/13	Kimball	SB	unknown
BY75	7/9/13	Kimball	SB	unknown
BY76	7/15/14	Oklee	SB	Conlon
BY77	7/15/14	Oklee	SB	Quest
BY78	7/15/14	Oklee	SB	Conlon

Appendix Table 2.1 continued

Sample ID	Date Collected	Location	Crop	Variety/Line
BY79	7/15/14	Oklee	SB	Celebration
BY80	7/18/14	Strathcona	SB	Rawson
BY81	7/18/14	Strathcona	SB	Celebration
BY82	7/18/14	Strathcona	HRSW	MN10281-1
BY83	7/15/14	Oklee	SB	M152
BY84	7/15/14	Oklee	SB	Stellar ND
BY85	8/1/13	Crookston	HRSW	MN68165-8
BY86	8/1/13	Crookston	HRSW	Norden
BY87	8/1/13	Crookston	HRSW	MN10281-1
BY88	8/1/13	Crookston	HRSW	ND816
BY89	8/1/13	Crookston	HRSW	MN10201-4
BY90	8/1/13	Crookston	HRSW	Knudson
BY91	8/1/13	Crookston	HRSW	SD189
BY92	8/1/13	Crookston	HRSW	WB Mayville
BY93	8/1/13	Crookston	HRSW	MN06075-4
BY94	8/1/13	Crookston	HRSW	Faller
BY95	8/11/14	Sabin	HRSW	Samson
BY96	8/11/14	Sabin	HRSW	Samson
BY97	8/11/14	Sabin	HRSW	Samson
BY98	8/11/14	Sabin	HRSW	Samson
BY99	7/14/14	Fergus Falls	HRSW	MN13317
BY100	7/8/13	Fergus Falls	HRSW	Vantage
BY101	7/8/13	Fergus Falls	HRSW	WB Digger
BY102	7/8/13	Fergus Falls	HRSW	ND819
BY103	7/8/13	Fergus Falls	HRSW	MN12496
BY104	7/8/13	Fergus Falls	HRSW	SD4189

Appendix Table 2.1 continued

Sample ID	Date Collected	Location	Crop	Variety/Line
BY105	7/8/13	Fergus Falls	HRSW	ND819
BY106	7/8/13	Fergus Falls	HRSW	ND819
BY107	7/8/13	Fergus Falls	HRSW	Marshall
BY108	7/8/13	Fergus Falls	HRSW	Vantage
BY109	7/8/13	Fergus Falls	HRSW	MN11473
BY110	7/15/14	Oklee	HRSW	Elgin-ND
BY111	7/3/14	Le Center	HRSW	Jenna
BY112	6/29/15	Oklee	HRSW	MN1202-6
BY113	6/29/15	Oklee	SB	Stellar-ND
BY114	7/29/15	Oklee	HRSW	MN11492-6
BY115	7/29/15	Oklee	HRSW	HRS3419
BY116	7/29/15	Oklee	SB	Celebration
BY117	7/29/15	Oklee	HRSW	RB07
BY118	7/29/15	Oklee	HRSW	MN14170
BY119	7/29/15	Oklee	SB	Rawson
BY120	7/29/15	Oklee	HRSW	LNR-0311
BY121	7/29/15	Oklee	HRSW	MN14327
BY122	7/29/15	Oklee	HRSW	MN12013W-4
BY123	7/29/15	Oklee	HRSW	MN14850
BY124	7/29/15	Oklee	HRSW	Linkert
BY125	7/29/15	Oklee	HRSW	MN12333-3
BY126	7/29/15	Stephen	SO	Souris
BY127	7/29/15	Stephen	SO	WIX9645-1
BY128	7/29/15	Stephen	HRSW	MN14648
BY129	7/29/15	Stephen	HRSW	RB07
BY130	7/29/15	Stephen	SB	S6M166

Appendix Table 2.1 continued

Sample ID	Date Collected	Location	Crop	Variety/Line
BY131	7/29/15	Stephen	SB	212
BY132	7/29/15	Stephen	SB	64
BY133	7/29/15	Stephen	SO	GMI423
BY134	7/29/15	Stephen	SB	214
BY135	7/29/15	Stephen	SO	Tack
BY136	7/29/15	Stephen	SO	MN10121
BY137	7/29/15	Stephen	SO	volunteer oat
BY138	7/29/15	Stephen	SB	M159(MS10S4013-019)
BY139	7/29/15	Stephen	SO	MN09103
BY140	6/30/15	Perley	HRSW	MN14802
BY141	6/30/15	Perley	HRSW	MN144436
BY142	6/30/15	Perley	SB	M152
BY143	6/30/15	Perley	HRSW	Linkert
BY144	6/30/15	Perley	SB	Stellar-ND
BY145	6/30/15	Perley	HRSW	MN14075
BY146	6/30/15	Perley	HRSW	MN14223
BY147	6/30/15	Perley	HRSW	MN14284
BY148	6/30/15	Perley	HRSW	MN14604
BY149	6/30/15	Perley	HRSW	Faller
BY150	6/30/15	Perley	HRSW	MN10201-4-B
BY151	6/29/15	Perley	HRSW	Oklee
BY152	6/30/15	Perley	SB	Tradition
BY153	7/13/15	unknown*	SO	unknown
BY154	7/13/15	unknown*	SO	unknown
BY155	7/13/15	unknown*	SO	unknown
BY156	7/13/15	unknown*	SO	unknown

Appendix Table 2.1 continued

Sample ID	Date Collected	Location	Crop	Variety/Line
BY157	7/13/15	unknown*	SO	unknown
BY158	7/13/15	unknown*	SO	unknown
BY159	7/24/15	Crookston	SO	unknown
BY160	7/24/15	Crookston	SB	unknown
BY161	7/24/15	Crookston	SB	unknown
BY162	7/24/15	Crookston	SB	unknown
BY163	7/24/15	Crookston	SB	unknown
BY164	7/24/15	Crookston	SB	unknown
BY165	7/24/15	Crookston	SB	unknown
BY166	7/27/15	Crookston	SB	unknown
BY167	7/31/15	Crookston	SB	EEBC233
BY168	7/31/15	Crookston	SB	M61
BY169	7/31/15	Crookston	SB	volunteer barley
BY170	7/31/15	Crookston	SB	volunteer barley
BY171	7/31/15	Crookston	SB	EEBC231
BY172	7/31/15	Crookston	SB	volunteer barley
BY173	7/31/15	Crookston	SB	volunteer barley
BY174	7/31/15	Crookston	SB	M61
BY175	7/31/15	Crookston	Grass	unknown
BY176	7/31/15	Crookston	SB	M61
BY177	7/31/15	Crookston	SB	M61
BY178	7/31/15	Crookston	SB	EEBC58
BY179	7/31/15	Crookston	SB	EEBC61
BY180	7/31/15	Crookston	Grass	unknown
BY181	7/31/15	Crookston	Grass	unknown
BY182	7/12/15	St. Paul	HRSW	unknown

Appendix Table 2.1 continued

Sample ID	Date Collected	Location	Crop	Variety/Line
BY183	7/12/15	St. Paul	HRSW	unknown
BY184	7/12/15	St. Paul	HRSW	unknown
BY185	7/12/15	St. Paul	HRSW	unknown
BY186	7/12/15	St. Paul	HRSW	unknown
BY187	7/12/15	St. Paul	HRSW	unknown
BY188	7/12/15	St. Paul	SB	unknown
BY189	7/12/15	St. Paul	SB	HR659B069
BY190	7/12/15	St. Paul	SB	unknown
BY191	7/12/15	St. Paul	SB	unknown
BY192	7/12/15	St. Paul	HRSW	unknown
BY193	7/12/15	St. Paul	SB	unknown
BY194	7/12/15	St. Paul	SB	unknown
BY195	7/12/15	St. Paul	SB	unknown
BY196	7/12/15	St. Paul	HRSW	Samson
BY197	7/12/15	St. Paul	HRSW	Samson
BY198	7/12/15	St. Paul	HRSW	Samson
BY199	7/12/15	St. Paul	HRSW	Samson
BY200	7/12/15	St. Paul	HRSW	Samson
BY201	7/12/15	St. Paul	HRSW	Samson
BY202	7/12/15	St. Paul	HRSW	Samson
BY203	7/12/15	St. Paul	SB	HR672S071
BY204	7/12/15	St. Paul	SO	unknown
BY205	7/12/15	St. Paul	SO	unknown
BY206	7/12/15	St. Paul	SO	unknown
BY207	7/12/15	St. Paul	SO	unknown
BY208	7/12/15	St. Paul	Grass	unknown

Appendix Table 2.1 continued

Sample ID	Date Collected	Location	Crop	Variety/Line
BY209	7/12/15	St. Paul	Corn	unknown
BY210	7/12/15	St. Paul	HRSW	MN10261-1
BY211	7/31/15	St. Paul	SB	unknown
BY212	7/10/15	St. Paul	IWG	unknown
BY213	7/10/15	St. Paul	IWG	unknown
BY214	7/10/15	St. Paul	IWG	unknown
BY215	7/10/15	St. Paul	IWG	unknown
BY216	7/10/15	St. Paul	IWG	unknown
BY217	7/10/15	St. Paul	IWG	unknown
BY218	7/10/15	St. Paul	IWG	unknown
BY219	7/10/15	St. Paul	IWG	unknown
BY220	7/10/15	St. Paul	IWG	unknown
BY221	7/10/15	St. Paul	IWG	unknown
BY222	7/10/15	St. Paul	IWG	unknown
BY223	7/10/15	St. Paul	IWG	unknown
BY224	7/10/15	St. Paul	IWG	unknown
BY225	7/10/15	St. Paul	IWG	unknown
BY226	7/10/15	St. Paul	IWG	unknown
BY227	7/10/15	St. Paul	IWG	unknown
BY228	7/10/15	St. Paul	IWG	unknown
BY229	7/10/15	St. Paul	IWG	unknown
BY230	7/10/15	St. Paul	HRSW	MN06075-4
BY231	7/10/15	St. Paul	HRSW	Linkert
BY232	7/10/15	St. Paul	WW	unknown
BY233	7/10/15	St. Paul	HRSW	MN11394-6
BY234	7/10/15	St. Paul	Grass	unknown

Appendix Table 2.1 continued

Sample ID	Date Collected	Location	Crop	Variety/Line
BY235	7/10/15	St. Paul	HRSW	MN10201-4-B
BY236	7/10/15	St. Paul	HRSW	Cromwell
BY237	7/10/15	St. Paul	HRSW	Alsen
BY238	7/10/15	St. Paul	HRSW	MN11394-6
BY239	7/10/15	St. Paul	HRSW	MN11325-7
BY240	7/10/15	St. Paul	HRSW	MN11325-7
BY241	7/10/15	St. Paul	HRSW	Bolles
BY242	5/29/15	Canningham	SO	Dean
BY243	5/29/15	Canningham	SO	unknown

[a] Abbreviations for hosts are; HRSW, hard red spring wheat; SB, spring barley; IWG, intermediate wheat grass; SO, spring oats; WW, winter wheat; and Grass, unidentified grass. Asterisks (*) indicate sample collected by field scouts.

Appendix Table 2.2 Plant samples, collected in Minnesota in 2013, 2014 and 2015, that tested positive for B/CYDV subgroups I and II by polymerase chain reaction (PCR). Samples that tested positive for subgroup I, were further differentiated into strains PAV, MAV and SGV by a second PCR test

Sample				Subgroup I					
ID	Year	Site	Host[a]	PAV	MAV	SGV	Unidentified	Subgroup II	Mixed[b]
BY9	2014	Le Center	HRSW	●[c]	⊖[d]	⊖		●	✖
BY11	2014	Le Center	HRSW	●	⊖	⊖		⊖	
BY18	2014	Le Center	HRSW	●	⊖	⊖		⊖	
BY22	2014	Perley	HRSW	●	⊖	⊖		⊖	
BY24	2014	Fergus Falls	HRSW	●	⊖	⊖		⊖	
BY29	2014	Le Center	HRSW	⊖	⊖	⊖		●	
BY71	2013	Kimball	SB	●	⊖	⊖		⊖	
BY72	2013	Kimball	SB	●	⊖	⊖		⊖	
BY73	2013	Kimball	SB	●	⊖	⊖		⊖	
BY74	2013	Kimball	SB	⊖	⊖	⊖		●	
BY75	2013	Kimball	SB	●	⊖	⊖		⊖	
BY95	2014	Sabin	HRSW	●	⊖	⊖		⊖	
BY96	2014	Sabin	HRSW	●	⊖	⊖		⊖	
BY97	2014	Sabin	HRSW	●	⊖	⊖		●	✖
BY98	2014	Sabin	HRSW	●	⊖	⊖		⊖	
BY102	2013	Fergus Falls	HRSW	●	⊖	⊖		⊖	
BY105	2013	Fergus Falls	HRSW	●	⊖	⊖		⊖	

Appendix Table 2.2 continued

BY ID	Year	Site	Host	Subgroup I				Subgroup II	Mixed
				PAV	MAV	SGV	Unidentified		
BY106	2013	Fergus Falls	HRSW	●	⊖	⊖		⊖	
BY109	2013	Fergus Falls	HRSW	●	⊖	⊖		⊖	
BY112	2015	Oklee	HRSW	●	⊖	⊖		⊖	
BY114	2015	Oklee	HRSW	●	⊖	⊖		⊖	
BY115	2015	Oklee	HRSW	●	⊖	⊖		⊖	
BY117	2015	Oklee	HRSW	⊖	⊖	⊖	□[e]	●	✖
BY118	2015	Oklee	HRSW	●	⊖	⊖		⊖	
BY119	2015	Oklee	SB	⊖	⊖	⊖		●	
BY121	2015	Oklee	HRSW	●	⊖	⊖		⊖	
BY124	2015	Oklee	HRSW	⊖	⊖	⊖	□	⊖	
BY132	2015	Stephen	SB	⊖	⊖	⊖		●	
BY138	2015	Stephen	SB	●	⊖	⊖		⊖	
BY140	2015	Perley	HRSW	●	⊖	⊖		⊖	
BY143	2015	Perley	HRSW	⊖	⊖	⊖	□	⊖	
BY144	2015	Perley	SB	●	⊖	⊖		⊖	
BY149	2015	Perley	HRSW	⊖	⊖	⊖		●	
BY150	2015	Perley	HRSW	●	⊖	⊖		⊖	
BY153	2015	Perley	SO	●	⊖	⊖		⊖	

Appendix Table 2.2 continued

BY ID	Year	Site	Host	Subgroup I PAV	Subgroup I MAV	Subgroup I SGV	Subgroup I Unidentified	Subgroup II	Mixed
BY154	2015	Perley	SO	●	⊖	⊖		⊖	
BY156	2015	Perley	SO	●	⊖	⊖		⊖	
BY157	2015	Perley	SO	⊖	⊖	⊖	◻	⊖	
BY158	2015	Perley	SO	⊖	⊖	⊖		●	
BY168	2015	Crookston	SB	●	⊖	⊖		⊖	
BY174	2015	Crookston	SB	●	⊖	⊖		⊖	
BY182	2015	St. Paul	HRSW	●	⊖	⊖		⊖	
BY183	2015	St. Paul	HRSW	⊖	⊖	⊖	◻	⊖	
BY184	2015	St. Paul	HRSW	●	⊖	⊖		⊖	
BY185	2015	St. Paul	HRSW	●	⊖	⊖		⊖	
BY186	2015	St. Paul	HRSW	●	⊖	⊖		⊖	
BY187	2015	St. Paul	HRSW	●	⊖	⊖		⊖	
BY188	2015	St. Paul	HRSW	⊖	⊖	⊖		●	
BY189	2015	St. Paul	SB	●	⊖	⊖		⊖	
BY191	2015	St. Paul	SB	⊖	⊖	⊖	◻	⊖	
BY192	2015	St. Paul	HRSW	●	⊖	⊖		⊖	
BY195	2015	St. Paul	SB	●	⊖	⊖		⊖	
BY196	2015	St. Paul	HRSW	●	⊖	⊖		⊖	

Appendix Table 2.2 continued

BY ID	Year	Site	Host	Subgroup I				Subgroup II	Mixed
				PAV	MAV	SGV	Unidentified		
BY199	2015	St. Paul	HRSW	●	⊖	⊖		⊖	
BY200	2015	St. Paul	HRSW	●	⊖	⊖		⊖	
BY201	2015	St. Paul	HRSW	⊖	⊖	⊖	◘	⊖	
BY202	2015	St. Paul	HRSW	●	⊖	⊖		⊖	
BY206	2015	St. Paul	SO	⊖	⊖	⊖		●	
BY207	2015	St. Paul	SO	⊖	⊖	⊖		●	
BY208	2015	St. Paul	Grass	⊖	⊖	⊖		●	
BY213	2015	St. Paul	IWG	⊖	⊖	⊖		●	
BY232	2015	St. Paul	WW[f]	●	⊖	⊖		⊖	

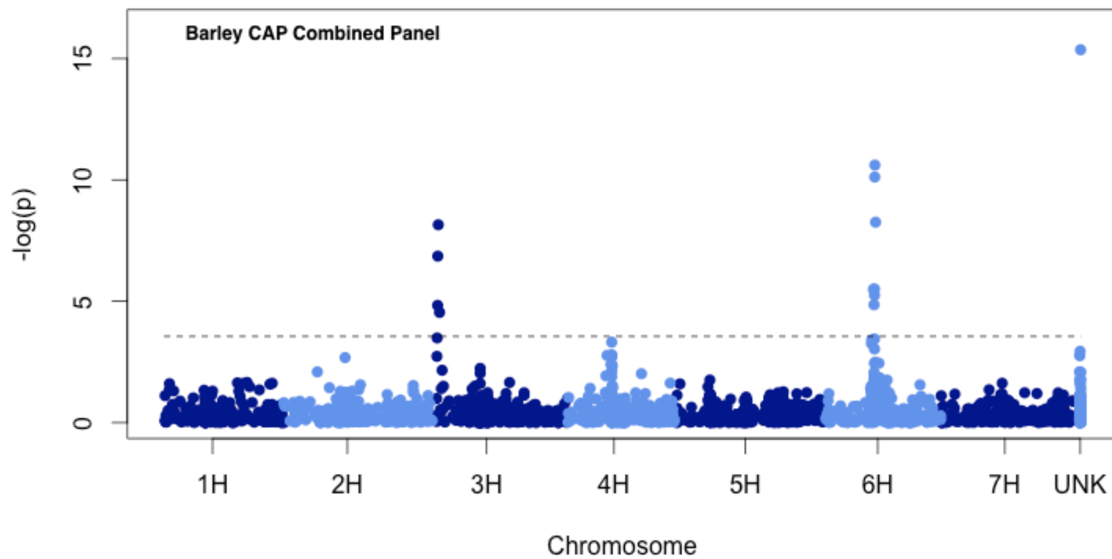
[a] Abbreviations for hosts are; HRSW, hard red spring wheat; SB, spring barley; IWG, intermediate wheat grass; SO, spring oats; WW, winter wheat; and Grass, unidentified grasses.

[b] Mixed (✖) = sample testing positive for both subgroups I and II.

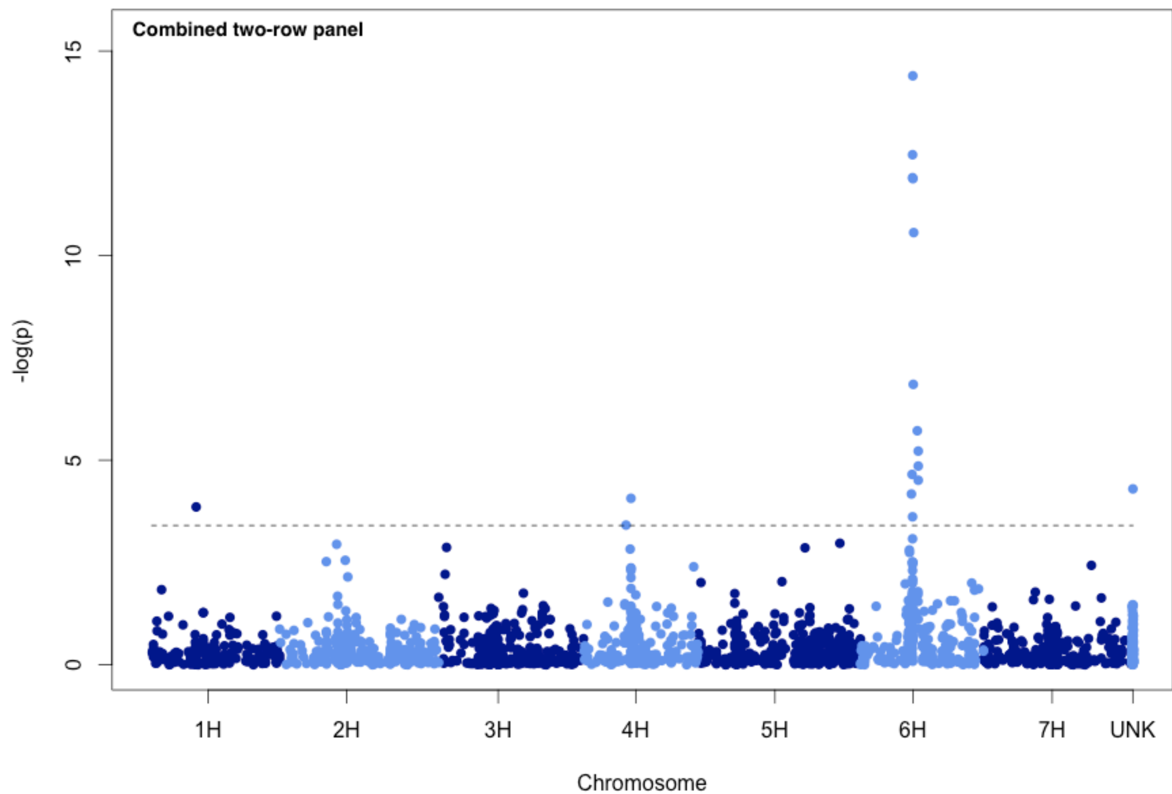
[c] ● = sample testing positive for strain or subgroup.

[d] ⊖ = sample testing negative for the particular test.

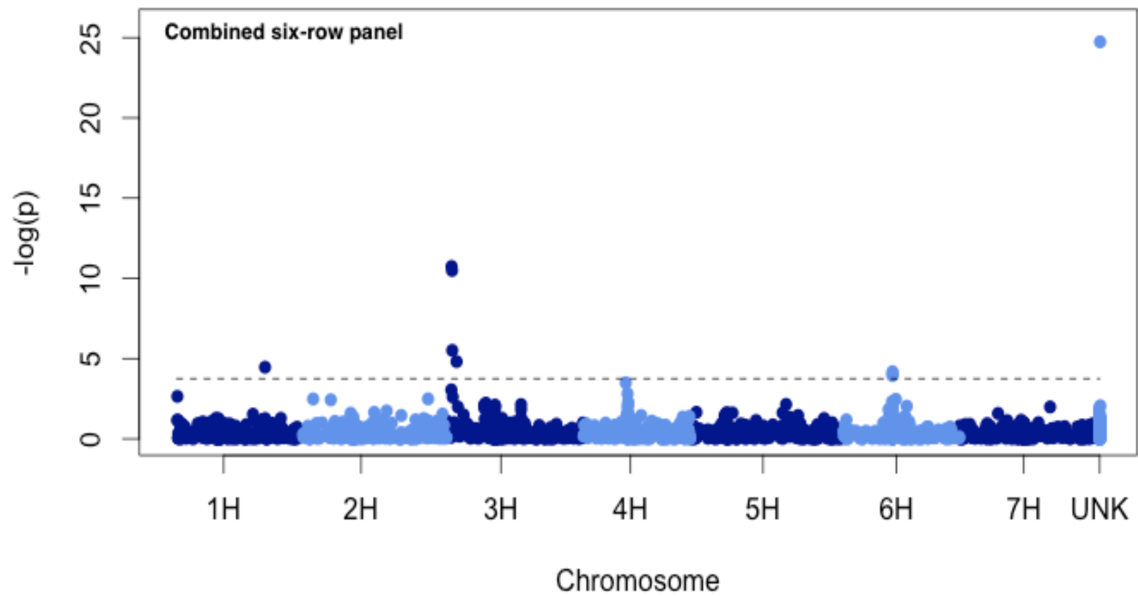
[e] ◘ = strain within subgroup I could not be determined.



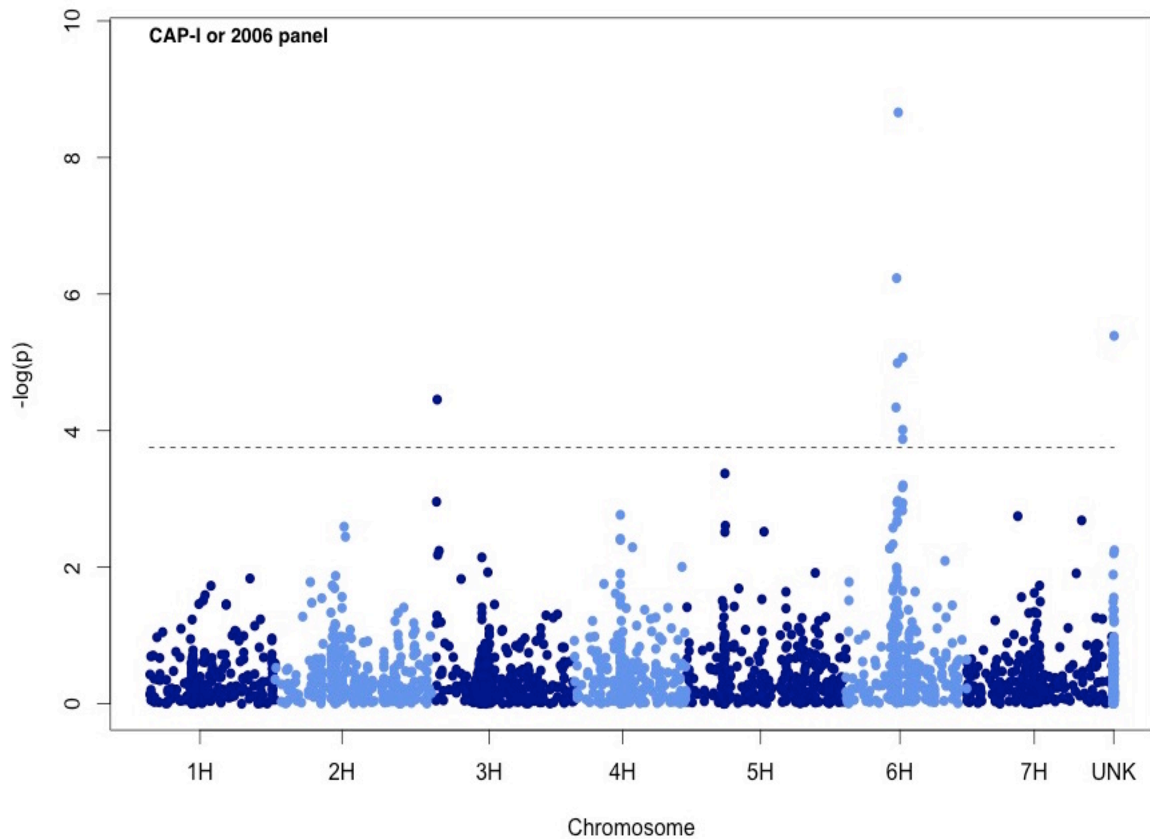
Appendix Fig. 4.1 Manhattan plot for markers used in a genome-wide association mapping study examining resistance to *Pyrenophora teres* f. *teres* in the combined panel with all barley breeding lines submitted to the Barley Coordinated Agricultural Project from 2006 to 2009. The x-axis shows the chromosomal positions of the markers and the y-axis the -log(p) values of the markers. UNK indicates markers with unknown map positions. The dotted line represents the significance threshold for a false discovery rate of 0.05.



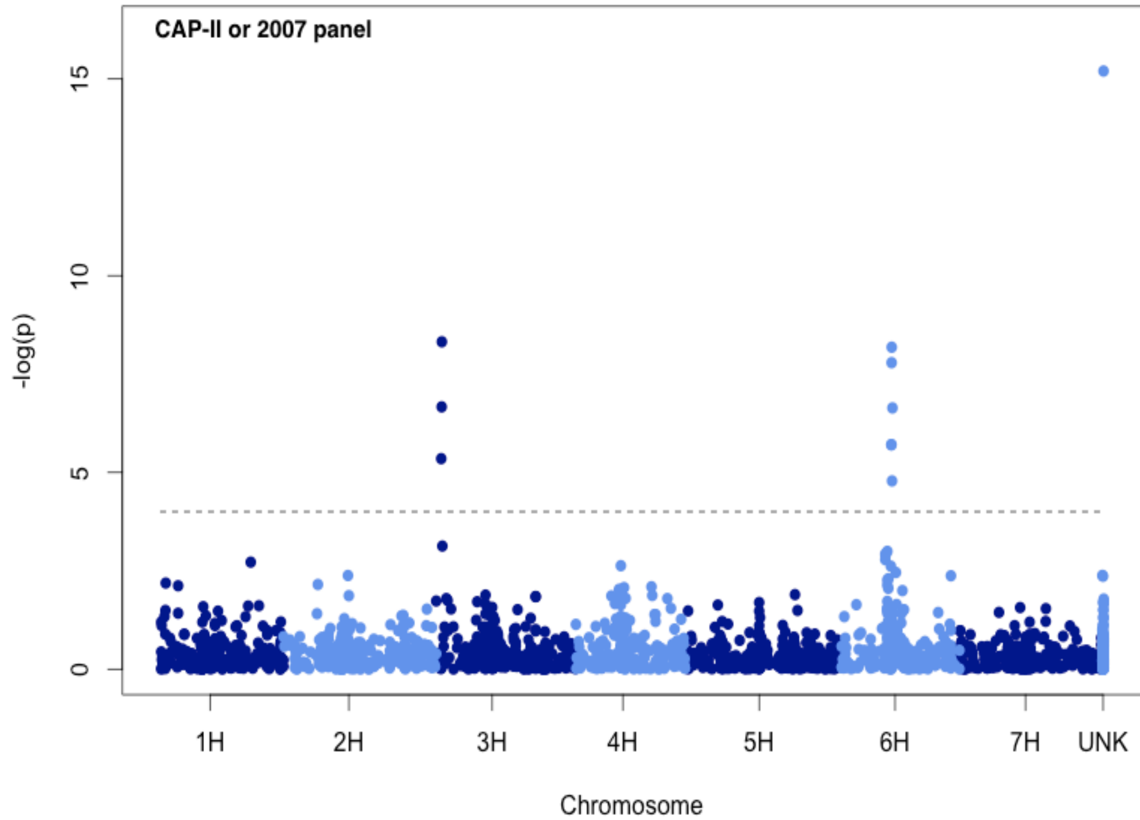
Appendix Fig. 4.2 Manhattan plot for markers used in a genome-wide association mapping study examining resistance to *Pyrenophora teres* f. *teres* in the two-row panel with all two-rowed barley breeding lines submitted to the Barley Coordinated Agricultural Project from 2006 to 2009. The x-axis shows the chromosomal positions of the markers and the y-axis the -log(p) values of the markers. UNK indicates markers with unknown map positions. The dotted line represents the significance threshold for a false discovery rate of 0.05.



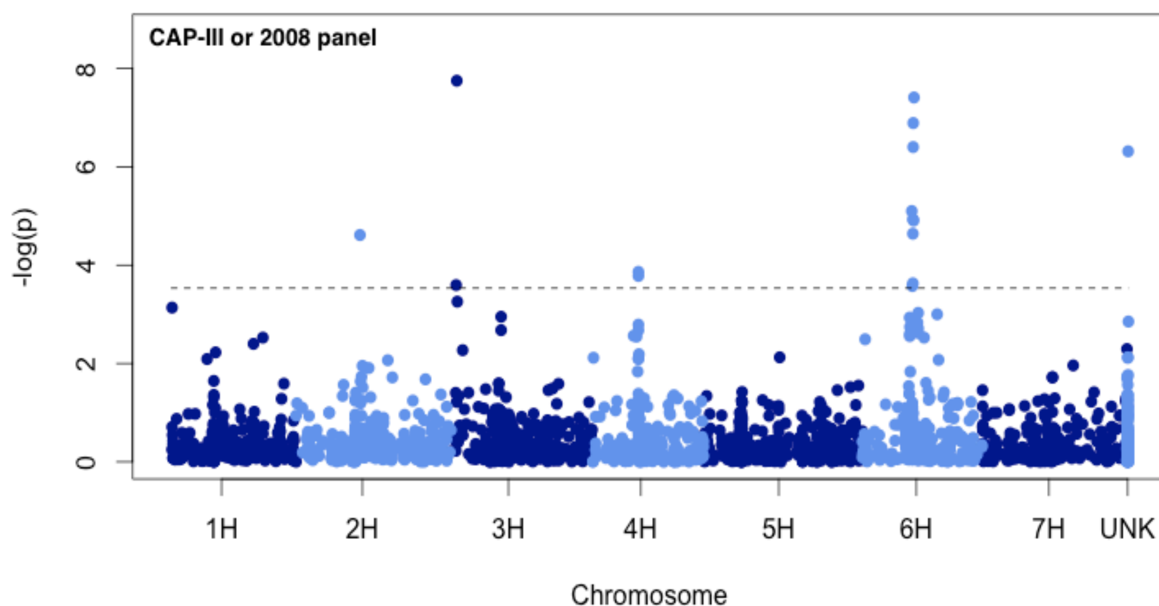
Appendix Fig. 4.3 Manhattan plot for markers used in a genome-wide association mapping study examining resistance to *Pyrenophora teres* f. *teres* in the six-row panel with all six-rowed barley breeding lines submitted to the Barley Coordinated Agricultural Project from 2006 to 2009. The x-axis shows the chromosomal positions of the markers and the y-axis the -log(p) values of the markers. UNK indicates markers with unknown map positions. The dotted line represents the significance threshold for a false discovery rate of 0.05.



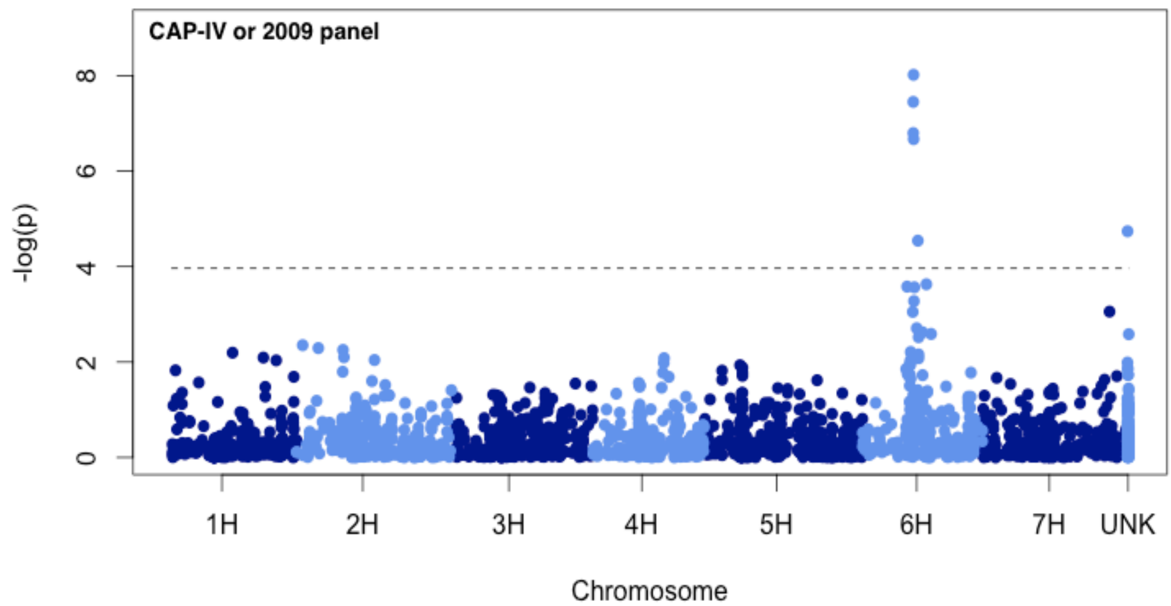
Appendix Fig. 4.4 Manhattan plot for markers used in a genome-wide association mapping study examining resistance to *Pyrenophora teres* f. *teres* in the CAP-I panel with all barley breeding lines submitted to the Barley Coordinated Agricultural Project in 2006. The x-axis shows the chromosomal positions of the markers and the y-axis the -log(p) values of the markers. UNK indicates markers with unknown map positions. The dotted line represents the significance threshold for a false discovery rate of 0.05.



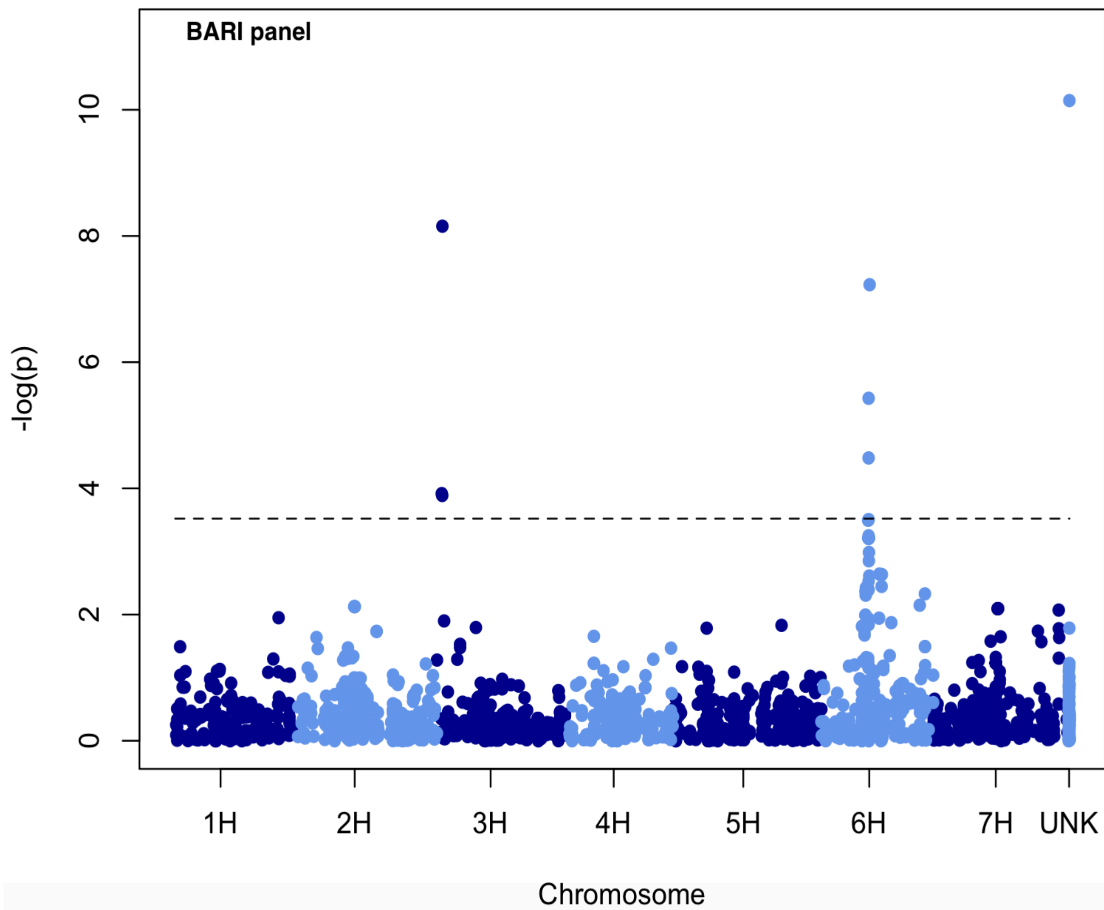
Appendix Fig. 4.5 Manhattan plot for markers used in a genome-wide association mapping study examining resistance to *Pyrenophora teres* f. *teres* in the CAP-II panel with all barley breeding lines submitted to the Barley Coordinated Agricultural Project in 2007. The x-axis shows the chromosomal positions of the markers and the y-axis the -log(p) values of the markers. UNK indicates markers with unknown map positions. The dotted line represents the significance threshold for a false discovery rate of 0.05.



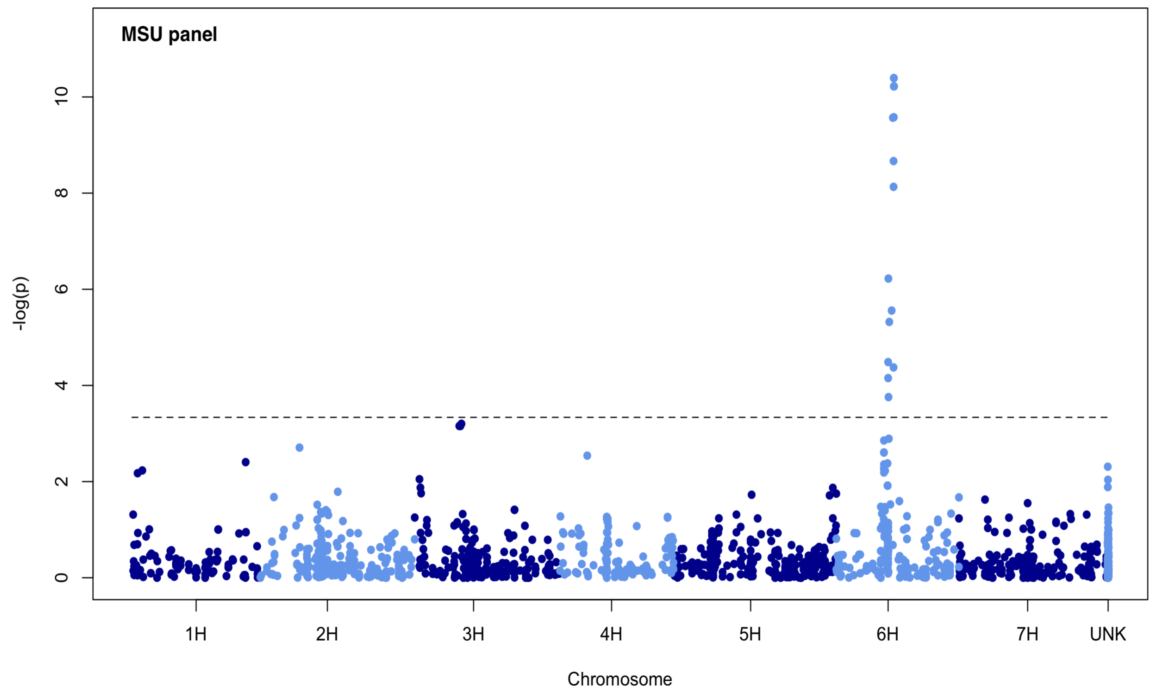
Appendix Fig. 4.6 Manhattan plot for markers used in a genome-wide association mapping study examining resistance to *Pyrenophora teres* f. *teres* in the CAP-III panel with all barley breeding lines submitted to the Barley Coordinated Agricultural Project in 2008. The x-axis shows the chromosomal positions of the markers and the y-axis the -log(p) values of the markers. UNK indicates markers with unknown map positions. The dotted line represents the significance threshold for a false discovery rate of 0.05.



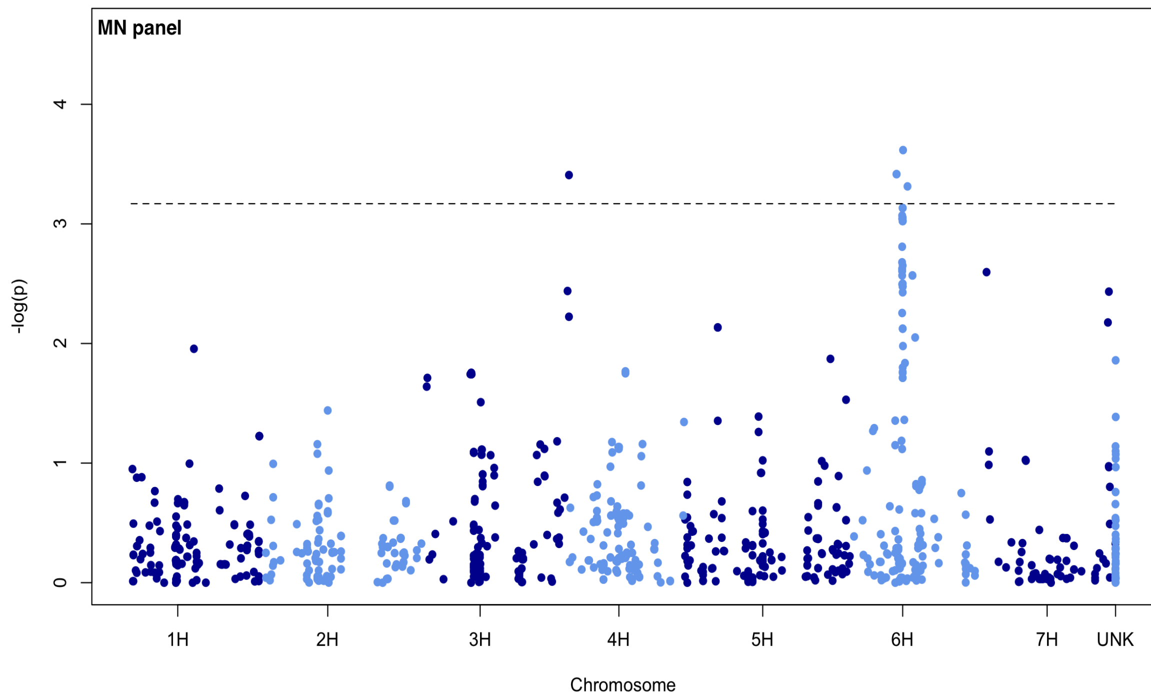
Appendix Fig. 4.7 Manhattan plot for markers used in a genome-wide association mapping study examining resistance to *Pyrenophora teres* f. *teres* in the CAP-IV panel with all barley breeding lines submitted to the Barley Coordinated Agricultural Project in 2009. The x-axis shows the chromosomal positions of the markers and the y-axis the -log(p) values of the markers. UNK indicates markers with unknown map positions. The dotted line represents the significance threshold for a false discovery rate of 0.05.



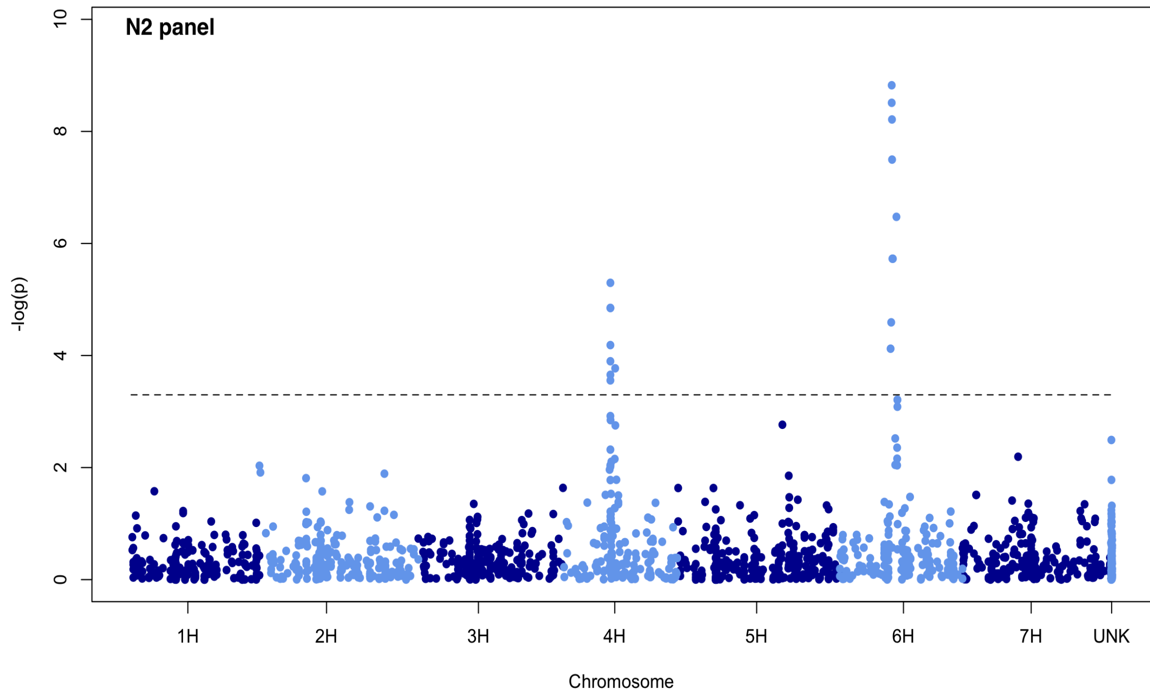
Appendix Fig. 4.8 Manhattan plot for markers used in a genome-wide association mapping study examining resistance to *Pyrenophora teres* f. *teres* in the BARI panel with all barley breeding lines submitted to the Barley Coordinated Agricultural Project by Busch Agricultural Resources, Inc. from 2006 to 2009. The x-axis shows the chromosomal positions of the markers and the y-axis the -log(p) values of the markers. UNK indicates markers with unknown map positions. The dotted line represents the significance threshold for a false discovery rate of 0.05.



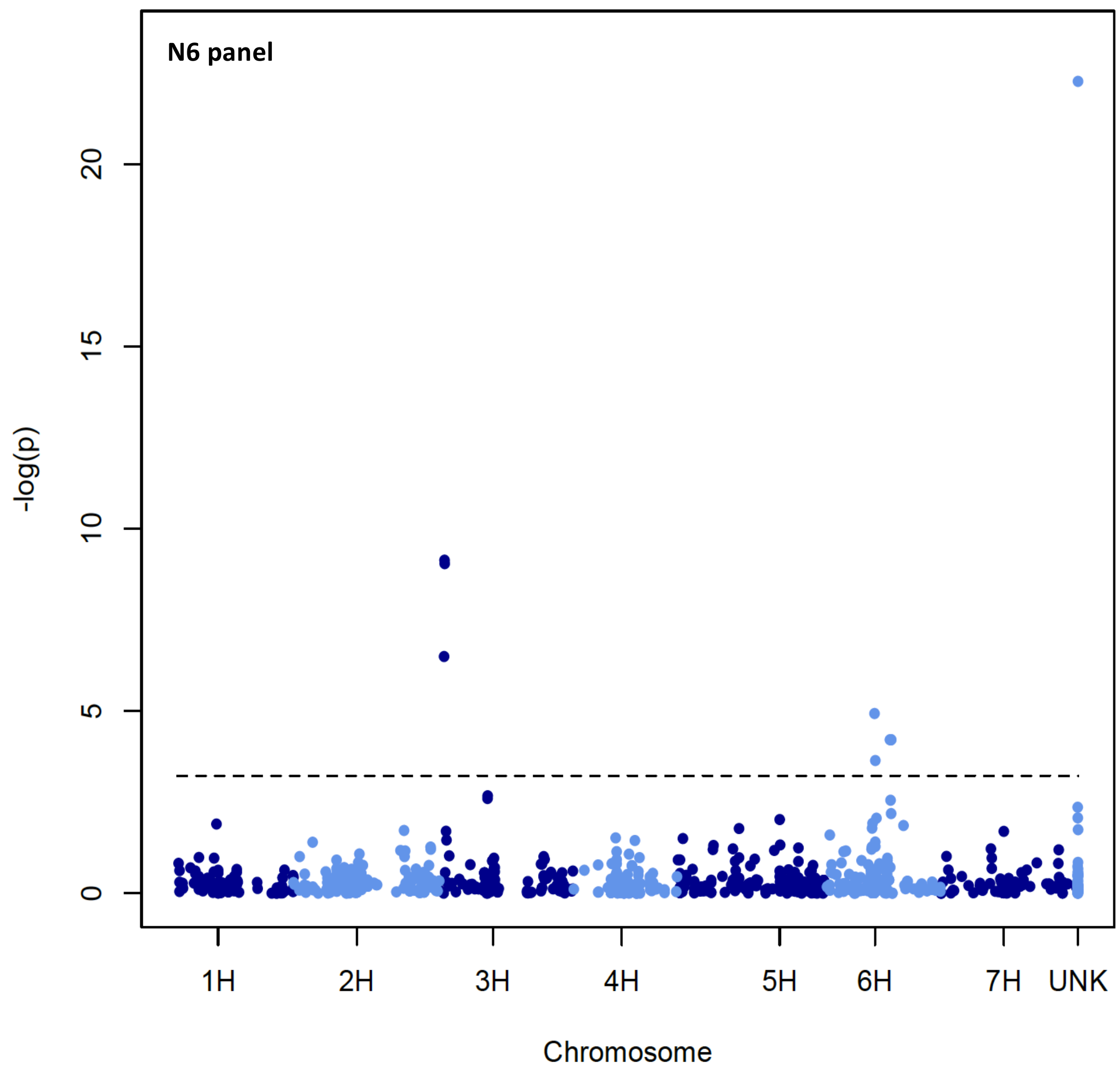
Appendix Fig. 4.9 Manhattan plot for markers used in a genome-wide association mapping study examining resistance to *Pyrenophora teres* f. *teres* in the MSU panel with all barley breeding lines submitted to the Barley Coordinated Agricultural Project by Montana State University from 2006 to 2009. The x-axis shows the chromosomal positions of the markers and the y-axis the -log(p) values of the markers. UNK indicates markers with unknown map positions. The dotted line represents the significance threshold for a false discovery rate of 0.05.



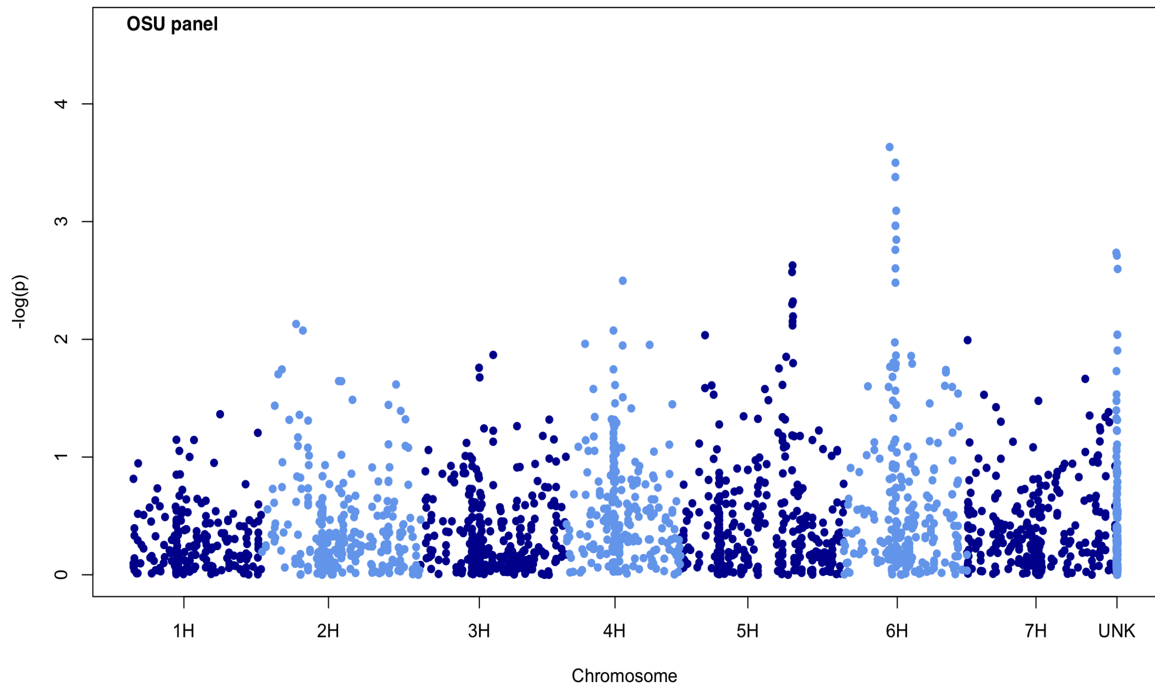
Appendix Fig. 4.10 Manhattan plot for markers used in a genome-wide association mapping study examining resistance to *Pyrenophora teres* f. *teres* in the MN panel with all barley breeding lines submitted to the Barley Coordinated Agricultural Project by the University of Minnesota from 2006 to 2009. The x-axis shows the chromosomal positions of the markers and the y-axis the -log(p) values of the markers. UNK indicates markers with unknown map positions. The dotted line represents the significance threshold for a false discovery rate of 0.05.



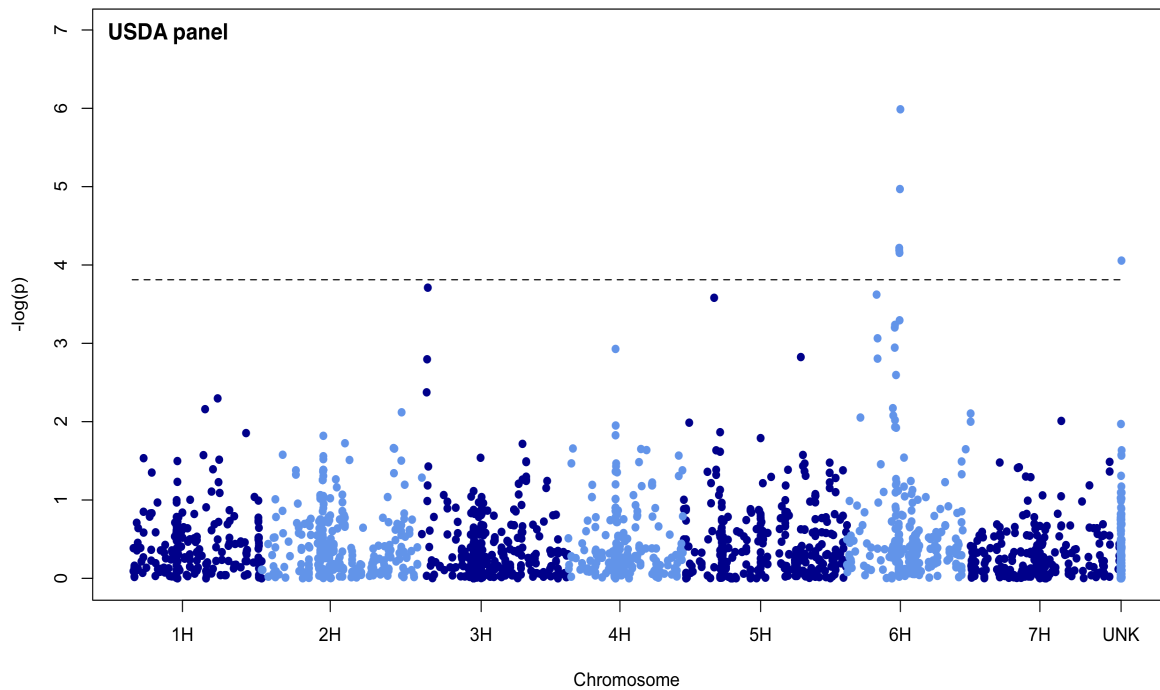
Appendix Fig. 4.11 Manhattan plot for markers used in a genome-wide association mapping study examining resistance to *Pyrenophora teres* f. *teres* in the N2 panel with all barley breeding lines submitted to the Barley Coordinated Agricultural Project by North Dakota State University's two-rowed barley breeding program from 2006 to 2009. The x-axis shows the chromosomal positions of the markers and the y-axis the -log(p) values of the markers. UNK indicates markers with unknown map positions. The dotted line represents the significance threshold for a false discovery rate of 0.05.



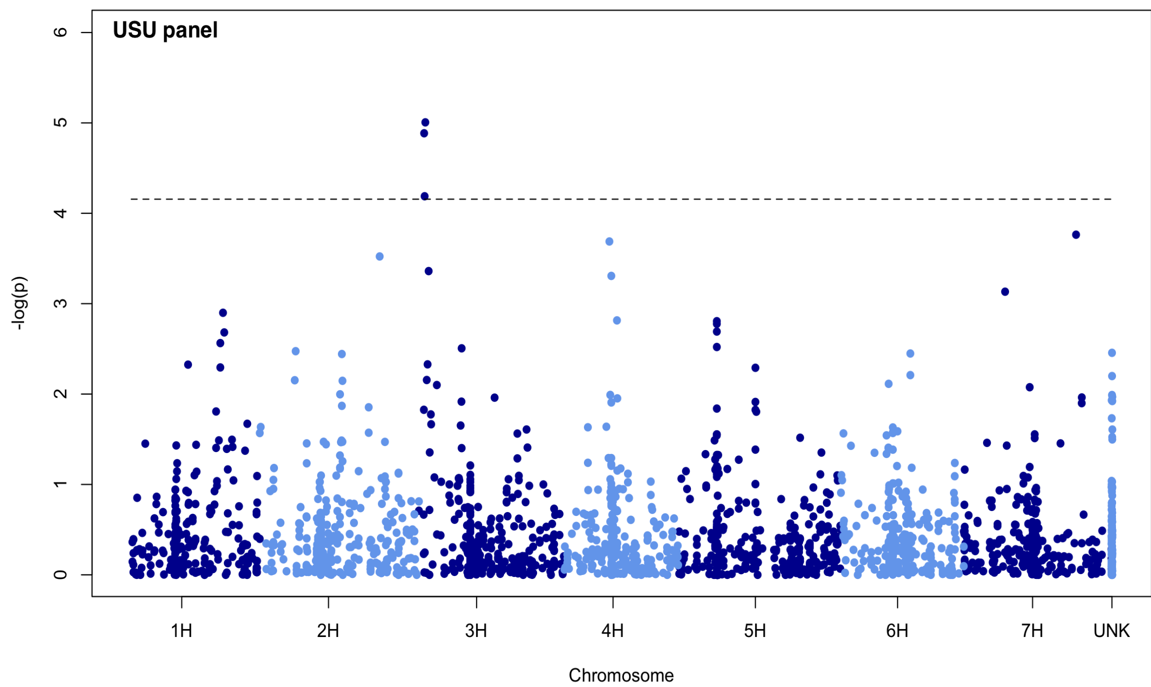
Appendix Fig. 4.12 Manhattan plot for markers used in a genome-wide association mapping study examining resistance to *Pyrenophora teres* f. *teres* in the N6 panel with all barley breeding lines submitted to the Barley Coordinated Agricultural Project by North Dakota State University's six-rowed barley breeding program from 2006 to 2009. The x-axis shows the chromosomal positions of the markers and the y-axis the -log(p) values of the markers. UNK indicates markers with unknown map positions. The dotted line represents the significance threshold for a false discovery rate of 0.05.



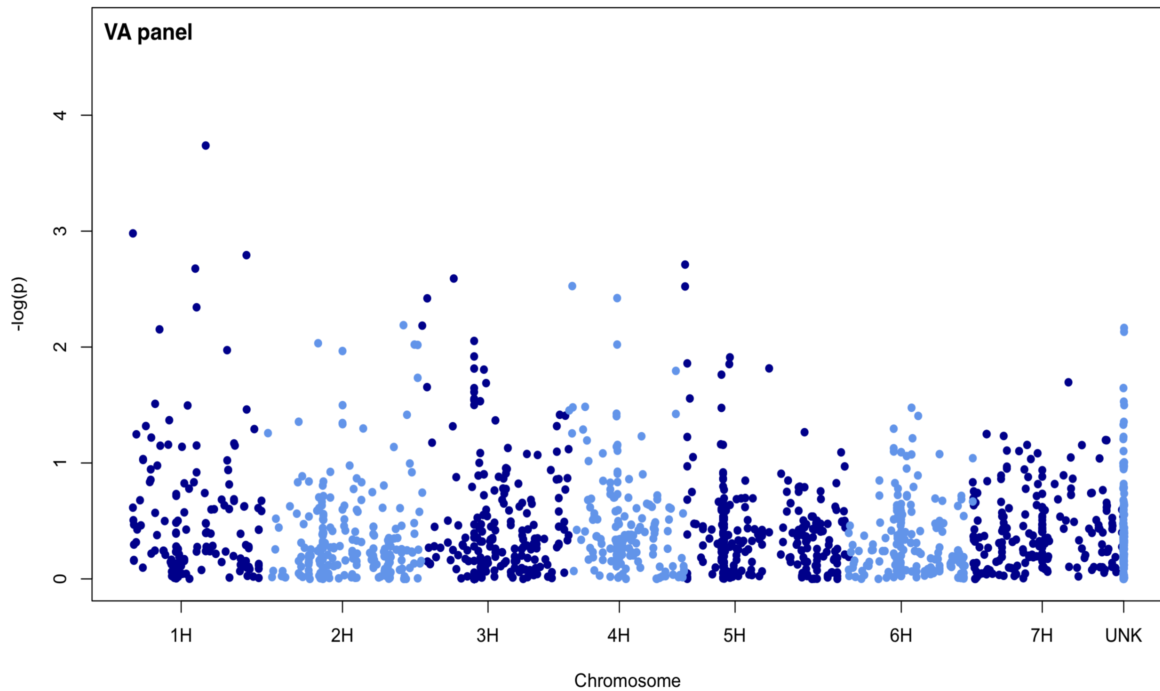
Appendix Fig. 4.13 Manhattan plot for markers used in a genome-wide association mapping study examining resistance to *Pyrenophora teres* f. *teres* in the OSU panel with all barley breeding lines submitted to the Barley Coordinated Agricultural Project by Oregon State University from 2006 to 2009. The x-axis shows the chromosomal positions of the markers and the y-axis the -log(p) values of the markers. UNK indicates markers with unknown map positions.



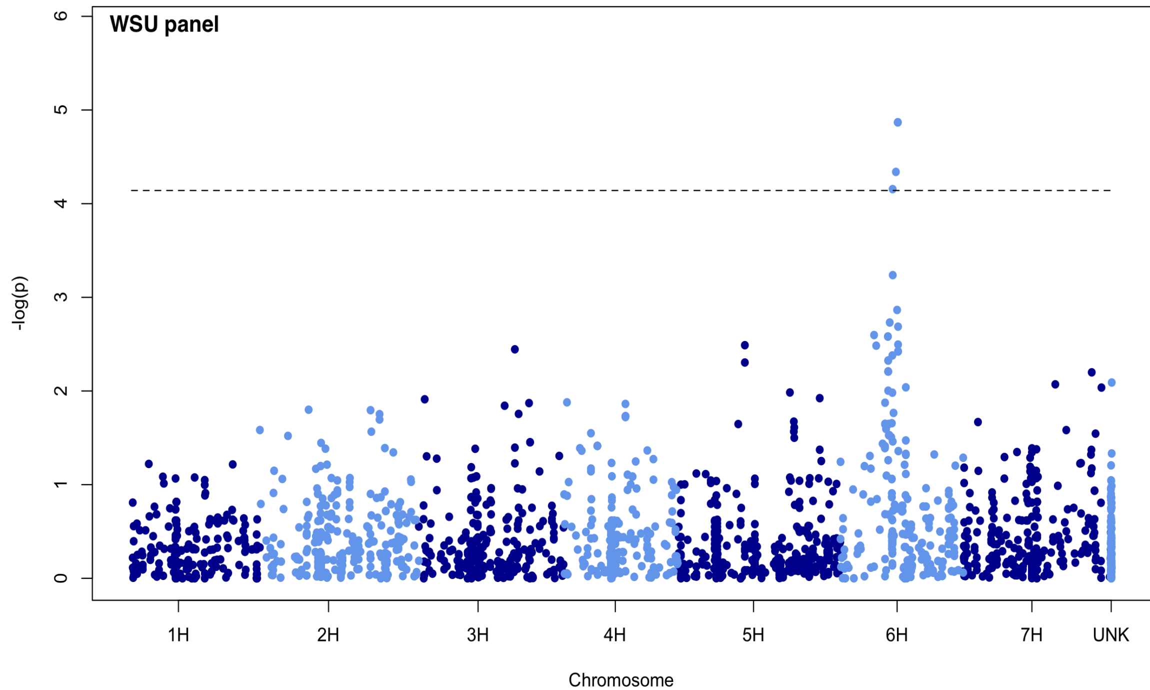
Appendix Fig. 4.14 Manhattan plot for markers used in a genome-wide association mapping study examining resistance to *Pyrenophora teres* f. *teres* in the USDA panel with all barley breeding lines submitted to the Barley Coordinated Agricultural Project by the United States Department of Agriculture, Agricultural Research Service (Aberdeen, ID) from 2006 to 2009. The x-axis shows the chromosomal positions of the markers and the y-axis the -log(p) values of the markers. UNK indicates markers with unknown map positions. The dotted line represents the significance threshold for a false discovery rate of 0.05.



Appendix Fig. 4.15 Manhattan plot for markers used in a genome-wide association mapping study examining resistance to *Pyrenophora teres* f. *teres* in the USU panel with all barley breeding lines submitted to the Barley Coordinated Agricultural Project by Utah State University from 2006 to 2009. The x-axis shows the chromosomal positions of the markers and the y-axis the -log(p) values of the markers. UNK indicates markers with unknown map positions. The dotted line represents the significance threshold for a false discovery rate of 0.05.



Appendix Fig. 4.16 Manhattan plot for markers used in a genome-wide association mapping study examining resistance to *Pyrenophora teres* f. *teres* in the VA panel with all barley breeding lines submitted to the Barley Coordinated Agricultural Project by Virginia Polytechnic Institute and State University from 2006 to 2009. The x-axis shows the chromosomal positions of the markers and the y-axis the -log(p) values of the markers. UNK indicates markers with unknown map positions.



Appendix Fig. 4.17 Manhattan plot for markers used in a genome-wide association mapping study examining resistance to *Pyrenophora teres* f. *teres* in the WSU panel with all barley breeding lines submitted to the Barley Coordinated Agricultural Project by Washington State University from 2006 to 2009. The x-axis shows the chromosomal positions of the markers and the y-axis the -log(p) values of the markers. UNK indicates markers with unknown map positions. The dotted line represents the significance threshold for a false discovery rate of 0.05.