GENETICS OF THE HOST-PATHOGEN INTERACTION IN THE BARLEY-NET FORM NET BLOTCH SYSTEM

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

By

Vaidehi Mohit Koladia

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

Major Department:
Plant Pathology

March 2017

Fargo, North Dakota

North Dakota State University
Graduate School

Title

Genetics of the Host-Pathogen Interaction in the Barley-Net Form Net Blotch System

By

Vaidehi Mohit Koladia

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

MASTER OF SCIENCE

SUPERVISORY COMMITTEE:

Robert S. Brueggeman
Chair

Timothy L. Friesen

Justin D. Faris

Xiwen Cai

Approved:

March 8, 2017
Date

Jack Rasmussen
Department Chair

ABSTRACT

Pyrenophora teres f. *teres* is a fungal pathogen that causes barley net form net blotch. To evaluate the genetics of resistance in barley, a RIL population was developed using resistant barley lines CI5791 and Tifang and tested against a global collection of nine *P. teres* f. *teres* isolates. QTL analysis indicated that CI5791 resistance mapped to chromosome 6H and was effective against all isolates. Additionally, CI5791 harbored resistance on chromosome 3H effective against two Japanese isolates. Tifang also had resistance that mapped to 3H and was effective against four of the isolates. To evaluate the genetics of virulence in *P. teres* f. teres, a fungal population was developed and evaluated against ten barley lines. 19 unique QTL were identified on 12 different linkage groups. 1 or 2 major loci were identified for a few of the barley lines whereas for most lines, virulence was contributed by several loci.

ACKNOWLEDGEMENTS

I would like thank my research advisor Dr. Timothy Friesen for allowing me to do research in his lab. Even though I had no background in plant pathology, he gave me a chance to develop my research interest in plant pathology and guided me through my research projects. I would like to thank Danielle Holmes for teaching me several techniques and guiding me through my experiments. I would like to take a chance to thank my academic advisor Dr. Robert Brueggeman for accepting me as his student and allowing me to pursue my degree in plant pathology. I would like to thank my committee members Dr. Justin Faris and Dr. Xiwen Cai for sparing time to review and edit my thesis and Dr. Justin Faris for guiding me in my data analysis.

I would like to thank all members (present and past) of the Friesen and Faris labs who have always been around to help me whether academically or in my research. These members include: Danielle Holmes, Rachel Shjerve, Steven Carlsen, Anjan Neupane, Jacob Jennings, Dr.Yuanyuan Gao, Nathan Wyatt, Dr. Jon Richards, Jason Axtman, Tylor Waln, Jessica Carpenter, Dr.Gongjun Shi, Dr. Zengcui Zhang, Jyoti Saini and Amanda Peters.

I would like to thank my parents, Nilesh Jha and Daksha Jha, my sister, Shivani Jha and my in-laws, Gordhan Koladia and Bhavna Koladia for supporting me and encouraging me to continue my studies. Last of all I would like to thank my dear husband, Mohit Koladia, who has provided me with tremendous support throughout my life and career. He has always encouraged me to face my difficulties from the front rather than running away from them.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Net blotch disease of barley is caused by the fungus *Pyrenophora teres* and has emerged as a major problem in many barley-growing areas of the world. The pathogen typically causes yield loss of 10 to 40% with the possibility of total loss when a susceptible cultivar is planted under environmental conditions conducive to disease epidemic (Mathre, 1997; Murray and Brennan, 2010). The pathogen was divided into two forms based on the difference in symptoms observed at the time of infection (Smedgard-Peterson, 1971). The two forms were identified as *P. teres* f. *teres* and *P. teres* f. *maculata* causing net form net blotch (NFNB) and spot form net blotch (SFNB), respectively (Smedegard-Peterson, 1971). *P. teres* f. *teres* produces individual net-like lesions on barley leaves that coalesce into large necrotic lesions as the infection progresses on susceptible barley lines. The pathogen is a necrotrophic fungus that directly penetrates the host cells without forming a feeding structure. *P. teres* f. *teres* kills its host as it progresses with its colonization (Reviewed in Liu et al., 2011). As observed in *Parastagonospora nodorum*, a necrotrophic pathogen of wheat (Friesen and Faris, 2010), *P. teres* f. *teres* also interacts with host genes in an inverse gene-for-gene manner resulting in susceptibility (Liu et al. 2015). Host triggered program cell death often helps a necrotrophic pathogen to proliferate instead of inhibiting its growth (Friesen and Faris, 2010). In order to better understand the life style of this pathogen a thorough study needs to be carried out on the host as well as pathogen side. Building upon previous information from studies performed on the avirulence/virulence genes of the pathogen (Lai et al., 2007; Shjerve et al., 2014; Liu et al., 2015) and resistance/susceptibility genes of the host (Friesen, 2006; Abu Qamar et al., 2008; Shjerve et al, 2014; Liu et al., 2015, Richards et al., 2016), we further investigated this pathosystem from both the host and the pathogen perspective to gain more insight into these host-parasite genetic

interactions. Host resistance was investigated using a barley population segregating for resistance and the genetics of virulence was characterized using a pathogen population derived from a cross between avirulent and virulent *P. teres* f. *teres* isolates. These studies have provided a better understand of the complex interactions occurring in this pathosystem.

Literature Cited

Abu Qamar, M., Liu, Z. H., Faris, J. D., Chao, S., Edwards, M. C., Lai, Z., Franckowiak, J.D. and Friesen, T.L. (2008) A region of barley chromosome 6H harbors multiple major genes associated with net type net blotch resistance. Theor. Appl. Genet. 117, 1261–1270

Friesen, T. L., and Faris, J. D. (2010) Characterization of the wheat-*Stagonospora nodorum* disease system: what is the molecular basis of this quantitative necrotrophic disease interaction? Canadian Journal of Plant Pathol. 32, 20-28

Friesen, T. L., Faris, J. D., Lai, Z. and Steffenson, B. J. (2006) Identification and chromosomal location of major genes for resistance to *Pyrenophora teres* in a doubled-haploid barley population. Genome, 49, 855–859

Lai, Z., Faris, J. D., Weiland, J. J., Steffenson, B. J. and Friesen, T. L. (2007) Genetic mapping of *Pyrenophora teres* f. *teres* genes conferring avirulence on barley. Fungal Genet. Biol. 44, 323–329

Liu, Z., Ellwood, S. R., Oliver, R. P and Friesen, T. L. (2011) *Pyrenophora teres*: profile of an increasingly damaging barley pathogen. Mol. Plant Pathol. 12, 1-19

Liu, Z., Holmes, D. J., Faris, J. D., Chao, S., Brueggeman, R. S., Edwards, M. C. and Friesen, T. L. (2015) Necrotrophic effector triggered susceptibility (NETS) underlies the barley-*Pyrenophora teres* f. *teres* interaction specific to chromosome 6H. Mol. Plant Pathol. 16, 188-200

Mathre, D. E. (1997) Compendium of Barley Diseases, 2nd edn. St. Paul, MN: *American* Phytopathological Society. Pg 28-32

Murray, G. M. and Brennan, J. P. (2010) Estimating disease losses to the Australian barley industry. Australas. Plant Pathol. 39, 85–96

Richards J, Chao S, Friesen T, Brueggeman R (2016) Fine mapping of the barley chromosome 6H net form net blotch susceptibility locus. G3 (Bethesda) 6(7):1809–1818. doi:10.1534/g3.116.028902

Shjerve, R. A., Faris, J. D., Brueggeman, R. S., Yan, C., Zhu, Y., Koladia, V. and Friesen, T.L. (2014) Evaluation of a *Pyrenophora teres* f . *teres* mapping population reveals multiple independent interactions with the barley 6H chromosome region. Fungal Genet. Biol. 70, 104-112

Smedegård-Petersen, V. (1971) *Pyrenophora teres* f. *maculata* f. nov. and *Pyrenophora teres* f. *teres* on barley in Denmark. Kgl. Vet. Landbohojsk. Arsskr. 124–144

CHAPTER 1: LITERATURE REVIEW

Host

Barley is a small-grain cereal belonging to the family *Poaceae*, the tribe *Triticeae* and the genus *Hordeum* (Mathre, 1997). The first records of barley domestication refer back to 8000 B.C. in the fertile crescent of Asia (Zohary et al., 2003, Mathre, 1997). Wide adaptability of barley has allowed it to be grown as a summer crop in tropical regions and as a winter crop in temperate regions of the world (www.barleyfoods.org/facts.html). Barley has been primarily used for animal feed, malting and brewing and also for human consumption in many parts of the world (Mathre, 1997). The US is among the leading producers of barley, ranking seventh in the world, with 27 states in production (www.barleyfoods.org/facts.html). North Dakota is typically the number one barley-producing state in the US. Since 1900, breeding efforts have focused on barley improvement in the areas of malting and feed quality, yield, straw strength, and disease resistance (Mathre, 1997). Yet, currently, most of the barley lines grown are susceptible to NFNB. Hence, it is important to develop resistant barley varieties to combat this necrotrophic pathogen.

The Causal Pathogen and Its Life Cycle

P. teres f. *teres* is an ascomycete belonging to class *Dothideomycete*, in the order *Pleosporales* and the family *Polyporacea*. As *P. teres* f. *teres* is heterothallic in nature, it requires opposite mating types to produce fertile pseudothecia (Mathre, 1997). Pseudothecia consist of asci that are club-shaped, bitunicate, and rounded at the apex. Each of the asci contain eight ascospores that are light brown in color and have three to four transverse septa and one or two longitudinal septa specifically in the median cells (Mathre, 1997). Under favorable environmental conditions ascospores are forcibly discharged from the pseudothecia in the

presence of moisture and dispersed long distances by wind serving as primary inoculum in the beginning of the growing season (Jordan, 1981). Asexual spores, known as conidia, are also produced at the tips of the conidiophores that are present either singly or in a group of two or three. The conidia are usually cylindrical with rounded apical cells, yellowish-brown in color and one to eleven septate (Mathre, 1997). Once the pathogen has colonized the host tissue, conidia are produced throughout the growing season, serving as secondary inoculum. Environmental factors such as leaf wetness, temperature and relative humidity play an important role in spore germination, dispersion and successful infection (van den Berg et al., 1990, 1991; Jordan, 1981). Under favorable environmental conditions there could be a development of high inoculum in the fields due to several secondary cycles. This increase in spores increases the disease severity on susceptible cultivars (Reviewed in Liu et al., 2011). Other factors contributing to disease severity are the continuous planting of barley in one field, minimum tillage practices, excessive nitrogen applications and movement of infested stubble from one field to another (Mathre, 1997). Seed borne mycelium also plays a role as inoculum in spreading the pathogen to fields previously free of the disease (Mathre, 1997). Finally, as the growing season ends, the fungus overwinters by forming pseudothecia and mycelium on barley stubble or as seed borne mycelium

Disease Symptoms

In NFNB disease there is an appearance of small circular dot-like lesions in the beginning of the infection that develop into longitudinal and transverse striations forming a net-like pattern on the leaves (Shipton, 1973; reviewed in Liu et al., 2011). Parts of the leaf affected by the disease turn brown due to necrosis, and the adjacent tissue becomes yellow due to chlorosis. The leaves that are severely affected by the disease become completely dried and necrotic (Mathre, 1997). In resistant varieties lesions do not expand but remain elliptical or spot-

like whereas in susceptible varieties the lesions expand over time forming the striated net-like patterns (Tekauz, 1985).

Disease Development

Breeding for resistant cultivars is one of the most effective ways to overcome this disease but to obtain complete and durable resistance, it is also important to study the factors playing a crucial role in the success of the pathogen in causing disease (reviewed in Liu et al., 2012). The development of a plant disease begins when a pathogen finds a way to interact with its host. In NFNB disease, the spore lands on the leaf surface and germinates by forming a germ tube. After growing along the host surface the germ tube penetrates the host cells by forming an appressoria. Direct penetration occurs through the cuticle between the epidermal cells by the high turgor pressure generated through the formation of a penetration peg (Van Caeseele and Grumbles, 1979; Keon and Hargreaves, 1983; Lightfoot et al., 2010). As the fungus grows, further colonizing the spaces between the mesophyll cells, it begins killing the plant cells it comes into contact with, leading to necrosis. Cells adjacent to the necrotic cells develop chlorosis, probably due to program cell death (PCD) triggered by the host in response to fungal infection (Lightfoot and Able, 2010). As the infection progresses the pathogen colonizes the host tissue, ultimately leading to dead necrotic leaves. No fungal growth was observed in the chlorotic cells surrounding the necrotic cells, indicating that the pathogen may release diffusible toxins/effectors that result in host cell death in the absence of direct contact with the fungal hyphae (Smedegard-Peterson, 1977; Keon and Hargreaves, 1983).

Necrotrophic pathogens are classified as necrotrophic generalists and necrotrophic specialists based on the host range they infect. Necrotrophic generalists have a broad host range whereas necrotrophic specialists have a more defined host range (Andrew et al., 2012) and

release host specific toxins now called necrotrophic effectors (NE) to facilitate colonization. Examples of necrotrophic specialists include *Parastagonospora nodorum, Pyrenophora tritici-repentis,* and several *Cochliobolus species* in which host-selective toxins have been identified that are specific to host sensitivity genes (Friesen et al., 2008; Ciuffetti et al., 2010; Condon et al., 2013).

Toxins have also been identified in *P. teres* f. *teres* but little conclusive work has been done to identify their role in disease (Smedegård-Petersen, 1977a; Bach et al., 1979; Sarpeleh et al., 2007, 2008). Liu et al. (2015) identified a small secreted necrotrophic effector protein (PttNE1) from the intercellular wash fluids of a susceptible barley line, Hector, inoculated with a virulent *P. teres* f. *teres* isolate. The sensitivity to PttNE1 mapped to a gene designated *SPN1* that was associated with a resistance/susceptibility QTL region of barley chromosome 6H in a recombinant inbred barley population produced from a cross of Hector and a resistant barley line NDB112 (Liu et al., 2015). This study indicated that the inverse gene-for-gene type of interaction between host gene products and necrotrophic effectors of the pathogen lead to susceptibility instead of resistance as observed in wheat-*Parastagonospora nodorum* system (Friesen et al., 2008; Friesen and Faris, 2010).

Pathogen Virulence

Several factors play a key role in pathogen infection. These factors include favorable environmental conditions, suitable spore attachment to the leaf surface, sensing physical cues such as topography of the leaf surface for penetration of the germ tube (Callow and Green, 1994) and release of degrading enzymes (Andrew et al., 2012) that are effective in dissolving the host cuticle and degrading epidermal cells to enter the host. Even after successful entry into the host, the pathogen has to evade or suppress the host defense mechanisms to be a virulent pathogen.

Several types of interactions are continuously occurring between hosts and pathogens in the ecosystem. Amongst them, the gene-for-gene type of interactions have been observed in biotrophic pathosystems, where interactions between the host and pathogen gene products lead to resistance (de Wit et al., 1992). Inverse gene-for-gene type interactions have been observed in necrotrophic pathosystems where interactions between the host and pathogen gene products lead to susceptibility (Friesen and Faris, 2010). *P. teres* f. *teres* is classified as a necrotrophic pathogen and although there is strong evidence for dominant susceptibility genes and necrotrophic effectors (Abu Qamar et al., 2008; Shjerve et al., 2014; Liu et al., 2015), strong dominant resistance genes have also been identified (Bockelman et al., 1977; Steffenson et al., 1996; Friesen et al., 2006 Koladia et al. 2017) suggesting that both interactions (gene-for-gene and inverse gene-for-gene) are playing a role in these host-pathogen genetic interactions.

Khan and Boyd (1969) performed studies to identify physiologic specialization among *P. teres* f. *teres* isolates collected from Western Australia and identified three physiological races WA-1, WA-2 and WA-3 using two barley differential lines Algerian and CI 7584. Tekauz (1990) performed virulence studies on 12 differential lines using *P. teres* f. *teres* isolates collected from Western Canada and found greater variability among the isolates as compared to the previous studies carried out in Western Canada (McDonald and Buchannon 1962). Steffenson and Webster (1992b) identified virulence diversity among 16 pathotypes collected from California using 22 barley differential lines. A similar barley differential set was used by Wu et al., 2003 to characterize virulence diversity among *P. teres* f. *teres* isolates collected from different parts of the world. Gupta and Loughman (2001) conducted studies to determine virulence diversity in Western Australia and identified different groups of isolates based on virulence and avirulence on the commonly used barley differential line, Beecher. Arabi et al.

(2003) evaluated virulence diversity among isolates collected from Syria and France. French isolates R5 and S5 were found to be highly virulent on resistant barley lines CI5791, 79-S10-10 and Arrivate. Arabi et al. (2003) evaluated the *P. teres* f. *teres* isolates using 11 barley genotypes and found continuous variability among isolates ranging from a high virulence pattern among French isolates S5, R5 and S6-2 to low virulence among Syrian isolates RICA31 and 12HAS-6. None of the barley genotypes were found to be highly resistant to all these isolates. Liu et al., (2012) conducted a study to determine virulence diversity among isolates collected in Langdon and Fargo, North Dakota. In this study, 22 barley differential lines including lines used by Steffenson and Webster (1999b) and Wu et al. (2003) were used. The study indicated a wide range of virulence diversity among the North Dakota isolates. Barley lines CI5791, Algerian, and Heartland were found to be resistant to all North Dakota isolates.

Weiland et al. (1999) performed studies to investigate the genetics of virulence in *P. teres* f. *teres* isolates by developing a bi-parental population of *P. teres* f. *teres* isolates 15A (California isolate) and 0-1 (Ontario isolate) based on the information obtained from previous studies (Steffenson and Webster, 1999b). A single major gene *AvrHar,* conferring avirulence on barley line Harbin, was mapped using molecular markers. Lai et al., (2007) performed further studies using the same bi-parental population used by Weiland et al., (1999) and identified two additional genes *AvrPra1* and *AvrPra2* conferring avirulence towards barley line Prato. *AvrPra2* mapped to the same locus as *AvrHar* but in repulsion as both the genes were identified to confer avirulence to different parents. Beattie et al. (2007) identified gene $Avr_{heartland}$ conferring avirulence on barley line Heartland by mapping another biparental population derived from a cross between Canadian isolates WRS 1906 and WRS 1607. Afanasenko et al. (2007) studied the genetics of the NFNB host parasite interactions using 12 barley differential lines. Afanasenko et

al. (2007) showed that resistance in the host and avirulence in the pathogen were determined by one or two genes and concluded that the *P. teres*. f. *teres*-barley system was a gene-for-gene type of interaction. Shjerve et al., 2014 developed a bi-parental population by crossing California isolates 15A and 6A and evaluating the population on the barley lines Rika and Kombar, which were selected based on their differential reactions to isolates 15A and 6A. Two loci, *VK1* and *VK2* were shown to confer virulence on Kombar and two distinct loci, *VR1* and *VR2*, were shown to confer virulence on Rika. Progeny isolates harboring only one of these genes were then selected and inoculated on a Rika × Kombar RIL population. Based on the results it was hypothesized that one or more tightly linked virulence target genes were located near the centromere of barley chromosome 6H that conferred susceptibility and this gene(s) was interacting with multiple virulence loci in the pathogen.

Host Resistance

Soon after a pathogen lands on the host surface, resistance mechanisms are activated via cell surface or plasma membrane anchored pattern recognition receptors (PRRs) that identify specific components of the pathogen called pathogen associated molecular patterns (PAMP) that activate PAMP-triggered immunity (PTI) to stop the infection (Jones and Dangl, 2006, Chisholm, 2006). However, the pathogen has the ability to evade or suppress PTI by releasing another set of weapons known as effectors. Effectors are the pathogen molecules that have the ability to modify the host cellular physiology, typically suppressing early PTI responses, leading to effector triggered susceptibility (ETS). In response to these pathogen effectors, the host evolved a second layer of defense known as resistance genes that typically have the nucleotide binding-leucine rich repeat (NB-LRR) protein domain architecture. The cytoplasmically localized NB-LRR proteins recognize the pathogen effectors (avirulent/virulent gene products)

directly or more commonly indirectly activating defense responses referred to as effector triggered immunity (ETI) (Jones and Dangl, 2006, Chisholm et al., 2006). In response, the pathogen can evade detection and ETI responses by mutation in or elimination of effectors that are recognized by host resistance genes returning back to the state of virulence on the host and disease susceptibility. Similarly, the natural host populations also select new alleles of genes with NB-LRR domains specific to the new effectors to obtain ETI (Jones and Dangl, 2006; Chisholm et al., 2006). Thus, along with the changing virulence strategies of the pathogen to cause infection, the host must also adapt its defense mechanisms to provide resistance to pathogen attack. The selection of effective host resistance genes is a major goal of disease resistance breeding in domesticated crops.

Resistance genes have been identified in some of the barley lines that are known to be effective against *P. teres* f. *teres*. Geschele (1928) reported that resistance to net blotch was inherited in a Mendelian fashion (Geschele, 1928). Later, three incompletely dominant resistant genes, Pt_1, Pt_2 and Pt_3 were shown to be effective against *P. teres* isolates collected in California (Schaller, 1955; Mode et al., 1958) . Net blotch resistance genes *Rpt1a, Rpt3d*, *Rpt1b* and *Rpt2c* were identified by trisomic analysis on barley chromosomes 3H of Tifang, 2H of CI7584 and 3H and 5H of CI9819, respectively (Bockelman et al., 1977). Several more recent NFNB studies have shown quantitative trait loci (QTL) to be present on each of the seven barley chromosomes (reviewed in Liu et al., 2011).

Multiple studies have shown that in several barley backgrounds, resistance to NFNB maps to a similar region on chromosome 6H (Steffenson et al., 1996; Cakir et al., 2003; Friesen et al., 2006; Grewal, 2008; St. Pierre et al., 2010) and other studies have shown that a similar region on chromosome 6H consists of multiple genes that confer dominant susceptibility to

different *P. teres* f. *teres* isolates (Abu Qamar et al., 2008; Shjerve et al., 2014; Liu et al., 2015, Richards et al., 2016). Collectively, the studies on the 6H chromosome of barley indicate that there are multiple genes or groups of genes present at the 6H chromosome that play a major role in the NFNB host-pathogen interaction.

Genome Sequencing

Genome sequencing is a process that provides information about the genetic composition of a particular species. The human genome project was a huge effort in the field of genomics that paved a way for sequencing of several species (International Human Genome sequencing consortium, 2001). Earlier the genomes were sequenced using the Sanger sequencing method (Sanger, 1977), which was tedious and costly. With the advent of new sequencing technologies, next generation sequencing (NGS) replaced the old sequencing methods with advantages of less cost and deeper coverage of genomes, in less time (Morozova et al., 2008; Haridas et al., 2011). NGS platforms, such as PAC BIO, Illumina, and Ion Torrent have revolutionized genome sequencing with their massive parallel sequencing feature, allowing sequencing of several individuals of a species at the same time (Marguerat et al., 2008). Applications of NGS technologies include whole genome sequencing and transcriptome sequencing (Chierico et al., 2015).

Baker's yeast, *Saccharomyces cerevisiae* was the first fungus to be sequenced, due to its simple eukaryotic organization and industrial applications in food, brewing and the pharmaceutical industry (Vassarotti and Goffeau, 1992; Mewes et al., 1997). Among the the cereal fungal pathogens that have been sequenced are *Magnaporthe oryzae*, the fungal pathogen of rice (Dean et al., 2005), *Parastagonospora nodorum*, the fungal pathogen of wheat (Hane et al., 2007), and *P. teres* f. *teres*, the fungal pathogen of barley (Ellwood et al., 2010). Sequencing

of *P. teres* f. *teres* was carried out using an Illumina Solexa mate pair platform. The total size of the *P. teres* f. *teres* genome was found to be 41. 95 Mbp with 11,799 gene models, much like the genomes of *M. oryzae* and *P. nodorum* (Ellwood et al., 2010). Further studies will be carried out for the functional analysis of the identified genes in the *P. teres* f. *teres* genome.

Molecular Markers

Molecular markers are the DNA sequences that are associated with known, as well as unknown regions of the genome (Liu and Cordes, 2004). Molecular markers have several applications that include mapping of genomes and identifying genetic variation among different individuals in a population. Restriction fragment length polymorphism (RFLP) markers (Bostein et al., 1980) were one of the first markers developed in the field of molecular biology. Later, PCR based markers such as random amplified polymorphic DNA (RAPD) markers (Welsh and McClelland, 1990; Williams et al., 1990), amplified fragment length polymorphism (AFLP) markers (Vos et al., 1995) and microsatellites also known as simple sequence repeat (SSR) (Hearne et al., 1992) markers were used. Single nucleotide polymorphisms (SNPs) are caused by point mutations, giving rise to different alleles, having alternate bases at a given nucleotide position within a locus. DNA sequencing has been the most useful approach for SNP discovery (Liu and Cordes, 2004). SNPs are important molecular markers as they are the most abundant polymorphism occurring in any organism.

Restriction associated DNA (RAD) are small sequences of DNA, adjacent to the restriction sites that can be used to generate genetic markers (Miller et al., 2007). Initially, the RAD based microarray technique was developed to identify RAD markers based on the variation observed in the restriction sites across the genome (Miller et al., 2007). However, it was found that the microarray method was more complicated and not useful for sequencing of several

species simultaneously (Baird et al., 2008). Baird et al. (2008) developed a new RAD based sequencing method that utilized the same principles of array technology combined with next generation sequencing technology. An Illumina platform was used instead of the microarray technique that allowed sequencing of several distinct individuals in a single run (Baird et al., 2008). High-through put sequencing of the RAD-tag libraries led to the discovery of thousands of SNPs across the genome. Moreover, this technique allowed for the discovery of SNPs outside the recognition site of a restriction enzyme as compared to AFLP technique.

As the name suggests, genotyping by sequencing (GBS) is an approach for genotyping a species along with the sequencing process (Deschamps et al., 2012). This approach eliminates the step of assay development for the generation of markers. SNPs identified across the genome, are directly used as genetic markers (Deschamps et al., 2012). The RAD-GBS method was adopted from a modified RAD method (Baird et al., 2008; Poland et al., 2012) and used for sequencing of several fungal populations using an Ion Torrent sequencer (Leboldus et al., 2014). The steps involved in this process included digestion, ligation, and size-selection followed by fragment sequencing.

Through the NGS process, thousands of SNPs are identified across the genome that can then be utilized for construction of a genetic map. Once, there is availability of phenotypic and genotypic data, quantitative trait locus (QTL) can be identified. QTL provide us with the information about the virulence/avirulence regions in the pathogen responsible for a particular phenotypic reaction. Further, deeper exploration of these regions through full genome sequencing will provide the knowledge about the genes responsible for a particular response. In conclusion, there are several things to unravel on the host-pathogen interaction to understand the role of each factor responsible in the development of disease.

Literature Cited

Abu Qamar, M., Liu, Z. H., Faris, J. D., Chao, S., Edwards, M. C., Lai, Z., Franckowiak, J.D. and Friesen, T.L. (2008) A region of barley chromosome 6H harbors multiple major genes associated with net type net blotch resistance. Theor. Appl. Genet. 117, 1261–1270

Afanasenko, O., Mironenko, N., Filatova, O., Kopahnke, D., Krämer, I. and Ordon, F. (2007) Genetics of host–pathogen interactions in the *Pyrenophora teres* f. *teres* (net form)–barley (*Hordeum vulgare*) patho- system. Eur. J. Plant Pathol. 117, 267–280

Andrew, M., Barua, R., Short, S. M., and Kohn, L. M. (2012) Evidence for a common toolbox based on necrotrophy in a fungal lineage spanning necrotrophs, biotrophs, endophytes, host generalists and specialists. Plos One 7, e29943

Arabi M. I. E., Al-Safadi B. and Charbaji T. (2003) Pathogenic variation among isolates of *Pyrenophora teres*, the causal agent of barley net blotch. J. Phytopathology 151, 376–382

Bach, E., Christensen, S., Dalgaard, L., Larsen, P.O. and Olsen, C.E. (1979) Structures, properties and relationship to the aspergillomarasmines of toxins produced by *Pyrenophora teres*. Physiol. Plant Pathol.
14, 41–46.

Baird, N. A., Etter, T. S., Atwood, M. C., Currey, A. L., Shiver, Z. A., Lewis, E. U., Selker, W. A., Cresko, W. A., Johnson, E. A. (2008) Rapid SNP discovery and genetic mapping using sequenced RAD markers, PLoS One. 3: e3376

Beattie, A. D., Scoles, G. J. and Rossnagel, B. G. (2007) Identification of molecular markers linked to a *Pyrenophora teres* avirulence gene. Phytopathology, 97, 842–849

van den Berg, C. G. J. and Rossnagel, B. G. (1990) Effects of temperature and leaf wetness period on conidium germination and infection of barley by *Pyrenophora teres*. Can. J. Plant Pathol. 12, 263–266

van den Berg, C. G. J. and Rossnagel, B. G. (1991) Epidemiology of spot- type net blotch on spring barley in Saskatchewan. Phytopathology, 81, 1446–1452

Bockelman, H. E., Sharp, E. L. and Eslick, R. F. (1977) Trisomic analysis of genes for resistance to scald and net blotch in several barley cultivars. Can. J. Bot. 55, 2142–2148

Bostein, D., White, R. L.,Skolnick, M., Davis, R. W. (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphism. Am. J. Hum. Genet. 32: 314- 331

Cakir, M., Gupta, S., Platz, G. J., Ablett, G. A., Loughman, R., Embiri, L. C., Poulsen, D., Li, C. D., Lance, R. C. M., Galwey, N. W., Jones, M. G. K., Appels, R . (2003) Mapping and validation of the genes for resistance to *Pyrenophora teres* f. *teres* in barley (*Hordeum vulgare* L.) Aust J Agric Res 54, 1369–1377

Callow, J. A., Green, J. R. (1994) Perspectives in plant cell recognition. Journal of Plant Physio. 2, 254-255

Chierico, F. D., Ancora, M., Marcacci, M., Camma, C., Putignani, L., and Conti, S. (2015) Choice of next-generation sequencing pipelines. Bacterial pangenomics: methods and protocols, methods in molecular biology. 1231: 31-47

Chisholm, S. T., Coaker, G., Day, B., and Staskawicz, B. J. (2006) Host-microbe interactions: shaping the evolution of the plant immune response. Cell 124, 803-814

Ciuffetti, L. M., Manning, V. A., Pandelova, I., Betts, M., F., and Martinez, J. P. (2010) Host-selective toxins, Ptr ToxA and Ptr ToxB, as necrotrophic effectors in the *Pyrenophora tritici-repentis*–wheat interaction. New Phytologist 187, 911–919

Condon, B. J., Leng, Y., Wu, D., Bushley, K. E., Ohm, R. A., Otillar, R., Martin, J., Schackwitz, W., Grimwood, J., MohdZainudin, N., Xue, C., Wang, R., Manning, V. A. , Dhillon, B., Tu, Z. J., Steffenson, B. J., Salamov, A., Sun, H., Lowry, S., K LaButti, K., Han, J., Copeland, A., Lindquist, E., Barry, K., Schmutz, J., Baker, S. E., Ciuffetti, L. M., Grigoriev, I.V., Zhong, S., Turgeon, B. G. (2013) Comparative genome structure, secondary metabolite, and effector coding capacity across *Cochliobolus* pathogens. PLOS genetics 9, e1003233

Cromey MG, Parkes RA (2003) Pathogenic variation in *Drechslera teres* in New Zealand. N Z Plant Protect 56:251-256

de Wit P. J. G. M. (1992) Molecular characterization of gene-for-gene systems in plant-fungus interactions and the application of avirulence genes in control of plant pathogens. Annu. Rev. Phytopathol 30, 391-418

Dean, R. A., Talbot, N. J., Ebbole, D. J., Farman, M. L., Mitchell, T. K., Orbach, M. J., Thon, M., Kulkarni R., Xu, J., Pan, H., Read, N. D., Lee, Y., Carbone, I., Brown, D., Oh, Y. Y., Donofrio, N., Jeong, J. S., Soanes, D. M., Djonovic, S., Kolomeits, E., Rehmeyer, C., Li, W., Harding, M., Kim, S., Lebrun, M. H., Bohnert, Coughlan, S., Butler, J., Calvo, S., Ma, L. J., Nicol, R., Purcell, S., Nusbaum, C., Galagan, J. E., Birren, B. W. (2005) The genome sequence of the rice blast fungus *Magnaporthe grisea*. Nature. 434: 980-986

Deshchamps, S., Llaca, V., May, G. D. (2012) Genotyping-by-sequencing in plants. Biology. 1: 460-483

Ellwood, S. R., Liu, Z., Syme, R. A., Lai, Z., Hane, J. K., Keiper, F., Moffat, C. S., Oliver, R. P., Friesen, T. L., (2010) A first genome assembly of the barley fungal pathogen *Pyrenophora teres* f. *teres*. Genome Biol. 11: R109

Friesen, T. L., and Faris, J. D. (2010) Characterization of the wheat-*Stagonospora nodorum* disease system: what is the molecular basis of this quantitative necrotrophic disease interaction? Canadian Journal of Plant Pathol. 32, 20-28

Friesen, T. L., Faris, J. D., Lai, Z. and Steffenson, B. J. (2006) Identification and chromosomal location of major genes for resistance to *Pyrenophora teres* in a doubled-haploid barley population. Genome. 49, 855–859

Friesen, T. L., Faris, J. D., Solomon, P. S. and Oliver, R. P. (2008) Host-specific toxins: effectors of necrotrophic pathogenicity. Cellular Microbiology 7, 1421–1428

Geschele, E. E. (1928) The response of barley to parasitic fungi *Helminthosporium teres* Sacc. Bull. Appl. Bot. Genet. Plant Breed. 19, 371– 384 (in Rev. Appl. Mycol. 8, 165)

Grewal, T. S., Rossnagel, B. G., Pozniak, C. J., and Scoles, G. J. (2008) Mapping quantitative trait loci associated with barley net blotch resistance. Theor. Appl. Genet. 116, 529-539

Gupta S, Loughman R (2001) Current virulence of *Pyrenophora teres* on barley in Western Australia. Plant Dis 85:960-966

Hane, J. K., Lowe, R. G.T., Solomon, P. S., Tan, K., Schoch, C. L., Spatafora, J. W., Crous, P. W., Kodira, C., Birren, B. W., Galagan, J. E., Torriani, S. F. F., Mc Donald, B. A, Oliver, R. P. (2007) Dothideomycete-plant interactions illuminated by genome sequencing and EST analysis of the wheat pathogen *Stagonospora nodorum*. Plant Cell. 19: 3347-3368

Haridas, S., Breuill, C., Bohlmann, J., Hsiang, T. (2011) A biologist's guide to de novo assembly using next-generation sequence data: A test with fungal genomes. Journal of Microbiol Methods. 86: 368-375

Hearne, C. M., Gosh, S., Todd, J. A. (1992) Microsatellites for linkage analysis of genetic traits. Trends Genet. 8: 288-294

International Human Genome Sequencing Consortium. (2001) Initial sequencing and analysis of the human genome. Nature 409: 860-921

Jones, J. D. G., and Dangl, J. L. (2006) The plant immune system. Nature 444, 323-329

Jordan, V. W. L. (1981) Aetiology of barley net blotch caused by *Pyrenophora teres* and some effects on yield. Plant Pathol. 30, 77–87

Keon, J.P.R. and Hargreaves, J.A. (1983) A cytological study of the net blotch disease of barley caused by *Pyrenophora teres*. Physiol. Plant Pathol. 22, 321–329

Khan, T. N. and Boyd, W. J. R. (1969) Physiologic specialization in *Drechslera teres*. Aust. J. Biol. Sci. 22, 1229–1235

Lai, Z., Faris, J. D., Weiland, J. J., Steffenson, B. J. and Friesen, T. L. (2007) Genetic mapping of *Pyrenophora teres* f. *teres* genes conferring avirulence on barley. Fungal Genet. Biol. 44, 323–329

Leboldus, J. M., Kinzer, K., Richards, J., Ya, Z., Yan, C., Friesen, T. L. and Brueggeman R. (2014) Genotyping-by-sequencing of the plant-pathogenic fungi *Pyrenophora teres* and *Sphaerulina musiva* utilizing Ion Torrent sequence technology. Mol. Plant Pathol. 6: 623-632

Lightfoot, D. J. and Able, A. J. (2010) Identification of *Pyrenophora teres* in planta during barley net blotch disease. Australas. Plant Pathol. 39, 499-507

Liu, Z. J and Cordes, J. F. (2004) DNA marker technologies and their applications in aquaculture genetics. Aquaculture. 238: 1-37

Liu, Z., Ellwood, S. R., Oliver, R. P and Friesen, T. L. (2011) *Pyrenophora teres*: profile of an increasingly damaging barley pathogen. Mol. Plant Pathol. 12, 1-19

Liu, Z., Holmes, D. J., Faris, J. D., Chao, S., Brueggeman, R. S., Edwards, M. C. and Friesen, T. L. (2015) Necrotrophic effector triggered susceptibility (NETS) underlies the barley-*Pyrenophora teres* f. *teres* interaction specific to chromosome 6H. Mol. Plant Pathol. 16, 188-200

Liu, Z. H., Zhong, S., Stasko, A. K., Edwards, M. C., Friesen, T. L. (2012) Virulence profile and genetic structure of a North Dakota population of *Pyrenophora teres* f. *teres*, the causal agent of Net form net blotch of barley. Phytopathology 102:539-546

Mcdonald, W. C., and Buchannon, K. W. (1962) The inheritance of variability in *Pyrenophora teres*. Barley Newsletter 6: 40

Marguerat, S., Wilhelm, B. T., Bahler, J. (2008) Next generation sequencing: applications beyond genomes. Biochem. Soc. Trans. 36: 1091-1096

Mathre, D. E. (1997) Compendium of barley diseases, 2nd edn. St. Paul, MN: American Phytopathological Society. Pg 28-32

Mewes, H. W., Albermann, K., Bahr, M., Frishman, D., Gleissner, A., Hani, J., Heumann, K., Kleine, K., Maierl, A., Oliver, S. G., Pfeiffer, F., Zollner, A. (1997) Overview of the yeast genome. Nature. 387:7-8

Miller, M. R., Dunham, J. P., Amores, A., Cresko, W. A., Johnson, E. A. (2007) Rapid and cost-effective polymorphism identification and genotyping using restriction site associated DNA (RAD) markers. Genome Research. 17: 240-248

Morozova, O. and Marra, M. A. (2008) Applications of next-generation sequencing technologies in functional genomics. Genomics. 92: 255-264

Poland, J. A., Brown, P. J., Sorrells, M. E., Jannink, J-L. (2012) Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach. PLoS ONE. 7: e32253

Richards J, Chao S, Friesen T, Brueggeman R (2016) Fine mapping of the barley chromosome 6H net form net blotch susceptibility locus. G3 (Bethesda) 6(7):1809–1818. doi:10.1534/g3.116.028902

Sanger, F., Nicklen, S. and Coulson, A. R. (1977) DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. U.S.A 74:5463-5467

Sarpeleh, A., Wallwork, H., Catcheside, D.E.A., Tate, M.E. and Able, A.J. (2007) Evidence of involvement of proteinaceous toxins from *Pyrenophora teres* in symptom development of net blotch of barley. Phytopathology, 97, 907–915

Sarpeleh, A., Wallwork, H., Tate, M.E., Catcheside, D.E.A. and Able, A.J. (2008) Initial characterization of phytotoxic proteins isolated from *Pyrenophora teres*. Physiol. Mol. Plant Pathol. 72, 73–79

Shipton, W. A., Khan, T. N. and Boyd, W. J. R. (1973) Net blotch of barley. Rev. Plant Pathol. 52, 269–290

Shjerve, R. A., Faris, J. D., Brueggeman, R. S., Yan, C., Zhu, Y., Koladia, V. and Friesen, T.L. (2014) Evaluation of a *Pyrenophora teres* f . *teres* mapping population reveals multiple independent interactions with the barley 6H chromosome region. Fungal Genet. Biol. 70, 104-112

Smedegård-Petersen, V. (1971) *Pyrenophora teres* f. *maculata* f. nov. and *Pyrenophora teres* f. *teres* on barley in Denmark. *Kgl.* Vet. Landbohojsk. Arsskr. 124–144

Smedegård-Petersen, V. (1977a) Isolation of two toxins produced by *Pyrenophora teres* and their significance in disease development of net-spot blotch of barley. Physiol. Plant Pathol. 10, 203–211

St. Pierre, S., Gustus, C., Steffenson, B., Dill-Macky, R. and Smith, K. P. (2010) Mapping net form net blotch and Septoria speckled leaf blotch resistance loci in barley. Phytopathology, 100, 80–84

Steffenson, B. J. and Webster, R. K. (1992a) Quantitative resistance to *Pyrenophora teres* f. *teres* in barley. Phytopathology, 82, 407–411

Steffenson, B. J., and Webster, R. K. (1992b) Pathotype diversity of *Pyrenophora teres* f. *teres*. Phytopathology 82, 170-177

Steffenson, B.J., Hayes, P.M. and Kleinhofs, A. (1996) Genetics of seedling and adult plant resistance to net blotch (*Pyrenophora teres* f. *teres*) and spot blotch (*Cochliobolus sativus*) in barley. Theor. Appl. Genet. 92, 552–558.

Tekauz A (1985) A numerical scale to classify reactions of barley to *Pyrenophora teres*. Can J Plant Pathol 7, 181–183

Tekauz, A. (1990) Characterization and distribution of pathogenic variation in *Pyrenophora teres* f. *teres* and *P. teres* f. *maculata* from western Canada. Can. J. Plant Pathol. 12, 141–148

Van Caeseele, L. and Grumbles, J. (1979) Ultrastructure of the interaction between *Pyrenophora teres* and a susceptible barley host. Can. Bot. 57, 40-47

Vassarotti, A., and Goffeau, A. (1992) Sequencing the yeast genome: The European effort. TIBTECH. 10: 15-18

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, Frijters, A., Pot, J., Peleman, J., Kuiper, M., Zabeay, M. (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 23: 4407:4414

Weiland, J. J., Steffenson, B. J., Cartwright, R. D. and Webster, R. K. (1999) Identification of molecular genetic markers in *Pyrenophora teres* f. *teres* associated with low virulence on 'Harbin' barley. Phytopathology, 89, 176–181

Welsh, J. and McClelland, M. (1990) Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. 18: 7213-7218

William, J. G.K., Kubelik, A. R., Livak, K. J., Rafalki, J. A., Tingey, S. V. (1990) DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18: 7213-7218

Wu, H-L, Steffenson, B. J., Zhong, S. (2003) Genetic variation for virulence and RFLP markers in *Pyrenophora teres*. Can J Plant Pathol 25:82-90

www.barley foods.org/facts.html

Zohary, D., Maria, H. (2003) Domestication of Plants in the Old World: The origin and Spread of cultivated Plants in West Asia, Europe, and the Nile Valley (3rd ed.). Oxford University Press. 59-69

CHAPTER 2: GENETIC ANALYSIS OF NET FORM NET BLOTCH RESISTANCE IN BARLEY LINES CIHO 5791 AND TIFANG AGAINST A GLOBAL COLLECTION OF *P. TERES* F. *TERES* ISOLATES[1]

Abstract

The barley line CI5791 confers high levels of resistance to *Pyrenophora teres* f. *teres*, causal agent of net form net blotch (NFNB), with few documented isolates overcoming this resistance. Tifang barley also harbors resistance to *P. teres* f. *teres* which was previously shown to localize to barley chromosome 3H. A CI5791 × Tifang F_6 recombinant inbred line (RIL) population was developed using single seed descent. The Illumina iSelect SNP platform was used to identify 2,562 single nucleotide polymorphism (SNP) markers across the barley genome, resulting in seven linkage maps, one for each barley chromosome. The CI5791 × Tifang RIL population was evaluated for NFNB resistance using nine *P. teres* f. *teres* isolates collected globally. Tifang was resistant to four of the isolates tested whereas CI5791 was highly resistant to all nine isolates. QTL analysis indicated that the CI5791 resistance mapped to chromosome 6H whereas the Tifang resistance mapped to chromosome 3H. Additionally, CI5791 also harbored resistance to two Japanese isolates that mapped to a 3H region similar to that of Tifang. SNP markers and RILs harboring both 3H and 6H resistance will be useful in resistance breeding against NFNB.

Introduction

Net blotch caused by *Pyrenophora teres* is present in most barley production regions of the world, including the Middle East, Australia, Asia, Europe, Africa, and South and North America (Mathre 1997). The pathogen is most prevalent where barley is planted under cool wet conditions; however, it also exists in warm dry areas (Shipton et al. 1973). *P. teres* exists in two forms, *P. teres* f. *teres* and *P. teres* f. *maculata*, causing net form net blotch (NFNB) and spot form net blotch (SFNB), respectively. The NFNB disease is initially observed as small circular and elliptical dot-like lesions that soon develop into dark brown blotches containing longitudinal and transverse striations forming a net-like pattern (Steffenson and Webster 1992; Mathre 1997). For highly resistant barley lines, dot-like lesions do not develop into the net-like pattern, but remain restricted.

Geschele (1928) showed that resistance to NFNB was inherited in a Mendelian fashion (Geschele 1928, Reviewed in Liu et al. 2011). Later, three incompletely dominant resistance genes reported as Pt_1, Pt_2 and Pt_3 were shown to be effective against *P. teres* isolates collected in California (Mode and Schaller 1958; Schaller 1955) and several other breeding lines have been reported to harbor single dominant resistant genes (Gray 1966; McDonald and Buchannon 1962). Khan and Boyd (1969a, b) were the first to report the physiological specialization of the pathogen which was useful in the evaluation of sources of resistance that correlated with differences in virulence. NFNB resistance genes *Rpt1a*, *Rpt3d*, *Rpt1b* and *Rpt2c* were identified by trisomic analysis on barley chromosomes 3H, 2H, 3H and 5H, respectively (Bockelman et al. 1977). As is often the case, inheritance of resistance in adult plants under field conditions was shown to be more complex as compared to seedling resistance (Arabi et al. 1990; Douglas and Gordon 1985; Steffenson and Webster 1992). Dominant susceptibility genes have also been

identified in seedlings (Ho et al. 1996; Abu Qamar et al. 2008; Liu et al. 2015), as well as the potential for the corresponding pathogen effectors (Liu et al. 2015; Shjerve et al. 2014), showing the complexity of this host pathogen interaction.

In several barley backgrounds, resistance to NFNB has mapped to chromosome 6H (reviewed in Liu et al. 2011) but other studies have shown that a similar region on chromosome 6H consists of multiple genes that confer dominant susceptibility to different pathotypes of *P. teres* f. *teres*. (Abu Qamar et al. 2008; Liu et al. 2011; Liu et al. 2015; Shjerve et al. 2014, Richards et al., 2016). Several studies have been performed using differential sets of barley lines that exhibited different resistance patterns when inoculated with NFNB isolates collected from different parts of the world (Steffenson and Webster 1992; Wu et al. 2003; Gupta and Loughman 2001; Cromey and Parkes 2003; Jalli 2004; Tekauz 1990; Jonsson et al. 1997; Khan and Boyd 1969b; Liu et al. 2011; Jalli and Robinson 2000). These studies indicated the presence of several different avirulence and/or virulence factors that theoretically correspond to different resistance/ susceptibility genes in these barley lines.

CI5791 is an Ethiopian breeding line reported to show high levels of resistance against *P. teres* f. *teres* isolates (Mode and Schaller 1958; Khan and Boyd 1969a,b; Khan and Boyd 1971; Tekauz 1990; Steffenson and Webster 1992; Wu et al. 2003; Cromey and Parkes 2003; Jalli 2004) and Tifang is a Manchurian line reported to show resistance against some *P. teres* f. *teres* isolates (Steffenson and Webster 1992; Wu et al. 2003; Cromey and Parkes 2003; Jalli 2004; Jonsson et al. 1997; Khan and Boyd 1969b; Jalli and Robinson 2000; Khan and Boyd 1971). Using trisomic analysis, Bockelman et al. (1977) reported that Tifang had resistance located on chromosome 3H (Bockelman et al. 1977). Based on the phenotypic differences observed in the resistance patterns against various *P. teres* f. *teres* isolates, CI5791 and Tifang

were selected to develop a population and study the genetics of resistance found in CI5791 and Tifang.

Materials and Methods

Biological Materials

One hundred and seventeen $F_{2:6}$ recombinant inbred lines (RILs) were developed by single seed descent from a cross between CI5791 (hereafter referred to as the CT population) and Tifang resulting in a CI5791 (female) × Tifang RIL population (hereafter referred to as CT). F_2 individuals were similarly derived from crosses of CI5791 and Tifang to be used in evaluating gene action. The CT population, F_2 individuals and the parents were evaluated for reactions to nine *P. teres* f. *teres* isolates that had diverse geographic origins, including LDNH04Ptt-19, Tra-A5, FGOH04Ptt-21, 15A, 6A, JPT0101, JPT9901, Br. Pteres and BB06 (Table 2.1). This is the most geographically diverse set of isolates that we have and several of these isolates have been used in other studies including as parents of mapping populations.

Table 2.1. Source and collection location information for each of the nine *P. teres* f. *teres* isolates used in this study.

Isolate	Location	References	Collector
LDNH04Ptt-19	North Dakota, USA	-	Tim Friesen
Tra-A5	Montana,USA	-	Tim Friesen
FGOH04Ptt-21	North Dakota,USA	-	Tim Friesen
JPT0101	Japan	-	Jack Rasmussen
JPT9901	Japan	Liu et al. 2015	Jack Rasmussen
15A	California, USA	Steffenson and Webster 1992; Wu et al. 2003; Shjerve et al. 2014; Liu et al. 2015	Brian Steffenson
6A	California, USA	Steffenson and Webster 1992; Wu et al. 2003; Shjerve et al. 2014; Liu et al. 2015	Brian Steffenson
Br.Pteres	Brazil	Liu et al. 2015	Flavio Santana
BB06	Denmark	Liu et al. 2015	Lise Nistrup Jorgensen

Genotypic Analysis

DNA was extracted from the CT population and parents using the Qiagen Biosprint 15 Plant Extraction kit (Shjerve et al. 2014). After obtaining DNA, the Illumina iSelect SNP platform (Comadran et al. 2012), including 7,824 SNP markers, was used to genotype the population. The Infinium SNP assay was performed following the manufacturer's instructions (Illumina Inc. 2010). Genotype calling was done using the genotyping module implemented in the GenomeStudio software v.2011.1 developed by Illumina (San Diego, CA). Genotype calls were then manually inspected for call accuracy.

Map Construction

The Microsoft Excel-based software program MapDisto version 1.7.5 (Lorieux 2012) was used to construct the genetic linkage maps. The 'find groups' command was used to identify linkage groups with LOD $_{min}$=3.0 and r_{max}= 0.3. The 'order sequence' command was used to establish the initial order of markers in each linkage group. The 'ripple order', 'check inversions', and 'drop locus' commands were used to refine and validate the final order of the markers. The 'draw all sequences' command was used to obtain a graphical representation of the maps for all the linkage groups. For QTL analysis, co-segregating markers were identified from the genetic maps, and a single marker within each set of co-segregating markers was retained while the remaining redundant markers were removed. Preference for the marker to be retained at each locus was given to the one with the least amount of missing data. The maps were then reconstructed in MapDisto and the data exported for QTL analysis using the computer program QGene v4.3 (Joehanes and Nelson 2008).

Genetic Map Comparison

Population sequencing (POPSEQ) positions (Mascher et al. 2013) of the Illumina iSelect SNPs previously described by Cantalapiedra et al. (2015) in the tool BARLEYMAP were utilized to determine map concordance. Data was imported into Microsoft Excel and the command 'vlookup' was executed to create a cross reference file containing all available POPSEQ positions of the markers utilized in the genetic map construction. If no POPSEQ position was available from this dataset for markers flanking a QTL, BLAST searches of the barley genome were conducted (http://webblast.ipk-gatersleben.de/barley/viroblast.php).

Phenotypic Analysis

All *P. teres* f. *teres* isolates were grown on V8-PDA (150 ml V-8 juice, 10g Difco PDA, 3g $CaCO_3$, 10g agar, and 850 ml distilled water). Petri plates were kept in a dark cabinet at room temperature for 5 days, followed by 24 h of light at room temperature, followed by 24 h of dark at 15°C. Plates were then flooded with distilled water and conidia were harvested from plates using an inoculating loop. The inoculum was collected and diluted with distilled water to obtain 2000 spores/ml. One drop of Tween 20 was added to every 50 ml of inoculum to reduce spore clumping (Abu Qamar et al. 2008).

Individual RILs were planted along with the parents in a rack containing 98 cone-tainers (Stuwe and Sons, Inc., Corvallis, OR) with 'Tradition' barley planted as the border to reduce any edge effect. The border was placed around each rack but cones were not randomized within each rack. Inoculations were done as described by Friesen et al. (2006). When the secondary leaves were fully expanded, plants were inoculated with a conidial solution of individual *P. teres* f. *teres* isolates using an air sprayer (Huskey, model # HDS790) until a heavy mist covered all the leaves before runoff occurred. After inoculations, plants were placed in 100% relative humidity in the light at 21° C for 24 h and then placed in a growth chamber under a 12 h photoperiod at 21° C. Disease reactions were evaluated seven days post-inoculation because, for this fungus, under these conditions, a 7 day evaluation was found to be optimal. These reactions were evaluated on a 1 to 10 scale as described by Tekauz (1985) where reaction type 1 was the most resistant and reaction type 10 was the most susceptible. Greater than or equal to 4 were considered as the susceptible rather than 5 as suggested by Tekauz (1985). Three un-randomized replicates with borders were completed for each isolate across the whole population. For F_2 analysis, F_2 individuals were planted in a single cone-tainer and inoculated with the nine *P. teres*

f. *teres* isolates separately, similar to the RIL population. Each plant was evaluated individually for disease reaction to each isolate.

For F_2 analysis, F_2 individuals were planted in a single container and inoculated with the nine *P. teres* f. *teres* isolate separately, similar to the RIL population. Each plant was evaluated individually for the disease reaction to each isolate.

QTL Analysis

The average of three replicates and the MapDisto marker data were exported to QGene software v 4.3.0 for QTL analysis (Joehanes and Nelson 2008). The critical logarithm of the odds ratio (LOD) threshold for each data set was calculated by performing 1000 permutations and the obtained value at the $\alpha_{0.01}$ level was used as the critical LOD threshold. Composite interval mapping (CIM) was performed by selecting the LOD value as the test statistic. QTL analysis was carried out by selecting a particular trait and looking across all the linkage groups for the significant QTL. The cofactor parameter was selected as a default parameter to identify the most significant marker underlying each QTL. The chromosome display command was used to view the marker loci on each linkage group.

Statistical Analysis

Least significant differences (LSD) were identified to determine separation between the average phenotypic reactions for genotypic classes identified in the CT population. SAS 9.4 (SAS Institute Inc., 2013) was used to perform the LSD tests at α=0.05.

Results

Linkage Mapping

The Barley iSelect chip used for marker identification featured 7824 SNP markers distributed across the barley genome. 2562 of the 7824 SNP markers were polymorphic in our

CT population and were, therefore, used for linkage mapping analysis. The markers were assembled into seven linkage groups corresponding to the seven barley chromosomes. The linkage groups spanned a total genetic distance of 1012.2 cM, with the chromosome 6H linkage group being the shortest (113.6 cM) and the chromosome 5H linkage group being the longest (184.6 cM) (Table 2.2). The number of SNP markers per chromosome ranged from 243 (chromosome 1H) to 503 (chromosome 5H). A total of 827 unique loci were detected by the 2562 SNP markers yielding an average density of 1.2 cM/locus. 19 gaps were identified on the linkage groups with sizes ranging from 5 cM to 10.8 cM and these gaps were located at different positions on the linkage groups. One marker from each of the 827 loci was chosen to derive a non-redundant marker set for subsequent QTL analysis.

Table 2.2. Summary of the seven linkage maps developed in the CI5791 × Tifang RIL population.

Chromosome	SNP markers	No. unique loci	Length (cM)	Marker Density (cM/locus)
1H	243	90	133.5	1.5
2H	480	143	161.2	1.1
3H	407	122	156.7	1.3
4H	250	91	115.3	1.3
5H	503	146	184.6	1.3
6H	323	103	113.6	1.1
7H	356	132	147.3	1.1
Total	2562	827	1012.2	1.2

Genetic Map Comparison

A total of 2562 markers that were identified as polymorphic between CI5791 and Tifang were compared to the barley genome via data from BARLEYMAP to obtain POPSEQ genetic

positions. Of the 2562 markers, a POPSEQ locus was obtained for 1938 markers and used for collinear comparison to the barley genome. POPSEQ chromosomal anchoring of the 1938 markers nearly perfectly correlated with the CI5791 × Tifang linkage groups, with the exception of marker SCRI_RS_180004, which was anchored to chromosome 7H via POPSEQ and to chromosome 6H in the CI5791 × Tifang population. However, upon further examination via BLAST searches of the barley genome, the second best BLAST hit (87% identity) for marker SCRI_RS_180004 was on chromosome 6H at 54.88 cM. Markers that flank SCRI_RS_180004 in the CI5791 × Tifang linkage map have POPSEQ positions ~55 cM on chromosome 6H, indicating that this marker may have been non-specific in this population. Scatterplots were constructed to compare the genetic positions of the remaining markers. General collinearity was observed, with only a few minor discrepancies between the CI5791 × Tifang and POPSEQ genetic positions.

Phenotypic Analysis

Homogeneity between the replicates was high with Pearson's correlation coefficients between replicates ranging from 0.6 to 0.9, therefore reps were averaged and used for analysis. CI5791 was highly resistant to all nine isolates (average disease reactions of less than 2.0) and Tifang was resistant to four of the nine isolates including 15A, 6A, Br. Pteres and BB06 (average disease reactions of less than 2) (Table 2.3, Fig.2.1). LDNH04Ptt-19, Tra-A5, FGOH04Ptt-21 and JPT9901 were virulent on Tifang with average disease reactions equal to or greater than 5.0. JPT0101 was more virulent on Tifang compared to CI5791, with average disease reaction types of 4.00 and 1.0, respectively, indicated a relatively lower level of virulence on Tifang for this isolate compared to the other virulent isolates (Table 2.3, Fig. 2.1).

Table 2.3. Disease reaction types of parents and genotypic classes of the CI5791×Tifang RIL population.

Isolates[a]	LSD[b]	CI 5791[c]	Tifang[c]	$6H_{CI5791}$/ $3H_{Tifang}$[d]	$6H_{CI5791}$/ $3H_{CI5791}$[d]	$6H_{Tifang}$/ $3H_{Tifang}$[d]	$6H_{Tifang}$/ $3H_{CI5791}$[d]
LDNH04Ptt-19	0.4220	1.00	7.17	1.12 A	1.10 A	5.99 B	6.21 B
Tra-A5	0.4897	1.00	7.33	1.25 A	1.10 A	6.47 B	6.82 B
FGOH04Ptt-21	0.4386	1.25	5.50	1.80 A	2.04 A	5.97 B	6.98 C
JPT0101	0.4572	1.00	4.00	1.13 A	1.01 A	4.85 C	1.77 B
JPT9901	0.4868	1.17	6.17	1.26 A	1.11 A	6.54 C	3.96 B
15A	0.5653	1.00	1.50	1.08 A	1.04 A	2.32 B	6.14 C
6A	0.6423	1.00	1.75	1.09 A	1.69 AB	1.89 B	4.94 C
Br. Pteres	0.3692	1.17	1.00	1.03 A	1.35 A	1.28 A	3.12 B
BB06	0.3592	1.00	1.17	1.00 A	1.12 A	1.16 A	3.35 B

[a] The nine *P. teres* f. *teres* isolates used in the analysis, which include the Northern Great Plains isolates (LDNH04Ptt-19, Tra-A5, FGOH04Ptt-21), the Japanese isolates (JPT0101 and JPT9901), the California isolates (15A and 6A), the Brazilian isolate (Br. Pteres) and the Danish isolate (BB06)

[b] Least significant difference (LSD) calculated at P=0.05 for each of the isolates

[c] Parents, CI5791 and Tifang, used to develop the CT mapping population

[d] The genotypic classes for the RIL population based on the presence of the most significant marker at the 3H and 6H resistance loci

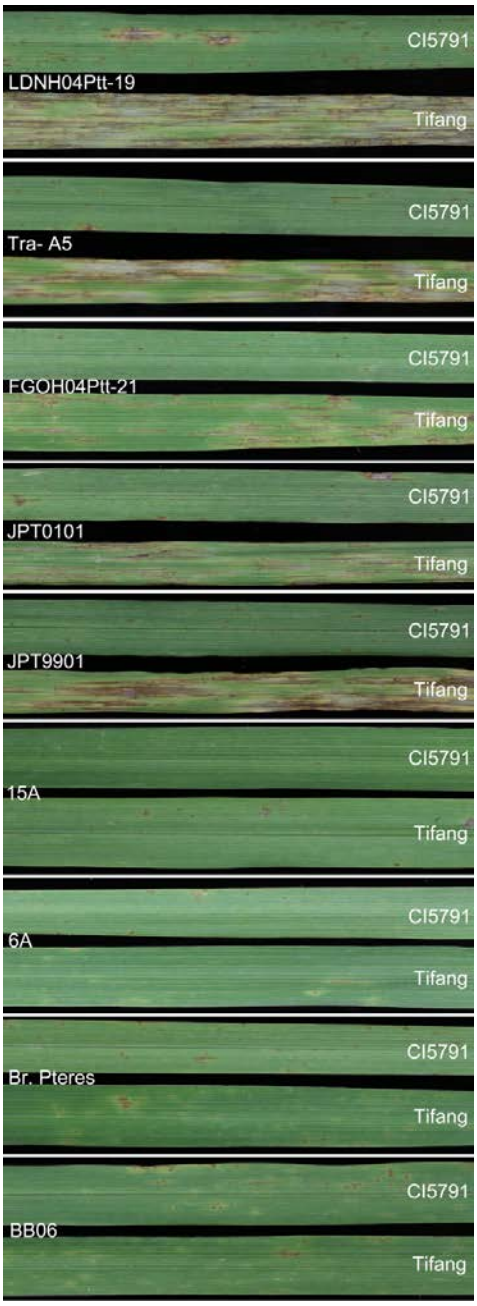

Fig. 2.1. Disease reactions on CI5791 and Tifang for each of the nine *P. teres* f. *teres* isolates. Barley parental lines are indicated on the right and *P. teres* f. *teres* isolates are indicated on the left. All tested isolates were avirulent on CI5791. 15A, 6A, Br. Pteres, and BB06 isolates were avirulent on both CI5791 and Tifang. The Northern Great Plains isolates (LDNH04Ptt-19, Tra-A5 and FGOH04Ptt-21) and the Japanese isolates (JPT0101 and JPT9901) were virulent on Tifang.

Across the CT population, similarities also arose among the members of three groups, i.e. the Northern Great Plains isolates (LDNH04Ptt-19, Tra-A5, FGOH04Ptt-21), Japanese isolates

(JPT0101 and JPT9901) and a geographically diverse group of isolates consisting of two California isolates (15A and 6A), a Brazilian isolate (Br. Pteres) and a Danish isolate (BB06). R:S segregation ratios of the three Northern Great Plains isolates that were virulent on Tifang (LDNH04Ptt-19, Tra-A5 and FGOH04Ptt-21) were not significantly different from a 1:1 when using a reaction type of 4.0 as the susceptible cutoff, indicating a single major gene conferring resistance or susceptibility (Table 2.4). For the Japanese isolates (JPT0101 and JPT9901) the R:S ratio was narrowly but still significantly different from a 3:1 ratio (χ^2=3.9; *P*=0.048 and χ^2=4.1; *P*=0.043, respectively) (Table 2.4).

Among isolates with avirulent phenotypes on both parents, inoculation of the California isolates (15A and 6A) resulted in a R:S segregation ratio that was not significantly different from a 3:1, indicating the presence of two resistance genes but with one coming from each parent. When using the same resistant/susceptible cutoff as we did for the other isolates, the Brazilian (Br. Pteres) and the Danish (BB06) isolates showed R:S ratios that were significantly different from a 3:1 ratio (χ^2 = 19.132; $P < 0.0001$ and χ^2 = 17.220; $P < 0.0001$) indicating the potential of at least one resistance gene coming from each parent but with additional genes resulting in a complex quantitative inheritance (Table 2.4).

Table 2.4. Resistant: susceptible (R:S) segregation ratios of the CI5791 × Tifang RIL population and F_2 individuals.

Isolates	Observed R:S ratio[e] (RILs)	Expected ratio	R:S ratio[e] (F_2)	Ratio
LDNH04Ptt-19	63:54	1:1	40:9	3:1
Tra-A5	63:51	1:1	35:15	3:1
FGOH04Ptt-21	59:58	1:1	31:14	3:1
JPT0101	97:20	3:1[a]	44:3	15:1
JPT9901	74:37	3:1[b]	51:3	15:1
15A	81:36	3:1	52:4	15:1
6A	92:24	3:1	48:2	15:1
Br. Pteres	99:7	3:1[c]	35:2	15:1
BB06	98:8	3:1[d]	43:4	15:1

[a] Significantly different from 3:1(P= 0.048)
[b] Significantly different from 3:1 (P=0.043)
[c] Significantly different from 3:1 (P<0.0001)
[d] Significantly different from 3:1 (P<0.0001)
[e] R:S ratios are based on a $\geq$ 4.0 cutoff for susceptibility

F_2 Analysis

To evaluate the resistance gene action conferred by both CI5791 and Tifang, F_2 analysis was performed using $\geq$ 4 as the susceptible cut off. CI5791 × Tifang F_2 individuals showed a 3:1 (R:S) ratio when inoculated with LDNH04Ptt-19, Tra-A5 and FGOH04Ptt-21 isolates (Table 2.4, Fig. 2.2), confirming the single resistance gene interpretation from the RIL population. For the isolates avirulent on both parental lines, including the California isolates (15A and 6A), the Brazilian isolate (Br. Pteres) and the Danish isolate (BB06), the F_2 individuals showed a R:S ratio not significantly different from 15:1 (Table 2.4, Fig. 2.2) indicating two dominant resistance genes. The Japanese isolates (JPT0101 and JPT9901) also showed a R:S ratio not

significantly different from a 15:1 (Table 2.4, Fig.2.2) indicating the presence of two resistance genes, both coming from CI5791, matching the results from the RIL population.

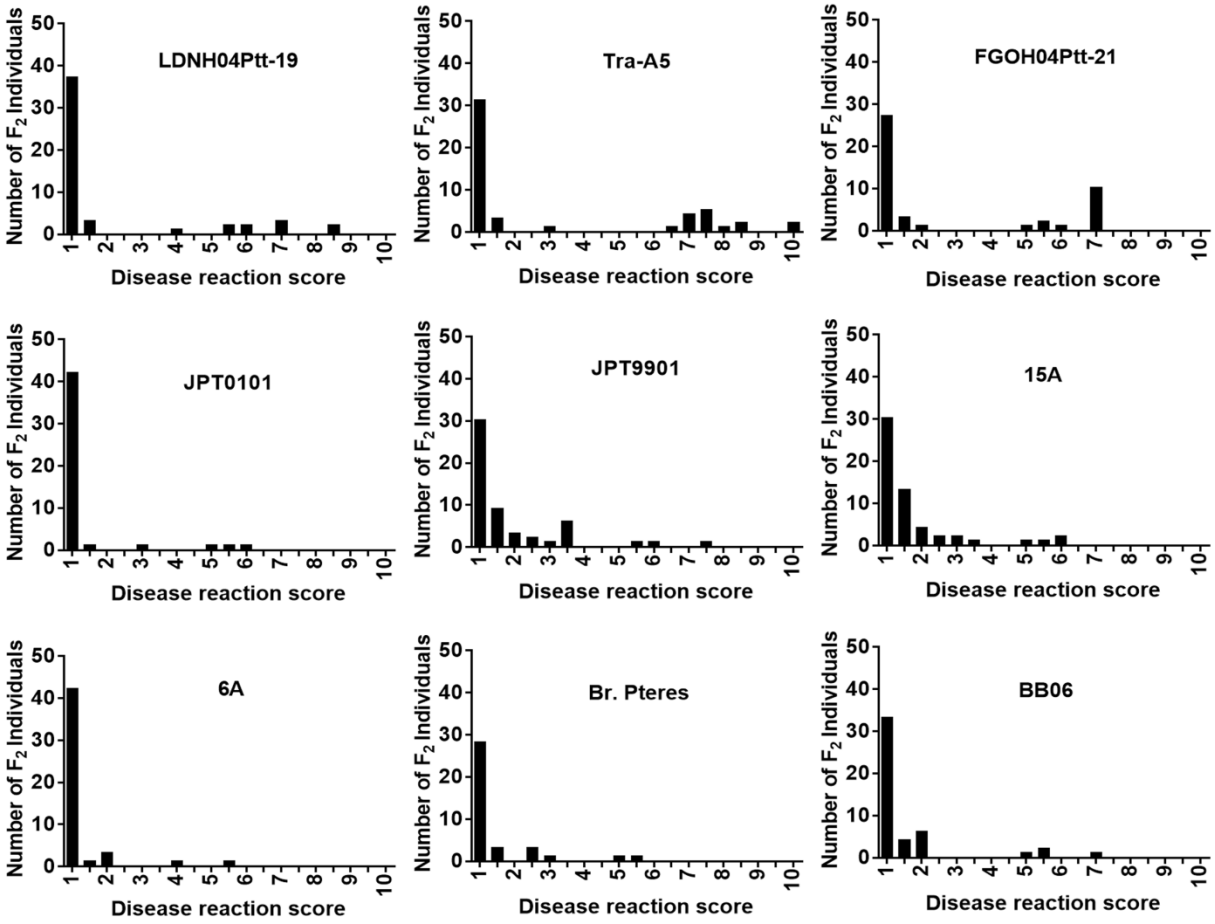

Fig. 2.2. Histograms showing the phenotypic reactions obtained for F_2 individuals of the CI5791×Tifang cross inoculated with the nine *P. teres* f. *teres* isolates (LDNH04Ptt-19, Tra-A5, FGOH04Ptt-21, JPT0101, JPT9901, 15A, 6A, Br. Pteres and BB06). The y-axis shows the number of the F_2 individuals and the x-axis shows the disease reaction score categories separated in 0.5 point intervals.

QTL Analysis

By performing 1000 permutations on each data set, LOD value thresholds (P=0.01) were obtained that ranged from 3.6-4.0. Hence, the highest stringency identified (4.0) was used as a critical LOD threshold value for identifying significant QTL.

A major resistance QTL located on chromosome 6H with resistance effects contributed by CI5791 was identified for all nine isolates tested (Table 2.5, Fig. 2.3). The three Northern Great Plains isolates, LDNH04Ptt-19, Tra-A5 and FGOH04Ptt-21 showed only the 6H QTL (Table 2.5, Fig. 2.3). However, for all isolates avirulent on both parents, including 15A, 6A, Br. Pteres, and BB06, a 3H resistance QTL was identified with resistance effects contributed by Tifang (Table 2.5, Fig. 2.3). A similarly located chromosome 3H QTL was also identified for JPT0101 and JPT9901 (Fig. 2.3), however, the 3H resistance to these two Japanese isolates was conferred by CI5791 (Table 2.5, Fig. 2.3). Additional relatively minor 1H and 3H QTL were also observed for 6A with a LOD value of 5.4 and 5.0, respectively.

The most significant markers at the major 3H and 6H QTL regions (i.e. SCRI_RS_140091 for 6H and SCRI_RS_221644 for 3H) were used to create four genotypic classes (Table 2.3). The genotypic classes consisted of $6H_{CI5791}/3H_{CI5791}$, $6H_{CI5791}/3H_{Tifang}$, $6H_{Tifang}/3H_{CI5791}$, and $6H_{Tifang}/3H_{Tifang}$ (Table 2.3) and were used to evaluate the data sets for each of the nine *P. teres* f. *teres* isolates. The Northern Great Plains isolates LDNH04Ptt-19, Tra-A5 and FGOH04Ptt-21 showed a QTL on chromosome 6H alone that was conferred by CI5791. The genotypic classes containing the $6H_{CI5791}$ marker type (i.e. $6H_{CI5791}/3H_{CI5791}$, $6H_{CI5791}/3H_{Tifang}$) were highly resistant regardless of the 3H genotype with the $6H_{CI5791}/3H_{CI5791}$ and the $6H_{CI5791}/3H_{Tifang}$ genotypes having disease reaction types ranging from 1.10 to 2.04 and 1.12 to 1.80, respectively, and the $6H_{Tifang}/3H_{Tifang}$ and $6H_{Tifang}/3H_{CI5791}$ genotypic classes showing phenotypic reactions ranging from 5.99 to 6.47 and 6.21 to 6.98, respectively (Table 2.3). Interestingly, for the FGOH04Ptt-21 data set, there was also a significant difference between the $6H_{Tifang}/3H_{CI5791}$ and $6H_{Tifang}/3H_{Tifang}$ genotypes, although, based on the QTL analysis, this is not explained by the 3H locus (Fig. 2.3).

For the Californian, Brazilian, and Danish isolates, the 3H resistance conferred by Tifang and the 6H resistance conferred by CI5791 were both highly effective. As observed for the Northern Great Plains isolates, the presence of the CI5791 type marker at the 6H locus ($6H_{CI5791}/3H_{CI5791}$ and $6H_{CI5791}/3H_{Tifang}$) showed complete resistance with reaction types ranging from 1.04 to 1.69 and 1.00 to 1.09, respectively (Table 2.3). When Tifang alleles were present at both 3H and 6H ($6H_{Tifang}/3H_{Tifang}$) the reaction types ranged from 1.16 to 2.32 showing the effectiveness of the 3H resistance being conferred by Tifang (Table 2.3) even in the absence of the 6H CI5791 resistance. When the Tifang allele at the 6H locus was combined with the CI5791 allele at the 3H locus ($6H_{Tifang}/3H_{CI5791}$), moderately susceptible to susceptible reactions were observed ranging from 3.12 to 6.14. For BB06 and Br. Pteres, no significant differences in resistance were identified between genotypes harboring Tifang alleles ($6H_{Tifang}/3H_{Tifang}$) and those harboring CI5791 alleles at both loci ($6H_{CI5791}/3H_{CI5791}$), however, the California isolate 15A showed a significant difference between these two genotypic groups, indicating that although both $6H_{CI5791}$ and $3H_{Tifang}$ confer resistance, the 6H resistance conferred by CI5791 is significantly more effective, at least to the California isolate 15A.

For the Japanese isolates, resistance was conferred by CI5791 only, with Tifang being significantly more susceptible (Table 2.3). Interestingly, the presence of the CI5791 allele at either the 3H or 6H locus (i.e. $6H_{CI5791}/3H_{CI5791}$ and $6H_{Tifang}/3H_{CI5791}$ and $6H_{CI5791}/3H_{Tifang}$) conferred a resistant reaction indicating that in addition to the CI5791 6H resistance, CI5791 harbours an isolate-specific resistance at a similar position on chromosome 3H as that of Tifang. Genotypes having the CI5791 6H allele were highly resistant as with the other isolates ranging from 1.01 to 1.11 for the $6H_{CI5791}/3H_{CI5791}$ genotype and 1.13 to 1.26 for the $6H_{CI5791}/3H_{Tifang}$ genotype. Unlike the California/Brazil/Denmark group, the $6H_{Tifang}/3H_{CI5791}$ was significantly

more resistant, ranging from 1.77 to 3.96 compared to the genotypic group containing Tifang alleles at both loci ($6H_{Tifang}/3H_{Tifang}$), which showed moderately susceptible to susceptible reactions ranging from 4.85 to 6.54. Additionally, as was seen with the California isolates, genotypes harboring the CI5791 6H resistance alone showed a significantly more resistant reaction than the genotypes harboring the CI5791 3H resistance (Table 2.3).

Table 2.5. Major quantitative trait loci associated with resistance to barley net form net blotch caused by *P. teres* f. *teres* isolates in the CI5791 × Tifang RIL population.

Isolates	LOD values (percent variation explained)			
	3H	Resistance source	6H	Resistance source
LDNH04Ptt-19	-	-	48.0 (83.0%)	CI5791
Tra-A5	-	-	47.0 (86.0%)	CI5791
FGOH04Ptt-21	-	-	35.0 (73.0%)	CI5791
JPT0101	11.0 (23.0%)	CI5791	18.0 (37.0%)	CI5791
JPT9901	6.2 (8.1%)	CI5791	29.0 (63.0%)	CI5791
15A	13.0 (18.0%)	Tifang	21.0 (45.0%)	CI5791
6A[a]	16.0 (23.0%)	Tifang	19.0 (30.0%)	CI5791
Br. Pteres	16.0 (28.0%)	Tifang	9.9 (25.0%)	CI5791
BB06	16.0 (26.0%)	Tifang	11.0 (26.0%)	CI5791

[a] Isolate 6A had additional QTL peaks on chromosomes 1H and 3H with LOD scores of 5.4 (11.0%) and 5.0 (8.0%), respectively

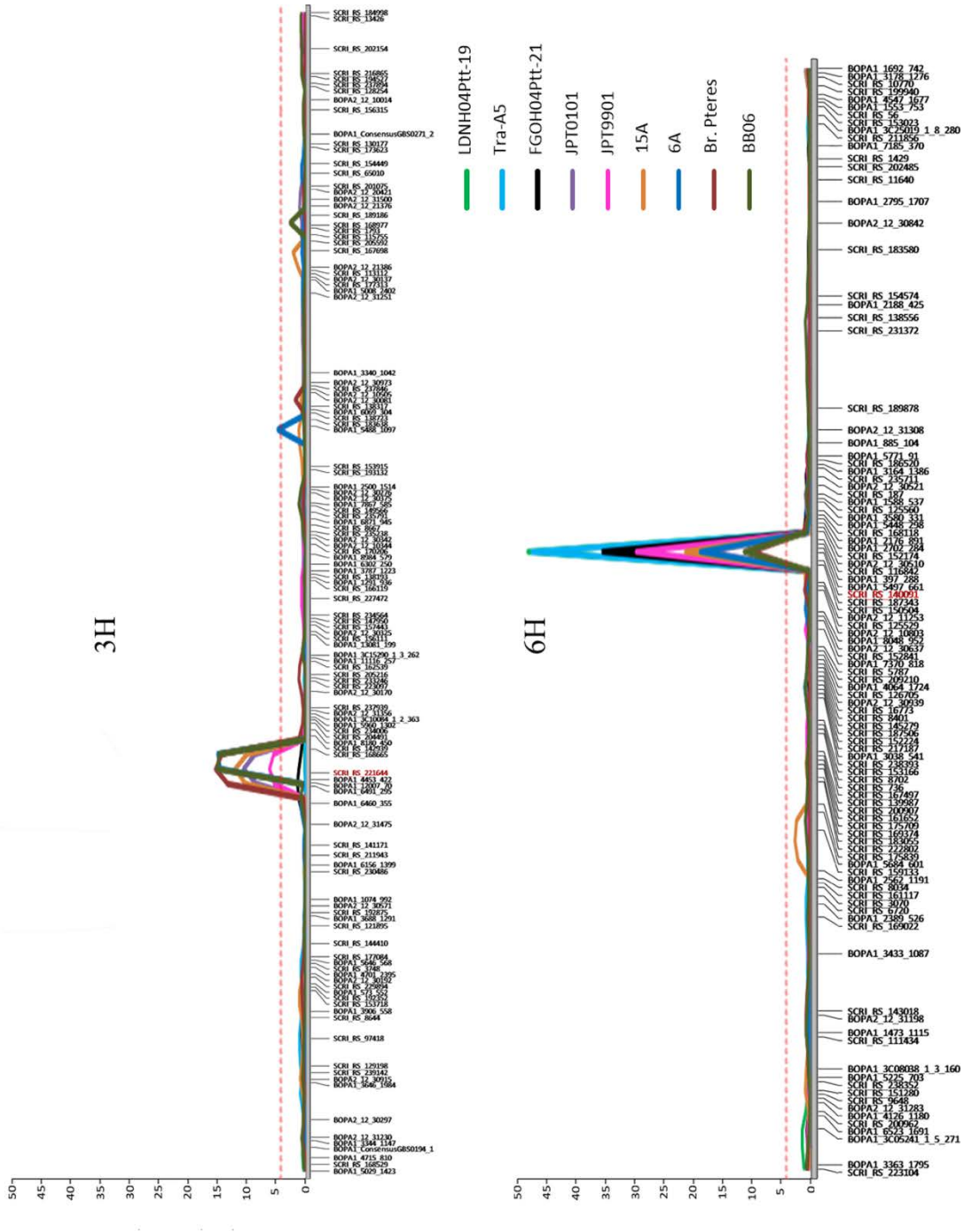

Fig. 2.3. QTL analysis of resistance in the CI5791×Tifang RIL population against a global collection of *P. teres* f. *teres* isolates. Chromosomes 3H and 6H of barley are shown with markers to the right of the corresponding QTL composite interval mapping curve. LOD scales (0-50) are shown on the x axis. The dotted line indicates the LOD threshold of 4.0 (P=0.01). The most significant marker for each QTL is shown in red.

Discussion

Several previous studies revealed the presence of both resistance and susceptibility genes at the centromeric region of barley chromosome 6H (reviewed in Liu et al. 2011). Here we showed that NFNB resistance conferred by CI5791 was effective against a global collection of *P. teres* f. *teres* and this resistance also mapped to a similar centromeric region on barley chromosome 6H. Unlike several of the other 6H studies, the 6H resistance conferred by CI5791 was highly effective with almost no disease-associated damage to the leaf, outside of a pinpoint dark brown lesion (Fig. 2.1). Based on phenotypic analysis of F_2 individuals and phenotypic and QTL analysis of an RIL population, it was clear that the 6H resistance conferred by CI5791 was dominant.

When dividing the population into genotypic classes that did or did not have the CI5791 6H alleles, the lines with CI5791 alleles ranged in disease reaction from 1.0 to 2.0 which are highly resistant reactions on the 1 to 10 Tekauz (1985) scale. The complete effectiveness of the CI5791 resistance to all the isolates tested indicates the potential usefulness and durability of this gene.

NFNB resistance/ susceptibility loci have also been identified on chromosome 3H (Graner et al. 1996; Raman et al. 2003; Gupta et al. 2004; Yun et al. 2005), including NFNB studies on Tifang (Schaller 1955) where resistance was located to chromosome 3H using trisomic analysis (Bockelman et al. 1977). However, no chromosome 3H map location was identified. Our current study mapped and genetically characterized the 3H locus and showed that resistance coming from chromosome 3H was effective against six of the nine isolates used in this study. Interestingly, 3H resistance effective against the Danish, the Brazilian, and the two California isolates was conferred by Tifang alleles, but the 3H resistance effective against the

two Japanese isolates was conferred by the CI5791 alleles, indicating the presence of allelic variation of a single resistance gene or two linked resistance genes, one in CI5791 and one in Tifang.

RIL population analysis did not clearly define resistance gene action for the Japanese, Brazilian or Danish isolates (Table 2.4). However, the F_2 results did indicate that the 3H resistance conferred by both Tifang and CI5791 as well as the 6H resistance conferred by CI5791 were dominant. It is possible that, similar to the 6H centromeric region identified here and by others, the 3H locus is also a complex region harboring different alleles of the same gene in Tifang and CI5791 or at least two closely linked resistance genes conferring resistance to different pathotypes. The generation of a larger population, fine mapping, and gene cloning will be necessary to characterize these regions.

Recently, several necrotrophic specialist pathogens have been shown to produce necrotrophic effectors (NEs) that are effective at triggering the host programmed cell death (PCD) response to induce necrosis for the purpose of extracting nutrients from the host (Liu et al. 2012; Lorang et al. 2012 and Ciuffetti et al. 2010). *P. teres* f. *teres* has also been defined as a necrotrophic pathogen (Liu et al. 2011) and we have shown that *P. teres* f. *teres* produces NEs that lead to NE-triggered susceptibility (NETS) (Liu et al. 2015). In this study, however, we have identified two single gene sources of dominant resistance, one of which (6H) was effective against all of the isolates tested and the other (3H) showing differential reactions across the set of isolates that we used, as well as showing resistance being conferred by different barley parental lines. We speculate that the level of resistance conferred by both the 6H and 3H loci is an early response in the host-pathogen interaction that limits either penetration altogether or any proliferation immediately after penetration. Further investigation including microscopy studies is

necessary to understand the temporal and spatial occurrence of this resistance mechanism. Additionally, characterization of the mode of pathogen recognition includes the identification of this resistance gene and the identification of the pathogen effector triggering this high level of resistance will be necessary to fully understand this host-pathogen interaction.

Literature Cited

Abu Qamar M, Liu ZH, Faris JD, Chao S, Edwards MC, Lai Z, Franckowiak JD, Friesen TL (2008) A region of barley chromosome 6H harbors multiple major genes associated with net type net blotch resistance. Theor and Appl Genet 117:1261-1270

Arabi MI, Sarrafi A, Barrault G, Albertini L (1990) Inheritance of partial resistance to net blotch in barley. Plant Breed 105:150-155

Bockelman HE, Sharp EL, Eslick RF (1977) Trisomic analysis of genes for resistance to scald and net blotch in several barley cultivars. Can J Bot 55:2142-2148

Cantalapiedra CP, Boudiar R, Casas AM, Igartua E, Contreras-Moreira B (2015) Barleymap: physical and genetic mapping of nucleotide sequences and annotation loci in barley. Mol Breeding 35: 1-11

Ciuffetti LM, Manning VA, Pandelova I, Betts MF, Martinez JP (2010) Host-selective toxins, Ptr ToxA and Ptr ToxB, as necrotrophic effectors in the *Pyrenophora tritici-repentis*-wheat interaction. New Phytol 187:911–919

Comadran J, Kilian B, Russell J, Ramsay L, Stein N, Ganal M, Shaw P, Bayer M, Thomas W, Marshall D, Hedley P, Tondelli A, Pecchioni N, Francia E, Korzun V, Walther A, Waugh R (2012) Natural variation in a homolog of Antirrhinum CENTRORADIALIS contributed to spring growth habit and environmental adaptation in cultivated barley. Nature Genet 44:1388-139

Cromey MG and Parkes RA (2003) Pathogenic variation in *Drechslera teres* in New Zealand. N Z Plant Protect 56:251-256

Douglas GB and Gordon LL (1985) Quantitative genetics of net blotch resistance in barley. N Z J Agric Res 28:157-164

Friesen TL, Faris JD, Lai Z, Steffenson BJ (2006) Identification and chromosomal location of major genes for resistance to *Pyrenophora teres* in a doubled-haploid barley population. Genome 49:855-859

Geschele EE (1928) The response of barley to parasitic fungi *Helminthosporium teres* Sacc. Bull. Appl. Bot. Genet. Plant Breed 19:371-384 (in Rev Appl Mycol 8:165)

Graner A, Foroughi-Wehr B, Tekauz A (1996) RFLP mapping of a gene in barley conferring resistance to net blotch (*Pyrenophora teres*). Euphytica 91:229-234.

Gray GG (1966) Genetic systems in the net blotch disease complex of barley. Phd Dissertation. North Dakota State University, Fargo, ND

Gupta S and Loughman R (2001) Current virulence of *Pyrenophora teres* on barley in Western Australia. Plant Dis 85:960-966

Gupta S, Wielinga C, Li C, Cakir M, Platz G, Loughman R, Lance R, Appels R (2004) Gene distribution and SSR markers linked with net type net blotch resistance in barley. In: Proceedings of the 9th International Barely Genetics Symposium (Spunar, J. and Janikova, J., eds.) Agricultural Research Institute Kromeriz, Ltd., Brno, Czech Republic, June 20-26. pp. 668-673

Ho KM, , Tekauz A, Choo TM, Martin RA (1996) Genetic studies on net blotch resistance in a barley cross. Can J Plant Sci 76:715-720

Illumina, Inc. (2010) Whole genome genotyping with the Infinium assay, Illumina, San Diego, CA

Jalli M (2004) Suitability of a selected barley differential set for *Pyrenophora teres* f. *teres* virulence screening. In: Proceedings of the 9th International Barely Genetics Symposium (Spunar, J. and Janikova, J., eds.) Agricultural Research Institute Kromeriz, Ltd., Brno, Czech Republic, June 20-26 pp. 266-269

Jalli M and Robinson J (2000) Stable resistance in barley to *Pyrenophora teres* f. *teres* isolates from the Nordic-Baltic region after increase on standard host genotypes. Euphytica 113:71-77

Joehanes R and Nelson JC (2008) QGene 4.0, an extensible Java QTL-analysis platform. Bioinformatics 24:2788-2789

Jonsson R, Bryngelsson T, Gustafsson M (1997) Virulence studies of Swedish net blotch isolates (*Drechslera teres*) and identification of resistant barley lines. Euphytica 94:209-218

Khan TN and Boyd WJR (1969a) Physiologic specialization in *Drechslera teres*. Aust J Biol Sci 22:1229-1235

Khan TN and Boyd WJR (1969b) Inheritance of resistance to net blotch in barley. II. Genes conditioning resistance against race WA-2. Can J Genet Cytol 11:592-597

Khan TN and Boyd WJR (1971) Genetics of host resistance to net blotch in Barley. Barley Genetics. II. R.A. Nilan, ed. Washington State University Press, Pullman. 484-492

Liu Z, Ellwood SR, Oliver RP, Friesen TL (2011) *Pyrenophora teres*: profile of an increasingly damaging barley pathogen. Mol Plant Pathol 12:1-19

Liu Z, Zhang Z, Faris JD, Oliver RP, Syme R, McDonald MC, McDonald BM, Solomon PS, Lu S, Shelver WL, Xu S, Friesen TL (2012). The cysteine rich necrotrophic effector SnTox1 produced by *Stagonospora nodorum* triggers susceptibility of wheat lines harboring Snn1. PLoS Pathog 8:e1002467

Liu Z, Holmes DJ, Faris JD, Chao S, Brueggeman RS, Edwards MC, Friesen TL (2015) Necrotrophic effector-triggered susceptibility (NETS) underlies the barley–*Pyrenophora teres* f. *teres* interaction specific to chromosome 6H. Mol Plant Pathol 16:188-200

Lorang J, Kidarsa T, Bradford CS, Gilbert B, Curtis M, Tzeng S-C, Maier CS, Wolpert TJ (2012) Tricking the guard: exploiting plant defense for disease susceptibility. Science 338:659-662

Lorieux M (2012) MapDisto: fast and efficient computation of genetic linkage maps. Mol Breed 30:1231-1235

Mascher M, Muehlbauer GJ, Rokhsar DS, Chapman J, Schmutz J, Barry K, Munoz-Amatriain M, Close TJ, Wise RP, Schulman AH, Himmelbach A, Mayer KFX, Scholz U, Poland JA, Stein N, Waugh R (2013). Anchoring and ordering NGS contig assemblies by population sequencing (POPSEQ) 76:718:727

Mathre DE (1997) Compendium of Barley Diseases, second ed. American Phytopathological Society. APS Press, St. Paul, MN

McDonald WC and Buchannon KW (1962) The inheritance of variability in *Pyrenophora teres*. Barley Newsl 6:40

Mode CJ and Schaller CW (1958) Two additional factors for host resistance to net blotch in barley. Agron J 50:15-18

Raman H, Platz GJ, Chalmers KJ, Raman R, Read BJ, Barr AR, Moody DB (2003) Mapping of genetic regions associated with net form of net blotch resistance in barley. Aust J Agric Res 54: 1359-1367

Richards J, Chao S, Friesen T, Brueggeman R (2016) Fine mapping of the barley chromosome 6H net form net blotch susceptibility locus. G3 (Bethesda) 6(7):1809–1818. doi:10.1534/g3.116.028902

SAS Institute Inc. (2013) 12.3 User's Guide: High-Performance Procedures. SAS Institute, Cary, NC

Schaller CW (1955) Inheritance of resistance to net blotch of barley. Phytopathology 45:174-176

Shipton WA, Khan TN, Boyd WJR (1973) Net blotch of barley. Rev Plant Pathol 52:269-290

Shjerve RA, Faris JD, Brueggeman RS, Yan C, Zhu Y, Koladia V, Friesen TL (2014) Evaluation of a *Pyrenophora teres* f. *teres* mapping population reveals multiple independent interactions with the barley 6H chromosome region. Fungal Genet and Biol 70:104-112

Steffenson BJ and Webster RK (1992) Quantitative resistance to *Pyrenophora teres* f. *teres* in barley. Phytopathology 82:407-411

Tekauz A (1985) A numerical scale to classify reactions of barley to *Pyrenophora teres*. Can J Plant Pathol 7:181–183

Tekauz A (1990) Characterization and distribution of pathogenic variation in *Pyrenophora teres* f. *teres and P. teres* f. *maculata* from western Canada. Can J Plant Pathol 12:141-148

Wu H-L, Steffenson BJ, Zhong S (2003) Genetic variation for virulence and RFLP markers in *Pyrenophora teres*. Can J Plant Pathol 25:82-90

Yun SJ, Gyenis L, Hayes PM, Matus I, Smith KP, Steffenson BJ, Muehlbauer GJ (2005) Quantitative trait loci for multiple disease resistance in wild barley. Crop Sci 45:2563–2572

CHAPTER 3: GENETIC ANALYSIS OF VIRULENCE/AVIRULENCE IN THE *PYRENOPHORA TERES* F. *TERES* POPULATION BB25 × FGOH04PTT-21 ACROSS A DIVERSE SET OF BARLEY LINES

Abstract

Pyrenophora teres f. *teres* is a necrotrophic pathogen responsible for causing net form net blotch of barley. In order to characterize the genetics of avirulence/virulence in the *P. teres* f. *teres* pathogen, a fungal population was developed using *P. teres* f. *teres* isolates BB25 (Denmark) and FGOH04Ptt-21 (North Dakota, USA). 109 progeny isolates were obtained from the cross between BB25 and FGOH04Ptt-21 that were used for NFNB disease evaluation across ten barley lines. BB25 was virulent on three of the barley lines and avirulent on seven of the barley lines whereas FGOH04Ptt-21 was virulent on all ten barley lines evaluated. Genetic maps were generated with single nucleotide polymorphism (SNP) markers obtained using a restriction associated DNA genotyping by sequencing (RAD-GBS) approach. Sixteen linkage groups were formed and used to identify major quantitative trait loci (QTL) associated with avirulence/virulence on the ten barley lines. Nineteen QTL were identified on twelve linkage groups out of which three QTL had major effects ($R^2 \geq 30\%$) while sixteen QTL were relatively minor ($R^2 < 30\%$). One or two major affect loci were identified for a few of the lines used regularly as differentials, conversely, variation in virulence on most of the local barley cultivars was associated with several loci that contributed quantitatively to disease.

Introduction

Net form net blotch (NFNB) of barley is caused by the fungal pathogen *Pyrenophora teres* f. *teres* and is prevalent in major barley-producing regions of the world. The pathogen causes yield losses of 10 to 40% with the possibility of total loss when a susceptible cultivar is

grown under disease-conducive environmental conditions (Mathre, 1997; Murray and Brennan, 2010). Initially, *P. teres* f. *teres* produces dot-like lesions on leaves that further develop into longitudinal striations, forming a net-like pattern. The pathogen directly penetrates the host cells without forming a feeding structure and kills its host as infection progresses (Reviewed in Liu et al., 2011). *P. teres* f. *teres* is closely related to other pathogens such as *Parastagonospora nodorum*, and *Pyrenophora tritici-repentis* that produce necrotrophic effectors (NEs) that interact with dominant host gene products in an inverse gene-for-gene manner (reviewed in Faris et al., 2013; Friesen and Faris, 2010). Liu et al. (2015) showed that *P. teres* f. *teres* also produces NEs, however, dominant resistance has also been identified in several barley backgrounds (Friesen et al., 2006; Koladia et al., 2017).

Khan and Boyd (1969) showed that *P. teres* f. *teres* isolates were cultivar-specific and had strong host genotype specificity. Weiland et al. (1999) were the first to perform avirulence mapping studies on a *P. teres* f. *teres* bi-parental population obtained from a cross of two *P. teres* f. *teres* isolates. The single gene *AvrHar* conferred low virulence on Harbin barley and was identified and mapped using molecular markers. Lai et al. (2007) used the same *P. teres* f. *teres* cross to show that two additional genes (*AvrPra1* and *AvrPra2*) conferred avirulence toward the barley line Prato where *AvrPra2* and *AvrHar* mapped to the same locus, but in repulsion. Beattie et al., (2007) developed a bi-parental mapping population by crossing two Canadian isolates, WRS 1906 (avirulent) and WRS 1607 (virulent) and identified a single gene $Avr_{heartland}$ conferring avirulence on Heartland barley. Afanasenko et al. (2007) showed that resistance in the host and avirulence in the pathogen were both controlled by one or two major genes. Specific host-pathogen interactions were shown to occur between barley lines and *P. teres* f. *teres* isolates and it was concluded that this system followed a gene-for-gene model (Afanasenko et al., 2007).

Shjerve et al., (2014) generated a cross of two California isolates to investigate the genetics of *P. teres* f. *teres* avirulence/virulence on barley lines Rika and Kombar, which were susceptible to 6A and 15A, respectively. Two loci, *VK1* and *VK2* conferred virulence on Kombar and two separate loci, *VR1* and *VR2* conferred virulence on Rika. Progeny isolates of the 15A × 6A population harboring only one of these loci were then inoculated on the Rika × Kombar population and susceptibility to these isolates corresponded to the same barley chromosome 6H region as the parental isolates (Shjerve et al., 2014; Abu Qamar et al. 2008) indicating major susceptibility genes located on barley chromosome 6H. Liu et al. (2015) reported a small, secreted NE protein PttNE1 from the intercellular wash fluids (IWFs) of Hector, a susceptible barley line, after being inoculated with a virulent isolate. The sensitivity to PttNE1 mapped to a gene designated *SPN1* that corresponded to a resistance/susceptibility QTL region of barley chromosome 6H in a recombinant inbred barley population derived from a cross between Hector and the resistant barley line NDB112 (Liu et al., 2015). This study showed the interaction between the host gene and NE of the pathogen led to susceptibility as observed in the wheat-*Parastagonospora nodorum* system (Friesen et al., 2008; Friesen and Faris, 2010). Collectively, these studies indicate that the barley- *P. teres* f. *teres* pathosystem belongs partially to the NE-triggered susceptibility (NETS) model and partially to an effector triggered immunity (ETI) model as dominant resistance genes have also been identified to be effective against the pathogen (Friesen et al., 2006; Steffenson and Webster, 1992b).

In the current study, the Danish *P. teres* f. *teres* isolate BB25 and the North Dakota *P. teres* f. *teres* isolate FGOH04Ptt-21 were chosen to develop a pathogen mapping population based on their phenotypic differences observed on both local and commonly used differential barley lines. This population was then used to genetically characterize this population for

virulence associated with net form net blotch on four local barley lines and six commonly used differential lines.

Materials and Methods

P. teres f. *teres Pathogen Population Development*

P. teres f. *teres* isolates BB25 (kindly provided by Lise Nistrup Jorgensen, Aarhus University, Denmark) and FGOH04Ptt-21 (FGO21) (collected from Fargo, North Dakota, USA) were used in a cross resulting in 109 progeny using methods described in Shjerve et al. (2014). Briefly, sterile wheat stems were placed on Sach's media (1 g $CaNO_3$, 0.25 g $MgSO_4$ $7H_2O$, trace $FeCl_3$, 0.25 g K_2HPO, 4 g $CaCO_3$, 20 g agar, ddH_2O to 1 L) and parents (BB25 and FGO21) were inoculated on opposite ends of wheat stems using 100 µl of inoculum containing 4000 spores/ml. Once the inoculum converged in the middle of the wheat stems, the media plates were incubated in the dark at 15º C for 12 days and then moved to 13º C with a 12 h photoperiod to produce pseudothecia-containing asci. When ascospores began to mature inside the pseudothecia, individual sterile wheat stems were transferred to lids of the water agar plates with the media facing opposite the wheat stems. These plates were then incubated at 13º C with a 12 h photoperiod and checked regularly for ascospore discharge on the water agar media. Individual ascospores were then picked from different spots on the water agar plates and plated on V8PDA media (150 mL V8 juice, 10 g difco PDA, 3 g CaCO3, 10 g agar, ddH_2O up to 1 L). These individual ascospores were then allowed to grow and sporulate and single conidia were isolated from cultures produced from each ascospore. Two rounds of single-sporing were done to ensure genetic purity. Mating type gene segregation ratios for all progeny isolates were evaluated as described in Lu et al. (2010).

Disease Evaluation on Barley Lines

The 109 progeny isolates of the BB25×FGO21 population along with the parents were used to perform phenotypic analysis on ten barley lines including the commonly used differential lines Manchurian, Tifang, CI9214, CI4922, Beecher, and Cape (Liu et al., 2012) as well as local barley cultivars Celebration, Pinnacle, Stellar and Hector.

All *P. teres* f. *teres* progeny isolates along with parental isolates were grown and inoculated under the same conditions as described in Shjerve et al (2014). Briefly, the pathogen isolates were inoculated on V8-PDA plates and the inoculated plates were kept in the dark for 5-7 days at room temperature. The plates were then placed in the light for 24 h at room temperature followed by dark for 24 h at 15º C. The plates were then flooded with distilled water and the spores were released using an inoculating loop. The inoculum was then collected and diluted to 2000 spores/ml. Tween 20 was added at a rate of one drop/50 ml inoculum to avoid spore clumping. The ten barley lines were planted in a rack containing 49 conetainers (Stuwe and Sons, Inc., Corvallis, OR, USA) with 'Stout' barley planted as the border to reduce any edge effect. Stout and Conlon were used as checks for all inoculations. Inoculations were done as described by Friesen et al. (2006) and plants were grown as described in Shjerve et al. (2014). Briefly, 14-16 day-old seedlings with secondary leaves fully expanded were inoculated with the inoculum of progeny and parental isolates individually. The isolates were inoculated using an air sprayer (Huskey, model # HDS790) until the leaves were covered with a heavy mist of inoculum but before run-off. The plants were then placed in mist chambers with 100% relative humidity at 21º C with 24 h of light. After 24 h, the plants were placed in a growth chamber at 21º C with a 12 h photoperiod until disease evaluation. Disease reactions were evaluated seven days post-inoculation on a 1 to 10 scale as described by Tekauz (1985) where reaction type 1 was resistant

and reaction type 10 was susceptible. Three replicates were performed for each parental and progeny isolate across the set of barley lines with the lines being randomized in replicates two and three.

Genotypic Analysis of the Pathogen Population

DNA was extracted from the 109 BB25 × FGO21 progeny lines using a modified CTAB extraction method similar to Shjerve et al., (2014). Genomic libraries were constructed using the RAD GBS method (Baird et al., 2008; LeBoldus et al., 2014; Shjerve et al., 2014). Size selection and amplification of the genomic libraries was carried out prior to performing sequencing runs on an Ion Torrent PGM system (Life Technologies). Once the sequencing was completed, the sequencing files obtained from the Ion Torrent Server were passed through a SNP calling pipeline to identify SNP markers useful for genetic mapping modified from LeBoldus et al., (2014) and Carlsen et al. (submitted). A low pass Ion Torrent sequence of the parental isolate FGO21 was used as the reference for SNP calling.

Marker files were then exported to an Excel sheet and filtered as described in Shjerve et al. (2014). Briefly, markers that did not meet the quality score threshold of 999 (assigned by SAM tools) or had greater than 30% missing data were removed. Allele frequency filter parameters were used to keep data within a 3:1 allele ratio, (i.e. useful markers were between 25 and 75% of each parental marker type).

Genetic Map Construction

The SNP markers obtained through the filtering process were exported to MapDisto version 1.7.5 (Lorieux 2012) for construction of genetic linkage maps. The “find groups” command was used for construction of linkage groups with LOD min= 7.0 and r_{max}=0.3. Functions such as “check inversions” and “ripple order”, were used to identify the best order of

the markers in each linkage group. The “drop locus” command was used to remove problematic markers that expanded the map size by greater than 3 cM. Co-segregating markers were identified for each linkage group and the marker with the least amount of missing data was retained from all the redundant markers. Linkage groups were named according to *P. tritici-repentis* chromosomes (Manning et al., 2013). Marker sequences that corresponded to each of the linkage groups were compared with the *P. tritici-repentis* chromosomes (Manning et al., 2013) using BLAST analysis (Altschul et al., 1990). If more than one linkage group showed correspondence to the same *P. tritici-repentis* chromosome they were renamed consecutively (i.e. if two linkage groups corresponded to chromosome 1, they were renamed as LG 1.1 and LG 1.2).

QTL Analysis

The genotypic and phenotypic data was exported to Q gene v 4.3.0 (Joehanes and Nelson 2008) for QTL analysis. QTL analysis was carried out as described in Shjerve et al. (2014). Briefly, critical logarithm of odds (LOD) was calculated by performing 1000 permutations. An $\alpha = 0.05$ level was used as the critical LOD threshold value. Composite interval mapping (CIM) was performed for identification of significant QTL as described in Koladia et al. (2017).

Results and Discussion

Phenotypic Analysis

In general, BB25 is less virulent than FGO21 on the barley lines used in this study (Figure 3.1), however, BB25 showed a higher virulence on CI9214, Cape, and Pinnacle showing average disease reactions greater than 4. FGO21 showed disease reactions of greater than 6 on all lines except CI9214, however, disease reactions across the progeny ranged from resistant (≤ 3) to susceptible (>6) for all the barley lines (Table 3.1, Figure 3.2). Transgressive segregation was

our first indication that BB25 and FGO21 had different complements of virulence and avirulence genes, likely because these isolates were collected on different continents and had the selection pressure of different resistance sources used by the respective local breeding programs.

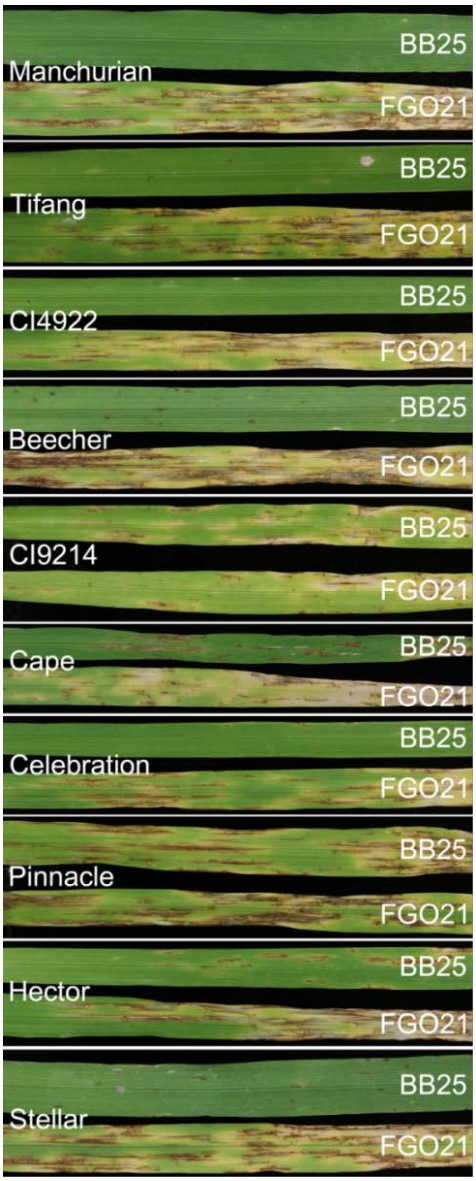

Figure 3.1. Disease reactions on ten barley lines. Parental isolates are indicated on the right and the ten barley cultivars are indicated on the left.

Table 3.1. Phenotypic reactions of ten barley lines when inoculated with parental isolates BB25 and FGO21.

Barley lines	BB25	FGO21	Range of population
Manchurian	1.00	7.83	1.00-7.83
Tifang	1.00	7.00	1.00-6.83
CI4922	1.33	7.33	1.00-7.66
Beecher	1.00	7.83	1.00-8.16
CI9214	5.33	4.83	1.00-8.25
Cape	4.16	7.83	2.33-8.33
Celebration	2.00	6.83	1.83-7.16
Pinnacle	6.16	7.00	2.50-8.33
Hector	3.33	8.16	3.00-9.16
Stellar	1.16	6.83	1.33-7.16

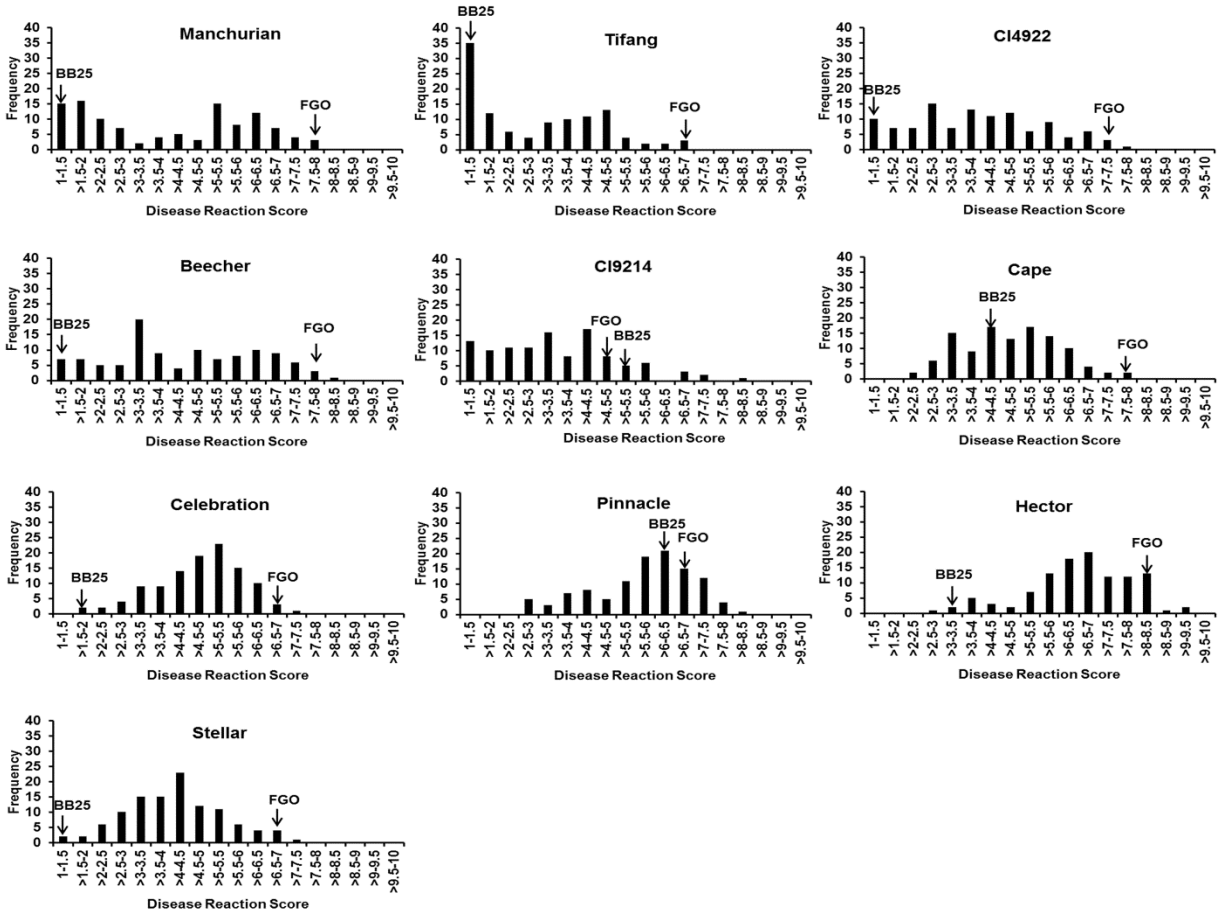

Figure 3.2. Histograms representing the average phenotypic reactions of progeny isolates of the BB25×FGO21 population when inoculated on ten barley lines. The y-axis represents the frequency of progeny isolates and x-axis represents the average disease reaction score of BB25×FGO21 progeny lines. Disease reactions for parents are shown on the histogram as BB25 and FGO and can be found in Table 3.1.

Marker Development and Linkage Mapping

A total of 60,185 sequence tags were identified through the SNP calling pipeline out of which 685 high quality SNP markers were obtained through the filtering process as described above. The 685 SNP markers were exported to MapDisto version 1.7.5 (Louriex, 2012) and 315 redundant markers were removed resulting in 370 high quality, non-redundant SNP markers to be used for construction of the linkage maps. Linkage mapping resulted in 16 linkage groups and a total map size of 1905.81 cM with the size of the linkage groups ranging from 32.77 to 230.71

cM (Table 3.2). Using only non-redundant markers, the average density was one marker locus every 5.15 cM with the largest gap being 19.8 cM (Table 3.2). Ellwood et al. (2010) estimated a minimum of nine and a maximum of eleven chromosomes for *P. teres* f. *teres* indicating that the smaller linkage groups are likely incomplete chromosomes. The maps presented in Ellwood et al. (2010) spanned 2477.7 cM, which may be explained by the difference in the quality of the different marker types used in the two studies because in our hands, SNP markers tend to be more reliable than AFLP markers.

Table 3.2. BB25×FGO21 linkage group summary.

Linkage group[a]	Marker loci	Linkage group size (cM)	Average marker density (cM/ marker)
LG 1.1	40	230.71	5.76
LG 1.2	40	219.22	5.48
LG 1.3	22	92.78	4.21
LG 1.4	9	39.45	4.38
LG 2.1	44	218.97	4.97
LG 3.1	29	149.54	5.15
LG 5.1	22	131.99	5.99
LG 6.1	13	95.73	7.36
LG 6.2	15	79.76	5.31
LG 7.1	29	111.06	3.82
LG 8.1	8	39.28	4.91
LG 9.1	23	160.66	6.98
LG 9.2	15	73.16	4.87
LG 9.3	7	32.77	4.68
LG 10.1	24	109.51	4.56
LG 11.1	30	121.23	4.04

[a] Linkage groups for BB25 × FGO21 population named according to the *Pyrenophora tritici repentis* chromosomes

QTL Analysis

A LOD value threshold (α=0.05) obtained after performing 1000 permutations on each of the data sets ranged from 2.9 to 3.1. The mean of all the LOD values (3.0) was used as the critical LOD threshold value when reporting significant QTL. Even if the maximum value of 3.1 were used, only a single QTL associated with virulence on Pinnacle would have been removed

and when evaluating the Pinnacle data, a LOD threshold of 3.0 was identified. Therefore, we used the 3.0 value. A total of 19 QTL were identified on 12 of the 16 linkage groups, and the number of significant QTL ranged from 1 to 5 for individual barley lines. The most significant QTL was identified on LG 10.1 and was identified on barley lines Manchurian, Tifang, and CI4922, accounting for 76%, 62% and 34% of the phenotypic variation, respectively (Figure 3. 3). These three lines have been used repeatedly in *P. teres* f. *teres* population virulence studies worldwide (Afanasenko et al., 2009; Cromey and Parkes, 2003; Liu et al., 2010, 2012; Steffenson and Webster, 1992a; Wu et al., 2003). A relatively minor QTL was also identified on LG 9.1 for Tifang, accounting for 3.3% of the phenotypic variation (Figure 3.3). No other QTL were identified for Manchurian or CI4922. All QTL identified for Manchurian, Tifang and CI4922 showed the virulence allele to be contributed by FGO21. It is likely that the QTL identified on LG 10.1 is underlied by an avirulence effector that is recognized by these three barley lines in a gene-for-gene manner. Support for this hypothesis comes from the Koladia et al. (2017) study where it was shown that Tifang harbors a major dominant resistance gene on chromosome 3H that was effective against another Danish isolate. The Tifang, Manchurian and CI4922 resistance could be an early recognition of an effector produced by BB25, resulting in an incompatible interaction.

For the commonly used differential line Beecher, unique QTL were identified on LG 1.1 and LG 2.1 accounting for 43% and 17% of the phenotypic variation, respectively (Figure 3.3). Virulence at both loci was contributed by FGO21. Beecher has been used several times as a differential line (Cromey and Parkes, 2003; Gupta and Loughman, 2001; Jonsson et al., 1997; Khan and Boyd, 1969; Khan, 1982; Liu et al., 2010, 2012; Steffenson and Webster, 1992a; Wu et al., 2003) and shows a strong differential response between parents (i.e. 1.0 for BB25 and 7.3

for FGO21). A continuum of reaction types was observed (1.0-8.16) (Table 3.1), however, a high number of highly resistant type reactions were also observed compared to the locally planted barley cultivars as seen below and in Table 1 and Figure 2. This strong differential response is likely why this and other commonly used differential lines like Manchurian, Tifang, and CI4922 have been popular in evaluating *P. teres* f. *teres* populations, resulting in an overly simplistic view of these interactions.

CI9214 has also been used as a differential line in several studies (Akhavan et al., 2016; Cromey and Parkes 2003; Gupta and Loughman 2001; Liu et al., 2010, 2012; Tekauz, 1990) and for this line, unlike the other differential lines, several relatively minor effect loci were observed on LG 1.3, LG 5.1, LG 8.1, LG 9.2 and LG11.1 accounting for 21.0%, 4.2%, 8.4%, 19.0% and 2.7% of the phenotypic variation, respectively. All QTL pertaining to CI9214 except the QTL on LG 8.1 were in unique genetic regions. Additionally, the virulence allele at each QTL was contributed by BB25 except that of the LG 8.1 QTL. The QTL region on LG 8.1 was also common to five of the ten lines used including CI9214, Cape, Celebration, Hector, and Steller and in each case the virulence allele was contributed by FGO21. (Figure 3.3). Typically, the differential lines that are commonly used provide a strong differential reaction; however, CI9214 likely has several quantitative resistance/susceptibility loci that correspond to the virulence loci identified here. CI9214 was likely commonly used as a differential line due to its unique reaction pattern across natural populations, e.g. in the set of 10 lines that we used in this study, CI9214 was the only line that was more susceptible to BB25 than FGO21.

For Cape, which has also been used in several studies (Liu et al., 2010, 2012; Steffenson and Webster 1992a), unique QTL were present on LG 6.1, 9.1, and 9.2 accounting for 4.8%, 5.9% and 8.9% of the phenotypic variation, respectively. The virulence allele was contributed by

BB25 for the QTL on LG 6.1 whereas the virulence allele was contributed by FGO21 for the QTL on LG 9.1 and 9.2. The common QTL with the virulence allele contributed by FGO21 on LG 8.1 (Figure 3.3) was also significant, accounting for 20% of the disease variation (Figure 3.3). Similar to CI9214, Cape has been used as a differential line but does not have a "differential" type response, but more typically shows an intermediate response due to the number of quantitative virulences that are effective against it (Liu et al. 2010, 2012). This results in a reaction type histogram with a bell shaped curve, unlike the other common differential lines but similar to the local cultivars (Figure 3.2)

The remainder of the lines, made up of locally planted cultivars, showed several relatively minor effect loci, similar to CI9214 and Cape. For Celebration, three QTL were observed, with unique QTL on LG 10.1 and 11.1 accounting for 6.1% and 15% of the phenotypic variation, respectively (Figure 3.3) and the common QTL on LG 8.1 that accounted for 15% of the phenotypic variation (Figure 3.3). The virulence alleles were contributed by FGO21 for QTL on LG 8.1 and LG 11.1 whereas BB25 contributed the virulence allele for the QTL on LG 10.1.

For Pinnacle, a major effect QTL was observed on LG 5.1, accounting for 41% of the phenotypic variation, with the virulence allele contributed by BB25. Additionally, a minor QTL was observed on LG 1.2 accounting for 6.0% of the phenotypic variation (Figure 3.3) with the virulence allele at this QTL being contributed by FGO21. Even though the only major QTL was observed on Pinnacle with the virulence allele contributed by BB25, a bell-shaped curve was still observed. Since FGO21 shows a higher disease reaction (7.0) than BB25 (6.16) (Table 3.1, Figure 3.1) and only a single minor virulence (accounting for only 6% of the disease variation) was contributed by FGO21, it is likely that other undetected loci are present in this population which are contributing to the bell-shaped curve.

For Hector, one of our most susceptible lines (Liu et al., 2010, 2012; Wu et al., 2003), minor effect QTL were observed on LG 1.2, LG 2.1, LG 8.1 and LG 11.1 accounting for 6.1%, 7.2 %, LG 8.6 % and 12.0% of the phenotypic variation, respectively (Figure 3.3) with all virulence alleles being contributed by FGO21. A Hector × NDB112 barley population was used to evaluate the genetics of resistance/susceptibility to *P. teres* f. *teres* (Liu et al., 2015) and showed that several susceptibility loci are present across the barley genome, including a necrotrophic effector sensitivity gene identified on chromosome 6H. It is likely that this necrotrophic effector gene corresponding to the 6H sensitivity underlies one of these four QTL.

Stellar had only the common QTL found on LG 8.1 that accounted for 12% of the phenotypic variation, however, four additional QTL peaks just under the significance threshold were also present (Figure 3.3).

In summary, nineteen virulence/avirulence QTL were identified on twelve of the *P. teres* f. *teres* linkage groups, out of which only three QTL accounted for greater than 30% of the phenotypic variation and sixteen QTL accounted for less than 30% of the phenotypic variation. Among the major effect QTL (> 30%), a common QTL was identified for Manchurian, Tifang and CI4922 on LG 10.1. For Beecher one unique major effect QTL was identified on LG 1.1 and one unique major effect QTL was identified for Pinnacle on LG 5.1 (Figure 3.3). This study clearly shows that the *P. teres* f. *teres* interaction is complex and that the differential lines that have been used in the past are not sufficient for capturing the virulence diversity of the *P. teres* f. *teres* population. In general, barley lines chosen for evaluating virulence diversity have been chosen based on major differential reactions of which many are available. The danger of choosing differentials in this way is that the interaction is over simplified as is seen in the current

study where differential lines show one or two major loci whereas the barley lines that are planted in the field have a more complex quantitative interaction with the pathogen.

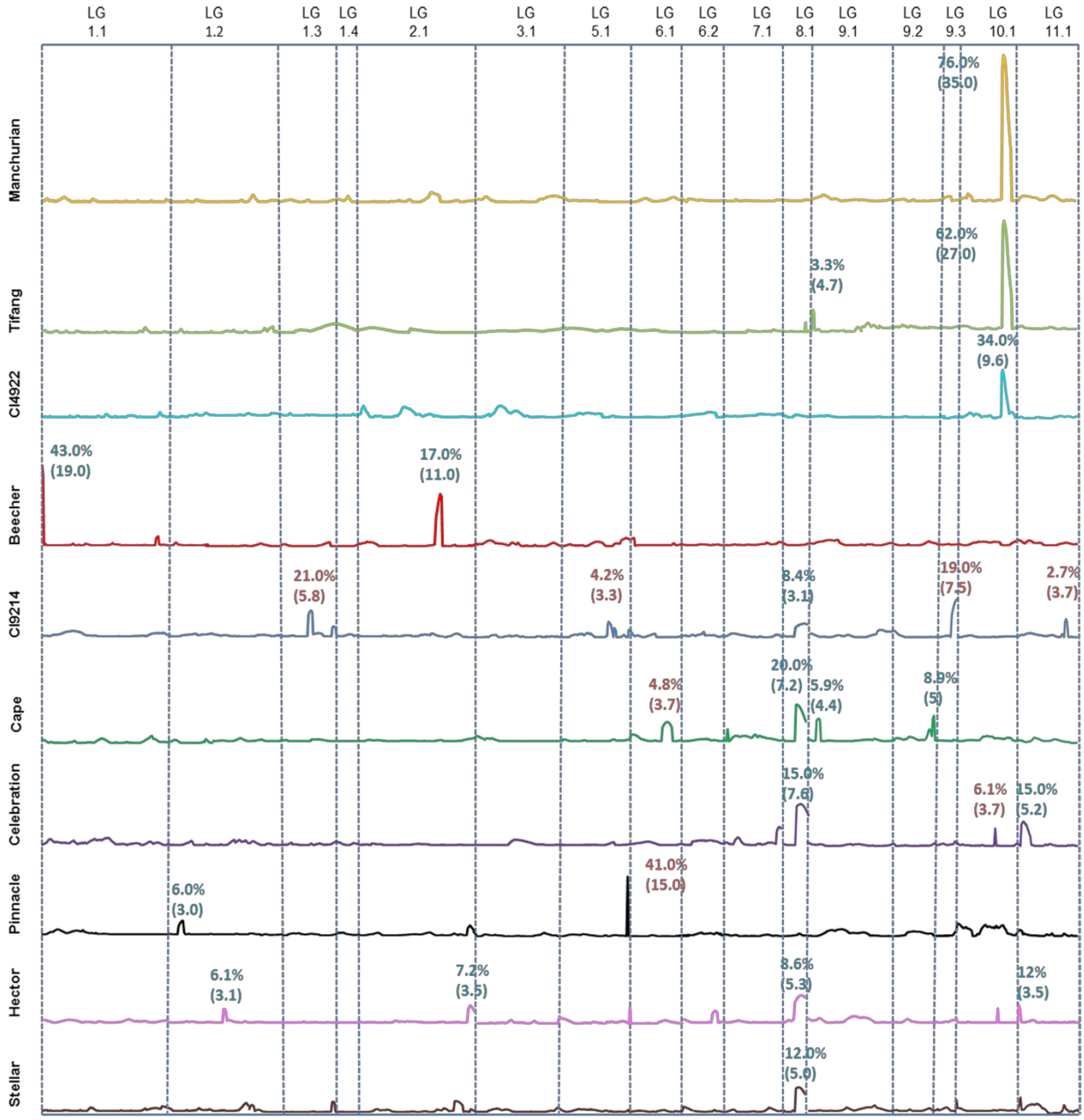

Figure 3.3. Quantitative trait loci (QTL) associated with disease reaction to each of the ten barley lines. Composite interval mapping of 16 linkage groups showing the presence of QTL significant at a logarithm of odds (LOD) threshold of 3.0. The LOD and R^2 values for each significant QTL are over or adjacent to the QTL peaks. Values in blue indicate that virulence is coming from FGO21 and values in red indicate that virulence is coming from BB25.

Conclusion

When combining this study with the current literature reporting on both NFNB resistance/susceptibility in barley and virulence/avirulence in *P. teres* f. *teres*, we can conclude that this interaction is highly complex with interactions spanning the spectrum from quantitative to qualitative. On the host side, the NFNB literature contains several examples of qualitative dominant resistance and quantitatively inherited susceptibility (reviewed in Liu et al., 2011). On the pathogen side, both active virulence, including necrotrophic effectors (Liu et al., 2015), and defense-inducing avirulence type effectors (Beattie et al., 2007; Lai et al., 2007; Weiland et al., 1999) that are likely triggering effective defense responses after recognition of the pathogen have been identified. Continued work on the cloning of the genes underlying these QTL is underway. Identification of these genes and their modes of action are critical to the understanding of the NFNB interaction.

Literature Cited

Abu Qamar, M., Liu, Z. H., Faris, J. D., Chao, S., Edwards, M. C., Lai, Z., Franckowiak, J. D., Friesen, T.L. (2008) A region of barley chromosome 6H harbors multiple major genes associated with net type net blotch resistance. Theoret. Appl. Genet. 117, 1261-1270

Afanasenko, O., Mironenko, N., Filatova, O., Kopahnke, D., Krämer, I. and Ordon, F. (2007) Genetics of host–pathogen interactions in the *Pyrenophora teres* f. *teres* (net form)–barley (*Hordeum vulgare*) patho- system. Eur. J. Plant Pathol. 117, 267–280

Afanasenko, O. S., Jalli, M., Pinnschmidt, H. O., Filatova, O., and Platz, G. J. (2009) Development of an international standard set of barley differential genotypes for *Pyrenophora teres* f. *teres.* Plant Pathol, 58,665-676 doi: 10.1111/j.1365-3059.2009.02062.x

Akhavan, A., Turkington, T. K., Askarian, H., Tekauz, A., Xi, K., Tucker, J. R., Kutcher, H. R. and Strelkov, S. E. (2016) Virulence of *Pyrenophora teres* populations in western Canada. Canadian Journal of Plant Pathology. 38, 183-196

Altschul, S. F., Gish, W., Miller, W., Myers, E.W. and Lipman, D. J. (1990) Basic local alignment search tool. J. of Mol. Biol. 215, 403-410

Baird, N.A., Etter, P.D., Atwood, T.S., Currey, M.C., Shiver, A.L., Lewis, Z.A., Selker, E.U., Cresko, W.A. and Johnson, E.A. (2008) Rapid SNP discovery and genetic mapping using sequenced RAD markers. PLoS ONE, 3, e3376

Beattie, A. D., Scoles, G. J. and Rossnagel, B. G. (2007) Identification of molecular markers linked to a *Pyrenophora teres* avirulence gene. Phytopathology, 97, 842–849

Carlsen, S. A, Neupane, A, Richards, J. K, Faris, J. D, Xu, S. S, Brueggeman, R. S and Friesen, T. L. Unraveling the complexities of the *Pyrenophora teres* f. *maculata* – barley interaction using pathogen genetics. G3- Gene Genom. Genet. (submitted)

Cromey, M. G. and Parkes, R. A. (2003) Pathogenic variation in *Drechslera teres* in New Zealand. N Z Plant Protect, 56, 251-256

Ellwood, S. R., Liu, Z., Syme, R. A., Lai, Z., Hane, J. K., Keiper, F., Moffat, C. S., Oliver, R. P., Friesen, T. L., (2010) A first genome assembly of the barley fungal pathogen *Pyrenophora teres* f. *teres*. *Genome Biol*. 11: R109

Faris, J. D. Zhaohui, L. and Xu S. S. (2013) Genetics of tan spot resistance in wheat. Theor Appl Genet 126, 2197–2217

Friesen, T. L., and Faris, J. D. (2010) Characterization of the wheat-*Stagonospora nodorum* disease system: what is the molecular basis of this quantitative necrotrophic disease interaction? Canadian Journal of Plant Pathol. 32, 20-28

Friesen, T. L., Faris, J. D., Lai, Z. and Steffenson, B. J. (2006) Identification and chromosomal location of major genes for resistance to *Pyrenophora teres* in a doubled-haploid barley population. Genome, 49, 855–859

Friesen, T. L., Faris, J. D., Solomon, P. S. and Oliver, R. P. (2008) Host-specific toxins: effectors of necrotrophic pathogenicity. Cellular Microbiol 7, 1421–1428

Gupta, S. and Loughman, R. (2001) Current virulence of *Pyrenophora teres* on barley in Western Australia. Plant Disease, 85, 960-966

Jonsson, R., Bryngelsson, T., Gustafsson, M. (1997) Virulence studies of Swedish net blotch isolates (*Drechslera teres*) and identification of resistant barley lines. Euphytica, 94, 209-218

Joehanes, R., Nelson, J.C. (2008) QGene 4.0, an extensible Java QTL-analysis platform. Bioinformatics 24, 2788-2789

Khan, T. N. and Boyd, W. J. R. (1969) Physiologic specialization in *Drechslera teres*. Aust. J. Biol. Sci. 22, 1229–1235

Khan, T.N. (1982) Changes in pathogenicity of *Drechslera teres* relating to changes in barley cultivars grown in Western Australia. Plant Disease, 66, 655-656.

Koladia, V. M., Faris, J. D., Richards, J. K., Brueggeman, R. S. Chao, S. and Friesen, T. L. (2017). Genetic analysis of net form net blotch resistance in barley lines CIho 5791 and Tifang against a global collection of *P. teres* f. *teres* isolates. Theoretical and applied genetics, 130,163-173. doi 10.1007/s00122-016-2801-4

Lai, Z., Faris, J. D., Weiland, J. J., Steffenson, B. J. and Friesen, T. L. (2007) Genetic mapping of *Pyrenophora teres* f. *teres* genes conferring avirulence on barley. Fungal Genet. Biol. 44, 323–329

Leboldus, J. M., Kinzer, K., Richards, J., Ya, Z., Yan, C., Friesen, T. L. and Brueggeman R. (2014) Genotyping-by-sequencing of the plant-pathogenic fungi *Pyrenophora teres* and *Sphaerulina musiva* utilizing Ion Torrent sequence technology. Mol. Plant Pathol. 6: 623-632

Liu Z, Faris J. D., Edwards M. C., and Friesen T. L. (2010) Development of expressed sequence tag (EST)–based markers for genomic analysis of a barley 6H region harboring multiple net form net blotch resistance genes. The Plant Genome 3, 41-52

Liu, Z., Ellwood, S. R., Oliver, R. P and Friesen, T. L. (2011) *Pyrenophora teres*: profile of an increasingly damaging barley pathogen. Mol. Plant Pathol. 12, 1-19

Liu, Z. H., Zhong, S., Stasko, A. K., Edwards, M. C., Friesen, T. L. (2012) Virulence profile and genetic structure of a North Dakota population of *Pyrenophora teres* f. *teres*, the causal agent of Net form net blotch of barley. Phytopathology 102, 539-546

Liu, Z., Holmes, D. J., Faris, J. D., Chao, S., Brueggeman, R. S., Edwards, M. C. and Friesen, T. L. (2015) Necrotrophic effector triggered susceptibility (NETS) underlies the barley-*Pyrenophora teres* f. *teres* interaction specific to chromosome 6H. Mol. Plant Pathol. 16, 188-200

Lorieux, M., 2012 MapDisto: fast and efficient computation of genetic linkage maps. Molecular Breeding 30, 1231–1235

Lu, S., Platz, G. J., Edwards, M. C., and Friesen, T. L. (2010) Mating type locus-specific polymerase chain reaction markers for differentiation of *Pyrenophora teres* f. *teres* and *P. teres* f. *maculata*, the causal agents of barley net blotch. Phytopathology 100, 1298-1306

Manning, V. A., Pandelova, I., Dhillon, B., Wilhelm, L. J., Goodwin, S. B., Berlin, A. M., Figueroa, M., Freitag, M., Hane, J. K., Henrissat, B., Holman, W. H., Kodira, C. D., Martin, J., Oliver, R. P., Robbertse, B., Schackwitz, W., Schwartz, D. C., Spatafora, J. W., Turgeon, B. G., Yandava, C., Young, S., Zhou, S., Zeng, Q., Grigoriev, I.V., Ma, L.J., Ciuffetti, L. M. (2013) Comparative genomics of a plant-pathogenic fungus, *Pyrenophora tritici-repentis*, reveals transduplication and the impact of repeat elements on pathogenicity and population divergence. G3-Genes Genom. Genet. (Bethesda) 3, 41–63

Mathre, D. E. (1997) Compendium of Barley Diseases, 2nd edn. American Phytopathological Society. APS Press, St. Paul, MN
Murray, G. M. and Brennan, J. P. (2010) Estimating disease losses to the Australian barley industry. *Australas. Plant Pathol.* 39, 85–96 *Pyrenophora teres*. Can. J. Plant Pathol.7, 181–183

Shjerve, R. A., Faris, J. D., Brueggeman, R. S., Yan, C., Zhu, Y., Koladia, V. and Friesen, T.L. (2014) Evaluation of a *Pyrenophora teres* f . *teres* mapping population reveals multiple independent interactions with the barley 6H chromosome region. Fungal Genet. Biol. 70, 104-112

Steffenson, B. J., Webster, R.K. (1992a) Pathotype diversity of *Pyrenophora teres* f. *teres* on barley. Phytopathology, 82, 170-177

Steffenson, B. J. and Webster, R. K. (1992b) Quantitative resistance to *Pyrenophora teres* f. *teres* in barley. Phytopathology, 82, 407–411

Tekauz A (1985) A numerical scale to classify reactions of barley to *Pyrenophora teres*. Can J Plant Pathol 7, 181–183

Tekauz A (1990) Characterization and distribution of pathogenic variation in *Pyrenophora teres* f. *teres* and *P. teres* f. *maculata* from western Canada. Can J Plant Pathol 12, 141-148

Weiland, J. J., Steffenson, B. J., Cartwright, R. D. and Webster, R. K. (1999) Identification of molecular genetic markers in *Pyrenophora teres* f. *teres* associated with low virulence on 'Harbin' barley. Phytopathology, 89, 176–181

Wu, H-L, Steffenson, B. J., Zhong, S. (2003) Genetic variation for virulence and RFLP markers in *Pyrenophora teres*. Can J Plant Pathol, 25, 82-90

CHAPTER 4: CONCLUSION

NFNB caused by *P. teres* f. *teres* is prevalent in most of the barley producing regions of the world and causes significant yield losses (10-40%). Previous studies indicate that the barley-*P. teres* f. *teres* pathosystem at least partially follows the NE-triggered susceptibility (NETS) model in which there is an interaction between dominant barley susceptibility genes and necrotrophic effectors of the pathogen (Shjerve et al., 2014; Liu et al., 2015) and partially to an effector triggered immunity (ETI) model, as dominant resistance genes and avirulence genes have been identified (Steffenson et al., 1992; Weiland et al., 1999; Friesen et al., 2006; Lai et al., 2007; Beattie et al., 2007; Afanasenko et al., 2007). The barley line CI5791 confers high levels of resistance to *P. teres* f. *teres* with few documented isolates overcoming this resistance. Tifang barley also harbors resistance to *P. teres* f. *teres* and using trisomic analysis, this resistance was previously shown to localize to barley chromosome 3H. To increase our knowledge about the resistance/susceptibility of barley to *P. teres* f. *teres*, a CI5791 × Tifang F_6 recombinant inbred line (RIL) population was developed using single seed descent. Utilizing the Illumina iSelect SNP platform 2,562 single nucleotide polymorphism (SNP) markers were identified across the barley genome, out of which 827 non redundant SNP markers were used to construct seven linkage maps that corresponded to the barley chromosomes. The CI5791 × Tifang RIL population was evaluated for NFNB resistance using nine *P. teres* f. *teres* isolates collected globally. Tifang was resistant to four of the isolates tested whereas CI5791 was resistant to all nine isolates. QTL analysis indicated that the CI5791 resistance mapped to chromosome 6H whereas the Tifang resistance mapped to chromosome 3H. Additionally, CI5791 also harbored resistance to two Japanese isolates that mapped to a 3H region similar to that of Tifang. SNP

markers and RILs harboring both 3H and 6H resistance will be useful in resistance breeding against NFNB.

In order to characterize the genetics of avirulence/virulence in the *P. teres* f. *teres* pathogen, a fungal population was developed using *P. teres* f. *teres* isolates BB25 (a Danish isolate) and FGOH04Ptt-21 (a North Dakota isolate). 109 progenies were obtained and used for virulence evaluation across 10 barley lines. BB25 was found to be virulent on three of the barley lines and avirulent on seven of the barley lines whereas FGOH04Ptt-21 was found to be virulent on all ten barley lines. 685 SNP markers were obtained using a RAD-GBS approach in conjunction with Ion Torrent sequencing. 370 non redundant SNP markers were obtained and used for construction of genetic linkage maps. 16 linkage groups were formed and used to identify QTL associated with avirulence/virulence on the ten barley lines. Nineteen unique QTL were identified on twelve linkage groups out of which three QTL had major effects with phenotypic variation greater than 30% while sixteen QTL were relatively minor with phenotypic variation less than 30%. One or two major affect loci were identified for a few of the lines used as differentials, however, virulence on most of the barley cultivars was contributed by several loci. This information will be further utilized in identifying genes underlying the major QTL.

Studies carried out in this thesis add more information to understand the complex interaction between barley and *P. teres* f. *teres*. The C15791×Tifang population will be useful in barley breeding programs. The 3H and 6H regions could be further studied to identify resistant genes underlying these regions. The major QTL regions identified in the BB25×FGOH04Ptt-21 pathogen population will be useful in identifying the genes conferring virulence. These genes could be further studied in order to determine the corresponding host regions.

Literature Cited

Afanasenko, O., Mironenko, N., Filatova, O., Kopahnke, D., Krämer, I. and Ordon, F. (2007) Genetics of host–pathogen interactions in the *Pyrenophora teres* f. *teres* (net form)–barley (*Hordeum vulgare*) patho- system. Eur. J. Plant Pathol. 117, 267–280

Beattie, A. D., Scoles, G. J. and Rossnagel, B. G. (2007) Identification of molecular markers linked to a *Pyrenophora teres* avirulence gene. Phytopathology, 97, 842–849

Friesen, T. L., Faris, J. D., Lai, Z. and Steffenson, B. J. (2006) Identification and chromosomal location of major genes for resistance to *Pyrenophora teres* in a doubled-haploid barley population. Genome, 49, 855–859

Lai, Z., Faris, J. D., Weiland, J. J., Steffenson, B. J. and Friesen, T. L. (2007) Genetic mapping of *Pyrenophora teres* f. *teres* genes conferring avirulence on barley. Fungal Genet. Biol. 44, 323–329

Liu, Z., Holmes, D. J., Faris, J. D., Chao, S., Brueggeman, R. S., Edwards, M. C. and Friesen, T. L. (2015) Necrotrophic effector triggered susceptibility (NETS) underlies the barley-*Pyrenophora teres* f. *teres* interaction specific to chromosome 6H. Mol. Plant Pathol. 16, 188-200

Shjerve, R. A., Faris, J. D., Brueggeman, R. S., Yan, C., Zhu, Y., Koladia, V. and Friesen, T.L. (2014) Evaluation of a *Pyrenophora teres* f . *teres* mapping population reveals multiple independent interactions with the barley 6H chromosome region. Fungal Genet. Biol. 70, 104-112

Steffenson, B. J., Webster, R.K. (1992a) Pathotype diversity of *Pyrenophora teres* f. *teres* on barley. Phytopathology, 82, 170-177

Weiland, J. J., Steffenson, B. J., Cartwright, R. D. and Webster, R. K. (1999) Identification of molecular genetic markers in *Pyrenophora teres* f. *teres* associated with low virulence on 'Harbin' barley. Phytopathology, 89, 176–181