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Genetic analysis of virulence in the *Pyrenophora teres* f. *teres* population BB25 × FGOH04Ptt-21

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ABSTRACT

Pyrenophora teres f. *teres* is the causal agent of net form net blotch (NFNB) of barley. In order to map the genetics of avirulence/virulence in *P. teres* f. *teres*, a fungal population was developed using *P. teres* f. *teres* isolates BB25 (Denmark) and FGOH04Ptt-21 (North Dakota, USA) due to these two isolates differing in virulence on several common barley lines. 109 progeny isolates were obtained from the BB25 by FGOH04Ptt-21 cross that were then used for NFNB disease evaluation across eight barley lines, four of which have been used commonly as NFNB differential lines as well as four cultivars commonly used in barley production in the Northern Great Plains. BB25 was virulent on one of the barley lines and avirulent on seven of the barley lines whereas, FGOH04Ptt-21 was virulent on all eight 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 were used to identify quantitative trait loci (QTL) associated with avirulence/virulence. Nine unique QTL were identified on eight linkage groups out of which three QTL had major effects ($R^2 \geq 45\%$) while the remaining six QTL were relatively minor ($R^2 < 20\%$). One or two major effect loci were identified for the lines commonly used as differentials. Conversely, variation in virulence on the local barley cultivars was mostly associated with small effect loci that contributed quantitatively to disease.

1. Introduction

In the last two decades, plant pathogen interaction research has made large strides. This work has included the characterization of several host and pathogen genes along with the development of useful models that help define the role of these genes (Cook et al., 2015; Toruño et al., 2016). Within the host-fungal interactions, the fundamental processes leading to both compatible and incompatible interactions have been described in detail. Much of the early work in this area was done on biotrophic interactions, especially the rusts and powdery mildews due to their host specific interactions (Flor, 1971). More recently, pathogens with a more necrotrophic interaction have received attention and significant strides have been made in these systems as well.

Pyrenophora tritici-repentis and *Parastagonospora nodorum* are pathogens of wheat that are described as necrotrophic specialist pathogens that produce necrotrophic effectors (NEs) (synonym: host selective toxins). These pathogens use NEs to trigger hallmarks of the defense response including programmed cell death (PCD). Both of these pathogens use *Tsn1*, a nucleotide binding, leucine rich repeat gene, as well as other host genes to induce PCD (Shi et al., 2016). Unlike in the biotrophic interactions, PCD in these interactions provides a nutrient source for the pathogen, allowing it to feed and ultimately sporulate (Faris et al., 2010).

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 10–40% 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 *P. nodorum*, and *P. tritici-repentis* that produce NEs (reviewed in Faris et al., 2013; Friesen and

Abbreviations: SNP, single nucleotide polymorphism; RAD-GBS, restriction associated DNA genotyping by sequencing; QTL, quantitative trait locus; LOD, logarithm of odds; CIM, composite interval mapping; NFNB, net form net blotch

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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) showing that the NFNB interaction is complicated in that it has the genetic hallmarks of both a gene-for-gene and an inverse gene-for-gene interaction.

The most predominant model describing host pathogen interactions was originally described as the gene-for-gene model (Reviewed in Flor, 1971). Based on our current understanding of several model systems, the gene-for-gene model has been updated to include the role of pathogen produced effectors and the mechanism of the host recognition of these effectors (Reviewed in (Dodds and Rathjen, 2010; Jones and Dangl, 2006; Chisholm et al., 2006; Cook et al., 2015). Typically, any given host is able to fend off the majority of microbes through the recognition of near-universal pathogen or microbe associated molecular patterns (PAMPs/MAMPs) leading to a basal defense response or PAMP triggered immunity (PTI). Pathogen produced effectors are known to manipulate the basal defense to gain entry and nutrient from the host, resulting in disease. Additionally, the host responds to these effectors through recognition, resulting in effector triggered immunity (ETI). Although all pathogens must overcome the same defense responses, different pathogens manipulate their host based on their specific set of tools, including effectors, developed through the evolution of each host-pathogen interaction. These effectors have different roles including the modulation of the effects of the defense response or spatial or temporal manipulation of plant innate immunity (Toruño et al., 2016).

Geschele (1928) was the first to show that resistance to NFNB could be inherited qualitatively (Reviewed in Liu et al., 2011). Mode and Schaller (1958) and Schaller (1955) later showed that three incompletely dominant genes conferred resistance to *P. teres* isolates collected in California. Several other early reports showed breeding lines harboring single dominant resistant genes (Gray, 1966; McDonald and Buchannon, 1962). Previous to molecular marker technology, trisomic analysis was used to identify the resistance genes *Rpt1a*, *Rpt3d*, *Rpt1b*, and *Rpt2c* on barley chromosomes 3H, 2H, 3H and 5H, respectively (Bockelman et al., 1977). Several reports have also identified dominant genes conferring susceptibility in this pathosystem (Ho et al., 1996; Abu Qamar et al., 2008; Liu et al., 2015), showing the complexity of this host-pathogen interaction.

Resistance and susceptibility to *P. teres* f. *teres* has often mapped to chromosome 6H (reviewed in Liu et al., 2011). Several recent studies have shown that chromosome 6H consists of multiple genes or alleles that confer dominant susceptibility to different pathotypes of *P. teres* f. *teres*. (Abu Qamar et al., 2008; Liu et al., 2011, 2015; Shjerve et al., 2014; Richards et al., 2016). Several studies have used differential sets of barley lines that exhibited different resistance patterns when inoculated with *P. teres* f. *teres* isolates collected from barley growing regions of the world to show variation in virulence (Steffenson and Webster, 1992a,b; Wu et al., 2003; Gupta and Loughman, 2001; Cromey and Parkes, 2003; Jalli, 2004; Tekauz, 1990; Jonsson et al., 1997; Khan and Boyd, 1969; Liu et al., 2011; Jalli and Robinson, 2000; Akhavan et al., 2016) where virulence is defined as the level of damage caused by a pathogen on its corresponding host plant. These studies indicated the presence of several different resistance/susceptibility genes in these barley lines that theoretically correspond to different *P. teres* f. *teres* avirulence/virulence factors.

Some of the most commonly used differential lines include Tifang, Manchurian, CI4922, and Beecher. These differential lines typically give a clean resistant or susceptible response and therefore have been used in multiple studies to characterize the *P. teres* f. *teres* virulence in several barley-growing regions (Steffenson and Webster, 1992a,b; Wu et al., 2003; Gupta and Loughman, 2001; Cromey and Parkes, 2003; Jonsson et al., 1997; Liu et al., 2011; Jalli and Robinson, 2000)

Khan and Boyd (1969) was the first to show that *P. teres* f. *teres* isolates had strong host genotype specificity. Weiland et al. (1999) performed 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 mapped a single gene $Avr_{Heartland}$ conferring avirulence on Heartland barley.

Afanasenko et al. (2007) generated several barley F_2 populations obtained from crosses of local barley lines and commonly used differential lines, including CI4922, Harbin, and CI9819. These populations were evaluated for resistance using several Russian, European, and North American *P. teres* f. *teres* isolates to postulate, based on segregation ratios, that one or two dominant or recessive genes controlled resistance in these barley lines. Additionally, Afanasenko et al. (2007) used phenotypic ratios of progeny from *P. teres* f. *teres* crosses of Russian and North American parents to postulate that one or two genes controlled virulence/avirulence. Specific host-pathogen interactions were proposed to occur between barley lines and *P. teres* f. *teres* isolates and it was concluded that this system may follow 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). The Liu et al. (2015) 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. BB25 is avirulent on the majority of the commonly used NFNB differential lines as well as on many of the cultivars grown in the Northern Great Plains. Conversely, FGOH04Ptt-21 is highly virulent on the majority of the same NFNB differential lines as well as being virulent on most locally planted barley cultivars. This population was used to genetically map virulence associated with NFNB on four commonly used differential lines and four local barley cultivars.

2. Materials and methods

2.1. 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