CHARACTERIZATION OF VIRULENCE IN *PYRENOPHORA TERES* f. *MACULATA*

A THESIS SUBMITTED BY

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ABSTRACT

Net blotches are common foliar diseases of barley caused by the fungus *Pyrenophora teres.* There are two forms of net blotch. The net form net blotch (NFNB), caused by *P. teres* f. *teres (Ptt)* and the spot form net blotch (SFNB), caused by *P. teres* f. *maculata (Ptm).* Spot form net blotch is a major foliar disease of barley worldwide. In Australia, annual losses to the barley industry caused by SFNB are estimated to be $192 million. Grain quality parameters such as kernel size, bulk density and plumpness are also negatively affected by SFNB. To date seven studies have been conducted to study the virulence/avirulence associated genomic regions in *Ptt* while only one study has been studied the virulence associated genomic regions in *Ptm*.

It is important to understand the genetics of *Ptm* virulence to manage SFNB resistance in barley varieties. Most of our current understanding of *Ptm*-barley interactions relates to the genomic regions involved in resistance of the barley host. Very little is known about the virulence factors of *Ptm*. This project investigated the genomic regions conferring virulence in *Ptm* isolates using genome wide association (GWAS) and quantitative trait loci (QTL) mapping. For GWAS, one hundred and twenty-one *Ptm* isolates from Australia were genotyped and phenotyped across 12 barley genotypes. Ten novel quantitative trait loci (QTL) associated with virulence in *Ptm* were identified. We also identified 125 candidate genes including, two genes encoding putative effector proteins. For QTL mapping, a bi-parental population was generated by crossing two Australian *Ptm* isolates. Progeny was genotyped and phenotyped across four barley genotypes. Genetic maps of 12 chromosomes were constructed and two QTL regions were found to be associated with virulence. Eight signal peptides were identified, of which five were small proteins. Five non-ribosomal peptide synthetase clusters were identified. These could have a potential role in the virulence and need further study.

Hybridisation between the two forms of *P. teres* has previously been documented but appears to be rare in nature. Hybrids may not be correctly identified as they cause symptoms similar to those of the parents or may be avirulent in nature. While rare, the occurrence of hybrids with novel virulences is of concern as they may overcome deployed sources of resistance. Production of hybrids in vitro is relatively easy as indicated by recent papers (Dahanayaka et al., 2021).To study the virulence of hybrids, we made four *in-vitro* crosses between *Ptt* and *Ptm*. The progeny populations were phenotyped across 20 barley genotypes. A total of 62

hybrid-barley interactions were virulent on barley genotypes to which parent isolates had avirulent responses. Twenty-seven progeny expressed greater average infection responses compared to the parental isolates. This study showed that novel virulences can be generated as a result of *Ptt* and *Ptm* hybridisation and could be a potential threat to sources of host-plant resistance in commercial varieties.

In summary, this study investigated genomic regions conferring virulence to *Ptm.* In addition, we also studied the virulence of hybrid isolates. Genomic sequence comparison of hybrids and parents can help us better understand the genetics of pathogenicity and provide insights into the genetic relationship between parents and hybrids.

CERTIFICATION OF THESIS

This Thesis is entirely the work of Rudrakshi Sharma except where otherwise acknowledged, with the majority of the authorship of the papers presented as a Thesis by Publication undertaken by the student. The work is original and has not previously been submitted for any other award, except where acknowledged.

Principal Supervisor: Dr. Anke Martin

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Student and supervisors signatures of endorsement are held at the University.

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STATEMENT OF CONTRIBUTION

Chapter 2: Genomic regions associated with virulence in *Pyrenophora teres* f. *maculata* identified using genome-wide association mapping. Sharma R, Poudel B, Tao Y, McLean M and Martin A. Manuscript submitted to Plant Pathology.

RS contributed 60% towards the concept of the manuscript, DNA extraction of the isolates, analysis of the obtained data, literature collection, writing and revision of the manuscript. MM contributed 20 % for collection of leaf samples and phenotyping. YT and AM contributed 10% towards the concept and design, critical revision of the manuscript and final editorial input.

Chapter 3: QTL mapping of the genomic regions conferring virulence in *Ptm.* Sharma R, Tao Y, McLean M and Martin A.

RS contributed 70% towards the concept of the manuscript, collection of ascospores, extracting the DNA, collecting the conidia, phenotyping, analysing and interpreting the data and writing and revising the manuscript. AM contributed 20% in setting up crosses, designing the experiment and critical review of manuscript. YT and MM contributed 5% in critical revision of the manuscript and final editorial input.

Chapter 4: New virulences identified in progeny of *P. teres* f. *teres* x *P. teres* f. *maculata* populations. Sharma R, McLean M, Tao Y and Martin A.

RS contributed 65% towards the concept of the manuscript, collecting the conidia, phenotyping, analysing and interpreting the results and writing and revising the manuscript. AM contributed 20% in setting up crosses, collecting ascospores and critical review of manuscript. MM contributed 10% in critical revision of the manuscript and final editorial input. YT contributed 5% in critical revision of the manuscript and final editorial input.

LIST OF PUBLICATIONS AND SUBMITTED ARTICLES

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List of Conferences attended

Sharma R, Tao Y, McLean M and Martin A (2019) "Genome wide association study to identify genomic regions associated with virulence in *Pyrenophora teres* f. maculata" Participated in poster presentation at Australian Plant Pathology Society Conference, November 27-1, Melbourne.

LIST OF ABBREVIATIONS AND UNITS

AFLP – Amplified fragment length polymorphism

DH– Doubled haploid

DAPC– Discriminant analysis of principal components

DArT – Diversity arrays technology

GWAS– Genome wide association study

HRF – Hermitage research facility

LD– Linkage disequilibirum

MAT – Mating type locus

MLG – Multi-locus genotype

NETS– Necrotrophic effector-triggered susceptibility

NFNB – Net form net blotch

PCD– Programmed cell death

PCR – Polymerase chain reactions

PDA – Potato dextrose agar

PCA – Principle component analysis

Ptt – *Pyrenophora teres* f. *teres*

Ptm – *Pyrenophora teres* f. *maculata*

RAD-GBS– Restriction associated DNA genotyping by sequencing

RAPD – Random amplified polymorphic DNA

RIL– Recombinant inbred line

SFNB – Spot form net blotch

SNP – Single nucleotide polymorphism

SSR – Simple sequence repeats

QTL –Quantitative trait loci

UPGMA – Unweighted pair group method with arithmetic mean

bp – base pair

cm – centimeter

°C – degree Celsius

g – gram

h – hour

ha – hectare

kg – kilogram

L – litre

m – meter

mm – millimetre

mM – micromolar

μm – micrometer

μL – microlitre

μM – micromolar

Mbp – Mega base pair

ng – nanogram

s – second

U – unit

V – volt

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CHAPTER 1

LITERATURE REVIEW

1.1 Barley

Barley (Hordeum vulgare) is the fourth largest grain crop grown globally (Giraldo et al., 2019). Net blotches are economically important foliar diseases barley worldwide. There are two forms of net blotch, including the net form of net blotch (NFNB) and spot form net blotch (SFNB) causing an estimated economic loss of $117 and $192 million per annum respectively in Australia (Murray and Brennan, 2010). Grain quality parameters such as kernel size, bulk density and plumpness are negatively affected by NFNB and SFNB (Carlsen et al., 2017; Khan, 1982).

1.2 *Pyrenophora teres* Drechsler [anamorph *Drechslera teres* (Sacc.) Shoem]

Net form net of blotch is caused by the fungus *Pyrenophora teres* f. *teres (Ptt)* and spot form net blotch SFNB is caused by *P. teres* f. *maculata* (*Ptm*) (McLean et al., 2009). The two forms of net blotch were named according to the symptoms they induce on barley (McLean et al., 2009). Net form of net blotch consists of dark brown net–like lesions, while SFNB consists of dark circular or elliptic brown lesions. Both *Ptt* and *Ptm* belong to the phylum *Ascomycota* within class *Dothideomycota* of order *Pleosporales*. The DNA markers and mating type sequences for *Ptt* and *Ptm* have indicated that the two forms are genetically closely related, however phylogenetic studies have shown that the two forms are divergent genetic groups and genetically independent (Liu et al., 2011, Rau et al., 2003, Rau et al., 2007).

Net form net blotch was first reported in United States by Atanasoff and Johnson (1920) and SFNB was first reported in the 1960s in Denmark (Smedegård-Petersen, 1971). Both have since been reported in most barley growing regions internationally, including Canada, North America, South Africa and Hungary (Tekauz, 1990, Liu et al., 2012, Ficsor et al., 2010, Louw et al., 1996). In Australia, SFNB was first recorded in 1977 in the Northern cereal belt of Western Australia (Khan and Tekauz, 1982) and since then SFNB has spread throughout Australia. Spot form net blotch is now a common disease of barley in Australia, (Liu et al., 2011, McLean, 2011).

1.3 Symptoms

Symptoms produced by NFNB and SFNB are initially very similar, appearing as small circular/elliptical lesions. For NFNB, these develop into fine dark lines that spread to produce a network extending across the entire leaf surface (Figure 1a) (Liu et al., 2011 , McLean et al.,

2009). For SFNB, symptoms on susceptible varieties appear as small pinpoint, dark brown necrotic spots which increase in size to form elliptical or fusiform lesions measuring 3-6 mm (McLean et al., 2009, Figure 1b). Lesions might be surrounded by chlorotic zones which can enlarge and merge to destroy an entire leaf (Smedegard-Petersen, 1977). On resistant varieties SFNB, symptoms appear as small, necrotic, dark brown lesions that do not increase in size. Phytotoxic low molecular weight compounds and proteinaceous metabolites secreted by *P. teres* are partially responsible for symptoms produced (Weiergang et al., 2002). Symptoms of NFNB and SFNB are mostly visible on leaves of barley plants but can also be found on leaf sheaths, stems, heads and kernels. Symptoms start to occur within 24 hours of infection, with symptom development and expression depending on pathogen virulence, host genotype and environmental conditions (Liu et al., 2011).

Similar mycelia growth is produced by *Ptt* and *Ptm* on agar and conidia production is identical. Symptoms of SFNB are identical to those of spot blotch caused by *Bipolaris sorokiniana*; sexual stage *Cochliobolus sativus* (Williams, 2001), therefore, characterisation at molecular level is required to distinguish between *Ptm* and *B. sorokiniana*. Symptoms produced by the fungus *Ramularia collo-cygni* are also similar however, the lesions are more rectangular with straight longitudinal sides compared to *Ptm* where the lesions are typically longer and irregular in shape (Havis et al., 2015).

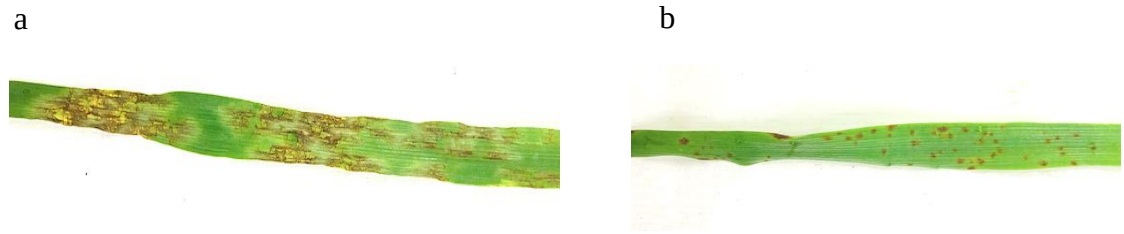

Figure 1: Symptoms of Net form net blotch caused by *Ptt* (a); symptoms of Spot form net blotch caused by *Ptm* (b)

Diffusible toxins and proteinaceous effectors secreted by *P. teres* play an important role in symptom development. Both forms of *P. teres* produce phytotoxins toxin A and toxin B initially described by Smedegard-Petersen (1977). Later Bach et al. (1979) identified a third toxin, toxin C. They showed that toxin A was N-(2-amino-2-carboxyethyl) aspartic acid, toxin B was anydroaspergillomarasmine A which could be a precursor of toxin C. Friis et al. (1991) showed that these toxins were non proteinaceous, low molecular weight metabolites which caused varied chlorosis and necrosis on susceptible plant varieties in the absence of the pathogen. Weiergang et al. (2002) showed that toxin A and toxin C produced different

responses in susceptible varieties within 120 hours. Toxin A produced yellow chlorotic symptoms with little necrosis, toxin C caused necrotic symptoms with little chlorosis. Toxin B produced little to no visible host response. Toxin A and C produced necrosis within 120 hours on resistant barley genotypes but no chlorosis. Therefore, it was proposed to use toxin A and C to screen barley germplasm for resistance to *P. teres*. However, these toxins produced only general symptoms associated with *P. teres* infection and were not associated with distinct necrotic lesions induced by *Ptt* and *Ptm*. Therefore, usage of these toxins for germplasm screening was limited.

Sarpeleh et al. (2007) found another proteinaceous metabolite secreted by both forms of *P. teres*. This metabolite was low in molecular weight and had characteristics similar to toxin B and toxin C, in terms of inducing broad host range, heat stability, staining, host range and electrophoretic properties. This metabolite caused necrosis only when injected into detached leaves and not when injected into intact leaves and induced symptoms only on barley plants. It produced more severe symptoms on variety Sloop and little symptoms on resistant barley variety CI9214. Presence of these host specific toxins indicated gene for gene or reverse gene for gene interactions.

Lightfoot and Able (2010) suggested that distinct symptoms caused by *Ptt* and *Ptm* could be due to lifecycle differences between the two forms. Where *Ptm* appeared to grow as biotroph first and then make a transition to necrotroph, *Ptt* appeared to spend most of the time as necrotroph. They also noticed that symptom development was only observed when the pathogen had reached the mesophyll cells of barley leaves when toxin production seemed most likely to happen. Severity of symptom production depended on the amount of toxin production as well as amount of fungal biomass on infected leaf tissue.

1.4 Life cycle

Both forms of *P. teres* are residue or stubble borne pathogens. Unlike other pathogens such as *Bipolaris* or *Helminthosporium, P. teres* cannot grow in soil or outside of host tissue. Stubble acts as primary source of inoculum. Retention of stubble for soil water conservation purposes has been linked to increased severity of disease (McLean et al., 2009). While seed-borne *Ptt* is also an important source of innoculum (Tervet, 1944). In contrast, no documentation of seed-borne transmission caused by *Ptm* has been recorded (McLean et al., 2009).

The life cycle of *P. teres* involves both a sexual and an asexual stage (Figure 2). *P. teres* is a heterothallic fungus that requires two opposite mating genotypes to undergo sexual reproduction (Liu et al., 2011). The sexual stage consists of pseudothecia which appear as small fruiting bodies on the surface of barley straw. They are usually 1-2 mm in diameter and are covered by dark, hair like setae (Mathre, 1997, McLean et al., 2009). Within the mature

pseudothecia, club shaped and bitunicate asci are present (Mathre, 1997). Generally, eight ascospores are present in each ascus. Ascospores are light brown in colour and often have 3-4 transverse and 1-2 longitudinal septa (Mathre, 1997). Under moist conditions of 95-100% relative humidity, ascospores are ejected into the air and are dispersed by wind. This serves as primary inoculum in the growing season and causes initial crop infection, although with *Ptt* primary inoculum can also be related to seed-to-seedling transmission (Jordan, 1981). The infection process of *P. teres* starts with germination of ascospore/conidia that lands on the host leaf surface. Both conidia and ascospore are able to germinate within few hours after landing on the leaf surface given suitable temperature and humidity conditions are met. A germ tube develops from conidial cells and hyphae from the germ tubes form a swollen, club shaped appressorial structure which is used by the fungus for penetration of the epidermal cell wall. Ruiz-Roldan et al. (2001) showed that formation of the appressorial structure is a complicated process and requires mitogen-activated protein kinase gene PTK1. The fungus grows

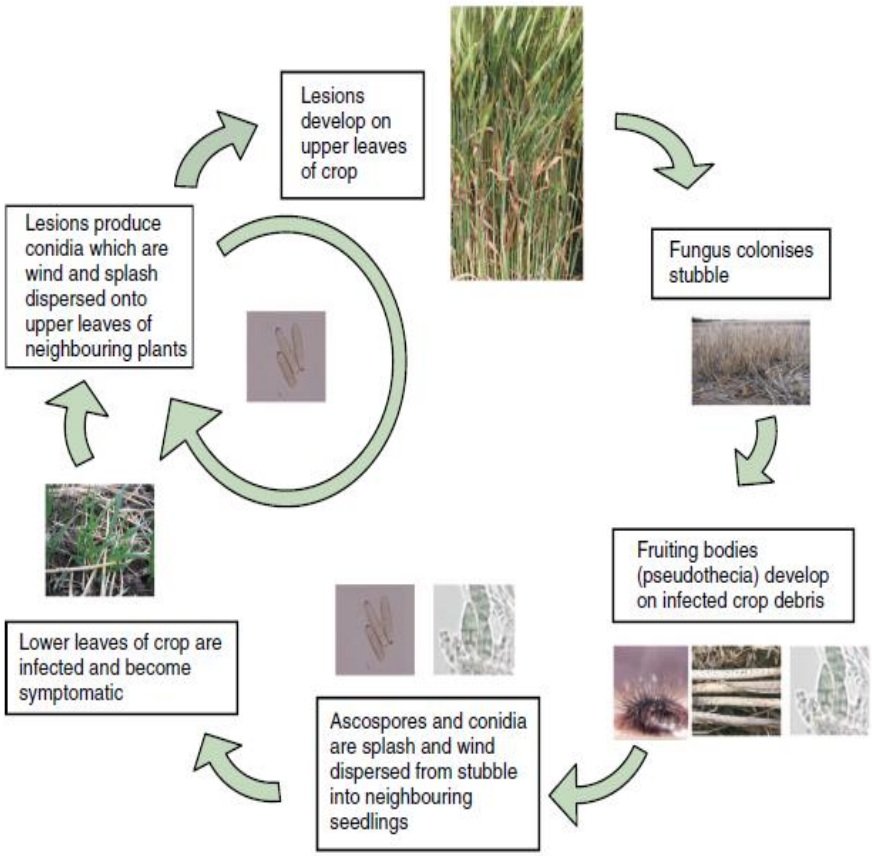

Figure 2: Life cycle of *Pyrenophora teres* f. *maculata* (McLean et al., 2009)

throughout the epidermal cell wall and colonizes the apoplast of the mesophyll tissue and eventually produces a lesion. Large intracellular vesicles develop from fungal hyphae known as primary vesicles. Secondary vesicles develop within the epidermal cell with simultaneous

disruption of infected as well as adjacent cells (Keon and Hargreaves, 1983). The asexual stage of *P. teres* consists of conidia which are produced from lesions or stubble (Figure 2). Conidia are usually straight and cylindrical in shape, round at both ends, sub-hyaline to yellowish brown in colour with 4-6 pseudosepta and are 15–23 x 30–175 μm in size (Smedegard-Petersen, 1971). Conidia develop on conidiophores, which are mild brown to olivaceous brown in colour. These can occur singly or in groups of 2-3. The conidia are dispersed by wind to cause new infections after adhesion to the leaf surface. Conidia are produced and dispersed throughout the barley growing season during favourable environmental conditions. After the growing season, pseudothecia develop on stubble, which acts as primary inoculum for next season's infection (McLean, 2011).

1.5 Sexual recombination

Sexual reproduction in *Pyrenophora teres* is controlled by a single mating type locus (MAT), which has two alternate forms known as idiomorphs named MAT1-1 and MAT1-2. Each gene within the idiomorphs regulates a pheromone. Haploid cells secreting the pheromones of opposite type will bind to each other. After binding two compatible cells will fuse and have two nuclei, known as the dikaryotic stage. This is followed by nuclei fusion forming a diploid nucleus. This diploid nucleus undergoes two meiotic divisions giving rise to four haploid nuclei. These nuclei then undergo meiotic divisions to produce eight haploid ascospores (Fincham and Stanley, 1971). Recombination through sexual reproduction influences the diversity and virulence profile of the pathogen (Milgroom, 1996). Previous studies have shown significant amounts of genetic variation within *Ptt* and *Ptm* populations, reflecting frequent sexual recombination (Harrabi and Kamel, 1990, Rau et al., 2003, Tekauz, 1990).

Sexual recombination between *Ptt* and *Ptm* is rare in nature (Poudel, 2018). Poudel et al. (2018) suggested reproductive isolation between *Ptt* and *Ptm* in the field and frequent recombination within forms. However, many studies have shown that *in vitro* recombination between the two forms is possible (Campbell et al., 1999, Campbell et al., 2002, Afanasenko et al., 2007, McDonald, 1963, Smedegard-Petersen, 1977). Smedegard-Petersen (1977) developed 11 crosses between *Ptt* and *Ptm* isolates and collected 478 ascospores. This was the first study to demonstrate that *in vitro* hybridisation between *Ptt* and *Ptm* is possible. Campbell et al. (1999) collected 23 ascospores from a cross between a *Ptt* and *Ptm* isolate. Novel DNA bands were confirmed with random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) markers. This study demonstrated that sexual recombination between *Ptt* and *Ptm* can result in substantial change in genetic recombination and disease expression in host and fungicide sensitivity in the pathogen (Campbell et al. 1999). Campbell and Crous (2003) observed that *Ptt/Ptm* hybrid progeny retained their virulence and fertility for a period

of more than two years. Jalli (2011) produced two *in vitro Ptt* and *Ptm* crosses. The virulence of progeny on five barley genotypes was studied. They observed that none of the progeny resembled their parents in terms of virulence. ElMor (2016) produced four *Ptt* and *Ptm* crosses *in vitro* and studied the virulence of progeny by detached leaf assay. Similar to Jalli (2011), this study also indicated that progeny exhibit virulence different to their parents.

Some studies have indicated the occurrence of sexual reproduction between *Ptt* and *Ptm* isolates in the field (Campbell et al., 2002, Leišova et al., 2005, McLean, 2011, Dahanayaka et al., 2021). Campbell et al. (2002) collected 36 net form isolates and 29 spot form isolates from two fields that were 30 km apart. The two fields represented net form and spot form populations. They found one net type isolate that did not cluster with any other net type isolates. This isolate contained unique spot form DNA bands. Leišova et al. (2005) analysed 37 *Ptt* and 20 *Ptm* Czech isolates using AFLP analysis. They found two isolates with a high number of shared markers of both forms of *P. teres*. McLean et al. (2014) studied 60 *Ptm* isolates using AFLP analysis. They found one isolate that contained both bands unique to *Ptt* and *Ptm*. In a recent study, Dahanayaka et al. (2021) studied 250 *Ptt* isolates collected from Australia, Canada, Hungary, Denmark, Japan, Sweden and Republic of South Africa. These isolates were collected during 1931-2018. Form specific amplification of DNA markers identified two hybrids. One hybrid (CBS281.31) was collected in Japan in 1931 and another hybrid (H-919) was collected in Hungary in 2018. These results indicate that hybrids produced by sexual recombination between the two forms may be present in the field. Perhaps they have not been reported because they cannot be distinguished from the parental isolates based on the symptoms. It is also possible that hybrids may be avirulent and have a low survival rate. Therefore, observation of virulence patterns of laboratory produced *Ptt* x *Ptm* hybrids is necessary to determine whether hybrids are mostly avirulent. Primers that are capable of distinguishing hybrids have been developed by Poudel et al. (2017).

1.6 Molecular Characterisation

Molecular markers can be used to reliably distinguish between *Ptt* and *Ptm* using polymerase chain reaction (PCR) assays. Primers that are capable of distinguishing between *Ptt* and *Ptm* have been designed by various researchers (Keiper et al., 2008, Leisova et al., 2006, Poudel et al., 2017, Williams, 2001). Two form specific mating type markers for *Ptt* (*Ptt*MAT1-1-1 and *Ptt*MAT1-2-1) and *Ptm* (*Ptm*MAT1-1-1 and *Ptm*MAT1-2-1) can be used to identify the mating type (Akhavan et al., 2015, Lu et al., 2010, Rau et al., 2005, Rau et al., 2007).

Techniques like RAPD and AFLP can be used to identify hybrids between the two forms of *P. teres* (Campbell et al., 1999, Campbell et al., 2002). However, these methods depend on gel electrophoresis resulting in low throughput. Diversity Arrays Technology (DArT) can also

prove to be useful for the detection of *Ptt* x *Ptm* hybrids (Dahanayaka et. al 2021). DArT is a genotyping method that uses array hybridisations technology to score hundreds of polymorphic markers across a whole genome in a single assay without the need for previous sequencing information (Jaccoud et al., 2001). DArT markers have been developed for fungal species, including *P. teres* (Sharma et al., 2014, Wittenberg et al., 2009, Martin et al., 2020, Dahanayaka et al., 2021), therefore, using DArT would be an ideal molecular marker system for producing markers across the whole genome of *Ptm* isolates and also for identifying the presence of hybrids.

1.7 Virulence

Significant pathogenic variation has been observed in *P. teres* (Gupta et al., 2011, McLean, 2012, Tekauz, 1990). Tekauz (1990), reporting the diversity in the Canadian *Ptm* population, characterized 219 isolates of *Ptm* and observed 20 pathotypes amongst 12 barley differentials. Gupta et al. (2011) characterised the diversity of *Ptm* isolates to understand the virulence in Australia. This study revealed a distribution of *Ptm* isolates with variation in their virulences throughout the agricultural region of Western Australia. McLean (2012) evaluated the disease response of 95 barley lines towards 19 *Ptm* isolates collected from Australia and Canada. Abundant pathogenic variation was observed within isolates from Australia and Canada and indicated that the development of SFNB resistant barley varieties may require a combination of multiple resistance genes conferring both major and minor effects. Akhavan et al., 2016 studied the virulence of 39 *Ptt* and 27 *Ptm* Canadian isolates. Cluster analysis revealed 13 and 16 distinct pathotypes of *Ptt* and *Ptm*, respectively. Fowler (2018) studied *Ptt* isolates collected from different states of Australia. The study revealed that isolates clustered into four different groups. The pathogenic variation of *P. teres* is a challenge for barley breeders to develop commercial varieties with resistance to *P. teres* pathotypes. To provide a standard method of discriminating pathotypes globally, McLean (2012) developed a standard set of differential barley lines for *Ptm* populations.

1.8 Tools for dissecting genetic architecture of complex trait

The regions within a genome that contain genes associated with a particular trait are known as quantitative trait loci (QTL) (Collard et al., 2005). Linkage analysis and genome wide association mapping are the most commonly used tools for dissecting the genetic architecture of complex traits. QTL mapping is powerful and well-established approach for identifying genomic regions associated with quantitative traits while genome wide association mapping is a recent development to dissect traits. Linkage mapping is based on the principle that genes and markers segregate during meiosis by chromosome recombination, thus allowing their

analysis in the progeny (Collard et al., 2005). Segregation of markers and QTL occur based on the proximity between the two. Marker and QTL will be inherited together in the progeny when they are in close proximity, however independent segregation will occur when they are further apart. Generally, linkage mapping requires construction of a segregating mapping population with 50 - 250 individuals. Larger population sizes are required to achieve higher mapping resolution. It is critical to have sufficient polymorphism between the parents in order to construct a genetic map.

Linkage disequilibrium is the key for genome wide association mapping (Kushwaha et al., 2017). Loci are considered to be in linkage disequilibrium when the frequency of association of their different alleles is higher or lower than what would be expected if the loci were independent and associated randomly. This is also referred to as non-random association of alleles between the genetic loci. Linkage disequilibrium removes the necessity of constructing large bi-parental populations. The power of GWAS to identify associations between SNP and trait of interest depends on the phenotypic variance explained by the SNP. Phenotypic variance is determined by how strongly two alleles differ in their frequency and effect size. The most straightforward approach for analysis of association between SNP and trait of interest is analysis of variance. However, one limitation of association mapping is generation of false positive or type one errors which may occur due to population structure, small sample size and low frequency of specific alleles (Kushwaha et al., 2017). False positives can also arise through spurious associations due to the large numbers of marker tests performed. One way to minimise false positives is to use mixed modelling approaches that accounts for kinship, population structure or both. Which model fits best depends on factors such as kinship, population structure and phenotype of the trait. Another approach is to apply multiple-comparison adjustment to p-value such as positive false decetection rates (pFDR) and or using the Bonferroni correction (Kushwaha et al., 2017).

Linkage analysis and association mapping techniques are complimentary to each other in providing knowledge, cross validation and statistical power (Kushwaha et al., 2017). Association mapping should not be regarded as a replacement for QTL mapping as both have advantages and disadvantages. Association mapping has lower power to detect rare alleles compared to linkage mapping, while QTL mapping can have poor mapping resolution compared to association mapping. When applied together both can lead to a better understanding of genetic architecture of quantitative traits.

1.8.1 Quantitative trait loci (QTL) mapping in barley

QTL analysis has been used to identify resistance genes against *P. teres* in host/barley. For *Ptt*, Koladia et al. (2017) developed a recombinant inbred line (RIL) mapping population by

crossing breeding lines CI5791 and Tifang and identified major resistance genes for *Ptt* on chromosome 3H and 6H in CI5791 and on 3H in Tifang. Liu et al. (2015) also identified major genes for *Ptt* resistance/susceptibility on chromosome 3H and 6H. Manninen et al. (2006) crossed barley line Rolfi with CI9819 and inoculated the doubled haploid progeny with four isolates of *Ptt* and four isolates of *Ptm*. A major *Ptt* resistance gene was identified on chromosome 6H and was designated as *Rpt5* while minor genes were reported on chromosomes 1H, 2H, 3H, 5H and 7H. For *Ptm*, a major gene, *Rpt6*, was identified on chromosome 5H, while no minor genes providing resistance to *Ptm* were reported in that study. Wonneberger et al. (2017) inoculated a mapping population of 109 doubled haploid (DH) lines with three isolates of *Ptt* at seedling stage in a greenhouse and adult plant stages under field conditions. Nine QTL associated with *Ptt* resistance were reported in this study suggesting that the disease is controlled by several genes. One significant QTL, *AL_QRptt5-2* located on chromosome 5H was observed in all environments and developmental stages. Eight other QTL present on 3H, 4H, 5H, 6H and 7H were present in at least one of the conditions tested. Another DH population developed from a cross between SM89010 and Q21891was genotyped using simple sequence repeat (SSR) and AFLP markers (Friesen et al., 2006). A major QTL for *Ptt* resistance at seedling stage was found on chromosome 6H while a major QTL providing *Ptm* resistance was found on chromosome 4H. A major QTL for *Ptt* resistance was also found on chromosome 6H by Pierre et al. (2010). Using a cross between barley lines Rika and Kombar seven QTL were identified at the adult plant stage including a major *Ptt* resistance gene on chromosome 6H (Steffenson et al., 1996).

1.8.2 QTL mapping in *Pyrenophora teres*

QTL mapping has been used to identify virulence/avirulence genes in different *Ptt*/*Ptt* populations (Koladia et al., 2017; Shjerve et al., 2014). Weiland et al. (1999) developed a cross between two *Ptt* isolates 0-1 and 15A. Isolate 0-1 was virulent while 15A was avirulent on barley cultivar Harbin. Virulences of 82 progeny were evaluated. The 15A allele designated as *AvrHar* was proposed to contribute avirulence on barley line Harbin. Later Lai et al. (2007) also developed a cross between *Ptt* isolate 0-1 and 15A to study the genetics of avirulence associated in barley lines Canadian Lake Shore (CLS), Tifang, and Prato. Isolate 15A was virulent on CLS and Tifang but was avirulent on Prato. Conversely 0-1 was avirulent on CLS and Tifang but was virulent on Prato. An AFLP based linkage map was constructed and *AvrHar* was found to be conferring avirulence on Tifang and CLS. They also found two additional QTL *AvrPra1* and *AvrPra2*, to be functionally redundant to confer avirulence on Prato. Both locus *AvrHar* and *AvrPra2* mapped to same locus on chromosome 5 of the W1-1 reference genome (Wyatt et al., 2018). But whether this locus contains two tightly linked genes or alleles of the

same gene is yet to be confirmed (Clare et al., 2020). The *AvrPra1* QTL is currently designated on chromosome 9 based on the 0-1 genome sequence assembly (Clare et al., 2020).

Beattie et al., (2007) developed a biparental mapping population with *Ptt* isolate WRS 1906 which was avirulent on barley cultivar Heartland and WRS 1607 which was virulent on Heartland. Sixty-seven progeny were phenotyped on Heartland. A single gene $Avr_{Heartland}$ was found to be responsible for determining avirulence of barley genotype Heartland. Shjerve et al. (2014) developed a biparental population between two California *Ptt* isolates 15A and 6A and developed a genetic map with SSR, SNP and AFLP markers. Two major QTL *VR1* and *VR2* were associated with virulence on barley genotype Rika. While two different loci *VK1* and *VK2* were shown to be associated with virulence on barley genotype Kombar. These loci were assigned chromosomal designations based on the updated genome reference assembly of the 0-1 isolate (Wyatt et al., 2018). The locus *VK1* is localised on chromosome 3, VK2 and *VR1* at different locations on chromosome 2 and *VR2* on chromosome 10 (Clare et al., 2020). Koladia et al. (2017b) developed a bi-parental fungal population consisting of 109 progeny by crossing the BB25 (Denmark) and FGOH04Ptt-21 (North Dakota,USA) *Ptt* isolates. A genetic map was obtained based on SNP markers and 16 linkage groups were formed. Nine unique QTL were found to be associated with virulence.

Carlsen et al. (2017) used a *Ptm/Ptm* population to develop a genetic map to identify QTL regions associated with virulence in *Ptm.* They developed a population with 105 progeny by crossing an Australia isolate (SG1) with one from the USA (FGO10Ptm-1). Phenotyping was conducted on four differential barley genotypes and genotyping was conducted using restriction associated DNA genotyping by sequencing (RAD-GBS). The size of the generated genetic map was 1807 cM and it involved 16 linkage groups. Six virulence related QTL were identified on five linkage groups. The data generated from this study suggested that the *Ptm*-barley interaction partially follows the necrotrophic effector-triggered susceptibility (NETS) model. This model states that the pathogen secretes necrotrophic effectors which interact with host targets to induce necrosis as a consequence of programmed cell death (PCD). This study conducted by Carlsen et al. (2017) demonstrated the complexity of virulence underlying the FGO10Ptm-1/SG1 bi-parental population, indicating that the intricacy present in the natural population is significant.

1.8.3 Genome wide association mapping in fungi

Genome wide association studies (GWAS) are used to identify genes in human and plant genomes (Dalman et al., 2013, Gao et al., 2016, Muller et al., 2011). However, only few studies have used GWAS in fungi. Most of the fungi undergo sexual reproduction, resulting in high

diversity between the individuals in the population. This reduces the linkage disequilibrium, making it a sound application for association studies.

To date, only one study has been conducted of the virulence in *P. teres* by GWAS (Martin et al., 2020). Martin et al. (2020) identified 14 QTL associated with virulence in *Ptt* from a population of 188 unrelated *Ptt* isolates. Four of these regions were identified and confirmed via QTL mapping. They also identified the presence of 20 effector proteins underlying these QTL regions. This study concluded that the genetic basis of *Ptt* virulence was quite complex as compared to other pure necrotrophs such as *Parastagonospora nordorum* and *P.tritici-repentis.* This study demonstrated the efficacy of applying GWAS to *P. teres* for dissecting complex traits. Association mapping studies have been conducted on other pathogenic fungi to understand their virulence mechanisms. Identification of genetic loci associated with pathogenesis in *Saccharomyces cerevisae* was conducted by Muller et al. (2011) using GWAS. They analysed 44 pathogenic and 44 non-pathogenic isolates and found that coding sequences of genes *VRP1, KIC1, SBE22* and *PDR*5 and upstream region of *YGR146C* are of high importance for pseudohyphal formation, cell wall maintenance and cellular detoxification and indicated that they might be involved in virulence. Virulence of *Heterobasidion annosum* on *Picea abies* and *Pinus sylvestris* was analysed by GWAS (Dalman et al., 2013). 23 haplotypes were sequenced out of 33,018 SNPs identified, 12 SNPs were associated with virulence. In *Parastagonospora nodorum* 2,983 SNP markers and gene markers for SnTox A and SnTox 3 were identified using 191 isolates (Gao et al., 2016). They identified a novel locus in the *P. nodorum* genome associated with virulence.

1.9 Effectors

The interaction between *Ptm* and barley is likely to have a gene for gene interaction. This means that there must be an avirulence gene in the pathogen which is recognised by the resistance gene in the host plant and triggers resistance reaction when expressed. Most host plants are able to resist a majority of pathogens through the recognition of microbe associated molecular patterns (PAMPs/MAMPs) leading to a basal defence response or PAMP triggered immunity (PTI) (Koladia et al., 2017b). Avirulence genes in a pathogen encodes proteins which when recognised by host, triggers resistance response (McLean, 2011). *P. teres* is a necrotrophic pathogen, which has many virulence/avirulence factors. Some of these virulence/avirulence factors have been mapped and genetically characterised. The first mapped avirulence gene identified by Weiland et al. (1999) was designated as *AvrHar*. It provided low virulence on barley genotype Harbin. Avirulence genes have been identified by two other studies (Lai et al., 2007, Beattie et al., 2007).

Inverse gene for gene interaction have also been proposed for *Ptm*-barley interaction. In this type of interaction, effectors are produced by pathogens to manipulate the basal defence, gain entry and to gain nutrients from the host resulting in disease (Koladia et al., 2017b). The host recognises and responds to these effectors which leads to effector triggered immunity (ETI). Programmed cell death is usually a defence mechanism of the host plants to restrict the growth of biotrophic plant pathogenic fungi in the plant. However, when this defence mechanism is hijacked by the necrotrophic plant pathogen, it provides the necrotroph with desired nutrients. Liu et al. (2015) isolated a proteinaceous necrotrophic effector PttNE1 from intracellular wash fluid of barley genotype Hector which was inoculated with *Ptt* isolate 0-1. Effector PttNE1 showed direct association with disease development. They concluded that necrotrophic effector triggered susceptibility is implicated in the *Ptt*-barley pathosystem and it is likely to play a role in the *Ptm*-barley pathosystem as well. However, the PttNE1 pathogen gene was not mapped in *Ptt.* Koladia et al. (2017b) identified nine QTL in *Ptt* that were associated with virulence. Effector candidate gene underlying these QTL are currently being evaluated (Clare et al., 2020). Martin et al. (2020) identified 20 effectors underlying 14 QTL regions in *Ptt*. According to the published literature, no effector genes have been cloned or characterised in *Ptm* and as a result the mechanism of virulence is unknown.

1.10 Bioinformatics

Different sequencing technologies are available for whole genome sequencing such as Roche/454 pyrosequencing (400-500 bp), Ilumina/Solexa sequencing (currently up to 100 bp) and Sanger sequencing (700-950 bp) (Ellwood et al., 2010). Sequencing technologies for long reads of up to 60 kb such as single molecule real time (SMRT) resequencing platforms and Pacific Biosciences have recently been developed (Goodwin, McPherson, & McCombie, 2016). The first genome assembly for *Ptt* with a genome size of 41.95 Mb was produced by Ellwood et al. (2010). They used Solexa sequencing (with 75 bp pair end reads) to assemble the genome and uploaded the initial draft genome to NCBI. This assembly predicted 11,799 genes. Wyatt et al. (2017) updated by long read sequencing and scaffolding with the assistance of genetic linkage maps. The reference genome resulted in a total genomic content of 46.5 Mb, with 86 contigs and 11541 genes. Comprehensive genome assemblies for *Ptt* and *Ptm* were constructed by Syme et al. (2018) who identified a difference in the genome sizes of *Ptt* and *Ptm*, with *Ptt* and *Ptm* having genome sizes of 49 Mbp and 41 Mbp, respectively. They found major genetic difference between the two forms in transposable element (TE) rich regions. Currently, whole genome assemblies of 11 *Ptt* isolates (W1-1, NB29, NB85, NB73, 15A, 6A, FGOH04Ptt-21 and BB25) and two *Ptm* isolates (FGBOB10Ptm-1 and SG1) are available

(Syme et al., 2018, Wyatt et al., 2020). The information within these sequences can help understand how pathogenesis is caused by *Ptm.*

1.11 Objectives of study

A total of six QTL mapping studies (Weiland et al., 1999, Lai et al., 2007, Beattie et al., 2007, Shjerve et al., 2014, Koladia et al., 2017a) and one GWAS (Martin et al., 2020) study have been conducted in identifying genomic regions associated with virulence/avirulence in *Ptt.* In contrast only one QTL mapping study has been conducted in *Ptm* (Carlsen et al., 2017*).* Most of our current understanding of *Ptm*-barley interaction is of the genomic regions involved in resistance of the barley host. Knowledge of genetics of *Ptm* virulence is necessary to better understand the host/pathogen interactions and to manage SFNB resistance in barley genotypes.

Although recombination between *Ptt* and *Ptm* is considered rare in nature, it is possible that hybrids are not being recognised or are misidentified as *Ptt* or *Ptm* as they produce symptoms similar to parents. It is also possible that hybrids produced are avirulent and cannot survive from one generation to the next. Six hybrids have been identified until now in fields. These isolates were only identified as hybrids after molecular diagnosis. Recombination between *Ptt/Ptm* can also lead to generation of novel pathotypes. This can be a major concern for barley industry. It is possible that pathotypes generated can overcome deployed resistances in barley genotypes and can be a potential threat to the barley industry. No information about the inheritance of virulence in hybrids is available to date.

The objectives of the study were to:

1. Identify genomic regions associated with virulence in *Pyrenophora teres* f. *maculata* using genome-wide association mapping (Chapter 2) and a bi-parental QTL mapping population (Chapter 3)

The virulence factors present in *Ptm* have not been characterized in Australia. Identification of makers associated with virulence will help to identify pathogen effectors. This will lead to thorough characterization of underlying mechanisms of the *Ptm*-barley genetic interaction.

2. Understand the virulence of *Ptt* x *Ptm* hybrids

Production of novel virulent *Ptt/Ptm* hybrids is of concern to the barley industry. To study the inheritance of virulence in hybrids four *in vitro* crosses between *Ptt* and *Ptm* were made. The

progeny from the cross were phenotyped across 20 barley genotypes and their infection responses were observed.

The results of the studies undertaken in this thesis will be useful in providing recommendations to better manage SFNB and to improve management strategies.

CHAPTER 2

GENOMIC REGIONS ASSOCIATED WITH VIRULENCE IN PYRENOPHORA TERES F. MACULATA IDENTIFIED USING GENOME-WIDE ASSOCIATION MAPPING

This study was conducted to better understand *Ptm*-barley interaction. In this study, genomic regions conferring virulence in Australian *Ptm* population were identified using genome wide association study. The identified regions were further investigated to find candidate genes. We also studied the genetic structure of Australian *Ptm* population.

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Note: Supplementary data associated with this chapter are given in the appendix

Genomic regions associated with virulence in *Pyrenophora teres* f. *maculata* identified using genome-wide association mapping

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Abstract

Spot form net blotch (SFNB) caused by *Pyrenophora teres* f. *maculata* (*Ptm*) is a major foliar disease of barley worldwide. Spot form net blotch can cause grain yield losses of up to 44% and can affect grain quality parameters such as kernel size, weight and protein content. Dissecting the genetic basis of *Ptm* virulence is important to understand the plant-pathogen interaction and to breed for durable resistance. This study aimed to identify the genomic regions conferring virulence in *Ptm* isolates by using a genome wide association study (GWAS). One hundred and twenty-one *Ptm* isolates from Australia were genotyped using DArT-seq™ and 751 high quality markers were obtained. Ten novel quantitative trait loci (QTL) associated with virulence in *Ptm* were identified on chromosomes 1, 2, 3, 4, 5, 6 and 7. In total, 125 candidate genes were identified including two genes encoding putative effector proteins. The large number of QTL identified in this study suggests that the *Ptm*-barley interaction is complex.

KeywordsDNA markers, effector proteins, genome wide association study, *Pyrenophora teres* f. *maculata* and virulence.

Introduction

The fungus, *Pyrenophora teres* Drechsler (anamorph *Drechslera teres* [Sacc.] Shoem.) causes net blotch of barley. This fungus occurs as two forms named according to the symptoms they induce on barley leaves. Net form net blotch (NFNB) caused by *Pyrenophora teres* f. *teres (Ptt)* produces dark brown net–like lesions and spot form net blotch (SFNB) caused by *P. teres* f. *maculata* (*Ptm*) produces dark circular or elliptic brown lesions. Lesions may be surrounded by chlorotic zones which can enlarge and merge to destroy entire leaves (Smedegård-Petersen, 1977). On resistant barley varieties, symptoms appear as small, necrotic, dark brown lesions that do not increase in size. Spot and net form net blotch disease affect grain yield and quality parameters such as kernel size, plumpness, weight and protein content (Khan and Tekauz, 1982; Carlsen et al., 2017). Yield losses of up to 44% have been observed for SFNB (Jayasena et al., 2007; McLean et al., 2016) while up to 40% yield losses were observed for NFNB (Khan,

1987; McLean and Hollaway, 2019). DNA markers and mating type sequences for *Ptt* and *Ptm* have been used to demonstrate that the two forms are closely related, while phylogenetic studies have shown that they are divergent and reproductively independent (Rau et al., 2003; Rau et al., 2007; Liu et al., 2011; Ellwood et al. 2012).

Understanding the genetic basis of virulence in *Ptm* is essential for the development of disease resistant barley varieties. Knowledge of the genetics of *Ptm* virulence is important to identify durable resistance sources and to characterise the genes. Six studies have identified quantitative trait loci (QTL) associated with virulence in *Ptt* (Weiland et al., 1999; Lai et al., 2007; Beattie, 2007; Shjerve et al., 2014; Koladia et al., 2017; Martin et al., 2020). Only one study to date has identified genetic regions conferring virulence in *Ptm*. Carlsen et al., (2017) developed a bi-parental population consisting of 105 progenies by crossing an Australian *Ptm* isolate (SG1) with one from the USA (FGO10Ptm-1). Six virulence related QTL were identified on five linkage groups. Their study provided strong evidence that the *Ptm*-barley interaction was not a simple gene-for-gene interaction and stated that further functional characterisation is required to understand the *Ptm*-barley interaction.

Bi-parental QTL mapping has been utilised to identify virulence genes in *P. teres* (Shjerve et al., 2014; Koladia et al., 2017; Carlsen et al., 2017). However, these studies can have limitations due to limited number of recombination events being captured in relatively small mapping populations (Kushwaha et al., 2017). Genome wide association mapping (GWAS) has emerged as a complimentary technique to QTL mapping with advantages such as increased mapping resolution and surveying a greater number of allelic variations (Yu and Buckler, 2006; Zhu et al., 2008; Chan et al., 2011). This approach also removes the necessity of constructing large bi-parental mapping populations. However, GWAS can be prone to false positives due to population structure and familial relatedness (Kushwaha et al., 2017). False positives can also arise through spurious associations due to the large numbers of marker tests performed. Improved statistical models such as the Mixed Linear Model (MLM) have been developed to incorporate population structure and relatedness to reduce false positives (Yu et al., 2006; Kushwaha et al., 2017). Genome wide association studies have been widely used to understand the genetic architecture of important traits in humans and plants (Muller et al., 2011; Dalman et al., 2013; Gao et al., 2016; Tao et al., 2019). Few studies have used GWAS in fungal genetic analyses (Gao et al., 2016; Korinsak et al., 2019; Martin et al., 2020).

Pyrenophora teres isolates reproduce sexually, resulting in a high frequency of recombination within the population and reducing the linkage disequilibrium, thus making them a sound application for association studies. To date only one study used the GWAS approach to study

virulence in *P. teres* (Martin et al., 2020). Diversity Arrays Technology (DArTseq^{TM}) markers were used to genotype 181 Australian *Ptt* isolates and the isolates were phenotyped across 20 different barley genotypes. Fourteen different genomic regions associated with virulence were identified with the majority being located on chromosomes 3 and 5 and one each present on chromosome 1, 6 and 9. This demonstrated the efficacy of applying GWAS to *P. teres* for dissecting complex traits and its potential for studying *Ptm*. The current study aimed to use GWAS to identify and characterise genomic regions in *Ptm* that are associated with virulence. Identified regions were located on the SG1 reference genome to provide gene candidates for further functional studies.

Materials and methods

2.1 Fungal isolates

In total, 121 single spore *Ptm* cultures were obtained from barley samples collected from different barley growing regions of Australia during 1996-2018 (Supplementary Table 1). Single spores were obtained by placing ~10mm^2 pieces of infected leaf tissue in 70% ethanol for 10s, 25% bleach solution for 60s and sterile reverse osmosis (RO) water for 30s. Sterile leaf pieces were placed onto potato dextrose agar (PDA) plates and incubated at 20°C, with 12-hour photoperiod until conidiophores developed. Plates were then placed in the dark at 16°C for 24 hours. Single conidia were transferred to PDA plates using a sterilize glass needle. These were incubated at 20°C, with 12-hour photoperiod for 5 days and freeze dried.

2.2. Virulence profiling of isolates

Seedling tests were conducted as described by McLean et al., (2014). In brief, three replicates of 12 barley genotypes, Arimont, Chebec, CI5286, CI5791, CI9214, CII6150, Galleon, Keel, Kombar, Skiff, Torrens and TR250 were sown in a complete randomised block design in the glasshouse at 20˚C±5. Each set of genotypes were grown to the 2-3 leaf stage and inoculated individually with a spore/mycelial suspension of ~20,000 parts/mL of the *Ptm* isolate. Inoculated plants were placed in a humidity chamber at 95-100% humidity for 32 hours in the dark and then returned to a glasshouse for symptom development for eight days. Lesion were visually assessed according to a 1 (resistant) to 9 (susceptible) scale based on Tekauz (1985). Kombar was used as the susceptible control. Each genotype was assigned a single score based on the median score of three replicates for the analysis.

2.3. Genotyping

Isolates were grown on PDA plates incubated at 22˚C for ~10 days to produce sufficient mycelium for DNA extraction. Mycelium was scraped from the culture plate into a 2 mL tube. DNA was extracted using a Wizard® Genomic DNA Purification kit (Promega Corporation,

Sydney, Australia). The quality of the extracted fungal DNA was determined by gel electrophoresis using a 1% agarose gel and quantified with an Implen Nanophotometer (Integrated Sciences, Sydney, Australia). The DNA was normalised to 50 ng/µL and was sent to DArT Pty Ltd, Canberra, Australia for DArTseq™ analysis. The samples were processed following the method described in Martin et al (2020) briefly, the samples were processed in ligation/digestion reactions using a single PstI compatible adaptor with two different adaptors. The processed samples were amplified in 30 rounds of PCR. Amplified samples were applied to c-Bot (Illumina) bridge PCR followed by sequencing on the Illumina Hiseq2500 platform. Sequences generated were processed using DaRArT analytical pipielines.

2.4 Multilocus genotype analysis

Isolates with the same combination of marker alleles were considered to be clones. Missing values and genotypic errors can increase the number of clones by assigning an individual of the same clone to separate multilocus genotypes (MLGs). Such errors were corrected using similarity based Prevosti's distance (Prevosti et al., 1975) 'farthest neighbour' algorithm in R package POPPR v. 2.0 (Kamvar et al., 2014). The maximum distance between the replicated samples was estimated as 0.07. Genotypes with genetic distances smaller than 0.07 were collapsed into the same clonal group. The MLGs and corrected Simpsons index to measure genotypic diversity within the *Ptm* population were estimated using Poppr v. 2.0 (Kamvar et al., 2014). Since sample size (N) of each population can have an effect on the lamda value, the Simpson index was adjusted by multiplying lamda with N/(N-1) (Arnaud-Haond et al., 2007).

2.5 Population structure

A discriminant analysis of principal components (DAPC) was conducted using the R package 'adegenet' (Jombart and Ahmed, 2011). The genetic data was initially transformed according to Principal Component Analysis (PCA). These components were then used to perform linear Discriminant Analysis (DA), which provided variables to describe the genetic groups that minimized the genetic variance within populations, while maximizing the variation between populations. The number of PCs to be retained was determined using the cross-validation method implemented within *adegenet*. The collection of PCs with "highest mean success" and lowest "root mean squared error" was retained. A scatterplot was generated in Rstudio using the scatter(dapc1) function to visualize the results.

2.6. Association mapping of virulence genes in *Ptm*

Linkage disequilibrium (LD) and association between molecular markers and *Ptm* virulence was analysed in TASSEL version 5.2.31 (Bradbury et al., 2007). The mixed linear model (MLM) was used to perform association analysis with the first four principal components from

PCA accounting for population structure and kinship for familial relatedness. A logarithm (base 10) of odds (LOD) score was calculated from the P-value (P) estimated by TASSEL using the equation LOD = -LOG10(P). Bonferroni correction was used to set the threshold for defining significant associations (LOD score >2.88). R version 3.3.1 was used to draw the LD decay graph by fitting a smooth spline of average r^2 over a physical distance. Memory-efficient, Visualization-enhanced, and Parallel-accelerated Tool (MVP) was used in R Studio (https://github.com/xiaolei-lab/rMVP) to draw Manhattan plots.

2.7 *Pyrenophora teres* f. *maculata* candidate gene analysis

Markers significantly associated with virulence were aligned on the *Ptm* SG1 reference genome (GCA_900231935.2) (Syme et al., 2018) using the NCBI Basic Alignment Search Tool (BLAST v2.9.0). Based on LD decay calculations, 15 kb on either side of the marker were searched for candidate effector genes. Bedtools intersect v2.29.0 (Quinlan and Hall, 2010) was used to identify regions in the annotated SG1 genome that overlapped with the QTL regions and Bedtools getfasta v2.29.0 was used to extract the sequences. The protein coding regions which were predicted to be secretory proteins by SignalP-5.0 (Armenteros et al., 2019) and have zero or one transmembrane domain as identified by TMHMM v.2.0 (Sonnhammer et al., 1998) were further employed to predict effectors using EffectorP 2.0 (Sperschneider et al., 2016). Secondary metabolite biosynthetic gene clusters were predicted using antiSMASH 5.0 (Blin et al., 2019).

3. Results

3.1 Phenotypic responses

Differences in phenotypic responses were observed between barley genotypes (Figure 1). The most resistant barley genotype was Keel with infection response ranging from 2 to 5 and an average infection response of 3.0. Barley genotype Kombar which was used as susceptible control, was the most susceptible barley genotype with infection responses ranging from 2 to 8 and an average infection response of 7. Barley genotype Chebec and Skiff had low average infection responses of 3.4 and 3.5 respectively. Barley genotypes CI5791, TR250, Arimont, Torrens, CII6150, CI5286, CI9214 and Galleon had average infection response ranging from 3.9 to 5.6. Differences in virulence responses were observed within isolates. Isolate *Ptm*13-136 was the most virulent isolate with average infection response of 5.8 across all barley genotypes tested while *Ptm* isolate 09-033 was the least virulent isolate with average infection response of 2 across all barley genotypes (Supplementary Table 2).

3.2 SNP discovery and LD decay

Genotyping by DArTseq™ analysis of 121 *Ptm* isolates provided 14,836 SilicoDArT and 6,440 SNP markers. Markers that could not be located in the SG1 reference genome and had

>20% missing data were removed, resulting in 5,475 markers. These markers were further filtered in TASSEL5 using a minor allele frequency > 0.05 and led to a final set of 751 markers used in the analyses. Using this marker set, LD was estimated to decay to half of the maximum at about 15 kb (Figure 2).

3.3 Genotypic analyses and population structure

Of the 121 *Ptm* isolates, 120 were unique MLGs (Table 1), with only one clone isolate found in Victoria. Genotypic diversity was high ($\lambda \geq 0.99$) at all locations (Table 1).

A set of 5,475 SilicoDArT and DArT SNP markers (combination of both monomorphic and polymorphic markers) were used for the 121 *Ptm* isolates to analyse the population structure using DAPC analysis. Two groups were present on the two main axes (Figure 3). Isolates from South Australia (SA) formed one cluster while isolates from Queensland (Qld), Western Australia (WA), New South Wales (NSW) and Victoria (Vic) clustered in another group.

3.4 Association mapping of virulence genes

A total of 751 high quality markers and disease reactions of 12 barley genotypes were used in the GWAS analysis. Ten markers significantly associated with virulence were detected (Figure 4 and Table 2) on chromosomes 1, 2, 3, 4, 5, 6 and 7 of the SG1 reference genome. The QTL Ptmv5 on chromosome 4 at the 2,164,661 bp position had the highest LOD score of 3.96 on barley genotype Kombar and explained 14.44% of phenotypic variance. Another QTL, Ptmv2 was significantly associated with virulence on varieties CI16150 and Kombar and explained between 9.65 and 18.11% of the phenotypic variance. Other significant associations on varieties Galleon, CI5286, Chebec, Torrens, TR250, CI5791, CII6150, Arimont had LOD scores ranging from 2.92 to 3.7. No markers associated with virulence were identified for genotypes CI9214, Keel and Skiff.

3.5 Analysis of QTL regions to identify candidate effector genes

The gene content within the 10 QTL regions was examined against the SG1 reference genome and a total of 125 genes were identified (Supplementary Table 3). Out of these, 10 genes were predicted to be signal peptides (Table 3). Two of the signal peptides were predicted to be effector proteins. Effector proteins corresponded to QTL Ptmv2 on chromosome 2 with probability of 0.75 and on QTL Ptmv7 on chromosome 5 with probability of 0.83. No potential biosynthetic gene cluster was identified using antiSMASH.

4. Discussion

This is the first study to identify virulence QTL present in a *Ptm* population using GWAS. Ten novel QTL associated with virulence in *Ptm* isolates were identified. Two putative effector proteins were also identified within two of the QTL regions. These may play an important role in understanding *Ptm* virulence.

A high level of genotypic diversity was observed within the Australian *Ptm* population with 120 unique MLGs detected. The high genetic diversity was also observed when the *Ptm* isolates were grouped based on barley growing regions. This confirmed that sexual reproduction occurs regularly in the field leading to rapid change in the genetic structure of *Ptm* populations. High levels of genetic diversity due to sexual reproduction have been observed in various studies (Serenius, 2006; Bogacki et al., 2010; McLean, 2011). McLean (2011) found high genetic diversity among Victorian *Ptm* isolates with abundance in both mating types, which indicated the occurrence of sexual reproduction between the isolates. Bogacki et al. (2010) found that sexual reproduction was predoiminant in South Australian fields among *Ptt* and *Ptm* populations and was contributing to genetic diversity. Similarly, Serenius et al. (2005) found high genotypic diversity and a 1:1 ratio of both sexual mating types of both forms of *P.teres* in Finnish isolates. This also indicates frequent sexual reproduction occuring among *P. teres* isolates in Finland. The DPAC analysis divided isolates into two clusters with isolates from SA distinct and clustered separately from Vic, NSW, WA and Qld isolates. This clustering may suggest adaption of particular populations of *Ptm* to local barley cultivars or environment or agricultural practices (Peever and Milgroom, 1994; McLean et al., 2014). Fowler et al. (2017) also found that the eastern Australia *Ptt* isolates grouped separately from the western Australia *Ptt* isolates. They suggested that cultivation of regionally adapted barley cultivars has led to the regional evolution of *Ptt.*

Linkage disequilibrium analysis provided an approximate LD half-decay value of 15 kb for Australian *Ptm* isolates. This value is similar to the LD half decay value of 20 kb calculated for *Ptt* (Martin et al., (2020). Within the ascomycetes the LD varies from 0.11 kb to 162 kb (Ellison et al., 2011; Talas and McDonald, 2015; Nieuwenhuis and James, 2016). The relatively low value of LD decay in our study could be due to sexual reproduction of *Ptm*.

We identified 10 significant marker trait associations for *Ptm*. Previously, Carlsen et al. (2017) reported six QTL associated with virulence in a bi-parental *Ptm* population using QTL mapping. Carlsen et al. (2017) constructed a bi-parental mapping population using a North Dakota and an Australian isolate. One common barley genotype (Skiff) was used in both our study and the Carlsen et al. (2017) study. Even though Carlsen et al. (2017) identified three virulence QTL on Skiff, our GWAS results did not indicate any significant marker-trait associations with Skiff. Skiff had a poor distribution of infection responses in our study while in the Carlsen et al. (2017) study Skiff had a phenotypic response ranging from 1.5 to 4.0 (on a scale of 1 to 5). So, detection of no significant marker trait association in our study was not surprising. It was interesting to observe that QTL Ptmv2 was associated with virulence in two barley genotypes i.e. CII6150 and Kombar. Fowler et al. 2018, suggested that pathogen

acquires virulence specific for genetic factors deployed in cultivars. It is also possible that CII6150 and Kombar carry similar resistance/susceptibility genes, however further studies are required to confirm this.

No significant marker-trait association was found for the CI9214, Skiff and Keel genotypes. Keel similar to Skiff did not have a differential response across isolates, so no marker-trait association detection was expected. Although a differential infection response was observed for CI9214 no significant marker-trait association was detected. A possible reason could be that the variance component associated with this genotype in MLM was low. Significant marker trait association was found for CI9214 when using the generalised linear model analysis (data not presented). We included only MLM analysis in this study as it controls false positives by taking into account population structure and kinship and hence is more reliable.

Among the candidate genes identified for the 10 QTL regions, 10 proteins were predicted to be secreted proteins. Nine of these putative proteins were small proteins and were about 85-596 aa in length. In fungi, these small proteins are the common virulence factors which facilitate infection or evoke the host response (Stewart et al., 2018). We also identified two effector proteins which could play a potential role in virulence. Effectors are considered to suppress plant defence response and modify plant physiology for fungal penetration and proliferation in host cells by providing them with nutrients (Lo Presti et al., 2015). To the best of our knowledge, no effector genes have been cloned or characterised in *Ptm* and as a result the mechanism of virulence is unknown. Further study of both small and effector proteins is important. It will provide us with further insight into how these proteins facilitate infection.

In conclusion, this is the first GWAS study undertaken to identify virulence loci in *Ptm*. Our study confirmed that virulence mechanisms of the *Ptm*-barley interaction is complex with multiple unique QTL identified. The two effector proteins identified within the QTL regions are potentially involved in host-pathogen interaction and warrant further functional characterisation. The number of genomic regions associated with virulence suggests that breeding strategies should introgress multiple resistance genes into barley cultivars and should monitor virulence to ensure resistance sources are effective.

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Data availability statement

The data that support the findings of this study are provided as supplementary.

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Pop	Number of isolates in each population	No. of unique multi locus genotypes (MLG)	Corrected Simpson's complement index (λ)
SA	9	9	1
Vic	68	67	0.99
NSW	22	22	1
QLD	8	8	1
WA	14	14	1
Total	121	120	0.99

Table 1: Indices of genetic diversity of 121 *Pyrenophora teres* f. *maculata* isolates

Table 2: Genome-wide association mapping results using MLM, indicating markers significantly associated with virulence in *Pyrenophora teres* f. *maculata*

QTL ID	[a]Chr	Marker	Trait	[a] Position	[b]PVE	[c]LOD score
Ptmv1	1	36347903	CI5791	2447,051	10.47	2.96
Ptmv2	2	28947849	CII6150	28,77,570	18.11	3.41
	2		Kombar		9.65	2.92
Ptmv3	2	100166310	Arimont	16,77,293	14.94	3.29
Ptmv4	3	28949188	Torrens	2,58,346	10.02	2.96
Ptmv5	4	28947302	Kombar	21,64,661	14.44	3.96
Ptmv6	5	36349000	CI5286	14,45,017	11.80	3.19
Ptmv7	5	28948414	Galleon	14,03,794	10.35	3.05
Ptmv8	6	28946826	TR250	20,49,816	12.45	3.70
Ptmv9	6	36345783	Chebec	17,86,405	10.21	2.89
Ptmv10	7	28947210	Chebec	30,57,293	9.92	2.92

[a]Chromosome on which marker is located and position according to SG1 reference genome (Syme et al. 2018)

[b]Percentage of phenotypic variance explained

[c] Logarithm (base 10) of odds

458 Table 3: Candidate gene analysis of significant QTL identified by GWAS in 121 *Ptm* isolates

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460

QTL	[a]Chr	[a]Start Position (bp)	[a]End Position (bp)	[b]Probability
Ptmv1	1	2450903	2451160	1
Ptmv1	1	2458166	2458509	1
Ptmv2	2	2876222	2877744	1
Ptmv2	2	2884956*	2885507	1
Ptmv3	2	1677055	1679047	1
Ptmv7	5	1391886	1393263	0.98
Ptmv7	5	1394882*	1395312	1
Ptmv7	5	1401230	1401881	0.95
Ptmv9	6	1787045	1788885	0.74
Ptmv10	7	3064328	3065473	0.88

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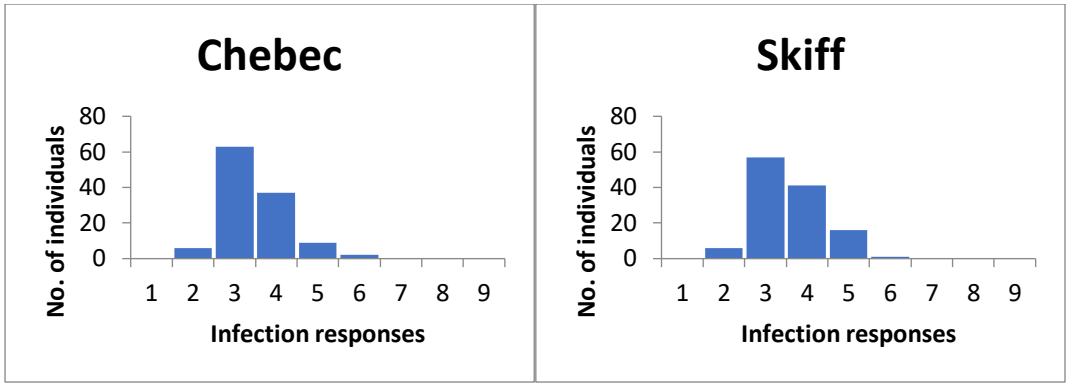

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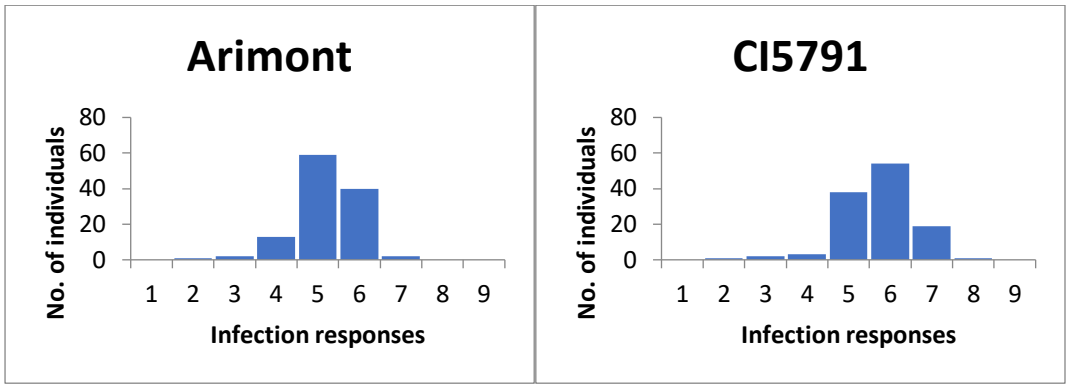

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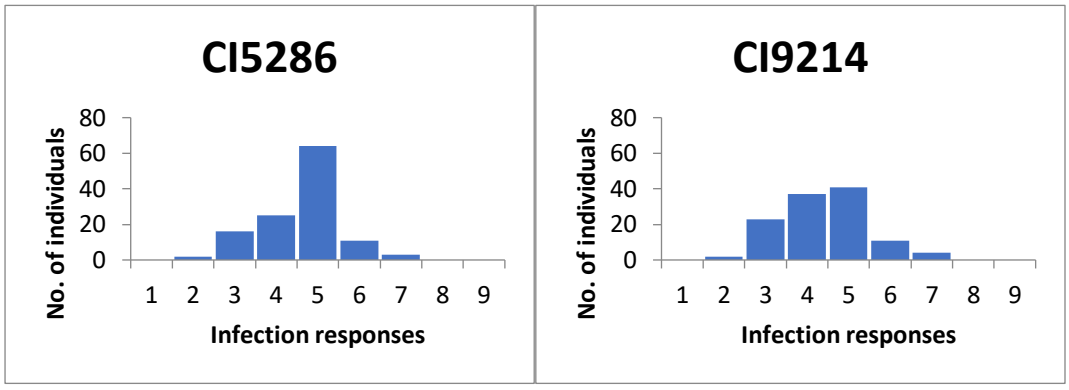

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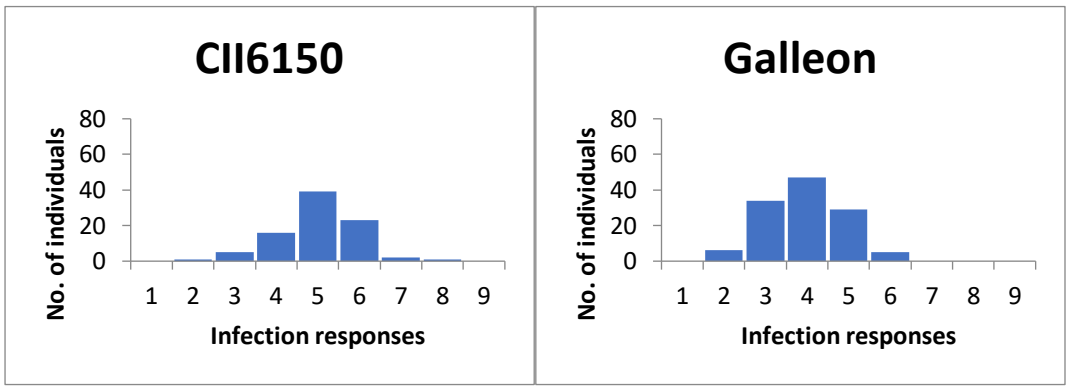

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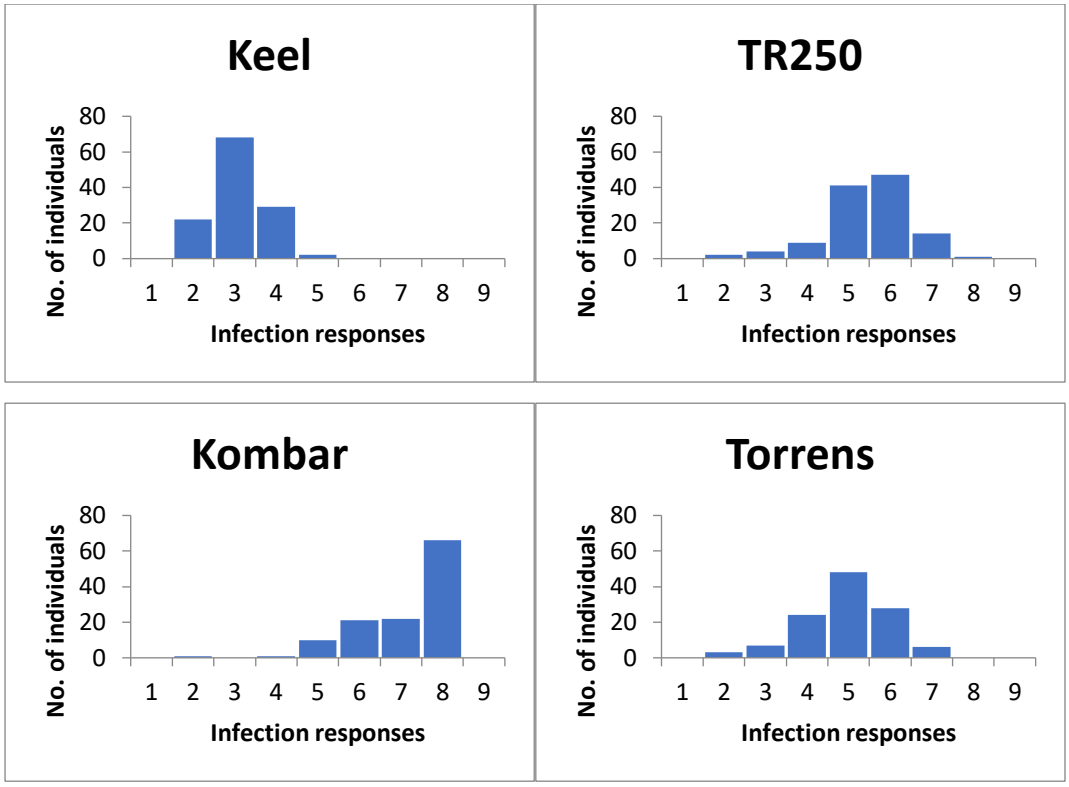


Figure 1: Distribution of infection responses of *Pyrenophora teres* f. *maculata* isolates across 12 different barley genotypes

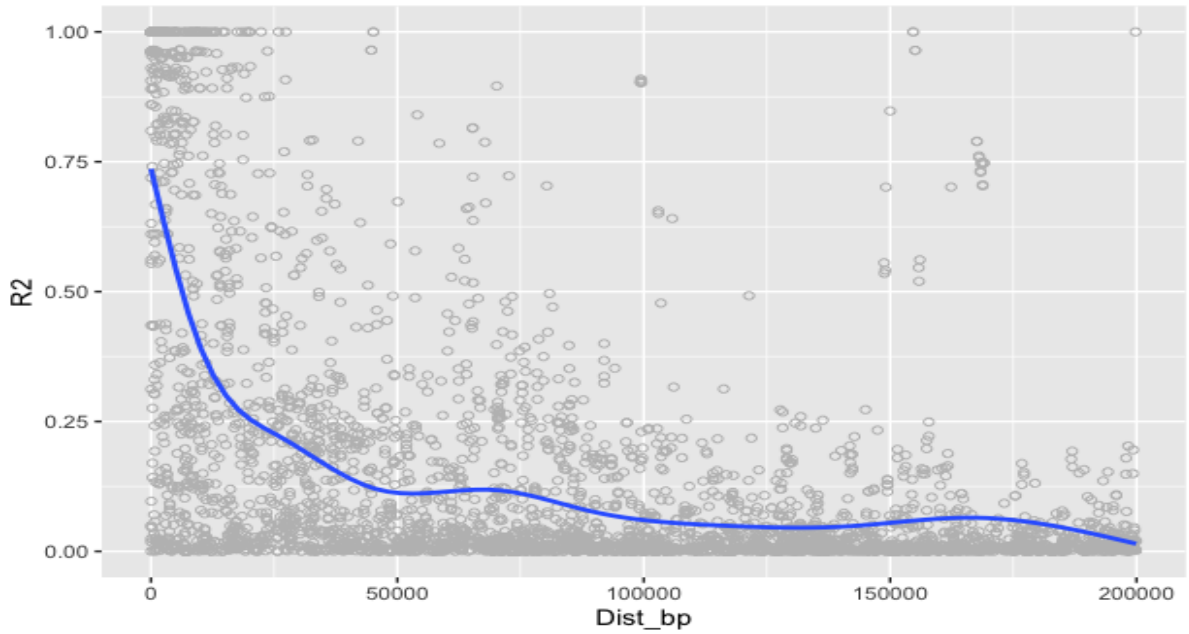
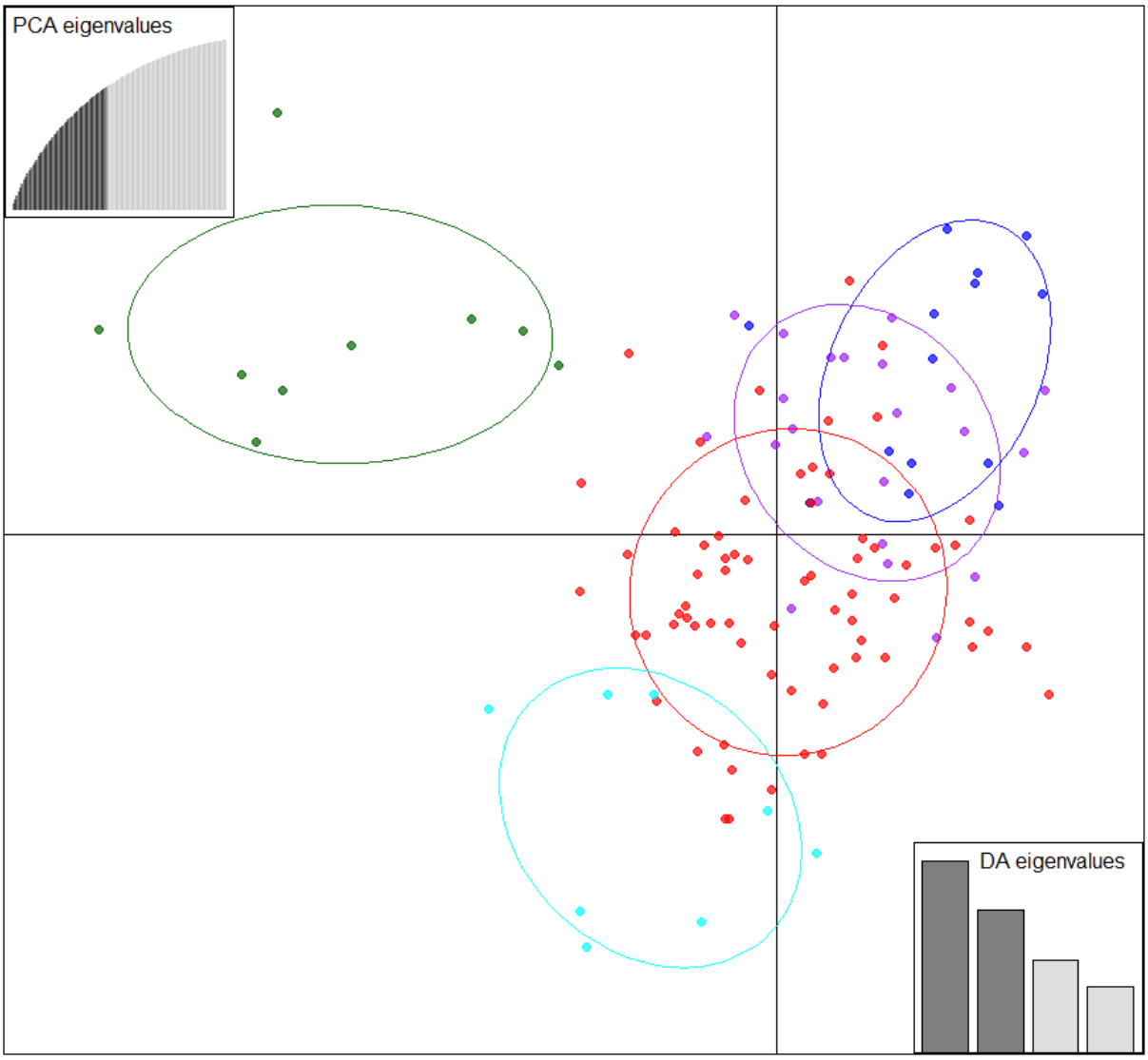


Fig. 2 Linkage disequilibrium (LD) decay based on 751 marker positions located on 12 chromosomes of *Ptm*



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477 Fig. 3 Genetic structure of 121 *Ptm* isolates as estimated by discriminant analysis of principal
478 components (DAPC). The colours and dots represents isolates from different states from where
479 they were collected i.e. green= South Australia, light blue= Queensland, red= Victoria, purple=
480 New South Wales, dark blue= Western Australia
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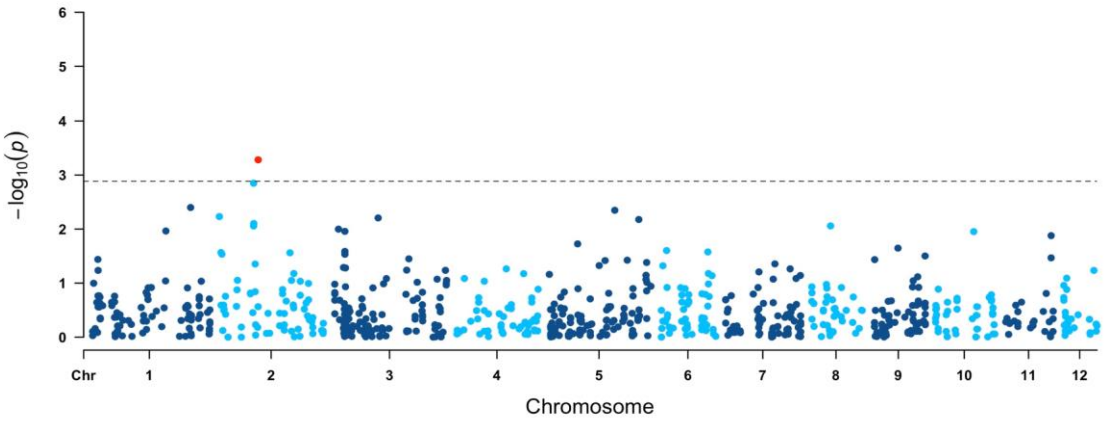
Arimont



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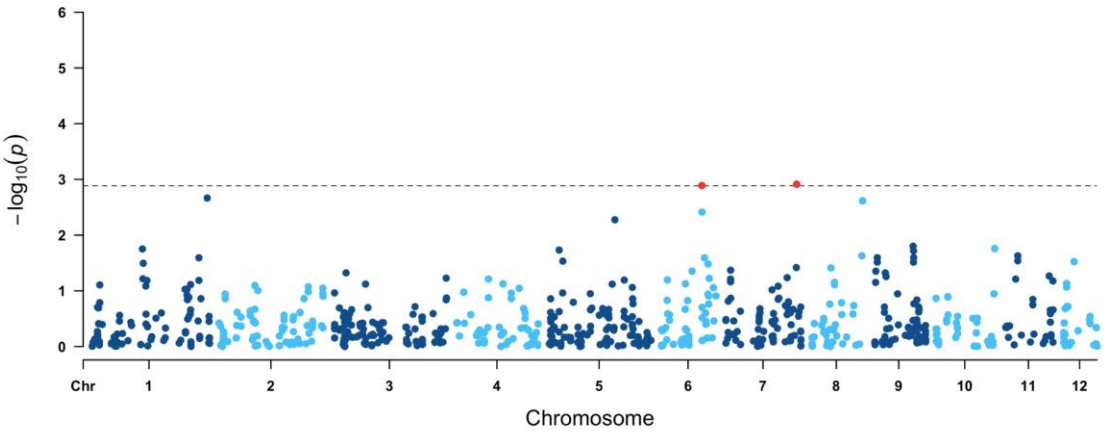

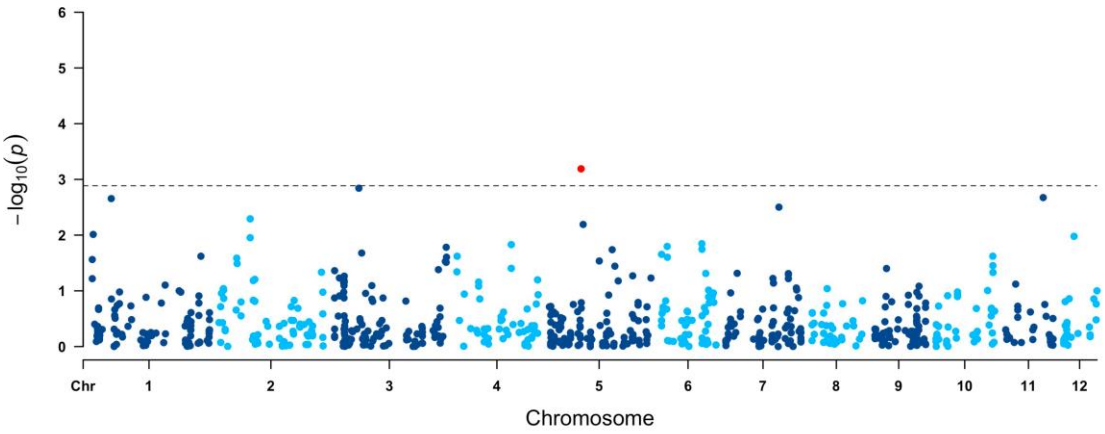

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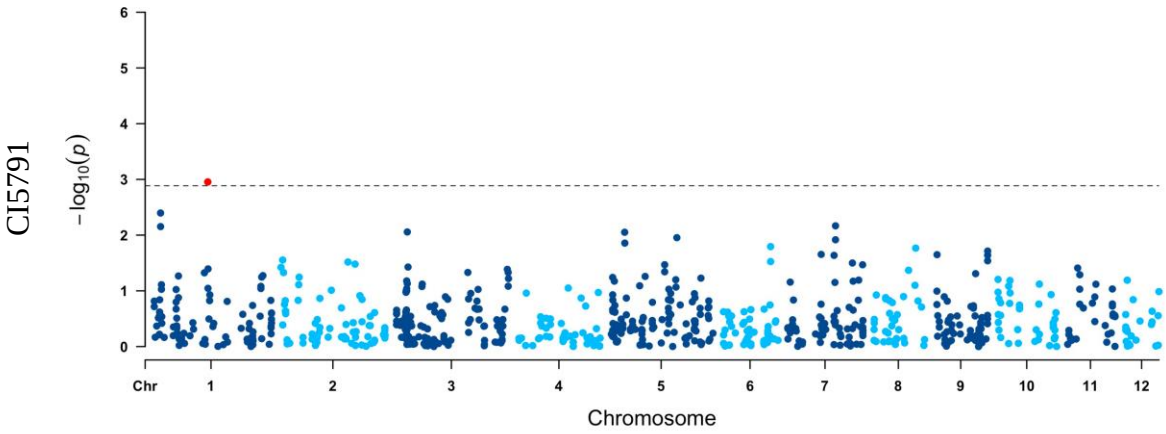

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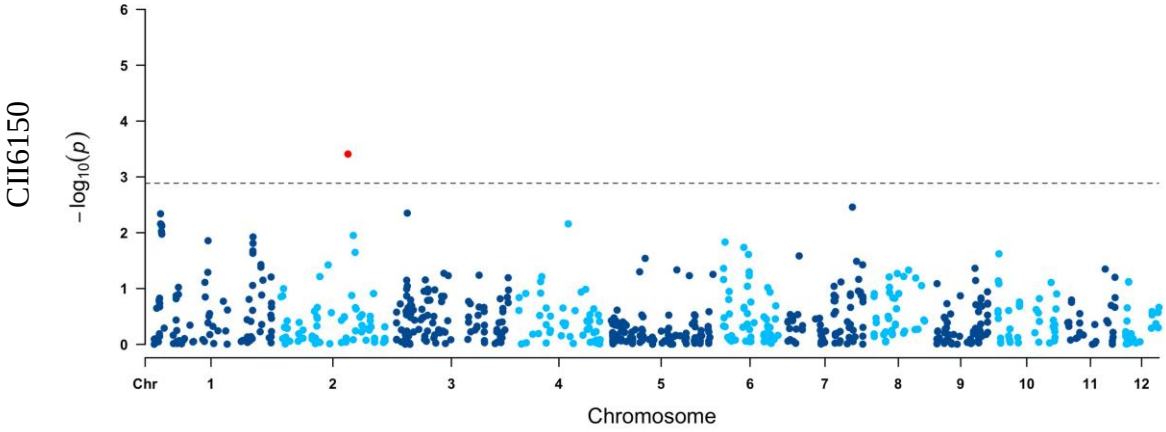

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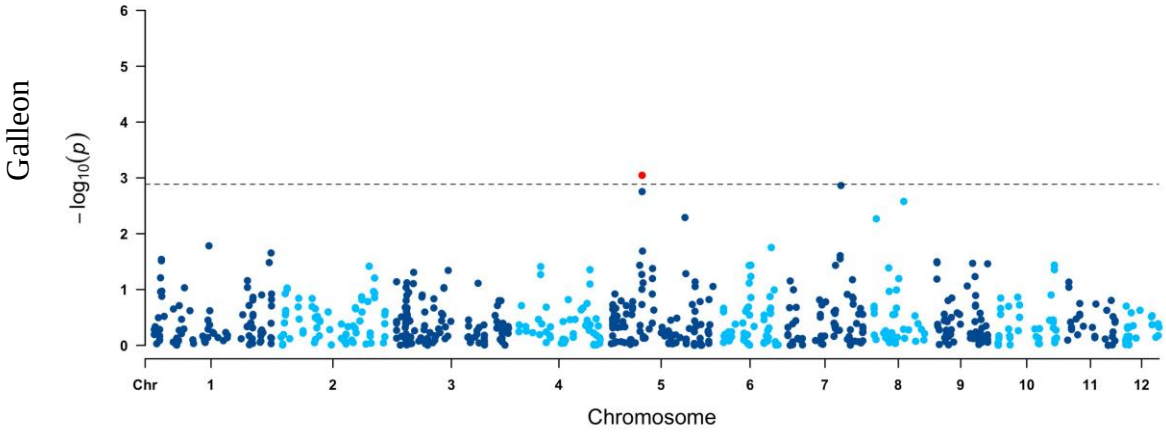

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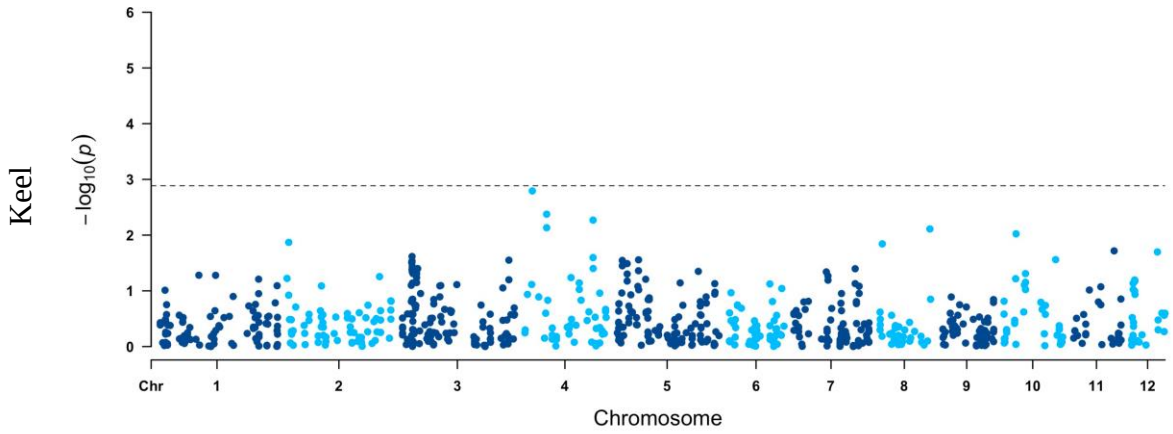

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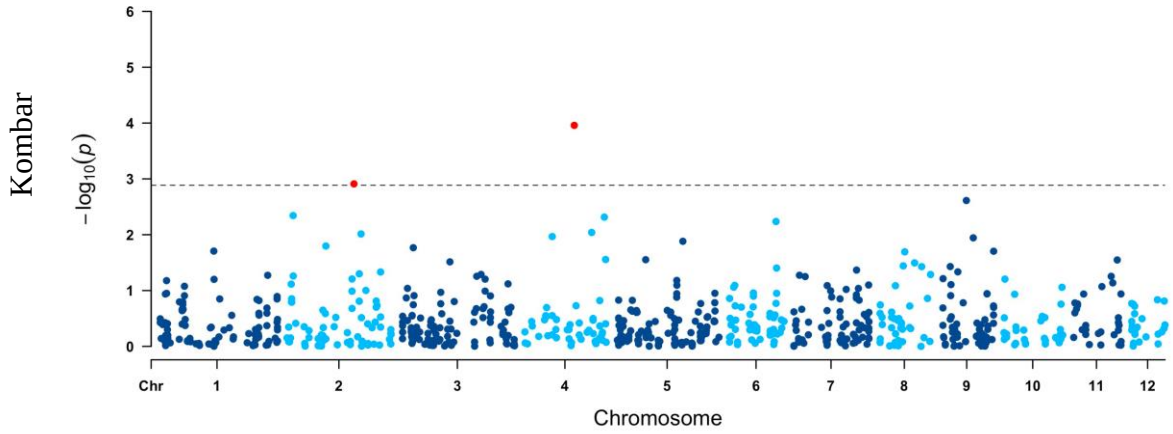

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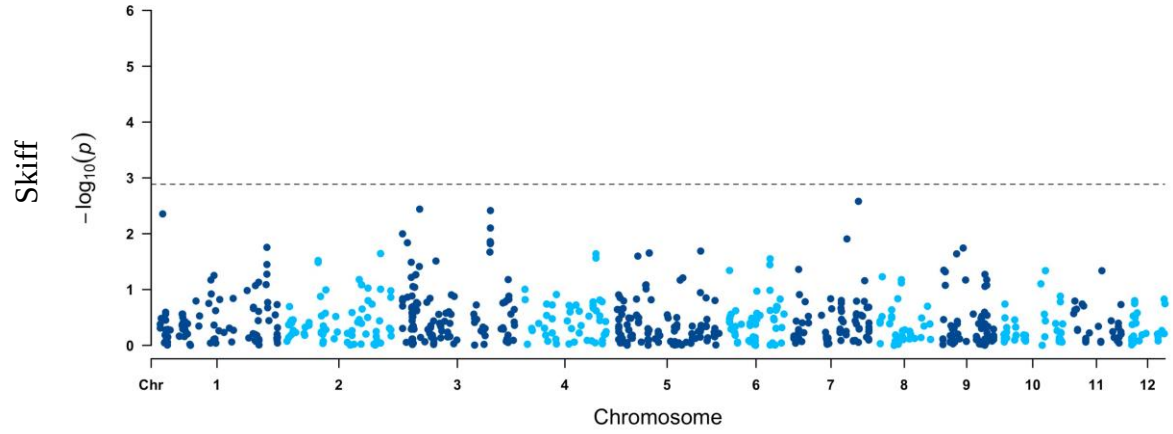

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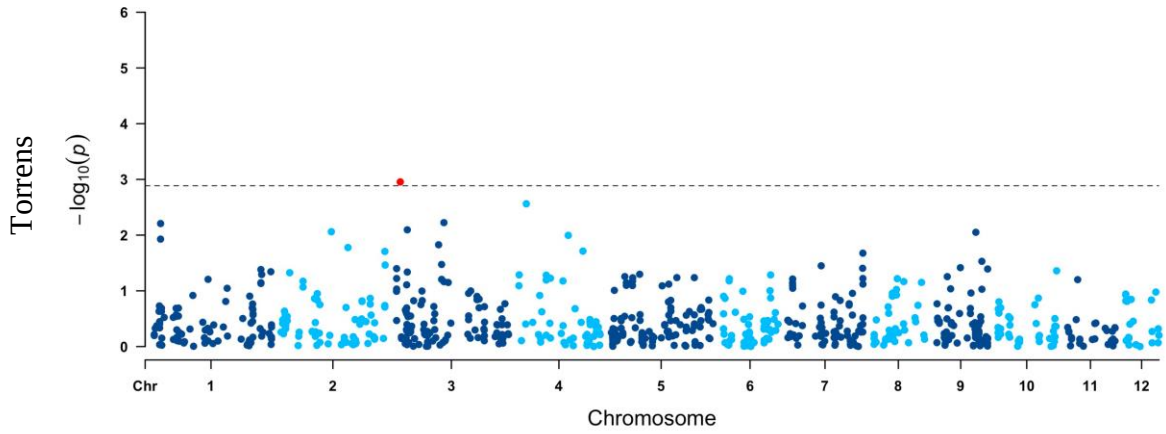

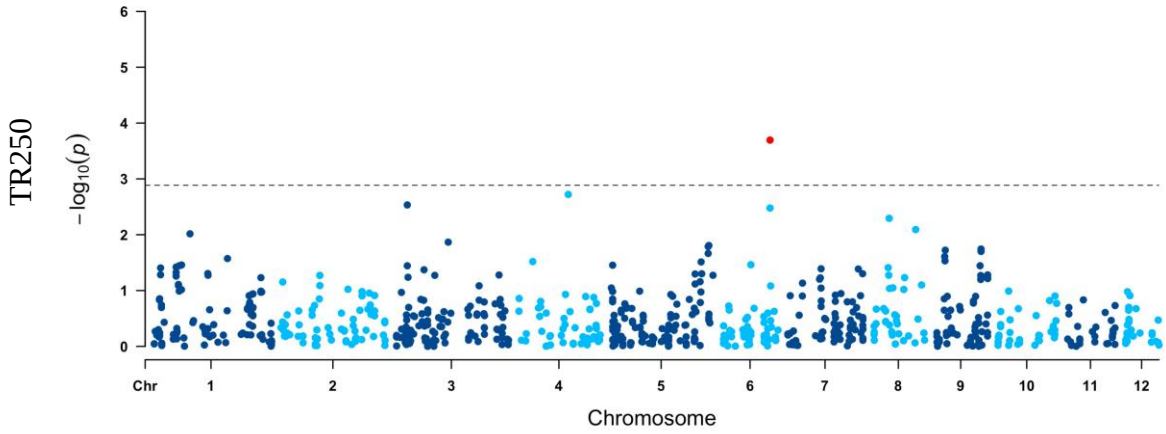

Figure 4: Manhattan plots of genome-wide analysis conducted to identify markers associated with virulence in *Pyrenophora teres* f. *maculate.*

CHAPTER 3

QTL MAPPING OF THE GENOMIC REGIONS CONFERRING VIRULENCE IN *PTM*

This study was conducted to understand the interaction between *Ptm* and barley. A bi-parental population was developed using two Australian *Ptm* isolates. The progeny was genotyped and phenotyped across four barley genotypes. One genetic map consisting of twelve chromosomes was constructed and QTL analysis was conducted. Candidate genes associated with virulence were also identified.

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Note: Supplementary data associated with this chapter are given in the appendix

QTL MAPPING OF THE GENOMIC REGIONS CONFERRING VIRULENCE IN *PTM*

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Abstract

Spot form net blotch (SFNB) caused by *Pyrenophora teres* f. *maculata* (*Ptm*) is a major foliar disease of barley worldwide. Knowledge of genomic regions associated with virulence and their genetic architecture is important to develop durable resistance to SFNB in barley varieties. This study conducted quantitative trait loci (QTL) mapping to identify genomic regions associated with virulence in an Australian bi-parental *Ptm* population. A total of 347 progeny were genotyped using DArTseq™ markers and phenotyped across four barley genotypes, Oxford, Skiff, TR250 and Gairdner. Two novel virulence QTL, *virPtm1* and *virPtm2*, were identified on chromosomes 2 and 3 respectively and explained 4% and 6% of the phenotypic variance. Further inspection of the QTL regions identified 381 candidate genes, out of which eight were predicted to be signal peptides. Five of these signal peptides were predicted to be small proteins, which may play a role in pathogen virulence. Five secondary metabolites i.e. non-ribosomal peptide synthetase clusters were also identified which may be associated with virulence response.

Keywords

Pyrenophora teres f. *maculata*, QTL analysis, virulence, barley, spot form net blotch

Introduction

Net blotches are major foliar diseases of barley, caused by the fungus *Pyrenophora teres* (*P. teres*). There are two forms of net blotch that are of importance. Net form net blotch (NFNB) is caused by *Pyrenophora teres* f. *teres* (*Ptt*) and spot form net blotch (SFNB) is caused by *Pyrenophora teres* f. *maculata* (*Ptm*). Spot form net blotch is a common foliar disease of barley in Australia and causes yield losses of 10-40% (Jayasena et al., 2007, McLean et al., 2009). Quality parameters such as kernel size, bulk density and plumpness are negatively affected by SFNB (Carlsen et al., 2017, Khan & Tekauz, 1982).

Spot form net blotch was first reported in the 1960s in Denmark (Smedegård-Petersen, 1971) and it has since been reported in most barley growing regions internationally, including

Canada, America, South Africa and Hungary (Ficsor et al., 2010; ZH Liu, Zhong, Stasko, Edwards, & Friesen, 2012; Louw, 1996; Tekauz, 1990). In Australia, SFNB was first recorded in 1977 in the Northern cereal belt of Western Australia (Khan, 1982) and has since spread throughout Australia.

Reproduction in *P. teres* can occur sexually or asexually. Sexual reproduction in *P. teres* is controlled by a single mating type locus (*MAT*), which has two alternate forms known as idiomorphs i.e. *MAT1-1* and *MAT1-2*. To undergo sexual reproduction, *P. teres* requires genotypes of opposite mating types. Pseudothecia appear as small fruiting bodies on barley straw during sexual reproduction. These are dark brown in colour and are covered with spiky setae. Eight club shaped asci are present in these pseudothecia (Liu et al., 2011). Under moist conditions, ascospores are ejected into the air and are dispersed by wind. This acts as primary inoculum in the growing season and causes initial crop infection (Jordan, 1981, Liu et al., 2011, McLean, 2011). To undergo asexual reproduction, *P. teres* produces conidia. These are the source of secondary infection and can also be a source of primary infection, being produced throughout the growing season (Lai et al., 2007).

New virulence profiles can be generated as a result of sexual reproduction. These newly generated virulences can multiply asexually and spread by wind dispersal Pathogens which have sexual and asexual reproduction as part of their lifecycle frequently overcome host genetic resistance. Several studies have described abundant genetic and pathogenic diversity in *Ptm* populations worldwide (Gupta et al., 2011, McLean, 2012, Tekauz, 1990, Akhavan et.al 2016). This makes it a challenge for barley breeders to deploy barley varieties with durable SFNB resistance.

Identifying genomic regions involved in the *Ptm*-barley interaction is vital for researchers and breeders when developing barley varieties with durable resistance to *Ptm*. Quantitative trait loci (QTL) mapping is a powerful and well-established statistical tool for genetic dissection of traits of interest. It is based on the principle that genes and markers segregate due to chromosome recombination during meiosis, thus allowing their analysis in the progeny (Collard et al., 2005). Genes and markers that are tightly linked to each other will be frequently transmitted together to the progeny as compared to genes and markers that are further apart (Collard et al., 2005).

Studies have reported major and minor resistance genes in barley genotypes against SFNB on all seven barley chromosomes (Ho et al., 1996, Steffenson et al., 1996, Williams et al., 1999, Williams et al., 2003, Molnar et al., 2000, Friesen et al., 2006, Grewal et al., 2008, Manninen et al., 2006, Cakir et al., 2011, Tamang et al., 2015, Tamang et al., 2019, Wang et al., 2015, Burlakoti et al., 2017). These studies illustrate that resistance to *Ptm* is complex and polygenic

in nature. To date only two studies (Carlsen et al., 2017,Sharma et al., 2021, chapter 2) have identified virulences related to QTL in *Ptm*. Carlsen et al. (2017) developed a bi-parental mapping population of 105 progeny using the North Dakota isolate FGOB10Ptm-1 and Australian isolate SG1. Four barley genotypes, Skiff, 81-82/033, TR326 and PI 392501, were used in phenotyping the progeny. Six QTL were identified on five different linkage groups. The study suggested that the *Ptm*-barley interaction is complex and does not follow a simple gene for gene model. Sharma et al.,2021, identified 10 virulence related QTL in 121 Australian *Ptm* isolates using genome wide association mapping.

Diversity Arrays Technology (DArT) is a genotyping method that uses array hybridisations technology to score hundreds of polymorphic markers across a whole genome in a single assay (Jaccoud et al., 2001). It requires no previous sequencing information. DArT markers have been developed for fungal species including *P. teres* (Syme et al., 2018, Poudel et al., 2019, Martin et al., 2020, Dahanayaka et al., 2021).

For this study, QTL mapping was undertaken to identify genomic regions conferring virulence in *Ptm* using a bi-parental mapping population derived from a cross between two Australian isolates. Knowledge of the virulence genes identified will provide important information to barley breeders while breeding for SFNB resistance.

Materials and methods

Pyrenophora teres f. *teres* isolates

A bi-parental population of 458 progeny was produced as described in Martin et al. (2020). Two Australian isolates SG1 and 16FRGO73 were used in the crossing. Isolate SG1 was used as a reference genome was developed using this isolate, while 16FRGO73 was chosen as it was virulent on barley genotype Oxford. The parent 16FRGO73 had the mating type *MAT1-1* and parent SG1 had *MAT1-2*. Briefly, 25mm^2 of mycelial plug from each isolate was placed on opposite side of wheat straw on Sach's media plate. Plates were incubated at 15˚C with 12hour light/dark period. Once pseudothecia started forming short cylindrical beaks or necks, 1% water agar was placed on the top of the Sach's media plate with water agar facing pseudothecia. Ascospores which were ejected by the pseudothecia onto agar plate were collected using a sealed glass needle. Each ascospore was transferred to a different potato dextrose agar (PDA) plate and was incubated at 22˚C for fungal mycelium growth.

Phenotypic evaluation of *Pyrenophora teres* f. *maculata* population

Four different barley genotypes (Oxford, Skiff, TR250 and Gairdner) were used to examine the virulence of progeny of the SG1/16FRGO73 cross. Barley genotype Oxford was susceptible to SG1, Skiff was resistant to both SG1 and 16FRGO73, TR250 was susceptible to

16FRGO73. Barley genotype Gairdner was used as the susceptible control. Phenotypic evaluation was performed using a completely randomised design as described in Martin et al. (2020). Briefly, four different genotypes were sown into one 15 cm diameter pot with groups of four seeds for each genotype evenly spaced around the circumference of the pot. Two replicates were grown for each isolate. Pots were placed in a controlled environment room under 12 hours day/night light at 22˚C Day/15˚C night and 75% humidity for 14 days prior to inoculation. A conidial suspension adjusted to 10,000 conidia/mL was sprayed at 3 mL/pot using a Preval sprayer (SEDL Agencies, Australia). Inoculated pots were placed in darkness at 95–100% relative humidity at room temperature for 48 hours. After 48 hours, the pots were transferred to a controlled environment room under 12 hours day/night light at 22˚C Day/15˚C night and 75% humidity for eight days to allow symptoms to develop. On the ninth day after inoculation, the second leaves of the barley seedlings were assessed for lesion infection response according to a 1 to 9 scale (Tekauz, 1985)

DArTseq™ analysis

Ascospores were grown on potato dextrose agar (PDA) for eight-ten days to obtain enough mycelial growth for DNA extraction. Mycelia were scraped from the culture plate and transferred into a 2 ml centrifuge tube. The DNA was extracted using a Wizard Genomic DNA Purification kit (Promega Corporation, Australia) following the protocol of the supplier. The quality of the extracted DNA was determined by gel electrophoresis using a 1% agarose gel. The DNA was normalised to 50 ng/µL and was sent to DArT Pty Ltd, Canberra, Australia for DArTseq™ analysis. The samples were processed following the method described in Martin et al (2020) briefly, the samples were processed in ligation/digestion reactions using a single PstI compatible adaptor with two different adaptors. The processed samples were amplified in 30 rounds of PCR. Amplified samples were applied to c-Bot (Illumina) bridge PCR followed by sequencing on the Illumina Hiseq2500 platform. Sequences generated were processed using DArT analytical pipelines.

Genetic map and QTL mapping

DArTseq ™ markers were partitioned into linkage groups using MapManager QTXb20 (Manly et al., 2001). RECORD (Van Os et al., 2005) was used to order the markers within the linkage groups. The Kosambi function was used to calculate map distances. Map figures were produced using MapChart version 2.1 (Voorrips, 2002). Windows QTL Cartographer version 2.5 (Wang et al., 2007) was used to conduct composite interval mapping. A permutation test run 1000 times at a significance level of 0.05 was used to determine the LOD threshold. Chromosomes were numbered according to the *Ptm* reference genome SG1 (Syme et al. 2018).

Pyrenophora teres f. *maculata* candidate gene analysis

QTL intervals that were significantly associated with virulence were aligned on the *Ptm* SG1 reference genome (GCA_900231935.2) (Syme et al., 2018) using the NCBI Basic Alignment Search Tool (BLAST v2.9.0). Bedtools intersect v2.29.0 (Quinlan & Hall, 2010) was used to identify regions in the annotated SG1 genome that overlapped with the QTL regions and Bedtools getfasta v2.29.0 was used to extract the sequences. The protein coding regions which were predicted to be secretory proteins by SignalP-5.0 (Armenteros et al., 2019) and have zero or one transmembrane domain as identified by TMHMM v.2.0 (Sonnhammer et al., 1998) were further employed to predict effectors using EffectorP 2.0 (Sperschneider et al.,2016). Secondary metabolite biosynthetic gene clusters were predicted using antiSMASH 5.0 (Blin et al., 2019).

Results

DArTseq™ genotyping

DArTseq™ analysis of the 372 *Ptm* isolates provided 2,858 SilicoDArT and 925 SNP markers. Markers with >20% missing data were removed from further analysis. This resulted in 833 SilicoDArT and SNP markers in total. Isolates with >20% missing data and clonal isolates were also removed. This resulted in total of 347 progeny that were used in genetic mapping analysis.

Phenotypic assessment of *Ptm* progeny

Disease ratings did not differ by more than two units across replications. The infection scores of 347 progeny ranged from 1-8.0 for Oxford, 1-6.5 for Skiff, 1-7.0 for TR250 and 1-7.5 for Gairdner (Figure 1). The average infection score was 3.7 for Oxford, 3.5 for Skiff, 3.1 for TR250 and 4.3 for Gairdner. Eight progeny were virulent on all barley varieties Oxford, Skiff, TR250 and Gairdner. Transgressive segregation was observed in the infection response ratings of progeny on all four barley genotypes.

Genetic map

A total of 833 high quality polymorphic SilicoDArT and SNP markers were used to construct the genetic map. The resultant genetic map of the population consisted of 12 linkage groups ranging in size from 120.5 to 289.8 cM (Figure 2) with a total map distance of 2,672 cM. The average distance between the markers was 1 cM. Based on the size of the *Ptm* SG1 genome estimated at 40 Mb, the physical to genetic distance ratio was 47.23 kb/cM.

A LOD value threshold of 3.1 for Oxford and Skiff, 3 for TR250, 2.9 for Gairdner was used to identify significant QTL after performing 1000 permutations on each of the data sets. Two

significant QTL *virPtm1* and *virPtm2* were identified (Table 1 and Figure 3). The QTL *virPtm1* was located on chromosome 2, had a LOD score of 3.2, explained a 4% of the phenotypic variance and provided virulence against TR250. The QTL *virPtm2* was located on chromosomes 3 and had LOD scores of 3.6, explained 6% of the phenotypic variance and provided virulence against Gairdner. QTL *virPtm1* was contributed by SG1 and *virPtm2* was contributed by parent 16FRGO73.

Analysis of QTL regions to identify candidate effector genes

The gene content within the two QTL regions between flanking markers was examined against the SG1 reference genome and a total of 381 genes were identified (Supplementary Table 1). Out of these, eight genes were predicted to be signal peptides (Table 2). None of the signal peptides were predicted to be effector proteins. Five potential biosynthetic gene clusters were identified using antiSMASH and all five were described to be non-ribosomal peptide synthetase cluster (NRPS) (Table 3).

Discussion

This study used a DArTseq marker based genetic map to identify two QTL associated with virulence in the bi-parental *Ptm* population SG1/16FRG073.

Genetic map constructed of *Ptm* population consisted of 12 chromosomes, this is in agreement with Syme et al., (2018). The size of the largest chromosome in our study was 289 cM. This was similar in size to the largest chromosome (221 cM) found in the Carlsen et al. (2017) study. This size is also similar to 290.85 cM and 227.39 cM of the largest chromosome in a recent QTL mapping study in *Ptt* (Martin et al., 2020).

Transgressive segregation in infection response was observed during phenotyping of the individual ascospores on barley genotypes as compared to parental isolates. Carlsen et al. (2017) also found that a total of 41 progeny showed transgressive segregation. Transgressive segregation has also been reported in *Ptt* bi-parental populations (Martin et al., 2020, Koladia et al., 2017, Weiland et al., 1999). This indicates the presence of multiple, different combinations of virulence genes in the parent. This also suggests that the progeny contained different gene combinations to those of the parents.

The QTL *virPtm2* associated with virulence on Gairdner and contributed by parent SG1was located on chromosome 3. The QTL *virPtm1* associated with virulence on TR250 was contributed by parent 16FRGO73 and located on chromosome 2. A QTL associated with virulence on TR250 was found in Sharma et al., 2021, chapter 2 on chromosome 6 of the reference genome of SG1 (Syme et. al 2018). Different isolates were used in the two studies and suggests that QTL governing virulence on the same barley genotype may not be conserved between different isolates. Both QTL identified in the current study explained only 4% and 6%

of the phenotypic variance and had low LOD scores. Some QTL could have been missed due to the low number of polymorphic markers present in the population. Of the 5473 markers obtained from DArTseq only 833 markers i.e. 15% were polymorphic. Both the QTL identified in the current study are different to the QTL identified by Carlsen et al. (2017). Carlsen et al. (2017) identified six QTL on five different linkage groups using a bi-parental population between isolates SG1 (Australia) and FGOB10Ptm-1 (North Dakota). Out of the six QTL identified, only one QTL was contributed by parent SG1 and five QTL were contributed by the parent FGOB10Ptm-1. Two major QTL contributed by parent FGOB10Ptm-1 were identified with the genotype Skiff and one minor QTL was identified with Skiff contributed by parent SG1. Although no QTL were observed for barley genotypes Skiff and Oxford in the current study, the phenotypic data was normally distributed. It is likely that undetected loci are present for this population which are contributing to the normal distribution.

Among the candidate genes identified for the two QTL regions, eight proteins were predicted to be secreted proteins. Five of these putative proteins were small proteins and were about 69-279 aa in length. In fungi, these small proteins are the common virulence factors which facilitate infection or evoke host response (Stewart et al., 2018). We also identified five NRPS clusters which could play a potential role in virulence. NRPS are a type of secondary metabolite secreted by fungi. Secondary metabolites are not important for growth, development, and reproduction like primary metabolites, but can contribute to plant virulence (Tünde et al., 2015). Further studies of both small and NRPS proteins is required to provide us with further insight into how these proteins facilitate infection.

During sexual recombination two compatible cells fuse followed by nuclei fusion forming a diploid nucleus. This diploid nucleus undergoes two meiotic divisions followed by two miotic divisions producing four pairs of haploid ascospores (Fincham & Stanley, 1971). Shjerve et al. (2014) proposed to collect single ascospores from a cluster/group of ascospores, to avoid clonal ascospores present within asci. On the contrary, Martin et al. (2020) suggested to collect as many ascospores as possible to obtain a higher number of useful individuals for a population. In this study we also collected as many ascospores as possible as suggested by Martin et al. (2020). DArTseq™ markers were used to identify clonal isolates. We collected 372 ascospores, out of which 25 (7%) were clones. Martin et al. (2020) also found 19% and 27% of the isolates in two different populations to be clonal. These results suggests that the collection of all ascospore leads to a higher number of isolates that can be used in the genetic mapping analysis.

In conclusion, this study identified two novel QTL associated with virulence in *Ptm*. Further studies of five identified small proteins and non-ribosomal peptide synthetase clusters could provide in depth knowledge of *Ptm*-barley interaction.

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Tables and Figures

Table 1: Summary of QTL identified in *Pyrenophora teres* f. *maculata* population SG1/16FRGO73

QTL name	Chr[a] no	Position of peak QTL (cM)	Genotype[b]	LOD score	R^{2c}	Parent contributing to QTL	Position on SG1 reference genome	[e]Position of flanking markers (bp)
virPtm1	2	75	TR250	3.2	4	SG1	1725249	1449769-2033747
virPtm2	3	54	Gairdner	3.6	6	16FRGO73	1378610	730470 - 1518361

[a]Chromosome number

[b]Genotype on which QTL was identified

[c]Percentage of phenotypic variance explained

LOD = Logarithm of odds

[d]Position of the QTL on SG1 reference genome (Syme et al.2018)

[e]Position of flanking markers on SG1 reference genome (Syme et al. 2018)

370

371 Table 2: Candidate gene analysis of significant QTL identified by QTL mapping

QTL	Chr[a]	Start[a] position (bp)	End[a] position (bp)	Probability[b]
virPtm1	2	1734250	1735007	0.594832
virPtm1	2	1758039	1758245	0.988414
virPtm1	2	1794580	1799902	0.589151
virPtm1	2	1924627	1929850	0.999308
virPtm2	3	1208069	1210621	0.794902
virPtm2	3	1313104	1313932	0.934085
virPtm2	3	1330524	1331315	0.978409
virPtm2	3	1511762	1512598	0.623665

372 [a]Chr, chromosome on which signal peptide is located and position according to SG1 reference
373 genome (Syme et al. 2018)
374 [b]Likelihood of being a signal peptide
375
376

Table 3: Secondary metabolities analysis identified five Non-ribosomal peptide synthetase cluster (NRPS)

Chr[a]	Start[a] position (bp)	End[a] position (bp)
3	730471	748888
3	963329	987170
3	1140937	1158447
3	1273830	1279457
3	1405192	1424210

[a]Chromosome on which NRPS is located on and position according to SG1 reference genome (Syme et al. 2018)

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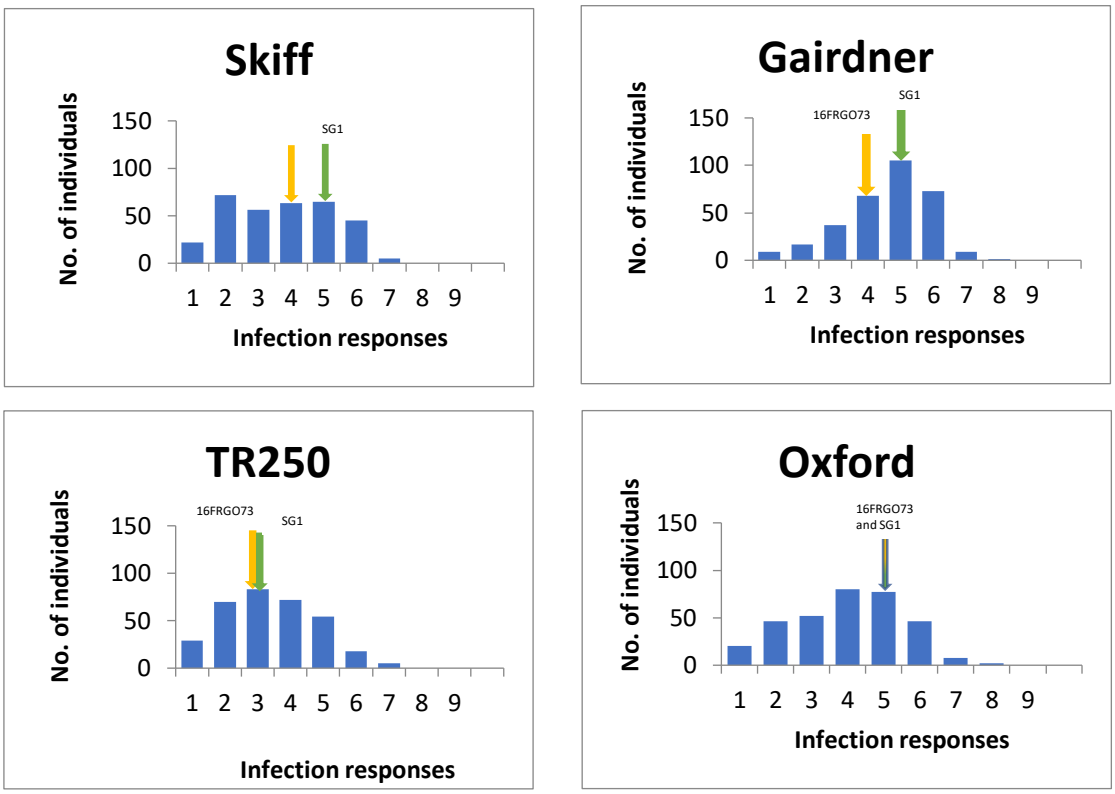
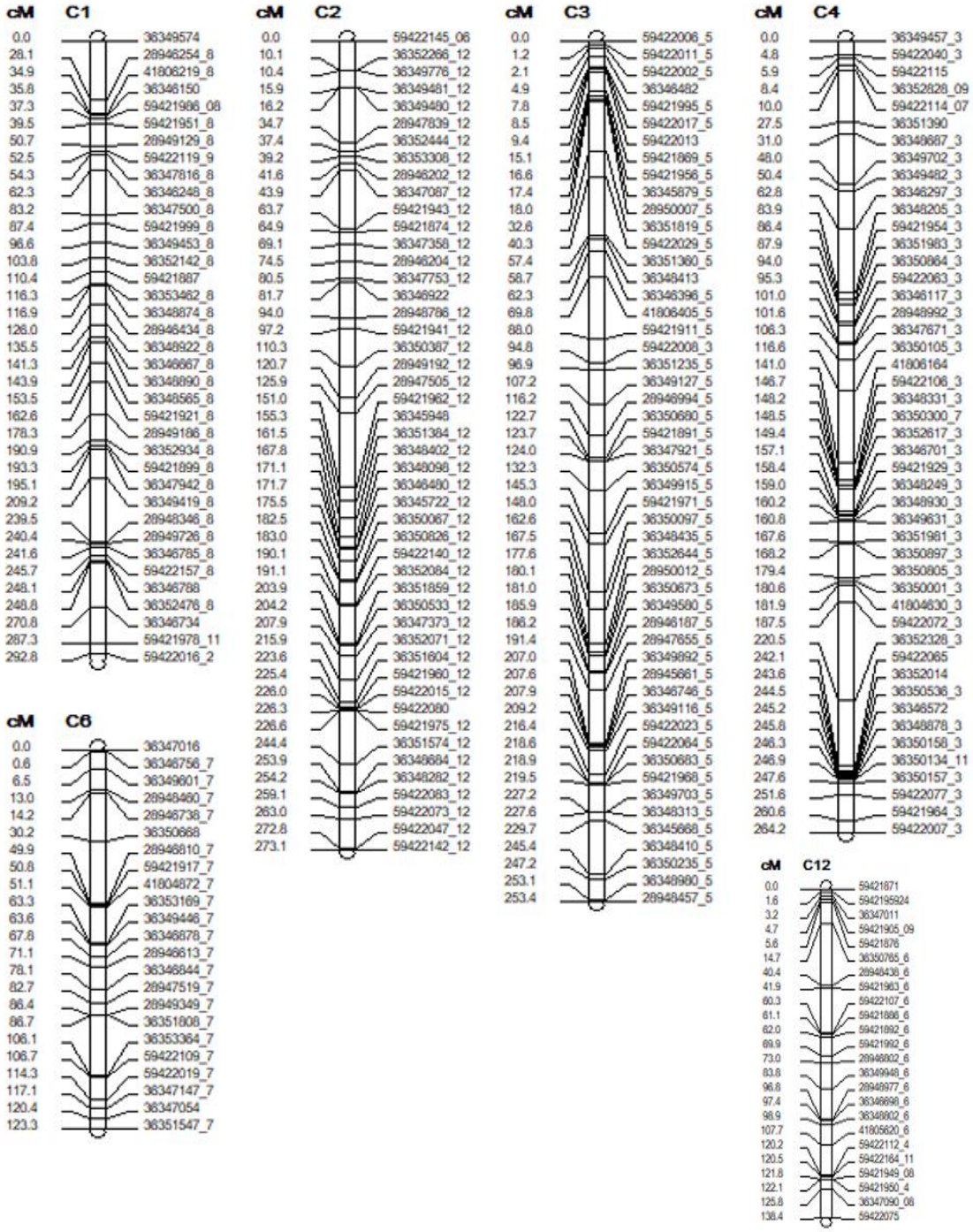


384 Figure 1: Infection response of progeny of *Ptm* population SG1/16FRGO73 on barley
385 genotypes Skiff, Gairdner, TR250 and Oxford. Parental isolate infection responses are
386 indicated.

387

388


cM C1
0.0 36349574
28.1 28946254_8
34.9 41806219_8
35.8 36346150
37.3 59421986_08
39.5 59421951_8
50.7 28949129_8
52.5 59422119_9
54.3 36347816_8
62.3 36346248_8
83.2 36347500_8
87.4 59421999_8
96.6 36349453_8
103.8 36352142_8
110.4 59421887
116.3 36353462_8
116.9 36348874_8
126.0 28946434_8
135.5 36348922_8
141.3 36346667_8
143.9 36348890_8
153.5 36348565_8
162.6 59421921_8
178.3 28949186_8
190.9 36352934_8
193.3 59421899_8
195.1 36347942_8
209.2 36349419_8
239.5 28948346_8
240.4 28949726_8
241.6 36346785_8
245.7 59422157_8
248.1 36346788
248.8 36352476_8
270.8 36346734
287.3 59421978_11
292.8 59422016_2
cM C2
0.0 59422145_06
10.1 36352266_12
10.4 36349776_12
15.9 36349481_12
16.2 36349480_12
34.7 28947839_12
37.4 36352444_12
39.2 36353308_12
41.6 28946202_12
43.9 36347087_12
63.7 59421943_12
64.9 59421874_12
69.1 36347358_12
74.5 28946204_12
80.5 36347753_12
81.7 36346922
94.0 28948786_12
97.2 59421941_12
110.3 36350387_12
120.7 28949192_12
125.9 28947505_12
151.0 59421962_12
155.3 36345948
161.5 36351384_12
167.8 36348402_12
171.1 36348096_12
171.7 36346480_12
175.5 36345722_12
182.5 36350067_12
183.0 36350826_12
190.1 59422140_12
191.1 36352084_12
203.9 36351859_12
204.2 36350533_12
207.9 36347373_12
215.9 36352071_12
223.6 36351604_12
225.4 59421960_12
226.0 59422015_12
226.3 59422090
226.6 59421975_12
244.4 36351574_12
253.9 36348684_12
254.2 36348282_12
259.1 59422083_12
263.0 59422073_12
272.8 59422047_12
273.1 59422142_12
cM C3
0.0 59422006_5
1.2 59422011_5
2.1 59422002_5
4.9 36346482
7.8 59421995_5
8.5 59422017_5
9.4 59422013
15.1 59421869_5
16.6 59421956_5
17.4 36345879_5
18.0 28950007_5
32.6 36351819_5
40.3 59422029_5
57.4 36351360_5
58.7 36348413
62.3 36346396_5
69.8 41806405_5
88.0 59421911_5
94.8 59422008_3
96.9 36351235_5
107.2 36349127_5
116.2 28946994_5
122.7 36350680_5
123.7 59421891_5
124.0 36347921_5
132.3 36350574_5
145.3 36349915_5
148.0 59421971_5
162.6 36350097_5
167.5 36348435_5
177.6 36352644_5
180.1 28950012_5
181.0 36350673_5
185.9 36349580_5
186.2 28946187_5
191.4 28947655_5
207.0 36349892_5
207.6 28945661_5
207.9 36346746_5
209.2 36349116_5
216.4 59422023_5
218.6 59422064_5
218.9 36350683_5
219.5 59421968_5
227.2 36349703_5
227.6 36348313_5
229.7 36345668_5
245.4 36348410_5
247.2 36350235_5
253.1 36348980_5
253.4 28948457_5
cM C4
0.0 36349457_3
4.8 59422040_3
5.9 59422115
8.4 36352828_09
10.0 59422114_07
27.5 36351390
31.0 36348687_3
48.0 36349702_3
50.4 36349482_3
62.8 36346297_3
83.9 36348205_3
86.4 59421954_3
87.9 36351983_3
94.0 36350864_3
95.3 59422063_3
101.0 36346117_3
101.6 28948992_3
106.3 36347671_3
116.6 36350105_3
141.0 41806164
146.7 59422106_3
148.2 36348331_3
148.5 36350300_7
149.4 36352617_3
157.1 36346701_3
158.4 59421929_3
159.0 36348249_3
160.2 36348930_3
160.8 36349631_3
167.6 36351981_3
168.2 36350897_3
179.4 36350805_3
180.6 36350001_3
181.9 41804630_3
187.5 59422072_3
220.5 36352328_3
242.1 59422065
243.6 36352014
244.5 36350536_3
245.2 36346572
245.8 36348878_3
246.3 36350158_3
246.9 36350134_11
247.6 36350157_3
251.6 59422077_3
260.6 59421964_3
264.2 59422007_3
cM C6
0.0 36347016
0.6 36346756_7
6.5 36349801_7
13.0 28948460_7
14.2 28946738_7
30.2 36350868
49.9 28946810_7
50.8 59421917_7
51.1 41804872_7
63.3 36353169_7
63.6 36349446_7
67.8 36346878_7
71.1 28946613_7
78.1 36346844_7
82.7 28947519_7
86.4 28949349_7
86.7 36351808_7
106.1 36353364_7
106.7 59422109_7
114.3 59422019_7
117.1 36347147_7
120.4 36347054
123.3 36351547_7
cM C12
0.0 59421871
1.6 5942195924
3.2 36347011
4.7 59421905_09
5.6 59421876
14.7 36350785_6
40.4 28948436_6
41.9 59421963_6
60.3 59422107_6
61.1 59421886_6
62.0 59421892_6
69.9 59421992_6
73.0 28946802_6
83.8 36349946_6
96.8 28948977_6
97.4 36346898_6
98.9 36348802_6
107.7 41805620_6
120.2 59422112_4
120.5 59422164_11
121.8 59421949_08
122.1 59421950_4
125.8 36347090_08
138.4 59422075

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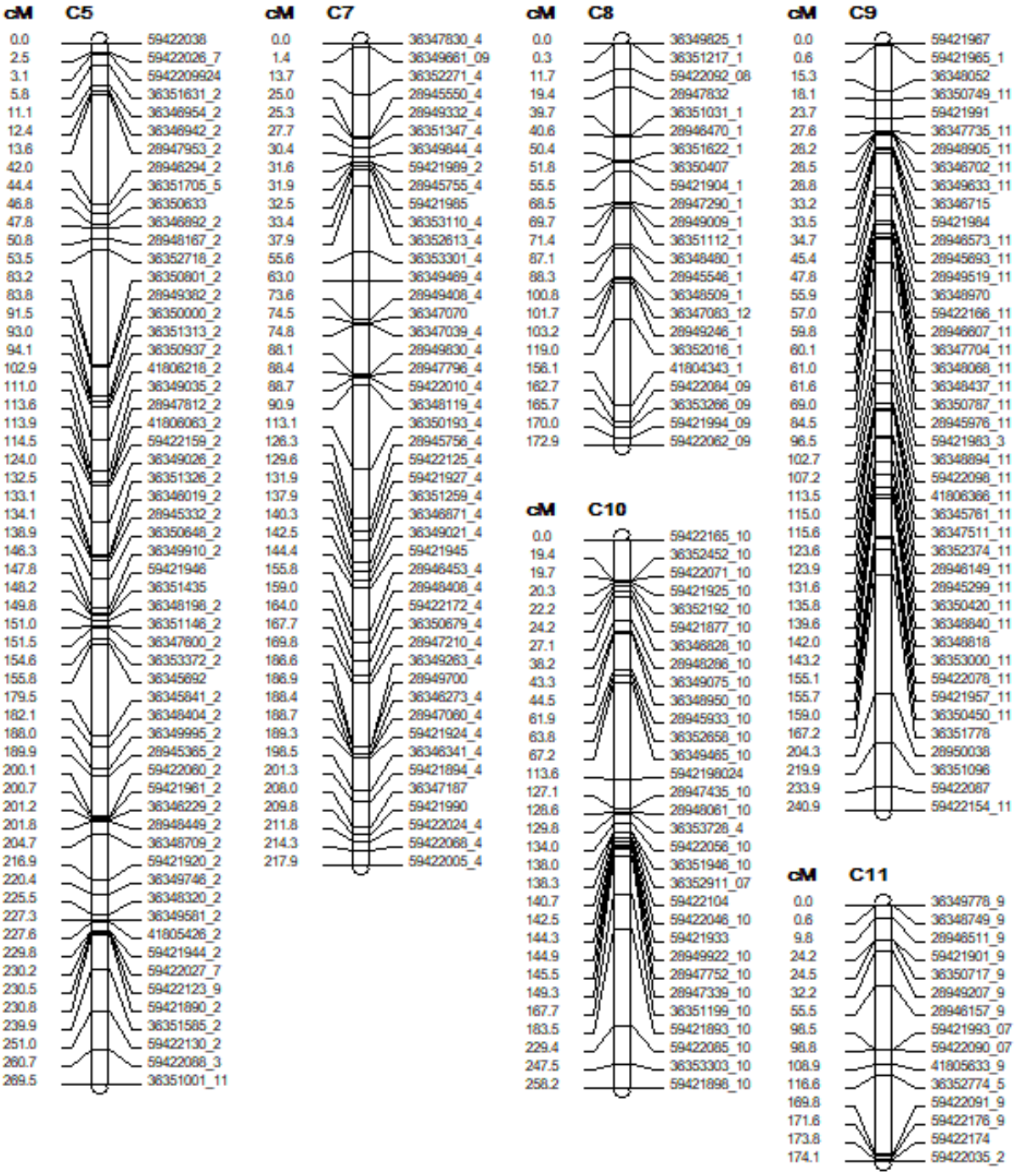

Figure 2: Genetic map of *Ptm* SG1/16FRGO73 population. Markers are presented on the right and distance (cM) in the left.

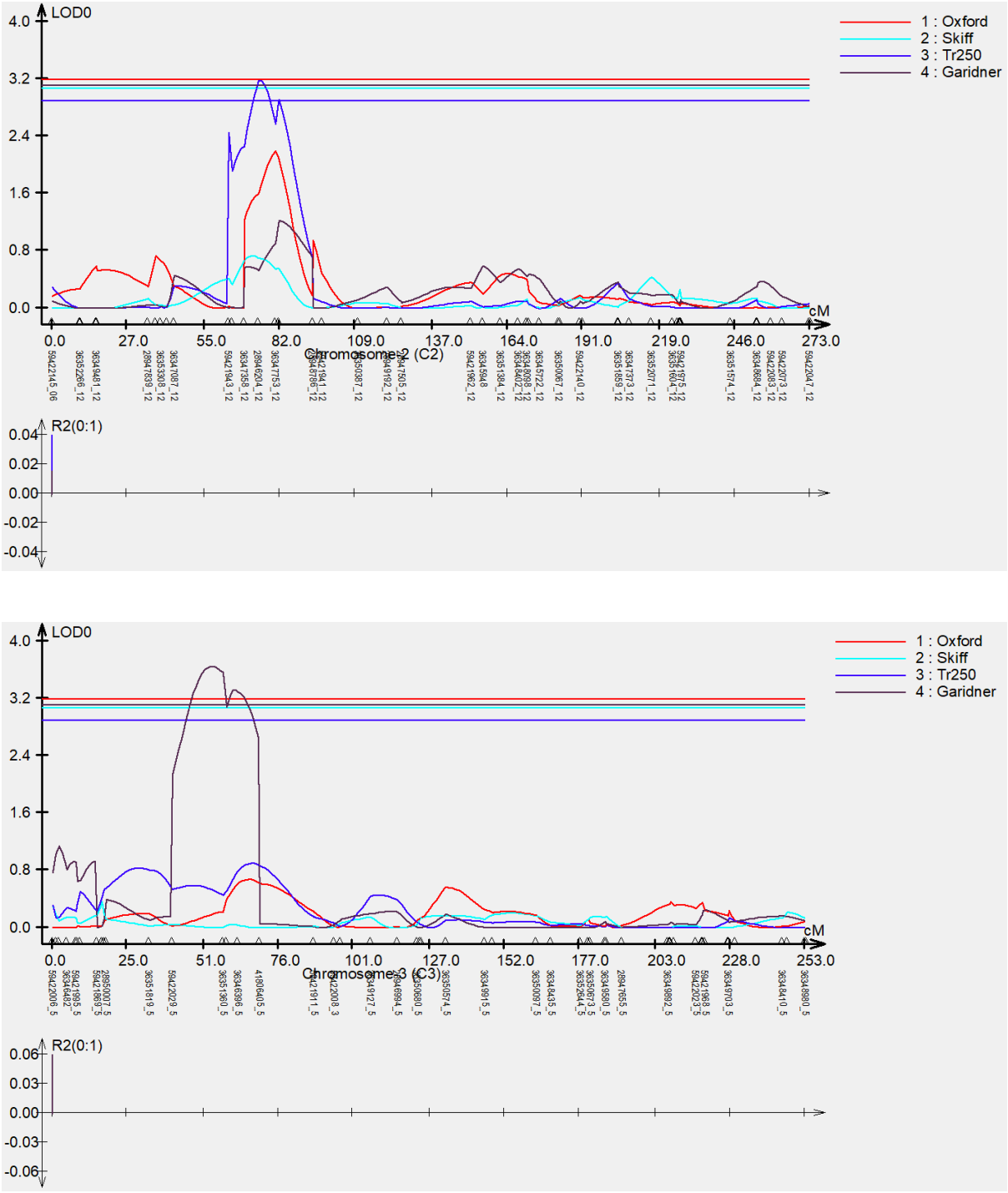

Figure 3: Composite interval mapping analysis of *Ptm* population SG1/16FRGO73 on four different barley genotypes (Oxford, Skiff, TR250, Gairdner). Markers with distances (cM) are presented on the X-axis. LOD (Logarithm of odds) values are given on theY-axis.

CHAPTER 4

NEW VIRULENCES IDENTIFIED IN PROGENY OF *P. TERES F. TERES* X *P. TERES F. MACULATA* POPULATIONS

This study was conducted to understand the virulence of hybrids. For this purpose, four in-vitro crosses between Ptt and Ptm were made. The progeny were phenotyped across 20 barley genotypes. Infection response of the progeny was observed.

Sharma R, McLean M, Tao Y and Martin A. New virulences identified in progeny of Ptt x Ptm hybrids.

Note: Supplementary data associated with this chapter are given in the appendix

NEW VIRULENCES IDENTIFIED IN PROGENY OF *P. TERES F. TERES* X *P. TERES F. MACULATA* POPULATIONS

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Abstract

The inheritance of virulence in *P. teres f. teres (Ptt) x P. teres f. maculata (Ptm)* hybrid isolates was studied using four *in vitro Ptt/Ptm* hybrid populations. Each population was phenotyped across 20 barley genotypes. Virulence profiles of the hybrid progeny varied significantly depending on the parents selected. The majority of progeny were virulent with some crosses producing avirulent progeny. Sixty-two progeny were virulent towards barley genotypes where parental isolates were avirulent. While twenty-seven progeny had greater average infection responses compared to the parental isolates. These findings demonstrated that novel virulences were generated from *Ptt* and *Ptm* hybridisation and could provide a threat to host plant resistance in the field.

Keywords: Hybrids, virulence, phenotyping, *Ptt/Ptm* cross, *P. teres*

Introduction

Net blotches are major foliar diseases of barley and consist of two forms; net form net blotch (NFNB) caused by *Pyrenophora teres* f. *teres* (*Ptt*) and the spot form net blotch (SFNB) caused by *Pyrenophora teres* f. *maculata* (*Ptm*). Symptoms of net form net blotch consist of long narrow lesions that may follow veins and with fine dark brown lines extending across the leaf surface to connect the longer lesions producing net like lesions. Conversely, symptoms of spot form net blotch consist of dark brown necrotic spots that increase in size to form elliptical or fusiform lesions (McLean, 2011). The two forms look morphologically similar but, phylogenetic studies have demonstrated that the forms are genetically distinct (Liu et al., 2011, Rau et al., 2003, Rau et al., 2007). Although both forms of *P. teres* are often present in the same field, one form is usually dominant depending on the cultivated barley genotype and climatic conditions (Clare et al., 2020).

The life cycles of *Ptt* and *Ptm* are very similar, with both being primarily stubble-borne and the only difference being that *Ptt* is able to carry over on infected seed (Jorgensen, 1980). *Ptm* has not been reported to be seedborne. The life cycle of *P. teres* involves both sexual and asexual stages. Sexual reproduction causes an increase in genetic variability by creating new

allelic combinations through meiosis and fertilization (Jonsson et al., 2000). The resultant progeny can have new combinations of virulence alleles can overcome deployed resistances (Tamang, 2017). To undergo sexual recombination, *P. teres* has a single mating type locus (*MAT*) with two alternative forms (*MAT-1* and *MAT-2*), that have to be opposite for individuals to mate (Ficsor et al., 2014). The sexual stage consists of pseudothecia which appear as small fruiting bodies on the surface of barley straw (Mathre, 1997; McLean et al., 2009). Within the mature pseudothecia club shaped and bitunicate asci are present (Mathre, 1997). Under moist conditions of 95-100% relative humidity, ascospores are ejected into the air and are dispersed by wind. An ascospore germinates and forms an appressorium, which penetrates the epidermal cell wall, colonizes the apoplast of the mesophyll tissue and eventually produces a lesion (Hargreaves & Keon, 1983). Asexual recombination is predominant, it produces a limited number of progeny as no exchange of genetic material is involved (Leišova et al., 2005). The asexual stage of *P. teres* consists of conidia which are produced from lesions or stubble. The conidia are dispersed by wind to cause new infections after adhesion to the leaf surface. Conidia are produced and dispersed throughout the barley growing season during favourable environmental conditions (Lai et al., 2007). After the growing season, *P. teres* produces pseudothecia on the stubble.

Previous studies have shown that the occurrence of sexual reproduction within individual forms of *P. teres* is common in fields (Akhavan et al., 2016, Liu et al., 2012, Peever & Milgroom, 1994, Serenius et al., 2007, Jonsson et al., 2000, Peng & Lapitan, 2005, Rau et al., 2003, Leišova et al., 2005, Campbell et al., 2002, Lehmensiek et al., 2010, Bogacki et al., 2010, McLean et al., 2009, McLean et al., 2010). However, sexual reproduction between *Ptt* and *Ptm* seems rare in nature. This has been demonstrated through the absence of hybrids and low number of shared markers between *Ptt* and *Ptm* reported in studies by Rau et al. (2003) and Poudel (2018). Rau et al. (2003) studied 150 *P. teres* isolates collected from fields in Sardinia and did not find any hybrids. Poudel (2018) investigated 223 isolates collected during three successive years and demonstrated that hybridisation occurred frequently within the forms but not between the forms. The reasons for this are not understood but were suggested that it may be due to reproductive isolation or poor fitness of hybrids (Poudel et al. 2018).

Four studies have found sexual reproduction between *Ptt* and *Ptm* in the field (Campbell et al., 2002, Leišova et al., 2005, McLean et al., 2014, Dahanayaka et al., 2021). Campbell et al. (2002) found one isolate that contained unique net form and spot form random amplified polymorphic DNA (RAPD) bands. This isolate did not cluster with net form or spot form isolates and formed a separate group on the phylogenetic tree. Leišova et al. (2005) found two isolates (*PTM* 15 and *PTM* 16) with a high number of shared markers between *Ptt* and *Ptm*.

McLean et al. (2014) found one isolate (WAC10721) that contained both AFLP bands unique to *Ptm* and *Ptt.* A recent study (Dahanayaka et al., 2021) identified another two hybrids, one from Hungary (H-919) and the other from Japan (CBS 281.31). The isolate from Japan was collected in 1931 while the isolate in Hungary was collected in 2018. These were confirmed to be hybrids only after the amplification with *Ptt* and *Ptm* specific DNA markers. These studies demonstrated that while rare, hybridisation between *Ptt* and *Ptm* does occur in the field.

In vitro recombination between the two forms has been demonstrated in various studies (Afanasenko et al., 2007, McDonald, 1963, Smedegård-Petersen, 1977, Jalli, 2011, Campbell et al., 1999, Campbell et al., 2002). Campbell et al. (1999) suggested that the two forms of *P. teres* could be hybridized to produce lesions that were morphologically intermediate between the spot and net forms. Campbell et al. (1999) also found that hybrids had reduced sensitivity to triazole fungicides as compared to their parents. Jalli (2011) and El-Mor et al. (2018) showed that the virulence pattern of the hybrids were different from those of the parents. Campbell and Crous (2003) observed that hybrids retained their virulence and fertility over generations. These results indicate that even though recombination between the two forms is rare, the resulting hybrids could have novel virulences or have combination of virulence of both parents that have the potential to overcome established sources of resistance.

Each form of *P. teres* has different sets of virulence genes (Clare et al., 2020). Isolates of *Ptt* and *Ptm* have different disease responses depending on the genotype. For example, barley genotype CIho5791 is universally known as a *Ptt* resistant variety (Koladia et al., 2017) but it is susceptible to *Ptm* (McLean et al., 2014). While barley genotype Keel which is considered resistant to *Ptm* (McLean et al., 2014) is susceptible to *Ptt* (Fowler et al., 2017). Occurrence of field hybridisation between *Ptt* and *Ptm* is of concern because this may introduce new pathotypes which may overcome deployed host resistances and fungicides. Therefore while breeding for resistance, pathogen genetics and virulence profiles present in local pathogen population must be considered to ensure that the durable sources of host-plant resistance are deployed in commercial varieties (Liu et al., 2011).

Recently, Turo et al., (2021) confirmed the occurrence of a natural recombination event between *Ptt* and *Ptm* in Western Australia with the hybrid isolate having acquired azole fungicide resistance through intraspecific sexual recombination . Although naturally occurring hybrids are considered to be rare knowledge of the genetic structure of *Ptt* and *Ptm* hybrids is important for researchers and breeders when developing net blotch resistant germplasm and breeding barley cultivars as they can have the ability to overcome resistance. The objective of this study was to investigate the virulence of four hybrid populations and to determine the proportion of novel virulences generated.

Materials and methods

In *vitro* mating

Single conidia of the parental isolates were grown for approximately 10 days on half-strength potato dextrose agar (PDA) (20 g/Litre; Merck Darmstadt, Germany). Four populations i.e. HRS07033/NB63, NB73/SNB171i, W1/16FRGO73, KO103/16FRGO73 were produced as described in Martin et al. 2020. A 3-mm agar plug with mycelium from each of two isolates were placed on opposite sides of a Sach's nutrient agar plate containing sterilized wheat or barley stems (Smedegård-Petersen, 1971). The plates were sealed in plastic bags to prevent desiccation of the agar and incubated at 15˚C with a photoperiod of 12 h of light and 12 h of darkness. Cultures were incubated until pseudothecia maturation for 3 to 6 months. To collect ascospores, the lids of the plate were replaced with a 2% water agar plate, sealed with Parafilm, and were incubated under the same conditions as above until the ascospores eject onto the water agar plate. Plates were checked daily and single ascospores were transferred to a PDA plate with a glass needle. Isolates HRS07033, SNB171i and 16FRGO73 were *Ptm* (MAT-1) parents while isolates NB63, NB73, W1 and KO103 were *Ptt* (MAT-2) parents.

Plant Material

A set of 20 barley lines; Arimont, Beecher, Cape, CI11458, CI5286, CIho5791, Commander, Corvette, Galleon, Hindmarsh, Keel, Kombar, Maritime, Prior, Rosalind, Skiff, Tallon, Torrens, TR250 and Vlamingh, with differential responses to *Ptm* and *Ptt* isolates were used for phenotyping of the populations. These lines have previously been used in Australian and international differential sets (Khan & Tekauz, 1982, Gupta & Loughman, 2001, Wu et al., 2003, Gupta et al., 2011, McLean, 2012, McLean et al., 2014).

Phenotyping

Four seeds of each barley cultivar were sown into 15 cm diameter pots with clusters of seeds of each genotype evenly spaced around the circumference of the pot containing Searles Premium Potting Mix. Three replicates were grown for each isolate using complete randomized design. Pots were placed in a random order on a bench in a glass house and grown under natural light at 20 ± 5˚C for 14 days (two to three leaf stage).

Isolates were grown on PDA plates and incubated at 22˚C with 12-h light/12-h dark photoperiod for 5-7 days. Five mycelium plugs of approximately 9 mm^2 were sub-cultured onto 1% water agar containing sterile sorghum leaves. The plates were grown at room temperature of approximately 22°C for six days and transferred to an incubator at 15˚C under 12 h of white fluorescent lights of ~200 µmoles/m^2/sec and 12 h of dark for a further six days. Spores were collected by adding 10 mL of distilled water (containing two drops of Tween®20/100 mL

water) to the plates, loosening conidia on plates with a paint brush and filtering the spore suspension through a 500-µm sieve. The spores were counted using a haemocytometer and adjusted to 10,000 spores/mL.

Barley seedlings were inoculated with 3 mL of the 10,000 spores/mL suspension by spraying using an aerosol-based spray system (Preval Sprayer, Chicago, US). The inoculated seedlings were incubated in the dark at 95–100% relative humidity at room temperature for 48 h. Seedlings were then transferred to a controlled environment room under 12 hours day/night light at 22˚C day/15˚ C night and 75% humidity for eight days to allow symptoms to develop. On the ninth day after inoculation, the secondary leaves of the barley seedlings were assessed for lesion infection response. Infection responses were rated using a 1 to 10 scale (Tekauz, 1985).

Results

In vitro mating

Ascospores were produced between 2 to 12 months after making the crosses. The success of the crosses varied between the parental isolates used in the cross. Crosses HRS07033/NB63, NB73/SNB171i, KO103/16FRGO73 and W1/16FRGO73 produced 400, 20, 27 and 13 ascospores respectively. No difference in the shape and size of the conidia was observed between those produced by the hybrids and the parents. From each cross, 15 randomly chosen progeny were selected for conidia production and inoculation, except for population W1/16FRGO73 which only produced 13 isolates in total. Since population HRS07033/NB63 produced around 400 ascospores, we selected ascospores that produced at least 10,000 spores/mL of conidia.

Virulence of progeny

HRS07033/NB63

Visual examples of hybrid and *Ptt* and *Ptm* symptoms and the average infection response of the parental isolates HRS07033 and NB63 was 4.7 and 4.6, respectively (Figures 1 and 2 and Supplementary Table 1). The hybrid populations infection response ranged from 4.7 to 6.5 (Figure 2). Fourteen progeny had average infection response greater than the parents. Parent HRS07033 and NB63 both had infection responses of ≤4 on barley genotype CI5791, Corvette and TR250. Seven hybrids had an infection response of ≥6 on CI5791, while five hybrids on Corvette and seven hybrids had infection responses of ≥6 on TR250. Kombar was the most susceptible barley genotype (Figure 2) with average hybrid infection response of 6.2 while Arimont was the least susceptible barley genotype with an average infection response of 4.9. Only one progeny produced net form symptoms, while nine progeny produced spot form

symptoms and five produced symptoms that were neither typically spot form or net form symptoms, referred to here as hybrid symptoms (Figure 1, Supplementary Table 1).

NB73/SNB171i

Visual examples of hybrid and *Ptt* and *Ptm* symptoms and the average infection response of parental isolates NB73 and SNB171i was 4.5 and 3.5, respectively (Figures 1 and 2 and Supplementary Table 1). The average response of progeny ranged from 1.8 to 6.6. Nine progeny had greater infection response than the parents (Supplementary Table 1). Parents NB73 and SNB171i had an infection response of ≤4 on barley genotypes Maritime, Rosalind, Torrens, TR250 and Vlamingh. Six hybrids of this population expressed infection response of ≥6 on Maritime, six hybrids on Rosalind, eight hybrids on Torrens, six hybrids on TR250 and eight hybrids on Vlamingh. Tallon was the most susceptible barley genotype (Figure 2) with an average infection response of 5.9 while Keel was least susceptible barley genotype with an average infection response of 4.5. Five progeny expressed net form symptoms, eight progeny expressed spot form symptoms and two expressed hybrid symptoms (Figure 1).

KO103/16FRGO73

Parents, KO103 and 16FRG073 had average reaction responses of 4.2 and 4.8 respectively (Supplementary table 1). The infection scores of progeny ranged from 1.7 to 6.8 (Supplementary Table 1). Three progeny had higher and nine progeny had a lower average infection response compared to the parents. Both the parents had an infection response of ≤ 4 on barley genotypes Beecher, Corvette and Vlamingh. Three hybrid progeny of this population had an infection response of ≥6 on Beecher, two hybrids on Corvette and four hybrids had an infection response of ≥6 on Vlamingh. Tallon was most susceptible barley genotype (Figure 2) with average infection response of 4.8 while Corvette was least susceptible barley genotype with average infection response of 2.9 (Supplementary Table 1). None of the progeny produced net form symptom, 14 progeny produced spot form lesions and only one progeny produced hybrid symptoms (Figure 1).

W1/16FRGO73

Parental isolates W1 and 16FRGO73 had an average response of 3.5 and 4.2, respectively (Supplementary Table 1). The average infection response of the progeny ranged from 1.8 to 4.5 (Supplementary Table 1). One progeny had a higher while 12 progeny had a lower average infection response as compared to the parents. Both the parental isolates had an infection response of ≤ 4 on barley genotypes Arimont, Beecher, Cape, Corvette, Hindmarsh, Rosalind and Vlamingh. None of the progeny had infection response of ≥6 on any of these barley genotypes. Tallon was the most susceptible barley genotype (Figure 2) with average infection response of 3.1 while Galleon was the least susceptible barley genotype with average infection

response of 1.9. Only one progeny showed net form symptoms and 11 progeny produced spot from symptoms and one isolate showed hybrid symptoms (Figure 1).

Discussion

In this study, infection responses caused by hybrid progeny of four *Ptt/Ptm* populations were investigated to determine whether progeny can produce novel virulences, different to those of their parents. A total of 62 interactions between the hybrid and barley genotypes were virulent to which parents expressed avirulent responses, demonstrating that the hybrids generated in this study possessed virulences which could overcome known host plant resistances. This confirms that hybridisation between *Ptt* and *Ptm* is possible and can lead to the emergence of new virulences not currently observed in the field.

All crosses used in this study produced viable ascospores which germinated on PDA plates. This indicates that the production of viable progeny from crosses between *Ptt* and *Ptm* isolates is easily achievable under laboratory conditions. Similarly, Jalli (2011) made two *in vitro* crosses between *Ptt* and *Ptm* and produced 50 and 100 viable ascospores from the two crosses, respectively. Smedegard-Petersen (1977) also generated 150 *Ptt/Ptm* hybrids which produced intermediate symptoms or fleck lesions. These studies demonstrate that there is strong genetic compatibility between the two forms and that the production of viable progeny between *Ptt* and *Ptm* can occur. However, further research is required to determine why hybrid progeny are uncommon in nature.

The average infection responses varied significantly when parents and progeny populations were compared. Crosses of HRS07033 and NB63 resulted in a primarily virulent population. A total of 27 progeny from all the populations had average infection responses greater than those of the parental isolates. Jalli (2011) also observed that the virulence profiles of *Ptt/Ptm* progeny were different to those of their parental isolates. Tekauz (1990) suggested that an increase in *P. teres* pathotypes could be due to a combination of factors, including sexual recombination between *Ptt* and *Ptm*. Turo et al. (2021) confirmed the occurrence of natural recombination events between *Ptt* and *Ptm* in Western Australia. The study also indicated that the hybrid isolate acquired azole fungicide resistance. These results indicate that although the occurrence of *Ptt/Ptm* isolates are rare they have the ability to overcome deployed resistance.

Most of the hybrid isolates of population W1/16FRGO73 were avirulent. A total of 24 progeny of all four crosses had an average infection response of less than four. This suggests that some parents do not produce pathogenically fit hybrid progeny and indicates they may not survive as they are avirulent. Leišova et al. (2005) also suggested that the presence of *Ptt/Ptm* hybrids in nature is possible but mentioned that they may not be able to survive due to selection pressure

or genetic drift. Syme et al. (2018) suggested that low viability of *Ptt/Ptm* hybrids maybe due to unequal recombination events leading to deleterious gene loss or gain, or a fitness penalty associated with incompatibility with new gene combinations. Comparative whole genome sequencing could help in identifying allele combinations that are contributing to hybrid fitness. Comparative whole genome sequencing is a powerful technique in which genome sequences of different organisms are compared. It is used to study the evolutionary changes in an organism, genes that are conserved and genes that make each organism unique (Touchman, 2010).

Each hybrid isolate expressed only one type of lesion on all barley genotypes i.e. net form, spot form or hybrid symptoms. This means the type of lesion developed was not influenced by the barley genotype and depended completely on hybrid isolate genes. Similarly, ElMor (2016) evaluated the virulence of three progenies arising from *Ptt* and *Ptm* crosses using a detached leaf assay. They also observed one type of lesion developed by each progeny on barley genotypes. Smedegård-Petersen (1977) studied the genetic factors for symptoms and pathogenicity in 478 progeny from *Ptt/Ptm* hybrids. The progeny was developed from 11 different crosses and demonstrated that lesion type incited by individual progeny depends on two independently segregating genes of the pathogen. The results of these studies suggest that the type of symptoms expressed on the barley leaves are controlled entirely by fungal genes independent of barley genotype.

Out of 58 *Ptt/Ptm* hybrid isolates, 43 isolates (74%) showed spot form symptoms and seven (12%) showed net form symptoms. Nine isolates (16%) produced hybrid lesions that could not be identified either as typical net form or spot form symptoms. This indicates that hybrids present in the field would likely be misidentified either as *Ptt* or *Ptm* as hybrids have symptoms similar to *Ptm* or *Ptt* on barley. To date, four isolates that were symptomatically identified as either *Ptt* or *Ptm* have been re-classified as hybrids using molecular markers. The Australian isolate (WAC10721) (McLean et al., 2014) and two isolates from the Czech republic (*PTM* 15 and *PTM* 16) (Leišova et al., 2005) were symptomatically classified as *Ptm* and a South African isolate (Campbell et al., 2002), was classified as *Ptt.* It is interesting as three out of four hybrids were classified as *Ptm* and only one isolate was classified as *Ptt.* Similarly, in our current study 74% of the isolates produced spot form symptoms. A possible explanation could be that at low infection levels the symptoms of *Ptt* infection are difficult to distinguish from those of *Ptm* and as a result, some of those symptoms could have been miss-classified as *Ptm*. Poudel et al. (2017) developed markers which clearly distinguish between *Ptt* and *Ptm* isolates and provide clear identification of hybrids. Usage of these diagnostic makers will provide more efficient

and reliable identification of *P. teres* isolates in the future, including determining the frequency of hybrids under field conditions.

In conclusion, this study has demonstrated that hybridisation between *Ptt/Ptm* can give rise to novel virulence profiles which can cause susceptibility to resistant barley genotypes. Although hybridisation between *Ptt/Ptm* is rare in nature, we suggest that regular monitoring of *P. teres* virulences present in the field should be conducted. In addition, molecular characterisation should be undertaken when identifying different forms of *P. teres* as identification based on symptom expression alone would not identify *Ptt/Ptm* hybrids.

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402 Figures

(a) Net form symptoms

(b) Spot form symptoms

(c) Hybrid symptoms

Figure 1 : Disease symptoms of (a) Net form isolate, (b) Spot form isolate and (c) *Ptt/Ptm* hybrid isolates

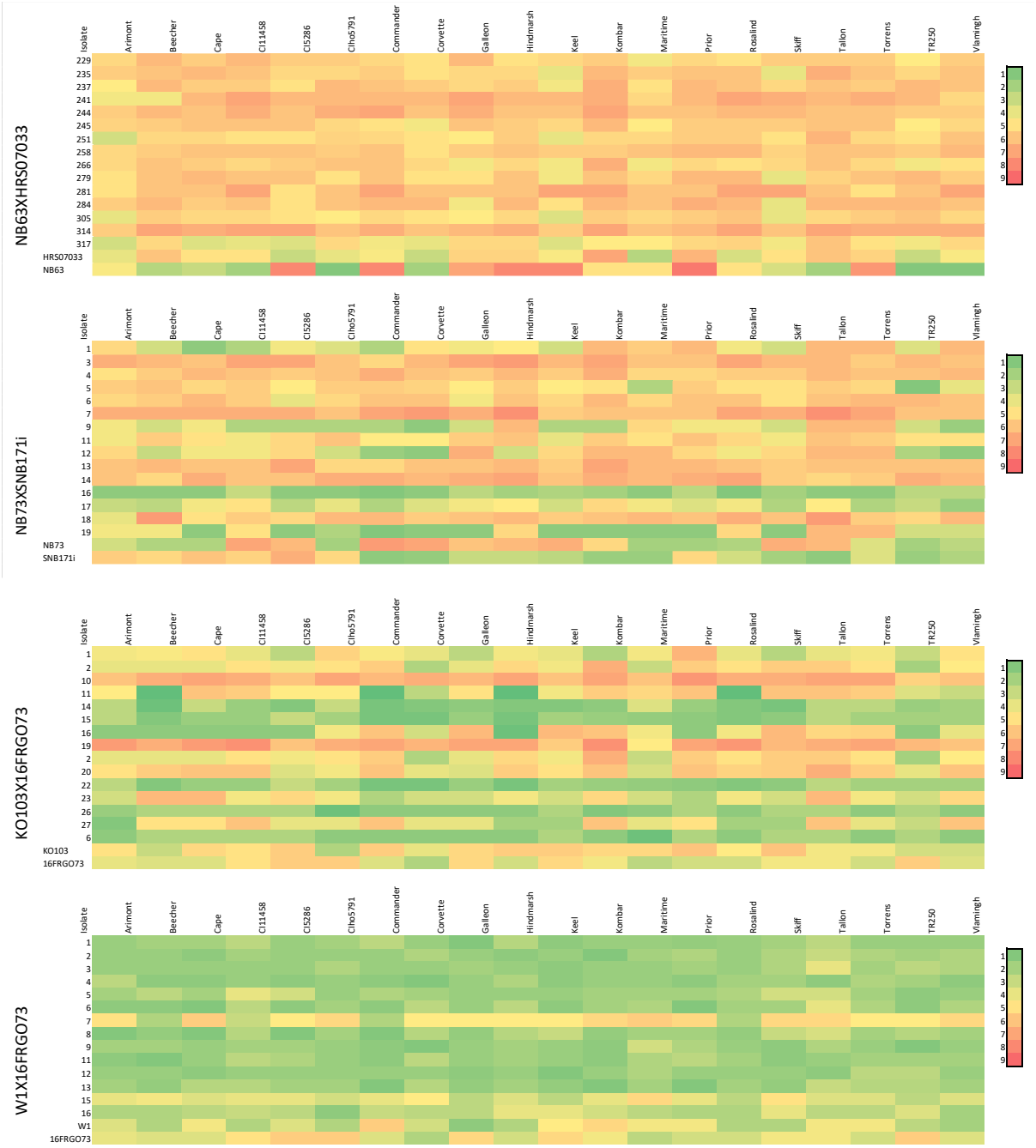

Figure 2 : Heatmaps of infection responses produced by hybrid isolates and their parents with a range of colour degradation from a green colour indicating an infection response of 1 and a red colour indicating an infection response of 9.

CHAPTER 5

KEY FINDINGS, FUTURE RECOMMENDATIONS AND CONCLUSION

5.1 Key findings of this study

Since the knowledge of genetics of pathogen virulence is required to better understand the host/pathogen interactions and to manage the disease resistance in host genotypes; the main objective of this thesis was to understand the virulence of *P. teres.* Multiple studies regarding the virulence of *Ptt* have already been published, so our research mainly focused on *Ptm* and *Ptt/Ptm* hybrids. Since understanding the genetic basis of virulence is important to developing resistant barley varities, two research chapters were dedicated to identifying the genomic regions conferring virulence in *Ptm.* On the other hand, since no information about the inheritance of virulence in hybrids is available to date. Third research chapter focused on studying the virulence profiles of the hybrid progenies and to see if novel virulences can be generated as a result of *Ptt/Ptm* and to see if novel virulences can be generated as a result of *Ptt/Ptm* hybridisation. Although, the occurrence of *Ptt/Ptm* hybrids is considered to be rare in nature, it is possible that even rare hybridisation events can lead to generation of virulent pathotypes. Once a virulent hybrids gets generated it can multiply easily through asexual recombination which can be of concern to barley industry.

The first objective of the study was to identify genomic regions associated with virulence by genome wide association mapping (GWAS). One hundred and twenty-one *Ptm* isolates collected from Victoria, New South Wales, Western Australia, South Australia and Queensland were genotyped using DArT-seq (Diverstiy Arrays Technology Pty Ltd) and 751 high quality markers were obtained. Isolates were phenotyped across twelve different barley genotypes. Ten novel quantitative trait loci (QTL) associated with virulence in *Ptm* were identified. This was the first study to identify virulence QTL in a *Ptm* population using GWAS. In total, 150 candidate genes were identified including two genes encoding putative effector proteins. A number of QTL identified in this study suggests that the *Ptm*-barley interaction is complex and does not follow a gene for gene interaction, which involves pathogen's avirulence factors and dominant host resistance. Liu et al. (2015) suggested that interaction between *Ptt* and barley follows a necrorophic effector triggered susceptibility (NETS) model. The NETS model includes secretion of necrotrophic effector molecules by the pathogen, which then are recognised by the host cells triggering programmed cell death in the host which ultimately helps the pathogen by providing it with desired nutrients. Other closely related pathogen

Pyrenophora tritici-repentis and *Parastagnospora nodorum* have been also suggested at least to follow a partial NETS model (Ciuffetti et al., 2010, Friesen et al., 2008).

The second objective of the study was to identify genomic regions conferring virulence in a *Ptm* bi-parental population using QTL mapping. A population containing 347 progeny was developed from a cross between Australian isolates SG1 and 16FRGO73. Two QTL were identified, one on chromosome 2 and the other on chromosome 3. Both QTL only explained only 4% and 6% of the phenotypic variance and had low LOD scores. Some QTL could have been missed due to the low number of polymorphic markers present in the population. Of the 5473 markers obtained from DArTseq only 833 markers i.e. 15% were polymorphic. We identified eight signal peptides within the QTL regions out of which five were small proteins. We also identified five non-ribosomal peptide synthetase clusters which could play a potential role in the virulence.

The third objective of our study was to identify novel virulences generated in *Ptt/Ptm* crosses. For this we made four *in vitro* crosses between *Ptt* and *Ptm* isolates, and the progeny were phenotyped across 20 barley genotypes. We observed that sixty-two progeny caused susceptibility in barley genotypes to which parental isolates had an avirulent response. This suggested that novel virulence in fields can also appear which can cause susceptibility to currently used resistant barley genotypes. Economic losses contributed by these virulent hybrids once they occur in fields is therefore anticipated. It was also observed that twenty-seven progeny expressed greater average infection responses compared to the parental isolates. Virulence profiles of the hybrid progeny varied significantly depending on the parents selected, with some crosses producing avirulent and others mainly virulent progeny. This study showed that *Ptt/Ptm* hybridisation can act as a potential threat to current sources of host plant resistance in commercial varieties. Turo et al., (2021) confirmed the occurrence of *Ptt/Ptm* hybrids and indicated that hybrid isolates acquired azole fungicide resistance. The results of this study suggests that occurrence of *Ptt/Ptm* isolates are not only possible but can also have the ability to overcome deployed resistance.

5.2 Future Recommendations

The goal of QTL mapping and GWAS was to identify genomic regions which are significantly associated with the trait of interest. Although GWAS and QTL mapping provide a statistical evidence that a particular region is likely associated with virulence, further studies are required to precisely pin-point genomic area associated with virulence. More precise identification of the genomic region associated with virulence can be done by fine mapping and machine

learning. Many functional and computational high throughput fine mapping methods have been developed. With further fine mapping, identification and characterisation of more genes, cloning of genes will become possible. Cloning of the virulence genes will prove to be very informative once the virulence regions have been identified and tagged. Once the genes have been thoroughly studied, target gene disruption can be done to develop mutants that are unable to cause virulence. Ruiz-Roldán et al. (2001) used the same approach to study *PTK1* which is responsible for appressoria formation in *P. teres*

Machine learning provides opportunity to further study the genomic regions identified in the post GWAS analysis phase. It provides an improved statistical foundation of evidence to support or enhance GWAS results (Nicholls et al., 2020). Models for GWAS prioritization vary greatly ranging from simple regression to complex ensemble approaches. (Nicholls et al., 2020). These models when paired with functional validation provide strong evidence-based approaches to direct post GWAS research.

Only two effector proteins were identified in *Ptm* to date (Sharma et al 2021, Chapter 2). Recent advances in genomics and bioinformatics along with evolving high-throughput tools allow the identification and characterisation of effectors. Effectors have a great potential to be employed in *Ptm* resistance breeding through mapping and cloning of resistance genes in barley. Understanding of the interaction between effectors and R genes have been employed by breeders for the identification and functional characterisation of R genes in potato against *Phytophthora infestans* (Vleeshouwers et al., 2008). Effectors can also be used to engineer disease resistance through host induced gene silencing (HIGS) of pathogen virulence effectors. In barley and wheat HIGS of effector *Avr* 10 resulted in reduced development of *Blumeria graminis* (Nowara et al., 2010).

Hybridisation is documented to be rare between *Ptt* and *Ptm* in nature (Poudel et al. 2018). There is a possibility that hybrids are present in nature and are not being reported because hybrids produce symptoms similar to the parents. It is also possible that hybrids are aviurlent and cannot survive from one generation to the next (Poudel et al. 2018). Novel virulences were observed in *in-vitro* hybrids in our study (Chapter 4). As a follow up sequencing of *Ptt/Ptm* hybrid genomes should be conducted. This could help to compare the genomes of hybrids with parents and identify genetic variations such as single nucleotide variations. This will provide us with insights regarding the genetic relationships between parents and hybrids and genetics of pathogenicity. It can also help us understand if there is any insertion/deletion in the key genes which are contributing to a loss or gain of virulence and to understand if there is a fitness penalty associated with new gene combinations which may contribute to the low survival rate of hybrids in nature.

5.3 Conclusion

We present here a study that provides information on the virulence profiles and genetic structure of *P. teres* f. *maculata* and *Ptt/Ptm* hybrid populations in Australia. Genomic regions associated with virulence in an Australian *Ptm* population were identified via GWAS and in an Australian bi-parental population via QTL mapping. Interaction between *Ptm* and barley appears to be complex. Sexual recombination between *Ptt* and *Ptm* can give rise to novel virulences and can potentially overcome deployed resistance; however given the rare occurrence of hybrids under field conditions it is not clear whether these findings represent a practical threat to the barley industry.

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APPENDIX

Chapter-2

Supplementary Table 1: List of 121 *Ptm* isolates used to investigate marker-trait associations including identification number, location and year collected

Isolate identification number	Location collected	Year collected
03-002	Streatham, Vic	2003
03-007	Horsham, Vic	2007
04-0018	Rainbow, Vic	2004
04-0073	Cavendish, Vic	2004
08-003	Coorong, SA	2008
08-032	Dimboola, Vic	2008
08-048	Nathalia, Vic	2008
09-002	Woomelang, Vic	2009
09-005	Irvingdale, QLD	2009
09-009	Mondure, QLD	2009
09-010	Denilequin, NSW	2009
09-011	Jandaryan, QLD	2009
09-015	NSW	2009
09-025	Peak Hill, NSW	2009
09-027	Parkes, NSW	2009
09-031	Tomingley, NSW	2009
09-033	The Rock, NSW	2009
09-035	Moree, NSW	2009
09-036	Goondiwindi, NSW	2009
09-037	Mooree, NSW	2009
09-038	Mooree, NSW	2009
09-054	Speed, Vic	2009

Isolate identification number	Location collected	Year collected
09-079	Kaniva, Vic	2009
09-084	St. Arnaud, Vic	2009
09-088	Rupanyup, Vic	2009
09-095	Geelong, Vic	2009
09-122	Euiston, SA	2009
09-124	Bute, SA	2009
09-128	Brentwood, SA	2009
09-129	Port Clinton, SA	2009
09-133	Cummius, SA	2009
09-139	Horsham, Vic	2009
09-151	Arthurton, SA	2009
09-o28	Wyalong, NSW	2009
15-056	Carwaro, Vic	2015
18-024	Dimboola, Vic	2018
18-026	Birchip, Vic	2018
18-136	Minimay, Vic	2018
23/96/7	Yeelanna, SA	1996
5/o3	Myponga, SA	2003
ptm#14060	Monto, QLD	2014
ptm12-026	Marnoo, Vic	2012
ptm13-105	Birchip, Vic	2013
ptm13-110	Quambatook, Vic	2013
ptm13-111	Birchip, Vic	2013
ptm13-125	Natimuk, Vic	2013
ptm13-126	Birchip, Vic	2013
ptm13-127	Birchip, Vic	2013
ptm13-13111	Chinchilla, QLD	2013
ptm13-13145	Gatton, QLD	2013
ptm13-133	Willenabrina, Vic	2013

Isolate identification number	Location collected	Year collected
ptm13-136	Talors lake, Vic	2013
ptm13-139	Horsham, Vic	2013
ptm13-140	Horsham, Vic	2013
ptm13-141	Horsham, Vic	2013
ptm13-149	Yalla Y Poora, Vic	2013
ptm13-150	Brim, Vic	2013
ptm13-151	Murrayville, Vic	2013
ptm13-159	Wonwondah, Vic	2013
ptm13-160	Narcoorte, Vic	2013
ptm13-168	Carmut, Vic	2013
Ptm13-200	Kangaroo Valley, WA	2013
ptm13-201	Lort River, WA	2013
ptm13-217	Medina, WA	2013
ptm13-218	Medina, WA	2013
ptm13-220	Lake King, WA	2013
ptm13-221	Moora, WA	2013
ptm13-223	Dandarago, WA	2013
ptm13-229	Kellerberrin, WA	2013
ptm13-233	Wongan Hills, WA	2013
ptm13#13205	Tuloona, NSW	2013
ptm14-014	Brim, Vic	2014
ptm14-017	Kalannie, WA	2014
ptm14-027	Birchip, Vic	2014
ptm14-028	Charlton, Vic	2014
ptm14-030	Northam, WA	2014
ptm14-031	South Toompup, WA	2014
ptm14-035	Hyden, WA	2014

Isolate identification number	Location collected	Year collected
ptm14-068	Wunghnu, Vic	2014
ptm14-078	Walpeup, Vic	2014
ptm14-097	Katamatite, Vic	2014
ptm14-100	Uranquinty, NSW	2014
ptm14-102	Elmore, Vic	2014
ptm14-112	Glenthompson, Vic	2014
ptm14-115	Hexham, Vic	2014
ptm14-117	Darlington, Vic	2014
ptm14-118	Derrinallum, Vic	2014
ptm14-120	Inverleigh, Vic	2014
ptm14-124	Buangor, Vic	2014
ptm15-030	Tempy, Vic	2015
ptm15-031	Kiamal, Vic	2015
ptm15-033	Coomalbidgup, WA	2015
ptm15-053	Merrinee, Vic	2015
ptm15-055	Robinvale, Vic	2015
ptm16-008	Murrayville, Vic	2016
ptm16-010	Rainbow, Vic	2016
ptm16-013	Warracknebeal, Vic	2016
ptm16-017	Charlton, Vic	2016
ptm16-018	Wycheproof, Vic	2016
ptm16-019	Ultima, Vic	2016
ptm16-020	Swan Hill, Vic	2016
ptm16-023	Meredin, Vic	2016
ptm16-024	Rupanyup, Vic	2016
ptm16-027	Shelfort, Vic	2016
ptm16-028	Beulah, Vic	2016

Isolate identification number	Location collected	Year collected
ptm16-029	Toobeah, QLD	2016
ptm16-034	Willaura, Vic	2016
ptm16-037	Streatham, Vic	2016
ptm16-038	Dooen, Vic	2016
ptm16-049	Boree Creek, NSW	2016
ptm16-054a	Finley, NSW	2016
ptm16-055a	Denilequin, NSW	2016
ptm16-061	Marrar, NSW	2016
ptm16-062	Methul, NSW	2016
ptm16-065	Narrandera, NSW	2016
Ptm16-068	Clifton, QLD	2016
ptm16-129	Duri, NSW	2016
ptm16-131	Tamworth, NSW	2016
ptm16-143	Skipton, Vic	2016
sf03-0133ss	Hamilton, Vic	2003
SNB113	Leeton, NSW	2007

Supplementary Table 2: Infection scores of 121 *Ptm* isolates on 12 barley genotypes.

Ptm isolate	Arimont	Chebec	CI5286	CI5791	CI9214	CII6150	Galleon	Keel	Kombar	Skiff	Torrens	TR250	Average
03-002	5.0	3.3	5.3	5.0	4.0	4.0	4.7	4.0	6.0	3.0	4.7	5.0	4.5
03-007	5.0	4.0	6.0	5.7	5.0	5.3	4.7	2.3	8.0	3.7	4.5	5.5	5.0
04-0018	5.3	3.5	5.0	5.0	4.0	4.8	4.8	3.0	7.5	2.8	4.0	5.8	4.6
04-0073	5.3	4.0	5.5	5.0	3.3	5.0	4.0	2.5	7.0	3.5	4.5	5.8	4.6
08-003	5.0	2.7	3.3	4.0	2.7	3.7	3.0	2.3	4.7	2.7	3.0	2.0	3.3
08-032	5.5	3.3	6.3	6.0	4.7	6.3	3.3	2.7	7.3	4.0	4.5	5.7	5.0
08-048	5.0	4.5	5.0	5.3	5.7	3.0	4.3	5.0	5.3	4.3	5.3	NA	4.8
09-002	5.0	3.0	5.0	5.0	3.7	5.0	4.0	2.7	5.7	2.7	4.7	6.0	4.4
09-005	5.3	4.0	4.7	6.3	5.0	5.7	4.3	3.0	6.7	3.3	4.3	6.0	4.9
09-009	6.0	3.0	5.7	6.7	5.0	6.0	5.7	4.0	6.0	3.3	5.3	5.5	5.2
09-010	5.3	3.3	5.0	6.7	5.3	5.0	3.7	3.3	7.7	4.0	5.0	6.3	5.1
09-011	5.7	3.3	5.0	5.0	4.3	5.0	5.0	3.3	6.0	3.0	4.0	4.7	4.5
09-015	5.0	4.0	5.3	4.7	4.3	5.0	4.3	3.3	5.3	3.0	NA	5.5	4.5
09-025	5.3	4.3	4.3	4.5	4.0	5.3	4.0	2.7	7.3	3.0	5.3	5.0	4.6
09-027	4.5	3.5	5.7	5.7	3.7	3.7	3.7	3.0	7.0	4.0	2.0	5.7	4.4
09-031	3.5	2.7	4.7	5.3	3.0	3.3	4.3	3.0	4.3	3.7	3.7	4.3	3.8
09-033	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
09-035	4.5	4.3	5.0	5.3	5.0	5.0	3.7	3.3	5.0	4.7	5.0	4.5	4.6
09-036	5.0	3.7	3.5	5.0	4.0	4.5	4.7	3.3	4.7	2.7	3.5	5.0	4.1
09-037	5.3	3.0	4.0	4.7	4.3	4.5	4.0	3.0	4.5	4.7	5.0	5.0	4.3
09-038	6.0	4.0	4.5	5.7	4.7	3.3	3.0	4.0	6.0	3.3	4.0	6.0	4.5
09-054	6.0	3.0	4.3	5.0	4.0	5.5	5.7	4.0	6.3	4.0	4.0	6.0	4.8
09-079	5.0	4.3	7.0	5.3	3.0	5.0	4.0	3.3	7.7	5.0	4.3	5.0	4.9
09-084	5.0	4.5	5.0	5.0	3.0	NA	3.3	3.0	6.7	4.3	3.5	4.5	4.3
09-088	6.0	3.3	4.3	5.3	3.0	4.3	5.0	3.7	4.3	5.3	5.0	5.5	4.6
09-095	4.3	2.5	4.0	4.3	4.7	5.5	5.7	2.7	4.3	4.3	3.5	5.5	4.3
09-122	4.0	3.7	4.0	4.3	3.3	4.0	4.0	3.3	5.0	4.0	3.0	4.0	3.9
09-124	4.3	3.3	4.0	5.0	5.0	4.3	4.7	2.3	5.0	2.7	4.0	4.7	4.1
	Ari	Che	CI5	CI5	CI9	CII	Gal	Kee	Ko	Ski	Tor	TR	Av

Ptm isolate													
09-128	3.7	3.0	4.3	3.7	3.0	4.0	3.0	2.0	3.5	2.7	NA	3.0	3.3
09-129	5.0	3.0	4.0	5.5	4.0	5.0	3.7	4.0	6.5	5.0	5.0	3.5	4.5
09-133	4.7	4.7	5.0	5.7	3.7	4.3	5.0	3.7	5.3	4.7	5.0	4.0	4.7
09-139	5.0	3.7	5.5	7.0	4.3	5.7	5.7	2.0	6.0	4.0	5.0	6.0	5.0
09-151	3.5	3.7	4.3	4.5	4.0	4.0	4.3	3.3	5.0	3.7	5.0	NA	4.1
09-o28	4.0	3.7	5.7	4.7	3.7	4.0	4.3	3.0	6.0	4.0	4.0	4.7	4.3
15-056	5.0	3.0	3.0	6.0	3.0	3.0	3.0	2.0	8.0	3.0	-	5.0	4.0
18-024	6.0	NA	5.0	NA	NA	NA	4.0	3.0	8.0	4.0	5.0	7.0	5.3
18-026	4.0	NA	3.0	NA	NA	NA	3.0	3.0	7.0	4.0	4.0	5.0	4.1
18-136	5.0	NA	5.0	NA	NA	NA	2.0	3.0	8.0	4.0	5.0	5.0	4.6
23/96/7	5.3	4.0	5.0	5.7	6.0	4.0	3.7	3.3	6.7	3.0	4.0	6.0	4.7
5/o3	3.0	2.0	3.3	3.0	2.3	2.3	2.0	2.0	5.3	2.0	2.7	2.3	2.7
ptm13-223	5.0	3.0	3.0	7.0	4.0	NA	5.0	4.0	6.0	3.0	5.0	6.0	4.6
ptm#14060	5.0	3.0	3.0	7.0	4.0	4.0	3.0	2.0	7.0	3.0	3.0	5.0	4.1
ptm12-026	-	3.0	4.0	6.0	6.0	4.0	3.0	3.0	7.0	4.0	5.0	6.0	4.6
ptm13-105	4.0	4.0	3.0	6.0	4.0	NA	3.0	4.0	7.0	5.0	5.0	4.0	4.5
ptm13-110	6.0	3.0	4.0	6.0	3.0	NA	4.0	3.0	7.0	2.0	5.0	5.0	4.4
ptm13-111	5.0	4.0	5.0	6.0	5.0	NA	3.0	3.0	8.0	3.0	6.0	5.0	4.8
ptm13-125	3.0	2.0	3.0	5.0	3.0	NA	2.0	2.0	8.0	3.0	4.0	5.0	3.6
ptm13-126	6.0	3.0	5.0	5.0	4.0	NA	3.0	3.0	7.0	4.0	5.0	5.0	4.5
ptm13-127	5.0	4.0	4.0	6.0	7.0	NA	4.0	4.0	8.0	3.0	6.0	7.0	5.3
ptm13-13111	7.0	3.0	3.0	5.0	6.0	NA	5.0	3.0	8.0	4.0	7.0	5.0	5.1
ptm13-13145	5.0	2.0	3.0	5.0	4.0	NA	2.0	3.0	6.0	3.0	2.0	4.0	3.5
ptm13-133	6.0	5.0	6.0	6.0	5.0	NA	4.0	4.0	8.0	4.0	6.0	5.0	5.4
ptm13-136	6.0	5.0	4.0	8.0	6.0	NA	6.0	4.0	8.0	4.0	6.0	7.0	5.8
ptm13-139	5.0	3.0	5.0	6.0	5.0	NA	4.0	4.0	6.0	4.0	6.0	6.0	4.9
ptm13-140	5.0	3.0	5.0	6.0	4.0	NA	4.0	5.0	8.0	3.0	6.0	3.0	4.7
ptm13-141	4.0	3.0	5.0	6.0	4.0	NA	4.0	3.0	8.0	3.0	6.0	5.0	4.6
ptm13-149	6.0	3.0	6.0	7.0	7.0	NA	5.0	3.0	7.0	4.0	6.0	7.0	5.5
ptm13-150	5.0	-	5.0	6.0	5.0	NA	3.0	4.0	8.0	3.0	5.0	-	4.9
Ptm isolate	Arimon	Chebec	CI5286	CI5791	CI9214	CII615	Galleon	Keel	Kombar	Skiff	Torrens	TR250	Averag

ptm13-151	5.0	2.0	3.0	3.0	5.0	NA	4.0	2.0	7.0	2.0	4.0	6.0	3.9
ptm13-159	5.0	4.0	7.0	6.0	4.0	NA	4.0	3.0	8.0	3.0	6.0	3.0	4.8
ptm13-160	-	4.0	4.0	7.0	4.0	NA	4.0	4.0	6.0	4.0	6.0	4.0	4.7
ptm13-168	5.0	3.0	5.0	5.0	6.0	NA	3.0	3.0	6.0	3.0	3.0	5.0	4.3
Ptm13-200	5.0	3.0	4.0	6.0	6.0	NA	4.0	3.0	6.0	3.0	5.0	6.0	4.6
ptm13-201	5.0	3.0	5.0	5.0	5.0	NA	3.0	3.0	8.0	3.0	5.0	5.0	4.5
ptm13-217	5.0	4.0	4.0	5.0	7.0	NA	5.0	4.0	8.0	5.0	6.0	7.0	5.5
ptm13-218	6.0	3.0	5.0	5.0	5.0	NA	3.0	2.0	7.0	3.0	6.0	6.0	4.6
ptm13-220	5.0	3.0	5.0	5.0	5.0	NA	5.0	3.0	8.0	3.0	4.0	6.0	4.7
ptm13-221	6.0	3.0	5.0	6.0	4.0	NA	4.0	3.0	7.0	2.0	5.0	6.0	4.6
ptm13-233	6.0	3.0	5.0	6.0	3.0	NA	3.0	3.0	8.0	4.0	7.0	7.0	5.0
ptm13-229	5.0	3.0	5.0	5.0	5.0	NA	3.0	3.0	8.0	3.0	6.0	5.0	4.6
ptm13#13205	5.0	3.0	5.0	6.0	6.0	NA	3.0	3.0	6.0	3.0	5.0	6.0	4.6
ptm14-014	4.0	3.0	4.0	6.0	4.0	5.0	4.0	3.0	8.0	3.0	6.0	6.0	4.7
ptm14-017	4.0	3.0	4.0	7.0	3.0	5.0	5.0	3.0	8.0	4.0	3.0	6.0	4.6
ptm14-027	6.0	3.0	3.0	6.0	4.0	5.0	2.0	2.0	8.0	3.0	5.0	5.0	4.3
ptm14-028	6.0	3.0	4.0	6.0	3.0	6.0	3.0	2.0	8.0	3.0	6.0	5.0	4.6
ptm14-030	5.0	3.0	3.0	6.0	3.0	4.0	3.0	2.0	7.0	3.0	4.0	6.0	4.1
ptm14-031	5.0	4.0	3.0	7.0	5.0	5.0	3.0	3.0	7.0	3.0	6.0	5.0	4.7
ptm14-035	5.0	3.0	5.0	7.0	5.0	4.0	4.0	3.0	7.0	3.0	5.0	5.0	4.7
ptm14-068	6.0	4.0	3.0	5.0	4.0	5.0	5.0	3.0	8.0	3.0	4.0	5.0	4.6
ptm14-078	6.0	4.0	4.0	7.0	2.0	6.0	5.0	3.0	8.0	3.0	6.0	5.0	4.9
ptm14-097	5.0	2.0	4.0	6.0	5.0	5.0	3.0	3.0	8.0	2.0	7.0	6.0	4.7
ptm14-100	5.0	3.0	4.0	6.0	5.0	5.0	3.0	3.0	6.0	3.0	5.0	5.0	4.4
ptm14-102	6.0	6.0	5.0	7.0	5.0	5.0	4.0	3.0	8.0	4.0	-	6.0	5.4
ptm14-112	5.0	3.0	5.0	6.0	3.0	6.0	3.0	3.0	8.0	3.0	5.0	5.0	4.6
ptm14-115	6.0	3.0	5.0	6.0	3.0	5.0	4.0	3.0	8.0	3.0	6.0	5.0	4.8
ptm14-117	5.0	3.0	5.0	6.0	5.0	5.0	3.0	4.0	8.0	3.0	5.0	6.0	4.8
ptm14-118	5.0	4.0	5.0	6.0	5.0	5.0	3.0	3.0	8.0	3.0	7.0	5.0	4.9
ptm14-120	5.0	3.0	3.0	5.0	4.0	3.0	3.0	2.0	6.0	3.0	4.0	5.0	3.8
Ptm isolate	Arimon	Chebec	CI5286	CI5791	CI9214	CII615	Galleon	Keel	Kombar	Skiff	Torrens	TR250	Averag
ptm14-124	5.0	3.0	5.0	7.0	5.0	5.0	3.0	3.0	8.0	3.0	4.0	6.0	4.8

ptm15-030	4.0	3.0	3.0	5.0	3.0	3.0	4.0	2.0	8.0	4.0	4.0	5.0	4.0
ptm15-031	-	4.0	2.0	5.0	4.0	4.0	3.0	3.0	7.0	3.0	3.0	4.0	3.8
ptm15-033	4.0	3.0	3.0	6.0	3.0	5.0	4.0	3.0	8.0	3.0	4.0	4.0	4.2
ptm15-053	-	3.0	5.0	7.0	5.0	4.0	3.0	3.0	8.0	3.0	7.0	7.0	5.0
ptm15-055	5.0	3.0	5.0	7.0	3.0	5.0	4.0	3.0	8.0	3.0	4.0	5.0	4.6
ptm16-008	5.0	3.0	5.0	7.0	4.0	5.0	3.0	3.0	8.0	3.0	5.0	6.0	4.8
ptm16-010	6.0	4.0	5.0	5.0	5.0	6.0	5.0	2.0	8.0	4.0	5.0	6.0	5.1
ptm16-013	5.0	3.0	4.0	6.0	5.0	5.0	4.0	3.0	8.0	4.0	5.0	6.0	4.8
ptm16-017	7.0	5.0	5.0	7.0	5.0	6.0	4.0	2.0	8.0	4.0	5.0	6.0	5.3
ptm16-018	5.0	4.0	4.0	6.0	4.0	5.0	4.0	3.0	6.0	4.0	5.0	6.0	4.7
ptm16-019	6.0	3.0	5.0	5.0	4.0	5.0	5.0	2.0	7.0	4.0	5.0	4.0	4.6
ptm16-020	4.0	3.0	4.0	6.0	3.0	6.0	4.0	2.0	8.0	4.0	5.0	5.0	4.5
ptm16-023	6.0	3.0	5.0	6.0	4.0	6.0	4.0	3.0	8.0	4.0	4.0	6.0	4.9
ptm16-024	5.0	3.0	5.0	6.0	5.0	6.0	5.0	3.0	8.0	3.0	5.0	6.0	5.0
ptm16-027	5.0	3.0	5.0	5.0	5.0	6.0	4.0	3.0	8.0	3.0	4.0	6.0	4.8
ptm16-028	6.0	4.0	5.0	6.0	3.0	6.0	5.0	3.0	8.0	5.0	5.0	7.0	5.3
ptm16-029	6.0	4.0	5.0	5.0	7.0	6.0	5.0	3.0	8.0	5.0	5.0	6.0	5.4
ptm16-034	6.0	3.0	5.0	6.0	5.0	5.0	4.0	2.0	8.0	5.0	6.0	6.0	5.1
ptm16-037	5.0	3.0	5.0	5.0	5.0	5.0	4.0	3.0	8.0	3.0	5.0	6.0	4.8
ptm16-038	6.0	3.0	5.0	6.0	6.0	6.0	3.0	3.0	8.0	4.0	6.0	7.0	5.3
ptm16-049	6.0	4.0	5.0	6.0	5.0	5.0	4.0	4.0	8.0	3.0	-	6.0	5.1
ptm16-054a	5.0	3.0	5.0	6.0	5.0	6.0	4.0	2.0	8.0	3.0	6.0	7.0	5.0
ptm16-055a	6.0	3.0	5.0	6.0	4.0	5.0	4.0	3.0	8.0	4.0	5.0	7.0	5.0
ptm16-061	5.0	3.0	5.0	6.0	4.0	7.0	4.0	3.0	8.0	4.0	6.0	6.0	5.1
ptm16-062	6.0	3.0	5.0	7.0	4.0	6.0	5.0	3.0	8.0	4.0	6.0	7.0	5.3
ptm16-065	5.0	3.0	5.0	6.0	4.0	6.0	3.0	3.0	8.0	3.0	6.0	6.0	4.8
Ptm16-068	6.0	4.0	5.0	6.0	4.0	5.0	4.0	2.0	8.0	4.0	5.0	5.0	4.8
ptm16-129	5.0	3.0	4.0	5.0	3.0	5.0	4.0	2.0	8.0	3.0	5.0	5.0	4.3
ptm16-131	5.0	4.0	5.0	5.0	5.0	6.0	3.0	4.0	8.0	5.0	5.0	6.0	5.1
Ptm isolate	Arimon	Chebec	CI5286	CI5791	CI9214	CII615	Galleon	Keel	Kombar	Skiff	Torrens	TR250	Averag
ptm16-143	5.0	3.0	5.0	6.0	5.0	5.0	3.0	3.0	8.0	3.0	5.0	6.0	4.8
sf03-0133ss	6.0	3.0	5.0	4.0	3.0	NA	5.0	3.0	8.0	5.0	6.0	7.0	5.0

SNB113	4.7	5.8	6.0	5.0	6.0	7.3	3.5	3.5	7.8	4.3	6.7	7.3	5.7
Average	5.1	3.4	4.5	5.6	4.3	4.9	3.9	3.0	7.0	3.5	4.9	5.4	

Supplementary Table 3: List of genes positions identified within QTL regions by Bedtools intersect v2.29.0.

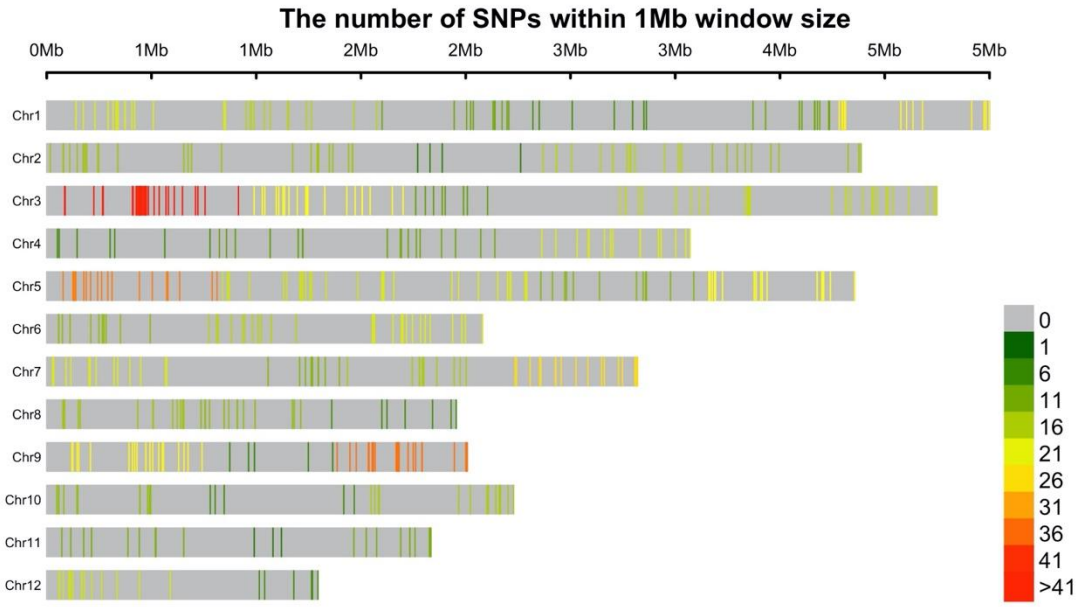
Chromosome	Start position (bp)	End position (bp)
1	2432065	2433159
1	2433352	2434167
1	2435214	2441609
1	2443002	2444073
1	2444883	2445566
1	2445724	2446683
1	2448822	2450101
1	2450903	2451160
1	2451678	2453300
1	2454758	2456027
1	2458166	2458509
1	2458879	2461152
2	1470120	1471565
2	1472537	1473892
2	1474685	1475977
2	1476682	1478405
2	1478767	1480610
2	1485280	1486878
2	1489076	1490140
2	1490483	1492592
2	1493162	1494858
2	1662418	1663143
2	1664018	1665897
2	1666692	1670896
2	1671885	1672892
2	1673526	1675492
2	1677055	1679047

Chromosome	Start position (bp)	End position (bp)
2	1679711	1683929
2	1684881	1685886
2	1687614	1687979
2	2865631	2867283
2	2870924	2871924
2	2873040	2875613
2	2876222	2877744
2	2879003	2882410
2	2884956	2885507
2	2887026	2887377
2	2889312	2890139
2	2891296	2892592
3	242776	244777
3	245185	246363
3	247021	248628
3	249634	250737
3	252522	255755
3	256939	257319
3	258787	259570
3	260425	260995
3	263592	264425
3	265038	266807
3	268151	269424
3	269675	270150
3	270502	271442
4	2147270	2155718
4	2156443	2158092
4	2159942	2160789
4	2161346	2163300

Chromosome	Start position (bp)	End position (bp)
4	2163680	2164531
4	2165295	2166263
4	2168028	2172170
4	2173707	2177231
4	2178332	2179363
5	1389830	1391727
5	1391886	1393263
5	1394882	1395312
5	1396777	1397187
5	1399427	1400315
5	1401230	1401881
5	1402933	1404502
5	1404606	1406546
5	1407000	1410479
5	1410888	1412195
5	1413192	1413407
5	1416228	1417685
5	1431494	1433641
5	1436738	1438838
5	1440006	1441201
5	1441749	1442930
5	1443128	1443616
5	1445441	1445908
5	1446368	1448030
5	1448778	1450128
5	1450385	1452451
5	1452820	1454379
5	1454497	1455849
5	1458469	1460913

Chromosome	Start position (bp)	End position (bp)
6	1771775	1772095
6	1774401	1775846
6	1778753	1779032
6	1781561	1782871
6	1784878	1786176
6	1787045	1788885
6	1789727	1793306
6	1798728	1801247
6	2038175	2038660
6	2039828	2040415
6	2042145	2043585
6	2044157	2045312
6	2047168	2047569
6	2048495	2050444
6	2050974	2055681
6	2057491	2059482
6	2060046	2060562
6	2061019	2061567
6	2062015	2062675
7	2285715	2286565
7	2287574	2289598
7	2289953	2291912
7	2292505	2293707
7	2294894	2295909
7	2295953	2297132
7	2298908	2302995
7	2305935	2306701
7	2306892	2307975
7	2308506	2309115

Chromosome	Start position (bp)	End position (bp)
7	2309842	2310836
7	2311318	2312286
7	3043027	3043632
7	3043761	3046901
7	3048229	3051994
7	3052707	3054981
7	3056644	3057483
7	3060393	3061064
7	3061969	3063423
7	3064328	3065473
7	3069774	3071936



Supplementary Fig. 1 Distribution and density of DArTseq™ markers used in genome-wide association mapping across the 12 *Pyrenophora teres* f. *maculata* chromosomes

Chapter-3

Supplementary Table 1: List of gene positions identified within QTL regions by Bedtools intersect v2.29.0

Chromosome	Start position (bp)	End position (bp)
2	1451228	1453021
2	1454560	1455180
2	1455563	1456523
2	1457276	1462909
2	1463093	1464545
2	1464973	1466060
2	1466354	1467688
2	1467919	1469350
2	1470120	1471565
2	1472537	1473892
2	1474685	1475977
2	1476682	1478405
2	1478767	1480610
2	1485280	1486878
2	1489076	1490140
2	1490483	1492592
2	1493162	1494858
2	1498197	1500209
2	1502473	1504236
2	1504834	1505939
2	1506977	1508038
2	1509193	1509828
2	1511900	1513809
2	1514878	1516077
2	1518418	1519421
2	1519872	1520706

Chromosome	Start position (bp)	End position (bp)
2	1521965	1525116
2	1526139	1528237
2	1529200	1529515
2	1530753	1531959
2	1535542	1537125
2	1537813	1538656
2	1539195	1541348
2	1541888	1542924
2	1545120	1545763
2	1547316	1547826
2	1549675	1552348
2	1552843	1553288
2	1553796	1555829
2	1559397	1561481
2	1562635	1563699
2	1564238	1566286
2	1567574	1568410
2	1568463	1569332
2	1569612	1570421
2	1573254	1574619
2	1575069	1576175
2	1576773	1579052
2	1579287	1580279
2	1583549	1583977
2	1584680	1585984
2	1586222	1587671
2	1589986	1592457
2	1596587	1598094
2	1602249	1603217

Chromosome	Start position (bp)	End position (bp)
2	1605148	1607165
2	1608301	1609146
2	1610706	1611565
2	1612126	1613638
2	1614496	1616344
2	1618553	1619820
2	1624254	1625390
2	1627105	1628117
2	1628333	1629892
2	1630326	1635041
2	1635333	1637048
2	1639648	1644811
2	1645533	1646898
2	1647570	1647907
2	1648505	1650898
2	1651877	1655679
2	1657412	1658008
2	1659717	1662001
2	1662418	1663143
2	1664018	1665897
2	1666692	1670896
2	1671885	1672892
2	1673526	1675492
2	1677055	1679047
2	1679711	1683929
2	1684881	1685886
2	1687614	1687979
2	1691942	1692859
2	1693007	1694099

Chromosome	Start position (bp)	End position (bp)
2	1694678	1696727
2	1696893	1698245
2	1699287	1699772
2	1700051	1700965
2	1703318	1706525
2	1707857	1710953
2	1713455	1714243
2	1714487	1715307
2	1717887	1719141
2	1719457	1720666
2	1721053	1722461
2	1723729	1724826
2	1729293	1732159
2	1732602	1733246
2	1734250	1735007
2	1735686	1736951
2	1737648	1740861
2	1742120	1744325
2	1744683	1747342
2	1747769	1749020
2	1749273	1749917
2	1750074	1751912
2	1752826	1754755
2	1758039	1758245
2	1758767	1761237
2	1762146	1762538
2	1764865	1771529
2	1772800	1775489
2	1776480	1778422

Chromosome	Start position (bp)	End position (bp)
2	1780987	1783398
2	1794580	1799902
2	1806415	1809345
2	1811514	1812837
2	1815054	1818004
2	1818461	1819078
2	1819460	1820836
2	1822380	1823931
2	1825833	1828069
2	1828346	1830382
2	1830931	1832178
2	1833686	1835521
2	1835867	1837021
2	1837843	1838709
2	1840164	1842122
2	1843247	1845451
2	1846633	1848438
2	1848864	1849667
2	1850487	1852248
2	1854013	1854802
2	1855642	1856661
2	1857365	1858577
2	1858886	1859889
2	1861017	1862747
2	1863691	1864862
2	1866085	1868982
2	1869664	1872779
2	1874003	1875311
2	1875945	1876500

Chromosome	Start position (bp)	End position (bp)
2	1876809	1878364
2	1879426	1881174
2	1882715	1882963
2	1883768	1884872
2	1885125	1885517
2	1886834	1888457
2	1889962	1891047
2	1891148	1892740
2	1893390	1894424
2	1895167	1896573
2	1897170	1897958
2	1897968	1900806
2	1901942	1903434
2	1904685	1905897
2	1906346	1908457
2	1910289	1910774
2	1911060	1913107
2	1914038	1915271
2	1917605	1919451
2	1920161	1921270
2	1921862	1924273
2	1924627	1929850
2	1930314	1930943
2	1932340	1933145
2	1933323	1934492
2	1935547	1939114
2	1940019	1941425
2	1942005	1945121
2	1947245	1948739

Chromosome	Start position (bp)	End position (bp)
2	1949241	1951952
2	1952450	1954954
2	1955143	1957032
2	1957512	1958534
2	1959257	1960622
2	1960897	1961277
2	1961821	1964281
2	1966427	1967095
2	1967997	1969254
2	1969850	1970738
2	1971181	1972813
2	1974519	1977572
2	1978234	1979646
2	1980118	1981201
2	1981834	1984395
2	1984699	1986265
2	1986763	1987878
2	1988287	1988771
2	1989119	1990780
2	1991278	1992604
2	1993841	1995898
2	1996819	1999268
2	2001038	2008971
2	2009696	2010788
2	2011494	2013146
2	2014374	2015432
2	2017042	2021298
2	2023046	2024719
2	2025117	2026475

Chromosome	Start position (bp)	End position (bp)
2	2028331	2028957
2	2029649	2032377
2	2032788	2033747
3	730471	748888
3	752327	753269
3	753476	754162
3	754520	756409
3	760219	764250
3	765240	767303
3	767666	769710
3	770481	772289
3	775120	776376
3	780987	782464
3	783047	784456
3	785022	787193
3	787833	788373
3	789014	789448
3	792019	792696
3	793780	795736
3	797566	799030
3	802167	803591
3	809257	810450
3	810643	811404
3	815380	815841
3	818231	819624
3	822398	824076
3	828824	829538
3	830569	831961
3	833307	836531

Chromosome	Start position (bp)	End position (bp)
3	839285	841794
3	843743	846562
3	846756	847980
3	848427	850881
3	852521	855188
3	855809	856231
3	857727	859433
3	863049	863736
3	864254	865483
3	865695	867222
3	867800	870898
3	871256	871864
3	872299	873430
3	878659	880798
3	889036	890880
3	896114	897144
3	897536	898994
3	900251	901997
3	908637	912345
3	915289	916164
3	916219	918545
3	919855	921434
3	932719	933231
3	936711	937229
3	943804	944952
3	945442	946134
3	953439	953957
3	957437	957949
3	963329	987170

Chromosome	Start position (bp)	End position (bp)
3	988434	988887
3	990841	992064
3	995970	997889
3	998640	999476
3	999761	1001885
3	1002592	1003140
3	1008532	1009881
3	1010450	1011894
3	1015363	1017728
3	1020757	1021903
3	1022468	1024899
3	1026402	1028584
3	1030884	1032038
3	1049315	1050817
3	1051838	1055330
3	1055958	1056815
3	1061804	1062344
3	1072669	1077957
3	1081115	1083298
3	1083670	1084313
3	1084891	1087170
3	1087664	1091444
3	1091817	1094721
3	1095171	1098525
3	1098811	1099267
3	1103111	1103524
3	1104086	1104943
3	1105708	1108301
3	1108690	1109415

Chromosome	Start position (bp)	End position (bp)
3	1109565	1111318
3	1111665	1114318
3	1114465	1116083
3	1116710	1118452
3	1119436	1121472
3	1123094	1124449
3	1124757	1126827
3	1127390	1132861
3	1135107	1135606
3	1140937	1158447
3	1178224	1183278
3	1183663	1185297
3	1185498	1186578
3	1191763	1192080
3	1192968	1194315
3	1201629	1202114
3	1204830	1206086
3	1208069	1210621
3	1211307	1212338
3	1212701	1214623
3	1215107	1216705
3	1221834	1222392
3	1225002	1225727
3	1226742	1227643
3	1228110	1228722
3	1229105	1230533
3	1232238	1234366
3	1234544	1235823
3	1236707	1238523

Chromosome	Start position (bp)	End position (bp)
3	1238880	1240768
3	1241585	1242612
3	1243137	1243696
3	1243959	1248478
3	1248951	1249883
3	1250347	1251396
3	1252982	1254336
3	1255784	1256243
3	1260262	1261137
3	1262221	1263204
3	1264618	1265994
3	1266405	1267115
3	1270956	1272779
3	1273830	1279457
3	1280710	1283076
3	1294979	1296076
3	1298456	1302229
3	1307273	1307720
3	1309973	1310740
3	1313104	1313932
3	1314680	1317619
3	1319521	1321114
3	1322706	1323371
3	1325928	1327780
3	1329404	1330224
3	1330524	1331315
3	1331560	1332041
3	1332279	1332971
3	1334137	1334430

Chromosome	Start position (bp)	End position (bp)
3	1335552	1337296
3	1339978	1340702
3	1341397	1343242
3	1343903	1344112
3	1345150	1347157
3	1347325	1348002
3	1349499	1351055
3	1352842	1356581
3	1357019	1357819
3	1362541	1363804
3	1371515	1374169
3	1377013	1377369
3	1378515	1379368
3	1380271	1382254
3	1387191	1387886
3	1392437	1394325
3	1401042	1402295
3	1405192	1424210
3	1428673	1429107
3	1432427	1435731
3	1437904	1438716
3	1438763	1441789
3	1442550	1443162
3	1444072	1447134
3	1447623	1451126
3	1451929	1453193
3	1454779	1459107
3	1460523	1463565
3	1463975	1465185

Chromosome	Start position (bp)	End position (bp)
3	1466372	1467847
3	1468475	1469486
3	1469706	1475366
3	1484805	1485346
3	1511762	1512598
3	1513804	1515528
3	1516640	1518249

Supplementary Table 2: Phenotypic scores of hybrid progeny and parents when inculated on four barley genotypes.

Isolate number	Oxford	Skiff	TR250	Gairdner
2	4	5	2	3.34
17	2.67	4.34	1.34	4.34
21	5	4	1.34	5.34
23	3.5	3	2.5	4.5
26	4	5.5	1.5	3
33	3.5	2.5	2.5	4.5
34	1	1.5	1.5	3.5
37	6.5	3.5	5	6
38	2.5	1	3.5	5.5
42	3	2	4.5	7.5
43	2	1.5	1.5	2
46	3.34	2.34	1.67	4.67
49	4	1.5	3.5	5.5
53	5.67	3.34	4.34	4.5
54	1	1	2.5	-
55	4.75	2	4.25	4.67
57	6.25	6.34	6.67	7
58	5.5	4.5	1	5.5
59	1.5	1.5	1.5	1.5
60	2.5	3.5	2	4.5
61	5	2	3	6
64	1	2.5	1	3
66	6	6	7	6
68	6.5	3	5.5	6
70	1.5	1.5	3	3
71	1	3.5	1	1
74	3.5	2	3.5	3
79	5.5	2.5	4	6
Isolate number	Oxford	Skiff	TR250	Gairdner

81	1	2	1	4
82	2.67	3	1	4
85	-	-	-	-
86	3.5	2	5	4
87	-	-	-	-
89	-	-	-	-
90	4	4	4	5.75
92	6.5	6.5	5.5	-
94	6.5	6.5	3.67	6.5
95	2	3.34	1	4
114	2.5	2.5	2.5	4
119	3.5	2	3.5	6
120	1	1.5	1	3
121	3	2	1	2
122	4	3	2	5
123	2	2	4.5	3
124	3	1.67	1	1
125	2.5	3.5	3.5	5
126	3.67	4	1	4
127	6	1	2.5	-
128	1.5	1.5	1.5	3
129	4	1.67	1	5
131	2	4.67	1	1.5
132	3.25	1.75	3.75	3.75
133	-	-	-	-
134	1.75	2.25	1.75	3.75
135	-	-	-	-
137	5	3.5	4.5	5.5
138	3	3.5	3	4.5
139	5.34	1.67	4.34	4
Isolate number	Oxford	Skiff	TR250	Gairdner
140	3.5	2	2	3.5

141	4.5	2.5	3.5	3.5
142	5	4	1.34	5.34
143	4	4	1	5
205	-	-	-	-
206	1	1	1.25	1.25
208	1.34	1.34	3	-
211	4	1.5	2.5	-
215	4	2	4	5
217	3	2	2.5	4.5
223	1	4	1	3
232	4	3	2	4
233	-	-	-	-
237	-	-	-	-
238	-	-	-	-
240	1.67	1.34	1	1.67
241	4	1	1	4
242	3	2	2	3
243	5.5	4.5	3.5	5.5
246	5.5	5.5	1.5	5.5
247	1.75	3	4.25	4.67
249	5	5.34	5.34	5.34
251	-	-	-	-
253	4	3	4	4
254	1	1	1	2.5
255	1.5	2.5	4.5	5.5
256	3.5	2.5	4	3.5
258	2	1.67	4	4
259	4	3	3	5
260	2.75	1.5	3.25	4.67
Isolate number	Oxford	Skiff	TR250	Gairdner
261	-	-	-	-
262	5.5	5.5	4	4.5

264	5	5.34	3	3
265	7.5	6	6.5	-
266	3	3	1.34	4
267	1.5	1	1.5	4.5
268	5	3	4.67	6
270	1	1	1	2
271	4.5	2	3	5.5
273	3.5	1	3.5	4.5
275	3.34	2.67	4	4.5
276	-	-	-	-
277	4.5	3	3	5.5
279	5	2	3.34	5.67
280	3	1.5	4	-
281	1.5	1.5	2.5	3.5
282	3	3.5	3.5	3.5
284	4	3.67	2	4.34
286	3.5	3	4	5
288	2	1	2	-
290	1	1	1	5
291	2	2	2	4.5
292	2	3	1	5
293	1	1	1	1.5
294	5	4	4	5
295	1.5	1	1	1
296	3.67	4	2	1
297	2.5	1.5	1.5	-
300	6	1.5	3.67	4.67
302	2.5	1.5	4.5	4.5
Isolate number	Oxford	Skiff	TR250	Gairdner
303	3.5	1	2.5	4.5
304	5	2.5	3	3.67
305	2	2	2	6

309	-	-	-	-
310	3.5	2	2.5	4.5
311	5.34	4	4.34	3
314	3	2	2.67	4.34
315	5	5.5	1.34	2.67
316	-	-	-	-
318	3.5	1.34	4	4
320	4	2	2.34	6.5
322	4	2.5	5.5	4.5
323	4.5	4.5	5	4.5
324	4	4	3.34	4.67
325	2.34	2	2	5.5
326	5.5	2	4.5	5.5
329	6	4.5	5	4.5
330	-	-	-	-
331	2	4	5	3.5
332	-	-	-	-
333	3.5	2.5	5	5.5
334	5.34	4.5	3.5	6
339	-	-	-	-
340	4.5	5.67	3	5
341	6	5	5.34	5
344	3.5	3.5	5.5	6
345	1.5	1.5	4	3.5
346	3.5	2	2.5	5
347	5.5	5.67	5.5	6.34
350	4	5	4	5
Isolate number	Oxford	Skiff	TR250	Gairdner
351	3.5	-	4.5	5
352	2	3.5	3	4.34
354	5.5	2	2	5
356	4	1.34	1.34	2.67

357	5.5	-	4	-
358	4	2.5	5.5	6
360	4.5	1	4.5	6
361	5	4	6	6
362	2	4.5	1.34	4
364	5.5	2	5.5	5.5
366	3	4	2.34	4
368	3	3	2.34	3
369	-	-	-	-
371	2	4.34	2	4.5
373	4.5	5	2.34	2.34
374	1	3.34	2	4
375	3	2.5	2.5	6.5
377	5.5	6.5	2.67	3
378	-	-	-	-
379	5.5	5.5	2.34	5
382	2.67	2	4	5.67
383	-	-	-	-
386	3	3.5	3	4
387	5	6	6	6
389	4.5	4	2	5
391	-	-	-	-
392	1.67	2.34	1.67	1
393	2.34	5	5.5	5
395	4.67	4	4	4.67
396	4	4.67	2.34	4
Isolate number	Oxford	Skiff	TR250	Gairdner
397	3	3.5	2.5	5.5
398	5	6	2.34	3
402	4	5.5	5.5	4
403	3.5	2.67	1.67	4
405	2.34	2.34	2.34	3

407	2	2	2	3
408	2.5	2	2	5.5
409	-	-	-	-
410	5.5	4.5	5	5
412	6	5	4.67	6
413	4.34	5	5	4.67
414	3.5	5.5	2	5.5
415	5.5	4	4	5
417	1	3	2	1
419	4.5	1.34	5	3.5
420	-	-	-	-
421	-	-	-	-
422	-	-	-	-
423	3	2	3	6
424	4	5	2	4
426	4.5	5	4.5	3
427	4.5	3.5	4	4
428	2.5	3.5	3.5	5.5
429	3	4.5	2.5	2.5
430	5.5	4.5	3	5
431	3	4	3	2
433	3.5	2	1.5	4.5
434	5.5	4.5	5.5	6.5
436	1	1	1	1
438	3.34	4	3.34	5
Isolate number	Oxford	Skiff	TR250	Gairdner
439	1.5	5.5	2.5	4.5
440	-	-	-	-
441	3.5	2.5	2	3.5
442	6	5.5	4.67	4.34
443	-	-	-	-
444	2	1	2.5	1.5

445	4	4	3.5	3.5
446	3.5	5.5	3.5	5.5
447	4	6	5	-
450	4.5	5.5	3	5.5
451	4.34	5.34	4	2.5
453	5	5.34	4.67	6
455	1	3	1.34	6.5
456	4.67	5.34	4.34	4.34
457	6	5.5	2.34	5.5
458	1	1	1	2
460	3.67	3	3	4
463	2	3.5	2.5	4.5
464	2	5.5	2.5	6
465	5.5	2.5	4.5	5.5
467	5.5	2	2.5	5.5
469	5.5	2.5	3	4.5
470	4.5	2.5	3	2.5
472	3.5	2.5	2.5	4
473	-	-	-	-
474	4.67	5	4.34	5
475	4.5	3	2.5	4
478	5.5	5	4	3.67
479	3.5	1.5	3.5	5.5
480	4.34	4.67	4	4.34
Isolate number	Oxford	Skiff	TR250	Gairdner
481	4.5	5.5	4	3.5
482	4.5	5.5	3	4.67
483	4.5	4.5	3.5	4.5
484	5.67	5.67	5.34	5.67
486	5	3	3	4
487	4.34	4.67	5	5
490	5.5	5.5	3	4

491	4.67	4.67	3.67	5.34
492	4.5	6	3.5	6
494	3	5	2	4
495	3.67	3	2	4
496	5.67	5.34	4	5
497	5	4.67	2	5.67
498	4.5	4.5	3	5.5
499	-	-	-	-
500	-	-	-	-
501	4.5	4.5	4.5	4.5
502	2.67	3.67	2.67	4.34
503	6	4.5	5	7
505	3.5	2	3.5	4.5
512	3.5	5.5	3.5	1
513	5	4	7	5.5
514	2.5	2.5	2	4.5
516	3.5	3.5	1.5	4.5
517	4.5	3.5	3.5	5.5
519	2	3.67	5.34	5.5
520	-	-	-	-
522	3.5	2.5	4.5	3.5
524	4.5	2	4.5	3.5
525	3.5	3	3.5	4.5
Isolate number	Oxford	Skiff	TR250	Gairdner
526	4.5	2.5	4.5	6
527	2.5	2	2	4.5
528	5.5	2	2.5	3.5
529	-	-	-	-
531	2	4	3	3
532	1.5	1	2	2.5
533	3.67	3.67	3.67	5
535	4.67	3.34	2.67	4.67

536	5	4	6	6
537	-	-	-	-
541	3.5	2	4.5	3
542	4.5	3.5	2.5	2.5
544	4	2.5	2	4.5
545	4.5	2.5	1.5	3
546	4	2	1.67	1.34
547	3.5	4.5	2.5	2.5
549	2	5.34	4	4.67
550	-	-	-	-
551	2.34	3	3.5	4
556	3	4	5	4
557	5.5	4	4.5	3.5
558	2.34	2.34	3	4.5
559	2.5	4.5	3.5	4.5
560	2	5	2	1
561	2.5	3.5	1.5	3.5
564	3	3.5	3	4.5
566	5.5	5	4.5	4.5
568	5.5	5.67	5	4.67
569	4.5	2.5	2.5	2.5
570	4	5.34	3	6
Isolate number	Oxford	Skiff	TR250	Gairdner
571	4.67	6	2.34	5.67
572	2.67	4	4	5
574	1	1	1	2
575	6.5	5.5	4	4.5
576	6	5	5	6
577	5	5	2.67	4
580	1.5	1	1.5	4.5
581	4.67	5.34	5	4
583	1	2	1	2

584	-	-	-	-
585	2	2	2	2.5
586	5	3.34	3	6
587	-	-	-	-
588	5	4	2	5
589	1	1	1	5
590	2.67	5.5	2.34	4.34
591	1.67	1.67	1.34	5.5
594	3	5	4	5.34
596	4	2.5	2	2.5
598	4.5	4.5	3.5	5.5
603	5.67	5	5	5
604	4.5	4.5	3	3.5
608	4	4.67	5	5
610	4.34	5.34	3	4
611	4.25	4.75	5.67	5
612	-	-	-	-
614	4.5	5.5	4.5	5
616	-	-	-	-
618	-	-	-	-
622	3.5	2	2	3.34
Isolate number	Oxford	Skiff	TR250	Gairdner
623	3	4.67	3.34	4.67
624	5	5	5	4
625	2.5	2	2	2.5
626	4.67	4.67	4	5
628	4.5	4.34	3.67	6
629	4	4.67	3	4.67
631	4	4.5	4	4.34
632	4.34	4	3.67	4.67
633	1.34	4.67	1.34	4
634	4.34	4.67	4.34	6

635	5.5	3.5	3	4.5
636	6	5	4	4
637	5.5	5.34	1.34	2
638	2	2	1.5	2
641	3.34	3.67	5	5.67
642	1	3.34	1.34	2
643	3	4.5	4.5	6
644	4	4.5	3.5	4.5
645	6.5	5	3	5.5
646	3.67	5.34	3.34	4.34
647	1.67	4.5	2	4
652	4	5	3	5.5
659	3	5	3	4
661	1.67	3.5	3	4
16frgo73	5.5	4.5	3	4
SG1	5.5	5.5	3.67	5.5

Chapter-4

Supplementary Table 1: Scores of four *in-vitro* Ptt/Ptm populations. Green colour indicates avirulent responses, yellow indicates intermediate responses and red indicates virulent responses.

	Isolate	Arimont	Beecher	Cape	CI11458	CI5286	CIhs5791	Commander	Corvette	Galleon	Hindmarsh	Keel	Kombar	Maritime	Prior	Rosalind	Skiff	Tallon	Torrens	TR250	Vlamingh	Average Lesion type*
HRS07033XNB63	229	5.3	6.4	5.7	6.4	5.7	5.7	5.4	5	6.4	5	5.4	5.7	4.4	5.4	5	5.7	5.7	5.7	4.7	5.7	5.5 H
	235	5.7	6	6.4	6	5.4	5.4	5.7	5	5.67	5.4	4	6.4	5.7	6	6	4	6.7	6	5.4	6	5.6 S
	237	4.7	6.4	5.7	5.7	5	6.4	6	5.7	5.33	5.7	4.4	6.7	5	6.4	6	5.4	6	5.7	6.4	6	5.7 H
	241	4.3	4.4	6.4	7	6.4	6.4	6.4	6.4	7	6.4	6.4	6.7	5.4	6.4	7	6.7	6.4	6.7	6.4	5.4	6.2 H
	244	5.7	6.4	6	6.7	6	6.7	7	6	6.67	6	6	7	6	6	6.4	6.4	6	6	5.7	5.7	6.3 H
	245	5.5	5.7	6	6	6	5.4	5	4.4	6	5.7	5.4	6.4	4.7	5.7	5.7	5.7	6	6	4.7	5.4	5.6 S
	251	3.3	5.4	5.4	5	5	5.5	5.4	5	4.67	5.7	4	5.4	5.4	5.7	5.7	5	6.5	5.4	5	6	5.2 H
	258	5.3	5.7	6	6	6	5.7	6	5	5.67	6	5.7	5.7	6	6.4	6.4	5.5	6	6	5.7	6.4	5.9 S
	266	5.3	6	5.7	5.4	5.7	6	6	5.4	4.33	5.4	4.4	6.7	4.4	5.4	5	5.4	6	5.4	4.4	5	5.4 S
	279	5	6	6.4	6	6	5	6	5	5	6	4.4	6	5.4	6.4	6.4	4	5.5	6	6.4	5.7	5.7 N
	281	5	6	6	7	5	6	7	6	6	6	7	7	6	6	7	7	6	5	6	7	6.2 S
	284	5.7	6.4	5.7	6	5	6	6.4	6.4	4.33	6.4	5	6.4	6	6.7	6.4	4	6.4	6.4	6.4	6	5.9 S
	305	4	5.7	5.4	5.4	5	4.7	5.4	5	4.67	5.4	3.7	5.7	5.4	5.7	6	4	5.4	5.7	6	5.4	5.2 S
	314	5.7	7	6.7	7	7	6	6.7	6	6.33	6.7	5.7	7	6	6.4	7	6	7	6.7	6.7	6.7	6.6 S
	317	3.3	5.4	3.7	4	3.7	5.4	4.4	4	5.33	5.4	3.7	4.7	4.7	5.4	5.5	4.4	6	5	4.4	5.4	4.7 S
	HRS07033	4	6	5	5	3	4	4.5	3	5.5	5.5	4.5	7	2.5	6.5	3.5	5	6	5.5	3	4.5	4.7 S
	NB63	4.5	2.5	3	2	8	1	8	2	7	8	8	5	5	8.5	5	3.5	2	7.5	1	1	4.6 N
NB73XSNB171i	1	5.3	3.4	1.4	2.4	4.4	3.7	2.4	5	4.33	4.7	3.4	6.4	5.7	6.4	4.4	3.4	6.4	6.4	3.7	6.4	4.5 N
	3	6.7	6.4	6	7	7	6	5.4	6.4	7	7.4	6.4	7	6	6	7	6.4	6.4	5.7	6.4	6	6.4 N
	4	5	5.7	6.4	6	5.7	6	6.7	6	5.67	6.4	5.7	6.7	5.4	5.4	5.7	5.7	6.4	6	6	6.4	6 S
	5	5.7	6	5.4	5.7	4.7	5.7	5.7	5.5	4.67	5.7	4.7	5	2.4	5.7	5	5	5.7	5.4	1	4	5 S
	6	5.3	5.7	6.4	5.7	4	5.4	6	6	4.33	6	5.4	6.4	5.4	6	6	5	6	6.4	6.4	6	5.7 N
	7	6.7	6.7	6.7	6.7	6.7	6	7	7.4	6.67	7.7	5.7	6	6	6	7	6.7	7.7	7	6	6	6.7 H
	9	4.3	3.4	4.4	2.4	2.4	2.4	2.4	1.4	3.33	6.4	2.4	2.4	5.4	4.4	4.4	3.4	6.4	6.4	3.4	1.7	3.6 S
	11	4.3	5.7	5	4.4	5.4	6	4.7	4.7	5.67	6	4	5.7	5	6	5.4	4.4	6	5.7	5	5	5.2 N
	12	5.3	3	4.4	4.4	5.4	3.4	1.7	1.4	6.67	3.4	5.4	6.4	6.4	5.4	4.4	5.4	6.4	6.4	2.4	1.4	4.4 S
	13	6	6.4	6	6	7	5.4	5.4	6	6	6.4	5.7	7	6.4	6.4	6	5.7	6	6	6	6	6.1 S
	14	6	5.4	6.7	6	6	6.7	6.7	6.4	6.67	7	6	6.7	6.4	6.7	7	5.7	5.4	5.7	6.7	6.4	6.3 S
	16	1.3	1.4	1.4	3	1.4	1.4	1	1.4	2.67	2	2.4	2	1.4	2.7	1	2	1.4	1.4	2.7	2.7	1.8 S
	17	3	2.7	4.4	5	2.7	4	2.4	3.7	4.33	4.7	3.4	5.4	4.4	3.7	4.4	2.4	4.7	2.4	3	1.7	3.6 H
	18	4	7.4	5	5.7	5.4	6.4	6.4	5.7	6	6.4	5.7	6	6.4	6	6.7	6	7.4	5.7	5.4	6.4	6 S
	19	4.3	4.4	1.4	5	1.4	2.4	1	1.4	1.33	5.4	1.4	1.4	1.4	1.4	5.4	1.4	6.4	6.4	3.4	3.4	3 N
	NB73	3.3	2.4	2.4	7	6.4	2	7.4	7	6	6.4	6.7	5.4	2	2	2.4	6.7	6.4	3.7	2	2.7	4.5 N
	SNB171i	5.7	5.4	6	5.5	6.7	5.4	1.4	1.5	3	3	2.7	1.7	1.7	5.4	3.4	2	1.4	3.7	1.7	2.4	3.5 S
KO103X16FRGO73	1	4.3	4.5	5	4	2.7	5.5	4.5	4	2.67	4.4	4	2.4	4.4	6.5	4	2.5	4	4.4	3	5	4.1 S
	2	4	4	4	5	4.4	5	5.7	2.4	4	5.4	4.4	6.7	3	5.7	5	5.7	5.7	5	2	4.7	4.7 S
	10	6	6.7	7	6.7	6	7	6.4	6.7	6.33	7	6	7	6	7.5	6.7	6.7	7	7	5.5	6	6.6 S
	11	4.7	1	6	5.7	4.7	4.7	1	2.7	5	1	4.4	5.7	5.4	6	1	6	6	5.7	3.7	3	4 S
	14	2.7	0.4	3	1.7	1	3	0.7	1	1.33	1	1	1.4	3.7	1.7	1	0.7	2.7	2.7	1.7	2	1.8 S
	15	2.7	1	1.7	1.7	3	2	0.7	0.7	1.67	0.7	2	1.7	1.5	1.4	1	1.4	2.7	2	2	2	1.7 S
	16	1.3	1.4	1.4	1.4	1.4	4.4	6	3.4	6.33	0.4	6.4	6	4.4	1.4	4.4	6.4	5.4	5.5	1.4	4	3.6 H
	19	7.3	6.7	7.4	7.7	6	6.7	7	6.5	7	7	5.7	7.7	4.7	7	7.5	6.4	6.7	7	6.4	6	6.8 S
	2	4	4	4	5	4.4	5	5.7	2.4	4	5.4	4.4	6.7	3	5.7	5	5.7	5.7	5	2	4.7	4.6 S
	20	5	5.7	6	6	3.7	4.4	6	4	3.67	5.7	5	6	3.5	6	5.5	5.7	6.7	5.7	4	6	5.3 S
	22	2.7	1	1.7	1.7	3	2	0.7	0.7	1.67	0.7	2	1.7	1.5	1.4	1	1.4	2.7	2	2	2	1.7 S
	23	3.3	6.4	6.4	4.4	5.4	4.4	2.4	3.4	3.33	4.4	3.4	5.4	3.4	2.4	4.4	3.4	6.4	4.4	3.4	5.4	4.3 S
	26	1.7	2.4	2.4	2.4	2.4	0.4	1.4	1.4	1.33	1.4	2.4	1	1.4	2.4	1.4	2.4	2.4	1.4	2.4	1.4	1.8 S
	27	1	5	5	6	4	4	6	4	4	2	2	6	4	5	2	2	6	4	3	6	4.1 S
W1X16FRGO73	6	1.3	2.4	2.4	2.4	2.4	1.4	1.4	1.4	1.33	1.4	2.4	1.4	0.4	2.4	1.4	2.4	2.4	1.4	2.4	1.4	1.8 N
	KO103	5	3	5.4	5	5.7	2.4	5.7	4	5.33	5.7	5.7	5.4	5.4	6	4.7	6	4.4	4.4	3.4	3.4	4.8 N
	16FRGO73	4	3.7	3.7	5	5.7	5.7	3.7	2.4	5.33	3.4	5.4	4.4	2.7	3.4	3.4	4.4	4.4	3.4	5.7	3.7	4.2 S
	1	1.7	2	2	2.7	1.7	2	2.7	1.7	1	2.5	1.4	1.7	1.7	1.5	1.7	2	2.7	1.7	1.7	1.7	1.9 S
	2	1.7	1.7	1.4	2	1.7	1.7	1.7	2.4	1.67	1.4	1.7	1	2	2.4	1.7	2.4	3	2.4	2	2.4	1.9 S
	3	1.7	1.7	1.7	1.7	1.7	2.4	1.7	1.7	2	1.7	1.4	1.7	1.7	2	1.7	2.4	4	2	2.7	2.4	2 S
	4	2.7	1.4	1.4	1.7	1.4	1.7	1.4	1	1.67	2.4	1.4	1.7	1.7	1.4	2	2	1.4	2.4	2	1.4	1.7 S
	5	2	2.7	2	4	3.4	1.7	2.4	2	1.67	1.7	1.7	2	2	2	2.4	3.4	3.4	2	1.4	1.7	2.3 S
	6	1.3	1.4	1	2.7	1.4	2	1.4	2.7	1.67	2.7	1.4	2	2.4	1	2	2	4	2.4	1.4	2.4	2 S
	7	5	2.4	5.7	3	4.7	5.4	2.4	4.7	4.67	4.7	4.7	5.4	5.7	5.4	2.4	5.4	5.4	4.7	4.7	5.4	4.6 S
	8	1	1.5	1	2.5	1	2	1	2.5	1.5	2.5	3	1.5	2	2	1.5	3	3.5	2	2.5	2	2 S
	9	2	2	1.7	2	2	1.7	1.4	1	1.67	2	1.7	1.4	3.4	2.4	1.7	1.4	2.4	1.7	1	1.7	1.8 S
	11	1.3	1	1.7	2.7	2.4	1.7	1.4	2.7	1.67	2	1.7	1.4	2.4	2.7	2	1.4	1.7	2	2	2	1.9 H
	12	1.7	1.7	1.4	1.7	1.7	1.7	1.7	1.7	1.33	1.7	1	1.7	2.4	1.7	1.7	1.7	1.4	2.7	2.7	1.7	1.8 S
	13	2	1.5	2.5	2.5	2	2	1	2.5	1.5	2	1.5	1	2	1	2	1.5	3	2.5	2.5	2	2 S
	15	4	4.4	3.7	4	4	3.4	4	4.7	2.67	3.7	2.7	4	5.4	4	2.7	5	3.7	3.7	2.7	3.7	3.8 S
	16	2.3	2.4	2.7	3	2.7	1.4	3	2.7	2.67	4	4	3.4	2.7	2.4	2.4	4	2.7	2.7	3.7	2	2.9 S
	W1	3.7	2.4	4.4	2.4	3.7	2.4	5.7	3.4	1.34	2.4	4.7	5.4	4.4	4.4	2.4	2.4	4.4	4.4	2.7	2	3.5 N
	16FRGO73	4	3.7	3.7	5	5.7	5.7	3.7	2.4	5.33	3.4	5.4	4.4	2.7	3.4	3.4	4.4	4.4	3.4	5.7	3.7	4.2 S

*H indicates hybrid symptoms, N indicates net form symptoms and S indicates spot form symptoms