CHARACTERIZATION OF *PYRENOPHORA TERES* ISOLATES AND MAPPING OF VIRULENCE GENES

A Thesis submitted by

Buddhika Amarasinghe Dahanayaka, (MPhil)

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ABSTRACT

Pyrenophora teres is the causative agent of net blotch of barley and one of the most economically important fungal pathogens affecting the Australian barley industry. The estimated yield losses due to net blotch exceed $300 million annually. It is a foliar pathogen that exists as two forms: *P. teres* f. *teres* (*Ptt*) and *P. teres* f. *maculata* (*Ptm*), causing net form-net blotch (NFNB) and spot form-net blotch (SFNB), respectively. In order to address some of the research gaps in our understanding of the *P. teres*-barley pathosystem, this project was designed to: 1. genetically characterise *Pyrenophora teres* f. *teres* populations collected from different continents; 2. investigate the mating preference between the two forms of *P. teres* and its hybrids; and 3. identify QTL (quantitative trait loci)/genes associated with virulence of *P. teres* using a hybrid population developed by crossing the two forms.

The genetic characterization of a pathogen population is important in understanding the genetic variation existing within a pathogen population. In this study we characterized the most geographically diverse *Ptt* population investigated in a single study using the genome-wide marker system DArTseq. Results obtained from different cluster analyses revealed that an Australian *Ptt* population shared more admixture with a Republic of South Africa (RSA) population than a Hungarian *Ptt* population. Neighbor-joining dendrogram analysis and form-specific PCR amplifications detected two field collected hybrids from Hungary (H-919) and Japan (CBS 281.31). CBS 281.31 was a historical isolate which was previously authenticated as *P. japonica*. Evidence for recent/ongoing gene migration among different continents was observed, which highlights the importance of practicing biosafety measures to prevent the introduction of a pathogenic gene pool from one geographical region to another.

After establishing that hybridization between the two forms of *P. teres* are more frequent than previously assumed, we investigated whether *Ptt* and *Ptm* have a mating preference for the same form over the opposite form *in vitro* when they were given the opportunity to mate with both forms. Results revealed that *Ptt* isolates preferred *Ptt* isolates at the early reproduction stage, however, later they did not have any preference but hybridized with *Ptm*. *Ptm* isolates did not have any preference toward isolates from the same form but underwent hybridization with *Ptt* isolates. Results also showed that *Ptt* isolates had greater reproduction vigour than *Ptm* isolates under the given laboratory conditions.

Progeny arising from a hybrid cross could have devastating effects on the barley industry in the absence of suitable resistant barely varieties as these hybrids may acquire combined virulence from both *Ptt* and *Ptm*. Hence, comprehensive knowledge of hybrids and the *P. teres*-

barley pathogen system would allow barley breeders to develop novel barley germplasms to withstand potential future outbreaks caused by hybrids. Therefore, as a part of this study we identified QTL/genomic regions associated with virulence and leaf symptoms of net blotch. Nine QTL associated with virulence and leaf symptoms across five linkage groups/chromosomes were identified. Phenotyping of selected highly virulent progeny isolates on net blotch-resistant barley genotypes revealed that some progeny isolates are highly virulent across all of the 20 tested current widely used net blotch-resistant barley varieties.

In conclusion, results obtained from this study give an insight into the *P. teres*-barley pathogen system, which can aid future development of disease resistant barley varieties. Future studies on cloning and gene expression of the identified genomic regions would improve our knowledge of the *P. teres*-barley pathosystem. Determining the mating preference of *Ptt* and *Ptm* under different environmental conditions would allow us to understand what environmental conditions favour hybrid production and to have control measures in place to prevent or reduce the occurrence of hybrids.

CERTIFICATION OF THESIS

This Thesis is entirely the work of Buddhika Amarasinghe Dahanayaka 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: A/Prof. Anke Martin

Associate Supervisor: Dr. Niloofar Vaghefi

Associate Supervisor: Dr. Lisle Snyman

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

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STATEMENT OF CONTRIBUTION

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Buddhika A. Dahanayaka (BD) contributed 60% for the concept of the manuscript, samples preparation, literature reviewing, data analysing and drafting of the manuscript. Niloofar Vaghefi (NV) and Anke Martin (AM) contributed 7.5% each towards data analysing and critical revision. Noel L. Knight, Renée Prins, József Bakony, Diána Seress, Lislé Snyman (LS) contributed 5% each towards critical revision of the article and final editorial editing for publication.

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LIST OF ABBREVIATIONS AND UNITS

AFLP – Amplified fragment length polymorphism

DArT – Diversity Arrays Technology

DAPC – Discriminant analysis of principal components

Fst – Fixation statistics

Gst – Nei's genetic distance

LMWC – Low molecular weight compounds

MAT – Mating type locus

MLG – Multi-locus genotype

NFNB – Net form net blotch

ORF – Open reading frame

PCR – Polymerase chain reactions

PDA – Potato dextrose agar

PCoA – Principal coordinates analysis

Ptt – Pyrenophora teres f. *teres*

Ptm – Pyrenophora teres f. *maculata*

qPCR– Quantitative polymerase chain reactions

QTL – Quantitative trait locus/loci

RAPD – Random amplified polymorphisms DNA

RSA – Republic of South Africa

SFNB – Spot form net blotch

SNP – Single nucleotide polymorphism

SSR – Simple sequence repeats

UPGMA – Unweighted pair group method with arithmetic mean

bp – base pair

cm – centimeter

cM – centi Morgan

°C – degree Celsius

g – gram

h – hour

ha – hectare

kb – kilo base pair

kg – kilogram

L – litre

m – meter

mM – micromolar

μL – microlitre

μM - micromolar

Mbp – Mega base pair

ng – nanogram

s- second

V – volt

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1
INTRODUCTION AND LITERATURE REVIEW

1.1 Barley and its importance

Barley [*Hordeum vulgare* L. (Linnaeus 1753)] is one of the main crops grown in Australia and is also grown in many other regions including Africa, Asia, Europe, and the United States (Zhang & Li 2010; FAOSTAT 2020). Barley is one of the founder crops of Old-World agriculture, dating back eight to ten thousand years (Badr et al. 2000). It has been suggested that the crop underwent several separate domestication events leading to the occurrence of modern landraces from different geographical areas (Badr et al. 2000). The major gene pool of Western and Eastern barley landraces was traced back to the archaeological remains found in the Fertile Crescent and Zagros Mountains, respectively, while Ethiopian and Eritrean landraces were traced back to the Horn of Africa (Morrell & Clegg 2007; Orabi et al. 2007).

Barley is classified under the genus *Hordeum* and tribe *Triticeae* in the *Poaceae*/grass family where wheat, oats and rye belong. Barley is a diploid inbreeding species with seven chromosome pairs carrying traits for extreme environment adaptations and pathogen resistance. As a result, barley is grown all over the world. Barley genotypes are divided into winter and spring barley according to which season they grow in.

With respect to global cereal production, in 2018 barley production was ranked fourth (Department of Health 2018), covering 48 million hectares worldwide (FAOSTAT 2020). In Australia, barley is the second most cultivated crop next to wheat, with a production of 13.5 million metric tonnes covering around 4.1 million hectares from Western Australia to Southern Queensland (Department of Agriculture and Water Resources Management 2018; FAO 2018) (Figure 1).

Barley grain is used for malt production, distillation and as feed for farm animals (Sun & Gong 2009). In the malt industry, barley grain is used to produce a wide range of beverages, including beer and whisky and many other malted drinks (Gupta et al. 2010). Barley is pearled for human consumption and found to contain bioactive phytochemicals for gut health (Idehen et al. 2017; Tosh & Bordenave 2020).

The cultivation of barley is limited by constraints which include abiotic stresses, such as drought and salinity, and biotic stresses, such as fungal diseases. Net blotches are major fungal foliar diseases of barley, causing devastating losses in barley production throughout the world (Mathre 1997).

Figure 1. Barley growing regions (>30 k ton/annum) in Australia (Based on Australian Bureau of Statistics 2016 census).

1.2 Net blotches

The net blotches can appear as two forms, i.e., net form net blotch (NFNB) and spot form net blotch (SFNB). In Australia, net blotches were identified as an important foliar disease of barley in the 1960s while in other regions of the world the net blotches have been considered as an economically important disease of barley since 1922 (McLean et al. 2009; Shipton 1966). Yield loss due to net blotches in susceptible barley varieties can range from 10 to 40% and total plant loss may occur in the absence of suitable fungicide treatments (Mathre 1997; Murray & Brennan 2010). Yield loss due to the net blotches is mostly coupled with significant reductions in seed weight and grain size (Khan, 1987; Shipton, 1966; Poulsen et al. 1999; Rees et al. 1999). In severe epidemics, NFNB can result in a 10 to 40% kernel weight reduction and destruction of the entire plant (Grewal et al. 2008; Shjerve et al. 2014) whereas outbreaks of SFNB can cause a seven percent reduction in grain weight (Khan 1989) and 44% yield loss (Jayasena et al. 2007). In Australia, yield loss due to NFNB is considered to be above 20%

(Shipton 1966; Khan 1987; Murray & Brennan 2010). During outbreaks of NFNB, the yield losses in the Queensland grown variety Gilbert and the South Australian grown variety Maritime of 60% (Poulsen et al. 1999) and 70% (Wallwork 2011), respectively were recorded. Yield losses due to SFNB are not well studied in Australia (McLean et al. 2009). However, Khan (1989) and (Jayasena et al. 2007) reported up to 44% yield loss caused by SFNB in barley grown in Western Australia, which varied depending on season, sowing date, and variety.

In Australia, yield loss of barley due to net blotches is estimated at AUD $60 million annually, which could increase to AUD $300 million per year under favourable disease conditions. The cost of annual net blotch control is reported to be AUD $246 million, ranking net blotch as Australia's most damaging barley disease (Murray & Brennan 2010).

1.3 *Pyrenophora teres*

Pyrenophora teres belongs to the Kingdom Fungi, Phylum Ascomycota, Class Dothideomycete, Order Pleosporales, Family Pleosporaceae, Genus *Pyrenophora*, and Species *teres*. *P. teres.* Previously, *P. teres* was classified under the genus *Helminthosporium* and later, due to the cylindrical morphological characterization of conidia, it was classified under *Pyrenophora* (Shoemaker 1959; Alcorn 1988). In addition to barley, *P. teres* can infect barley grass (*Hordeum murinum L. ssp. leporinum*), an annual weed, and other crops like wheat (*Triticum aestivum*) and oat (*Avena sativa*) (Van den Berg 1988). These ancillary plants, especially barley grass which cohabited with barley, may act as a source of inoculum for *P. teres* (Linde and Smith 2019; MacNish 1967).

The two types of net blotches SFNB and NFNB in barley are caused by two forms of the fungus. The two forms produce different disease symptoms on barley leaves: SFNB is caused by *P. teres f. maculata* (*Ptm*) and NFNB is caused by *P. teres f. teres* (*Ptt*) (Smedegård-Petersen 1971). In Western Australia, *Ptm* was first detected in the 1970s (Khan 1982, 1987). Symptoms of SFNB on susceptible varieties are characterized by dark circular or elliptic brown patches surrounded by a yellowish chlorotic region in the host, which are predominant on the leaf lamina and the leaf sheath (Figure 2a) (Smedegård-Petersen 1971; McLean et al. 2009).

Net form net blotch was first reported by Atanasoff and Johnson (1920) and can be identified by streaks or net like dark brown necrotic lesions on leaves of susceptible hosts. In mature leaves, lesions can extend up the vein (Figure 2b). The complete death of infected leaves exhibiting a dry appearance can be observed in severe infections (Mathre 1997). In resistant barley varieties, symptoms can be seen as minute brown dots (Liu et al. 2011).

The two forms of *P. teres* are structurally identical and, therefore, cannot be distinguished using morphological characteristics (McLean et al. 2009). In the early 2000s Williams et al. (2001), using PCR (polymerase chain reaction), were able to differentiate *Ptt* and *Ptm* by two amplicons, 411 bp and 378 bp, respectively. However, based on population genetics and genetic characterization, *Ptt* and *Ptm* have been identified as genetically distinct as they form two distinguished clusters (Williams et al. 2001; Keiper et al. 2008; McLean et al. 2009; Liu et al. 2011; Poudel et al. 2017). Recently, 12 primer pairs specific to the two forms of *P. teres* were developed to differentiate *Ptt* and *Ptm* by PCR amplification followed by polyacrylamide gel electrophoresis (Poudel et al. 2017).

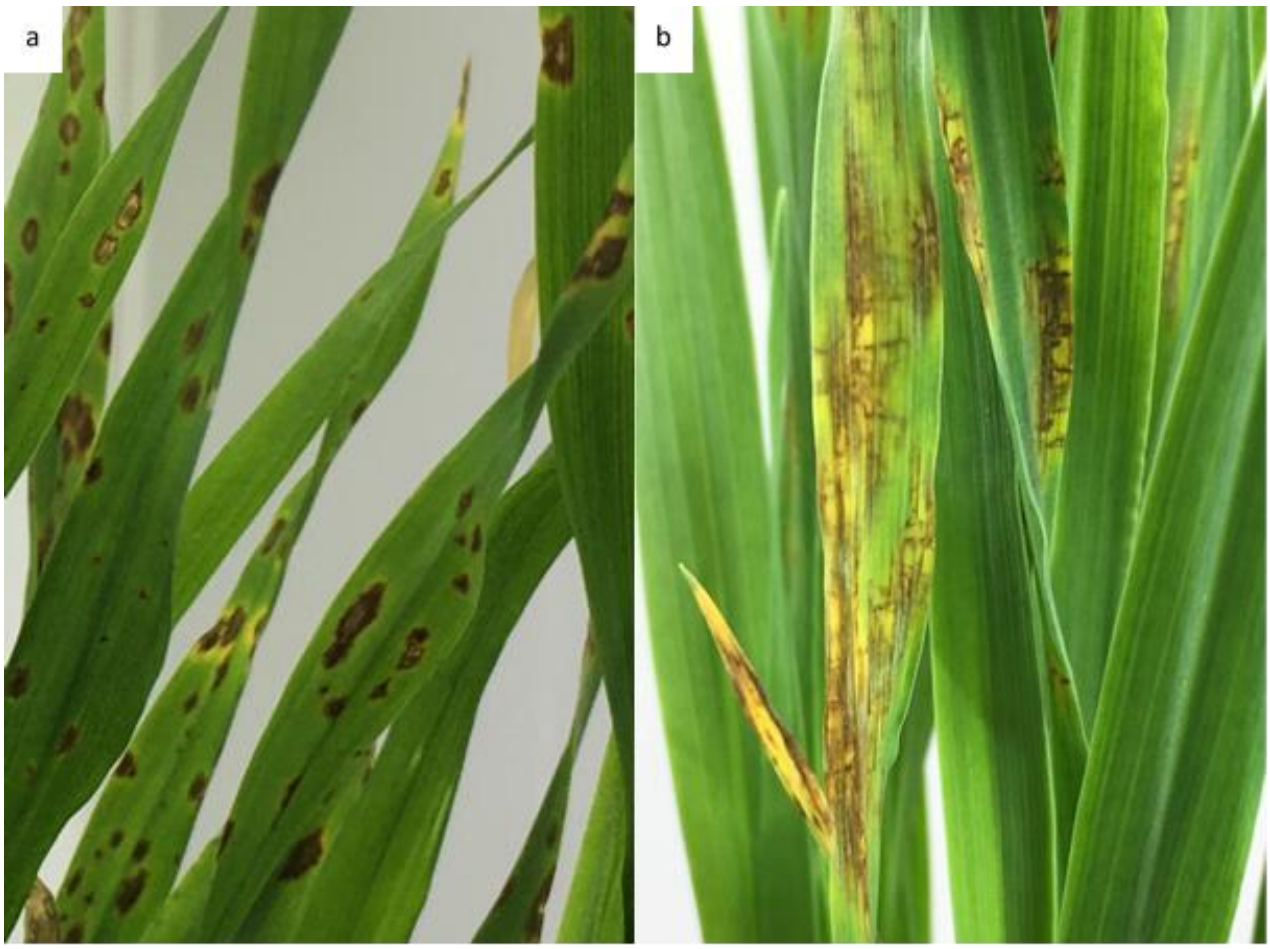

Figure 2. Symptoms of net blotch caused by *P. teres* a) SFNB symptoms caused by *Ptm* and b) NFNB symptoms caused by *Ptt* on barley.

1.4 Life cycle of *Pyrenophora teres*

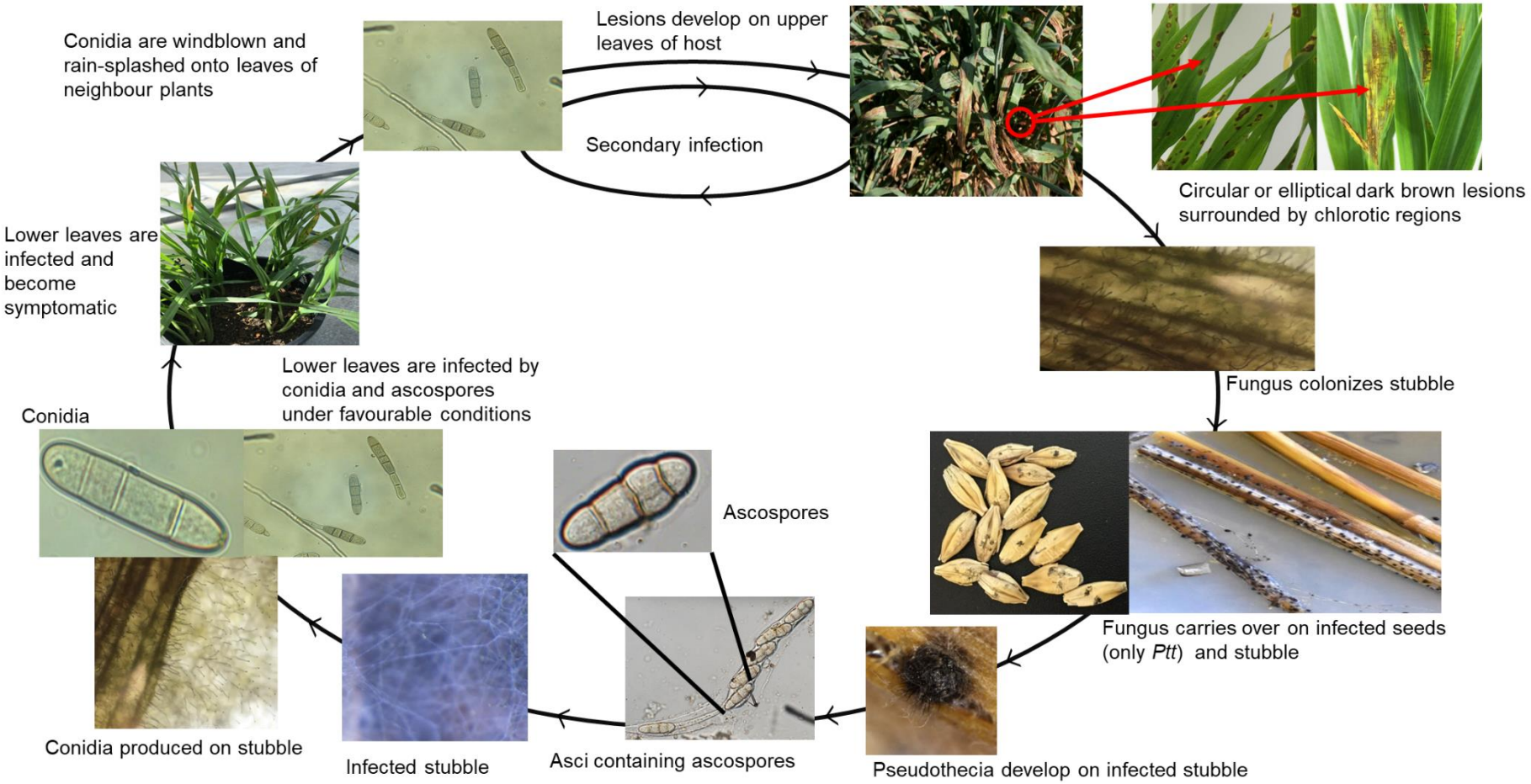

Figure 3. Life cycle of *Pyrenophora teres*.

The life cycles of *Ptt* and *Ptm* (Figure 3) are identical except that *Ptt* can be transmitted from one season to another via seeds, whereas *Ptm* is not considered to be seed-borne (McLean et al. 2009). Primary inoculum or pseudothecia (fruiting bodies) of the fungus can be seen as 1-2 mm diametric dark spots on barley stubble (Mathre 1997; McLean et al. 2009; Liu et al. 2011). Due to the heterothallic nature (requiring two thalli of opposing mating type to produce a fertile sexual reproductive structure) of *P. teres*, isolates from two opposite mating types (idiomorphs which regulates the compatibility in mating; mating type I and mating type II) are necessary to develop the sexual fruiting bodies (Rau et al. 2005) along with favourable environmental conditions (Kenneth 1962). The asci which develop in mature pseudothecia are bitunicate (Mathre 1997). Each ascus comprises of eight ascospores of 18 to 28 µm × 43 to 61 µm in size, three to four transverse septa and one or two longitudinal septa can be observed in median cells of the ascospores (Mathre 1997). These eight ascospores exist as four groups, with each group containing a genetically identical pair due to mitosis taking place after meiosis II (Finchman 1971). Once the ascospores are mature, they are discharged and dispersed by wind (Jordan 1981).

After reaching a suitable host plant, colonization of the fungus occurs, and large numbers of conidia are produced throughout the growing season as the secondary inoculum (Mathre 1997) (Figure 3). It has been suggested that conidia discharged from infected stubble or other hosts could act as the primary inoculum (Jordan & Allen 1984; Louw 1996; McLean et al. 2009; Liu et al. 2011). Conidiophores develop as single structures or in a cluster of two or three and are yellowish brown, straight, smooth, cylindrical and round-ended structures with four to six pseudosepta (Webster 1951; Mathre 1997; McLean et al. 2009). The width of a conidium varies from 15 to 30 µm and the length ranges from 23 to 174 µm (Webster 1951).

The development of the mycelium is highly dependent on weather conditions and the susceptibility of the host. High relative humidity (>80%), temperature (15-25 °C) and wetness of the leaf are some of the factors that can affect the distribution and germination of the fungus (Jordan 1981; Van den Berg & Rossnagel 1990). The severity of the disease in the field may increase due to the production of conidia during several cycles (McLean et al. 2009). Pseudothecia are produced upon the completion of the growing season by infecting the decaying tissues and act as protective and spore storing structures for the next seasons (Liu et al. 2011).

1.5 Infection process of *Pyrenophora teres*

Once the conidia or ascospores land on the surface of barley leaves, the germination of the spores begins within a few hours of favourable temperature and humidity (Kenneth 1962; Shipton 1966; Van den Berg & Rossnagel 1990). Hyphae are initiated by the formation of germ tubes which differentiates into appressoria to invade host cells (Caeseele & Grumbles 1979). The penetration of the appressorium into a host epidermal cell occurs through the cuticle of the leaf (Keon & Hargreaves 1983; Jørgensen et al. 1998), and is facilitated by the enzymatic hydrolysis of the cuticle layer and the outer epidermal cell wall of the host.

The differential disease symptoms, i.e., net like lesions and spot like lesions, which appear on the host plant due to *Ptt* and *Ptm*, respectively were explained by Lightfoot and Able (2010). *Pyrenophora teres* f. *maculata,* considered as a hemibiotroph, initiates the infection process with the formation of a haustorial-like primary intracellular vesicle in the outer epidermal cell wall. Secondary intracellular vesicles are then formed within the epidermal cells, which lead to the destruction of the infected epidermal cells as well as adjacent epidermal cells (Keon & Hargreaves 1983). The extended hyphal growth of *Ptm* to mesophyll tissue is intercellular and leads to the destruction of host cells attached to the hypha causing spot like necrotic lesions (Lightfoot & Able 2010). *Pyrenophora teres* f. *teres* develops as a necrotroph, where it infects intercellularly throughout the infection process, resulting in disruption of intact cells and adjacent cells, which leads to net like brown lesions on the leaf surface (Lightfoot & Able 2010). After initial infection, yellowish chlorotic regions start to appear around the brown necrotic lesions. Studies conducted by Keon and Hargreaves (1983) showed that there were no fungal hyphae in this chlorotic area, however, the chloroplasts of these regions had been disrupted.

After inoculation, *P. teres* rapidly causes chlorotic and necrotic lesions on plant tissue, which may be due to an array of chemical compounds secreted by the fungus invading the host (Liu et al. 2011). Two phytochemical compounds, toxin A and toxin B, purified from *Ptt* and *Ptm* by Smedegård-Petersen (1977), were able to cause necrotic/chlorotic lesions on healthy leaf samples. Another compound, toxin C was detected from the same isolates in 1979 (Bach et al. 1979). The chemical structures of toxins A, B and C were found to be *N*-(2-amino-2-carboxyethyl) aspartic acid, anhydroaspergillomarasmine A, and aspergillomarasmine A, respectively (Liu et al. 2011). Out of these three toxins, toxin C is more abundant in cultures. Under low pH, toxin C is converted to toxin B (Liu et al. 2011). A study conducted in 2007 identified low-molecular weight compounds and proteinaceous metabolites from *P. teres*

culture filtrates, which could induce yellow chlorotic regions and brown necrotic lesions on susceptible barley varieties, respectively (Sarpeleh et al. 2007). The toxic compounds recognized so far do not show any specificity to isolates or the barley variety and each of these compounds have been observed with a range of sensitivities (Sarpeleh 2008b, 2008a). Low-molecular weight compounds identified *in planta* were non-host selective but were temperature and light-dependant, proposing their ability to regulate the host cell metabolism and chloroplast regulation like light-dependant organelles (Sarpeleh 2008b). It was suggested that the target protein of host plants differs among barley genotypes in quantity, type or availability and hence, toxic proteins of *P. teres* may bind to specific molecules only available in susceptible plants and induce reactions (Sarpeleh 2008a).

A study conducted by Ruiz-Roldán et al. (2001) cloned a mitogen activated protein kinase (MAPK) gene (*PTK1*) involved in cellular signal transduction pathways, which appeared to be critical for the formation of the appressorium in disease infection processes during conidium germination. Also, high levels of expression of a plant host gene, *HvS40*, after the infection of chlorotic and necrotic leaf tissues, have been identified and this gene has been reported to play a major role in leaf senescence which leads to disease symptom development in the host (Krupinska et al. 2002).

1.6 Pathotypes of *Pyrenophora teres*

A pathotype of *P. teres* is a group of isolates which have the same pathogenicity on a specific barley variety or varieties. When considering local and global *P. teres* populations, there is a broad spectrum of variation in their pathogenicity (Serenius et al. 2007; McLean et al. 2010). Both *Ptt* and *Ptm* show a wide range of pathotype diversity, hence, different isolates of *Ptt* and *Ptm* are capable of developing different levels of disease severity against different barley varieties (Oğuz & Karakaya 2017) and overcome resistance in barley (Linde & Smith 2019). Therefore, pathotype diversity is an important factor to consider in plant breeding programmes when developing resistant varieties (Tekauz 1990; Liu et al. 2011).

Pathotype variation/physiological specialization was first reported in 1969 in Western Australia by Khan and Boyd (1969), who showed that the pathogenic *Ptt* population was virulent on the popular variety Beecher. Beecher was thus predominantly replaced by Dampier as the popular and the most widely grown variety followed by the NFNB-resistant variety Clipper, released in the 1970s. *Pyrenophora teres* f. *teres* isolates collected after 1976 showed no virulence on Beecher (Khan 1982) until the 1990s (Gupta & Loughman 2001). Khan (1982)

concluded that the change in the barley variety towards NFNB resistance was rapidly followed by an alteration in virulence of the *Ptt* population in Western Australia.

Pathotype variation/diversity is detected using a set of barley varieties, these sets are called differential sets. A number of subsequent studies have identified different *P. teres* pathotypes using a wide range of differential lines across the world, including Australia (Khan & Boyd 1969; Khan 1982; Gupta & Loughman 2001; McLean, M. et al. 2010; Wallwork et al. 2016; Fowler et al. 2017), Canada (Tekauz 1990; Liu et al. 2012; Akhavan et al. 2016), Europe (Jonsson et al. 1997; Arabi et al. 2003), Middle East Asia (Douiyssi et al. 1998; Jebbouj & El Yousfi 2010; Bouajila et al. 2011; Bouajila et al. 2012; Boungab et al. 2012; Oğuz & Karakaya 2017), New Zealand (Cromey & Parkes 2003) and the United States (Steffenson & Webster 1992). The number of isolates and the barley varieties used in each study ranged from three to 1,000 and one to 38, respectively. The results of these studies indicated that increasing the number of isolates and varieties being tested increased the number of pathotypes detected (Sato & Takeda 1993). However, in these studies' different sets of isolates and differential barley varieties were used to detect the pathotype variations. In 2009, a universal set of differential barley varieties were developed using an international collection of 1000 *Ptt* isolates and 14 barley varieties (Afanasenko et al. 2009). Even though more studies have focused on *Ptt*, a few studies have reported on the pathotype diversity in *Ptm* from Australia (Gupta & Loughman 2001; McLean et al. 2014), Canada (Tekauz 1990), Turkey (Oğuz & Karakaya 2017) and The United states (Wu et al. 2003).

A study conducted by Fowler et al. (2017) using 123 *Ptt* isolates collected from five states of Australia suggested that the widespread growing of locally adapted barley varieties has resulted in regional evolution and long-term survival of virulence gene combinations of *Ptt.* Hence, regular monitoring of pathogenic variation in a population is crucial for determining better varieties to grow for minimum yield losses due to *P. teres*.

1.7 Disease management

Management of net blotches can be achieved by a number of methods, including using resistant varieties, fungicide application, control barley grass and other alternate hosts, destruction of primary inoculum by crop rotation and destroying stubble (Liu et al. 2011; GRDC 2018). Crop rotation is a practice where growers are advised not to repeatedly cultivate barley, but instead rotate with a non-host crop (Rees et al. 1999). Growing barley repeatedly in the same field and cultivating the same variety may increase the pathotype incidences through evolution by speeding up the rate of adaptation to the resistance genes. Prolonged existence of stubble could

carry the pathogen inoculum from barley plants grown several years earlier (Jordan & Allen 1984). Hence, cultivation of crops which cannot act as a host for the pathogen, for example legumes and oilseeds, is recommended for better disease management (GRDC 2017). However, as *P. teres* persists on plant residue, the widely adopted practice of reduced- or zero-tillage in recent years is likely to have caused increased incidence of net blotch diseases (McLean et al. 2009).

The long-distance dispersal of seed-borne inoculum or wind-borne ascospores is important for the spread of the pathogen. The survival of the pathotypes depends on many factors including the barley varieties grown (Jonsson et al. 1999). Hence, increased cultivation of susceptible varieties increases outbreaks and the severity of the disease (McLean et al. 2010; McLean 2016). Cultivation of high yielding and resistant barley varieties is considered the most effective, environmentally friendly and long-term disease management method (Shipton 1966; Mathre 1997). However, the severity of the symptoms of net blotch on barley genotypes can depend on the pathogenic diversity of *P. teres* isolates (Liu et al. 2011). Current commercial barley varieties in Australia are annually monitored for diseases resistance including to the net blotch diseases (Table 1) (GRDC 2018, 2020) which helps growers to choose suitable barley varieties to grow.

Table 1. Disease severity levels of net blotches on different barley varieties (GRDC 2020)

Cultivar	Spot-form net blotch	Net-form net blotch
Banks	MS-S	R-MS
Buff	S	MR-MS
Commander	MS-S	MS-VS
Compass	MS	MR-S
Fairview	S	MS-VS
Fathom	MR	MS-VS
Flinders	MR-S	MR-MS
La Trobe	MS-S	MR-S
Maximus CL	MR-MS	MR-MS
Oxford	MS-S	MR-VS
RGT Planet	S-VS	MR-VS
Rosalind	MS-S	MR-S
Spartacus CL	S-VS	MR-VS
Westminster	S	MR-VS
Laperouse	MR-MS	MR-S

R, resistant; R-MR, resistant to moderately resistant; MR, moderately resistant; MR-MS, moderately resistant to moderately susceptible; MS, moderately susceptible; MS-S, moderately susceptible to susceptible; S, susceptible; S-VS, susceptible to very susceptible; VS, very susceptible

Unlike *Ptm*, *Ptt* is a seed-borne pathogen hence, NFNB can be dispersed not only over short distances but also over long distances by infected seed. Seed-treatment with a suitable fungicide can be used as a protection method against seed-borne NFNB. During the application of a fungicide, it is important to use a registered fungicide for net blotches following the recommend rate to ensure effective treatment (Rees et al. 1999). A wide range of fungicides are used to control net blotches across the world including quinone outside inhibitors (QoI,

group 11), succinate dehydrogenase inhibitors (SDHI, group 7) and azole or demethylase inhibitors (DMI, group 3) (Mair et al. 2016; FRAC 2020a, 2020b; Lammari et al. 2020). Foliar fungicides can be applied to protect the upper leaf layers of the plant. To prevent the pathogen becoming fungicide resistant, fungicide application should be limited by applying only when required, following the recommended doses and rotating fungicides groups.

1.8 Development of fungicide resistance in *Pyrenophora teres* populations

Due to the lack of barley varieties with high levels of resistance to net blotches especially spot form, application of fungicides is the predominant method used for the disease management worldwide (Sierotzki et al. 2007).

Quinone outside inhibitors were introduced in 1996 as an effective fungicide for net blotches (Lammari et al. 2020). This group of fungicides controls the pathogen by hindering mitochondrial respiration. Upon the successful binding of the fungicide to the Qo site of the cytochrome *bc1* complex, the electron transport chain in the mitochondrion is disrupted (Fernández-Ortuño et al. 2008). As a result, ATP synthesis is discontinued leading to the inhibition of spore germination and mycelia development of the pathogen.

Fungicides belonging to the SDHI group are also associated with fungal respiration. The target site of these fungicides is the succinate dehydrogenase enzyme, a key enzyme that acts as the bridge between the tricarboxylic acid cycle and electron transport chain (Rehfus et al. 2016). SDHI fungicides inhibit the respiration of the pathogen by binding to the ubiquinone-binding sites of the enzyme in the mitochondrial complex II.

The specific target site of demethylase inhibitors (DMI) is the cytochrome P450 (*CYP51*) sterol 14α-demethylase enzyme (Lamb et al. 1999). The selective binding of the fungicide to the active site of *CYP51* leads to disruption of the biosynthesis of ergosterol which is a vital component of the pathogen membrane (López-Ruiz et al. 2010).

Fungicides with the same mode of action are widely used as a disease management strategy to control net blotches, hence, the pathogen has successfully developed resistance over the years. As a result, the efficacy and the performance of the fungicides have been affected (Mair et al. 2016; Lammari et al. 2020). Two mutations, F129L (at 129 position) and G137R (at 137 position), in *P. teres* at the specific target site of cytochrome *b* have been detected as a response to QoI fungicides (FRAC 2020a).

A number of mutations in *P. teres* responsible for the reduced sensitivity to SDHIs have been reported since 2012 (Stammler et al. 2014; FRAC 2020b). The first reported mutation in response to SDHI was found to be *SdhB*-H277Y in Europe (Stammler et al. 2014) and then

mutations in subunit C (N75S, G79R, H134R, S135R) and subunit D (D124N, D124E, H134R, D145G, E178K) in the SDH complex of *P. teres* populations were reported throughout the world to reduce the level of sensitivity to SDHIs (FRAC 2020b). Reduced resistance to DMIs was detected *in vitro* in *Ptt* isolates collected from Western Australia, with these isolates reported to possess an overexpressed copy of the *Cyp51A* gene carrying mutation F489L (Mair et al. 2016). *Ptm* isolates with the same mutation at F489L coupled with a 134 bp insertion in the promoter of the *Cyp51A* gene conferred increased resistance to DMI *in vitro* (Mair et al. 2020).

Successful mutations and reduced sensitivity to fungicides in *P. teres* populations in Australia and around the world suggests the rapid evolution and adaptation of the pathogen. The rapid evolution of *P. teres* to challenging environments, including exposure to fungicides, could be facilitated by clonal reproduction and natural selection.

1.9 Recombination and hybridization of *Pyrenophora teres*

Sexual recombination in *P. teres* is controlled by a single mating type locus (*MAT1*), which exists as two alternative forms or idiomorphs, i.e., MAT1-1 and MAT1-2 (McDonald 1963). Successful mating between two thalli possessing opposite mating types results in fertile pseudothecia containing asci. Ascospores in these asci are genetically different to their parental isolates (Finchman 1971). *In vitro* progeny isolates resulting from sexual recombination were reported to be highly genetically diverse (McDonald 1963; McLean et al. 2009; Liu et al. 2011) and developed different levels of virulence than those of the parental isolates (Afanasenko et al. 2007).

Even though *Ptt* and *Ptm* are considered to be two genetically diverse groups (Smedegård-Petersen 1971; Rau et al. 2007; McLean et al. 2009; Lehmensiek et al. 2010; Liu et al. 2011), mating within (recombination) and between (hybridization) forms has been induced *in vitro* (Smedegård-Petersen 1971; Crous et al. 1995; Louw et al. 1995; Campbell et al. 1999; Jalli 2011). Isolates resulting from these crosses showed net-like, spot-like or intermediate disease symptoms on barley (Smedegård-Petersen 1971; Campbell et al. 1999). These laboratory produced hybrids were fertile (Campbell & Crous 2003) and virulent over years and showed reduced sensitivity to triazole fungicides compared to parental isolates (Campbell et al. 1999). Studies conducted by Ellwood et al. (2012) and Serenius et al. (2007) suggested that hybridization between the two forms is rare under field conditions due to their genetic isolation. A previous study, aimed at inducing hybridization between two forms in field conditions showed that the two forms preferred to undergo sexual recombination within forms (Poudel et

al. 2018). However, six hybrid isolates collected from barley fields have been reported to-date from South Africa [*n* = 1: Campbell et al. (2002)], Czech Republic [*n* = 2: Leišova et al. (2005) and Australia [*n* = 2: McLean et al. (2014) and Turo et al. (2021)]. The hybrid reported by Turo et al. (2021), collected from the Western Australian barley belt, was reported to have increased resistance to some of the group 3 fungicides (azole or demethylase inhibitor) and also showed rapid asexual propagation. This suggests that hybrid isolates found in the field could be genetically stable, fertile and possess increased virulence similar to *in vitro* hybrid isolates.

Hybridization and/or recombination of *P. teres* forms during sexual reproduction can lead to the development of novel pathotypes. More importantly, hybrids possessing virulence and fungicide resistance traits from both *Ptt* and *Ptm* and rapid evolution through accelerated sexual recombination could result in more complex novel pathotypes overcoming host resistance. This would affect disease management methods of the pathogen and increase the genetic diversity in the population (Syme et al. 2018). Therefore, it is necessary to study and broaden the knowledge of the behaviour of such hybrids and recombinants that could occur through sexual reproduction.

1.10 Population genetics and genetic diversity of *Pyrenophora teres*

Population genetics refers to the study of the genetic makeup of biological populations, and the variations in genetic makeup which result mainly from mutation, gene flow, recombination, drift and selection. Genetic variation/diversity within a population is a vital factor for the evolution of a pathogen. Characterization of the amount and distribution of genetic diversity in a pathogen population is important as it reflects the evolutionary potential of the pathogen (McDonald & Linde 2002). Continuous exposure of the pathogen to the same resistance genes or fungicides causes evolution of the pathogen population in order to overcome the resistance, rendering the disease management strategies ineffective against the pathogen (McDonald & Linde 2002). Hence, comprehensive knowledge on the genetic diversity of the pathogen populations is important for better disease management.

Barley is grown in a wide range of climates across the world and outbreaks of both forms of *P. teres* occur in all barley growing regions (Van den Berg 1988). However, the predominant form of net blotch differs among regions (Mäkelä 1972; Arabi et al. 2003). In Europe, *Ptt* outbreaks are more prominent in susceptible spring barley varieties while infection levels of *Ptm* isolates are higher in winter barley varieties (Minarikova & Polisenska 1999). Previous reports revealed that temporal changes in population dynamics of net blotch have caused the dominant form of

net blotch to change across different regions throughout Australia (McLean et al. 2010). Previously, *Ptt* was considered the major cause for net blotch in Australia (Khan 1982), however, in recent years, the prevalence of *Ptm* has increased due to increased planting of susceptible varieties. The occurrence of *Ptm* was first reported in Western Australia in 1977 (Khan 1982), after which it was identified in South Australia and in the Eastern states (Wallwork et al. 1992; McLean et al. 2009). The occurrences of net blotches worldwide have increased the necessity of understanding the population diversity and genetic structure in order to achieve efficient disease management strategies such as the successful development of resistant barley varieties (Liu et al. 2011).

Previous studies conducted using different molecular markers including simple sequence repeats (SSR), random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) have reported high genetic diversity within the *P. teres* populations collected from different countries including Australia, Republic of South Africa, Canada and Hungary (Peltonen et al. 1996; Campbell et al. 2002; Rau et al. 2003; Bogacki et al. 2010; Lehmensiek et al. 2010; McLean et al. 2010; Leišová et al. 2014; Linde and Smith 2019). A recent study conducted with SSR using 50 *Ptt* isolates collected from Western Australia demonstrated high genetic and genotypic diversity (Ellwood et al. 2019). Another study conducted using 15 sequence-tagged microsatellite primers with 44 *Ptm* isolates collected from Victoria, Australia, also revealed high levels of diversity (McLean et al. 2010). Studies have also shown that the genetic diversity of *Ptt* populations is higher than *Ptm* populations (Ho et al. 1996; Rau et al. 2003; Serenius et al. 2007; Lehmensiek et al. 2010). Furthermore, studies conducted by Serenius et al. (2007) and Lehmensiek et al. (2010), using AFLP markers to analyse Australian isolates with other *Ptt* and *Ptm* populations from different geographical regions, showed significant genetic differentiation among sampled locations and also between the two forms of *P. teres*. Since 2010, there has been a lack of published data on temporal population differences among Australian isolates and other geographical regions.

Use of conventional markers, like AFLP markers in some of the aforementioned studies, limits the reproducibility of data. More recently, novel marker systems have become available, which produce a large number of genome-wide molecular markers, some of which are located in gene regions. Diversity Arrays Technology (DArT) is one such high throughput molecular marker technology which does not require prior knowledge of whole genome sequences (Wenzl et al. 2004). Furthermore, it has been developed for fungal species including *P. teres* (Syme et al. 2018). In this technology, polymorphisms are generated at restriction enzyme recognition sites

and the presence or absence of individual DNA fragments in the genome is detected through fluorescent labelled microarray hybridization and scores are given accordingly (Jaccoud et al. 2001). DArTseq™ is a cost-effective novel modification of initial DArT. It deploys Next Generation Sequencing (NSG) platforms for genotyping-by-sequencing and compared to the previous microarray version of DArT, DArTseq™ provides higher number of markers (Kilian et al. 2012). Therefore, usage of DArTseq™ data would be an ideal molecular marker system for a meaningful comparison of the diversity and structure of *P. teres* populations.

1.11 *Pyrenophora teres* genome

Ellwood et al. (2010) conducted the first genome assembly of *P. teres* (*Ptt* isolate 0-1) using 75-bp paired-end Illumina reads that resulted in an assembly of 6,412 contigs and a total size of 41.95 Mb. The genome was validated by comparing sequences with bacterial artificial chromosome (BAC) sequences, expressed sequence tags (ESTs), orthologous genes and PCR amplification with simple sequence repeat (SSR) markers along with cytogenetic karyotyping. Ellwood et al. (2010) reported that the *Ptt* genome contained a rich diversity in genes especially related to protein and carbohydrate hydrolases, efflux pumps, cytochrome P450 genes, siderophores, tetraspanins, nonribosomal peptide synthetases, polyketide synthases and a complex secretome which could be attributed to the lifestyle of the pathogen.

Using long-read sequencing (Pacific Biosciences single-molecule real-time sequencing) and scaffolding, Wyatt et al. (2018) updated the genome sequence of isolate 0-1 to produce a high quality reference genome assembly comprised of 86 contigs and a total size of 46.5 Mb. The genome was annotated following an evidence-based method consisting of assembled transcripts using an Illumina platform and multiple *ab initio* gene predictions. The protein evidence for the annotation was taken from the closely related species *P. tritici-repentis* (Manning et al. 2013) and the previous genome annotation of 0-1 (Ellwood et al. 2010). The updated genome assembly and the annotation contained a substantial amount of the repetitive content, which plays a major role in the evolution of other filamentous fungal plant pathogens (Dong et al. 2015).

A recent genome assembly study by Syme et al. (2018) using five *Ptt* (W1-1, Stir9-2, NB29, NB73 and NB85) and four *Ptm* isolates (SG1, Cad6-4, M2 and FGOB10Ptm-1) revealed that genomes of the two forms are highly collinear and comprised of 12 chromosomes. Out of these two forms, *Ptt* has a larger and more repetitive genome (ranged from 46.31 to 51.76 Mbp) compared to *Ptm* (ranged from 39.27 to 41.48 Mbp) and both forms contain a large complement of secondary metabolite gene clusters, suggesting an ability to produce different molecules that

allow the pathogen to invade the host (Syme et al, 2018). Genic variations detected between *Ptt* and *Ptm* genomes were predominantly found in gene-sparse regions near or within transposable elements (TE) rich regions harbouring fungal effectors.

A recent comparative genomic analyses of five *Ptt* isolates conducted to examine the genomic organization, structural variation, and core and accessary genomic content using a Pan-genome approach revealed that sub-telomeric accessory genomic compartments harbor virulence loci (Wyatt et al. 2020). These sub-telomeric regions were proposed to have the capability of evolving rapidly.

1.12 Genetics of pathogen host interaction

Various hosts show differential reactions to different isolates exhibiting the specific relationship between the host and the pathogen (Khan & Boyd 1969; Liu et al. 2011). Early classical genetic studies showed that the virulence of *P. teres* isolates depends on the variety and that the resistance or the susceptibility of barley varieties to fungal isolates depends on one or two genes or qualitative traits (Khan & Boyd 1969; Ho et al. 1996; Afanasenko et al. 2007). It was reported that the qualitative traits of virulence or avirulence of the pathogen towards the host plant follows the gene-for-gene model when qualitative traits or dominant genes are involved in the pathosystem (Flor 1956; Person 1959; Flor 1971; Weiland et al. 1999; Friesen et al. 2006). The expression of avirulence gene/s of the pathogen would be recognised by the corresponding resistance gene/s in the plant which leads to the activation of resistance in the plant towards the pathogen (Leach & White 1996; Laugé & De Wit 1998; Beattie et al. 2007; Lai et al. 2007).

However, recent studies indicated that the virulence/susceptibility and avirulence/resistance of the pathogen/host do not necessarily follow the gene-for-gene model and that the interaction is highly complex and governed by both qualitative and quantitative trait loci (QTL) (Liu et al. 2015; Koladia et al. 2017). Identification of QTL or genes which are responsible for the virulence/avirulence of *P. teres* to induce net blotch in barley is an important step towards development of resistant barley varieties.

1.13 QTL/genes associated with virulence in *Pyrenophora teres*

Resistance/susceptibility of barley varieties to *P. teres* has been identified to be associated with both qualitative (gene-for-gene hypothesis) and quantitative traits. Necrotic effectors (NEs)/host selective toxins and multiple dominant genes have also been found to play a major role in disease development in barley varieties (Liu, 2011), suggesting that in addition to the gene-for-gene hypothesis, an inverse process of gene-for-gene interaction may also occur

through NEs mediated programmed cell death (Friesen et al. 2008; Ciuffetti et al. 2010; Faris et al. 2010).

Several studies, including six bi-parental populations and two genome wide association mapping studies, have been conducted to identify QTL/genes associated in the *P. teres* (Weiland et al. 1999; Beattie et al. 2007; Lai et al. 2007; Shjerve et al. 2014; Kinzer 2015; Carlsen et al. 2017; Koladia et al. 2017; Martin et al. 2020) (Table 2, Figure 4 and 5). The first mapping study using RAPD markers in *P. teres* by Weiland et al. (1999) identified the locus *AvrHar* using progeny from a cross between the two *Ptt* isolates 0-1 (Ontario, Canada) and 15A (California, USA). The locus *AvrHar* was recognized in 15A, conferring low virulence/avirulence on the barley line Harbin. Another study using AFLP markers with the same cross, 0-1/15A, reported two loci, *AvrPra2* and *AvrPra1*, conferred avirulence on CLS and Tifang, and Prato respectively (Lai et al. 2007). A unique avirulence gene, $Avr_{Heartland}$, was identified from a cross between two Canadian isolates WRS1906 and WRS1607 (Beattie et al. 2007). The locus was responsible for the avirulence on the barley variety Heartland and identified using AFLP markers. Using AFLP, SSR and single nucleotide repeat (SNP) markers, another QTL mapping study with two USA *Ptt* isolates, 15A and 6A, reported four virulent loci, *VK1*, *VK2*, *VR1* and *VR2*, with *VK1* and *VK2* being virulent on barley Kombar and *VR1* and *VR2* virulent on Rika (Shjerve et al. 2014). Nine QTL associated with virulence in *P. teres* were identified by Koladia et al. (2017) using a *Ptt* population developed by crossing a Danish isolate, BB25, with a USA isolate FGOH04Ptt-21. SNPs data generated by genotyping by sequencing using restriction site-associate DNA (RAD-GBS) were used in the study and these QTL were associated with virulence on eight barley genotypes including commonly used differential varieties Manchurian, Tifang, CI4922 and Beecher, and locally (North Dakota) grown Pinnacle. A study by Martin et al. (2020) reported 14 different genomic regions associated with virulence in Australian *Ptt* isolates using genome wide association mapping with 20 barley varieties. The identified genomic regions were then confirmed by QTL analysis of two bi-parental mapping populations, NB029/HRS09122 and NB029/NB085. The DArTseq™ marker system was implemented for both GWAS and QTL mapping.

The number of studies conducted using *Ptm* bi-parental populations are limited. The first mapping study associated with virulence of *Ptm* identified six QTL using a cross between FGOB10Ptm-1 (USA) and SG1 (Western Australia) (Figure 5) (Carlsen et al. 2017). These QTL were detected using the RAD-GBS marker system and phenotyping the progeny developed by crossing FGOB10Ptm-1 and SG1 on Skiff, 81-82/033, TR326, and PI 392501.

The only association mapping study conducted on 82 *Ptm* isolates collected from Northern United States using 30 barley genotypes identified 45 significant SNP loci associated with virulence or avirulence and the most significant locus, 01700_198, was found to be associated with four barley genotypes: CI3576, CI9819, MXB468, and CI7854. These studies suggest a high degree of complexity involving the *P. teres*-barley pathosystem.

Table 2. Summary of QTL/genes reported for *P. teres* using bi-parental mapping populations (Martin et al. 2021; Clare et al. 2020).

Locus	Marker type	No. pro[a]	Cross	Vir/Avi[b]	Chro[c]	Position[d] Starting	Position[d] Ending	Marker at peak of QTL	Genotype[e]	LOD score[f]	R^2[g]	Parent contributing the QTL	Reference
QTL identified in *P. teres* f. *teres*													
AvrHar	RAPD	82	0-1/15A	Avi	5	4193688	-	-	Harbin	36	72	15A	Weiland et al. (1999)
AvrPra2	AFLP	78	0-1/15A	Avi	5	3008702	-	M11E13190-M12E11250	Tifang, Canadian Lake Shore	5.3	-	0-1	Lai et al. (2007)
AvrPra1	AFLP	78	0-1/15A	Avi	9	-	1256349	M15E20400-M12E11250	Prato	7.2	-	0-1	Lai et al. (2007)
$Avr_{Heartland}$	AFLP	67	WRS1607/WRS1906	Avi	1	-	-	GTTA285-CGAA1600	Heartland	-	-	WRS1906	Beattie et al. (2007)
VR1	SNP, SSR, AFLP	118	6A/15A	Vir	2	2066532	3939100	07628_18	Rika	5-10	35	6A	Shjerve et al. (2014)
VR2	SNP, SSR, AFLP	118	6A/15A	Vir	10	1516021	2300448	10177_27	Rika	10-15	20	6A	Shjerve et al. (2014)
VK1	SNP, SSR, AFLP	118	6A/15A	Vir	3	1041300	1650040	18850_67	Kombar	15-20	26	15A	Shjerve et al. (2014)
VK2	SNP, SSR, AFLP	118	6A/15A	Vir	2	442489	507296	03948_8	Kombar	10-15	19	15A	Shjerve et al. (2014)
PttTif1*	SNP	109	BB25/FGOH04Ptt-21	Vir	1	1519813	2279473	1579_4251	CI4822,Tifang, Manchurian	11,30, 35	45,67, 74	FGOH04Ptt-21	Koladia et al. (2017)
PttTif2*	SNP	109	BB25/FGOH04Ptt-21	Vir	8	-	593132	547_32651	Tifang	4.4	3	FGOH04Ptt-21	Koladia et al. (2017)
PttBee1*	SNP	109	BB25/FGOH04Ptt-21	Vir	1	-	2776486	1588_12100	Beecher	24.0	56	FGOH04Ptt-21	Koladia et al. (2017)
PttBee2*	SNP	109	BB25/FGOH04Ptt-21	Vir	5	316575	525281	752_3220	Beecher	7.0	17	FGOH04Ptt-21	Koladia et al. (2017)
PttPin1*	SNP	109	BB25/FGOH04Ptt-21	Vir	3	5804230	-	1667_1175	Pinnacle	14.0	49	BB25	Koladia et al. (2017)
PttPin2*	SNP	109	BB25/FGOH04Ptt-21	Vir	12	1044631	1438885	2428_2378	Pinnacle	3.1	11	FGOH04Ptt-21	Koladia et al. (2017)
PttCel1*	SNP	109	BB25/FGOH04Ptt-21	Vir	8	2843621	3029139	1454_3802	Tifang, Celebration	3.2,7.0	7,17	FGOH04Ptt-21	Koladia et al. (2017)
PttCel2*	SNP	109	BB25/FGOH04Ptt-21	Vir	9	2562601	2806018	994_25330	Celebration	5.1	17	FGOH04Ptt-21	Koladia et al. (2017)
PttHec1*	SNP	109	BB25/FGOH04Ptt-21	Vir	8	2652633	-	252_25719	Hector, Stellar	3.1,6.5	11,18	FGOH04Ptt-21	Koladia et al. (2017)
PttSki_3	DArTseq	78	NB29/HRS09122	Vir	3	114611	-	28946459	Skiff	6.6	24	HRS09122	Martin et al. (2020)
PttBee_5	DArTseq	78	NB29/HRS09122	Vir	5	5183980	5208563	28948016	Beecher	4.0	15	NB29	Martin et al. (2020)
PttSki_5	DArTseq	78	NB29/HRS09122	Vir	5	3980200	4457075	28948170	Skiff	4.8	19	HRS09122	Martin et al. (2020)
PttBee_9	DArTseq	78	NB29/HRS09122	Vir	9	1073073	1122817	28945299	Beecher	3.0	11	NB29	Martin et al. (2020)
PttBee_3	DArTseq	72	NB29/NB85	Vir	3	796216	970812	28945535	Beecher	12.0	36	NB29	Martin et al. (2020)
PttBee_7	DArTseq	72	NB29/NB85	Vir	7	2166986	2928737	28946946	Beecher	3.9	11	NB85	Martin et al. (2020)
PttPri_7	DArTseq	72	NB29/NB85	Vir	7	2166986	2928737	28949493	Prior	3.6	18	NB85	Martin et al. (2020)
PttBee_8	DArTseq	72	NB29/NB85	Vir	8	1883966	1972858	28949931	Beecher	3.0	7	NB29	Martin et al. (2020)
QTL identified in *P. teres* f. *teres*													
VQTL1A						117776	117958	SNP_11381_87	TR326, Skiff	8.4,5.8	21,23		
VQTL1B	SNP	105	SG1/FGOB10Ptm-1	Vir	1	170676	170836	SNP_2207_88	81-82/033	5.5	21	FGOB10Ptm-1	Carlsen et al. (2017)
VQTL1C						316665	316851	SNP_16439_27	PI 392501	9.4	34		
VQTL2	SNP	105	SG1/FGOB10Ptm-1	Vir	3	3154965	3155149	SNP_12879_149	Skiff	5.5	22	FGOB10Ptm-1	Carlsen et al. (2017)
VQTL3	SNP	105	SG1/FGOB10Ptm-1	Vir	5	2709841	2710025	SNP_41831_15	Skiff	5.3	20	FGOB10Ptm-1	Carlsen et al. (2017)
VQTL4	SNP	105	SG1/FGOB10Ptm-1	Vir	2	1108007	1108161	SNP_21264_143	81-82/033 PI 392501	8.0,11.0	30,37	FGOB10Ptm-1	Carlsen et al. (2017)
VQTL5	SNP	105	SG1/FGOB10Ptm-1	Vir	3	2419962	2420099	SNP_2673_169	81-82/033, TR326,	6.0,6.6,	33,26,	FGOB10Ptm-1	Carlsen et al. (2017)

									PI 392501	9.3	34			
VQTL6	SNP	105	SG1/FGOB10Ptm-1	Vir	4	1618736	1618905	SNP_26064_6	PI 392501	5.0	20	FGOB10Ptm-1	Carlsen et al. (2017)	

[a] Number of progeny, [b] virulence nature of the allele (Vir= virulent; Avi=avirulent), [c&d] chromosome location according to W1-1 reference genome, [e]name of the genotype that the allele is virulent/avirulent on, [f]logarithm of odds, [g]percentage of phenotypic variation explained by the QTL

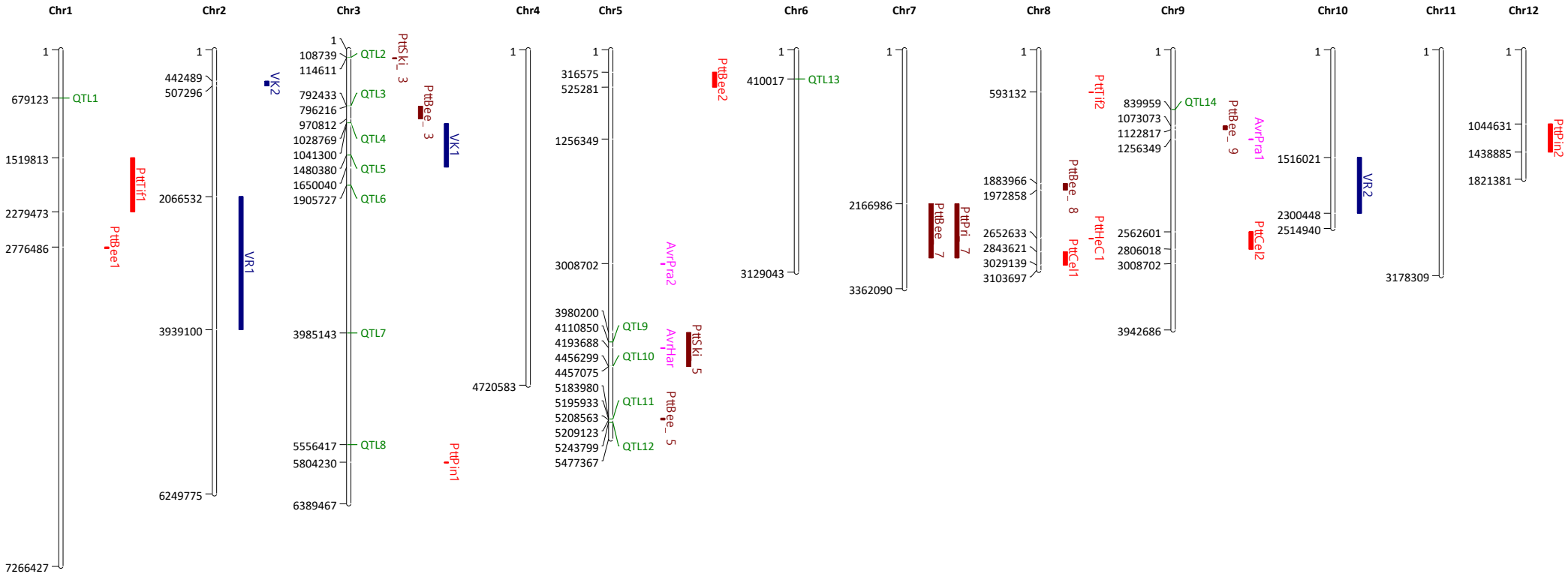

Figure 4. Map of the reference genome W1-1 showing genomic regions/QTL identified for avirulence/virulence of *Pyrenophora teres* f. *teres* by QTL mapping and GWAS (green). Positions in base pairs are given on the left. (Martin et al. 2021).

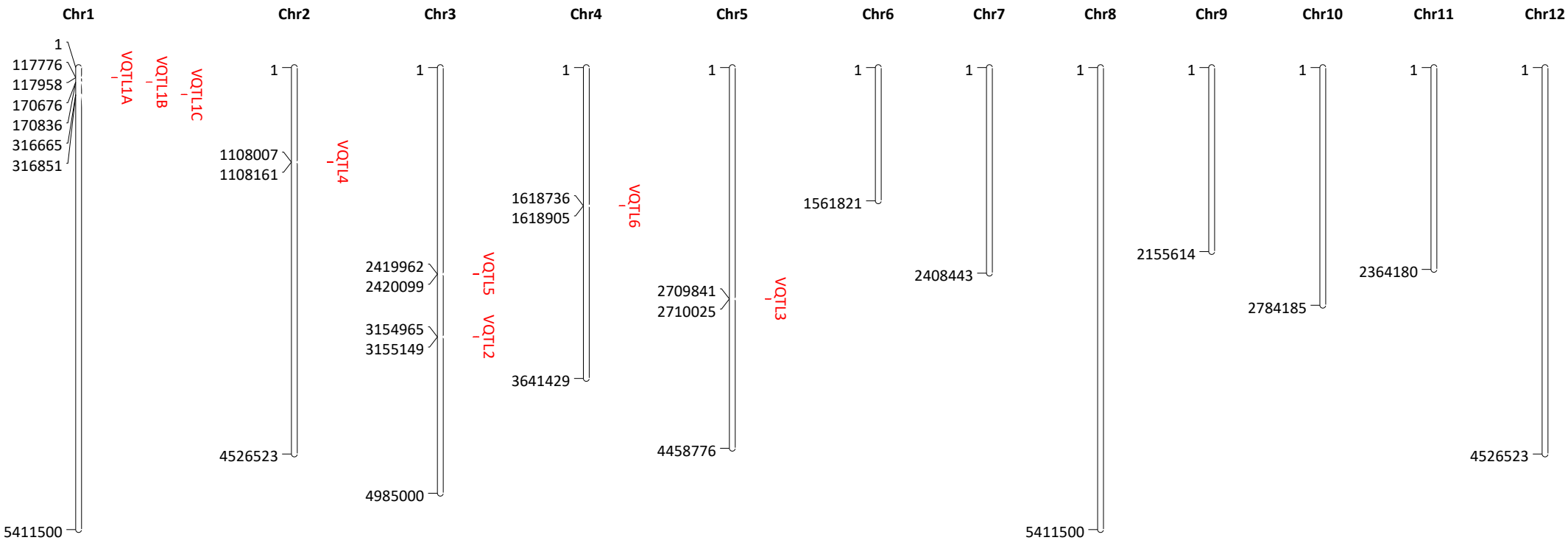

Figure 5. Map of the reference genome SG1 showing QTL identified for the virulence of *Pyrenophora teres* f. *maculata* by QTL mapping (Martin et al. 2021).

1.14 Aims and objectives of the study

1) Understanding the genetic diversity of *P. teres* populations from different continents.

Genetic characterization of *P. teres* populations is important to enhance our understanding of genetic differences in isolates from different regions of the globe. Although the genetic diversity of the Australian *P. teres* population has been studied in the past, no studies have revealed the recent changes in the genetic diversity and genetic structure of the Australian population collected from all barley growing regions (Serenius et al. 2007; Lehmensiek et al. 2010). Also, comparing the Australian *P. teres* population with collections from overseas will provide us with valuable information regarding global patterns of diversity and dispersal. Previous genetic diversity studies performed using *P. teres* population across the world have used conventional markers like SSR, RFLP or RAPD (Peltonen et al. 1996; Campbell et al. 2002; Rau et al. 2003; Bogacki et al. 2010; Lehmensiek et al. 2010; McLean et al. 2010; Leišová et al. 2014; Linde and Smith 2019). Usage of different marker systems in these available studies limits the comparison of genetic diversity of *P. teres* populations in different geographical areas. Therefore, in this research project we aimed to characterise the most geographically diverse population of *P. teres* f. *teres* using a genome-wide marker system DArTseq™ to provide an extensive genetic background for these isolates for use in future studies. Previous studies have only compared 23 (Bakonyi & Justesen 2007), 60 (Lehmensiek et al. 2010) and 84 (Serenius et al. 2007) *P. teres* f. *teres* isolates from different geographical continents. Investigating the genetic diversity and genetic distances across continents may give us an insight into the virulence profile and possible gene migration which subsequently results in the adaptation and evolution of the pathogen. The knowledge of potentially different virulences present in other countries is vital as these could spread to Australia.

2) Investigation of mating preference between or within two forms of *P. teres* and its hybrids

A previous study suggested that *Ptt* and *Ptm* isolates preferred to mate within the same form (recombination) rather than between the two forms (hybridization), in the field (Poudel et al. 2018). To date however, preferences of *P. teres* isolates and its hybrids for mating between or within have not been studied *in vitro.* Discovery of an increased number of field hybrids in the past few years has increased the necessity of understanding their occurrence and behaviour. Identification of the mating preferences of both forms of *P. teres* and its hybrids during sexual reproduction is important as it might give rise to novel pathotypes. The occurrence of new pathotypes with different levels of disease severities and symptoms would further complicate

disease management. Therefore, as a part of this study, preferences for sexual reproduction of two forms of *P. teres* and its hybrids (produced in laboratory) will be examined to broaden the understanding of mating patterns of *P. teres*.

3) Identification of genomic regions associated with virulence using a *Ptt*/*Ptm* mapping population

To date, virulence genes/QTL have been identified using either *Ptt/Ptt* or *Ptm/Ptm* crosses. Hybrid *Ptt/Ptm* populations have not been used even though they could be more polymorphic and therefore, result in the production of more marker dense genetic maps. Usage of a genome-wide molecular marker system like DArTseq™ would also enable the generation of a large number of molecular markers and would be an ideal genotyping method to use in the detection of polymorphism in *Ptm* and *Ptt* hybrids and development of a comprehensive genetic map which can be used to identify QTL in the progeny. This, in turn, may lead to the identification of markers closer to the identified virulence genes. Using a hybrid population for QTL mapping will enable the identification of genomic regions associated with leaf symptoms, net and spot form, as well as expand the knowledge on the virulence performance of hybrids. As per the authors' knowledge there are no studies which have investigated the genes responsible for the leaf symptoms and whether these are the same as the genes causing virulence. Using a *Ptt*/*Ptm* population enables us to map these genes. Hence, a mapping population consisting of hybrid progeny from a cross between a *Ptt* and a *Ptm* isolate will be used to identify the genomic regions associated with virulence and leaf symptoms of *P. teres* in barley.

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CHAPTER 2

POPULATION STRUCTURE OF *PYRENOPHORA TERES* F. *TERES* BARLEY PATHOGENS FROM DIFFERENT CONTINENTS

In this study we characterized the genetic structure of *Pyrenophora teres* f. *teres* isolates collected mainly from Australia, Hungary and Republic of South Africa along with five isolates from Canada and five historical isolates. Genotyping was carried out by DArTseq™ and the genetic structure was detected by model based and multivariate cluster analyses. Through the genetic structure and genetic differentiation among populations we deduced the potential long dispersion of *Pyrenophora teres* f. *teres.*

Buddhika A. Dahanayaka, Niloofar Vaghefi, Noel L. Knight, Renée Prins, József Bakony, Diána Seress, Lislé Snyman and Anke Martin. *Phytopathology*. (*In press*) DOI:10.1094/PHYTO-09-20-0390-R

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Population structure of *Pyrenophora teres* f. *teres* barley pathogens from different continents

Buddhika A. Dahanayaka[1], Niloofar Vaghefi[1], Noel L. Knight[1], József Bakonyi[2], Renée Prins[3,4], Diána Seress[2], Lislé Snyman[5], Anke Martin[1,*]

[1] Centre for Crop Health, University of Southern Queensland, Toowoomba, QLD, 4350, Australia.
[2] Plant Protection Institute, Centre for Agricultural Research, 15 Herman Ottó Street, Budapest, 1022, Hungary.
[3] CenGen (Pty) Ltd, 78 Fairbairn Street, Worcester, 6850, South Africa.
[4] Stellenbosch University, Department of Genetics, Private Bag X1, Matieland, Stellenbosch, 7602, South Africa.
[5] Department of Agriculture and Fisheries Queensland, Hermitage Research Facility, 604 Yangan Rd, Warwick, QLD, 4370, Australia.
[*]Corresponding author: Anke Martin; anke.martin@usq.edu.au

Keywords: Australia, Diversity Arrays Technology, Hungary, Hybrids, Net form net blotch, South Africa.

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ABSTRACT

Net-form net blotch disease caused by *Pyrenophora teres* f. *teres* (*Ptt*) results in significant yield losses to barley industries. Up-to-date knowledge of the genetic diversity and structure of pathogen populations is critical for better understanding the disease epidemiology and unravelling pathogen survival and dispersal mechanisms. Thus, this study investigated long distance dispersal and adaptation by analysing the genetic structure of 250 *Ptt* isolates collected from Australia, Canada, Hungary and Republic of South Africa (RSA), and historical isolates from Canada, Denmark, Japan and Sweden. The population genetic structure detected by discriminant analysis of principal component, using 5890 Diversity Arrays Technology (DArT) markers, revealed the presence of four clusters. Two of these contained isolates from all regions, and all isolates from RSA were grouped in these two. Australia and Hungary showed three clusters each. One of the Australian clusters contained only Australian isolates. One of the Hungarian clusters contained only Hungarian isolates and one Danish isolate. STRUCTURE analysis indicated that some isolates from Australia and Hungary shared recent ancestry with RSA, Canada and historical isolates and were thus admixed. Subdivisions of the Neighbor-joining network indicated that isolates from distinct countries were closely related, suggesting multiple introduction events conferred genetic heterogeneity in these countries. Through a Neighbor-joining analysis and amplification with form-specific DNA markers two hybrid isolates, CBS 281.31 from Japan and H-919 from Hungary collected in 1931 and 2018, respectively, were detected. These results provide a foundation for exploring improved management of disease incursions and pathogen control through strategic deployment of resistances.

Keywords: Australia, Canada, Diversity Arrays Technology, Historical isolates, Hungary, Hybrids, Net form net blotch, Republic of South Africa.

The net blotch diseases, caused by *Pyrenophora teres*, are major fungal foliar diseases of barley, causing devastating losses to barley production throughout the world (Mathre 1997). Yield loss due to *P. teres* in susceptible barley varieties can range from 10 to 70% (Jayasena et al. 2007; Wallwork et al. 2016). Additionally, total plant death may occur in the absence of suitable fungicide treatments (Steffenson et al. 1991; Mathre 1997; Murray & Brennan 2010). Net blotch can appear as two forms, net form net blotch (NFNB), caused by *P. teres* f. *teres* (*Ptt*), and spot form net blotch (SFNB), caused by *P. teres* f. *maculata* (*Ptm*). Phylogenetically these two forms are closely related to each other (Marin-Felix et al. 2019) while in terms of population genetic analyses, the two forms represent two genetically distinct populations (McLean et al. 2009; Liu et al. 2011; Ellwood & Wallwork 2018). Even though hybrids between *Ptt* and *Ptm* have been produced successfully under laboratory conditions (Smedegård-Petersen 1971), hybrids in the field are considered to be absent or rare due to the genetic distance between these two forms (Lehmensiek et al. 2010; Ellwood et al. 2012; Poudel et al. 2017).

Net form net blotch is characterised by streaks or net-like dark brown necrotic lesions along barley leaf veins, comprising longitudinal and transverse striations (Smedegård-Petersen 1971; Liu et al. 2011). Outbreaks of *Ptt* have occurred across a wide range of barley growing regions and climates (Van den Berg 1988). Short distance dispersal of *Ptt* by air turbulence and water splashing (Deadman & Cooke 1989) can occur through ascospores and conidia produced during sexual and asexual reproduction, respectively (Liu et al. 2011). Since *Ptt* is a seed-borne fungus (Liu et al. 2011), long distance transmission of *Ptt* could result from exchange of infected seeds among geographically remote areas (Shipton 1966; Martin & Clough 1984). Furthermore, as sexual recombination is known to play a major role in the life cycle of *Ptt*, integration and adaptation of novel *Ptt* pathotypes into local areas from another geographical region is possible. Introduction of a novel pathotype may greatly shape the local *Ptt* genetic structure.

Knowledge of population diversity and structure is essential for understanding population dynamics and improving disease control methods. The genetic structure of a *Ptt* population depends on a number of factors such as mutations, genetic drift, gene flow, selection and the relative significance of sexual versus asexual stages in the life cycle of the pathogen (Akhavan et al. 2016). With the advent of molecular genotyping technologies, *Ptt* populations from different geographical locations have been characterized using molecular markers such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLP) and simple sequence repeats (SSR). Genetic characterization studies in Australia

(Serenius et al. 2007; Bogacki et al. 2010; Lehmensiek et al. 2010; Ellwood et al. 2019), Europe (Jonsson et al. 2000; Rau et al. 2003; Serenius et al. 2005; Bakonyi & Justesen 2007; Ficsor et al. 2014), North America (Peever & Milgroom 1994; Jonsson et al. 2000; Akhavan et al. 2016) and the Republic of South Africa (RSA) (Campbell et al. 2002; Lehmensiek et al. 2010) have detected high genetic diversity within *Ptt* populations.

Studies conducted on Australian *P. teres* populations using AFLP and SSR markers revealed high genetic variation within *P. teres* isolates collected from New South Wales, Queensland, South Australia, Victoria and Western Australia (Lehmensiek et al. 2010; McLean et al. 2010; Ellwood et al. 2019). To date, two studies have characterized the genetic structure of *P. teres* populations from the RSA using AFLP and RAPD markers (Campbell et al. 2002; Lehmensiek et al. 2010), which revealed high genetic diversity in the *Ptt* populations. Ficsor et al. (2014) used RAPD markers to detect greater genotypic variability and genetic diversities within sampling units than between sampling units (mating type, field type, geographical region and year), and significant temporal genetic differentiation between seasons in Hungarian *Ptt* populations. While each of these studies provide valuable information on the biology and epidemiology of *Ptt* in the respective regions, it is not possible to compare the genetic diversity and structure of *Ptt* populations among these geographical areas as different studies have used different marker and analysis systems. Hence, application of a single marker system is necessary to enable valid comparisons of the genetic diversity and structure in *Ptt* populations from different parts of the world.

Use of less efficient markers such as AFLPs and RAPDs limits the reproducibility of the results (Mondini et al. 2009). Alternative marker systems, such as Diversity Arrays Technology (DArT), have become available, which produce a large number of reproducible genome-wide markers, some of which are located in gene regions (DArTseq 2020). Diversity Arrays Technology is a high throughput efficient molecular marker technology which, unlike SSR markers, does not require prior knowledge of the genome sequence (Wenzl et al. 2004). With DArT, polymorphisms are detected at restriction enzyme recognition sites and the presence or absence of individual DNA fragments in the genome is detected through microarray hybridization (Jaccoud et al. 2001). The advanced DArT technology also identifies single nucleotide polymorphisms (SNPs) within sequences. This technology has been previously implemented for genetic population analysis of fungal species including *P. teres* (Syme et al. 2018; Poudel et al. 2019; Martin et al. 2020).

The genetic diversity of a pathogen can affect its ability to adapt to host resistances and control strategies (McDonald & Linde 2002). Therefore, pathogens that are genetically more

diverse may also have a higher diversity profile of virulence (Linde & Smith 2019) and an increased ability to respond to environmental changes and control measures, which may affect the resistance to fungicides or pathogenicity on the host (Peltonen et al. 1996). A recent study revealed rapid changes in the genetic structure of *Ptt* populations collected over three years from barley fields in Australia, suggesting potential adaptation and underlining the necessity of using multiple sources of host-plant resistance for defence against the pathogen (Poudel et al. 2019). The continued evolution of fungal pathogen populations driven by the selection pressure applied by host resistance will likely lead to a decline in the efficiency of the deployed resistance (Suffert et al. 2018).

The worldwide occurrence of *Ptt* in barley and its potential for rapid genetic change through sexual recombination over a short period of time demonstrates the necessity of understanding its population diversity and structure in order to achieve efficient disease management strategies, including the development of resistant barley varieties (McDonald & McDermott 1993; Liu et al. 2011). Hence, this study was designed to characterize the genetic diversity and structure of *Ptt* populations from Australia, Canada, Hungary and the RSA, and explore the potential for long distance dispersal and geographic adaptation of the pathogen.

MATERIALS AND METHODS

Sample collection and fungal isolation. The terms entire collection, population and subpopulation in this study refer to the isolates from all countries included in the study, a collection of isolates from a country and a collection of isolates from a region/state within a country, respectively. All the isolates used in this study were monoconidial isolates and collected randomly. Isolates were mostly originated from barley leaves (except two isolates: H-374 and H-376 from Hungary originated from wheat and one: CG16015 from RSA originated from rye grass) exhibiting NFNB symptoms collected from Australia, Canada, Hungary and RSA. Five additional historic isolates were included in this study from Canada [WRS858; Serenius et al. (2007)], Denmark [Pt-Pastorale; Justesen et al. (2008)], Japan [CBS 282.31 and CBS 281.31; Bakonyi and Justesen (2007)] and Sweden [UPSC1838; Bakonyi and Justesen (2007)].

The Australian population included 118 isolates collected between 1985 and 2017 from New South Wales (NSW, n = 20), Queensland (QLD, n = 43) South Australia (SA, n = 24), Victoria (VIC, n = 6) and Western Australia (WA, n = 25), including the previously reported hybrid WAC17021 (McLean et al. 2014) (Supplementary Table S1 and Fig. 1). Sample collection and fungal isolation of Australian samples were performed following the method

described by Martin et al. (2020). Six isolates from Canada, collected by Akhavan et al. (2016) in 2010 and 2011 from Alberta, Manitoba and Saskatchewan, were also included in the study.

The Hungarian population consisted of 85 isolates derived from naturally infected barley (n = 83) and wheat leaves (n = 2) collected from 2006 to 2018 (Supplementary Table S1 and Fig. 1). Seventy-eight isolates were collected from experimental fields at the Centre for Agricultural Research or National Food Chain Safety Office (NFCSO) in the Martonvásár region (n = 31), Fleischmann Rudolf Research Institute, Eszterházy Károly University, Kompolt (n = 17), Institute for Agricultural Research and Educational Farm, University of Debrecen, Karcag (n = 22) and a commercial field or experimental plots of NFCSO and Cereal Research Non-Profit Ltd in the Szombathely region (n = 8). The remaining seven isolates were collected from five commercial and two NFCSO barley fields (Bőny: n = 1, Kölcse: n = 3, Márok: n = 2 and Székkutas: n = 1).

Fungal isolation of Hungarian isolates was performed by inducing conidiogenesis. Leaf segments with necrotic lesions were placed in glass Petri plates and kept on a laboratory bench at ambient temperature or incubated under white light (OSRAM model L36W/640) for 16/8 hour light/dark cycles for 1 to 3 days at 18 to 20°C. Monoconidial isolates were then made by transferring single conidia from the conidiophores to V8-juice agar medium (16 g agar, 3 g $CaCO_3$, 177 mL Campbell's V8-juice and 900 ml distilled water) (Miller 1955) with a sterile needle, using a Leica MZ6 stereomicroscope at 300 to 400× magnification in a laminar air flow cabinet. Single-conidial isolates were incubated for 10 to 14 days in the dark at 18 to 20°C and used as inocula for stock and pea broth cultures. Stock cultures were grown on V8-juice agar slants for 7 to 10 days in the dark at 20 to 22°C, then kept under mineral oil at 15°C. Mycelium for DNA extraction was grown on pea-broth (Erwin & Ribeiro 1996) in steady cultures for 7 to 10 days at 18 to 20°C in the dark. Liquid cultures were then harvested by filtration, washed with deionised water, freeze-dried and ground in liquid nitrogen. Pulverized mycelia were kept at -70°C for DNA extraction.

The RSA population contained 72 isolates collected from leaves of barley (n = 71) and rye grass (n = 1) from eight regions (Bredasdorp: n = 11, Caledon: n = 28, Greyton: n = 6, Klipdale: n = 8, Napier: n = 12, Protem: n = 4, Rietpoel: n = 2 and Riviersonderend: n = 1) around the Western Cape Province of RSA during October 2016 (Supplementary Table S1 and Fig. 1). Fungal isolation was performed by sterilizing the surface of leaf samples in 70% (vol/vol) ethanol for 5 seconds, 5 g/liter NaOCl for 2 minutes and washing three times in sterile water. These were placed on water-agar (10 g/liter) or moist filter paper (×2) and incubated at room temperature and natural day/night light conditions for 1 to 4 days to allow the growth of

conidia. Monoconidial culture production was performed by transferring single conidia to potato dextrose agar (39 g/liter PDA; Biolab Merck, Modderfontein, RSA) and Solustrep (0.3 ml/liter) plates. Plates were incubated for 4 to 5 days, and a single colony was subcultured onto a new PDA plate. After 7 days, agar plugs were collected and stored in 15% glycerol at -80°C and the remaining mycelium was harvested for DNA extraction.

DNA extraction for DArTseq™. DNA from Australian isolates was extracted from single-conidium cultures using the method described by Martin et al. (2020). DNA of Hungarian isolates was extracted from lyophilized mycelium powder using the Cetyl Trimethyl Ammonium Bromide (CTAB) method (Richards et al. 1997) and DNA of all other isolates was extracted using a similar CTAB method (Saghai-Maroof et al. 1984).

The integrity of DNA extracted from each isolate was assessed under ultraviolet light (Fusion FX, VILBER, Marne-la-Vallée, France) after electrophoresis at 100V for 30 min on a 0.8 g/litre agarose gel (Bioline, London, United Kingdom) containing 0.03% GelRed® (Biotium Inc, California, USA). DNA quantity was measured using a NanoPhotometer P300® (IMPLEN, Munich, Germany). For each isolate, 20 µl of DNA solution (> 50 ng/µl) was submitted to Diversity Arrays Technology Pty. Ltd. (Canberra, ACT, Australia) for DArTseq™.

Data filtering and clone correction. Data obtained from DArTseq™ consisted of SNPs and SilicoDArTs (equivalent to microarray markers scored for the presence or absence of sequences obtained from genomic representations). Both forms of data were filtered manually using 10% as the cut off value for the maximum number of missing data points for markers and isolates. Markers with a minimum allele frequency of less than one percent were removed from the data set (Vaghefi et al. 2017). Reproducibility (the proportion of technical replicate assay pairs for which the marker score is consistent) and the CallRate (the proportion of samples for which the genotype call is either present or absent rather than missing) of each marker was evaluated and markers with reproducibility of <1 and CallRate less than 85% were removed. SNPs and SilicoDArTs were combined for further analyses.

A small number of genotyping errors may occur whilst generating DArTseq™ marker data, and this may result in clonal isolates being identified as unique multilocus genotypes (MLGs). In order to remove potential genotyping errors, all genotypes were contracted using the furthermost bitwise distance (Kamvar et al. 2015) among five control DNA samples from the same isolate (NB63i; extracted from an original culture using five different samples of single-conidium derived mycelia) by the *bitwise.dist* function in *poppr* package version 2.8.3 (Kamvar et al. 2014) in R version 3.0.2 (R 2013). The furthermost bitwise distance among five

control samples (0.000925) was set as the threshold value to contract genotypes within the entire population. All populations were clone corrected at the subpopulation stratum using the *clonecorrect* function in *poppr* to collapse clonal groups into a single MLG for all subsequent analyses except for the estimation of genetic diversity indices. Multilocus genotypes shared among subpopulations were calculated by the *cross.pop* function in *poppr*.

Dendrogram construction. All isolates were assigned to genetic clusters without *a priori* assumptions using DARwin version 6.0.021 (Perrier & Jacquemoud-Collet 2006). A dendrogram was produced based on the Jaccard similarity coefficient following the unweighted neighbor-joining clustering method. Bootstrap analysis with 1,000 replicates was used to test the support of the branches on the dendrogram.

Form specific primer amplification to confirm hybrids. After assessing the dendrogram, two isolates forming a group with the previously reported *Ptt-Ptm* hybrid isolate WAC17021 were subjected to PCR amplification using six *Ptt* and six *Ptm* specific primer pairs following Poudel et al. (2017) with modifications. A combination of both *Ptt* and *Ptm* specific primer pairs are expected to be amplified in hybrid isolates (Poudel et al. 2017). DNA of three *Ptt* isolates (NB63i, NB29 and NB50) (Lehmensiek et al. 2010; Martin et al. 2020), three *Ptm* isolates (HRS06033, SNB113 and HRS07033) (Lehmensiek et al. 2010; McLean et al. 2014) and three laboratory produced hybrids (37.1, 37.4 and 37.16) (unpublished data) were also amplified with the primer pairs as positive controls. Each real time PCR reaction was prepared with 2 µl (~ 50 ng/µl) of DNA, 5 µl of SsoAdvanced™ Universal Inhibitor-Tolerant SYBR® Green Supermix (BIORAD, California, USA), 0.25 µM of each primer and 2 µl of molecular water (MilliporeSigma™, Fisher Scientific, Massachusetts, USA) to a final volume of 10 µl. Amplifications were conducted in a CFX384 Touch Real-Time PCR Detection System™ (BIORAD, California, USA) with an initial denaturation at 98°C for 3 min followed by 35 cycles of denaturation at 98°C for 15 s and annealing at 60°C for 30 s. A melt curve analysis was performed after PCR completion by ramping the temperature from 65°C to 95°C, rising by 0.5°C with each step. The presence/absence of specific loci in isolates were assessed by comparing the quantitative data generated by the melt curves and the melt temperatures of the positive controls.

Analysis of molecular variance. In order to identify significant variation among populations and subpopulations, the *amova* function in *Ade4* version 1.7.13 (Dray & Dufour 2007) in R was used. Analysis of molecular variance (AMOVA) was conducted on the combined Australia, Hungary and RSA populations using the *poppr.amova* function in *poppr* with 1,000 permutations. Isolates were stratified based on the country of origin, region/state

and year of collection. Analysis was conducted to identify the amount of genetic variation within and among countries, year of collection, and region/state within countries. When conducting AMOVA for the separate Australian, Hungarian and RSA populations, subpopulations consisting of less than five isolates were removed. Analysis was performed for genetic variation within and among states/fields and year of collection for Australia and Hungary populations.

Population structure by multivariate cluster analyses. Two multivariate analyses, principal component analysis (PCA) followed by discriminant analysis of principle components (DAPC) were conducted to identify the genetic structure of the entire clone-corrected collection without *a priori* assumptions. For PCA, the optimum number of principal components and principal coordinates were found and plots were drawn using the *pcadapt* function in *pcadapt* version 4.3.3 package (Luu et al. 2017). Discriminant analysis of principle components was calculated using the *dapc* function in the R package *adegenet* version 2.1.2 (Jombart 2008) and was performed for individual populations in order to detect the population structure and number of clusters within countries. The optimum number of clusters in the population was obtained using the Bayesian information criterion function *find.clusters* and the optimal number of principal component axes to retain in DAPC were estimated via the *xvalDapc* function in *adegenet.*

Population structure by model-based cluster analyses. Population structure without *a priori* assumption was investigated using STRUCTURE version 2.3.4 (Pritchard et al. 2000), in which the Bayesian unsupervised genetic clustering algorithm was implemented for the entire clone-corrected collection (100 Australian, 78 Hungarian, 59 RSA, six Canadian and one historical isolate each from Canada, Japan, Sweden and Denmark). The analysis was conducted following an admixture model with a burn-in period of 10,000 Markov chain Monte Carlo and 100,000 iterations. Ten independent runs were conducted for each potential number of genetic clusters (*K*), where *K* ranged from 1 to 10. The analysis was performed independently for Australian, Hungarian and RSA populations with the above-mentioned criteria to identify the genetic structure within populations. Values extracted from STRUCTURE HARVESTER version 0.6.94 (Earl & vonHoldt 2012) were used to identify the optimal number of clusters for the entire clone-corrected collection as well as Australian, Hungarian and RSA populations (Evanno et al. 2005). Each replicate for the optimal delta *K* (ΔK) value was entered into CLUMPAK version 1.1 (Kopelman et al. 2015) to generate the graphical representation of the optimal *K*. A cut off value of 70% was considered as the minimum value of an individual to be included in each population.

Population structure based on phylogenetic network. A Neighbor-net phylogenetic network was built for the entire collection using SplitsTree version 4.13 (Huson 1998) to identify the subdivisions of the clone corrected *P. teres* population. The Neighbor-net network was produced based on neighbor-joining (NJ) algorithm described by Saitou and Nei (Saitou & Nei 1987) following the method depicted by Bryant and Moulton (2004). Bootstrap analysis with 1,000 replicates was used to test the support of branches on the network.

Identification of mating type and sexual recombination. Amplification of mating type primer pairs *pttMAT1-1* and *pttMAT1-2* (Lu et al. 2010) was assessed across all isolates. A chi square test of the ratio of *pttMAT1-1* and *pttMAT1-2* was manually calculated for *Ptt* clusters identified by individual DAPC analyses from Australia, Hungary and RSA to determine whether there was a significant deviation from the expected 1:1 ratio under panmixia. In order to identify the mating type of the hybrids, all mating type primer pairs (*pttMAT1-1, pttMAT1-2, ptmMAT1-1* and *ptmMAT1-2* (Lu et al. 2010) were amplified across hybrids.

Pairwise homoplasy index (PHI) test which tests the null hypothesis of no recombination available in SplitsTree 4.13 was also implemented for the same clusters detected in individual DAPC analyses for Australia, Hungary and RSA to identify the potential sexual recombination within the countries as described by Bruen et al. (2006).

Genetic diversity of populations. The non-clone corrected data set was used to calculate the number of MLGs, expected MLGs (eMLG) after rarefaction, Simpson's complement index of multilocus genotypic diversity (1-λ) and Nei's unbiased gene diversity (genetic variation within the population defined as the probability that two randomly sampled alleles are different) (Nei 1973; Nei & Chesser 1983) using *poppr*. The normalised Shannon-Wiener index (H) was calculated manually following the method described by Spellerberg and Fedor (2003). Simpson's complement index is given based on the probability of two random isolates drawn from a subpopulation to be of a different genotype (Simpson 1949; Morris et al. 2014) and Shannon-Wiener index measures the genotypic diversity of the population by richness (number of MLGs in the population) and relative abundance in a defined location (Shannon 2001; Spellerberg & Fedor 2003). Expected MLG, Simpson's complement index of multilocus genotypic diversity (1-λ), Nei's unbiased gene diversity and the normalised Shannon-Wiener index were also calculated for the clusters identified from individual DAPC analyses of Australia, Hungary and RSA.

Variant annotation and associated genes. Markers with the largest contribution to the genetic variation detected in DAPC analysis of the entire clone-corrected collection were detected using the function *loadingplot* in *adegenet* (Jombart et al. 2010). The largest

contributing markers for the genetic clusters in PCA for the entire collection were also determined at the 0.0001 significance level using the function *outliers.pcadapt* in the *pcadapt* package, and compared to the markers detected from DAPC analysis. Sequences (68 bp reads produced by DArTseq™) harbouring markers significantly ($P < 0.0001$) responsible for the genetic variation were aligned by NCBI-BLAST (NCBI) and NBLSTX (*EnsemblFungi*) to the reference genomes of *Pyrenophora teres* f. *teres* isolates W1-1 (GenBank accession number: OCTH00000000 and BioProject: PRJEB18107) and 0-1 (GenBank accession number: AEEY01000000 and BioProject: PRJNA66337), and partial genomic regions of 13A (GenBank accession numbers: JQ837863 and JQ582646). This enabled identification of possible genes linked to markers with the largest contribution to the genetic clustering during DAPC and PCA analyses. The putative proteins for the respective genes were predicted using Universal Protein knowledgebase (UniProt).

RESULTS

Genetic data and marker filtering. Across 286 isolates, a total of 6,440 SNPs and 14,829 SilicoDArTs were reported, with 891 SNPs and 4,999 SilicoDArTs retained for the analysis after filtering (Supplementary Material_2). After contraction (collapsing genotypes by genetic distance in order to remove genotypes identified as unique due to genotyping errors) of the entire collection, 286 genotypes were contracted to 250 genotypes. No clonal genotypes were identified after clone correction of 250 MLGs and no MLGs were shared across any regions/states within a country. Of these, 101 MLGs were from the Australian population collected from 1985 to 2017 (including a previously reported hybrid WAC17021), seven were Canadian isolates collected in 2010 and 2011 including one historical isolate collected in 1973, 59 were RSA isolates collected in 2016 and 79 were Hungarian isolates (16 collected from 2006 to 2009 and 63 in 2017/8). Four historical isolates representing four different MLGs, two from Japan (collected in 1931) and one each from Denmark (1976) and Sweden (1986) were also included (Supplementary Table S1).

Dendrogram construction. The distance-based dendrogram obtained from DARwin showed the presence of a distinct group of three isolates (Supplementary Fig.1). This group showed distinct genetic separation from the rest of the *Ptt* isolates and contained the previously reported hybrid WAC10721 from Australia along with H-919 from Hungary and CBS 281.31 from Japan, thus suggesting that these two isolates may also be hybrids.

Form specific primer amplification to confirm hybrids. PCR amplification of six *Ptt* and six *Ptm* specific primer pairs (Poudel et al. 2017) confirmed the hybrid identity of isolates H-919 and CBS 281.31. PCR results of the isolate H-919 with 12 primer pairs showed

amplification for *PttQ1, PttQ3, PttQ5, PtmQ7, PtmQ8* and *PtmQ12* while CBS 281.31 showed amplification for *PttQ1, PttQ2, PttQ5, PtmQ7, PtmQ8, PtmQ9.* The *Ptt* positive control isolates NB63i, NB29 and NB50 and the *Ptm* positive controls HRS06033, SNB113 and HRS07033 showed amplification for the six *Ptt* specific primers pairs and the six *Ptm* specific primer pairs, respectively. Isolate WAC10721 and the laboratory produced hybrid isolates used as controls amplified a mixture of both *Ptt* and *Ptm* specific primer pairs. The two hybrid isolates H-919 and CBS 281.31, along with the previously reported hybrid WAC10721, were removed from subsequent analyses characterizing the genetic structure and genetic diversity of *Ptt*.

Analysis of molecular variance. AMOVA showed significant genetic variation among countries, accounting for 19.13% (P = 0.001) of the total genetic variation, while variation among isolates within populations was 82.59% (P = 0.001) (Table 1). Within population, among regions/states variation accounted for 17.40% (P = 0.001) of the total genetic variation. Considering the country and the year of collection, no significant genetic variation (P = 0.259) was observed among populations (0.52%). Out of the total genetic variation in Australia, 7.01% (P = 0.001) was observed among states in Australia, while genetic variation among regions in Hungary (2.08%) and RSA (1.78%) was not significant (P = 0.072). The variation for the year of collection of *Ptt* isolates for the total genetic variation in Australia (0.12%) and Hungary (0.99%) were not significant (P = 0.415 and 0.192 respectively).

Population structure based on multivariate cluster analyses. In the PCA plot, principal component 1 (PC1) separated a group of Australian isolates (n = 45) and another cluster of Hungarian isolates (n = 55) along with the historical Danish isolate Pt-Pastorale from the rest of the collection. Separation of the 45 Australian isolates from the rest of the collection was further supported by PC2 (Fig. 2).

DAPC without *a priori* population assignment indicated the presence of four clusters for the entire clone-corrected collection (Fig. 3). All isolates in cluster 1 (n = 46) were from Australia, while cluster 3 consisted of 55 Hungarian isolates and isolate Pt-Pastorale from Denmark. Cluster 4 consisted of isolates from Australia (n = 44), Canada (n = 6), Hungary (n = 5) and RSA (n = 40). Cluster 2 contained isolates from Australia (n = 10), Hungary (n = 18), RSA (n = 19) and one each from Canada (MB05), Japan (CSB 282.31) and Sweden (UPSC1838).

Individual DAPC results obtained for each population from Australia, Hungary and RSA showed three clusters each (Supplementary Fig. S2A, B and C). These clusters contained isolates from different regions/states within the respective countries, except for cluster 3 in the

Australian population which contained isolates only from QLD, SA and WA (Supplementary Fig. S2A). Cluster_1 and cluster_3 obtained from the individual DAPC plot of Australian isolates consisted of isolates present in cluster 1 from the entire clone-corrected DAPC plot. Cluster_2 contained isolates present in cluster 2 and cluster 4 from the entire clone-corrected DAPC plot. Cluster_1 and cluster_3 isolates from the individual Hungarian DAPC plot contained isolates present in cluster 3 from the entire clone-corrected DAPC plot and cluster_2 contained isolates present in cluster 2, cluster 3 and cluster 4 from the entire clone-corrected DAPC plot. Cluster_1 and cluster_2 from the individual RSA DAPC plot contained isolates present in cluster 4 and cluster 2 from the entire clone-corrected DAPC plot, respectively, while cluster_3 contained isolates present in both cluster 4 and 2 from the entire clone-corrected DAPC plot.

Population structure based on model-based cluster analyses. STRUCTURE analysis of 247 isolates determined that three clusters best described the data (Supplementary Fig. S3A). In the three-clusters STRUCTURE model, genotypes from Australia tended to have intermediate membership in multiple clusters, while genotypes from RSA and Hungary tended to have high membership proportions in a single cluster. Using a 70% cutoff on membership proportions to assign a genotype into a cluster, a first cluster (cluster I) consisted of 46 isolates from Australia, a second cluster (cluster II) consisted of 55 isolates from Hungary and 1 isolate (Pt-Pastorale) from Denmark and a third cluster (cluster III) consisted of 145 isolates from Australia (n = 54), Canada (n = 6), Hungary (n = 23), RSA (n = 59) and historical isolates (n = 3) (Supplementary Table S1 and Fig. 4). Many genotypes from Australia (cluster III) had shared ancestry with genotypes from RSA and are thus, admixed in the three-cluster model. The six Canadian isolates along with the historical Canadian isolate were also found to be admixed (cluster III). At K=3, historical isolates from Japan and Sweden had high membership in the cluster present in RSA, Hungary and Australia, while the historical isolate from Denmark had high membership in the cluster specific to Hungary.

Genetic structure was also analysed independently for each population to identify further subdivision within countries. The mode of ΔK was observed at K=2 for the Australian, Hungarian and RSA populations (Supplementary Fig. S3B, C and D). The individual STRUCTURE analysis for Australian isolates showed that 50% and 43% of the isolates clustered into either cluster_I or cluster_II, with membership proportions of >70% for the respective clusters, while 7% of isolates were considered admixed due to membership proportions of <70% for both clusters (Supplementary Fig. S4A). The Hungarian isolates showed two clusters, cluster_I and cluster_II) containing 71% and 29% of the isolates,

respectively, with no admixed individuals. The two clusters, cluster_I and cluster_II, from the STUCTURE analysis of RSA isolates contained 68% and 17% of the isolates with membership proportions of >70% for the respective clusters and 9% admixed isolates that were not assigned to either of the clusters. The clusters obtained for the Australian, Hungarian and RSA populations were compared to the year and field/state of collection and no association was found.

Cluster_I and cluster_II obtained from the individual Australian STRUCTURE analysis consisted of isolates present in cluster III and I from the entire clone-corrected STRUCTURE analysis, respectively. Cluster_I and cluster_II from the Hungarian STRUCTURE analysis contained isolates present in cluster II and cluster III from the entire clone-corrected STRUCTURE analysis, respectively. Both cluster_I, cluster_II and admixed isolates from the individual RSA STRUCTURE analysis contained isolates present in cluster III from the entire clone-corrected STRUCTURE analysis.

The DAPC and STRUCTURE analyses of the entire clone-corrected collection resulted in identification of four and three clusters, respectively. Cluster 1 and cluster 3 from the DAPC analysis corresponded to cluster I and cluster II from the STRUCTURE analysis, respectively. Isolates present in cluster 2 and cluster 4 from the DAPC analysis corresponded to the isolates in cluster III from the STRUCTURE analysis. Therefore, DAPC analysis characterized the population subdivision in the dataset with higher resolution than STRUCTURE analyses (Jombart et al. 2010), thus, clusters detected by DAPC were further used to calculate the sexual recombination and genetic diversity.

Population structure based on phylogenetic network. The Neighbor-net phylogenetic network inferred using Splitstree showed extensive reticulation connecting all isolates (Fig. 5), consistent with a history of recombination. The structure of the network indicated that genotypes from different countries could be closely related (Fig. 5.). Historical Danish isolate Pt-Pastorale, Japanese isolate CBS282.31 and Swedish isolate UPSC1838 grouped with Hungarian genotypes.

Identification of mating type and sexual recombination. Amplification of *Ptt* isolates with mating type primers indicated that 47 Australian isolates had the *MAT1-1* idiomorph (mating type 1) while the remaining 53 carried the *MAT1-2* idiomorph (mating type 2) (Supplementary Table S3). For Hungary, 37 isolates were found to be *MAT1-1*, and 41 isolates were *MAT1-2*. Out of 59 RSA isolates, 39 were *MAT1-1* and 21 were *MAT1-2*. Mating type ratios calculated for populations from Australia, Hungary and RSA based on clusters identified with country-specific DAPC analyses (Supplementary figure S2) showed that except cluster_2

from RSA (P = 0.021) the chi square values for the clusters from Australia, Hungary and RSA did not significantly differ from the expected ratio of 1:1 under panmixia. PHI rejected the null hypothesis of clonality in cluster_2 (P = 0.014) in Australia and cluster_1 (P = 4.8E-4) and 3 (P = 0.007) in Hungary while other clusters from Australia (cluster_1 and cluster_3), Hungary (cluster_2) and RSA (cluster_1, cluster_2 and cluster_3) did not show evidence for recombination (Supplementary Table S3).

Genetic diversity. The number of eMLGs calculated for Australia, Hungary and RSA was 10. The highest genetic diversity indices among three countries for the non-clone corrected data set were observed for the population from Hungary, with a normalised Shannon-Wiener index and Nei's unbiased gene diversity index of 0.992 and 0.184, respectively (Table 2). The lowest normalised Shannon-Wiener index, 0.973, and Nei's unbiased gene diversity index, 0.143, were calculated for the population from RSA. The highest value for Simpson's complement index of multilocus genotypic diversity was 0.991, exhibited by the Australian population, while the lowest value, 0.986, was reported for the population from RSA. However, the overall genetic diversity within the populations was high.

The highest genetic diversity indices for the clusters detected by DAPC were observed for Hungarian isolates with a normalised Shannon-Wiener index and Nei's unbiased gene diversity index of 0.935 and 0.279, respectively (Supplementary Table S4). The lowest total normalised Shannon-Wiener index, 0.859, and Nei's unbiased gene diversity index, 0.224, were observed for the clusters from RSA. The highest Simpson's complement index of multilocus genotypic diversity was 0.990, exhibited by the Australian population, while the lowest value, 0.983, was reported for the population from RSA.

Variant annotation and associated genes. Out of 5,890 markers used for the DAPC and PCA analyses of the entire clone-corrected collection, 66 were found to be significantly associated with the genetic differences of clusters and subdivisions (P < 0.0001) detected by DAPC and PCA respectively. Out of 66 markers, 34 were aligned with reference genomes with the E-values (expected value) ranging from $8.4E^{-33}$ to 1.7. Out of these 34 markers, four markers aligned with known genes, another four were not situated near genes, five aligned with genes of uncharacterized proteins and 21 aligned with genes for hypothetical proteins in the reference *Ptt* genomes (Supplementary Table S2). The four markers aligned with genes were associated with ND89-9 nonribosomal peptide synthetase 2 (GenBank accession number: JQ582646), glyceraldeyde-3-phosphate dehydrogenase-like protein (*GPD1*) gene (GenBank accession number: JQ837863), endo-1,4-beta-xylanase A mRNA (GenBank accession number: JX900133) and cytochrome P450 lanosterol 14 alpha-demethylase (*CYP51A*) gene (GenBank

accession number: KX578221). The identified hypothetical genes represented seven different hypothetical proteins: ANK_REP_REGION domain-containing protein, DDE-1 domain-containing protein, SET domain-containing protein, DUF1996 domain-containing protein, Peptidase A1 domain-containing protein, AAA domain-containing protein and MFS domain-containing protein in *Ptt*.

DISCUSSION

The present study investigates the most geographically diverse collection of *Ptt* isolates analysed in a single study to date. It provides a comprehensive investigation of the genetic structure of *Ptt* populations from different geographical areas through the implementation of the genome-wide marker system, DArTseq™, and inclusion of a higher number of isolates compared to previous studies. In this study, 247 *Ptt* MLGs, predominantly from Australia, Hungary and RSA, were assessed in order to describe the genetic structure of *Ptt* isolates among distinct geographical areas.

The genetic structure of the entire clone-corrected collection detected by the DAPC analysis revealed the presence of four clusters. Two clusters contained some isolates from Australia and Hungary, and all the isolates from Canada, RSA and all the historical isolates except Pt-Pastorale from Denmark. The other two clusters were specific to Australian isolates and Hungarian isolates along with the historical Danish isolate. STRUCTURE analysis also revealed the presence of two distinct clusters for Australia (n = 46) and Hungary (n = 55) reflecting their genetic isolation from each other based on geographical origin. Furthermore, Neighbor-net phylogenetic network showed a distinct Hungarian cluster. In the Neighbor-net phylogenetic network, the *Ptt* isolates from Australia, Canada, Hungary and RSA formed more than one subdivision per country. The isolates from these subdivisions did not relate to their year of collection or the region/state of origin. Therefore, the underlying factor for the genetic isolation of *Ptt* populations from the same geographical area might include other variables such as varietal differences (Fowler et al. 2017), fungicide regimes, geographical isolation or environmental factors.

A number of different analyses used in this study identified the admixed nature of multiple isolates mainly from Australia. STRUCTURE based cluster analysis revealed that there were population subdivisions in Hungary and Australia, and that one of the clusters present in each of these countries shared recent ancestry with the cluster containing the Canadian, RSA and most of the historical isolates. Cluster analyses results also showed more admixture in Australia than in Hungary. DAPC and highly reticulated Neighbor-net phylogenetic network also gave evidence that these isolates are of mixed origin. In the

519 Neighbor-net phylogenetic network, some of the isolates from the same countries were closely
520 related to isolates from other countries. Even though some isolates from subdivisions of
521 Australia and RSA showed mixed origin/multiple origins, others showed ancestry in a single
522 group, suggesting that these isolates could have evolved from a common ancestor or an
523 introduction of isolates from a common population and then adapted to the respective
524 environments through sexual reproduction. The admixed origin of isolates could have resulted
525 from gene flow among countries. Gene flow is one of the main evolutionary forces affecting
526 in the genetic structure of a pathogen (Rogers & Rogers 1999). As *Ptt* is a seed borne pathogen
527 (Liu et al. 2011), gene flow/introduction of isolates from one geographical area to another is
528 possible through seed exchange and then adaptation to local environments. This may have
529 occurred in the case of Australian *Ptt* isolates, which have been suggested by Fowler et al.
530 (2017) to have evolved and adapted to regional barley cultivars in Australia.
531 Individual STRUCTURE analyses of Australian, Hungarian and RSA isolates indicated
532 that some of the isolates from Australia and RSA were admixed while isolates from Hungary
533 showed no admixture. The potential admixture found within Australian and RSA isolates could
534 have resulted from the dispersion of the pathogen through sexual reproduction and lack of
535 varietal specialization within the country. The absence of admixed in Hungarian isolates might
536 have been caused due to physical and reproduction barriers in the dispersion of the pathogen,
537 host specialization and/or recent introduction of isolates.
538 The genetic structure of *Ptt* populations detected in model-based cluster analyses did not
539 correspond to the region/state or the year of collection of the isolates, hence, factors
540 contributing to the genetic structure of *Ptt* populations were investigated by identifying the
541 markers underlying the genetic structure detected in DAPC and PCA. One of these markers
542 was located within the gene responsible for the nonribosomal peptide synthetases protein. The
543 nonribosomal peptide synthetases are responsible for the production of nonribosomal peptides,
544 which are bioactive secondary metabolites known to be involved in cellular development,
545 pathogenicity and stress responses in plant fungal pathogens (Keller et al. 2005; Sayari et al.
546 2019). The potential role of this locus in differential aggressiveness of *Ptt* isolates requires
547 further investigation. Other markers that were significantly associated with genetic structuring
548 of the *Ptt* populations included a glyceraldeyde-3-phosphate dehydrogenase-like protein
549 (*GPD1*) gene, an endo-1,4-beta-xylanase A mRNA gene, and a cytochrome P450 lanosterol 14
550 alpha-demethylase (*CYP51A*) gene. The *GPD1* gene has been frequently used as a genetic
551 marker in phylogenetic studies to differentiate fungal pathogens including *Pyrenophora teres*
552 (Zhang & Berbee 2001; Andrie et al. 2008; Lu et al. 2013). *GPD1* plays a major role in fungal

553 metabolic pathways like energy synthesis and biomass synthesis (Larsson et al. 1998). It has
554 been suggested that mutations in the glyceraldeyde-3-phosphate dehydrogenase gene
555 contribute to the nutrient uptake of phytopathogenic *Colletotrichum* spp. during their
556 biotrophic phase in the infection process on many perennial plants including olive, citrus and
557 tomato (Wei et al. 2004; Materatski et al. 2019). The enzyme endo-1,4-beta-xylanase plays a
558 vital role in the breakdown of xylan, a major component of plant cell walls (Nguyen et al.
559 2011), and the degradation of the plant cell wall has been correlated with virulence and
560 pathogenicity of phytopathogenic *Fusarium* spp. and *Valsa* spp. on tomato and apple (Gómez-
561 Gómez et al. 2001; Wang et al. 2014). Cytochrome P450 lanosterol 14 alpha-demethylase is
562 important for the biosynthesis of ergosterol, a primary fungal cell membrane sterol that is
563 responsible for maintaining membrane fluidity and stability (Rodriguez et al. 1985; Parks &
564 Casey 1995; Luo & Schnabel 2008; Koch et al. 2013). Mutations of this gene have been
565 associated with the demethylase inhibitor (DMI) or group 3 fungicide resistance in *P. teres*
566 (Ellwood et al. 2019; Mair et al. 2019). Considering the importance of these genes for fungal
567 virulence/pathogenicity, it is plausible that mutations at these loci are due to external effects
568 such as environmental factors and fungicide regimes. These factors may have driven local
569 and/or host adaptation of *Ptt* isolates in different regions, resulting in the distinct genetic sub-
570 structuring detected in this study.

571 Sexual recombination plays a major role in the evolution and adaptation of a pathogen
572 which may influence the genetic structure (Lee et al. 2010). *Ptt* is a well-known sexually
573 reproducing fungus (Liu et al. 2011). A mating type ratio of 1:1 is expected in the absence of
574 segregation distortion and clonal selection among mating types and the two mating types ratio
575 is equalized through sexual recombination in *P. teres* (Milgroom 1996; Rau et al. 2005). In the
576 current study, except for cluster_2 from RSA, other clusters collected from Australia, Hungary
577 and RSA did not deviate from the expected 1:1 ratio. Studies of Finish, Australian and
578 Canadian *Ptt* populations reported that the mating type ratio did not deviate from the expected
579 1:1 ratio (Rau et al. 2005; Serenius et al. 2005; Akhavan et al. 2016; Linde & Smith 2019),
580 while studies of *Ptt* populations from Czech Republic and Slovakia, and Krasnodar, Russia
581 deviated from a 1:1 ratio (Leišová al. (2014); Serenius et al. (2007)). Deviation of mating type
582 ratio in cluster_2, RSA and absence of sexual recombination evidence for cluster 1 and 3 from
583 Australia, cluster 2 from Hungary and all clusters from RSA based on PHI test results might
584 have occurred due to unsystematic sampling or introduction of primary inoculum like
585 contaminated seeds/conidia to the fields. In the current study, *Ptt* isolates from Australia and
586 Hungary have been collected from different years. Therefore, further studies are necessary with

587 a higher number of isolates and intensive sampling methods to confirm the evidence for sexual
588 reproduction of Australian and Hungarian *Ptt* populations.

589 Previous studies have suggested that hybridization between the two types of *P. teres* is
590 rare or absent under field conditions due to the apparent genetic isolation of both forms
591 (Lehmensiek et al. 2010; Ellwood et al. 2012). Prior to this study, only four naturally occurring
592 putative hybrids had been detected from barley fields: one putative hybrid from the south-
593 western Cape of RSA (Campbell et al. 2002), two from Tovacov, Czech Republic (PTM-15
594 and PTM-16) (Leišova et al. 2005), and one from a barley field in Western Australia
595 (WAC10721) (McLean et al. 2014). In the current study, additional isolates from Hungary (H-
596 919) and Japan (CBS 281.31) were identified as putative hybrids based on distinct genetic
597 subdivision compared to the *Ptt* population and genetic similarity to the previously identified
598 hybrid WAC10721 in the Neighbor-net phylogenetic network. Amplification using *Ptt* and *Ptm*
599 specific DNA markers confirmed that these two isolates were hybrids. The isolate CBS 281.31
600 was originally identified as *Pyrenophora japonica* by Ito (Crous et al, 1995). Crous et al. (1995)
601 found a high degree of homology in restriction digestion (*Hae* III and *Msp* I) DNA banding
602 patterns and similar symptom expression on differential cultivars when comparing CBS 281.31
603 with *Ptm* isolates. In addition, similar morphological characterizations between these isolates
604 led Crous et al. (1995) to conclude that *P. japonica* was a synonym of *P. teres*. A recent study
605 by Marin-Felix et al. (2019) also referred to isolate CBS 281.31 as *P. japonica* and found that
606 the isolate grouped together with *P. teres* based on phylogenetic similarities. Marin-Felix et al.
607 (2019) agreed with the conclusion of Crous et al. (1995) that *P. japonica* was a synonym of *P.*
608 *teres* based on CBS 281.31 as the sole representative of *P. japonica*. A previous distance based
609 cluster analysis study, using seven RAPD markers and complemented with the two *P. teres*
610 form specific PCR markers developed by Williams et al. (2001), identified CBS 281.31 as a
611 *Ptt* isolate (Bakonyi & Justesen 2007). The types and small number of markers used might be
612 the reason for not detecting this isolate as a hybrid in previous studies. The Japanese isolate,
613 CBS 281.31 collected in 1931 was found to be a hybrid nearly a century after it was collected.
614 During the 89 years since it was collected, this hybrid could have crossed with many other
615 Japanese *P. teres* isolates, potentially influencing the genetic structure of the population. Sexual
616 recombination/hybridization between and within the forms of *P. teres* can potentially lead to
617 the generation of novel pathotypes. This may increase the genetic diversity of the population
618 and make disease management more challenging through changes in traits such as fungicide
619 resistance of the pathogen (Syme et al. 2018). Therefore, further population genetics studies
620 and pathotyping of *Ptt* populations are warranted.

In conclusion, the genetic structure and the genetic relationships of *Ptt* isolates collected from different continents reported in this study indicated that some isolates from Australia, Canada, Hungary and RSA shared ancestry with other countries while some of the isolates from Australia and Hungary showed no admixture. Admixed origin among populations provide crucial evidence for the spread of the pathogen. Identification of naturally occurring hybrids supports the fact that the hybridisation between two forms of *P. teres* is possible, which may lead to novel and more complex pathotypes and may cause unpredicted yield losses to the barley industry. Hence, up to date knowledge about genetic structure and the genetic diversity of geographically diverse *P. teres* populations is important to predict and implement efficient disease management strategies and to develop resistant barley cultivars.

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909 TABLE 1. Analysis of molecular variance of *Pyrenophora teres* f. *teres* isolates from Australia,
910 Hungary and the Republic of South Africa (RSA).

Source of variance	Degrees of freedom	Variation (%)	Sum square	Mean square
Australia, Hungary and RSA				
Among countries	2	19.13[a]	6788.27	3394.13
Year among countries	4	0.52[ns]	846.12	211.53
Among regions/states within countries	13	17.40[a]	10088.81	776.06
Among isolates within populations	211	82.59[a]	38143.65	180.78
Australia				
Among states	4	7.01[a]	1794.25	448.56
Within states	95	92.99[a]	17670.53	186.01
Year within Australia	3	0.12[ns]	601.16	200.39
Hungary				
Among fields	3	2.08[ns]	775.19	258.40
Within fields	67	97.92[a]	12822.33	191.38
Year within Hungary	1	0.99 [ns]	244.96	244.96
RSA				
Among fields	4	1.78 [ns]	731.09	182.77
Within fields	49	97.90 [a]	9576.68	195.44

911 [a] Significant at $P \leq 0.001$

912 [ns] Not significant

913 TABLE 2. Indices of genetic diversity for *Pyrenophora teres* f. *teres* populations from
914 Australia, Hungary and Republic of South Africa (RSA).

Population	*n*[a]	MLG[b]	eMLG[c]	H[d]	1-λ[e]	H_{exp}[f]	CF[g]
Australia							
NSW	20	17	10	0.974	0.941	0.164	0.150
QLD	43	37	10	0.981	0.973	0.172	0.140
SA	24	23	10	0.995	0.957	0.184	0.042
VIC	6	6	6	1.000	0.833	0.167	0
WA	24	17	10	0.633	0.941	0.177	0.292
Australia total	117	100	10	0.986	0.991	0.183	0.145
Hungary							
Bony	1	1	1	NaN[h]	NaN[h]	NaN[h]	NaN[h]
Karcag	22	19	10	0.610	0.947	0.162	0.136
Kompolt	16	14	10	0.511	0.929	0.188	0.125
Kölcse	3	3	3	NaN[h]	NaN[h]	NaN[h]	NaN[h]
Martonvásár	31	30	10	0.996	0.967	0.186	0.032
Márok	2	2	2	NaN[h]	NaN[h]	NaN[h]	NaN[h]
Székkutas	1	1	1	NaN[h]	NaN[h]	NaN[h]	NaN[h]
Szombathely	8	8	8	1.000	0.875	0.190	0
Hungary total	84	78	10	0.992	0.988	0.184	0.071
RSA							
Bredasdorp	11	6	6	0.960	0.833	0.120	0.455
Caledon	28	26	10	0.992	0.962	0.149	0.071
Greyton	6	5	5	0.970	0.800	0.145	0.167
Klipdale	8	8	8	1.000	0.875	0.157	0
Napier	12	9	9	0.973	0.889	0.150	0.250
Protem	4	3	3	NaN[h]	NaN[h]	NaN[h]	NaN[h]
Rietpoel	2	2	2	NaN[h]	NaN[h]	NaN[h]	NaN[h]
Riviersonderend	1	1	1	NaN[h]	NaN[h]	NaN[h]	NaN[h]
RSA total	72	59	10	0.973	0.986	0.143	0.181
Total	273	237	10	0.987	0.996	0.202	0.132

915 [a] Number of isolates

916 [b] Number of multilocus genotypes (MLG)

[c] The number of expected MLG based on rarefaction at the smallest sample size of ≥10

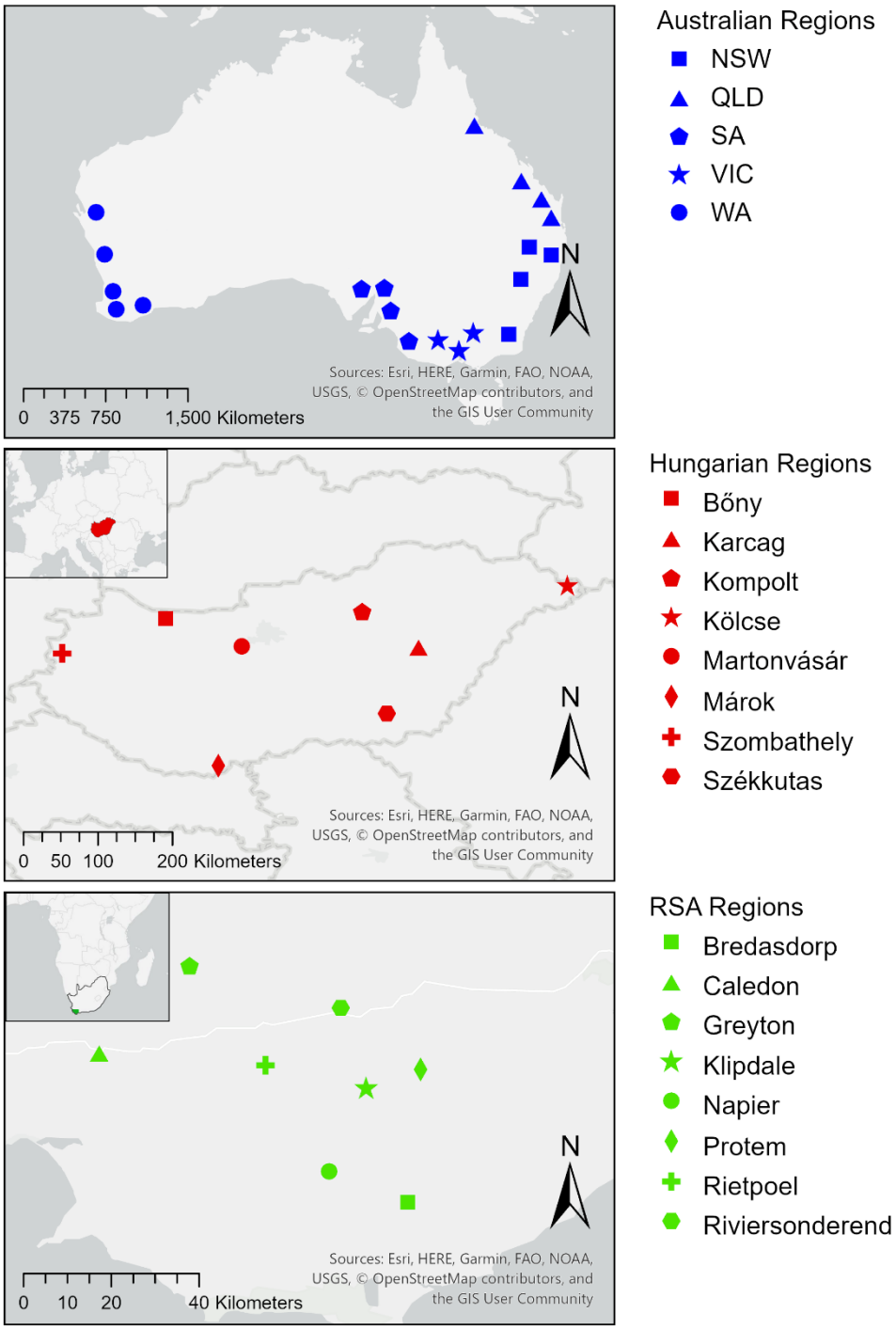
[d] Normalised Shannon-Wiener index of MLG genotypic diversity, the genotypic diversity of the population by richness and relative abundance in a defined location

[e] Simpson's complement index of multilocus genotypic diversity, the probability of two random isolates drawn from a subpopulation to be of a different genotype

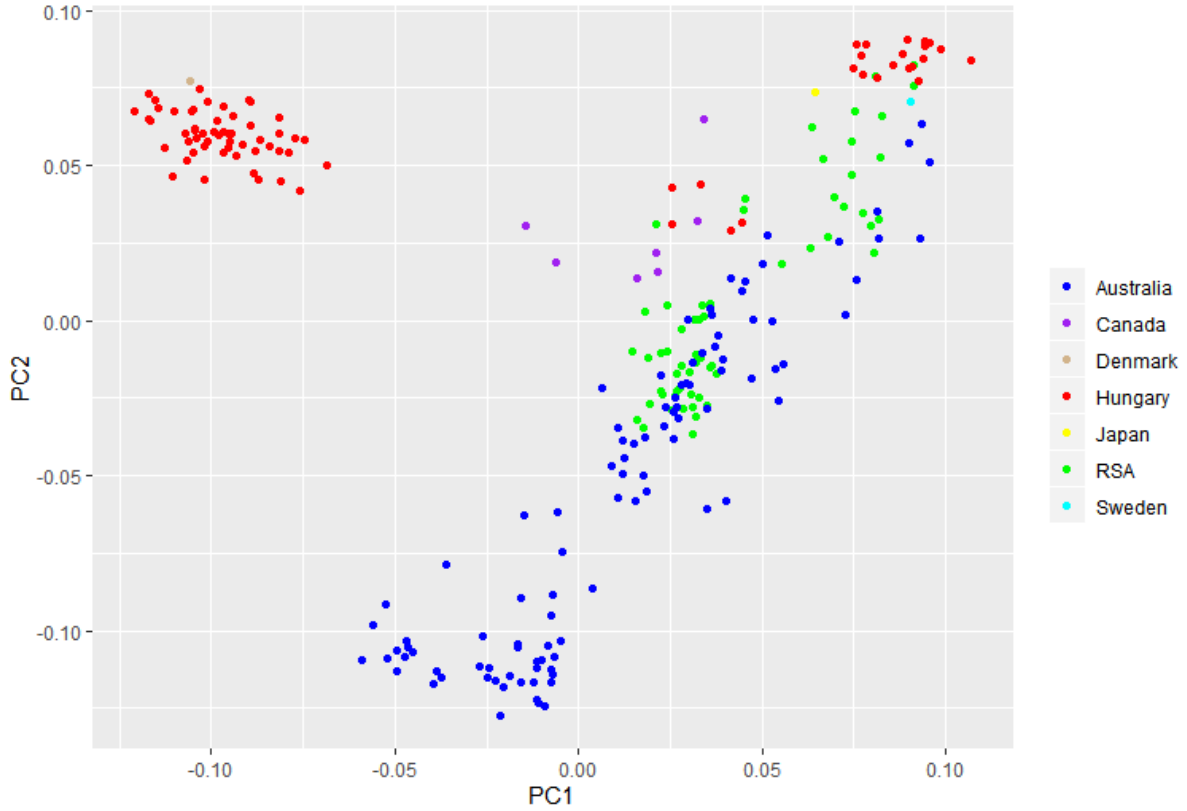
[f] Nei's unbiased gene diversity, the probability that two randomly chosen alleles are different

[g] Clonal fraction (CF), (1-MLG/*n*) where, MLG equals to number of MLGs and *n* equals the number of isolates of the population/subpopulation

[h] Not calculated due to < 5 isolates



954 **Fig. 1.** Sample collection regions of *Pyrenophora teres* f. *teres* isolates in (A) Australia, (B)
955 Hungary and (C) Republic of South Africa (RSA). (ArcGISPro version 2.3, Esri, California,
956 USA).
957



958

959 **Fig. 2.** Principal components analysis of *Pyrenophora teres* f. *teres* isolates collected from
960 Australia, Canada, Denmark, Hungary, Japan, Republic of South Africa (RSA) and Sweden.
961 Principal component axis 1 (PC1) and principal component axis 2 (PC2) explained 13.6% and
962 9.3% variation, respectively, for the genetic clusters.

963

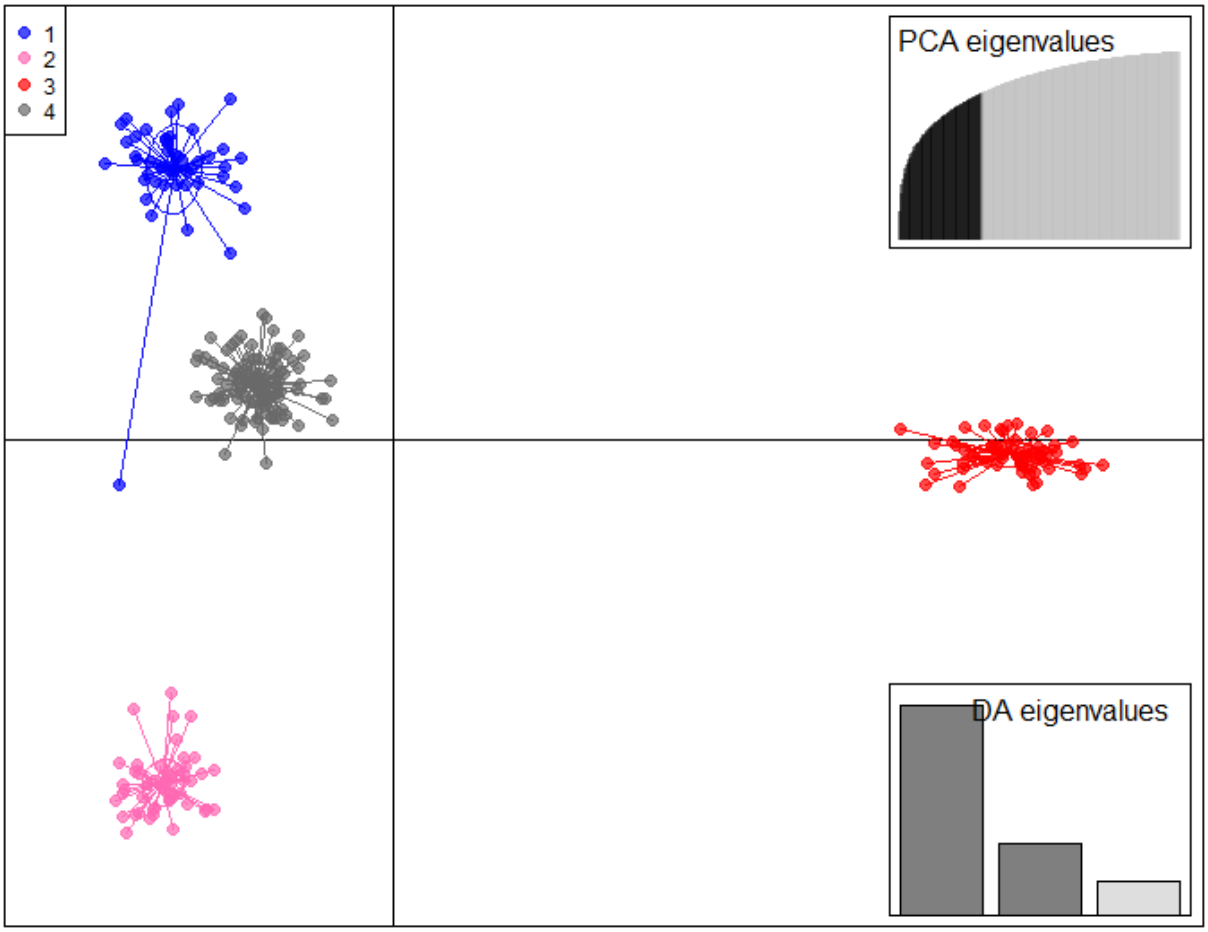


Fig. 3. Discriminant analysis of principal components of the entire collection of *Pyrenophora teres* f. *teres* from Australia (Aus), Republic of South Africa (RSA), Hungary (Hun), Canada (Can), Japan (Jap), Sweden (Swe) and Denmark (Den). The distribution of the eigenvalues of principal component analysis (PCA) and discriminant analysis (DA) indicate that the first two principal components explain 25% of the genetic structure of the clusters.

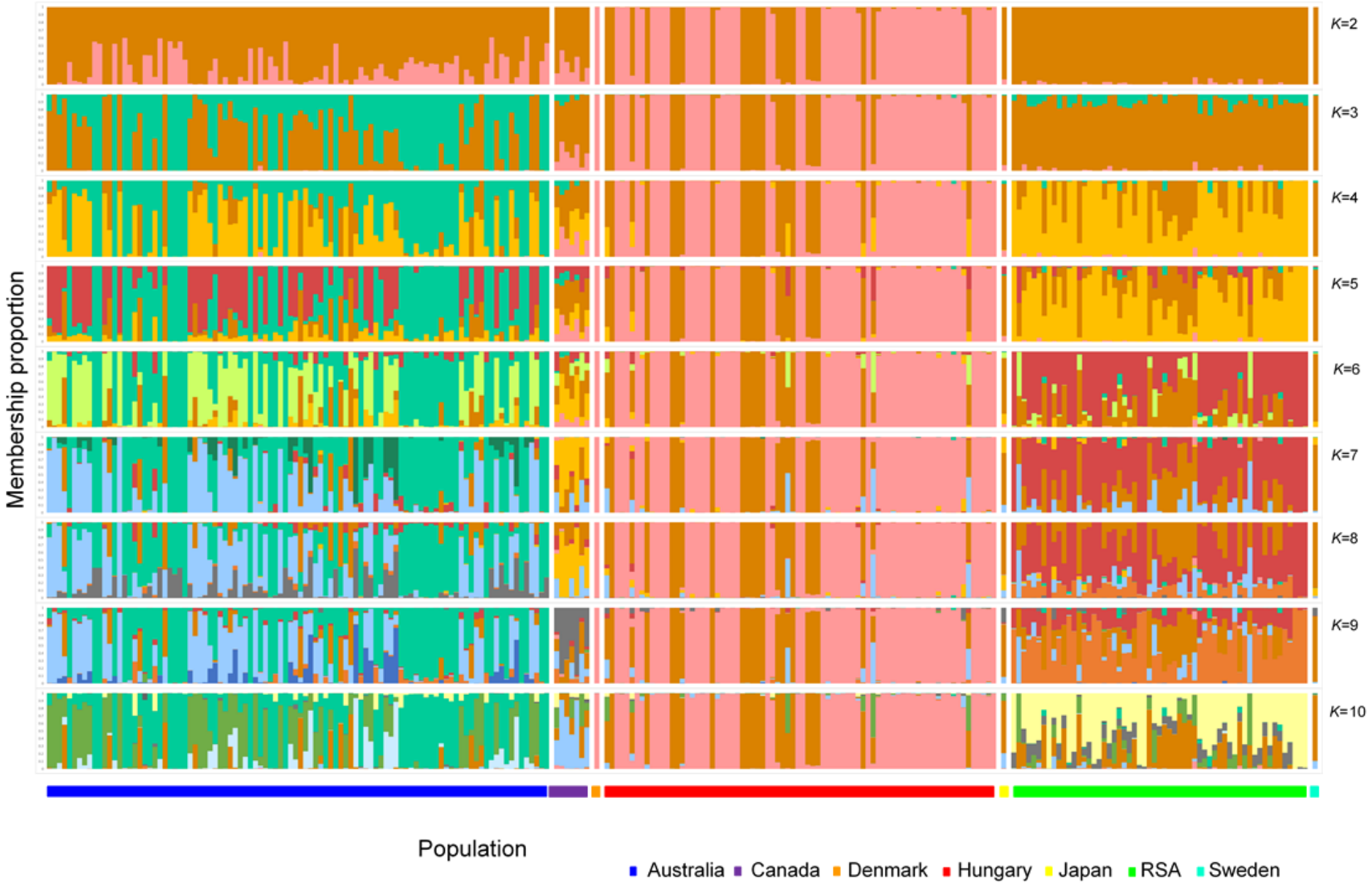

Membership proportion
K=2
K=3
K=4
K=5
K=6
K=7
K=8
K=9
K=10
Population
Australia
Canada
Denmark
Hungary
Japan
RSA
Sweden

Fig.4. Estimates of genetic structuring in the entire clone-corrected *Pyrenophora teres* f. *teres* collection grouped into clusters (K = 2-10) using the model-based clustering method in STRUCTURE. Population color bars represent isolates from Australia (n = 100), Canada (n = 7; including the historical Canadian isolate), Denmark (n = 1), Hungary (n = 78), Japan (n = 1), Republic of South Africa (n = 59) and Sweden (n = 1) respectively. Bars represent individual isolates and the color and height of each bar depicts the estimated membership fraction of each individual into the corresponding cluster.

981

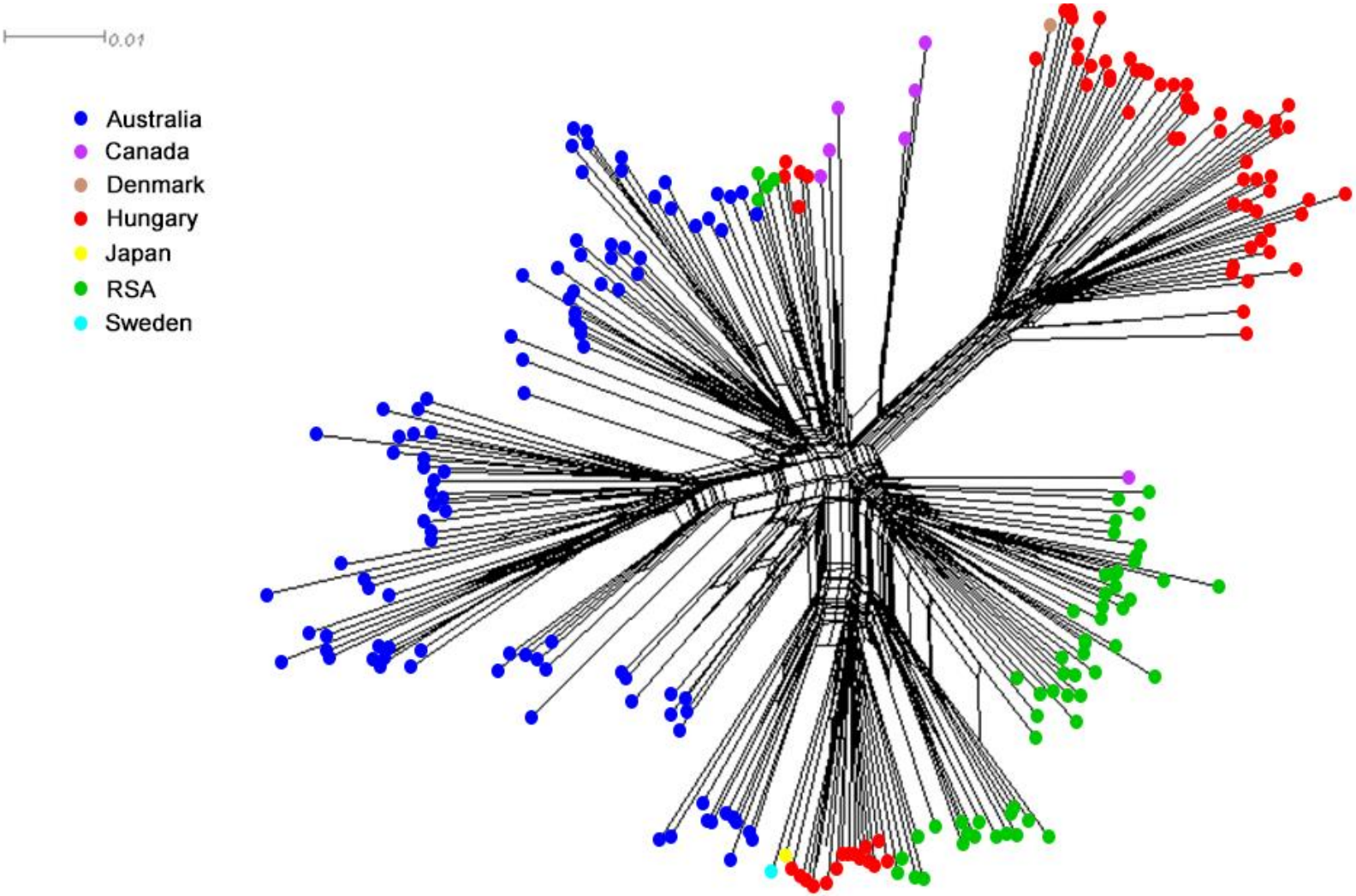


Fig. 5. Neighbor-net phylogenetic network based on DArTseq™ data for *Pyrenophora teres* f. *teres* isolates from Australia (*n* = 100), Canada (*n* = 7; including the historical Canadian isolate), Denmark (*n* = 1), Hungary (*n* = 78), Japan (*n* = 1), Republic of South Africa (*n* = 59) and Sweden (*n* = 1).

CHAPTER 3

INVESTIGATING *IN VITRO* MATING PREFERENCE BETWEEN OR WITHIN THE TWO FORMS OF *PYRENOPHORA TERES* AND ITS HYBRIDS

Previously, the mating preference of *P. teres* was examined under field conditions, which revealed that isolates preferred to undergo sexual recombination with the same form rather than undergoing hybridization with the opposite form. In our study we established different sets of crosses where *Ptt* and *Ptm* were given opportunities to mate with the same or opposite form simultaneously. Additionally, another set of crosses with laboratory-hybrids, *Ptt* and *Ptm* was also established to identify the mating preference of laboratory-hybrids. Mating preferences of the crossed isolates were checked by assessing the progeny isolates using form-specific primer pairs and a Neighbor-net phylogenetic network developed by DArTseq™.

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Investigating *in vitro* mating preference between or within the two forms of *Pyrenophora teres* and its hybrids

Buddhika A. Dahanayaka[1], Niloofar Vaghefi[1], Lislé Snyman[2], Anke Martin[1,*]

[1] Centre for Crop Health, University of Southern Queensland, Toowoomba, QLD, 4350, Australia.

[2] Department of Agriculture and Fisheries Queensland, Hermitage Research Facility, 604 Yangan Rd, Warwick, QLD, 4370, Australia.

[*]Corresponding author: anke.martin@usq.edu.au; Phone +61 7 4631 2261

Keywords: Back crosses, Hybridisation, Introgression, Recombination, Reproduction vigour, Sexual reproduction.

ABSTRACT

Net blotch diseases result in significant yield losses to barley industries worldwide. They occur as net-form and spot-form net blotch caused by *P. teres* f. *teres* (*Ptt*) and *P. teres* f. *maculata* (*Ptm*), respectively. Hybridisation between the forms was proposed to be rare, but recent identifications of field hybrids has renewed interest in the frequency and mechanisms underlying hybridisation. This study investigates the mating preference of *Ptt*, *Ptm* and laboratory-produced hybrids *in vitro*, using 24 different isolates and four different experimental setups. Two crosses in our study produced ascospores during two intervals separated by a 32-35 day period of no ascospore production. For these crosses *Ptt* isolates mated with isolates of the same form during the early ascospore production interval and produced hybrids during the later interval. *Ptm* isolates did not mate with isolates of the same form, instead hybridised with *Ptt* isolates. Analyses based on DArTseq™ markers confirmed that laboratory-produced hybrids, when given the choice to mate with both *Ptt* and *Ptm*, mated with *Ptt* isolates. These results unravel a novel concept that *Ptt* seems to have a greater reproduction vigour than *Ptm*, which could lead to increased prevalence of hybrid incidences *in vivo.*

Keywords: Back crosses, Hybridisation, Introgression, Recombination, Reproduction vigour, Sexual reproduction.

Barley net blotches caused by the fungal pathogen *Pyrenophora teres* (syn. *Drechslera teres*) are important foliar diseases resulting in yield losses and reduced grain quality of barley (Smedegård-Petersen 1974). The pathogen exists as two forms: *P. teres* f. *teres* (*Ptt*) and *P. teres* f. *maculata* (*Ptm*), causing net form net blotch (NFNB) and spot form net blotch (SFNB) of barley, respectively. Lesions caused by *Ptt* are characterized by dark-brown narrow, net-like transverse and longitudinal necrotic striations while *Ptm* is distinguished by dark-brown circular to elliptic lesions on the infected leaf sheaths (Smedegård-Petersen 1971). Both forms can co-exist in the same field and there is no clear evidence of morphological or life cycle differences except that *Ptt* can be transmitted through infected seeds while *Ptm* is not known to be seed-borne (McLean et al. 2009; Liu et al. 2011).

Pyrenophora teres reproduces both sexually and asexually. Asexual reproduction of *P. teres* occurs via the production of genetically identical conidia. As *P. teres* is a heterothallic Ascomycetous fungus, two opposite mating types are needed for sexual reproduction (McDonald 1963). Sexual reproduction in *P. teres* is controlled by a single mating type locus (*MAT1*), which exists as two alternative forms or idiomorphs, i.e., *MAT1-1* and *MAT1-2* (McDonald 1963). During successful mating between *MAT1-1* and *MAT1-2* genotypes, fertile ascomata are formed. Ascomata contain asci and each ascus contains four pairs of ascospores with each pair being genetically identical (Finchman 1971). *In vitro* progeny isolates resulting from sexual recombination (sexual reproduction within form) were reported to have a great genetic diversity (McDonald 1963; McLean et al. 2009; Liu et al. 2011) and exhibited different levels of virulence to those of the parental isolates (Afanasenko et al. 2007).

Hybridisation, i.e. successful genetic crossing between non-conspecific individuals, plays an important role in the exchange of genetic material between species. Hybridisation is proposed as one of the major factors shaping the evolution of fungal plant pathogens, which has resulted in the emergence and adaptation of novel crop pathogens (Brasier 2001). In fungi, hybridisation may occur between species due to both sexual recombination and asexual fusion of hyphae (Kohn 2005). However, hybrids are found to be rare in nature as a result of reduced fitness compared to parental isolates (Stukenbrock 2016) and genetic incompatibilities like Dobzhansky-Muller interaction (negative epistatic interactions) (Kondrashov et al. 2002; Kohn 2005). Occurrence of hybrids, including ascomycetous species in natural conditions indicates that both genetic incompatibilities and reduced fitness could be overcome under certain environmental conditions, potentially enhancing adaptive diversity and accelerating adaptive evolution of crop pathogens (Stukenbrock 2016).

Many population genetic studies have identified *Ptt* and *Ptm* as genetically distinct groups, and sexual reproduction between the two *P. teres* forms, also known as hybridisation, has been reported to be rare (Campbell et al. 2002; Rau et al. 2003; Leišova et al. 2005; Serenius et al. 2005; Bakonyi & Justesen 2007; Lehmensiek et al. 2010). Seven hybrid isolates have thus far been reported in barley fields, including one each from South Africa (Campbell et al. 2002), Japan (Dahanayaka et al. 2021) and Hungary (Dahanayaka et al. 2021), and two from the Czech Republic (Leišova et al. 2005) and Australia (McLean et al. 2014; Turo et al. 2021). A hybrid isolate collected from barley fields in Western Australia showed increased resistance to some group 3 fungicides (azole or demethylase inhibitor) and was also found to be rapidly propagated by asexual reproduction suggesting that field hybrid isolates could also be genetically stable, fertile, and potentially possess increased virulence similar to the *in vitro* hybrid isolates (Turo et al. 2021). The recent increase in the identification of hybrids in nature indicates the necessity to broaden the knowledge of the sexual reproduction pattern of this pathogen. Acquiring virulence from both *Ptt* and *Ptm* could lead to the development of complex host and fungicide resistant pathotypes through evolutionary changes, as was recently seen in Western Australia (Turo et al, 2021).

A number of studies have produced hybrid isolates following mating between *Ptt* and *Ptm in vitro* (Smedegård-Petersen 1971; Crous et al. 1995; Louw et al. 1995; Campbell et al. 1999; Jalli 2011). The progeny resulting from these hybrid crosses have produced net-like, spot-like or intermediate disease symptoms described as jagged-type spots on the host (Smedegård-Petersen 1971; Campbell et al. 1999). Laboratory-hybrids retained their fertility and virulence, and were genetically stable under laboratory conditions over the course of years (Campbell & Crous 2003). Some laboratory-hybrids were less sensitive to triazole fungicides compared to parental isolates while others were reported to show virulence patterns different to both the parental isolates, with some hybrid isolates being virulent on barley cultivars on which both parents were avirulent (Jalli 2011). Exchange of genetic material between *Ptt* and *Ptm* due to hybridisation can, therefore, lead to enhanced virulence and novel pathotypes that may overcome available host resistances. This could directly challenge existing disease management strategies which may become ineffective. Thus, having a better understanding of mating preference of *P. teres* would help in predicting the virulence profile of the pathogen and the development of novel disease resistant varieties.

Hybridisation of *Pyrenophora* forms within barley fields may be more prevalent than previously assumed as hybrid isolates may not have been identified in the past due to a lack of an appropriate molecular marker system. For example, isolate CBS 282.31 (collected in 1931

in Japan), previously described as *P. japonica* and recently synonymised with *P. teres* based on multi-locus phylogenetic analyses (Crous et al. 1995; Marin-Felix et al. 2019), was revealed to be a hybrid between *Ptt* and *Ptm* based on genome wide DArTseq™ markers (Dahanayaka et al. 2021). The availability of molecular markers specific to *P. teres* hybrids (Poudel et al. 2017) provides the opportunity to rapidly and more reliably screen *P. teres* populations for occurrence of hybrids in the field. Interestingly, previously identified field hybrids, including CBS 282.31, H-919 (Dahanayaka et al. 2021) and WAC10721 (McLean et al. 2014) seemed to be genetically more closely related to *Ptm* than *Ptt*, having a larger percentage of *Ptm* alleles. This would suggest that hybrid isolates themselves more frequently undergo sexual reproduction with *Ptm* rather than *Ptt* in subsequent matings.

In order to assess the prevalence of natural hybridisation between the two forms of *P. teres* in the field, a field study was designed where a barley variety susceptible to both forms was inoculated with *Ptt* and *Ptm* isolates of opposite mating types (Poudel et al. 2018). Results indicated that *Ptt* and *Ptm* isolates preferred to undergo sexual recombination within their respective forms as no hybrids were collected from the field during three years of trials. A number of reasons were given for the lack of sexual reproduction between the two forms, including pre-mating barriers like sexual selection and temporal difference, and post-mating barriers like gametic compatibility (Kohn 2005; Giraud et al. 2008). It was suggested that competition mating assays involving individuals of both *Ptt* and *Ptm* of opposite mating types need to be conducted under laboratory conditions to observe competition in mating within and between forms.

Hence, this study was designed to use molecular assays and genotyping-by-sequencing approaches to i) test the hypothesis that there is a preference of *Ptt* and *Ptm* isolates to undergo sexual recombination within forms rather than hybridising between forms *in vitro*; and ii) investigate whether hybrid isolates have a greater preference for *Ptm* isolates than *Ptt* isolates for mating *in vitro*.

MATERIALS AND METHODS

Fungal material. For this study**,** 10 *Ptt* (NB50, NB29, NB81, NB63, NB85 HRS09127, NB90, HRS11093, NB73 and 97NB1) (Lehmensiek et al. 2010; Martin et al. 2020), eight *Ptm* (HRS07033, 07-047, 16FRG073, SNB320, SNB113, SG1, SNB 171 and U7) (Lehmensiek et al. 2010; McLean et al. 2014; Ellwood et al. 2019) and six laboratory-hybrids (unpublish data) were used. For conidia production, infected barley leaf samples of *Ptt* and *Ptm* isolates were incubated in Petri plates with sterile moist filter paper at 15 ± 1°C under 12 hours of white

fluorescent light and 12 h of dark for two days in the incubator (Thermoline, New South Wales, Australia). Single conidium from each isolate was transferred aseptically using a sterile glass needle onto Petri plates containing potato dextrose agar (PDA) medium (20 g/litre; Biolab Merck Darmstadt, Germany). Petri plates were incubated at 25 ± 1°C for ten days to produce mycelium. The six laboratory-hybrids were retrieved from 15% glycerol tubes stored at -80°C and were cultured on PDA.

Establishment of crosses. To investigate the preference of *Ptt* and *Ptm* for mating**,** three experiments each with two *Ptt* and two *Ptm* isolates belonging to alternate mating types were conducted (Experiments 1 to 3; Table 1). An additional experiment (experiment 4) was conducted using six laboratory-hybrids, crossed with seven *Ptt* and six *Ptm* isolates (Table 2) to investigate the preference of hybrids mating with either *P. teres* form.

Each of the experiments 1 to 3 consisted of two *Ptt* (selected from NB50, NB29 and NB81: *MAT1-1*; and NB63, NB85 and HRS09127:*MAT1-2*) and two *Ptm* (selected from HRS07033, 07-047 and 16FRG073: *MAT1-1*; and SNB320, SNB113 and SG1:*MAT1-2*) isolates and comprised of 15 different crosses, including five competition (Cm) crosses, four positive control crosses (Co), and six negative control crosses. Competition crosses (Cm) were established to assess the mating preference of *Ptt* or *Ptm* isolates, where opportunity for both within-form and between-form mating was provided by crossing three or four isolates of different forms and mating types. Positive control crosses (Co) were used to i) confirm the reproductive viability of isolates used in competition crosses under given laboratory conditions by crossing alternate mating types of the same form and ii) establish the ability of isolates to produce hybrids (Table 1). Negative control crosses were established in two settings (data not shown): i) the same isolate was used as both the maternal and paternal isolate and ii) two isolates possessing the same *MAT1* idiomorph from different forms were placed on to the same crossing plate (*Ptt MAT1-1* and *Ptm MAT1-1*, *Ptt MAT1-2* and *Ptm MAT1-2*). The crosses of experiment 1 and 2 were established on the 28th of April 2019 and experiment 3 on the 23rd of May 2019.

Experiment 4 was established according to Table 2 on the 12th of September 2019, with 12 competition hybrid crosses (H) and six control crosses (HCo). For hybrid crosses, laboratory- hybrid isolates, previously confirmed though real time PCR form-specific markers (Dahanayaka et al. 2021) and mating type markers (Lu et al. 2010), were placed on the crossing plate along with a *Ptt* and *Ptm* isolate of opposite mating type to the hybrid. Positive control contained hybrids with one of the parents used in the original cross.

Crosses were established according to the method described by Martin et al. (2020). Five 50 mm long autoclaved pieces of wheat straw were placed onto Sach's agar (Hebert 1971) plates before the agar had set. An approximately 25 mm^2 mycelial plug was taken from isolates grown on PDA plates and placed adjacent to the barley straw. Mycelial plugs with different isolates were placed at equal distance from each other mycelial plug. The plates were then kept in transparent plastic bags to prevent desiccation and placed inside an incubator at 15°C with a 12 h light/12 h dark photoperiod.

Ascospore collection. Once ascomata had matured (when ascomata formed a short cylindrical beak or neck), lids of Petri plates were replaced with 2% water agar (Sigma LifeScinece, María de Molina, Spain) plates for ascospore collection. After establishing crosses, crosses of experiment 1 to 3 were monitored daily for one year while those for experiment 4 were monitored for 8 months. The number of days taken from producing the crosses to the production of the first ascospore and the ascospore production time period were recorded for each cross. Single ascospores were collected under a dissecting microscope (Nikon SMZ 745, New York, USA) and transferred to PDA plates using a sterile glass needle. Water agar plates were replaced with new plates each day after collecting ascospores. All ascospores produced from the competition crosses were collected. A maximum of 12-13 ascospores from control crosses were collected. Plates inoculated with ascospores were placed in an incubator at 20°C to facilitate mycelial growth for fungal DNA extraction.

DNA extraction. Aerial mycelium of each isolate was scraped aseptically from two-week-old PDA isolates and used to extract DNA using a Wizard Genomic DNA Extraction Kit (Promega Corporation, New South Wales, Australia) according to manufacturer's instructions.

PCR amplification for hybrid identification. DNA of all the ascospores obtained from experiment 1, 2 and 3 plus positive controls (*Ptt* isolates NB29 and NB50 (Lehmensiek et al. 2010; Martin et al. 2020) and *Ptm* isolates HRS06033 and SNB113 (Lehmensiek et al. 2010; McLean et al. 2014) and two laboratory-produced hybrids Pop37.1 and Pop37.8 (unpublished data) were amplified using six *Ptt* and six *Ptm* specific PCR markers (Poudel et al. 2017). Amplification was conducted as described in Dahanayaka et al. (2021)

DArTseq genotyping and data analyses. DNA samples extracted from progeny and parents in experiment 4 were confirmed for integrity as described by Dahanayaka et al. (2021) and submitted to Diversity Arrays Technology Pty. Ltd., Canberra, Australian Capital Territory, Australia, for DArTseq™ genotyping. DArTseq™ data (SNP and SilicoDArTs) were filtered following the method described by Dahanayaka et al. (2021) using 10% as the cut off value for the maximum number of missing data points for loci and isolates. SilicoDArTs

and SNPs were combined for further analyses. Clonal isolates of each cross were identified by the function *clonecorrect* in *poppr* package (Kamvar et al. 2014) in RStudio version 4.0.1 and were removed.

Phylogenetic relationship among isolates. A neighbor-net network was used in this study in order to depict the relationship among isolates, which provides information about the exchange of genetic material among isolates (Bryant & Moulton 2004). From this information, the respective parental isolates for each progeny were identified. Based on clone-corrected DArTseq™ data, a neighbor-net network was constructed for isolates obtained from competition crosses in experiment 4 using SplitsTree version 4.16.2 (Huson 1998). Networks were produced for each of the hybrid competition crosses based on neighbor-joining (NJ) algorithm described by Saitou and Nei (Saitou & Nei 1987) following the method depicted by Bryant and Moulton (2004). Bootstrap analysis with 1000 replicates was used to test the support of branches on the network.

Multivariate cluster analyses. Discriminant analysis of principle components (DAPC) is a multivariate cluster analysis which has been developed to detect clusters of genetically related individuals (Jombart 2008). Hence, in this experiment, DAPC was used to identify the structure and the clustering of the progeny isolates and parental isolates to reveal their genetic relatedness. DAPC was calculated from the clone-corrected DArTseq™ data of the isolates using the *dapc* function in the R package *adegenet* version 2.1.2 (Jombart 2008) in the RStudio. The optimum number of clusters in each cross was obtained using the Bayesian information criterion function *find.clusters* and the optimum number of principal component axes to include in the DAPC analysis were calculated via the *xvalDapc* function in *adegenet.*

RESULTS

Ascospore collection. Ascomata emerged four to six weeks after establishing the crosses. In experiment 1, four out of five competition crosses (Cm18-1, Cm18-2, Cm18-4 and Cm18-5) produced 2-63 ascospores between 65-190 days after crossing (Table 1). The period of ascospore production of these crosses varied from 41 to 152 days. Out of the four control crosses, two (Co18-1 and Co18-5) produced ascospores 56-83 days after the crosses were established (Table 1).

In experiment 2, three competition crosses (Cm18-6, Cm18-7 and Cm18-9) produced 8-63 ascospores between 65-186 days after crossing. The ascospore production period ranged from 11 to 225 days. Three control crosses (Co18-7, Co18-10 and Co18-11) produced ascospores between 76-108 days after establishing crosses (Table 1).

In experiment 3, all competition crosses (Cm19-1, Cm19-2, Cm19-3, Cm19-4 and Cm19-5) produced 12-100 ascospores between 50-163 days after crosses were established. The ascospore production period varied from 132 to 73 days. Except for the crosses Cm19-1 and Cm19-2, all other crosses produced ascospores continuously throughout their respective ascospore production period. Crosses Cm19-1 and Cm19-2 had a period of 32 and 35 days, respectively, after the collection of the first ascospores where no ascospores were produced (Table 1). After this period, 12 and 39 further ascospores were collected from crosses Cm19-1 and Cm19-2, respectively. Three control crosses (Co19-6, Co19-7 and Co19-10) produced ascospores 56-162 days after establishing the crosses (Table 1).

In experiment 4, three crosses (H9, H10 and H12) and two control crosses (HCO-14 and HCo-17) produced 3-16 ascospores. The first ascospore of each cross was observed 141-172 days after establishing the crosses (Table 2).

PCR amplification of *Ptt* and *Ptm* specific markers. PCR amplification of DNA samples obtained from experiment 1 with *Ptt* and *Ptm* specific markers indicated that all ascospores collected from competition crosses Cm18-4 (n = 2) and Cm18-5 (n = 37) amplified both *Ptt* and *Ptm* markers. On the other hand, DNA samples obtained from competition crosses Cm18-1 (n = 63) and Cm18-2 (n = 18) resulted in amplification of only *Ptt* specific markers (Table 1). DNA samples from isolates of control cross Co18-1 amplified only *Ptt* specific markers while Co18-5 amplified both form-specific markers.

In experiment 2, DNA of all isolates from crosses Cm18-6 and Cm18-7 showed amplification only for *Ptt* specific markers. Seven out of eight isolates from Cm18-9 showed amplification for both *Ptt* and *Ptm* markers while the remaining isolate showed amplification only for *Ptm* markers. Isolates of control crosses Co18-7 and Co18-8 of experiment 2 amplified *Ptt* specific markers while Co18-11 amplified both *Ptt* and *Ptm* specific markers.

Many isolates from competition crosses in experiment 3 [Cm19-1 (n = 5), Cm19-2 (n = 39), Cm19-3 (n = 8), Cm19-4 (n = 24) and Cm19-5 (n = 10)] showed amplification for both *Ptt* and *Ptm* markers, while 64 isolates from Cm19-1, 61 isolates from Cm19-2 and two isolates from Cm19-5 amplified only *Ptt* markers. Additionally, one isolate from Cm19-3 and 26 isolates from Cm19-4 amplified only *Ptm* specific markers. Isolates from control crosses Co19-6 and Co19-7 amplified only *Ptt* markers while isolates from Co19-10 amplified both *Ptt* and *Ptm* specific markers.

Overall, more crosses were observed involving a *Ptt* isolate than a *Ptm* isolate and the crosses involving *Ptt* isolates yielded 63, 18, 63, 39, 64 and 61 ascospores, whereas those

involving *Ptm* isolates yielded 1, 1 and 26 ascospores. We therefore, consider *Ptt* isolates to have a higher reproduction vigour than *Ptm* isolates.

Clone correction and data filtering. After quality filtering of DArTseq™ data obtained for progeny isolates of experiment 4, 1,444 markers (273 SNPs and 1,171 SilicoDArTs) were retained. Clone-correction results showed that two pairs of isolates from cross H9 and one pair from H12 were clonal, hence, one clonal isolate from each pair was removed for subsequent analyses.

Phylogenetic relationship among isolates. The neighbor-net network results for *P. teres* and parental hybrid isolates from experiment 4 divided *Ptt* and *Ptm* isolates into two distinct groups. Hybrid isolates used as the parental isolates were positioned in between *Ptt* and *Ptm* isolates (Fig. 1). The neighbor-net network of the progeny from experiment 4 showed two distinct subdivisions each for the isolates from hybrid crosses H9 (n = 13), H10 (n = 10) and H12 (n = 15) (Fig. 2A, B and C) and were highly reticulated. All progeny from the hybrid crosses grouped close to the parental *Ptt* and hybrid isolates.

Multivariate cluster analyses. The optimum number of clusters in the DAPC analysis was found to be four for the progeny isolates of H9 cross (Fig. 3A). One cluster (red: cluster 3) out of four consisted of the *Ptm* isolate SG1 used in the H9 cross and cluster 1 (blue: n = 7) and cluster 2 (green: n = 6) consisted of progeny isolates along with the *Ptt* isolate 97NBi of the H9 cross and the parental hybrid isolate 37_416, respectively. Cluster 4 contained two progeny isolates: H9_3 and H9_12. Cross H10 showed three distinct clusters in DAPC analysis. Six progeny isolates clustered with crossed hybrid isolate 37_416 in cluster 2 (green: n = 7) and four progeny isolates clustered with the crossed *Ptt* isolate HSR09127 in cluster 3 (blue: n = 5) (Fig. 3B). Cluster 1 (red: n = 1) contained the *Ptm* isolate SNB320 of the cross. The progeny isolates of cross H12 and its crossed isolates NB81, U7 and 37_407 were best fit into four clusters according to the DAPC analysis. Cluster 1 (blue: n = 6) and cluster 2 (green: n = 4) consisted of progeny isolates with the crossed *Ptt* and hybrid isolates, respectively. Cluster 4 (cyan: n = 7) consisted of only progeny isolates while the crossed *Ptm* isolate formed a separate cluster, cluster 3 (Fig. 3C).

DISCUSSION

In this study for the first time, we investigated the sexual reproduction patterns and preference of *P. teres* and its hybrids for mating *in vitro*. A previous study aimed at inducing natural hybridisation between two *P. teres* forms under field conditions reported that *Ptt* and *Ptm* isolates preferred to mate within their respective forms (Poudel et al. 2018). Therefore, the

current study was established to investigate whether there is a preference of *P. teres* isolates for mating *in vitro*.

Some of the crosses in experiments 1, 2, and 3, established to identify the preference of mating within or across *Ptt*, showed that *Ptt* isolates preferred to undergo recombination within the same form *in vitro* rather than hybridizing with *Ptm* at early stages of ascospore production. A similar observation was made in a field trial, where originally *Ptt* and *Ptm* isolates were co-inoculated in a barley field to facilitate hybridisation, but all the progenies were detected as *Ptt* or *Ptm* (Poudel et al. 2018). It was suggested that this was due to *Ptt* and *Ptm* being reproductively isolated, which prevented the exchange of genetic material between the two forms (Giraud et al. 2008). It was proposed that the genetic isolation could have resulted from pre-mating barriers like sexual selection (Fernández-Meirama et al. 2017), temporal difference, and post-mating barriers like gametic compatibility, which lead to unfit or nonviable hybrids (Kohn 2005; Giraud et al. 2008) or ineffectual meiosis occurring in the crosses between *Ptt* and *Ptm* (Serenius et al. 2005). Furthermore, sexual incompatibility between the two forms might have arisen due to the environmental conditions, such as temperature and rain fall which favoured *Ptt* to mature while hindering *Ptm* maturity (Giraud et al. 2008).

The crosses in this study showed that mating preference for some *Ptt* isolates changed over time. Some crosses (Cm19-1 and Cm19-2) in experiment 3 were established to assess whether there is a preference of *Ptt* to recombine with the same form rather than hybridizing with *Ptm*. Even though the majority of progeny were found to be *Ptt* isolates, many progeny isolates produced in the second round of ascospore production were hybrids, confirming that the preference of *Ptt* under *in vitro* conditions could change with time. In the current study, even though *Ptt* and *Ptm* were crossed at the same time, *Ptm* mycelia involved in sexual recombination might have matured later than *Ptt* mycelia. Late maturity of *Ptm* mycelia for mating in the first round of ascospore production may have led to the generation of only *Ptt* offspring. Hybrid offspring found in Cm19-1 and Cm19-2 crosses in the second round of ascospore production could have resulted from the availability of mature *Ptm* mycelium for sexual reproduction., This suggests that pre-mating genetic isolation barriers/genetic incompatibility could be overcome over time (Stukenbrock 2016).

Progenies of some crosses revealed that *P. teres* isolates do not possess any preference to mate within the same form when both forms and mating types were present. Progeny isolates from cross Cm18-5 and more than 80% of isolates from cross Cm19-5 were found to be hybrids, indicating that in these crosses *Ptt* and *Ptm* did not have any preference for the same form but sexual reproduction occurred randomly under *in vitro* conditions. Furthermore, these

crosses needed longer to produce the first ascospores compared to other crosses, suggesting that maturity of mycelia for sexual reproduction for some of the parents in these crosses could have been delayed.

Our results showed that *Ptm* isolates preferred *Ptt* isolates for mating when given the choice between *Ptm* and *Ptt* isolates of opposite mating type. Of the crosses established to identify the mating preference of *Ptm in vitro*, only three (Cm18-9, Cm19-3 and Cm19-4) produced ascospores. Majority of progeny isolates collected from all three crosses produced progenies of hybrid isolates, suggesting that crossed *Ptm* isolates do not have a preference for the respective *Ptm* isolate to recombine *in vitro* conditions. In comparison to most of the *Ptt* crosses (Cm18-1, Cm18-6, Cm18-7, Cm19-1 and Cm19-2), *Ptm* crosses (Cm18-4, Cm18-9 and Cm19-4) required a longer period to produce the first ascospore. The laboratory conditions set in this study could have delayed the maturity of the *Ptm* mycelium. Also, delaying of the mycelium for sexual reproduction could be the reason behind the absence of ascospores from the *Ptm* cross Cm18-8. Hybrid formation in these *Ptm* crosses could also be due to *Ptt* having greater vigour to recombine/hybridise with a suitable mating type regardless of the form of the isolate. A similar observation was demonstrated for strains of *Microbotryum violaceum*, an anther smut fungus, taken from two *formae speciales* of *Silene latifolia* and *S. dioica* (Van Putten et al. 2003). Of the strains from the two *formae speciales*, strains from *S. latifolia* outcompeted and had higher frequency of conjugation than strains from *S. dioica* in both male hosts of *S. latifolia* and *S. dioica*, which was similar to how *Ptt* outcompeted *Ptm* in the presence of both *Ptt* and *Ptm*. Further studies are warranted with different laboratory conditions, *e.g.*, different temperature or light intensities to determine whether *Ptm* isolates prefer to mate with *Ptm* in the presence of *Ptt* under different laboratory conditions or whether these hybrids were the result of high *Ptt* reproduction vigour.

Results of the current study showed that the preference of laboratory-produced hybrid isolates was to mate with *Ptt*. Previous field collected hybrids from Leišova, et al. (2005) (PTM-15 and PTM-16), McLean et al. (2014) (WAC17021), Dahanayaka et al. (2021) (H-919 and CBS 281.31), and Turo et al. (2021) were genetically closer to *Ptm* than *Ptt*. Hence, experiment 4 was established to get an insight into the nature of progeny arising from crosses between hybrids in the presence of both *Ptt* and *Ptm*. Clustering of all progeny isolates of H9, H10 and H12 with the respective *Ptt* parent of the cross indicated the preference of hybrid isolates to mate with *Ptt* isolates rather than *Ptm* isolates. These results also suggest that *Ptt* isolates have a higher ability or higher reproduction vigour compared to *Ptm* under the given laboratory conditions. Environmental conditions used in the experiment could have favoured

the crossing between hybrid and *Ptt* isolates. Successful production of ascospores of these crosses confirms the fertility of hybrid isolates and their ability to integrate with the *P. teres* population.

Progeny isolates from crosses H9, H10 and H12 showed evidence for introgressive hybridization of laboratory-hybrids to parental forms of *P. teres*, namely *Ptt*. Repeated introgression/backcrossing of hybrids in oomycetes proposed to "dilute" or reduce the genetic material of hybrids and change the hybrid genome towards the parental species while retaining adaptive traits from both species (Baack & Rieseberg 2007). The neighbor-net networks and DAPC analyses of this study showed that genomic characters of these laboratory-hybrid progenies shifted toward the *Ptt* genome and reduced the hybrid genetic characters. Repeated introgression of *P. teres* hybrids with their parental forms in fields may have left these unrecognised in nature. Introgression/backcrossing may accelerate the adaptive evolution through descending heritable/adaptive genetic characters between species (Arnold 2004) and result in novel pathogenic fungi (Menardo et al. 2016). Progeny of natural hybrids, along with introgressive hybrids, occurred between *Melampsora medusae* and *M. occidentalis*, two rust pathogens of *Populus deltoides* and *P. trichocarpa*, respectively (Newcombe et al. 2000). These progeny isolates were found to be virulent on a hybrid population of *Populus deltoides* and *P. trichocarpa*, which was originally developed against *M. occidentalis* (Newcombe et al. 2000). Hybrids and introgressive hybrids of *P. teres* could also have devastating effects on barley varieties which have been developed against either *Ptt* or *Ptm*. Identifying heritable genes of *P. teres* through developing backcrosses would allow us to recognize inheritable genes/genomic regions and expand the knowledge of this challenging pathogen.

The identification of field hybrids in recent studies has led to the understanding that field hybrids may not have been detected in previous studies due to the absence of an appropriate marker system and not due to the absence of field hybrids (Dahanayaka et al. 2021). The possibility of retaining the fertility, virulence and genetic stability of laboratory-produced hybrids (Campbell & Crous 2003) and decreased fungicide sensitivity and rapid asexual reproduction of field hybrids (Turo et al. 2021) suggest potential for integration with the local *P. teres* population. Thus, regular monitoring of *P. teres* isolates in barley fields is vital. Also, further studies should be conducted under different laboratory conditions including temperature, light intensities, culture medium and field conditions, including glasshouse experiments, to gain comprehensive knowledge on the sexual reproduction patterns and reproduction vigour of *P. teres* and its hybrids.

In conclusion, the *P. teres* crosses that were established to identify the mating preference of *Ptt* isolates in this study revealed that *Ptt* isolates preferred to undergo recombination with the respective *Ptt* isolates at the early stages of their maturity but over time *Ptt* preferred to undergo hybridisation with *Ptm* isolates. In contrast to *Ptt*, *Ptm* isolates did not have preference to undergo recombination with *Ptm* and instead showed preference towards hybridisation with *Ptt*. The laboratory-hybrids preferred to undergo sexual reproduction with *Ptt* rather than *Ptm* isolates. These results suggest that *Ptt* isolates have a greater reproduction vigour than *Ptm* hence, *Ptm* and hybrid isolates were forced to undergo sexual reproduction with *Ptt*. These findings indicate the high potential for production of hybrids *in vitro* and would support the development of a reproductive model and a better understanding of speciation/form differentiation and evolution of *P. teres.* The potential for more frequent occurrences of field hybrids under suitable environmental conditions, could lead to novel, more complex and highly virulent pathotypes with both *Ptt* and *Ptm* characteristics. Thus the development of novel barley lines which can withstand both *Ptt* and *Ptm* infections is vital.

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543 Table 1. Meta data of *Pyrenophora teres* crosses of experiment 1 to 3.The number of ascospores produced per cross, results of PCR amplification
544 with the form-specific markers and ascospore production time are given

Cross ID[a]	*Ptt 1*[b]	*Ptt 2*[c]	*Ptm 1*[d]	*Ptm 2*[e]	No. of ascospores[f]	*Ptt* specific markers[g]	*Ptm* specific markers[h]	Both types of markers[i]	Days to produce the first ascospore[j]	Production period (Days)[k]
Experiment 1										
Cm18-1	NB50	NB63	HRS07033	-	63	63	0	0	65	125
Cm18-2	NB50	NB63	-	SNB320	18	18	0	0	190	44
Cm18-3	NB50	-	HRS07033	SNB320	0	0	0	0	NA	0
Cm18-4	-	NB63	HRS07033	SNB320	2	0	0	2	190	41
Cm18-5	NB50	NB63	HRS07033	SNB320	37	0	0	37	185	152
Co18-1	NB50	NB63	-	-	11	11	0	0	56	NA
Co18-2	-	-	HRS07033	SNB320	0	0	0	0	NA	NA
Co18-4	NB50	-	-	SNB320	0	0	0	0	NA	NA
Co18-5	-	NB63	HRS07033	-	3	0	0	3	83	NA
Experiment 2										
Cm18-6	NB29	NB85	07-047	-	63	63	0	0	65	225
Cm18-7	NB29	NB85	-	SNB113	39	39	0	0	65	31
Cm18-8	NB29	-	07-047	SNB113	0	0	0	0	NA	NA
Cm18-9	-	NB85	07-047	SNB113	8	0	1	7	186	11
Cm18-10	NB29	NB85	07-047	SNB113	0	0	0	0	NA	NA

Co18-7	NB29	NB85	-	-	12	12	0	0	76	NA
Co18-8	-	-	07-047	SNB113	12	0	12	0	107	NA
Co18-10	NB29	-	-	SNB113	0	0	0	0	NA	NA
Co18-11	-	NB85	07-047	-	2	0	0	2	108	NA
Experiment 3										
Cm19-1	NB81	HRS09127	16FRG073	-	69	64 (57+7[l])	0	5[l]	50	131 (1-76 and 109-131)[m]
Cm19-2	NB81	HRS09127	-	SG1	100	61	0	39[n]	55	132 (1-71 and 107-132)[o]
Cm19-3	NB81	-	16FRG073	SG1	9	0	1	8	82	80
Cm19-4	-	HRS09127	16FRG073	SG1	50	0	26	24	101	119
Cm19-5	NB81	HRS09127	16FRG073	SG1	12	2	0	10	163	73
Co19-6	NB81	HRS09127	-	-	13	13	0	0	56	NA
Co19-7	-	-	16FRG073	SG1	11	0	11	0	91	NA
Co19-9	NB81	-	-	SG1	0	0	0	0	NA	NA
Co19-10	-	HRS09127	16FRG073	-	12	0	0	12	162	NA

[a] Identity of cross

[b] Mating type 1 *Ptt* isolates used in the experiment

[c] Mating type 2 *Ptt* isolates used in the experiment

[d] Mating type 1 *Ptm* isolates used in the experiment

[e] Mating type 2 *Ptm* isolates used in the experiment

[f] Number of ascospores produced by the respective cross

[g] Number of isolates which only amplified with *Ptt* specific markers

[h] Number of isolates only amplified with *Ptm* specific markers

[i] Number of isolates amplified with both *Ptt* and *Ptm* specific markers

[j] Number of days to produce the first ascospore

[k] Number of days ascospores were produced and collected

[l] Ascospores were produced in the second round (n = 12)

[m] Ascospores were produced in two time periods, day 1 to day 75 first period, day 76 to day 108 no ascospore production and day 109 to day 131 second period

[n] Ascospores were produced in the second round (n = 39)

[o] Ascospores were produced in two time periods, day 1 to day 71 first period, day 72 to day 106 no ascospore production and day 107 to day 132 second period

NA Not available

565 Table 2. Meta data for *Pyrenophora teres* isolates used in experiment 4 with the number of ascospores produced by each cross and ascospore
566 production time given

Cross ID[a]	Hybrid[b]	Mat[c]	Hybrid cross[d]	*Ptt*[e]	Mat[f]	*Ptm*[g]	Mat[h]	No. of ascospores[i]	Days to produce the first ascospore[j]	Production period (Days)[k]
H1	30_1	*Ptm*1	NB73 × SNB171	97NB1	2	SG1	2	0	NA	NA
H2	30_1	*Ptm*1	NB73 × SNB171	HRS09127	2	SNB320	2	0	NA	NA
H3	30_3	*Ptt*2	NB73 × SNB171	HRS11093	1	16FRG073	1	0	NA	NA
H4	30_3	*Ptt*2	NB73 × SNB171	NB81	1	U7	1	0	NA	NA
H5	34_8	*Ptm*1	NB90 × HRS07033	97NB1	2	SG1	2	0	NA	NA
H6	34_8	*Ptm*1	NB90 × HRS07033	HRS09127	2	SNB320	2	0	NA	NA
H7	34_18	*Ptt*2	NB90 × HRS07033	HRS11093	1	16FRG073	1	0	NA	NA
H8	34_18	*Ptt*2	NB90 × HRS07033	NB81	1	U7	1	0	NA	NA
H9	37_416	*Ptm*1	NB63 × HRS07033	97NB1	2	SG1	2	15[l]	141	48
H10	37_416	*Ptm*1	NB63 × HRS07033	HRS09127	2	SNB320	2	10	155	52
H11	37_407	*Ptt*2	NB63 × HRS07033	HRS11093	1	16FRG073	1	0	NA	NA
H12	37_407	*Ptt*2	NB63 × HRS07033	NB81	1	U7	1	16[m]	146	49
HCo-13	30_1	*Ptm*1	NB73 × SNB171	NB73	2	-	-	0	NA	NA
HCo-14	30_3	*Ptt*2	NB73 × SNB171	-	-	SNB171i	1	3	172	NA
HCo-15	34_8	*Ptm*1	NB90 × HRS07033	NB90	2	-	-	0	NA	NA
HCo-16	34_18	*Ptt*2	NB90 × HRS07033	-	-	HSR07033	1	0	NA	NA

HCo-17	37_416	*Ptm*1	NB63 × HRS07033	NB63i	2	-	-	8	170	NA
HCo-18	37_407	*Ptt*2	NB63 × HRS07033	-	-	HSR07033	1	0	NA	NA

[a] Identity of the crosses

[b] Hybrid isolates used in the crosses

[c] Mating type of the hybrid isolates used

[d] Parental genotypes of the hybrid isolates

[e] *Ptt* isolates used in experiment 4

[f] Mating type of the *Ptt* isolates used

[g] *Ptm* isolates used in experiment 4

[h] Mating type of the *Ptm* isolates used

[i] Number of ascospores produced by the respective cross

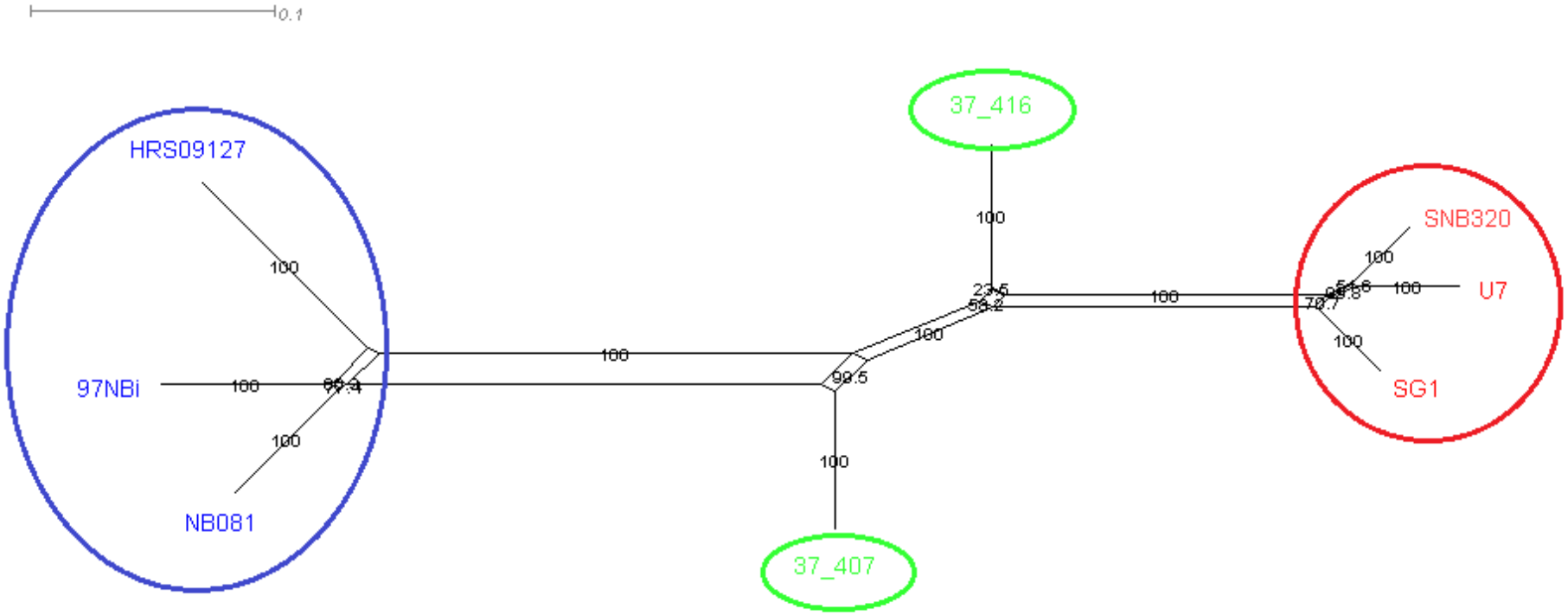
[j] Number of days to produce the first ascospore

[k] Number of days ascospores were produced by the respective cross

[l] Out of 15, 13 isolates were retain as two pairs of isolates were clones based on DArTseq™

[m] Out of 16, 15 isolates were retain as one pair of isolates were clones based on DArTseq™

- Not applicable



582 **Fig. 1.** Neighbor-net network constructed for *Pyrenophora teres Ptt* (blue), *Ptm* (red) and the parental hybrid isolates (green) used for hybrid
583 competition crosses H9, H10 and H12, with 1000 bootstrap replicates, based on DArTseq™ data.

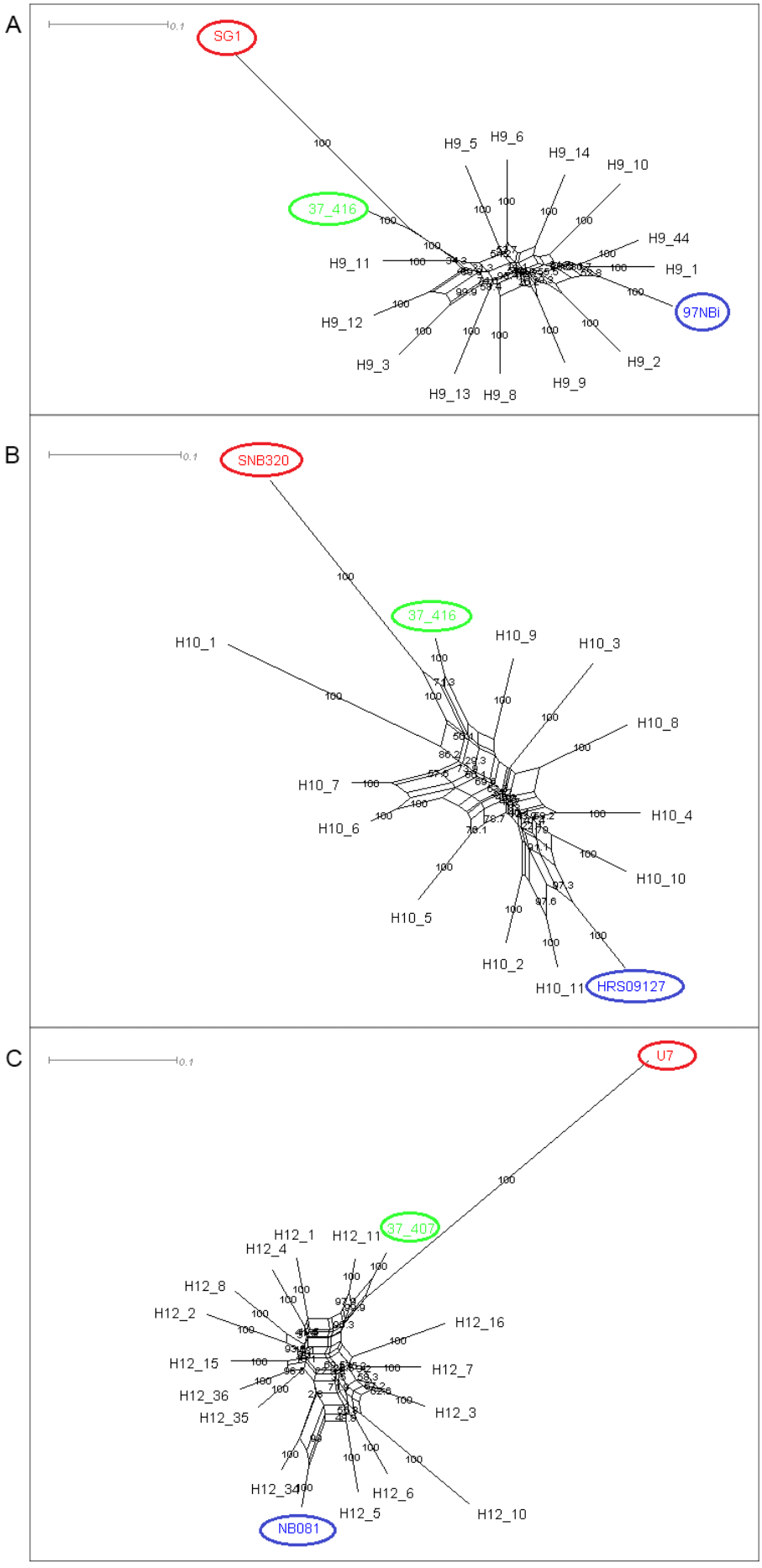

A
0.1
SG1
37_416
97NBi
H9_1
H9_2
H9_3
H9_4
H9_5
H9_6
H9_8
H9_9
H9_10
H9_11
H9_12
H9_13
H9_14
B
0.1
SNB320
37_416
HRS09127
H10_1
H10_2
H10_3
H10_4
H10_5
H10_6
H10_7
H10_8
H10_9
H10_10
H10_11
C
0.1
U7
37_407
NB081
H12_1
H12_2
H12_3
H12_4
H12_5
H12_6
H12_7
H12_8
H12_10
H12_11
H12_15
H12_16
H12_34
H12_35
H12_36

Fig. 2. Neighbor-net networks constructed using neighbour-net distance matrix with 1000 bootstraps for progeny isolates of *Pyrenophora teres* hybrid competition crosses (A) H9 (B) H10 and (C) H12 based on DArTseq™ data. Different colours depict progeny (black), parental *Ptt* (blue), parental *Ptm* (red), and parental hybrid (green) isolates used to establish the cross.

CHAPTER 4

IDENTIFICATION OF GENOMIC REGIONS OF *PYRENOPHORA TERES* ASSOCIATED WITH VIRULENCE USING A *PTT*/*PTM* MAPPING POPULATION

In this study, we detected QTL responsible for the virulence of *P. teres* using laboratory-produced hybrid progeny developed by crossing a *Pyrenophora teres* f. *teres* with a *Pyrenophora teres* f. *maculata* isolate. Genotyping of the progeny was carried out using the whole-genome marker system DArTseq™ to identify a large number of polymorphic markers. A seedling assay of the population was conducted to assess virulence of the progeny on eight barley genotypes. Identified QTL were mapped on the reference genomes and candidate genes for novel QTL associated with virulence were identified in order to facilitate future studies into molecular determinants of virulence and leaf symptoms caused by *P. teres* forms.

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Note: Supplementary data materials of the manuscript are available in the appendix

Genomic regions of *Pyrenophora teres* associated with virulence identified in a *Ptt*/*Ptm* mapping population

Buddhika A. Dahanayaka[1], Lislé Snyman[2], Niloofar Vaghefi[1], Anke Martin[1]

[1] Centre for Crop Health, University of Southern Queensland, Toowoomba, QLD, 4350, Australia.

[2] Department of Agriculture and Fisheries Queensland, Hermitage Research Facility, 604 Yangan Rd, Warwick, QLD, 4370, Australia.

[*]Corresponding author: Anke Martin; anke.martin@usq.edu.au

Abstract

Net blotches caused by *Pyrenophora teres* are important foliar fungal diseases of barley and result in significant yield losses of up to 40%. Two types of net blotches; net-form net blotch and spot-form net blotch are caused by two forms of *P. teres* namely *P. teres* f. *teres* (*Ptt*) and *P. teres* f. *maculata* (*Ptm*). This study is the first to use a cross between the two forms of *P. teres*, *Ptt* and *Ptm*, to identify quantitative trait loci (QTL) associated with virulence and leaf symptoms in eight barley cultivars. Progeny isolates were genotyped by the whole-genome marker system DArTseq™. After filtering for clonal isolates, 351 isolates were used to construct the genetic map consisting of 3,996 markers. Eight barley cultivars showing differential reactions to the parental isolates were used to phenotype the hybrid progeny isolates. This study is the first to report QTL associated with the leaf symptoms (net form/spot form) of the pathogen. Nine QTL associated with virulence and leaf symptoms were identified across five linkage groups. Phenotypic variation explained by these QTL ranged from 6 to 16%. Further phenotyping of selected progeny isolates on 12 other barley cultivars revealed that some progeny isolates are highly virulent across widely used cultivars. The results of this study suggest that accumulation of QTL in hybrid isolates can result in enhanced virulence. Results also showed the complexity of genetic mechanisms underlying virulence in the *P. teres*–barley pathosystem and that virulence may differ among hybrids.

Keywords: Hybrids, quantitative trait loci, barley, candidate genes, leaf symptoms, net form net blotch, spot form net blotch

Introduction

Pyrenophora teres [syn: *Drechslera teres*] is a haploid ascomycetous pathogen that causes net blotches in barley (*Hordeum vulgare* L.). Net blotches have been reported in all barley-growing areas of the world including regions in Europe (Jonsson et al. 2000; Rau et al. 2003; Serenius et al. 2005; Bakonyi & Justesen 2007; Ficsor et al. 2014), Middle East (Bouajila et al. 2011), Far-East (Sato & Takeda 1997), North America (Peever & Milgroom 1994; Jonsson et al. 2000; Akhavan et al. 2016), South America (Moya et al. 2020), South Africa (Campbell et al. 2002; Lehmensiek et al. 2010), and Oceania (Serenius et al. 2007; Bogacki et al. 2010; Lehmensiek et al. 2010; McLean et al. 2010; Ellwood et al. 2019). Barley net blotches are economically important foliar fungal diseases worldwide with average yield losses ranging between 10% and 40% with complete destruction of plants possible in susceptible barley cultivars (Martin 1985; Khan 1987; Steffenson et al. 1991; Jayasena et al. 2007; Jebbouj & El Yousfi 2009, 2010; Moya et al. 2020). In Australia alone, the potential annual economic losses due to *P. teres* have been estimated to be over AUD $300 million (Murray & Brennan 2010).

Net blotches occur as two types based on the symptoms: net-form net blotch (NFNB) caused by *Pyrenophora teres* f. *teres* (*Ptt*) and spot-form net blotch (SFNB) caused by *P. teres* f. *maculata* (*Ptm*) (Smedegård-Petersen 1971). Symptoms caused by both *Ptt* and *Ptm* initially appear as chlorotic spots. In NFNB, chlorotic regions later extend into longitudinal and transverse net-like necrotic streaks. In SFNB, initial chlorosis develops into circular spot-like necrotic lesions. The two forms have identical morphology and can only be differentiated using molecular markers (Williams et al. 2001; Keiper et al. 2008).

Molecular studies have shown that the two forms of *P. teres* are phylogenetically independent and divergent groups (McLean et al. 2009; Liu et al. 2011; Ellwood & Wallwork 2018; Syme et al. 2018; Marin-Felix et al. 2019; Clare et al. 2020), hence, sexual reproduction between the two forms is suggested to be rare (Serenius et al. 2005; Lehmensiek et al. 2010; McLean et al. 2014; Akhavan et al. 2015). However, identification of *Ptt*/*Ptm* hybrids collected from barley fields (Campbell et al. 2002; Leišova et al. 2005; McLean et al. 2014; Dahanayaka, et al. 2021b; Turo et al. 2021) and successful establishment of laboratory-based hybrids (Smedegård-Petersen 1971; Crous et al. 1995; Louw et al. 1995; Campbell et al. 1999; Jalli 2011) suggest that the two forms can overcome sexual reproduction barriers under certain environmental conditions (Dahanayaka, et al. 2021a). Hybridisation between the two forms may result in hybrids harbouring both *Ptt* and *Ptm* virulence genes, which may cause devasting yield losses in the absence of barley cultivars resistant to both *P. teres* forms. Therefore, comprehensive

knowledge of possible pathotypes that could arise from a hybrid population and identification of genomic regions associated with virulence would help accelerate the development of new hybrid resistant barley cultivars.

It has been suggested that the barley-*P. teres* pathosystem fits the gene-for-gene model, where qualitative traits or dominant genes are involved in the infection process (Flor 1956; Mode & Schaller 1958; Weiland et al. 1999; Friesen et al. 2006; Afanasenko et al. 2007; Friesen et al. 2008). However, with the identification of host selective toxins, also known as necrotic effectors (NEs), it was proposed that in addition to the gene-for-gene interaction, an inverse process of gene-for-gene interaction may also occur in the *P. teres*-barley pathosystem through NEs mediated programmed cell death, similar to the one found in the wheat pathogen *P. tritici-repentis* (Friesen et al. 2008; Ciuffetti et al. 2010; Faris et al. 2010).

Fungal effectors are proteins that act as either avirulence/virulence factors or both (Kamoun 2007). Pathogens have evolved to manipulate their effectors as a response to the host defence mechanism (Białas et al. 2018). To verify the long-term endurance of the pathogen, constant development of novel effectors may be needed to allow recognition of new host targets (Möller & Stukenbrock 2017). In order to undergo rapid evolution and alteration of effectors according to the host response, genomic regions associated with effectors reside in low complexity regions, which often harbor transposable elements (TEs) and repeat-rich regions of the pathogen genome (Raffaele & Kamoun 2012; Dong et al. 2015). As a result, these genomic regions show increased point mutagenesis (Rouxel et al. 2011), extensive chromosomal rearrangements and structural polymorphism (de Jonge et al. 2013; Möller & Stukenbrock 2017).

Recent *P. teres* secretome analyses, including in *planta* analyses, highlighted the significant role of effectors/NE in the infection process and virulence mechanisms (Ismail & Able 2016, 2017). Several genomic regions associated with virulence/avirulence of *P. teres* have been identified and mapped using bi-parental and genome-wide association mapping populations (Weiland et al. 1999; Beattie et al. 2007; Lai et al. 2007; Shjerve et al. 2014; Kinzer 2015; Carlsen et al. 2017; Koladia et al. 2017; Clare et al. 2020; Martin et al. 2020). As *P. teres* is a haploid fungus, it is difficult to determine the dominance of the genes responsible for virulence/avirulence. However, some of these genomic regions identified in *P. teres* may encode effectors/NEs that have been reported previously (Martin et al. 2020). Identification of QTL/genes associated with effectors would expand the knowledge on genomic regions that drive rapid evolution and adaptation of *P. teres*.

Both *Ptt* and *Ptm* show high pathogenic variations, challenging breeding for disease resistance (Liu et al. 2011). Pathogenic variation in *P. teres* was first recorded in 1949 with the detection of differences in pathogenicity towards different barley cultivars (Pon 1949). Since then, a number of studies have reported complex and high pathogenic variation among *P. teres* populations worldwide (Khan 1982; Tekauz 1990; Steffenson & Webster 1992; Jonsson et al. 1997; Douiyssi et al. 1998; Wu et al. 2003; Jebbouj & El Yousfi 2010; McLean et al. 2010; Wallwork et al. 2016; Fowler et al. 2017). Identification of large numbers of pathotypes using a differential set of barley cultivars indicates that a number of host specific effectors are involved in the *P. teres*-barley pathosystem (Carlsen et al. 2017). This suggests that a genomic region responsible for the virulence of *P. teres* on a specific barley cultivar may not be responsible for the virulence on another barley cultivar. Hence, identification of more genomic regions associated with virulence on a large number of barley cultivars is warranted in order to understand the *P. teres*-barley pathosystem.

Previously reported bi-parental mapping studies have been conducted using mapping populations developed by crossing *Ptt/Ptt* (Weiland et al. 1999; Beattie et al. 2007; Lai et al. 2007; Shjerve et al. 2014; Koladia et al. 2017; Martin et al. 2020) or *Ptm/Ptm* isolates (Carlsen et al. 2017). Using a mapping population developed by crossing *Ptt* and *Ptm* would enable the development of a high-density genetic map due to higher frequency of polymorphism between *Ptt*/*Ptm* isolates than between *Ptt/Ptt* or *Ptm/Ptm* isolates. High-density genetic maps better facilitate the identification of candidate genes (Collard et al. 2005). Furthermore, using hybrid progeny for QTL mapping may allow the identification of QTL from both *Ptt* and *Ptm* genomes and genes responsible for the different leaf symptoms caused by *Ptt* and *Ptm*, which have not been reported previously.

To comprehensively understand the *P. teres*-barley pathosystem, the current study was conducted using a bi-parental mapping population developed from a *Ptt/Ptm* cross. The aims of the current study were to: 1. identify genomic regions associated with virulence in *P. teres*; 2. detect genomic regions responsible for leaf symptoms of net blotch; 3. identify candidate genes encoding predicted effector-like proteins using protein information repositories; and 4. identify different virulence levels of the hybrid population across eight barley cultivars. Gaining knowledge of the genomic regions associated with virulence and leaf symptoms of net blotch will expand the information on the *P. teres*-barley pathosystem.

Materials and methods

Biological materials

A hybrid population (*Pop37*) consisting of 406 isolates developed by crossing strains NB63 (*Ptt*: *MAT1-2*) and HRS07033 (*Ptm*: *MAT1-1*) was used in this study (Supplementary Table S1). Crosses were done as indicated in Poudel et al. (2017). Eight barley cultivars, including the universal *Ptm* susceptible cultivar Kombar along with Gairdner, Prior, Dampier, Fleet, Flagship, Grimmette and Ciho 5791, were used in phenotyping assays for the QTL analyses. Another 12 novel barley cultivars (Ciho 11458, Vlamingh, Spartacus CL, Rosalind, Compass, RGT Planet, Fathom, Navigator, Harbin, Keel, Beecher and Schooner) known to be resistant to either *Ptt* or *Ptm* were used to phenotype 10 selected highly virulent progeny isolates to examine their virulence levels.

DNA extraction and DArTseq™

Hybrid progeny cultures stored in -80°C were grown on potato dextrose agar (PDA) medium (20 g/liter PDA; Biolab Merck, Darmstadt, Germany) at 22°C for 10 days. The DNA of the parental isolates and ascospores was extracted using the Wizard® Genomic DNA Purification kit following the protocol of the supplier (Promega Corporation, Wisconsin, USA). The integrity of DNA was assessed (Dahanayaka et al. 2021b) and sent to Diversity Arrays Technology Pty. Ltd. (Canberra, ACT, Australia) for DArTseq™.

PCR amplification using mating type primer pairs

In order to identify the mating type of progeny isolates, PCR with two mating type primer pairs amplifying *Ptt*: *MAT1-2* and *Ptm*: *MAT1-1* alleles was conducted as in (Ellwood et al. 2010). The amplified PCR products were electrophoresed on 1% agarose gel stained with GelRed® and the mating type of each isolate was determined according to the amplicon size (*Ptt: MAT1-2*: 1421 bp and *Ptm*: *MAT1-1*: 194 bp). A Chi square test was conducted to examine whether the progeny has a segregation distortion.

Phenotypic evaluation and disease assessment

Phenotypic assessment was conducted following a completely randomized design in a controlled environment room at the University of Southern Queensland, Australia, using the method described by Martin et al. (2020) with three replicates. The eight barley cultivars were grown in pots with 5 cm diameter and 14 cm height. Each pot contained four plants each from four barley cultivars. Barley cultivars were grown in a glass house at 20 ± 5°C for 14-15 days. The conidial suspension for plant inoculation was prepared as follows. Agar plugs from each isolate growing on PDA (2 mm diameter each) were grown on peanut oatmeal agar (Speakman and Pommer 1986) plates at 15 ± 1°C under white fluorescent lights for ten days to induce

conidia production (Fowler et al. 2017). Conidia were recovered as described by Martin et al. (2020) and diluted to 10000 conidia/mL using a Haemocytometer. Three millilitres of the suspension was used for each pot. Conidia suspensions were stored at -80°C (up to 1-3 months) until inoculation.

Fourteen to fifteen days after planting, plants in each pot were sprayed with the 3 mL of conidial suspension. Two hybrid isolates and parental isolate NB63 were used as control isolates for each cycle of inoculation to monitor differences across cycles. Inoculated pots were incubated in the dark for 48 hours at 95% humidity with a temperature of 20 ± 1°C. After 48 hours, plants were transferred to the controlled environment room for nine days with diurnal light at 75% humidity with a temperature of 20 ± 1°C. Nine days after inoculation, disease severity on the second leaf was scored according to Tekauz (1985) and the disease symptoms, i.e. net-like or spot-like symptoms, recorded for each cultivar.

The 10 progeny isolates showing the highest virulence reaction scores on the initial eight tested barley cultivars were assessed on another 12 resistant barley cultivars following the method described above.

Genetic map construction

Molecular marker data resulting from DArTseq™ were qualitatively filtered using Microsoft Excel (Dahanayaka et al. 2021b). Markers with more than 10% missing data and non-polymorphic markers for the parental isolates (Minor allele frequency < 0) were removed. Clonal isolates of the progeny were detected using the *clonecorrect* function in *poppr* package version 2.8.3 (Kamvar et al. 2014) in RStudio version 3.0.2 (R 2013). Both DArTseq™ markers were grouped into linkage groups using the make linkage groups function in MapManager QTXb20 version 2.0 (Manly et al. 2001) with a $P = 0.05$ search linkage criterion. Markers were ordered using RECORD (Van Os et al. 2005). The final genetic map of the population was obtained by manual map curation (Lehmensiek et al. 2009). To confirm the order of the markers within linkage groups, marker positions of the resulting genetic map were compared with marker positions of the *Ptt* and *Ptm* reference genomes: W1-1 (BioSample SAMEA4560035 available under PRJEB18107 BioProject) and SG1 (BioSample SAMEA4560037 available under PRJEB18107 BioProject), respectively. DArTseq™ marker sequences (~ 62 bp) were aligned with the two reference genomes using the *bowtie2* (Langmead & Salzberg 2012) function in Galaxy (*https://usegalaxy.org.au/*).

QTL analysis

Disease symptom scores of the progeny isolates NB63 and HRS07033 (*Pop37*) were combined with genotypic data to detect QTL associated with *P. teres* virulence using the composite interval mapping method in Windows QTL Cartographer version 2.5 (Wang 2007). Experiment-wise LOD threshold values at the 0.05 significance level were estimated based on 1000 permutation tests for each trait (Churchill & Doerge 1994; Doerge & Churchill 1996). Additive effects of QTL and the phenotypic variances explained (R^2) were calculated. The resulting QTL figures were drawn using MapChart version 2.32 (Voorrips 2002).

The nomenclature of the identified QTL was formatted as follows: the abbreviation of the institute where the QTL were detected (University of Southern Queensland) followed by the trait that the QTL is associated with and ending with the chromosome number. Where more than one QTL was identified on the same chromosome and for the same trait, a decimal value was added to the chromosome number according to the order in which they were found along the chromosome.

Identification of candidate genes

A 20kb flanking region on either side of the markers at the peak of the QTL regions was used to identify candidate genes within the QTL regions (Martin et al. 2020). These regions were aligned with the two respective reference genome assemblies (W1-1 and SG1) in the NCBI data repository. Identified candidate genes were further analysed for predicted effector genes by EffectorP version 1 and 2 (*http://effectorp.csiro.au/*) (Sperschneider et al. 2016; Sperschneider et al. 2018). Candidate genes were also compared with the published gene expression profiles of net blotch in barley during the infection process for effector identification (Ismail & Able 2016, 2017).

Results

Filtering of genetic data and clonal isolates

A total of 6,441 SNPs and 14,549 SilicoDArT were obtained by DArTseq™. After filtering markers for 10% missing values and non-polymorphism, 1,428 SNPs and 2,579 SlicoDArT markers were retained for the identification of clonal isolates and the construction of the genetic map. Out of 406 isolates, 351 hybrid isolates were unique isolates. In the sexual reproduction of filamentous ascomycetous fungi, karyogamy occurs followed by meiosis. Meiosis gives rise to four haploid unique nuclei and later these four nuclei undergo mitosis to produce eight cells/ascospores. As a result of the mitosis, each ascus contains four pairs of ascospores and the ascospores of each pair are identical (Finchman 1971). Hence, these identical isolates were

removed, and 351 unique isolates were used for the phenotypic evaluation and genetic map construction.

PCR amplification

PCR amplification of 351 progeny isolates with mating type primer pairs revealed that 166 isolates had the *Ptt MAT1-2* idiomorph (mating type 2) while the remaining 185 carried the *Ptm MAT1-1* idiomorph (mating type 1). The segregation of the population was in a 1:1 ratio (chi square 0.74; $P = 0.390$).

Phenotypic evaluation and disease assessment

Out of 351 progeny isolates, 172 hybrid isolates produced conidia, which was 49% of the total number of isolates. Only these isolates were used for the phenotypic evaluation. Disease reaction scores of the 172 hybrid isolates across eight barley cultivars ranged from avirulent to virulent with transgressive segregation observed for most cultivars (Table 1 and Fig. 1). Of the isolates showing symptoms (n = 148), 13 resulted in net-like leaf symptoms and 135 in spot-like leaf symptoms. The rest of the 24 isolates were avirulent thus, no symptoms were detectable on the leaves of any of the cultivars tested. Ten of the progeny isolates (*Pop37*_41, *Pop37*_48, *Pop37*_52, *Pop37*_63, *Pop37*_74, *Pop37*_237, *Pop37*_245, *Pop37*_249, *Pop37*_339 and *Pop37*_362) having scores ≥ 6 on all of the eight barley cultivars tested (Supplementary Table S2) were further evaluated on another 12 barley cultivars known to be resistant to *Ptt* or *Ptm*. Three (*Pop37*_41, *Pop37*_63 and *Pop37*_339) of the 10 isolates had scores ≥ 6 on all 20 cultivars tested (Fig. 2).

Genetic map and QTL analysis

Out of 4007 SNP and SilicoDArT markers, 1,965 high-quality markers were retained for the construction of the genetic map of NB63/HRS07033. The genetic map of *Pop37* consisted of 12 linkage groups spanning from 79.7 to 254.3 cM (Supplementary Table S3). The total length of the genetic map was 1816.3 cM with 1432 non-redundant markers (Table 2). The average distance between flanking markers ranged from 1.152 to 1.627 per linkage group with an average distance between flanking markers of 1.268 for the entire genetic map. The physical distance to genetic map distance ratio for *Pop37* with respect to W1-1 and SG1 genomes was 28.5 kb/cM and 22.7 kb/cM, respectively. The marker order of the genetic map of *Pop37* was mostly in agreement with the marker positions of W1-1 and SG1 (Supplementary Table S3).

Four QTL associated with the qualitative trait of having either net-like or spot-like symptoms were identified (*USQNB5.1, USQNB5.2, USQNB11 and USQNB12*), with LOD values ranging

from 3.0 to 3.9. The phenotypic variance explained by these QTL ranged from 7% to 9% (Table 3 and Fig. 3).

Five QTL associated with disease on different genotypes were identified. The QTL *USQV12* was associated with Dampier, Grimmet, Kombar and Prior, phenotypes with LOD values of 5.5, 3.3, 6.9 and 5.7, respectively. The phenotypic variation explained by this QTL was 13%, 8% 16% and 14%, for Dampier, Grimmet, Kombar and Prior, respectively. The QTL *USQV9*, identified on chromosome 9, was associated with the disease reaction on Ciho 5791 and Flagship with LOD scores of 3.2 and 3.6, respectively and explained 7% and 8% of the phenotypic variance, respectively. Both QTL *USQV9* and *USQV12* were contributed by the *Ptm* parent HRS07033. The QTL *USQV2* was responsible for the variation in the disease reaction score of Flagship and Kombar with LOD 3.0 and LOD 3.8, respectively and explained 7 to 8% of the variation in the disease reaction score of Flagship and Kombar, respectively. The QTL, *USQV8* was responsible for the variation in disease reaction score on Gairdner and had a LOD score of 3.4 explaining 10% of the phenotypic variance. The QTL, *USQV5* was responsible for the variation in disease reaction score of Fleet with LOD score of 3 and explained 6% of the phenotypic variance. The QTL *USQV2*, *USQV5* and *USQV8* were contributed by the *Ptt* parent NB63.

Out of eight barley cultivars only Flagship and Kombar were associated with more than two QTL, hence QTL accumulation effects of progeny isolates on Flagship (*USQV2* and *USQV9*) and Kombar (*USQV2* and *USQV12*) were detected. Progeny isolates harbouring either *USQV2* or *USQV9* showed average disease reaction scores of 3.4 and 3.2, respectively on Flagship. Isolates harbouring both *USQV2* and *USQV9* had a disease reaction score of 4.7 on Flagship. Progeny isolates harbouring either *USQV2* or *USQV12* showed average disease reaction scores of 3.8 and 4.4 on Kombar, respectively. Isolates harbouring both QTL associated with Kombar had an average disease reaction score of 5.8 on Kombar. QTL accumulation curves observed for the two QTL associated with the virulence on Kombar and two QTL associated with Flagship revealed that progeny isolates harbouring both QTL from each cultivar had a positive significant (P = 0.05) correlation with increased disease reaction scores on the respective cultivar (Fig. 4).

Candidate genes and effectors genes

The QTL regions (20 kb flanking regions on both side of the peak marker) were aligned with both reference genome annotations. Sixty-eight candidate genes were detected for five of the nine QTL regions (Supplementary Table S4). No candidate genes were found for the other four

QTL. Out of 68 candidate genes, 12 genes were effector candidate genes with a score > 0.8 estimated by EffectorP and gene expression profile (Ismail & Able 2016, 2017). Effector PTTW11_06577 associated with QTL *USQV5* is a known protein (G27; XP_003303420) that is expressed during net-form net blotch disease of barley (Ismail & Able 2016, 2017). This effector gene was also reported to be associated with thioredoxin (PTTW11_06577; PF00085) (Finn et al. 2016). Candidate gene PTTW11_06585 was also associated with QTL *USQV5* and was found to be an effector gene (G154; XP_003301637) by gene expression profiling. This gene is associated with the peptidase A4 family (PTTW11_06585; PF01828) (Finn et al. 2016). The candidate gene PTMSG1_09710 found in *USQNB11* QTL region was responsible for Dolichol-phosphate mannosyltransferase production (PTMSG1_09710; PF00535). The candidate gene PTMSG1_10204 located within the region of QTL *USQV12* was associated with glycoside hydrolase family 45 proteins (PTMSG1_10204; PF02015). Only four predicted effector genes PTTW11_06577, PTTW11_06585, PTMSG1_09710 and PTMSG1_10204 had known protein domains according to the protein family database pfam (Finn et al. 2016). The other eight predicted effector genes were identified as hypothetical proteins.

Discussion

To the author's knowledge, this is the first study to use a hybrid population of *Pyrenophora teres* f. *teres* and *Pyrenophora teres* f. *maculata* in a QTL analysis study. Recent identification of an increasing number of hybrids in barley fields indicates the importance of understanding the virulence patterns of hybrid isolates. This prepares the global barley industry for possible future outbreaks.

Most of the QTL identified in the current study are unique and novel QTL. To date, seven bi-parental mapping studies for *Ptt* and *Ptm*, and one genome-wide association mapping study for *Ptt* had been conducted using different barley cultivars to detect genomic regions associated with avirulence/virulence of *P. teres* (Weiland et al. 1999; Beattie et al. 2007; Lai et al. 2007; Shjerve et al. 2014; Carlsen et al. 2017; Koladia et al. 2017; Martin et al. 2020). Most of the QTL identified in previous studies were not detected in the current study. Except for Kombar and Prior, all other barley cultivars used in the current study were different from previous studies. Absence of previously reported QTL in the current study might have resulted from the host/cultivar specificity of *P. teres.* During the infection process, different host specific effectors/NEs are secreted by *P. teres* (Liu et al. 2011). Genomic regions associated with a set of NEs specific to one barley cultivar might not be available/active for another cultivar. Hence,

genomic regions identified for one barley cultivar might not be detectable in different barley cultivars.

Some of the cultivars used in the present study, like Kombar and Prior, were common among previous studies (Shjerve et al. 2014; Koladia et al. 2017; Martin et al. 2020), however, QTL in this study associated with these cultivars were different from other studies. A cross between two Californian *Ptt* isolates (15A and 6A) with different virulence reactions to Rika and Kombar, detected two virulence loci, VK1 and VK2, for the Kombar cultivar and another two loci, VR1 and VR2, for the Rika cultivar, with single nucleotide polymorphism (SNP), simple sequence repeats (SSR) and AFLP markers (Shjerve et al. 2014). Even though the cultivar Kombar was common to the current study and the aforementioned study (Shjerve et al. 2014), none of the above QTL identified for the virulence of Kombar were identified in the current study. Fourteen different genomic regions associated with virulence of *Ptt* were detected using a genome wide association mapping study of Australian *Ptt* isolates collected from five states in Australia (Martin et al. 2020). The study was conducted using the DArTseq™ marker system and phenotyping 20 barley lines. The identified genomic regions were confirmed by QTL analysis of two bi-parental mapping populations, NB029/HRS09122 and NB029/NB085, with Beecher, Skiff and Prior barley cultivars. Four regions identified by GWAS, which were responsible for phenotypic variation in Beecher, Skiff and Prior, were confirmed by bi-parental QTL mapping. The genomic regions associated with Prior in the current study and Martin et al. (2020) study were located in different locations of the *P. teres* genome. The QTL associated with Prior virulence in the current study was contributed by the *Ptm* parent, while the QTL identified in the previous study (Martin et al. 2020) was contributed by a *Ptt* parent. A similar observation was detected in two bi-parental mapping studies (Koladia et al. 2017; Martin et al. 2020), which both used the cultivar Beecher. One study used a Denmark/USA cross and the other a cross made from Australian isolates. The QTL reported for Beecher in these two studies were located in different locations. Therefore, the authors suggested that genomic regions controlling the virulence of the same barley cultivar may not be conserved among geographically distant isolates (Martin et al. 2020).

Using a hybrid mapping population in this study enabled the development of a high-density genetic map due to higher frequency of polymorphism between *Ptt/Ptm* isolates than between *Ptt/Ptt* or *Ptm/Ptm* isolates (Weiland et al. 1999; Beattie et al. 2007; Lai et al. 2007; Shjerve et al. 2014; Carlsen et al. 2017; Koladia et al. 2017; Martin et al. 2020). Also, it enabled the detection of QTL present in both *Ptt* and *Ptm* genomes. Furthermore, using a hybrid population

allowed identification of genomic regions associated with the development of leaf symptoms caused by *Ptt* and *Ptm*.

One of the aims of this study was to identify the genomic regions associated with the net blotch leaf symptoms. However, most of the progeny isolates showed spot-like disease symptoms and only 13 of the progeny isolates could be clearly identified as having net-like symptoms. The same observation was made for field collected hybrids which all showed spot-like disease symptoms (Campbell et al. 2002; McLean et al. 2014; Turo et al. 2021). To differentiate between spot and net form symptoms at the lower infection range is difficult and thus some of these progeny isolates could have been miss-classified as spot-form instead of net-form. In this study, four genomic regions associated with leaf symptoms were detected. The infection process and development of disease symptoms of the pathogen have been proposed to be complex events (Lightfoot & Able 2010; Liu et al. 2011), indicating that, there could be a number of genes associated with *P. teres* infection and disease development on barley.

A study conducted with SNP markers using the *Ptt* population BB25/FGOH04Ptt-21 reported nine unique QTL responsible for the virulence on eight different barley cultivars, with QTL on chromosomes 1, 3, 5, 8, 9 and 12 conferring a major effect (Koladia et al. 2017). One of the QTL, *PttBee2*, which was detected in the Koladia et al. (2017) study to be responsible for the virulence on Beecher was co-localized with leaf symptom QTL *USQNB5.2* in our study. Two QTL detected by Martin et al. (2020) from GWAS, QTL11 and QTL12 on chromosome 5, were co-located with QTL, *PttBee_5*, identified in a bi-parental mapping population in the same study, and were also found to be co-localized with *USQNB5.2* in the current study. Co-localization of the leaf symptom QTL with those for virulence suggests that some genomic regions responsible for virulence in *P. teres* may have effects on determining the leaf symptoms of the pathogen or that these genes could be closely linked to each other and were identified as a single QTL in the current study. QTL *VK2*, associated with the virulence on Kombar and detected on chromosome 2 (Shjerve et al. 2014), and QTL *PttSki_5*, associated with the virulence on Skiff (Koladia et al. 2017), were also located closely to QTL *USQV2* and *USQV5*, which were associated with virulence on Kombar and Fleet, respectively, in the current study. Similar to Martin et al (2020) three QTL, *USQV2, USQV9* and *USQV12* identified in this study were associated with the virulence of more than one barley cultivar. Identification of common QTL regions (*USQV2, USQV9* and *USQV12*) responsible for the virulence of more than one cultivar in the current study confirms that some genomic regions are less host specific

compared to unique QTL regions which were responsible for virulence on only one barley cultivar.

Three hybrid isolates were virulent on all 20 genotypes tested including currently used net blotch-resistance cultivars (*Pop37*_41, *Pop37*_63 and *Pop37*_339). Out of five QTL associated with virulence, isolates *Pop37*_41, *Pop37*_63 and *Pop37*_339 harbour three (*USQV2, USQV5* and *USQV8*), two (*USQV5* and *USQV8*) and four (*USQV2,USQV8,USQV9* and *USQV12*) QTL associated with virulence identified in this study, respectively. The QTL accumulation curves observed for the disease reaction scores of Kombar and Flagship also confirms that QTL accumulation can significantly increase the disease severity. These disease assessment results indicate the potential devastating damages hybrid progenies can have on the barley industry in the absence of suitable resistant barley cultivars. Furthermore, most of the current cultivars are susceptible to spot-form (GRDC 2020). Hence, in order to develop suitable cultivars resistance to *P. teres* hybrids, barley breeders will need to incorporate both net-form and spot-form resistance QTL into one cultivar.

This is the first study to report QTL associated with disease symptoms of net blotches of barley. Different QTL including unique QTL identified in this study point to a complex interaction between *P. teres* and its barley host. Detection of highly virulent hybrid isolates suggests that the current net-blotch resistant barley cultivars will be ineffective during a hybrid outbreak. Hence, it is necessary to introgress multiple barley resistance genes for *P. teres* into new barley germplasm. Furthermore, these results demonstrate the need for the development of novel barley cultivars capable of withstanding destructive yield losses due to hybrid isolates.

Author Contributions

BD: designing experiment, data analysing, writing, revising. LS: revising. NV: revising. AM: designing experiment and revising.

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687 Table 1. Disease reaction scores for the eight barley cultivars/lines used for QTL analysis and
688 virulence percentage of the hybrid population for each barley cultivar/line

Cultivar [a]	Average[b]	SE[c]	Avirulent[d]	Virulent[e]	Virulent %[f]
Ciho 5791	4.26	0.184	135	37	21.51
Dampier	4.80	0.192	124	48	27.91
Flagship	3.48	0.175	157	15	8.72
Fleet	3.17	0.173	159	13	7.56
Flagship	3.48	0.175	157	15	8.72
Grimmett	4.56	0.181	133	39	22.67
Kombar	4.35	0.201	132	40	23.26
Prior	4.60	0.216	127	45	26.16

689

690 [a] Barley cultivar

691 [b] Average disease reaction score showed by progeny isolates for the respective barley cultivar

692 [c] Standard error

693 [d] Number of avirulent isolates (< 7) out of 172

694 [e] Number of virulent isolates (> 7) out of 172

695 [f] Percentage of virulent isolates

696

697 Table 2. Genetic map information of *Pop37*

Linkage group/Chromosome	Number of markers	Non-redundant markers	Size cM	Average distance between flanking markers
1	108	86	107.9	1.255
2	203	154	200.1	1.299
3	183	120	169.7	1.414
4	154	114	136.4	1.196
5	279	212	254.3	1.200
6	67	49	79.7	1.627
7	115	86	121.2	1.409
8	276	193	230.1	1.192
9	107	79	95.6	1.210
10	96	75	97.3	1.297
11	122	81	113.2	1.398
12	255	183	210.8	1.152
Total	1965	1432	1816.3	1.268

698

699 Table 3. List of QTL for virulence and leaf symptom identified using *pop37*

QTL[a]	Trait[b]	Chr[c]	Genetic map[d]					W1-1[e]			SG1[f]			LOD[g]	R^{2h}	Parent[i]
			Start[j] cM	End cM[k]	peak_1[l] cM	peak_2 CM	Marker name[m]	Start (bp)	End (bp)	Peak marker (bp)	Start (bp)	End (bp)	Peak marker (bp)			
QTL for virulence																
USQV2	Flagship	2	1	8	2	NA	NA	280936	346503	337069	125561	192984	NA	3.0	7.0	*Ptt*
	Kombar	2	1	7	6	NA	28946283	280936	346503	337069	125561	192984	182038	3.8	8.0	*Ptt*
USQV5	Fleet	5	156	168	162.9	NA	36346592	3626679	3745439	3719823	3043251	3161843	3136235	3.0	6.0	*Ptt*
USQV8	Gairdner	8	178	187	180	NA	36349857	5999571	6189805	6007171	4711299	4919725	4768710	3.4	10.0	*Ptt*
USQV9	Ciho 5791	9	31	42	37	NA	36348095	987772	1196189	1171258	734586	901217	876223	3.2	7.0	*Ptm*
	Flagship	9	31	48	35	NA	36350521	987772	1273721	1055386	734586	978963	876223	3.6	8.0	*Ptm*
USQV12	Dampier	12	1	12	1	11.4	36348695	361269	508637	508637	63893	213609	128854	5.5	13.0	*Ptm*
	Grimmet	12	1	13	1	NA	36346885	361269	508637	361269	63893	213609	128854	3.3	8.0	*Ptm*
	Kombar	12	1	12	2	NA	36346885	361269	508637	361269	63893	213609	128854	6.9	16.0	*Ptm*
	Prior	12	1	11	1	11.2	36348695	361269	508637	508637	63893	213609	128854	5.7	14.0	*Ptm*
QTL for Leaf symptom																
USQNB5.1	Form	5	2	25	13	NA	28945886	256820	700266	440934	224725	470158	313831	3.2	7.0	*Ptm*
USQNB5.2	Form	5	195	217	207.3	213	36349981 & 36349583	4709550	5579160	5100294 5151650	3768230	4530001	4362659 4413961	3.9	9.0	*Ptm*
USQNB11	Form	11	6	18	12	NA	36347703	200745	593148	241491	250568	394230	318802	3.4	7.0	*Ptm*
USQNB12	Form	12	78	91	78	NA	36349475	2328147	2630739	2328147	1916192	2219650	1916192	3.0	7.0	*Ptm*

[a] Name of the QTL

[b] Barley cultivar

[c] Chromosome number according to W1-1 and SG1 reference genomes

[d] *Pop37* genetic map information

[e] W1-1 reference genome

[f] SG1 reference genome

[g] Logarithm of the odds based on WinQTLCartographer V2.5

[h] Phenotypic variation described by the respective QTL based on WinQTLCartographer V2.5

[i] Parental isolate contributing the QTL

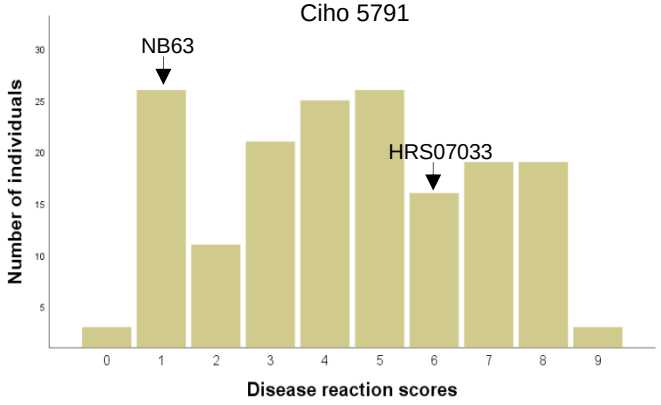
[j] Starting position of the QTL

[k] Ending position of the QTL

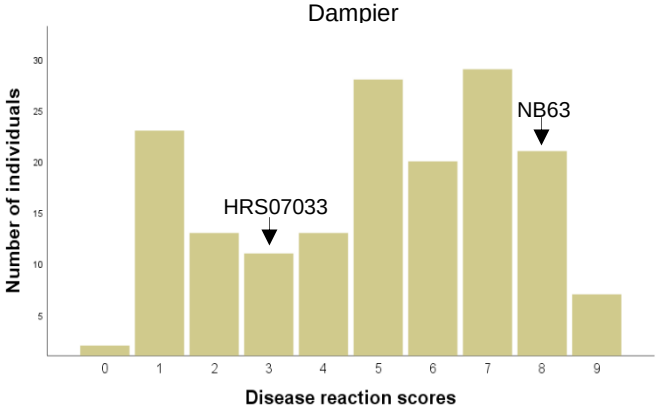
[l] Peak position of the QTL

[m] Peak position marker name of the QTL

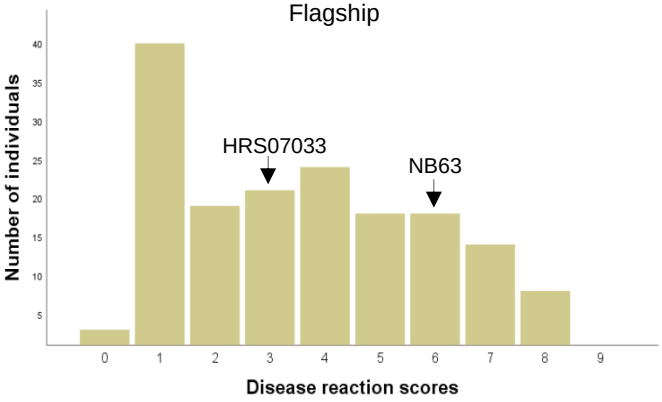
Ciho 5791



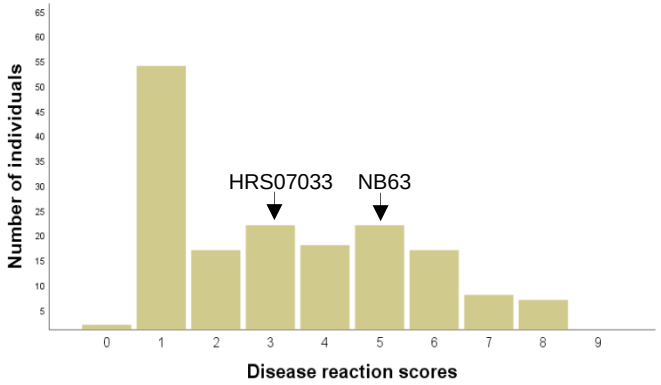
Dampier



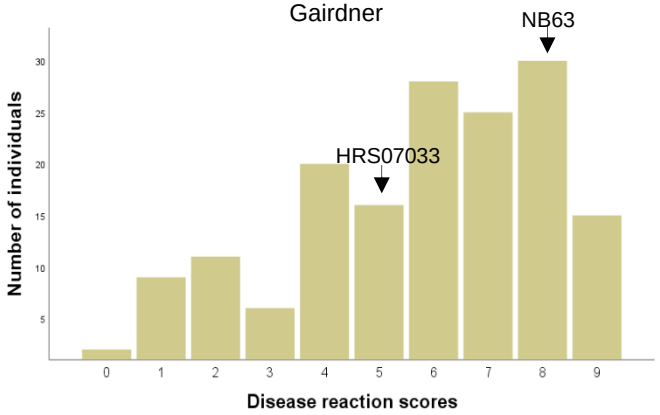
Flagship



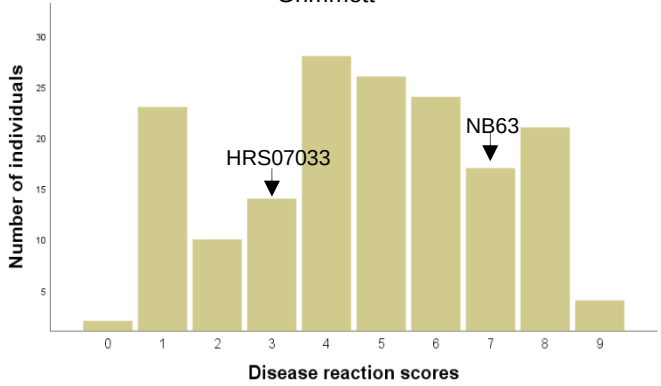
Fleet



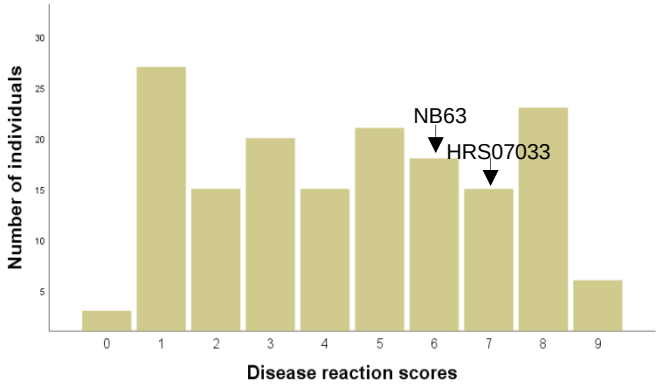
Gairdner



Grimmett

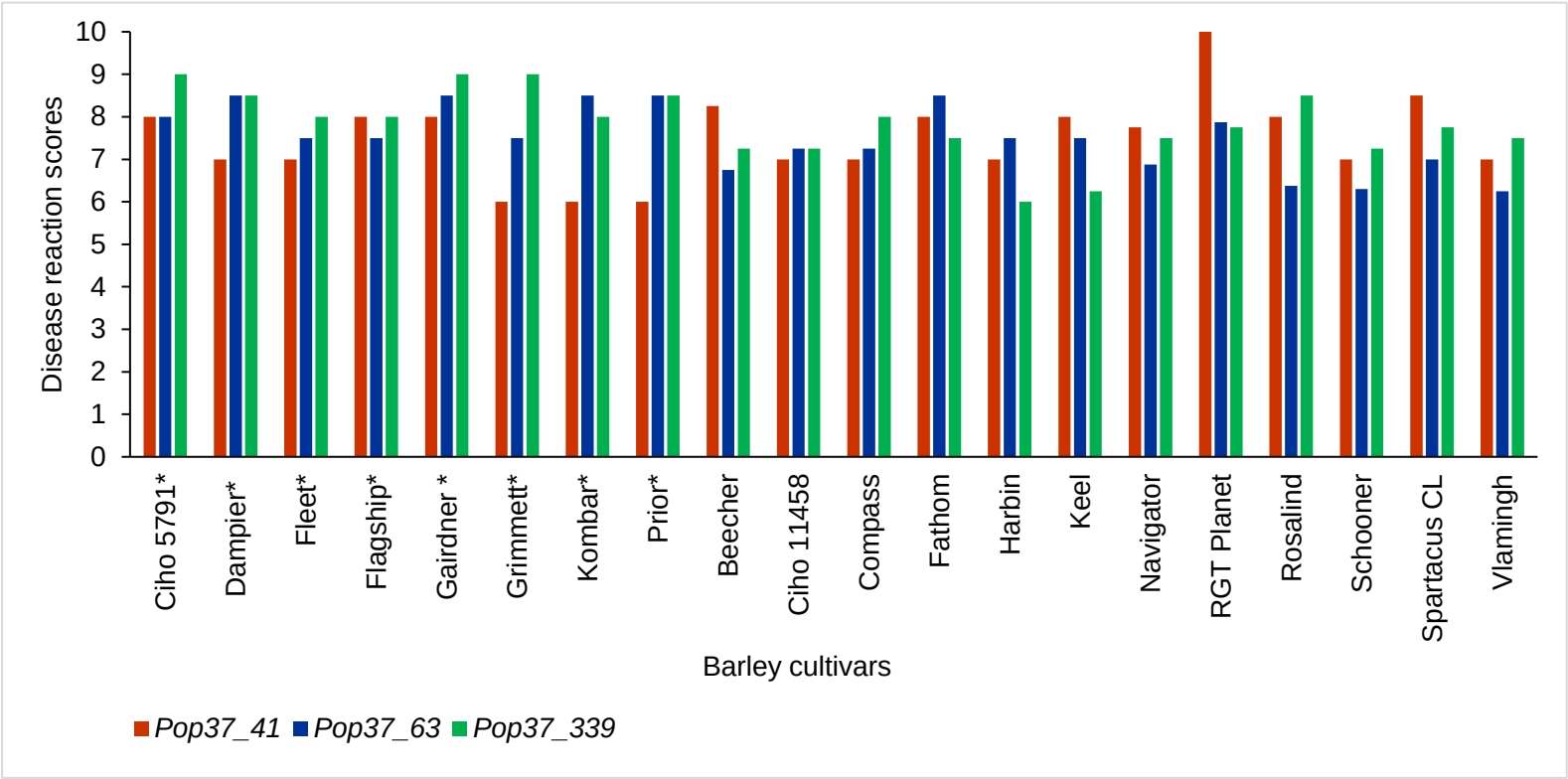


Kombar



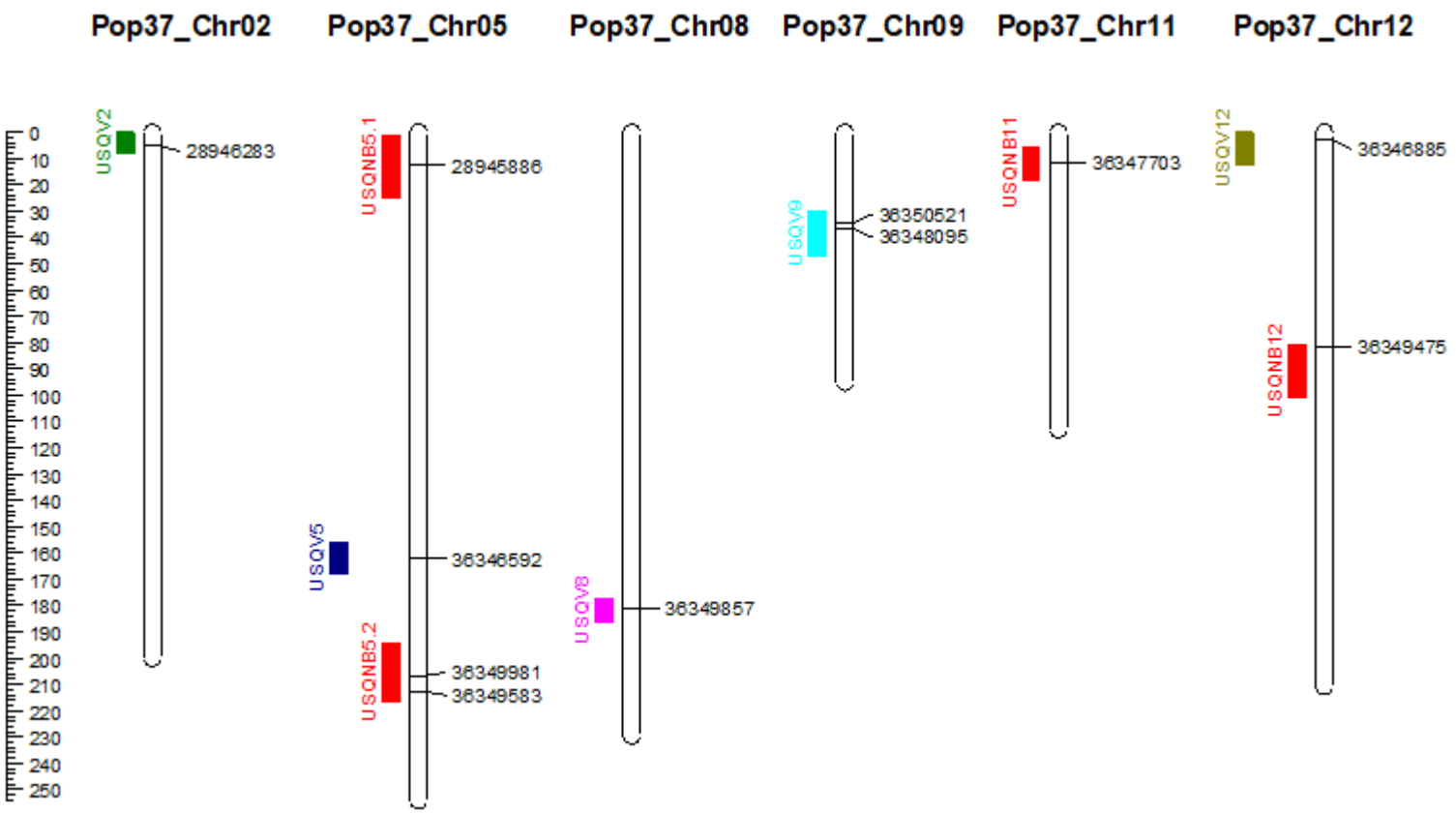
Prior

714 Figure 1. Disease reaction scores of progeny isolates of *Pop37* on eight barley cultivars/lines used in QTL analysis. Disease reaction scores of
715 parental isolates are indicated with arrows.
716



*Cultivars used for QTL mapping

Figure 2. Disease reaction scores of highly virulent progeny isolates (*Pop37*_41, *Pop37*_63 and *Pop37*_339) of population *Pop*37 on all 20 barley cultivars/lines.



753

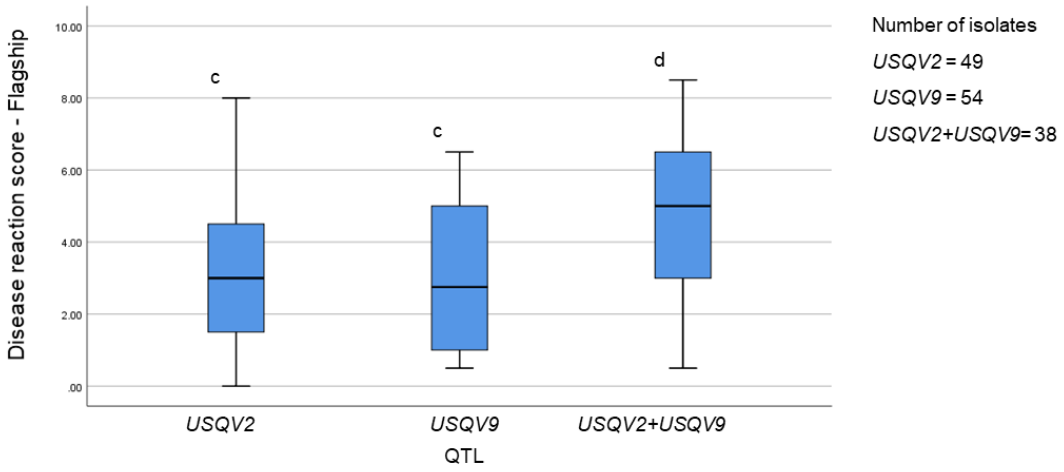
754

755 Figure 3. Genetic map of *Pop37* (*Ptt*-NB63 × *Ptm*-HRS07033) showing identified QTL on the left of the chromosome and markers at the peak of

756 the QTL on the right. Distance in cM is indicated on the left.

757

A



B

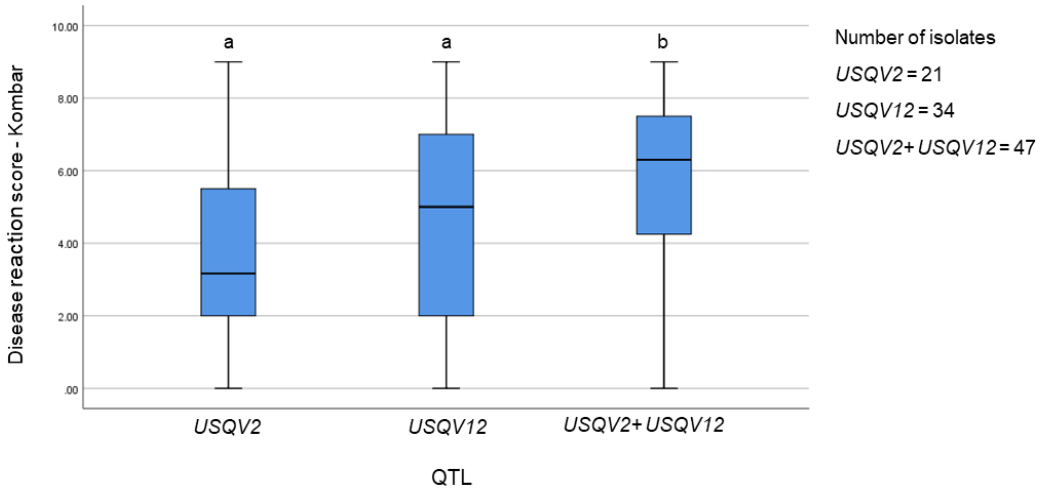


Figure 4. Pyramiding of QTL associated with virulence of *P. teres* for Flagship (A) and Kombar (B). Boxes with similar letters are not significantly different (*P*=0.05).

CHAPTER 5

GENERAL DISCUSSION, FUTURE RECOMMENDATIONS AND CONCLUSION

5.1 General discussion

The main objectives of this PhD study were to 1. characterize *Pyrenophora teres* f. *teres* populations collected from different continents, 2. identify the mating preference between or within the two forms of *P. teres*, *Ptt* and *Ptm*, and its hybrids *in vitro* and 3. recognize genomic regions of *P. teres* associated with virulence using a mapping population developed by crossing *Ptt* and *Ptm* isolates.

An important finding of this study was detection of two natural *Ptt*/*Ptm* hybrids collected from barley fields. One of these hybrids was a historical isolate, CBS 281.31, which was recorded in 1931 and previously identified as *P. japonica*/*P. teres* f. *maculata* and *P. teres* f. *teres* based on morphological characteristics and molecular markers (Crous et al. 1995; Williams et al. 2001; Bakonyi & Justesen 2007; Marin-Felix et al. 2019). However, in this study, we found that CBS 281.31 is a hybrid. This highlights the inefficiency of using morphological features to identify *Pyrenophora* species and the importance of using appropriate molecular markers to characterise fungal cultures. This also suggests that the occurrence of *P. teres* hybrids might not be as rare in nature as previously assumed, but hybrids may be left unrecognised due to the lack of proper marker systems used for hybrid identification. A set of form-specific markers was recently developed to differentiate the two forms of *P. teres* as well as *Ptm*/*Ptt* hybrids using polyacrylamide gel electrophoresis to visualize fragments (Poudel et al. 2017). In the current study, these previously published markers were converted into non-gel-based markers using quantitative PCR, thus omitting the need for gel electrophoresis and thereby making identification faster and more efficient (Dahanayaka et al. 2021b).

Pyrenophora teres hybrids in barley fields may remain undetected not only due to the use of inappropriate marker systems but also due to the potential avirulent nature of some hybrids. Avirulent hybrids will not be selected for in barley fields and thus may not survive from one generation to the next. However, the laboratory-based hybrid population from *Pop37* produced in this study included both highly virulent and avirulent isolates. Recognition of hybrid isolates by visual appearance is impractical as they frequently show either net-like or spot-like symptoms. In the field, this could be another reason for hybrid isolates to be left undetected. Repeated mating of hybrid isolates with either *Ptt* or *Ptm* as shown in this study may also lead to the accumulation of *Ptt* or *Ptm* genetic material in the hybrid gene pool and leave these undetected.

Hybrid isolates acquiring virulence from both *Ptt* and *Ptm* could result in more complex virulence profiles than the existing virulence profiles. The results of our study confirmed that *Ptt* isolates had greater reproduction vigour than *Ptm* and the sexual reproduction barriers between *Ptt* and *Ptm* could be overcome under suitable conditions, allowing hybridisation between *Ptt* and *Ptm*, and production of hybrids (Dahanayaka et al. 2021a). A wide range of virulence profiles were observed in the hybrid population used in the current study with some of the hybrid isolates being virulent on all 20 tested commonly used net blotch-resistant barley cultivars. Potential natural occurrence of such hybrids in the field could lead to devastating yield losses to the barley industry, in the absence of resistant barley cultivars. This further emphasizes the need for commercial barley varieties resistant to both spot- and net-form net blotch.

Sexual recombination/mating plays a major role in the life cycle of fungal pathogens including *P. teres* (Liu et al., 2011; Stukenbrock 2016). Our study evidenced sexual recombination and exchange of genetic material between *P. teres* f. *teres* populations collected from different regions (Dahanayaka et al. 2021b) and also in *P. teres* hybrids (Dahanayaka et al. 2021a). Repeated sexual recombination between virulent isolates could result in the accumulation of genomic regions associated with virulence, which could cause significant yield losses to the barley industry in the absence of barley varieties with resistance to multiple virulence genes.

As per the authors' knowledge, this study is the first attempt to identify and map genes associated with the symptoms of *P. teres*. Identification of QTL responsible for the different symptoms caused by *P. teres* forms was made possible via production of a *Ptt/Ptm* hybrid population. However, phenotyping the progeny isolates based on displaying *Ptt* (net-like) and *Ptm* (spot-like) symptoms showed that the population was not segregating with a 1:1 ratio but instead was skewed toward spot-like leaf symptoms. The segregation distortion observed for leaf symptoms might be due to misclassification of progeny isolates. Disease symptoms caused by both *Ptt* and *Ptm* initially appear as dot-like necrotic lesions and gradually develop into either net-like elongated or spot-like circular lesions, respectively. Hence, the skewed ratio toward spot-like leaf symptoms might have resulted from classifying initial net-form symptoms as spot-form symptoms. As avirulent progeny isolates do not produce any disease symptoms, the inability of classifying them into net-like or spot-like symptoms might have also led to segregation distortion of the leaf symptom trait.

5.2 Future recommendations

Identification of naturally occurring hybrids in this study suggests that the hybridization between *Ptt* and *Ptm* is a possible phenomenon, hence, regular monitoring and genetic characterization of *P. teres* populations using a suitable marker system should be undertaken. Periodical monitoring and genetic characterization along with phenotypic assessment of *P. teres* populations in Australia should be conducted to reveal potential temporal changes of the population at a genetic and phenotypic level.

The identified greater reproduction vigour of *Ptt* compared to *Ptm* observed in this study could be due to the environmental conditions used during the test. Therefore, repeating the experimental setup under different temperatures and light conditions would confirm whether *Ptt* naturally had greater reproduction vigour or if the laboratory conditions resulted in an increase in vigour of *Ptt* compared to *Ptm.* More experiments using different conditions in the laboratory, such as different temperature ranges or photoperiod, may provide further insights into the conditions needed for the reproduction barriers between *Ptt* and *Ptm* to be overcome. These could be used to predict the increased occurrence of hybrids in the field. It may also be worthwhile to investigate the mating preference of *Ptt* and *Ptm* in a glasshouse environment as it would provide an *in planta* environment for isolates with fewer numbers of variables than field conditions. Furthermore, future studies on growth, sporulation and the infection process of hybrid isolates compared to *Ptt* and *Ptm* isolates would also provide useful information on the fitness of hybrid isolates.

High resolution mapping of the QTL identified in this study using a large number of isolates would assist in narrowing down the QTL and facilitate detection of the most probable/exact candidate gene. Cloning and expression of the candidate genes identified in this study would expand our knowledge of the function of these genes. Studying the possible mutations of these regions through cloning would also allow us to understand the function of these genes and their involvement in the infection process. Whole-genome sequencing and comparative genomic analysis of highly virulent hybrid isolates and parental isolates would allow identification of more genomic regions associated with virulence of *P. teres*.

As per the authors' knowledge, all previously reported QTL and association mapping studies conducted to identify genomic regions of *P. teres* associated with virulence examined the QTL at the seedling stage of barley. Previously, Lehmensiek et al. (2007) reported that the set of QTL identified for *P. teres* resistance of barley seedlings were different to the QTL identified in the same barley genotypes at the adult stage. This suggests that the genomic regions

associated with the virulence of *P. teres* at the seedling and adult stages could also be different. Hence, repeating the QTL mapping analysis at the adult stage with the hybrid progeny would allow a greater understanding of the adaptation and behaviour of *P. teres* during infection of adult barley genotypes. Furthermore, the three highly virulent hybrid isolates identified in this study could be included in barley breeding programs when developing net-blotch resistant barley cultivars.

Even though this study detected four QTL associated with leaf symptoms of *P. teres* using a *Ptt/Ptm* population, there was segregation distortion for the trait. Hence, in order to verify the segregation distortion of hybrid isolates for the leaf symptom trait and confirm the identified QTL associated with leaf symptom of this study, it is important to conduct future studies using multiple-population QTL analysis. Also, to overcome the misclassification of leaf symptoms either as net-like or spot-like leaf symptom at the early stage, the inoculum concentration could be increased or the inoculated barley seedlings may be left until they develop distinct disease symptoms.

5.3 Conclusion

This thesis aimed to improve the understanding of the *P. teres*-barley pathosystem and the possible evolutionary adaptation of this pathogen through various methods. Results of this study highlighted the importance of deploying a genome-wide marker system to understand the host pathogen interaction. Employing DArTseq™ markers in our study identified field isolates as hybrids, which were reported to be rare. Our results also confirmed the high probability of occurrence of *P. teres* hybrids under suitable environmental conditions due to overcoming sexual reproduction barriers and that these resulting hybrids could possess high virulence in commercially available net blotch-resistant cultivars. It suggests the potential rapid evolution of the pathogen in response to changes in environmental conditions. Our study emphasizes the need of developing novel barley germplasms in order to withstand future outbreaks which could occur not only due to highly virulent hybrids but also due to highly virulent *Ptt* or *Ptm* isolates occurring as a result of rapid adaptation. Hence, this study provides novel and highly valuable knowledge for understanding the complex *P. teres*-barley pathosystem.

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APPENDIX

Chapter 2

Supplementary Table S1. Meta data for *Pyrenophora teres* isolates genotyped in this study

Isolate	Mat[a]	Year	Host	Region/State	Country	Reference[e]	DAPC	STR
HRS07013[d]	2	2007	Unknown	NSW	Australia	Martin et al. (2020)	4	III
HRS08046[d]	2	2008	NRB07572	QLD	Australia	Martin et al. (2020)	4	III
HRS08117[d]	2	2008	Unknown	QLD	Australia	Martin et al. (2020)	4	III
nf25/08[d]	1	2007	Fleet	SA	Australia	Martin et al. (2020)	2	III
nf49/07	1	2007	Keel	SA	Australia	Martin et al. (2020)	NA	NA
nf55/07[d]	1	2007	Keel	SA	Australia	Martin et al. (2020)	1	I
HRS08194	1	2008	Net form net blotch differential	SA	Australia	Martin et al. (2020)	NA	NA
HRS08195[d]	2	2008	Unknown	NSW	Australia	Martin et al. (2020)	4	III
HRS09015[d]	1	2009	Barley Stubble	QLD	Australia	Martin et al. (2020)	4	III
HRS09042[d]	1	2009	Skiff	QLD	Australia	Martin et al. (2020)	4	III
HRS09092[d]	2	2009	Shepherd	QLD	Australia	Martin et al. (2020)	4	III
nf47/09A3[d]	2	2009	Maritime	SA	Australia	Martin et al. (2020)	1	I
nf48/09A3[d]	2	2009	Maritime	SA	Australia	Martin et al. (2020)	1	I

HRS09120	2	2009	Shepherd	QLD	Australia	Martin et al. (2020)	NA	NA
HRS09121[d]	1	2009	TR129/Skiff	NSW	Australia	Martin et al. (2020)	4	III
HRS09122[d]	2	2009	TR129/Skiff	NSW	Australia	Martin et al. (2020)	2	III
HRS09123[d]	2	2009	Vlamingh	WA	Australia	Martin et al. (2020)	1	I
HRS09127	2	2009	TR129/Skiff	NSW	Australia	Martin et al. (2020)	NA	NA
03-0006[d]	1		Unknown	VIC	Australia	Martin et al. (2020)	4	III
nf08/007ss[d]	2	2008	Unknown	SA	Australia	Martin et al. (2020)	1	I
HRS09136[d]	2	2009	Barley	WA	Australia	Martin et al. (2020)	1	I
nf09/136[d]	1	2009	Barque	VIC	Australia	Martin et al. (2020)	4	III
nf09/140[d]	1	2009	Barque	VIC	Australia	Martin et al. (2020)	2	III
nf122/09b[d]	1	2009	Fleet	SA	Australia	Martin et al. (2020)	1	I
HRS10004	1	2010	Grimmett	QLD	Australia	Martin et al. (2020)	NA	NA
HRS10015[d]	1	2010	NRB06059	QLD	Australia	Martin et al. (2020)	1	I
ptt09-120[d]	1	2009	Unknown	SA	Australia	Martin et al. (2020)	4	III
ptt09-154	1	2009	Baudin	WA	Australia	Martin et al. (2020)	NA	NA
ptt09-155[d]	1	2009	Vlamingh	WA	Australia	Martin et al. (2020)	1	I

HRS10033	1	2010	Keel	QLD	Australia	Martin et al. (2020)	NA	NA
nf32/98[d]	2	1998	Unknown	SA	Australia	Martin et al. (2020)	2	III
nf57/09[d]	2	2009	Unknown	SA	Australia	Martin et al. (2020)	1	I
nf66/09[d]	2	2009	Unknown	SA	Australia	Martin et al. (2020)	1	I
nf70/09[d]	1	2009	Unknown	SA	Australia	Martin et al. (2020)	1	I
nf123/09[d]	2	2009	Unknown	SA	Australia	Martin et al. (2020)	1	I
HRS10077[d]	1	2010	Unknown	QLD	Australia	Martin et al. (2020)	4	III
HRS10097[d]	1	2010	NRB06059	QLD	Australia	Martin et al. (2020)	4	III
HRS10108[d]	1	2010	Tallon	QLD	Australia	Martin et al. (2020)	4	III
HRS10109[d]	1	2010	Unknown	QLD	Australia	Martin et al. (2020)	4	III
HRS10121[d]	2	2010	Grout	QLD	Australia	Martin et al. (2020)	1	I
HRS10122[d]	2	2010	Shepherd	QLD	Australia	Martin et al. (2020)	1	I
HRS10131	1	2010	Barley	NSW	Australia	Martin et al. (2020)	NA	NA
HRS10135[d]	2	2010	Mackay	NSW	Australia	Martin et al. (2020)	4	III
HRS10137[d]	2	2010	Shepherd	NSW	Australia	Martin et al. (2020)	4	III
HRS10138[d]	2	2010	Commander	NSW	Australia	Martin et al. (2020)	2	III

HRS10142[d]	1	2010	Grout	NSW	Australia	Martin et al. (2020)	4	III
HRS10159[d]	2	2010	Bass	NSW	Australia	Martin et al. (2020)	4	III
HRS10164[d]	2	2010	Grimmett	QLD	Australia	Martin et al. (2020)	4	III
HRS10167[d]	1	2010	Grout	QLD	Australia	Martin et al. (2020)	1	I
HRS10185[d]	2	2010	Hindmarsh	QLD	Australia	Martin et al. (2020)	4	III
HRS10189[d]	1	2010	Mackay	QLD	Australia	Martin et al. (2020)	1	I
HRS10190a[d]	1	2010	Tallon	QLD	Australia	Martin et al. (2020)	4	III
HRS10193[d]	2	2010	Bass	WA	Australia	Martin et al. (2020)	1	I
HRS10194[d]	2	2010	Baudin	WA	Australia	Martin et al. (2020)	1	I
HRS10220[d]	2	2010	Commander	NSW	Australia	Martin et al. (2020)	4	III
HRS13164a[d]	2	2013	Fathom	SA	Australia	Martin et al. (2020)	1	I
HRS13175a[d]	2	2013	Unknown	QLD	Australia	Martin et al. (2020)	4	III
HRS13182a[d]	2	2013	Henley	QLD	Australia	Martin et al. (2020)	4	III
HRS13199a[d]	2	2013	Scope CL	WA	Australia	Martin et al. (2020)	2	III
HRS13209a[d]	2	2013	Barley	QLD	Australia	Martin et al. (2020)	4	III
HRS13217a[d]	1	2013	Unknown	QLD	Australia	Martin et al. (2020)	4	III

nf018/13[d]	2	2013	Fleet	SA	Australia	Martin et al. (2020)	4	III
ptt14-007[d]	2	2014	Unknown	VIC	Australia	Martin et al. (2020)	4	III
ptt14-057[d]	1	2014	Unknown	VIC	Australia	Martin et al. (2020)	1	I
ptt14-110[d]	1	2014	Fairview	VIC	Australia	Martin et al. (2020)	2	III
nf65/14a[d]	2	2014	Maritime	SA	Australia	Martin et al. (2020)	1	I
nf71/14a[d]	2	2014	Fleet	SA	Australia	Martin et al. (2020)	2	III
nf117/14a[d]	2	2014	Barque	SA	Australia	Martin et al. (2020)	4	III
87/15a[d]	2	2015	Alstar	SA	Australia	Martin et al. (2020)	2	III
HRS16025a[d]	1	2016	Shepherd	QLD	Australia	Martin et al. (2020)	4	III
HRS16026a[d]	1	2016	Compass	NSW	Australia	Martin et al. (2020)	1	I
HRS16031a[d]	2	2016	Shepherd	QLD	Australia	Martin et al. (2020)	4	III
HRS16033a[d]	1	2016	Unknown	QLD	Australia	Martin et al. (2020)	4	III
HRS16041a[d]	1	2016	Compass	SA	Australia	Martin et al. (2020)	1	I
HRS16043a[d]	2	2016	Compass	QLD	Australia	Martin et al. (2020)	1	I
HRS16051a[d]	1	2016	Shepherd	QLD	Australia	Martin et al. (2020)	4	III
HRS16083a[d]	1	2016	Shepherd	QLD	Australia	Martin et al. (2020)	4	III

HRS17058[d]	1	2017	Shepherd	QLD	Australia	Martin et al. (2020)	4	III
HRS17066a[d]	2	2017	Commander	NSW	Australia	Martin et al. (2020)	1	I
HRS17080a[d]	1	2017	Commander	QLD	Australia	Martin et al. (2020)	1	I
HRS17081a[d]	1	2017	Barley	QLD	Australia	Martin et al. (2020)	1	I
HRS17082a	1	2017	Commander	NSW	Australia	Martin et al. (2020)	NA	NA
HRS17083a[d]	1	2017	Commander	NSW	Australia	Martin et al. (2020)	1	I
HRS17084a[d]	1	2017	Commander	QLD	Australia	Martin et al. (2020)	1	I
HRS17085a[d]	1	2017	Commander	NSW	Australia	Martin et al. (2020)	1	I
HRS17087a[d]	1	2017	Commander	NSW	Australia	Martin et al. (2020)	1	I
HRS17088a[d]	2	2017	Commander	QLD	Australia	Martin et al. (2020)	1	I
HRS17090a[d]	2	2017	Commander	QLD	Australia	Martin et al. (2020)	1	I
62/17a[d]	2	2017	Fathom	SA	Australia	Martin et al. (2020)	1	I
NB2015-024[d]	1	2015	Navigator	WA	Australia	Ellwood et al. (2019b)	1	I
NB2015-027[d]	1	2015	Fleet	WA	Australia	Ellwood et al. (2019b); Mair et al. (2019)	1	I

NB2015-032[d]	1	2015	CMP	WA	Australia	Ellwood et al. (2019b)	4	III
NB2015-033	2	2018	Barley	WA	Australia	Ellwood et al. (2019b)	NA	NA
NB2016-045[d]	1	2016	Oxford	WA	Australia	Ellwood et al. (2019b)	1	I
NB2016-048[d]	1	2015	Unknown	WA	Australia	Ellwood et al. (2019b)	2	III
NB2016-051[d]	2	2015	Unknown	WA	Australia	Ellwood et al. (2019b)	4	III
NB2016-052[d]	1	2016	Unknown	WA	Australia	Ellwood et al. (2019b)	4	III
Ko103-3[d]	2	2013	Barley	WA	Australia	Ellwood et al. (2019b)	1	I
NB029[d]	1	1985	Beecher	WA	Australia	Martin et al., 2019)	1	I
NB033[d]	1	1989	Grimmett	QLD	Australia	Martin et al. (2020)	4	III
NB034[d]	2	1989	Corvette	QLD	Australia	Martin et al. (2020)	1	I
NB035[d]	2	1993	Gilbert	QLD	Australia	Martin et al. (2020)	1	I
NB050	1	1994	Barley	QLD	Australia	Martin et al. (2020)	NA	NA
NB053[d]	2	1994	Tallon	SA	Australia	Martin et al. (2020)	4	III

NB085	1	1995	Cape	QLD	Australia	Martin et al. (2020)	NA	NA
NB102[d]	1	1995	Gilbert	QLD	Australia	Martin et al. (2020)	4	III
NB2015-021[d]	2	2015	Barley	WA	Australia	Ellwood et al. (2019b)	1	I
NB223[d]	1	1996	Beecher	SA	Australia	Martin et al. (2020)	1	I
NB270[d]	2	1996	Grimmett	NSW	Australia	Martin et al. (2020)	4	III
NB330a[d]	2	2003	Binalong	NSW	Australia	Martin et al. (2020)	4	III
NB63-1	2	1994	Unknown	WA	Australia	Martin et al. (2020)	NA	NA
NB63-2	2	1994	Unknown	WA	Australia	Martin et al. (2020)	NA	NA
NB63-3 [d]	2	1994	Unknown	WA	Australia	Martin et al. (2020)	1	I
NB63-4	2	1994	Unknown	WA	Australia	Martin et al. (2020)	NA	NA
NB63-5	2	1994	Unknown	WA	Australia	Martin et al. (2020)	NA	NA
NB73	2	1994	Gilbert	QLD	Australia	Martin et al. (2020)	NA	NA
W1-1[d]	2	2009	Unknown	WA	Australia	Syme et al. (2018)	1	I
WAC10721[b]	1[c]	2002	Unknown	WA	Australia	McLean et al. (2014)	NA	NA
AB11[d]	1	2010	Unknown	Alberta	Canada	Akhavan et al. (2016a)	4	III

AB34[d]	2	2010	Unknown	Alberta	Canada	Akhavan et al. (2016a)	4	III
MB05[d]	2	2010	Unknown	Manitoba	Canada	Akhavan et al. (2016a)	2	III
MB11[d]	1	2011	Unknown	Manitoba	Canada	Akhavan et al. (2016a)	4	III
MB14[d]	1	2011	Unknown	Manitoba	Canada	Akhavan et al. (2016a)	4	III
WRS858[d]	1	1973	Barley	Manitoba	Canada	Serenius et al. (2007)	4	III
SK52[d]	1	2011	Unknown	Saskatchewan	Canada	Akhavan et al. (2016a)	4	III
Pt-Pastorale[d]	1	1976	Barley	Unknown	Denmark	Justesen et al. (2008)	3	II
CBS282.31[d]	2	1931	Unknown	Unknown	Japan	Bakonyi and Justesen (2007)	2	III
CBS281.31[b]	2[c]	1931	Barley	Unknown	Japan	Bakonyi and Justesen (2007)	NA	NA
H-114-1[d]	2	2006	Pasadena	Szombathely	Hungary	This study	4	III
H-137[d]	1	2006	Adagio	Kompolt	Hungary	This study	2	III
H-186[d]	1	2007	Petra	Kölcse	Hungary	This study	3	II
H-190[d]	2	2007	Barley	Kölcse	Hungary	This study	3	II
H-191[d]	2	2007	Barley	Kölcse	Hungary	This study	3	II

H-196[d]	2	2007	Spring barley	Szombathely	Hungary	This study	4	III
H-288[d]	2	2008	20899YH2-PETRA	Martonvásár	Hungary	This study	3	II
H-289[d]	2	2008	F74-82-MANAS-SZD0205	Martonvásár	Hungary	This study	3	II
H-306-1[d]	2	2008	Henley	Szombathely	Hungary	This study	2	III
H-308-2[d]	2	2008	Barley	Székkutas	Hungary	This study	3	II
H-309-2[d]	1	2008	Barley	Márok	Hungary	This study	3	II
H-322[d]	2	2008	Barley	Martonvásár	Hungary	This study	3	II
H-323-2 (CBS123931)[d]	1	2008	Barley	Martonvásár	Hungary	This study	3	II
H-374[d]	2	2008	Wheat	Bőny	Hungary	This study	2	III
H-376[d]	2	2008	Wheat	Márok	Hungary	This study	2	III
H-386-1[d]	2	2009	GK Habzó	Szombathely	Hungary	This study	2	III
H-529[d]	2	2017	Petra	Martonvásár	Hungary	This study	3	II
H-540[d]	1	2017	Mv Initium	Martonvásár	Hungary	This study	3	II
H-546[d]	1	2017	Laverda	Martonvásár	Hungary	This study	3	II
H-547[d]	2	2017	Laverda	Martonvásár	Hungary	This study	3	II
H-618[d]	1	2017	KH Zsombor	Martonvásár	Hungary	This study	3	II
H-620[d]	2	2017	KH Hunor	Martonvásár	Hungary	This study	2	III
H-623[d]	2	2017	KH Anatólia	Martonvásár	Hungary	This study	3	II
H-627[d]	1	2017	KG Apavár	Martonvásár	Hungary	This study	3	II
H-630[d]	1	2017	Mv Initium	Martonvásár	Hungary	This study	3	II
H-632[d]	1	2017	Mv Initium	Martonvásár	Hungary	This study	3	II
H-638[d]	2	2017	Patina	Martonvásár	Hungary	This study	3	II
H-641[d]	1	2017	KG Puszta	Martonvásár	Hungary	This study	2	III
H-642[d]	2	2017	KG Puszta	Martonvásár	Hungary	This study	2	III
H-645[d]	2	2017	KH Tas	Martonvásár	Hungary	This study	2	III
H-647[d]	1	2017	KH Tarna	Martonvásár	Hungary	This study	2	III
H-651[d]	1	2017	Su Ellen	Martonvásár	Hungary	This study	2	III
H-656	1	2017	Monique	Martonvásár	Hungary	This study	NA	NA
H-660[d]	1	2017	Faktor	Martonvásár	Hungary	This study	3	II

H-665[d]	2	2017	GKH 3015	Martonvásár	Hungary	This study	3	II
H-668[d]	2	2017	KH Malko	Martonvásár	Hungary	This study	2	III
H-672[d]	1	2017	KH Kárpátia	Martonvásár	Hungary	This study	2	III
H-675[d]	1	2017	KH Korsó	Martonvásár	Hungary	This study	4	III
H-679[d]	2	2017	Antonella	Martonvásár	Hungary	This study	2	III
H-690[d]	1	2017	Mv Initium	Kompolt	Hungary	This study	3	II
H-732[d]	1	2017	KH Tas	Kompolt	Hungary	This study	3	II
H-733	1	2017	KH Tas	Kompolt	Hungary	This study	NA	NA
H-746[d]	1	2017	KH Hunor	Kompolt	Hungary	This study	2	III
H-747[d]	2	2017	KH Hunor	Kompolt	Hungary	This study	2	III
H-748[d]	1	2017	KH Hunor	Kompolt	Hungary	This study	2	III
H-771[d]	1	2017	KH Korsó	Karcag	Hungary	This study	3	II
H-774[d]	1	2017	KG Puszta	Karcag	Hungary	This study	3	II
H-778[d]	2	2017	Patina	Karcag	Hungary	This study	3	II
H-784	2	2017	KG Konta	Karcag	Hungary	This study	NA	NA
H-785[d]	2	2017	KH Tas	Karcag	Hungary	This study	3	II
H-786[d]	1	2017	KH Tas	Karcag	Hungary	This study	3	II
H-788[d]	1	2017	Mv Initium	Karcag	Hungary	This study	3	II
H-791[d]	2	2017	KH Anatólia	Karcag	Hungary	This study	3	II
H-798[d]	1	2017	KH Zsombor	Karcag	Hungary	This study	3	II
H-802	2	2017	Antonella	Karcag	Hungary	This study	NA	NA
H-804	1	2017	KH Hunor	Karcag	Hungary	This study	NA	NA
H-815[d]	1	2017	KH Tarna	Karcag	Hungary	This study	2	III
H-826[d]	2	2017	GKH 3015	Karcag	Hungary	This study	3	II
H-835[d]	2	2017	KH Zsombor	Szombathely	Hungary	This study	4	III
H-848[d]	2	2017	GKH 3815	Szombathely	Hungary	This study	3	II
H-850[d]	2	2017	GKH 3815	Szombathely	Hungary	This study	3	II
H-855[d]	1	2017	LGBB14W232-11	Szombathely	Hungary	This study	3	II
H-867[d]	1	2018	KG Puszta	Karcag	Hungary	This study	3	II
H-874[d]	1	2018	KH Tas	Karcag	Hungary	This study	3	II
H-883[d]	1	2018	KH Korsó	Karcag	Hungary	This study	3	II
H-890[d]	2	2018	KH Zsombor	Karcag	Hungary	This study	3	II

H-893[d]	1	2018	Patina	Karcag	Hungary	This study	3	II
H-897[d]	2	2018	Mv Initium	Karcag	Hungary	This study	3	II
H-906[d]	1	2018	GKH 3015	Karcag	Hungary	This study	3	II
H-912[d]	1	2018	Siberia	Karcag	Hungary	This study	3	II
H-919	1[c]	2018	KG Konta	Kompolt	Hungary	This study	NA	NA
H-920[d]	2	2018	KG Apavár	Kompolt	Hungary	This study	3	II
H-922[d]	2	2018	KG Apavár	Kompolt	Hungary	This study	3	II
H-932[d]	1	2018	Patina	Kompolt	Hungary	This study	3	II
H-936[d]	2	2018	KH Tarna	Kompolt	Hungary	This study	3	II
H-944[d]	2	2018	Siberia	Kompolt	Hungary	This study	3	II
H-949[d]	2	2018	KWS Meridian	Kompolt	Hungary	This study	3	II
H-955	2	2018	Faktor	Kompolt	Hungary	This study	NA	NA
H-958[d]	2	2018	GKH 3015	Kompolt	Hungary	This study	3	II
H-961[d]	2	2018	KWS Meridian	Karcag	Hungary	This study	4	III
H-970[d]	2	2018	Boreale	Kompolt	Hungary	This study	3	II
H-974[d]	1	2018	KH Kárpátia	Martonvásár	Hungary	This study	3	II
H-977[d]	2	2018	KG Konta	Martonvásár	Hungary	This study	3	II
H-981[d]	2	2018	KG Puszta	Martonvásár	Hungary	This study	3	II
H-995[d]	1	2018	KH Zsombor	Martonvásár	Hungary	This study	3	II
CG16001	1	2016	Disa	Napier	RSA	This study	NA	NA
CG16002	1	2016	Aghulas	Napier	RSA	This study	NA	NA
CG16004[d]	2	2016	Aghulas	Napier	RSA	This study	4	III
CG16005[d]	2	2016	Aghulas	Napier	RSA	This study	4	III
CG16006[d]	2	2016	Aghulas	Napier	RSA	This study	4	III
CG16007[d]	1	2016	Aghulas	Napier	RSA	This study	4	III
CG16008	1	2016	Aghulas	Napier	RSA	This study	NA	NA
CG16009[d]	1	2016	Erica	Caledon	RSA	This study	4	III
CG16010[d]	2	2016	Erica	Caledon	RSA	This study	4	III
CG16011[d]	1	2016	Erica	Caledon	RSA	This study	2	III
CG16013[d]	1	2016	Erica	Caledon	RSA	This study	4	III
CG16014[d]	1	2016	Erica	Caledon	RSA	This study	4	III
CG16015[d]	1	2016	Rye grass	Caledon	RSA	This study	4	III
CG16016[d]	2	2016	Erica	Caledon	RSA	This study	2	III

CG16017[d]	1	2016	Erica	Caledon	RSA	This study	4	III
CG16018[d]	1	2016	Erica	Caledon	RSA	This study	4	III
CG16019[d]	1	2016	Erica	Caledon	RSA	This study	2	III
CG16021[d]	1	2016	Erica	Caledon	RSA	This study	4	III
CG16023[d]	1	2016	Erica	Caledon	RSA	This study	4	III
CG16024[d]	1	2016	Erica	Caledon	RSA	This study	4	III
CG16028[d]	2	2016	1070	Caledon	RSA	This study	4	III
CG16029[d]	1	2016	1069	Caledon	RSA	This study	4	III
CG16030[d]	1	2016	1065	Caledon	RSA	This study	4	III
CG16031	1	2016	1055	Caledon	RSA	This study	NA	NA
CG16032[d]	1	2016	1005	Caledon	RSA	This study	4	III
CG16034[d]	1	2016	999	Caledon	RSA	This study	2	III
CG16035[d]	2	2016	1000	Caledon	RSA	This study	4	III
CG16036[d]	1	2016	995	Caledon	RSA	This study	4	III
CG16037[d]	2	2016	992	Caledon	RSA	This study	2	III
CG16038[d]	1	2016	744	Caledon	RSA	This study	4	III
CG16040[d]	2	2016	736	Caledon	RSA	This study	4	III
CG16041	1	2016	722	Caledon	RSA	This study	NA	NA
CG16043[d]	1	2016	4	Caledon	RSA	This study	2	III
CG16044[d]	2	2016	394	Caledon	RSA	This study	4	III
CG16047[d]	1	2016	407	Caledon	RSA	This study	2	III
CG16048[d]	1	2016	Erica	Rietpoel	RSA	This study	2	III
CG16049[d]	2	2016	LE 18	Rietpoel	RSA	This study	2	III
CG16050	2	2016	LE 12	Riviersonderend	RSA	This study	NA	NA
CG16051[d]	2	2016	Erica	Greyton	RSA	This study	2	III
CG16052[d]	2	2016	Hessequa	Greyton	RSA	This study	2	III
CG16054	1	2016	Elim	Greyton	RSA	This study	NA	NA
CG16055[d]	1	2016	Elim	Greyton	RSA	This study	2	III
CG16056[d]	1	2016	S16	Greyton	RSA	This study	2	III
CG16057[d]	1	2016	LE 16	Greyton	RSA	This study	4	III
CG16061[d]	1	2016	Erica	Napier	RSA	This study	4	III
CG16062[d]	1	2016	Elim	Napier	RSA	This study	4	III
CG16063[d]	1	2016	S16	Napier	RSA	This study	4	III

CG16064[d]	2	2016	LE 3	Napier	RSA	This study	4	III
CG16065[d]	1	2016	LE 16	Napier	RSA	This study	2	III
CG16067[d]	2	2016	Erica	Protem	RSA	This study	2	III
CG16068[d]	1	2016	Nemesia	Protem	RSA	This study	4	III
CG16070	1	2016	LE 12	Protem	RSA	This study	NA	NA
CG16072[d]	1	2016	LE 17	Protem	RSA	This study	4	III
CG16073[d]	1	2016	Erica	Klipdale	RSA	This study	2	III
CG16075[d]	1	2016	Nemesia	Klipdale	RSA	This study	4	III
CG16076[d]	2	2016	LE 8	Klipdale	RSA	This study	4	III
CG16077[d]	2	2016	LE 10	Klipdale	RSA	This study	4	III
CG16078[d]	1	2016	LE 12	Klipdale	RSA	This study	4	III
CG16079[d]	2	2016	LE 18	Klipdale	RSA	This study	2	III
CG16081[d]	1	2016	LE 22	Klipdale	RSA	This study	4	III
CG16082[d]	1	2016	LE 25	Klipdale	RSA	This study	2	III
CG16083[d]	2	2016	Erica	Bredasorp	RSA	This study	2	III
CG16084[d]	1	2016	Nemesia	Bredasdorp	RSA	This study	4	III
CG16086	1	2016	Elim	Bredasdorp	RSA	This study	NA	NA
CG16088	1	2016	LE 9	Bredasdorp	RSA	This study	NA	NA
CG16089[d]	2	2016	LE 13	Bredasdorp	RSA	This study	4	III
CG16090[d]	1	2016	LE 13	Bredasdorp	RSA	This study	4	III
CG16091	1	2016	LE 15	Bredasdorp	RSA	This study	NA	NA
CG16092	1	2016	LE 16	Bredasdorp	RSA	This study	NA	NA
CG16093[d]	1	2016	LE 25	Bredasdorp	RSA	This study	4	III
CG16094	1	2016	LE 23	Bredasdorp	RSA	This study	NA	NA
CG16095[d]	1	2016	LE 3	Bredasdorp	RSA	This study	4	III
UPSC1838[d]	1	1986	Oat	Unknown	Sweden	Bakonyi and Justesen (2007)	2	III

[a] Mating type of the isolate

[b] Included only in distance based cluster analysis and hybrid specific PCR amplification

[c] *Ptm* mating type

[d] MLGs included in all analyses except hybrid specific PCR amplification

[e] Original research article describing the isolates

DAPC The cluster number resulted from DAPC analysis

STR The cluster number assigned to clusters resulted from the K=3 STRUCTURE model

NA Not included in Neighbor-net network, DAPC and STRUCTURE analysis

Supplementary Table S2. Chi square and PHI test values for subdivisions in Australia, regions in Hungary and RSA

Country/subdivisions based on DAPC	Number of isolates	Number of *MAT1-1*	Number of *MAT1-2*	Chi square value	*P* value	PHI test mean	*P* value
Australia	100	47	53				
Cluster_1	31	15	16	0.133	0.715	0.576	0.358
Cluster_2	53	24	29	0.472	0.492	0.641	0.014 [a]
Cluster_3	16	15	11	2.250	0.134	0.518	0.806
Hungary	78	37	41				
Cluster_1	23	9	14	1.087	0.297	0.614	4.8E-4 [c]
Cluster_2	23	13	10	0.391	0.532	0.695	0.221
Cluster_3	32	13	19	1.125	0.289	0.696	0.007 [b]
RSA	59	39	20				
Cluster_1	19	10	9	0.053	0.819	0.610	0.511
Cluster_2	12	10	2	5.333	0.021[a]	0.553	0.246
Cluster_3	28	18	10	2.286	0.131	0.649	0.310

[a] Significant at $P \leq 0.05$

[b]Significant at $P \leq 0.01$

Supplementary Table S3. Indices of genetic diversity for *Pyrenophora teres* f. *teres* populations from Australia, Hungary and Republic of South Africa (RSA) based clusters detected in DAPC

Country/subdivisions based on DAPC	*n*[a]	eMLG[b]	H[c]	1-λ[d]	H_{exp}[e]
Australia					
Cluster_1	31	16	0.745	0.968	0.200
Cluster_2	53	16	0.863	0.981	0.213
Cluster_3	16	16	0.702	0.938	0.187
Australia total	100	16	0.890	0.990	0.255
Hungary					
Cluster_1	23	23	0.730	0.958	0.188
Cluster_2	23	23	0.710	0.955	0.207
Cluster_3	32	23	0.797	0.969	0.204
Hungary total	78	23	0.935	0.987	0.279
RSA					
Cluster_1	19	12	0.694	0.941	0.251
Cluster_2	12	12	0.629	0.923	0.173
Cluster_3	28	12	0.627	0.966	0.255
RSA total	59	12	0.859	0.983	0.224

[a] Number of isolates

[b] The number of expected MLG based on rarefaction at the smallest sample size of ≥10

[c] Normalised Shannon-Wiener index of MLG genotypic diversity, the genotypic diversity of the population by richness and relative abundance in a defined location

[d] Simpson's complement index of multilocus genotypic diversity, the probability of two random isolates drawn from a subpopulation to be of a different genotype

[e] Nei's unbiased gene diversity, the probability that two randomly chosen alleles are different

Supplementary Table S4. Details of the most contributing DArTseq™ marker annotations for the DAPC and PCA

Marker	Marker Sequence	E value	Gene/locus	Protein	Accession of reference genome	*Ptt* strain
41804355	TGCAGATCCTGTCTGACTTTGCAATTCGAGTTGATCG CAAGCGCTAGTTGTAGTTCTTGAGTGCTGAGA	3.30E-08	PTT_13375	Hypothetical protein (ANK_REP_REGION domain-containing protein)	AEEY01000000	0-1
28945199	TGCAGATCGCCAGCTATTGCGAGCGGCAAACGCCTT GCCTGCATGCAACTAACCGGCAGCTCACGTGAC	NA	NA	NA		
28945202	TGCAGCCCTGCGACGTCGCCGTGTTTGCACCTCTGAA AGCAGCTTACCGGGAGCAAGTCGAATGACTTG	6.50E-07	PTT_17416 PTT_06721	Hypothetical protein (DDE-1 domain-containing protein)	AEEY01000000	0-1
41804358	TGCAGGCTGATGTATAAGTCTGTGTACTCAGTCTCAG AGCAGTCGTACTGCCATCCAGAAGTGGGGAAC	1.60E-04	PTT_17416 PTT_06721	Hypothetical protein (DDE-1 domain-containing protein)	AEEY01000000	0-1
36347108	TGCAGGTCAAGAAGATACCAAGGCCAAAGTGTGACG CTACAATAGACCACCTTCTGCCGATCACCTTGT	NA	NA	NA	NA	NA
36347128	TGCAGTAGCAGAGCAGGAGAGACCCTAAACCGCGAC AGCTTCTGTGTCGAGACGCGGTAAGAGCCTTCA	NA	NA	NA	NA	NA
41804360	TGCAGTGAGCTTTTGTCCAGCATGAACGGAGCCTTCG ATCAAAGCCACCAGACCAATTATGCTATGCAT	NA	NA	NA	NA	NA
28946425	TGCAGCAAGACACAATGTCCCTGAACTTACAGATCG GAAGAGCGGTTCAGCAGGAATGCCGAGACCGAT	NA	NA	NA	NA	NA
28945458	TGCAGTCGCAACTCACCTTTGGTAAGGACGCGATGC CTATCTAGGGCTAGCACTGTTTACGGTCACCCA	NA	NA	NA	NA	NA
28945448	TGCAGATCTATTGCTCCGCGCTCGTGTTCGCACCAGA GAGGAGCCTGATTCGACAAACCTTTGTAGACC	NA	NA	NA	NA	NA
28945774	TGCAGCACCACCTTGACGTACTGCTGCATTCTGTGCA GTCGCTGCATTTCGACTTCTCCAGAAAGGTTG	NA	NA	NA	NA	NA
28945775	TGCAGCACTAAGTTACGTTTCTTGCCGTCCACGAGTG GTTCTACACTAGCGGACTTGCATCAAGGATAG	NA	NA	NA	NA	NA

28946079	TGCAGCTATATGGGTGTGTATAATAATAAAGTGTGG TAGCGATAGCCGTACCTGAGTAGGTCTTAGCAA	7.00E-04	PTT_07236	Uncharacterized protein	AEEY01000000	0-1
28945457	TGCAGGTTTTGTCTCCTTGTCCTGTCAAGAGTACGAG CATCCTGCTTCATGATCAGATTGGGTAGCGAC	0.017	PTT_06709	Uncharacterized protein	AEEY01000000	0-1
28945782	TGCAGTATTAGGACTGCTTTCTGAAAATTGTGAACCG AGTAGTCCGGGGCAACCAGCGTCGCATGATTA	NA	NA	NA	NA	NA
28945785	TGCAGTGCGTTGCCGTAGTATTCACCCTGCGCGTTGA TGTCGGCGTGCATGTCAATCAACACCTGAGCA	2.10E-09	PTT_13375	Hypothetical protein (ANK_REP_REGION domain-containing protein)	AEEY01000000	0-1
36347130	TGCAGTTCGATGGACTGGCGACATGAGCTCAGTAAG CGGAATATCTGTGAGTGCATTTACACCCATAAC	NA	NA	NA	NA	NA
28948860	TGCAGCAAGGACTCTCCATAGGTATTATTACAGATC GGAAGAGCGGTTCAGCAGGAATGCCGAGACCGA	NA	NA	NA	NA	NA
36347037	TGCAGCTATCACGACTGCTTCTAAGCTATATACTAGT GGTCGGCAAGGCCGAATACCGTAAGACTATGT	NA	NA	NA	NA	NA
28945446	TGCAGAAGCAGAGCAGGAGACCCCAAACTGTGACA GTACAAGATGTAGTGAAAAAATAAGTTTGGTATC	NA	NA	NA	NA	NA
28946771	TGCAGGACTTACTAGCGCAGTCAATCGACTCCTTGA GGCAGGATGCGACATCAACGAGAAAGACAGCAA	2.20E-08	PTT_13375	Hypothetical protein (ANK_REP_REGION domain-containing protein)	AEEY01000000	0-1
28949273	TGCAGGCAGCTTCAGTTAGAGGCCACGAGCAGGTGG TCAAGATGCTGCTCGACGCGGGCGCCGAAGTTA	1.6E-5	PTT_17957 PTT_08880	Hypothetical protein (ANK_REP_REGION domain-containing protein)	AEEY01000000	0-1
36347080	TGCAGAACCACTATAGTTCAGGCAATTACAGATCGG AAGAGCGGTTCAGCAGGAATGCCGAGACCGATC	NA	NA	NA	NA	NA
28946327	TGCAGTAACACCATCCATAGGTACCTCCCACTTACCC GTAACCTGCGTTTCCAGCTCCCTAGACCGAAT	NA	NA	NA	NA	NA
28946069	TGCAGAAAAGCTCTTCCTGTAATCCACTGCGATTTCC ATGCCATCCCATATATCTCGTCGCGCGCGGAG	4.40E-10	PTT_07238	Hypothetical protein (SET domain-containing protein)	AEEY01000000	0-1
28946772	TGCAGGAGCGGGCCATAAAGGCTGGTGCTGTGTCAG GAGTGAGAAAAGACACAATGGTCAACATTGCAG	7.90E-08	PTT_16779	Uncharacterized protein	AEEY01000000	0-1

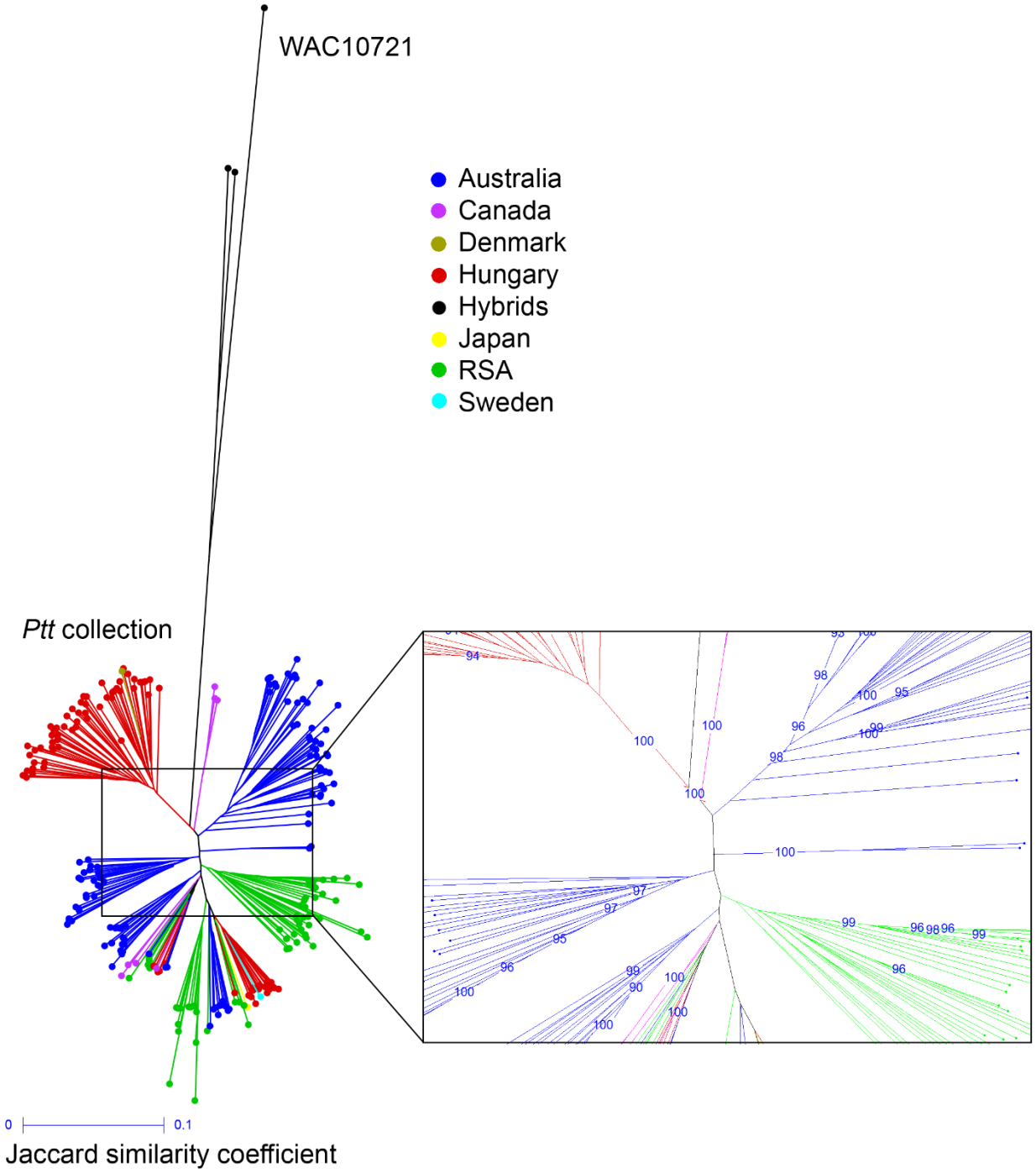
28945780	TGCAGGGGCTAAGTTGAAACTCAAAAGATAGCAGCA CTCCTACGAACGCATCAAAAGTAACTTTCTATA	NA	NA	NA	NA	NA
28947083	TGCAGGTTGCCGCGTGCCAAGGAGCTGCTAGTTGCG CACCGGCAGTCAGACATATTCTACGACCTTGCT	0.75	JQ582646	Pyrenophora teres f. teres isolate ND89-9 nonribosomal peptide synthetase 2 (NPS2) gene	NA	13A
28945784	TGCAGTGCCAGCCAGAAAGTCGTTTTCGTTATTCGCT AGAATACTAGAGCTATACTTGCAACGTTTCAT	NA	NA	NA	NA	NA
28948294	TGCAGCTGCGTCGGACTTAGACGCGTCCGACTCATCC ACCATAGACGATCCGGAGATAAATATAGCGCA	NA	NA	NA	NA	NA
28948801	TGCAGGTAGTCAAGACACTGTTCGACGCGGGCGCCG AAGTCAACGCGCAGGGTGGATACTACGGCAACG	6.90E-06	PTT_06711	Hypothetical protein (ANK_REP_REGION domain-containing protein)	AEEY01000000	0-1
28946767	TGCAGATGAAAAGTGTTTGTGCGCGACATGTAGCAA GAGCGTAGCATCGACGAATACTGTAGAACAAGC	4.80E-09	PTT_07238	Hypothetical protein (SET domain-containing protein)	AEEY01000000	0-1
28945777	TGCAGCTCTTATTCTCCTAGCACGTTAGTTTCCGACG CTAAAAAAGCGCATCGTTGACCGTGCTGTCGC	NA	NA	NA	NA	NA
28946773	TGCAGGCAGCTTCAGCTGAAGGCCACGAGCAGGTGG TCAAAATGCTGCTCGACGCGGGCGCCGACGTCA	3.4E-5	PTT_17957 PTT_08880	Hypothetical protein (ANK_REP_REGION domain-containing protein)	AEEY01000000	0-1
28946082	TGCAGGGTCTCTCACTATTATAGACCTGACTGATCCT GTCATCGACGCTGATTCTACCTGCGTGCTCTT	7.60E-05	PTT_09544	Uncharacterized protein	AEEY01000000	0-1
28947077	TGCAGAAAAAAAAGGCGGCACTGCGTCAGGAGACT GCTCCACGCCACCGACTAGGGTTCCAGATCTAAT	NA	NA	NA	NA	NA
28947744	TGCAGACTACAAGACTCGAATTCCGGCTCTATTTTTT GAAACGATTTGGGATACTTCGGTCTTTCGTAA	0.0018	PTT_19103	Hypothetical protein (DUF1996 domain-containing protein)	AEEY01000000	0-1
28946403	TGCAGGTACCCACATTGTAAGGGGTGAGGACTAGAG TAAGACTAGGTACGCATCTACATAATCCTTATT	NA	NA	NA	NA	NA
36349731	TGCAGAACCTGCCTGCACCTCTTCAATGAACATGATG AGAGAAAGAACTGGCACATTGCTTTCGATATC	NA	NA	NA	NA	NA
28947411	TGCAGCTAGGACGCTGATTATACCGAGTAGACTAGG CTTGAGGTAAGAGTGAAAAAGCCCGGTAGAGCT	NA	NA	NA	NA	NA

36349088	TGCAGGGTCTGTTATGCGACCTATGAGCGATGCAGA GAAGAGGGAGCTAGGGTTCAGATGAGATTTTAT	NA	NA	NA	NA	NA
28947086	TGCAGCGCCAGATTAGATGAGGTCTAATGGGATCAA TGCCCATAACTAGCAGGTGATTGCTGAGTATAT	NA	NA	NA	NA	NA
28946426	TGCAGTGCGTTGCCGTAATCTCCACCCTGCGCGTTGA CGCCCGCGTCGAACAGTGTCTTGACTACCAGC	0.0011	PTT_06711	Hypothetical protein (ANK_REP_REGION domain-containing protein)	AEEY01000000	0-1
36350466	TGCAGCAGAAAGAGATGCGTTGTGTCTCTATCAACA ATTTCTTGCTGACTCTGCTTACAGATCGGAAGA	NA	NA	NA	NA	NA
36350467	TGCAGGCTGGGGAGCATGGAAAGACGCTCTACGTTA CTAATGATAGACTTACAGATCGGAAGAGCGGTT	1.80E-05	PTT_13375	Hypothetical protein (ANK_REP_REGION domain-containing protein)	AEEY01000000	0-1
28947745	TGCAGTCGATGCCGTCTAAGGCTTTATTACAGATCGG AAGAGCGGTTCAGCAGGAATGCCGAGACCGAT	NA	NA	NA	NA	NA
28946404	TGCAGAGCAAACGATGATATACAATAGTCAGTCCTG TAGCAAGCACCATTATGCATGCCTGTCCAATAC	NA	NA	NA	NA	NA
28946073	TGCAGTAAGCGGAGACCGATCGGAGAGTAACCCCGC CTCTATTGCAAAGGCGAATGTGTCCGTGCTAAT	NA	NA	NA	NA	NA
28948009	TGCAGATCCCAGCCGAGCGCCCTGCTTACGCCACCTC AACGTCGGCCAAGACAAACAACGGGCGCATCA	NA	NA	NA	NA	NA
28946407	TGCAGGCTACCCCCACACGCCAAGAACCAATCGGTT CCAGTCTGAAAACCGTACAGCCAGTCTTGAATG	NA	NA	NA	NA	NA
28948861	TGCAGTCGCTACTCACCTTTGGTAATGGAACGATGCC TATCCAGGACTAGTGCTGTTTACGGTCACTCA	8.40E-33	NA	NA	NA	NA
36349342	TGCAGCCCTAAATTAGAGGCTAAAATGTATGATTCCT ATGAGTCTTACAGATCGGAAGAGCGGTTCAGC	0.023	PTT_17763	Hypothetical protein (Peptidase A1 domain-containing protein)	AEEY01000000	0-1
28948597	TGCAGGCTTGAAACCCGACTTATCGAAGATTACAGA TCGGAAGAGCGGTTCAGCAGGAATGCCGAGACC	0.023	PTT_17763	Hypothetical protein (Peptidase A1 domain-containing protein)	AEEY01000000	0-1
28948331	TGCAGACACGAACTATAGCCTATCTTTATTACAGATC GGAAGAGCGGTTCAGCAGGAATGCCGAGACCG	9.70E-08	NA	NA	NA	NA

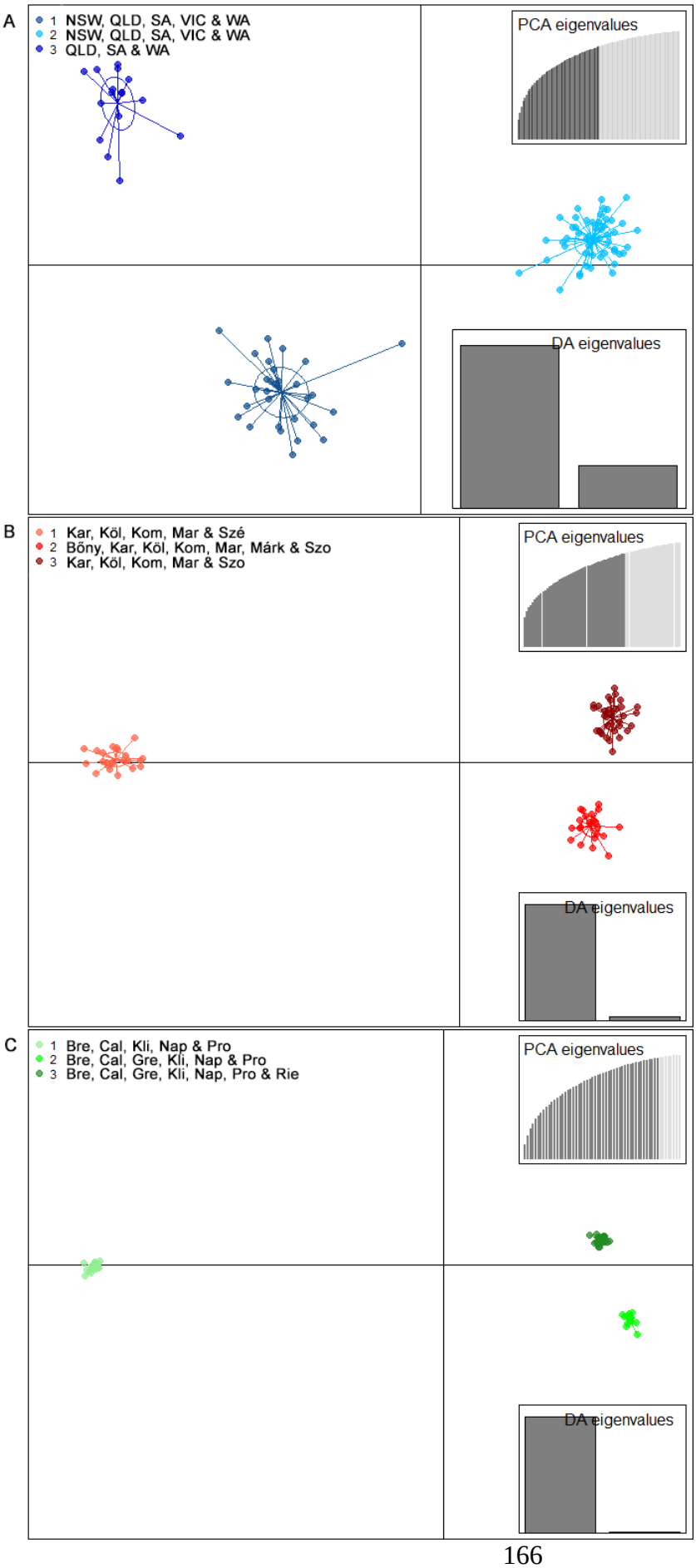
28947406	TGCAGTGTGTGGATGAGATCGGATCTCCTCACGTTCT TGACTCACTTACAGATCGGAAGAGCGGTTCAG	0.023	PTT_17763	Hypothetical protein (Peptidase A1 domain-containing protein)	AEEY01000000	0-1
28946408	TGCAGCATTTCAACCTGATTGCGAGCGAAAGTCTCG ATGTCGGCAGTGACGTTCTTGGTCTGGATCTGG	8.40E-33	PTT_13375	Hypothetical protein (ANK_REP_REGION domain-containing protein)	AEEY01000000	0-1
28949340	TGCAGTCTGCGTTGTGCACTCTCCTGTCCTTCGCCAT ACGCGGTGGGCATAGAGACACCAAGAATCCCA	7.20E-21	PTT_08524	Hypothetical protein (AAA domain-containing protein)	AEEY01000000	0-1
36350313	TGCAGATGTAGGAAGCACAAGCCTAAAGCTATATTA CAGATCGGAAGAGCGGTTCAGCAGGAATGCCGA	0.09	PTT_17763	Hypothetical protein (Peptidase A1 domain-containing protein)	AEEY01000000	0-1
28948798	TGCAGATCTAAAGCCCGTTCTGGCATTATCGTGTCGA TTTAGAGGTCCAGAAATCCGGAACTTACAGAT	0.023	PTT_01845	Hypothetical protein (MFS domain-containing protein)	AEEY01000000	0-1
36351780	TGCAGCTTGAGCCATTTGAAAGTGAGTGCCGTGCAG GAAGGCAACTCGTGAAGGACGTAGAGACGAAGA	8.40E-33	PTT_16779	Uncharacterized protein	AEEY01000000	0-1
28948601	TGCAGTATGCGTGTCGTTATTGGGTCGATCATCTTAC AGATCGGAAGAGCGGTTCAGCAGGAATGCCGA	3.80E-07	NA	NA	NA	NA
28949991	TGCAGTGCTTGGGTTTCTCTAGATAAACGAGGAATA GCTAGGGTTTACAGATCGGAAGAGCGGTTCAGC	2.80E-17	NA	NA	NA	NA
36347554	TGCAGAAAGAGAGACGGAAGCTACAAATGAGCTTAC AGATCGGAAGAGCGGTTCAGCAGGAATGCCGAG	0.0015	PTT_17763	Hypothetical protein (Peptidase A1 domain-containing protein)	AEEY01000000	0-1
28948607	TGCAGACCTATCAATTGTAGACTCCGAGAAAGAGAG AGAGAGAGAGAGAAAGAGTGGGAGACTTACAAC	1.7	JQ837863	Pyrenophora teres f. teres isolate 13A glyceraldeyde-3-phosphate dehydrogenase-like protein (GPD1) gene	NA	13A
28947400	TGCAGAAGTATCAATTGTAGACTCAGGGGAAGAGAG AGAGAGAGAAAGAGTGGGAGACTTACAACAACA	0.12	JX900133	Endo-1,4-beta-xylanase A mRNA	AEEY01000000	0-1
36351843	TGCAGCCACCGGTTGAAGTTAGCCCGCCTAGTTACG CGCGACGCAACCAGGCGCTCACCAATACAACTA	0.061	KX578221	Cytochrome P450 lanosterol 14 alpha-demethylase (CYP51A) gene	OCTH00000000	W1-1

E Expected value indicating the possibility of finding an alignment with the reference genome by random chance

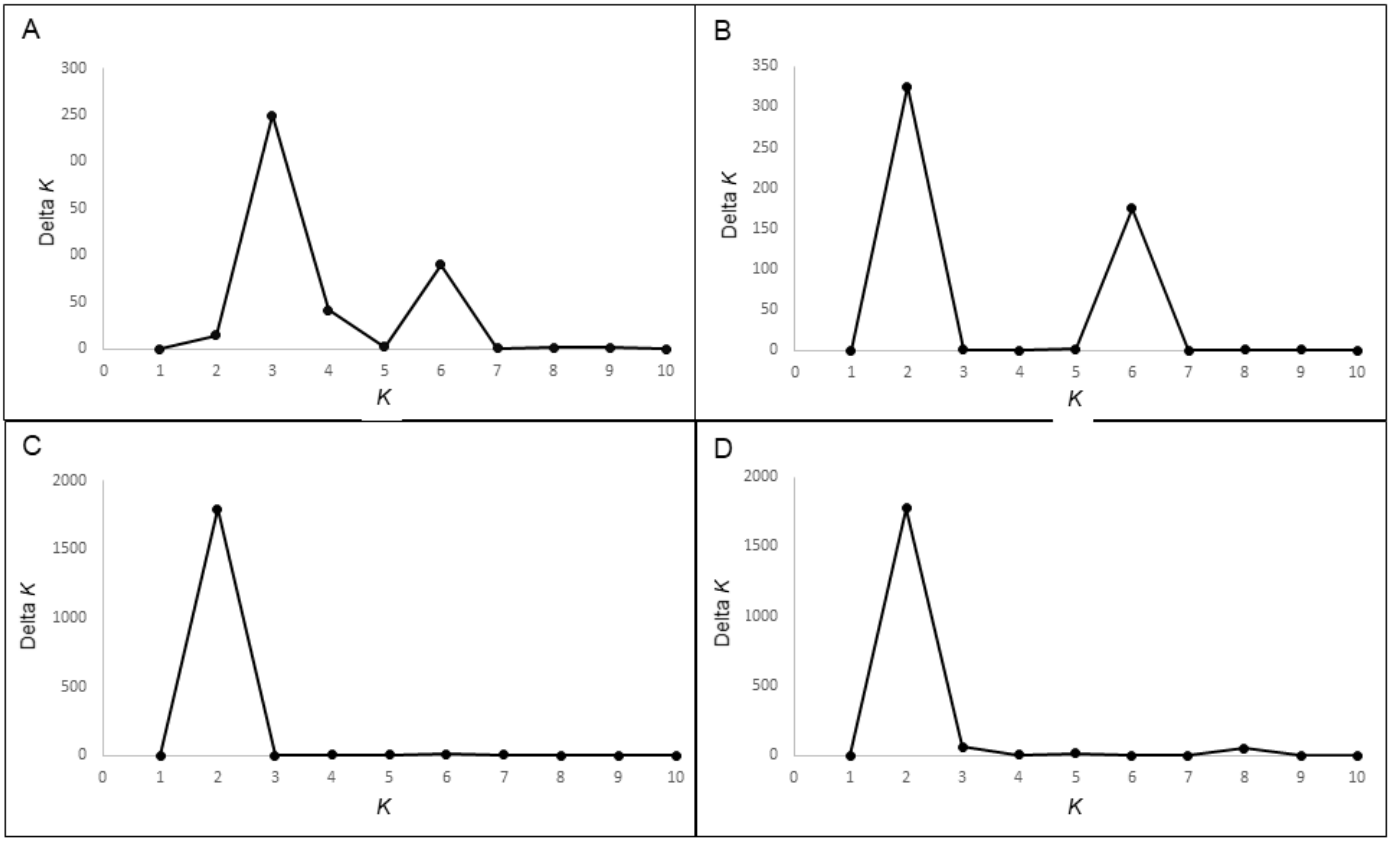
NA No significant alignment was observed with the reference genome



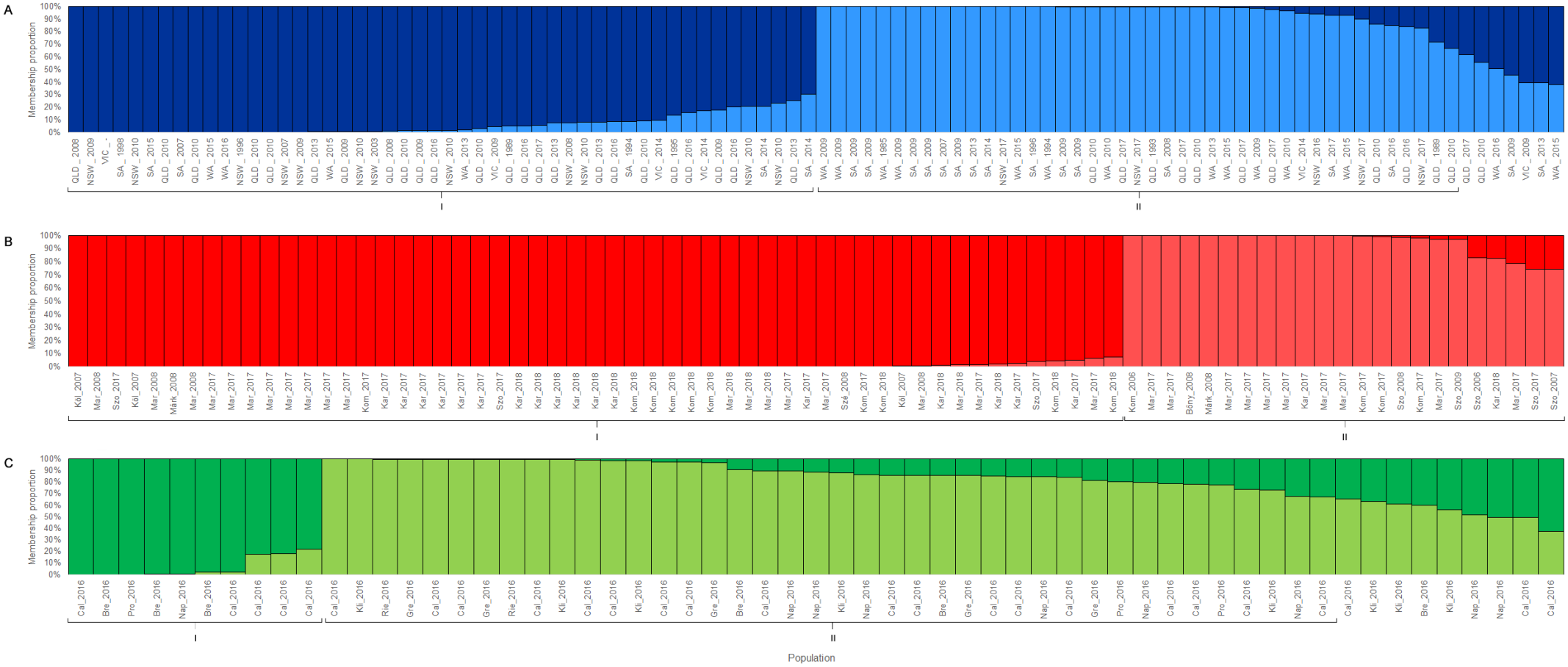
Supplementary Fig. S1. Neighbor-joining clustering with bootstrapping (≥90%) based on DArTseq™ data following Jaccard similarity coefficient for *Pyrenophora teres* f. *teres* isolates from Australia (*n* = 101), Canada (*n* = 7), Denmark (*n* = 1), Hungary (*n* = 79), Japan (*n* = 2), Republic of South Africa (RSA) (*n* = 59) and Sweden (*n* = 1).


A
1 NSW, QLD, SA, VIC & WA
2 NSW, QLD, SA, VIC & WA
3 QLD, SA & WA
PCA eigenvalues
DA eigenvalues
B
1 Kar, Köl, Kom, Mar & Szé
2 Bőny, Kar, Köl, Kom, Mar, Márk & Szo
3 Kar, Köl, Kom, Mar & Szo
PCA eigenvalues
DA eigenvalues
C
1 Bre, Cal, Kli, Nap & Pro
2 Bre, Cal, Gre, Kli, Nap & Pro
3 Bre, Cal, Gre, Kli, Nap, Pro & Rie
PCA eigenvalues
DA eigenvalues

Supplementary Fig. S2. Discriminant analysis of principal components of *Pyrenophora teres* f. *teres* populations collected from (A) Australia, (B) Hungary and (C) Republic of South Africa (RSA). The distribution of the eigenvalues of principal component analysis (PCA) and discriminant analysis (DA) indicate that the first two principal components adequately explain > 50% of the genetic structure of the clusters. States of Australia; NSW- New South Wales, QLD-Queensland, SA- South Australia, VIC-Victoria and WA- Western Australia. Regions of Hungary; Kar- Karcag, Köl-, Kölcse, Kom- Kompolt, Mar- Martonvásár, Márk- Márok, Szé- Székkutas and Szo- Szombathely. RSA regions; Bre- Bredasdorp, Cal- Caledon, Gre- Greyton, Kli- Klipdale, Nap- Napier, Pro- Protem and Rie- Rietpoel.



Supplementary Fig. S3. The optimum number of clusters (*K*) for (A) the entire *Pyrenophora teres* f. *teres* collection, populations from (B) Australia, (C) Hungary and (D) RSA based on delta *K* (Δ*K*) estimated over 10 independent runs.


A
B
C
Membership proportion
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
I
II
Population

Supplementary Fig. S4. Estimates of genetic structuring in (A) Australia (blue, $K = 2$) (B) Hungary (red, $K = 2$) and (C) RSA (green, $K = 2$) grouped into optimal clusters using the model-based clustering method in STRUCTURE. Bars represent individual isolates and the colour and height of each bar depicts the estimated membership fraction of each individual into the corresponding cluster. States of Australia; NSW- New South Wales, QLD-Queensland, SA- South Australia, VIC-Victoria and WA- Western Australia. Regions of Hungary; Kar- Karcag, Köl-, Kölcse, Kom- Kompolt, Mar- Martonvásár, Márk- Márok, Szé- Székkutas and Szo- Szombathely. RSA regions; Bre- Bredasdorp, Cal- Caledon, Gre- Greyton, Kli- Klipdale, Nap- Napier, Pro- Protem and Rie- Rietpoel.

Chapter 4

Supplementary Table 1. *Pyrenophora teres* progeny isolates used in this study with their mating type idiomorph and leaf symptom

	Isolate ID	Mating type*	Leaf symptom
1	*Pop37*_1	2	Spot-form net blotch
2	*Pop37*_2	2	Spot-form net blotch
3	*Pop37*_3	1	Spot-form net blotch
4	*Pop37*_4	2	-
5	*Pop37*_5	2	Spot-form net blotch
6	*Pop37*_7	1	Spot-form net blotch
7	*Pop37*_8	2	Spot-form net blotch
8	*Pop37*_9	2	Spot-form net blotch
9	*Pop37*_10	2	Spot-form net blotch
10	*Pop37*_11	1	-
11	*Pop37*_12	1	-
12	*Pop37*_13	1	Spot-form net blotch
13	*Pop37*_14	2	Spot-form net blotch
14	*Pop37*_15	1	Spot-form net blotch
15	*Pop37*_16	2	-
16	*Pop37*_17	2	Spot-form net blotch
17	*Pop37*_18	1	Spot-form net blotch
18	*Pop37*_19	1	-
19	*Pop37*_20	1	Spot-form net blotch
20	*Pop37*_21	1	Spot-form net blotch
21	*Pop37*_22	1	Spot-form net blotch
22	*Pop37*_23	2	Spot-form net blotch
23	*Pop37*_24	2	Net-form net blotch
24	*Pop37*_25	1	Spot-form net blotch
25	*Pop37*_26	1	Spot-form net blotch
26	*Pop37*_27	2	Net-form net blotch
27	*Pop37*_28	2	Net-form net blotch
28	*Pop37*_29	2	Net-form net blotch
29	*Pop37*_30	1	-
30	*Pop37*_31	2	Spot-form net blotch
31	*Pop37*_34	1	Spot-form net blotch
32	*Pop37*_35	1	Spot-form net blotch
33	*Pop37*_37	2	-
34	*Pop37*_38	2	Net-form net blotch
35	*Pop37*_40	2	-
36	*Pop37*_41	2	Net-form net blotch
37	*Pop37*_42	2	Spot-form net blotch
38	*Pop37*_43	2	-
39	*Pop37*_44	2	Spot-form net blotch

40	*Pop37*_45	2	-
41	*Pop37*_46	2	-
42	*Pop37*_47	1	Spot-form net blotch
43	*Pop37*_48	2	Spot-form net blotch
44	*Pop37*_49	1	Spot-form net blotch
45	*Pop37*_50	2	-
46	*Pop37*_51	2	Spot-form net blotch
47	*Pop37*_52	2	Spot-form net blotch
48	*Pop37*_53	2	Spot-form net blotch
49	*Pop37*_54	1	Spot-form net blotch
50	*Pop37*_55	2	Spot-form net blotch
51	*Pop37*_56	2	Spot-form net blotch
52	*Pop37*_57	1	Spot-form net blotch
53	*Pop37*_58	2	-
54	*Pop37*_59	1	Spot-form net blotch
55	*Pop37*_60	1	Spot-form net blotch
56	*Pop37*_62	1	Spot-form net blotch
57	*Pop37*_63	1	Spot-form net blotch
58	*Pop37*_64	1	-
59	*Pop37*_65	2	Spot-form net blotch
60	*Pop37*_66	1	Spot-form net blotch
61	*Pop37*_67	2	-
62	*Pop37*_69	1	Spot-form net blotch
63	*Pop37*_70	2	-
64	*Pop37*_71	1	-
65	*Pop37*_72	1	-
66	*Pop37*_73	1	-
67	*Pop37*_74	1	Spot-form net blotch
68	*Pop37*_76	1	Spot-form net blotch
69	*Pop37*_77	2	Spot-form net blotch
70	*Pop37*_78	2	Net-form net blotch
71	*Pop37*_79	1	-
72	*Pop37*_80	1	Spot-form net blotch
73	*Pop37*_81	1	Spot-form net blotch
74	*Pop37*_82	2	-
75	*Pop37*_83	2	Spot-form net blotch
76	*Pop37*_84	2	Spot-form net blotch
77	*Pop37*_85	2	-
78	*Pop37*_86	1	Spot-form net blotch
79	*Pop37*_87	2	Spot-form net blotch
80	*Pop37*_88	1	-
81	*Pop37*_89	2	-
82	*Pop37*_90	1	Spot-form net blotch
83	*Pop37*_91	2	Spot-form net blotch

84	*Pop37*_92	2	-
85	*Pop37*_93	1	Spot-form net blotch
86	*Pop37*_94	1	Net-form net blotch
87	*Pop37*_95	1	-
88	*Pop37*_96	2	-
89	*Pop37*_97	1	-
90	*Pop37*_101	2	-
91	*Pop37*_102	1	-
92	*Pop37*_103	2	-
93	*Pop37*_104	1	-
94	*Pop37*_105	1	-
95	*Pop37*_106	2	-
96	*Pop37*_107	2	-
97	*Pop37*_108	2	-
98	*Pop37*_111	2	-
99	*Pop37*_113	2	-
100	*Pop37*_114	2	Spot-form net blotch
101	*Pop37*_115	2	-
102	*Pop37*_117	2	-
103	*Pop37*_118	2	-
104	*Pop37*_119	1	-
105	*Pop37*_120	1	-
106	*Pop37*_121	1	-
107	*Pop37*_122	2	-
108	*Pop37*_123	1	-
109	*Pop37*_124	1	-
110	*Pop37*_125	2	-
111	*Pop37*_126	2	-
112	*Pop37*_127	1	-
113	*Pop37*_128	1	-
114	*Pop37*_129	1	-
115	*Pop37*_130	1	-
116	*Pop37*_131	2	-
117	*Pop37*_132	2	-
118	*Pop37*_133	2	-
119	*Pop37*_134	2	-
120	*Pop37*_135	1	Spot-form net blotch
121	*Pop37*_137	1	-
122	*Pop37*_139	1	-
123	*Pop37*_140	1	-
124	*Pop37*_141	2	Spot-form net blotch
125	*Pop37*_142	2	-
126	*Pop37*_143	1	-
127	*Pop37*_144	2	-

128	*Pop37*_145	2	-
129	*Pop37*_146	1	-
130	*Pop37*_147	2	-
131	*Pop37*_148	2	-
132	*Pop37*_149	1	-
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144	*Pop37*_161	2	-
145	*Pop37*_162	2	-
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148	*Pop37*_165	2	-
149	*Pop37*_166	2	-
150	*Pop37*_167	1	-
151	*Pop37*_169	2	-
152	*Pop37*_170	1	-
153	*Pop37*_171	1	-
154	*Pop37*_173	2	-
155	*Pop37*_174	2	-
156	*Pop37*_175	1	-
157	*Pop37*_177	2	-
158	*Pop37*_178	1	-
159	*Pop37*_179	2	-
160	*Pop37*_180	2	-
161	*Pop37*_181	2	-
162	*Pop37*_182	2	-
163	*Pop37*_183	2	-
164	*Pop37*_184	2	-
165	*Pop37*_185	1	-
166	*Pop37*_186	1	-
167	*Pop37*_187	1	-
168	*Pop37*_188	2	-
169	*Pop37*_189	1	-
170	*Pop37*_190	1	-
171	*Pop37*_192	1	-

172	*Pop37*_193	1	-
173	*Pop37*_194	1	-
174	*Pop37*_195	1	-
175	*Pop37*_196	2	-
176	*Pop37*_197	1	-
177	*Pop37*_198	2	-
178	*Pop37*_199	1	-
179	*Pop37*_200	1	Spot-form net blotch
180	*Pop37*_202	1	Spot-form net blotch
181	*Pop37*_203	1	-
182	*Pop37*_204	2	-
183	*Pop37*_205	2	-
184	*Pop37*_206	2	-
185	*Pop37*_207	2	Spot-form net blotch
186	*Pop37*_208	2	-
187	*Pop37*_209	2	Spot-form net blotch
188	*Pop37*_210	1	Spot-form net blotch
189	*Pop37*_211	2	-
190	*Pop37*_212	1	Spot-form net blotch
191	*Pop37*_213	1	-
192	*Pop37*_214	1	Spot-form net blotch
193	*Pop37*_216	2	-
194	*Pop37*_217	2	Spot-form net blotch
195	*Pop37*_219	1	-
196	*Pop37*_220	2	-
197	*Pop37*_221	1	-
198	*Pop37*_222	1	-
199	*Pop37*_223	2	Net-form net blotch
200	*Pop37*_224	1	Spot-form net blotch
201	*Pop37*_226	1	Spot-form net blotch
202	*Pop37*_227	1	-
203	*Pop37*_228	2	Spot-form net blotch
204	*Pop37*_230	2	-
205	*Pop37*_231	2	-
206	*Pop37*_232	1	Spot-form net blotch
207	*Pop37*_233	1	Spot-form net blotch
208	*Pop37*_234	2	-
209	*Pop37*_235	2	Spot-form net blotch
210	*Pop37*_236	1	Spot-form net blotch
211	*Pop37*_237	1	Spot-form net blotch
212	*Pop37*_238	1	-
213	*Pop37*_239	1	-
214	*Pop37*_240	2	-
215	*Pop37*_241	1	Spot-form net blotch

216	*Pop37*_242	1	-
217	*Pop37*_243	2	Spot-form net blotch
218	*Pop37*_244	2	Spot-form net blotch
219	*Pop37*_245	2	Net-form net blotch
220	*Pop37*_246	1	Spot-form net blotch
221	*Pop37*_247	1	-
222	*Pop37*_248	2	-
223	*Pop37*_249	1	Spot-form net blotch
224	*Pop37*_250	2	-
225	*Pop37*_251	2	Spot-form net blotch
226	*Pop37*_254	1	Spot-form net blotch
227	*Pop37*_255	1	Spot-form net blotch
228	*Pop37*_256	1	Spot-form net blotch
229	*Pop37*_258	1	Spot-form net blotch
230	*Pop37*_259	2	Spot-form net blotch
231	*Pop37*_261	1	-
232	*Pop37*_262	2	-
233	*Pop37*_263	2	-
234	*Pop37*_265	1	Spot-form net blotch
235	*Pop37*_266	2	Spot-form net blotch
236	*Pop37*_267	1	-
237	*Pop37*_268	1	-
238	*Pop37*_269	1	Spot-form net blotch
239	*Pop37*_270	1	Spot-form net blotch
240	*Pop37*_271	2	Net-form net blotch
241	*Pop37*_272	2	-
242	*Pop37*_273	1	-
243	*Pop37*_274	2	-
244	*Pop37*_276	1	-
245	*Pop37*_277	1	-
246	*Pop37*_279	2	Spot-form net blotch
247	*Pop37*_280	1	-
248	*Pop37*_281	1	Spot-form net blotch
249	*Pop37*_282	1	-
250	*Pop37*_284	2	Spot-form net blotch
251	*Pop37*_285	1	-
252	*Pop37*_286	2	Spot-form net blotch
253	*Pop37*_288	1	-
254	*Pop37*_289	1	-
255	*Pop37*_291	2	-
256	*Pop37*_294	2	-
257	*Pop37*_295	1	-
258	*Pop37*_297	2	-
259	*Pop37*_301	1	-

260	*Pop37*_303	1	-
261	*Pop37*_305	1	Spot-form net blotch
262	*Pop37*_306	1	Spot-form net blotch
263	*Pop37*_307	1	-
264	*Pop37*_308	2	-
265	*Pop37*_309	2	-
266	*Pop37*_310	1	-
267	*Pop37*_311	2	-
268	*Pop37*_312	1	-
269	*Pop37*_314	1	Spot-form net blotch
270	*Pop37*_315	1	-
271	*Pop37*_316	1	Spot-form net blotch
272	*Pop37*_317	1	Spot-form net blotch
273	*Pop37*_318	1	Spot-form net blotch
274	*Pop37*_319	2	-
275	*Pop37*_320	1	Spot-form net blotch
276	*Pop37*_321	1	Spot-form net blotch
277	*Pop37*_322	1	Spot-form net blotch
278	*Pop37*_323	1	Spot-form net blotch
279	*Pop37*_328	2	-
280	*Pop37*_329	1	Spot-form net blotch
281	*Pop37*_330	1	-
282	*Pop37*_332	2	Spot-form net blotch
283	*Pop37*_333	2	Spot-form net blotch
284	*Pop37*_334	2	Net-form net blotch
285	*Pop37*_335	1	-
286	*Pop37*_336	1	-
287	*Pop37*_337	1	Spot-form net blotch
288	*Pop37*_338	1	-
289	*Pop37*_339	2	Spot-form net blotch
290	*Pop37*_340	1	Spot-form net blotch
291	*Pop37*_342	2	-
292	*Pop37*_343	2	Spot-form net blotch
293	*Pop37*_344	1	Spot-form net blotch
294	*Pop37*_345	1	Spot-form net blotch
295	*Pop37*_346	1	Spot-form net blotch
296	*Pop37*_347	2	Spot-form net blotch
297	*Pop37*_350	2	-
298	*Pop37*_351	1	-
299	*Pop37*_352	1	-
300	*Pop37*_354	1	-
301	*Pop37*_355	2	Spot-form net blotch
302	*Pop37*_356	1	Spot-form net blotch
303	*Pop37*_359	1	Spot-form net blotch

304	*Pop37*_360	2	Spot-form net blotch
305	*Pop37*_361	1	Spot-form net blotch
306	*Pop37*_362	1	Spot-form net blotch
307	*Pop37*_364	2	-
308	*Pop37*_365	2	-
309	*Pop37*_366	2	Spot-form net blotch
310	*Pop37*_367	1	Spot-form net blotch
311	*Pop37*_368	1	-
312	*Pop37*_369	1	-
313	*Pop37*_370	2	-
314	*Pop37*_371	1	Spot-form net blotch
315	*Pop37*_372	2	Spot-form net blotch
316	*Pop37*_373	2	Spot-form net blotch
317	*Pop37*_374	2	-
318	*Pop37*_376	2	-
319	*Pop37*_377	1	-
320	*Pop37*_380	2	Spot-form net blotch
321	*Pop37*_383	2	Spot-form net blotch
322	*Pop37*_396	2	Net-form net blotch
323	*Pop37*_402	2	-
324	*Pop37*_403	2	-
325	*Pop37*_404	2	Spot-form net blotch
326	*Pop37*_405	1	-
327	*Pop37*_406	1	-
328	*Pop37*_408	2	-
329	*Pop37*_411	2	-
330	*Pop37*_412	2	Spot-form net blotch
331	*Pop37*_413	2	Spot-form net blotch
332	*Pop37*_414	2	-
333	*Pop37*_415	1	-
334	*Pop37*_416	1	Spot-form net blotch
335	*Pop37*_417	1	-
336	*Pop37*_418	1	-
337	*Pop37*_420	2	-
338	*Pop37*_421	1	-
339	*Pop37*_422	2	Spot-form net blotch
340	*Pop37*_425	2	-
341	*Pop37*_427	1	-
342	*Pop37*_430	2	-
343	*Pop37*_431	1	-
344	*Pop37*_432	1	Spot-form net blotch
345	*Pop37*_433	1	-
346	*Pop37*_434	1	Spot-form net blotch
347	*Pop37*_435	1	-

348	*Pop37*_436	2	-
349	*Pop37*_437	1	-
350	*Pop37*_438	1	-
351	*Pop37*_440	1	-

* Mating type idiomorph of the isolate -Not available

Supplementary Table 2. *Pyrenophora teres* progeny isolates showed the highest disease reaction scores for the eight used for QTL analysis and additional twelve barley genotypes

Isolates	*Pop37*_41	*Pop37*_48	*Pop37*_52	*Pop37*_63	*Pop37*_74	*Pop37*_237	*Pop37*_245	*Pop37*_249	*Pop37*_339	*Pop37*_362
Ciho 5791*	8	7	8	8	7	6.5	8	8	9	8.5
Dampier*	7	6	8	8.5	7	7	9	7	8.5	7
Flagship*	8	6	7.5	7.5	6.5	6	6	6	8	8
Fleet*	7	6	8	7.5	6	6.5	7	8	8	8
Gairdner*	8	7	8.5	8.5	7	8	8	7.5	9	8.5
Grimmett*	6	7	8	7.5	8	6.5	7	6.5	9	8
Kombar*	6	7	7	8.5	6.5	7.5	7	7	8	8
Prior*	6	8	8.5	8.5	-	7	8.5	7.5	8.5	8.5
Beecher	8.3	6	4	6.8	2.3	5	4.3	3.5	7.3	4.5
Ciho11458	7	5.8	5	7.3	2.5	5.5	4.8	4.78	7.3	6
Compass	7	2.8	4.3	7.3	2	4.8	5	3.5	8	5
Fathom	8	4	6.3	8.5	0.5	4.8	3.8	4	7.5	4.5
Harbin	7	5.8	4.5	7.5	1	5.5	2.5	3	6	4.8
Keel	8	4.3	5.8	7.5	4	4.8	4.8	5.3	6.3	5
Navigator	7.8	3.5	4.8	6.8	0.5	4.5	1.3	3.8	7.5	4.5
RGT Planet	10	4.3	5.8	7.8	1.8	5	4	4.8	7.8	4.8
Rosalind	8	5.5	6	6.3	2.5	5.5	4	4	8.5	5
Schooner	7	4.5	3	6.3	2	4.5	2.8	3.3	7.8	4.78
Spartacus CL	8.5	5	5	7	2	4.5	4	3	7.8	5.5
Vlamingh	7	5.5	5	6.3	1.3	5	3.3	4	7.5	5.5

* Eight barley genotypes used in QTL analysis.

Supplementary Table S3. The corresponding marker order and marker position of *Pop37* genetic map to the two reference genomes; W1-1 and SG1

*Pop37*_Chr01			W1-1		SG1	
Marker order	Marker ID	cM	Marker order	Base pair	Market order	Base pair
1	36350590	0.6006	1	411511	1	173836
2	36348241	0.9083	2	418770	2	181292
3	36346381	4.4752	3	421039	3	183562
4	36348174	5.1101	4	426173	4	188692
5	36346923	7.0158	9	691885	9	302244
6	28946156	9.0284	6	740484	6	350269
7	28947716	11.0468	10	950620	10	574331
8	36350940	13.7935	7	974975	11	595968
9	36345988	15.1096	12	997285	7	596033
10	36346005	17.6434	13	1007502	12	618218
11	36349470	19.6735	14	1020669	14	641623
12	36347906	21.2463	15	1046383	15	667353
13	36349009	21.5638	16	1049138	16	670136
14	28947304	22.4436	19	1111357	18	731745
15	28948967	23.0251	20	1192023	19	731810
16	36349901	28.2615	21	1198439	20	812414
17	28948849	28.8412	22	1209462	21	818822
18	36351550	29.4844	23	1225957	22	829620
19	36350228	34.386	25	1272246	23	846089
20	36350818	34.6881	26	1294707	24	892358
21	28947570	35.5578	30	1329420	25	892358
22	36349690	36.43	31	1334999	27	910592
23	28946600	39.1111	33	1366387	29	940863
24	28946431	39.4276	34	1378930	30	940928
25	36350781	42.243	35	1387434	31	946497
26	36346614	43.1895	36	1431642	33	977882
27	36348886	43.5285	38	1539703	34	990428
28	28947277	45.1847	39	1548277	35	998927
29	28945144	45.4963	40	1559727	36	1043140
30	36349862	45.7887	41	1574641	37	1086327
31	36348761	47.5646	42	1587421	38	1151046
32	36346711	47.88	43	1599594	39	1159635
33	36346060	48.8206	44	1632148	40	1171085
34	28950112	49.4037	45	1635557	42	1198468
35	28947631	49.6919	48	1708275	43	1210704
36	28945654	52.2804	49	1715021	44	1243256
37	36349698	59.4079	50	1743107	45	1246666
38	28949848	60.0104	47	1757376	48	1319365
39	36349464	60.6039	46	1763532	49	1326117
40	28947928	61.5186	51	1838680	50	1354121
41	28947665	61.8235	52	1851358	47	1368394
42	28948613	62.7085	53	1939879	46	1374549
43	36347564	63.0181	54	2135723	51	1449648
44	36348123	63.3249	55	2153712	52	1462318
45	36348303	66.3487	56	2177813	53	1550897

46	36346308	66.6832	57	2187759	54	1634989
47	28946720	67.2856	58	2202265	55	1652984
48	36350777	67.6124	59	2230958	56	1677062
49	36348805	67.9329	60	2231536	57	1766266
50	36346671	74.053	61	2273753	58	1780690
51	36350728	74.6628	62	2318176	59	1809391
52	28947958	76.7048	63	2337452	60	1809969
53	36348509	76.9921	66	2405233	61	1852080
54	36350564	77.5669	67	2425603	62	1872591
55	28947354	77.8542	69	2465787	63	1891705
56	28946536	78.7239	70	2469467	64	1959411
57	28947997	80.182	73	2506394	66	1959411
58	36348278	81.0671	71	2506580	67	1979787
59	28947683	81.4004	75	2726335	68	2020315
60	36347905	83.4354	76	2802176	69	2020315
61	36348578	83.7549	77	2814354	70	2024000
62	28946967	84.3939	78	2826644	72	2060948
63	36348917	87.0592	79	2847888	73	2060948
64	28948372	87.644	80	2849439	71	2061134
65	36346710	88.2671	81	2857252	75	2107857
66	36349426	88.9102	82	2865471	77	2198030
67	36346757	90.8529	83	2876240	78	2210321
68	36348337	91.1645	84	2909402	79	2231615
69	28946626	91.829	85	2915942	80	2233158
70	36346303	92.8291	86	2925445	81	2240939
71	28945792	93.134	#N/A		82	2249135
72	36349585	93.4325	#N/A		83	2259967
73	36349586	93.731	#N/A		84	2296816
74	28949757	96.0651	#N/A		85	2303279
75	28946368	99.1083	#N/A		86	2312772
76	28945704	99.4179	#N/A		#N/A	
77	36349628	100.9244	#N/A		#N/A	
78	36348772	101.5759	#N/A		#N/A	
79	36346518	102.219	#N/A		#N/A	
80	36348777	102.5149	#N/A		#N/A	
81	36348544	102.8099	#N/A		#N/A	
82	28948128	106.1381	#N/A		#N/A	
83	36346553	107.3541	#N/A		#N/A	
84	28948925	107.6456	#N/A		#N/A	
85	28948453	107.9398	#N/A		#N/A	
86	28948149	107.99	#N/A		#N/A	
*Pop37*_Chr02			W1-1		SG1	
Marker order	Marker ID	cM	Marker order	Base pair	Marker order	Base pair
1	28947153	0.2976	3	280936	2	125561
2	36348929	0.5917	4	284271	3	125730
3	28945265	0.8885	1	317210	4	129288
4	28947085	4.7537	5	337069	1	162250
5	28946283	5.3617	7	346503	5	182038
6	28950221	5.9887	8	358195	7	192984
7	36346791	8.4446	10	359343	8	204769
8	36350689	9.0417	11	360380	10	205912

9	36347802	9.3358	9	743099	11	206970
10	36351297	10.2734	13	1408024	9	416116
11	36345801	10.5909	15	1457316	14	512990
12	36346079	12.7661	16	1523474	13	575919
13	36347767	13.3493	17	1542208	15	625155
14	28947926	17.3694	18	1546108	16	691329
15	36348200	19.3936	19	1558514	17	710130
16	28949827	19.6843	21	1575337	18	714026
17	36347686	19.9901	20	1699790	19	724954
18	36350559	20.9104	22	1738535	21	741922
19	36349602	21.2063	25	1745663	20	742763
20	36346294	21.5084	26	1745728	22	781470
21	28948170	23.7181	23	1753677	25	788622
22	36350656	24.6736	28	1766167	26	788687
23	36349936	24.9871	24	1767074	23	796527
24	36348713	25.2948	27	1784625	24	809943
25	36347999	25.5987	29	1807136	27	828089
26	36346070	25.9142	30	1868620	29	850672
27	36350035	26.8667	31	1900673	30	911273
28	28947535	30.0866	32	1906896	31	943302
29	36348633	31.4807	33	1913846	32	949526
30	36349740	33.3624	35	1953511	33	956501
31	28948448	34.2321	37	2001297	35	996142
32	36348938	34.5228	36	2005683	37	1045131
33	36349782	35.6691	38	2114007	36	1049517
34	28947429	36.2422	43	2114007	38	1140232
35	36351140	38.8233	41	2126839	43	1140232
36	28947789	41.2568	42	2144424	41	1153109
37	36345731	44.6289	39	2149202	42	1170713
38	36349865	45.6324	44	2149202	39	1175638
39	36348404	48.4045	40	2181946	44	1175638
40	28947848	49.2617	45	2238961	40	1208398
41	28948996	52	46	2239738	45	1265769
42	36350117	53.6785	47	2258613	46	1266546
43	28946286	54.0175	48	2325602	47	1285736
44	28946301	58.5342	50	2388838	48	1352609
45	28947010	58.8721	51	2485443	50	1418031
46	36350122	59.6129	52	2507344	51	1514464
47	36351343	62.1958	55	2547471	52	1536364
48	28947318	62.4865	53	2584949	54	1571181
49	36346720	64.571	56	2629640	55	1576654
50	28947826	66.9538	58	2667501	53	1614089
51	36350069	67.9003	59	2668973	56	1658657
52	36347668	69.1624	57	2670777	58	1696461
53	36348780	70.0771	60	2725996	59	1697861
54	36346222	71.0118	61	2730966	60	1754684
55	28948625	72.4828	62	2747624	61	1759694
56	36348198	75.18	63	2790313	62	1776361
57	28947583	75.4777	64	2830947	63	1819092
58	36348147	75.7798	65	2888165	64	1859731
59	36346387	79.7394	67	2906333	65	1918084
60	28948472	80.8924	68	2910208	67	1935218

61	28948344	81.5293	66	2912044	68	1938717
62	28945168	84.513	69	2984626	66	1940554
63	28946469	84.8198	70	2996453	69	2013104
64	36347514	86.6443	71	2997719	70	2024899
65	36348153	87.8906	72	3004141	71	2026165
66	36345763	88.2002	73	3004141	72	2032597
67	36350002	88.7902	75	3029889	73	2032597
68	36350512	91.5285	76	3068500	74	2050741
69	36348631	93.6446	77	3068500	75	2058336
70	28946700	93.9336	78	3084971	76	2096968
71	28948174	94.2312	79	3119385	77	2096968
72	28946852	94.5361	80	3196781	78	2113439
73	28946072	94.8373	81	3524805	79	2147877
74	28945449	96.0646	82	3558798	80	2225353
75	36349026	96.3732	83	3588931	81	2627877
76	28949961	96.68	84	3600707	82	2661884
77	28949960	97.8532	85	3611361	83	2692041
78	36349742	98.7356	86	3642207	84	2703793
79	28947553	100.1981	87	3683334	85	2714473
80	28948113	100.4922	88	3687987	86	2745344
81	36349872	101.3746	89	3720531	87	2786491
82	28948245	106.0257	90	3732294	88	2791138
83	28950092	106.8928	92	3779722	89	2823654
84	36348646	107.8246	91	3784489	90	2835434
85	28947216	108.4596	93	3797497	92	2882941
86	36348348	109.3633	102	3824585	91	2887707
87	36347826	109.6583	100	3843731	93	2900657
88	36349508	110.5486	101	3858796	102	2926223
89	28945656	111.1917	95	3880610	101	2960754
90	36346910	112.1352	94	3890886	95	2982345
91	28945831	112.4419	96	3890886	94	2992594
92	36347592	113.4522	99	3898369	96	2992594
93	36346109	117.4474	98	3909480	99	3000088
94	36347670	118.3705	97	3916870	98	3011202
95	28948650	118.684	103	3958419	97	3018592
96	36346883	119.323	104	4035616	103	3060021
97	36349000	119.6252	110	4035616	104	3137308
98	28948703	121.1594	109	4035681	110	3137308
99	41805310	122.7472	105	4091367	105	3193111
100	28946282	123.9745	108	4091367	108	3193111
101	36349752	125.1341	106	4112186	106	3348900
102	28947900	132.9331	113	4169857	107	3363998
103	28949382	136.8687	112	4176511	113	3406671
104	36349418	137.4937	111	4181342	112	3413331
105	36347987	137.8024	114	4181342	111	3418130
106	41805581	138.0906	115	4223591	114	3418130
107	36349816	138.3763	117	4251254	115	3460024
108	28948349	140.9182	118	4251254	117	3486843
109	28947468	141.2515	119	4278442	118	3486843
110	100303497	143.8597	121	4278507	119	3513899
111	28945689	144.7944	120	4329776	121	3513964
112	28946336	145.9851	122	4382494	120	3557417

113	36349373	146.9376	123	4495923	123	3721573
114	36349973	149.4795	124	4496550	124	3722200
115	28947980	150.9505	125	4512707	125	3738383
116	28949869	151.266	126	4545576	126	3771352
117	36347525	151.6096	127	4567816	127	3793563
118	36346821	152.2327	128	4597394	128	3821996
119	28947560	152.8333	129	4646821	129	3884435
120	36348717	154.072	130	4662018	130	3899623
121	36345798	154.9839	135	4668142	135	3905743
122	28945270	164.7145	133	4683101	133	3920713
123	36348296	165.0185	132	4696164	131	3928239
124	36346829	166.567	131	4696448	132	3928239
125	28948167	166.862	136	4866741	136	4071006
126	28945286	168.0284	134	4869891	134	4074158
127	28947869	168.3182	137	4909395	137	4113687
128	28949006	168.9047	138	4922090	138	4126272
129	28948054	169.7795	139	4957903	139	4162245
130	28946961	170.4045	140	4957903	140	4162245
131	36351189	170.722	141	4963245	141	4167577
132	28949400	171.0161	142	4975852	142	4180029
133	28946813	172.2586	143	4980474	143	4184651
134	36346835	172.5843	145	5003764	145	4208099
135	36346897	173.2173	144	5018828	144	4223168
136	28947381	176.8163	146	5042270	148	4272804
137	36347980	177.1268	148	5068491	150	4314045
138	28945871	180.443	149	5099713	151	4354315
139	28947280	180.7938	150	5109848	152	4374395
140	36349024	182.0599	151	5147358	153	4384257
141	36348243	182.9529	152	5167364	#N/A	
142	28946241	183.8621	153	5177243	#N/A	
143	36349515	185.7498	154	5205986	#N/A	
144	36349547	186.673	#N/A		#N/A	
145	28948440	187.5739	#N/A		#N/A	
146	28946983	187.8761	#N/A		#N/A	
147	36350430	189.7878	#N/A		#N/A	
148	36346924	190.8296	#N/A		#N/A	
149	28949187	191.4547	#N/A		#N/A	
150	28947953	193.4966	#N/A		#N/A	
151	28949396	197.0009	#N/A		#N/A	
152	28949880	198.7357	#N/A		#N/A	
153	36350509	200.092	#N/A		#N/A	
154	28946936	200.093	#N/A		#N/A	
*Pop37*_Chr03			W1-1		SG1	
Marker order	Marker ID	cM	Marker order	Base pair	Marker order	Base pair
1	36349905	0.304	3	149605	1	137557
2	36349027	0.6061	2	892055	5	162916
3	36349032	1.5098	4	1098678	6	192636
4	28949140	1.8119	5	1559717	7	206984
5	36350224	2.7584	6	1589628	11	250412
6	36346345	3.3776	7	1604004	9	260631
7	28946232	4.3065	11	1650412	10	267213

8	36349309	4.9219	9	1660628	12	267213
9	28947142	6.4801	10	1667224	13	270578
10	36349693	7.1253	12	1667224	15	288035
11	36347966	8.7387	15	1687975	14	298555
12	36349692	9.3819	14	1698505	17	326490
13	36348628	10.3721	16	1704527	19	387418
14	41804653	11.9351	17	1726459	18	400386
15	36347908	12.2266	19	1794205	20	403103
16	28948401	14.3686	18	1807169	21	443534
17	28946463	20.5731	20	1809890	22	475794
18	28945560	20.8743	22	1880484	23	475794
19	36351333	21.1719	23	1880484	24	520324
20	36347663	22.7448	24	1925537	26	583155
21	28945773	23.0553	26	1988242	25	611026
22	28945370	23.7005	25	2016098	27	635611
23	28945371	24.9469	27	2040663	28	654086
24	28948357	27.2947	28	2059179	30	672483
25	36347534	27.6052	30	2077575	29	688974
26	36351278	31.3389	29	2094066	31	710849
27	28946351	31.6504	31	2115939	32	718179
28	36346834	36.1936	32	2123272	33	747505
29	36348269	36.5081	33	2152599	36	850192
30	36345903	38.6834	36	2237422	34	873722
31	36350705	39.5632	34	2261191	35	876768
32	28949718	40.7296	35	2264239	37	882826
33	36348077	42.8848	37	2270297	38	893547
34	36346908	43.1887	38	2281024	39	914942
35	36348020	43.8278	39	2302434	40	924907
36	28946216	44.1462	40	2330021	41	1184513
37	28948381	47.048	41	2337700	43	1223188
38	28948143	48.2043	43	2519106	44	1243483
39	36349573	48.784	44	2539338	45	1248790
40	28948941	51.6719	45	2544648	42	1250645
41	36348552	52.6621	42	2546504	46	1304242
42	36346072	52.9921	46	2599878	47	1329433
43	36348026	55.5179	47	2625034	48	1350995
44	36351564	55.8053	48	2646613	50	1358305
45	28948734	57.2425	49	2672012	49	1376219
46	36349395	58.9723	51	2673875	51	1378075
47	28946975	60.1286	52	2700983	52	1405177
48	28946845	60.4201	53	2702770	53	1406958
49	28948008	62.6297	56	2729873	56	1427298
50	36345901	63.2791	54	2755999	54	1450540
51	36348487	63.9481	57	2787476	57	1482032
52	36350650	66.8359	58	2912654	58	1507102
53	36348718	67.4073	61	2924391	61	1518768
54	28947634	67.7095	60	2928552	60	1522930
55	36351066	68.0191	59	2937879	59	1532236
56	36346322	72.1583	62	2937880	62	1532237
57	28945730	76.2017	63	2962716	63	1556829
58	36350106	76.8059	64	3020573	64	1614757
59	28946551	77.4194	65	3076085	65	1670036

60	36349835	79.3251	66	3102260	66	1702500
61	36345797	79.6509	67	3127208	67	1727440
62	36345983	82.8501	68	3135042	68	1735268
63	36346338	84.9347	69	3135042	69	1735268
64	36349884	85.222	70	3141284	70	1741497
65	28947635	86.0917	71	3157822	71	1758028
66	28947690	87.8571	73	3186100	72	2168989
67	28949352	88.4419	72	3417238	75	2168989
68	36348081	88.7478	75	3417238	74	2185736
69	28946559	92.4814	74	3433968	77	2237312
70	28949368	93.0875	77	3485530	76	2251644
71	28947992	95.9029	76	3499868	78	2290922
72	36348722	96.8585	78	3539429	79	2349655
73	36348210	98.7055	79	3598142	80	2409173
74	28949964	101.1997	80	3657667	82	2421006
75	36348721	105.6846	82	3669484	81	2431464
76	36346471	106.3361	81	3679937	83	2553167
77	28945385	107.6022	83	3801654	84	2612158
78	28946532	108.4693	84	3891615	85	2673817
79	28945917	114.0479	85	3953289	86	2697711
80	28948731	114.9654	86	3977184	88	2730988
81	36348206	115.2819	88	4010412	89	2763932
82	28945152	118.9247	89	4043295	90	2803347
83	28947059	119.2146	90	4082701	91	3075436
84	28948232	121.4035	91	4338379	92	3075775
85	28948210	122.0285	92	4338714	93	3135960
86	28947666	122.9135	93	4398983	94	3140049
87	36346713	123.2085	94	4403127	95	3177003
88	28947623	124.3648	95	4440274	96	3182195
89	28949971	124.9412	96	4445467	97	3185744
90	28948077	125.2294	97	4449013	99	3192509
91	28948267	125.5235	99	4455802	98	3192608
92	36348871	127.7614	98	4455900	100	3204920
93	36346530	128.0769	100	4468209	101	3207294
94	28947605	130.7109	101	4470583	102	3224663
95	28946578	132.2404	102	4487954	103	3263082
96	36349591	133.1959	103	4526327	104	3270631
97	28945863	133.8249	104	4533911	106	3296740
98	36346329	134.7369	105	4539369	107	3296740
99	28947945	137.3632	106	4560083	108	3323311
100	28949939	137.669	107	4560083	109	3347157
101	28947616	141.0277	108	4586639	111	3368355
102	36349629	144.6928	109	4610437	110	3368420
103	36346531	145.6103	111	4631687	113	3385892
104	28945559	147.7789	110	4631752	112	3386220
105	28945700	148.4039	113	4649075	114	3398072
106	28946468	148.7125	112	4649403	115	3421327
107	36350549	151.6004	114	4662225	120	3468219
108	28945166	157.3954	115	4685328	3	3517117
109	36350851	158.6041	#N/A		#N/A	
110	28947301	158.9466	#N/A		#N/A	
111	28946652	160.2758	#N/A		#N/A	

112	36347738	160.6125	#N/A		#N/A	
113	36346203	162.6136	#N/A		#N/A	
114	28947884	167.271	#N/A		#N/A	
115	36346097	167.5844	#N/A		#N/A	
116	28945757	168.8005	#N/A		#N/A	
117	28948041	169.1017	#N/A		#N/A	
118	36349207	169.402	#N/A		#N/A	
119	28949965	169.7041	#N/A		#N/A	
120	36345919	169.7042	#N/A		#N/A	
*Pop37*_Chr04			W1-1		SG1	
Marker order	Marker ID	cM	Marker order	Base pair	Marker order	Base pair
1	36348166	0.3268	1	44565	2	39178
2	36346339	1.2388	3	68653	1	59946
3	36348686	4.5975	4	72477	3	81467
4	36350916	7.6223	5	82261	4	85234
5	36347830	12.5515	7	142625	5	95127
6	28946183	12.8439	6	142900	6	155645
7	28946122	15.3227	9	172367	9	185295
8	36347960	15.6304	8	172930	8	185857
9	36348723	16.2101	10	197730	10	210660
10	36351214	21.6367	12	221542	12	237637
11	28949332	22.5773	13	227434	13	244300
12	28945424	24.1452	14	235638	14	252525
13	36345921	24.4558	15	254368	15	271257
14	28947335	27.3623	16	274739	16	291452
15	36346457	29.6219	17	315305	17	313967
16	28945630	30.1999	18	365858	18	364327
17	36348274	33.0066	19	377528	19	375988
18	36345658	34.253	20	390882	20	389375
19	36348666	35.4126	21	433286	21	431868
20	36350277	37.4605	22	437647	22	436225
21	28946946	37.759	23	441521	23	440100
22	36346430	39.2432	24	481340	24	479751
23	28948993	42.6969	25	522310	25	522984
24	28947014	43.5641	26	546627	26	547293
25	28949507	44.1438	27	566043	27	566720
26	28946681	44.7355	28	581129	28	581822
27	36347557	45.3238	29	598204	29	599079
28	28947385	46.761	30	621502	30	622388
29	36351295	48.2023	35	719696	35	720760
30	28946629	48.777	31	939848	31	1015391
31	28947682	49.678	36	946822	36	1089206
32	36349861	49.9783	34	1029710	34	1172085
33	28948732	50.5632	32	1071050	32	1213392
34	28949896	50.8573	33	1092947	33	1233766
35	28948099	52.1194	37	1106375	37	1247280
36	36346761	52.7688	38	1144507	38	1284085
37	28945579	57.2826	39	1178014	40	1400214
38	36348232	58.2261	40	1205584	41	1427165
39	28947260	58.516	41	1232498	42	1457623
40	28946672	58.8041	42	1262949	43	1496590

41	28945650	59.9505	43	1301905	44	1537933
42	28946034	61.2246	44	1343236	45	1563235
43	28945254	63.1487	45	1368538	47	1615281
44	36348510	66.6529	47	1420746	48	1615346
45	28948881	67.8508	48	1420811	49	1627734
46	36352001	68.1466	49	1433461	53	1644246
47	36349894	68.446	53	1449973	55	1644246
48	28945680	69.0614	55	1449973	54	2005057
49	28946177	70.3316	54	1555668	56	2034817
50	36348707	71.2781	56	1585458	50	2035408
51	28946264	71.5829	50	1586049	52	2077479
52	28948468	71.8711	52	1684307	51	2077544
53	28947134	72.1635	51	1684372	57	2091843
54	36352286	72.482	57	1698100	59	2091843
55	36347561	72.812	59	1698100	58	2118049
56	28947017	73.116	58	1724096	60	2129703
57	28948368	73.4285	60	1735720	61	2138876
58	28945319	74.0516	61	1745005	63	2176154
59	36347493	76.9534	63	1782292	62	2191530
60	28945881	77.5348	62	1797701	64	2197645
61	28946046	82.2687	64	1803813	65	2230676
62	36348837	82.9016	65	1836804	66	2244788
63	36348583	83.9017	66	1850870	67	2260332
64	28948865	85.1599	67	1866408	68	2266385
65	36347653	86.0346	68	1872458	70	2342439
66	36348648	88.2099	70	1947235	69	2370794
67	28946904	88.8291	69	1975608	72	2388207
68	28947601	90.2663	72	1993013	71	2393239
69	28947463	92.0908	71	1998045	73	2424226
70	36346050	92.3994	73	2029044	74	2433177
71	36348088	94.2753	74	2037995	76	2455309
72	36349292	95.4877	76	2060148	75	2510663
73	36347983	95.7944	75	2144835	78	2537231
74	36346902	96.6954	78	2171182	77	2560879
75	28949236	97.3146	79	2204237	79	2570156
76	36346900	98.597	80	2264846	80	2630693
77	36346894	98.9075	81	2282917	81	2648516
78	28947123	99.1991	82	2330005	82	2695541
79	28946864	100.7524	83	2343732	83	2708943
80	36346476	101.4147	84	2377469	84	2742686
81	36350266	106.7811	85	2502837	85	2791506
82	36349587	107.1133	86	2512844	86	2801519
83	36348366	108.0939	89	2531845	88	2820396
84	28949247	109.2435	88	2531910	87	2844462
85	28946814	109.5292	87	2555560	94	2852384
86	28949404	109.8166	94	2563487	95	2852824
87	28946453	112.3504	95	2563926	90	2855434
88	36346846	112.6894	90	2566528	93	2869684
89	36346819	113.3473	93	2580770	92	2873051
90	36346868	115.2291	92	2584269	96	2899195
91	36348300	116.3689	96	2610526	91	2901431
92	36351582	117.5739	91	2612759	97	2913946

93	28945843	117.8845	97	2625303	100	2941425
94	28948408	118.195	98	2642603	99	2954991
95	28946717	119.7728	100	2652945	102	3029138
96	28946587	120.1018	99	2666508	104	3042219
97	36347775	120.7939	102	2740587	105	3057293
98	28947019	121.4518	103	2753668	106	3064310
99	28946118	122.3778	105	2768772	107	3101988
100	28947314	125.8719	106	2777302	108	3115282
101	36349322	126.4533	107	2815003	109	3134844
102	36350679	127.3998	108	2828306	110	3142488
103	28947734	128.7424	110	2855139	111	3153008
104	36346451	129.6571	111	2865677	114	3154307
105	28947210	130.2335	114	2866934	112	3172936
106	28948542	131.9485	112	2885588	113	3172936
107	28947625	132.2479	113	2885588	#N/A	
108	36349677	132.871	#N/A		#N/A	
109	36348118	133.496	#N/A		#N/A	
110	36346331	133.8125	#N/A		#N/A	
111	36346433	134.123	#N/A		#N/A	
112	36347845	135.721	#N/A		#N/A	
113	28947201	136.4155	#N/A		#N/A	
114	36346879	136.4156	#N/A		#N/A	
*Pop37*_Chr05			W1-1		SG1	
Marker order	Marker ID	cM	Marker order	Base pair	Marker order	Base pair
1	28946097	0.2985	4	256820	4	224725
2	28947600	1.2361	3	283618	3	237821
3	36345879	1.8534	5	296106	5	248461
4	36347894	2.1433	6	302694	6	255079
5	28949134	3.0104	7	307530	7	259926
6	36350089	4.2228	8	318123	8	270466
7	36346232	7.8356	10	440934	11	309976
8	36346350	11.6647	9	449886	13	311842
9	36350279	11.9651	11	526041	12	313831
10	28945886	12.8715	13	527908	14	339126
11	36346955	14.7242	12	529896	16	347324
12	28947163	15.0282	14	554487	15	369504
13	36348263	16.748	16	562706	17	406499
14	28948483	17.0362	15	594546	20	470158
15	36348562	18.5799	17	630067	25	504519
16	28945244	19.2129	20	700266	26	507256
17	28945890	21.7792	49	758240	24	520669
18	36350382	25.1482	21	925473	27	522433
19	36351483	25.4494	22	925752	28	530814
20	36346747	25.7515	24	941067	31	531558
21	36348992	26.0501	27	948596	23	537886
22	36350236	26.3459	23	958734	33	588897
23	28948839	28.8402	#N/A	987937	32	602290
24	36348221	29.1577	#N/A	988266	49	626855
25	36346428	29.7926	33	990704	#N/A	635449
26	36351143	30.1091	32	1004247	30	663749
27	28945159	30.4187	63	1032493	29	678451

28	36348546	31.0854	30	1037367	36	708981
29	36346139	31.408	29	1052863	35	751206
30	36351222	31.703	36	1086026	34	796590
31	28947405	31.9988	35	1114747	37	797464
32	28946964	33.2569	34	1160777	38	852087
33	36346227	34.5602	37	1161558	39	985717
34	36348037	35.557	38	1229548	41	1028318
35	36348534	36.7995	39	1459682	43	1062109
36	28947188	37.3965	41	1527390	44	1124033
37	28946070	41.3202	43	1603621	45	1140442
38	28948427	46.2494	44	1657828	46	1195810
39	36348163	46.5427	45	1674365	47	1252372
40	28945776	47.1525	46	1760263	51	1333822
41	28946329	48.682	47	1826941	52	1333822
42	36351741	49.8621	54	1905727	55	1364907
43	28949137	54.1148	51	1950384	57	1416743
44	36345649	54.4115	52	1950384	59	1425041
45	36349474	55.2712	55	1993119	56	1427456
46	36349917	55.8493	56	2027138	61	1454203
47	28946656	69.0772	61	2052267	#N/A	1493675
48	28950033	72.8501	62	2133316	62	1513935
49	36351675	82.1984	64	2168056	64	1548577
50	36351057	84.0627	65	2174517	65	1555040
51	36349490	84.3832	67	2229846	67	1610441
52	28949126	86.7874	66	2248958	66	1628608
53	36346814	87.1059	70	2291947	70	1671649
54	28945161	91.3093	72	2311485	72	1691128
55	28948051	91.6209	73	2317219	73	1696844
56	28945521	92.266	74	2331749	74	1711362
57	36348259	92.5664	71	2331965	71	1711578
58	36348413	93.1914	75	2341512	75	1721134
59	36346047	95.085	76	2393259	77	1775079
60	36346058	96.8557	77	2395396	80	1824761
61	28947055	99.4668	78	2401437	79	1829714
62	28948480	100.3815	80	2446362	81	1873683
63	28945441	100.6911	79	2451316	82	1901317
64	28948354	100.9978	81	2495247	83	1903654
65	28948792	103.8133	82	2522683	84	1939092
66	36347807	104.1248	83	2525020	86	1979660
67	36347737	104.4216	84	2560433	87	2023038
68	28949082	105.0258	86	2600984	88	2037794
69	28948045	106.5648	87	2644308	89	2069882
70	36346768	108.5395	88	2659080	90	2075930
71	28946943	108.8674	89	2691216	91	2104412
72	28948240	109.1572	90	2697274	92	2149014
73	36349576	109.7543	91	2726338	93	2184712
74	36351363	110.6553	92	2770922	95	2279937
75	28946521	110.9511	93	2806612	94	2285045
76	36351587	111.8829	95	2903506	96	2296566
77	36346896	112.2208	94	2908613	99	2314033
78	36349314	114.9079	96	2920145	97	2317124
79	36346891	116.17	99	2937673	98	2317124

80	28947323	117.0346	97	2940780	103	2348520
81	28946361	119.96	98	2940780	102	2378370
82	28945870	120.289	103	2972226	101	2396316
83	36349879	121.1741	102	3002077	100	2404916
84	28945399	122.0944	101	3019976	104	2416715
85	36349310	123.0945	100	3028592	105	2470773
86	28945502	123.7503	104	3040389	106	2512461
87	28948397	124.0552	105	3094483	107	2574226
88	28947132	124.935	106	3136364	109	2599192
89	28946994	126.1048	109	3232477	108	2878600
90	28948136	127.014	108	3461560	115	2891671
91	28946952	127.915	115	3474630	114	2907177
92	36348483	129.1771	114	3490159	110	2931117
93	36348662	133.889	110	3514596	113	2944250
94	28945588	134.2035	113	3527685	112	2981984
95	28948693	134.4917	112	3565412	116	2994162
96	36349730	136.51	116	3577591	111	2997249
97	36350745	136.8095	111	3580752	117	3025939
98	36350746	137.7589	117	3609441	118	3043251
99	36349050	140.1914	118	3626679	119	3047913
100	36346777	141.7128	119	3631341	120	3063488
101	28947125	142.0407	120	3646873	121	3064403
102	28945620	142.3392	121	3647788	124	3078124
103	36345805	143.3632	124	3661729	122	3078458
104	36349803	147.1636	122	3662063	123	3086916
105	36346516	147.7946	125	3667045	126	3107425
106	28947252	150.2281	123	3670524	127	3136235
107	36346581	150.5257	126	3691016	128	3149319
108	28947799	151.1488	127	3719823	132	3161843
109	36347879	151.4663	128	3732926	130	3176027
110	36346683	151.7721	132	3745439	135	3212765
111	36349949	152.3638	130	3759621	134	3214011
112	100332252	152.6562	131	3777585	133	3259709
113	28948263	152.9799	135	3802272	136	3308405
114	36347878	153.3004	134	3803509	137	3352813
115	28947556	153.5869	133	3849330	138	3356241
116	28946245	154.7636	136	3985143	139	3400743
117	28946035	155.3571	137	4024670	140	3402563
118	28946951	155.9706	138	4028099	142	3451235
119	36349588	156.3006	139	4072755	143	3484601
120	36347594	156.6212	140	4074577	147	3484601
121	36350725	157.5195	141	4099350	146	3485292
122	36348879	157.8262	142	4124938	145	3488468
123	36346691	158.132	143	4158103	144	3503878
124	28948410	158.7068	147	4158103	149	3519392
125	28946874	160.1357	146	4158808	148	3534044
126	36349945	161.9438	145	4161901	150	3534044
127	36346592	162.5535	144	4177324	151	3567852
128	28946240	163.755	148	4207551	152	3582971
129	36349287	164.3556	150	4207551	153	3635238
130	36350213	165.5791	151	4241364	154	3660673
131	36351724	166.6173	152	4341342	155	3675705

132	36345712	168.3014	153	4528823	156	3705144
133	36346113	169.2303	154	4554341	157	3713899
134	36349462	169.5262	155	4569376	158	3729840
135	36346686	171.3021	156	4598795	161	3752558
136	36347868	172.7393	157	4607583	160	3754143
137	28948978	173.0588	158	4623536	162	3768230
138	36348524	174.7095	159	4626128	163	3786621
139	36350798	175.0191	161	4646135	164	3795772
140	28945586	177.6531	160	4647719	168	3852145
141	28945364	178.2397	162	4709550	165	3852282
142	36350672	178.5436	163	4727951	167	4162428
143	36350816	179.1553	164	4737000	166	4179717
144	28947659	179.4574	168	4793710	169	4220888
145	28945354	179.766	165	4793847	171	4237553
146	36348428	180.1145	167	4956981	172	4312116
147	36350817	182.9645	166	4974230	173	4329209
148	28946187	184.2306	169	5015459	180	4340150
149	36350589	184.8138	171	5032183	174	4348410
150	36349580	186.574	172	5049744	175	4359322
151	28948407	187.1554	173	5066839	179	4359322
152	36348623	187.4428	180	5077773	178	4360735
153	28946737	188.3049	174	5086039	176	4362659
154	28948883	188.8932	175	5096957	177	4362659
155	36348451	190.6744	179	5096957	181	4369396
156	28949113	191.5568	178	5098370	183	4378506
157	36348120	192.1349	176	5100294	184	4411475
158	28948251	192.4388	177	5100294	186	4413961
159	36346053	193.3649	181	5107041	188	4467086
160	28946554	193.6697	183	5116230	189	4486932
161	36350840	193.9647	184	5149165	190	4530001
162	36350397	194.2597	186	5151650	191	4530001
163	28947041	194.8732	188	5515494	193	4538865
164	36348406	198.2526	189	5535344	194	4543437
165	28945706	198.552	192	5578635	196	4580442
166	28946440	198.8735	190	5579160	197	4595508
167	36351620	199.222	191	5579160	199	4608928
168	28947367	200.6064	193	5588048	198	4609564
169	28946009	200.9239	194	5592616	201	4612134
170	36350385	201.8115	195	5603364	200	4612135
171	36350631	203.8358	196	5629842	202	4618195
172	36348308	204.713	197	5644909	203	4636011
173	36348708	205.008	199	5658313	205	4644421
174	28945341	205.6234	198	5658947	204	4666990
175	36349941	206.2408	201	5661512	206	4707799
176	36349980	206.5484	200	5661513	207	4721580
177	36349981	206.869	202	5667613	209	4792253
178	36349434	207.5424	203	5685635	210	4803470
179	28945671	208.5041	205	5694061	212	4853167
180	36349614	209.0838	204	5716646	#N/A	
181	36347759	209.3762	206	5757394	#N/A	
182	28950152	209.6848	207	5780239	#N/A	
183	36350501	210.0238	208	5824748	#N/A	

184	28947977	210.3605	209	5850941	#N/A	
185	28949056	211.2456	210	5863752	#N/A	
186	28947272	212.7845	212	6137080	#N/A	
187	36349583	213.1258	#N/A		#N/A	
188	28945984	214.126	#N/A		#N/A	
189	28947377	217.5689	#N/A		#N/A	
190	36351267	217.8785	#N/A		#N/A	
191	28947316	218.1834	#N/A		#N/A	
192	28945644	218.4855	#N/A		#N/A	
193	28948454	219.0844	#N/A		#N/A	
194	28947358	220.3268	#N/A		#N/A	
195	36348512	224.0722	#N/A		#N/A	
196	100281708	225.3185	#N/A		#N/A	
197	36346753	226.2305	#N/A		#N/A	
198	36351160	227.7461	#N/A		#N/A	
199	36348410	229.0003	#N/A		#N/A	
200	28945746	229.984	#N/A		#N/A	
201	36346810	231.1927	#N/A		#N/A	
202	36348217	232.0674	#N/A		#N/A	
203	28949866	232.6506	#N/A		#N/A	
204	36347639	233.5882	#N/A		#N/A	
205	28945950	236.0206	#N/A		#N/A	
206	36346156	236.7224	#N/A		#N/A	
207	28948984	240.4561	#N/A		#N/A	
208	28948268	246.2139	#N/A		#N/A	
209	36347618	248.6623	#N/A		#N/A	
210	28948057	251.031	#N/A		#N/A	
211	36346914	254.3095	#N/A		#N/A	
212	36350252	254.3096	#N/A		#N/A	
*Pop37*_Chr06			W1-1		SG1	
Marker order	Marker ID	cM	Marker order	Base pair	Marker order	Base pair
1	28947322	0.304	1	36435	1	81983
2	36346717	0.6126	4	173298	4	97290
3	41805818	4.1328	5	184861	6	109709
4	36346529	7.8664	6	185736	9	120250
5	28945753	9.157	9	196338	8	132404
6	36345819	13.1389	8	208480	7	132833
7	28949784	16.2789	7	208910	11	193210
8	36350872	16.6361	11	269363	10	195160
9	28946061	22.0529	10	271316	12	207217
10	36348536	23.327	12	283392	13	213739
11	28946447	23.6273	13	289914	14	222842
12	36351294	23.925	14	320319	15	247386
13	28947511	27.127	15	344718	16	261066
14	28950076	29.9873	16	358403	17	266397
15	36349572	32.5291	17	363732	18	272036
16	28947767	33.6854	18	369154	19	281180
17	28946871	34.2989	19	378330	20	285429
18	36345802	34.9125	20	382583	22	347511
19	28947346	35.2183	22	445282	23	357965
20	36346766	35.5269	23	455736	24	384880

21	36352236	41.122	24	482637	26	423808
22	28947286	41.411	27	586702	27	508578
23	28946688	41.7051	25	700912	25	585334
24	28945947	41.9967	28	767821	28	652232
25	36347649	43.7993	29	780541	29	664959
26	36345908	44.4302	30	880427	30	752809
27	36346016	46.0181	31	1346732	31	1118628
28	36348019	46.9245	32	1357243	32	1129152
29	28947935	49.2316	33	1393607	33	1165329
30	28945633	49.8047	34	1399552	35	1171268
31	28947382	51.8289	40	1423047	40	1194740
32	28946854	52.7219	41	1428713	41	1200395
33	36347958	53.0268	38	1430476	38	1202167
34	36348946	53.3494	37	1431001	37	1202692
35	36345685	56.2843	36	1443505	36	1215198
36	36348379	56.621	39	1443505	39	1215198
37	36348261	57.3564	42	1458378	42	1230068
38	36346437	60.8369	43	1518752	43	1290358
39	36348378	63.57	44	1536875	44	1308846
40	28945304	65.6119	48	1558514	48	1330546
41	28948465	66.7682	47	1560246	47	1332253
42	28949628	72.6779	46	1571301	46	1343333
43	36350794	74.6206	49	1581117	45	1363004
44	36346447	75.6343	45	1591102	#N/A	
45	36348500	75.9569	#N/A		#N/A	
46	36345676	78.4747	#N/A		#N/A	
47	36348991	79.4008	#N/A		#N/A	
48	36346730	79.7265	#N/A		#N/A	
49	36346765	79.7266	#N/A		#N/A	
*Pop37*_Chr07			W1-1		SG1	
Marker order	Marker ID	cM	Marker order	Base pair	Marker order	Base pair
1	28946604	0.3068	1	612111	1	192936
2	36349817	2.6895	2	612176	2	193001
3	28949762	3.6271	4	626170	4	206978
4	28948134	6.9858	5	657312	7	265121
5	36346353	7.8814	9	728943	9	309397
6	28950108	10.9528	10	745063	10	325456
7	36346950	12.5256	11	750815	11	363720
8	28948096	13.1606	12	757968	12	386867
9	36351321	13.7876	13	813200	13	420148
10	36346770	15.6289	14	867415	14	474749
11	28945255	16.2224	15	901498	15	564421
12	28947338	17.0772	16	935352	17	598273
13	28947956	22.2788	17	935352	19	621342
14	36346952	22.5846	18	947506	20	650519
15	28948639	24.0556	19	958432	21	698273
16	28949724	24.3596	20	987645	23	759159
17	36346936	24.962	21	1035393	22	766565
18	36350393	25.5451	23	1106110	25	869151
19	28948409	26.1404	22	1113516	27	877710
20	28949415	30.6984	24	1206108	26	899982

21	36348590	36.5733	25	1216763	28	940019
22	28946513	37.2042	27	1225313	30	1043363
23	28948749	42.0113	26	1247571	31	1066171
24	36346272	46.5693	28	1287851	32	1084798
25	36346926	47.8558	30	1390960	33	1111276
26	28949782	50.4304	31	1413762	34	1127346
27	28949127	52.7921	32	1432253	35	1160344
28	36349734	53.6931	33	1547585	36	1225554
29	36351745	55.1728	34	1563660	37	1241680
30	28947155	56.0399	35	1596720	38	1272349
31	36347545	56.6572	36	1661973	39	1300527
32	28946613	57.5833	37	1678132	40	1318554
33	36348394	57.8967	38	1708877	47	1358288
34	36346858	58.8523	39	1737112	48	1360080
35	36346878	61.2279	40	1755154	50	1384331
36	28948518	61.5153	47	1795112	41	1642547
37	36347665	61.8202	48	1796909	49	1780680
38	28948278	62.1438	50	1821250	42	1792382
39	36350843	62.7592	41	1950024	44	1822863
40	36347842	63.339	49	2328709	43	1824688
41	28945985	63.6272	42	2340292	51	1858505
42	28948660	64.2103	45	2345384	52	1892931
43	28947444	64.5504	44	2371067	54	1906035
44	36348651	67.0797	43	2372892	53	1944298
45	36346323	67.7269	51	2406751	55	1950905
46	28948595	69.5739	52	2441127	56	1950907
47	28947326	71.7358	54	2454001	57	1962273
48	36346637	73.0606	53	2492166	58	2008484
49	41805640	73.3928	55	2498776	59	2042410
50	36346771	77.7081	56	2498778	60	2118522
51	28945849	81.4186	57	2510167	61	2123019
52	28946875	81.7119	58	2556386	63	2129292
53	28948928	82.0254	59	2590866	62	2137268
54	36345860	83.6337	60	2666924	68	2137273
55	36349791	83.9648	61	2671421	64	2154471
56	36349792	84.2725	63	2677694	67	2154471
57	28947469	84.559	62	2685655	66	2154536
58	28948434	85.1338	68	2685660	65	2156605
59	36349897	91.3235	64	2702850	69	2161781
60	28949730	91.6194	67	2702850	71	2206436
61	36348782	93.1035	66	2702915	72	2206436
62	36346408	93.4151	65	2704980	73	2225964
63	36345705	94.0602	69	2710159	74	2247883
64	36350677	94.7452	71	2754842	76	2254200
65	36346198	95.0617	72	2754842	77	2256056
66	28946498	95.3822	73	2774435	78	2272302
67	28945928	96.0445	74	2796345	79	2276017
68	36346805	97.9441	76	2803366	83	2295438
69	28947843	100.5476	77	2805222	82	2297554
70	28949951	103.3898	78	2821942	80	2302444
71	28945295	103.7043	79	2825657	81	2305006
72	36347835	107.524	83	2845079	84	2331710

73	28946738	108.6938	80	2852062	85	2335131
74	36351186	109.2838	81	2854639	86	2341934
75	28947400	110.4502	84	2881396	#N/A	
76	28948460	110.7677	85	2885031	#N/A	
77	36345844	111.7387	86	2891845	#N/A	
78	28949379	112.0592	#N/A		#N/A	
79	36348990	113.749	#N/A		#N/A	
80	28949631	114.378	#N/A		#N/A	
81	36347533	114.9715	#N/A		#N/A	
82	36348955	115.29	#N/A		#N/A	
83	36346756	119.6053	#N/A		#N/A	
84	36348608	120.57	#N/A		#N/A	
85	28948910	121.1514	#N/A		#N/A	
86	28949551	121.1515	#N/A		#N/A	
*Pop37*_Chr08			W1-1		SG1	
Marker order	Marker ID	cM	Marker order	Base pair	Marker order	Base pair
1	36346627	0.637	4	474200	1	54382
2	28945978	0.9466	2	668580	5	182255
3	28946817	1.5383	1	778534	8	244875
4	36346579	2.1266	5	803753	7	259871
5	28950166	4.7529	8	887585	6	260781
6	36350523	5.0616	7	905417	9	274993
7	28948972	5.6885	6	910024	10	301182
8	28947643	6.2965	9	948668	11	359153
9	28945819	7.4461	10	975086	15	658058
10	36350740	7.7402	11	1031764	14	730910
11	36347897	9.3433	13	1048360	13	749924
12	28949846	11.231	12	1265810	12	913590
13	28946729	11.5313	17	1284872	17	932683
14	36345758	11.8381	18	1312890	18	960698
15	36346057	13.9478	20	1348148	20	995955
16	36346479	14.8767	19	1376229	19	1023975
17	28945509	16.7124	21	1391665	21	1039411
18	28949106	20.7557	22	1397973	22	1045737
19	28949209	21.0682	23	1403381	23	1051145
20	36348042	22.3185	24	1494301	24	1133320
21	28947787	22.8949	25	1513615	25	1152621
22	28947058	23.7595	26	1563392	26	1202284
23	28946907	30.1901	27	1567984	27	1207189
24	28947674	31.6786	28	1586920	28	1226116
25	36347839	33.2082	29	1602031	29	1241226
26	36345660	33.5049	30	1604896	30	1244095
27	36351136	33.8089	31	1616206	31	1255428
28	36350071	34.1137	32	1631873	32	1271093
29	28946265	35.0067	33	1667661	33	1312199
30	36345703	38.0133	34	1685112	34	1329643
31	36349684	39.2294	35	1703251	35	1347775
32	28947937	42.8186	37	1741918	37	1378118
33	28945657	45.8022	36	1743746	36	1379920
34	36345970	46.9965	38	1745431	38	1381605
35	36348680	48.4255	39	1776477	39	1412597

36	36350034	48.7162	40	1779226	40	1415342
37	28946530	49.022	41	1816580	41	1453923
38	36346930	50.5657	42	1827833	42	1465154
39	28947885	52.2846	43	1851297	43	1487752
40	36346154	53.288	45	1878861	45	1516573
41	36349770	55.4907	46	1897588	46	1535108
42	36345899	57.7724	47	1904662	47	1542164
43	36345894	58.7159	48	1911414	48	1548927
44	36346498	59.3008	49	1937875	49	1575416
45	28947818	59.8788	50	2111921	50	1689418
46	28948275	61.316	54	2111921	54	1689418
47	36348689	61.6093	55	2997497	55	1889160
48	36348591	63.3799	53	3068419	53	1959878
49	28946642	64.5923	52	3080636	52	1972120
50	28947236	64.9019	57	3170375	57	2041430
51	36349042	65.1986	58	3179065	58	2049815
52	36346099	65.5161	59	3185886	59	2056636
53	36348589	66.1676	64	3204662	64	2075473
54	36345951	66.7985	60	3211603	60	2082587
55	28949194	67.3851	62	3214341	62	2085283
56	36350438	68.2701	63	3216214	65	2099413
57	28948406	68.8549	61	3217251	66	2128805
58	36347911	69.1517	65	3228485	67	2141715
59	28945556	69.7505	66	3257916	68	2155149
60	36350185	70.3855	67	3270847	69	2188069
61	36346949	71.0328	68	3284281	70	2188069
62	36349556	71.3472	69	3315525	73	2204154
63	36346927	71.6422	70	3315525	74	2209608
64	36346394	71.9321	73	3331661	77	2209608
65	28947140	72.2219	74	3337162	75	2264758
66	36349453	72.5126	77	3337162	76	2282999
67	28948754	74.2729	75	3392311	78	2463181
68	28945966	76.0647	76	3410533	72	2479584
69	36347659	76.3606	78	3780665	71	2502539
70	36347658	78.1105	72	3797043	79	2543330
71	28947610	78.4127	71	3819947	81	2574601
72	36350697	79.0377	79	3860724	82	2575663
73	36346580	79.3542	82	3907344	80	2578595
74	36346706	79.6657	80	3910282	83	2693889
75	28945896	79.9589	85	3953116	84	2703475
76	28946856	80.2714	84	3962680	86	2723704
77	28946381	80.8907	86	3982857	87	2764005
78	28947618	81.4877	87	4023277	88	2777293
79	36348065	85.3761	88	4036560	89	2779985
80	36349715	85.6755	89	4039245	90	2786993
81	36350904	86.0755	90	4046257	91	2818792
82	28946207	88.5265	91	4077797	93	2889353
83	36348927	89.14	92	4114650	94	2892572
84	36353420	89.4421	93	4152060	95	2925360
85	28946348	89.7452	94	4155277	97	2973928
86	28945743	90.9466	95	4188014	96	2976572
87	28945903	91.2469	97	4230004	98	2985137

88	28946732	91.5351	96	4232648	101	2985137
89	28947672	92.2842	98	4241148	99	2985352
90	41804206	94.9382	101	4241148	103	3005327
91	28946492	97.417	99	4241363	104	3034700
92	28950231	98.6443	103	4261339	102	3060013
93	28946434	99.2752	104	4291670	105	3077698
94	36347660	101.801	102	4316786	106	3092215
95	36349437	102.0876	106	4349967	107	3130281
96	36347597	102.3962	107	4388051	108	3179690
97	36346664	102.7219	108	4437614	109	3182117
98	36349686	103.0404	109	4440045	110	3206937
99	28946840	103.3311	110	4464918	112	3243925
100	36351016	103.9409	112	4501979	113	3246774
101	28946557	105.4942	113	4504817	114	3287906
102	28945554	106.729	114	4545917	115	3294723
103	36347661	107.0206	115	4552938	116	3300840
104	28945288	107.3138	116	4559078	117	3315875
105	36348890	109.3618	117	4574134	118	3348740
106	28948907	110.2264	118	4607456	119	3389258
107	28948082	111.2037	119	4647983	120	3399030
108	36345662	111.5284	120	4657753	121	3403537
109	28946288	112.6847	121	4662258	122	3474270
110	36349566	113.2747	122	4685287	123	3475593
111	36346712	113.8612	123	4686610	124	3579553
112	28948231	115.2984	124	4790660	125	3584617
113	36348202	116.4547	125	4795705	126	3644762
114	28945422	116.7454	126	4855795	127	3669736
115	28947704	117.9016	127	4880727	128	3698159
116	28946919	118.5427	128	4909159	129	3763750
117	36346386	119.1838	129	4974708	132	3809818
118	36347694	121.7873	132	5020732	130	3820176
119	36348044	122.0797	130	5031094	134	3933380
120	36348049	122.7047	133	5121704	135	3970227
121	36349452	123.3458	134	5137703	136	3984579
122	36348897	123.6573	135	5174522	138	4056673
123	28947972	124.5476	136	5190513	139	4080568
124	28947539	125.1256	137	5223069	141	4080568
125	28946205	125.4305	138	5262592	140	4133804
126	36347607	125.7373	#N/A	5276503	147	4195622
127	36350543	126.8969	139	5309158	148	4243562
128	28947590	127.4733	141	5309158	145	4251168
129	36348234	129.7938	140	5353602	144	4263440
130	36347718	130.0837	147	5529872	149	4269221
131	28946792	132.2794	148	5577943	143	4284027
132	28945363	134.2348	145	5585546	146	4316628
133	36349596	136.0933	144	5600584	150	4331596
134	36348334	136.3814	143	5620971	152	4352777
135	28946861	137.2486	142	5645050	151	4358380
136	36349046	138.9784	146	5653572	153	4385751
137	28949891	143.0119	150	5668550	154	4420785
138	36345872	143.3355	152	5689810	155	4545015
139	36347964	144.9233	151	5698704	157	4647899

140	28946175	148.3236	153	5725945	156	4658799
141	36347965	153.2253	154	5761207	160	4711299
142	36350274	153.5586	155	5792850	161	4768710
143	28945708	156.1249	157	5936127	162	4788442
144	28949835	158.3276	156	5947033	163	4811243
145	36347585	160.5029	160	5999571	165	4905817
146	28946704	161.3651	161	6007171	167	4916765
147	28947925	161.9881	162	6026730	164	4919725
148	36348014	162.3006	163	6049592	166	4919725
149	36348415	163.2531	165	6175967	170	4941696
150	36350611	166.5803	167	6186842	172	4959218
151	36346938	166.9125	164	6189805	171	4976837
152	36346460	167.5435	166	6189805	174	5082263
153	36351265	169.864	170	6211777	176	5082267
154	36348740	170.7438	172	6229303	173	5089755
155	28946560	173.2538	175	6333503	177	5099367
156	28945919	173.8828	174	6349330	178	5112821
157	36348627	175.4312	176	6349334	181	5136504
158	36350386	176.363	173	6356824	180	5142055
159	36349244	177.5328	177	6366404	184	5161262
160	28950119	178.1176	178	6379857	183	5166196
161	36350090	179.5974	179	6388412	182	5170996
162	36349857	181.4443	181	6403501	185	5216727
163	28948445	186.6142	180	6409053	186	5257520
164	36348791	186.9032	184	6428226	187	5262112
165	28948106	187.2237	183	6433153	188	5273404
166	36348790	187.556	182	6437931	189	5283735
167	36346034	189.4556	185	6483545	190	5285988
168	36350428	190.0599	186	6523529	191	5303874
169	36349239	190.9321	187	6528292	192	5330958
170	28948346	191.5571	188	6539533	193	5335567
171	36348993	191.8766	189	6549854	#N/A	
172	36346785	193.5722	190	6552101	#N/A	
173	36348226	194.5893	191	6569974	#N/A	
174	36345945	195.4743	192	6596227	#N/A	
175	36348767	196.0804	#N/A		#N/A	
176	36346758	197.6146	#N/A		#N/A	
177	36349852	198.4971	#N/A		#N/A	
178	28948652	199.3718	#N/A		#N/A	
179	36350545	202.6021	#N/A		#N/A	
180	28947300	202.9107	#N/A		#N/A	
181	28946215	204.8661	#N/A		#N/A	
182	36349531	206.784	#N/A		#N/A	
183	28949012	207.0907	#N/A		#N/A	
184	36350783	216.8398	#N/A		#N/A	
185	36347716	219.4893	#N/A		#N/A	
186	36348347	220.0707	#N/A		#N/A	
187	28948112	220.659	#N/A		#N/A	
188	28947481	221.5415	#N/A		#N/A	
189	36348325	222.7011	#N/A		#N/A	
190	28946815	223.0165	#N/A		#N/A	
191	36348963	229.7447	#N/A		#N/A	

192	36350229	230.0612	#N/A		#N/A	
193	36346769	230.0613	#N/A		#N/A	
*Pop37*_Chr09			W1-1		SG1	
Marker order	Marker ID	cM	Marker order	Base pair	Marker order	Base pair
1	36348957	0.6452	7	476594	7	75371
2	28949238	1.8015	6	492038	5	132254
3	28946611	2.388	5	533347	4	146991
4	28947473	2.9745	4	548061	3	161078
5	36350573	4.5135	3	561231	9	188180
6	28949660	4.8632	9	588000	10	202990
7	28947207	5.208	10	614084	11	245928
8	36349284	8.1613	11	628463	2	246206
9	28947554	8.7444	2	628741	1	248949
10	28948150	9.3896	1	631483	14	330985
11	28949657	9.7143	12	645242	13	356760
12	36350663	10.3095	14	685053	21	467875
13	28949093	11.552	13	710685	15	473133
14	36347982	17.0402	21	807352	20	476518
15	36345667	17.3577	15	812576	19	497735
16	36346286	17.6771	20	815968	18	502895
17	36349712	17.9766	19	837176	17	505180
18	28946882	18.2623	18	842329	22	507712
19	36348559	18.5505	17	844614	16	519093
20	28945838	19.4408	22	847146	23	548578
21	28946276	20.6174	16	858531	24	699097
22	28946157	21.561	23	887995	25	702117
23	36347985	22.532	24	952297	27	734055
24	36350769	23.4844	25	955322	28	734586
25	36346635	28.8851	27	987231	26	769405
26	36346755	29.5141	28	987772	29	780598
27	36348803	29.8126	26	1021939	30	802843
28	36350779	30.6698	29	1033141	31	841710
29	28946661	31.8396	30	1055386	32	876223
30	36350521	35.0604	31	1094283	33	901217
31	28947147	36.5402	32	1171258	34	916568
32	36348095	37.139	33	1196189	36	956496
33	36349927	42.1057	34	1211494	37	956496
34	28945218	47.2829	36	1251226	35	978963
35	36346542	47.6326	37	1251226	38	1001753
36	28948536	47.9311	35	1273721	41	1005083
37	36352131	48.8716	38	1297272	43	1020229
38	36345766	49.487	41	1300607	39	1020599
39	28948243	50.3492	43	1315764	42	1029905
40	100311996	51.2342	39	1316134	40	1055319
41	28945996	51.5953	42	1325452	45	1066889
42	36346129	51.9769	40	1351129	47	1077937
43	28945563	52.9906	45	1362697	46	1083729
44	36346772	54.3608	47	1373719	49	1110041
45	36350800	57.194	46	1379511	48	1126703
46	36349435	57.7771	49	1405838	52	1188238
47	28948667	59.2226	52	1443820	50	1212268

48	36350717	60.1374	50	1467848	51	1221718
49	41804965	61.9674	51	1477298	53	1244966
50	36348476	62.2548	53	1500532	54	1264166
51	28947727	63.8081	54	1519737	55	1282682
52	36346119	65.0782	55	1538259	56	1327233
53	28950088	66.5938	56	1584653	62	1337030
54	36347887	68.375	62	1594426	57	1337033
55	36347691	73.3476	57	1594429	63	1354675
56	28948223	73.95	63	1612081	58	1354676
57	28947498	74.2737	58	1612082	61	1367397
58	36345840	75.2606	61	1624799	60	1379708
59	36346098	76.8434	60	1637151	59	1398506
60	28947636	77.1511	59	1655945	68	1430456
61	28946702	78.0659	66	1679498	66	1450708
62	28945447	78.7195	65	2184617	65	1803453
63	36346557	81.7231	67	2238857	67	1856543
64	36346629	82.0531	64	2270283	64	1888050
65	28945908	83.0179	70	2270348	70	1888112
66	28946982	83.3061	71	2309120	71	1906757
67	28947961	83.6028	72	2342946	72	1937866
68	36346011	83.9031	74	2603650	74	2005272
69	28949077	84.1955	76	2616297	76	2017918
70	28949346	85.0702	75	2643211	75	2043505
71	36349736	87.1063	78	3008642	79	2082777
72	28947115	88.5948	#N/A		78	2093550
73	28946113	90.1385	#N/A		#N/A	
74	36347728	91.7314	#N/A		#N/A	
75	36349716	92.3684	#N/A		#N/A	
76	28948413	92.6582	#N/A		#N/A	
77	36351047	94.6825	#N/A		#N/A	
78	28947470	95.5916	#N/A		#N/A	
79	36346461	95.5917	#N/A		#N/A	
*Pop37*_Chr10			W1-1		SG1	
Marker order	Marker ID	cM	Marker order	Base pair	Marker order	Base pair
1	36346903	0.6645	2	58194	2	144146
2	36346882	1.608	4	88073	4	185718
3	28947075	2.1861	5	93453	5	191098
4	28947397	2.5056	6	93851	6	191496
5	36346674	2.8705	1	97694	1	195309
6	36346752	4.7441	7	97694	7	195309
7	36346404	7.0258	8	103577	9	201196
8	28948447	7.3442	10	150815	10	248983
9	36350675	20.4918	11	189893	11	287613
10	28949410	24.1457	12	193527	12	291249
11	36346082	24.4469	13	195379	13	293101
12	28947224	25.0386	16	216083	15	301065
13	28948270	28.0007	17	216554	14	301130
14	36348965	28.2992	18	223611	16	313937
15	36346754	29.1975	19	224088	17	314409
16	28948094	29.489	20	233394	18	321474
17	36349500	32.3908	21	278381	19	321951

18	28945894	32.6815	22	322497	20	331290
19	36349465	34.7355	23	343860	21	375422
20	28948384	41.5016	24	487028	22	419610
21	28948435	43.5259	25	513101	25	478075
22	28946487	46.1676	26	576578	26	537812
23	28948546	47.0553	30	610936	31	539882
24	36345936	49.6893	29	616109	30	571987
25	28946659	50.604	27	727908	29	577101
26	28945841	51.2775	34	863213	28	587688
27	36346524	51.6119	35	893280	27	927208
28	36346560	51.9304	33	932363	34	946364
29	36346660	53.1652	36	936605	35	966640
30	28948257	55.0237	37	962811	33	1005848
31	36345992	56.3354	32	965450	36	1010075
32	41804214	57.9386	38	973679	37	1036270
33	28947265	58.2267	39	985425	32	1038905
34	28947687	58.5166	40	1024844	38	1047134
35	28948154	58.8206	44	1024844	39	1058828
36	36348760	59.7297	43	1029421	40	1098043
37	28947603	60.03	41	1080743	44	1098043
38	36346506	60.9338	45	1082707	43	1102621
39	28947006	62.4223	49	1141250	41	1153568
40	28946220	63.3315	46	1197438	45	1155527
41	28945257	63.6318	48	1208744	49	1438794
42	28949616	63.9242	51	1210399	46	1762763
43	28947851	66.6877	50	1211809	48	1774083
44	36348005	67.0156	47	1236125	51	1775738
45	36345888	67.6846	52	1236125	50	1777151
46	36346367	68.3256	53	1241815	47	1801497
47	36350070	69.8231	54	1256159	52	1801497
48	28947189	70.4113	55	1285941	53	1807203
49	36348078	71.7019	56	1295097	54	1821427
50	28946331	72.0235	57	1386733	55	1851182
51	28946040	72.6564	58	1453355	56	1860412
52	28945724	72.9719	59	1508413	57	1913423
53	28946051	73.2731	61	1691272	58	2008283
54	36350127	74.1795	62	1713224	59	2063095
55	28949383	74.4745	64	1719522	61	2239921
56	28947339	75.6209	63	1924661	62	2261814
57	28946683	77.3358	65	1952836	64	2268112
58	28946131	79.0858	66	1970888	63	2571955
59	28949452	80.2695	67	1994211	65	2600099
60	28949317	81.7943	68	2003917	66	2618205
61	28947389	82.0955	69	2023119	67	2641541
62	28947128	82.3977	70	2030321	68	2651243
63	36351591	83.0001	71	2043344	69	2670129
64	28947894	83.5782	72	2043344	70	2677393
65	28947630	84.4428	73	2061493	71	2690571
66	36349067	85.6195	74	2069129	72	2690571
67	36348013	86.2256	75	2150276	73	2708659
68	36347832	87.7693	#N/A		75	2720455
69	36349797	91.8407	#N/A		#N/A	

70	36348729	93.705	#N/A		#N/A	
71	36346967	94.3339	#N/A		#N/A	
72	36347849	95.5574	#N/A		#N/A	
73	28948651	96.1474	#N/A		#N/A	
74	28945403	97.3206	#N/A		#N/A	
75	36350050	97.3207	#N/A		#N/A	
*Pop37*_Chr11			W1-1		SG1	
Marker order	Marker ID	cM	Marker order	Base pair	Marker order	Base pair
1	28945322	0.3135	1	109004	4	58446
2	36346728	4.8995	4	111740	5	60306
3	36346663	5.5056	5	113600	1	65129
4	28945717	5.8152	#N/A	149048	6	250568
5	28945140	6.1146	7	200745	7	277988
6	36351344	8.1746	8	235921	8	313236
7	28948432	10.4817	9	241491	9	318802
8	36349809	11.6904	10	252339	10	329457
9	36347703	12.3174	13	256488	13	333596
10	36346565	13.2321	14	264779	14	341889
11	36346575	13.5219	12	277816	12	354911
12	28947032	14.7198	19	363304	11	358480
13	36351335	15.0466	17	487067	15	394230
14	36348458	18.3408	15	593148	20	432173
15	28945461	18.6573	16	930042	21	432173
16	36345831	19.5637	20	955736	22	500250
17	36352440	19.8649	21	955736	24	514245
18	36352928	20.1643	22	1043466	23	516338
19	36351841	21.3992	24	1057421	25	524965
20	36350556	21.704	23	1059514	26	538775
21	28947807	24.2851	25	1068112	28	563634
22	28946735	24.8876	26	1081905	27	585461
23	36346059	25.1924	28	1106805	29	604482
24	36348250	28.137	27	1128633	30	660163
25	36348297	30.5444	29	1147649	31	677385
26	36347800	32.6962	30	1203353	32	700846
27	28947593	33.0068	31	1220591	33	790932
28	28946555	33.8832	32	1244034	34	877376
29	28945715	36.4943	33	1363227	35	902737
30	36350583	36.7992	34	1393963	36	934854
31	36349781	39.0515	35	1419351	37	974406
32	36348645	41.22	36	1451414	38	995873
33	36349639	43.0012	37	1593053	39	1060315
34	36348853	48.1642	38	1614534	41	1111240
35	36345715	51.9911	39	1679092	42	1124714
36	36346796	54.2434	41	1730005	43	1141001
37	36347680	54.5384	42	1743461	44	1143578
38	28947305	55.1115	43	1759754	45	1185000
39	36350741	55.698	44	1762334	47	1262481
40	36349275	57.7888	45	1801400	49	1262481
41	36352496	61.8223	47	1870507	48	1301658
42	36345723	62.1428	49	1870507	50	1322309
43	36345913	62.4623	48	1917523	51	1387549

44	36350400	66.0398	50	1941303	52	1440997
45	36351194	69.0555	51	2099740	53	1517245
46	36346815	70.3673	52	2153190	54	1536430
47	36350787	72.9254	53	2527472	56	1541420
48	36347662	75.3739	54	2546677	55	1572820
49	36350786	77.8681	56	2551673	57	1594074
50	28947865	79.4868	55	2583074	58	1607028
51	36347610	80.1558	57	2604083	59	1632338
52	36348989	81.0818	58	2617003	60	1649243
53	28946130	81.7068	59	2642483	61	1684761
54	36351306	84.549	60	2659323	63	1700819
55	28949788	86.1072	61	2694750	67	1714788
56	36346795	88.7586	62	2710864	65	1728566
57	36346762	90.1242	67	2724828	64	1735445
58	36349427	91.419	65	2738609	66	1787710
59	36350233	92.3365	64	2745498	68	1795774
60	28949088	94.9782	66	2797750	80	1826003
61	28950234	95.2804	68	2805583	81	1831952
62	36347696	95.5968	80	2836029	69	1841730
63	36346540	96.8916	81	2841983	74	1886133
64	36346608	97.2195	69	2912239	79	1903835
65	36346675	97.5452	74	2956651	70	1911472
66	36348816	98.4977	79	2973982	71	1917962
67	28947834	101.3827	70	2981643	72	2225238
68	36349498	104.1463	71	2988118	75	2227605
69	36346586	104.7694	73	3451446	#N/A	
70	36351571	105.1678	78	3483218	#N/A	
71	28947175	106.3777	72	3567170	#N/A	
72	36346742	107.0271	75	3569620	#N/A	
73	36350118	107.3161	76	3772727	#N/A	
74	28948778	107.8942	77	3772792	#N/A	
75	36350073	109.3523	#N/A		#N/A	
76	36348520	109.9813	#N/A		#N/A	
77	36347586	111.6	#N/A		#N/A	
78	36345739	112.5585	#N/A		#N/A	
79	36346360	113.1955	#N/A		#N/A	
80	28946573	113.5032	#N/A		#N/A	
81	36346512	113.5033	#N/A		#N/A	
*Pop37*_Chr12			W1-1		SG1	
Marker order	Marker ID	cM	Marker order	Base pair	Marker order	Base pair
1	36352523	1.1301	5	361269	3	63893
2	36348996	1.4269	6	367388	5	128854
3	36346906	1.7236	7	418227	6	134954
4	36349007	2.6301	8	442373	7	175165
5	36346885	3.2307	9	508637	8	199339
6	28950051	5.5512	10	529047	9	213609
7	28945529	9.3125	11	566373	10	234032
8	36347701	9.6586	12	596200	11	271327
9	36348695	12.3885	13	612740	12	293547
10	28948204	18.4237	14	623738	13	310095
11	36352145	19.0298	15	639344	14	321080

12	36350526	19.9201	17	648662	15	336682
13	28947913	20.5083	19	661168	17	346000
14	36348515	21.4093	18	669249	16	347190
15	36347711	21.7199	21	684446	19	358577
16	36347688	22.0276	22	692386	18	366664
17	28948663	22.609	26	694967	21	381868
18	36350785	23.5322	23	730801	22	389733
19	36348898	24.4817	27	730806	26	392312
20	36346646	26.4057	28	746404	23	428171
21	36347902	27.7089	25	768266	27	428176
22	36345910	28.6768	24	775512	28	443760
23	36348429	29.298	29	1136825	25	465587
24	36345775	30.2071	30	1216250	24	472782
25	28945829	30.7954	31	1266408	29	806777
26	28948924	31.119	32	1280178	30	875014
27	28950055	31.7642	33	1333853	31	924900
28	36348814	32.07	34	1346274	32	938687
29	36350077	33.7998	35	1374977	33	964078
30	28946466	34.9462	36	1415143	34	976406
31	28946127	35.2335	37	1422520	35	1005108
32	36348568	38.3528	38	1430276	36	1045260
33	36345654	40.2893	39	1463568	37	1052637
34	28945368	41.5053	40	1478980	38	1060396
35	36348368	42.1114	41	1518212	39	1093708
36	28945544	43.9086	42	1530623	40	1109128
37	28945284	44.201	43	1568200	41	1150374
38	28946307	45.0656	44	1597109	42	1162752
39	28945975	45.6404	45	1665394	43	1200238
40	28946998	46.5025	46	1669454	44	1229340
41	36349757	46.8047	48	1705718	45	1297398
42	36348266	47.4071	49	1736659	46	1301449
43	36346842	48.56	51	1770898	47	1337680
44	28946844	53.6218	50	1771530	49	1368668
45	36348615	54.226	52	1778025	51	1403203
46	36348073	55.1908	54	1783089	50	1403836
47	36347957	55.5043	53	1783154	52	1410350
48	28947218	57.2907	55	1802152	55	1434468
49	36347973	57.9217	56	1821362	56	1453687
50	36345694	58.2313	57	1847612	57	1479940
51	28946592	58.5399	58	1868300	58	1500599
52	36346887	59.7748	59	1950748	59	1539070
53	36346880	60.3845	60	2010851	60	1598755
54	28949713	60.9763	61	2017807	61	1605695
55	28945639	61.2704	62	2038186	62	1626318
56	36351366	61.5689	63	2089372	63	1677534
57	36348063	62.1677	64	2126574	64	1714814
58	36346857	63.6344	67	2220873	65	1730150
59	28948072	64.5016	68	2267317	67	1809061
60	28947836	64.8093	69	2294491	68	1855476
61	36348355	66.4492	70	2328147	69	1882661
62	36346848	67.7193	71	2406014	70	1916192
63	36348208	68.6096	73	2417726	71	1994076

64	36347608	68.9108	72	2450723	73	2005786
65	36347753	69.8633	75	2490539	72	2038648
66	36346922	71.1254	76	2540344	75	2077775
67	36349645	76.5357	77	2630739	76	2127596
68	28948182	77.7264	78	2662166	77	2219650
69	36348399	79.2466	79	2670938	78	2251098
70	36349475	81.7409	80	2687972	79	2259874
71	36345949	82.3451	82	2752741	80	2276908
72	36348715	82.9741	83	2786347	81	2285865
73	36349012	86.4279	84	2816173	82	2341676
74	100313000	87.0358	85	2859887	83	2375268
75	36345953	88.2518	86	2859887	84	2405374
76	28947915	89.7448	89	2880221	85	2449172
77	28946305	90.7127	87	2882883	86	2449172
78	36349669	91.9868	88	2907544	89	2469505
79	36347633	92.2827	92	3006058	87	2472167
80	28949110	92.5876	94	3034660	88	2496874
81	36351920	94.3741	97	3042427	91	2559876
82	28948986	95.5405	95	3042492	92	2595227
83	36348137	96.7068	96	3042492	94	2624403
84	28949142	96.9942	99	3066444	97	2632173
85	28945690	97.3097	98	3090586	95	2632238
86	36348529	98.6172	100	3123264	96	2632238
87	36346379	98.9429	104	3165953	100	2712990
88	36346223	99.5902	102	3188012	101	2726629
89	28945362	101.0655	105	3416220	103	2726694
90	36349242	102.3119	107	3416220	104	2757798
91	36348023	103.2644	106	3449474	102	2780047
92	36347962	103.8705	109	3473998	108	2792967
93	36346501	104.4502	110	3488885	105	2801332
94	28947578	104.7418	113	3547083	107	2801332
95	36350066	105.0523	112	3551874	106	2834582
96	28946719	105.3781	111	3801870	109	2859109
97	36348103	107.3335	114	3818573	110	2873965
98	28945799	109.8356	115	3853964	113	2932063
99	28946877	112.6422	116	3877130	112	2936833
100	36346188	113.9715	117	3902907	111	2944457
101	36346128	114.3082	118	3917272	114	2961119
102	36348233	114.6393	119	3945395	115	2988569
103	36346078	114.9405	120	3984365	117	3028236
104	28948653	118.0598	125	4053742	118	3042604
105	28948236	118.3704	127	4073344	119	3070733
106	28948866	118.6553	121	4087083	120	3109705
107	36350815	118.9556	129	4161369	125	3169251
108	28945408	120.1791	122	4217916	127	3185912
109	36346366	121.4025	123	4253394	121	3199655
110	28947845	121.9724	124	4253394	129	3255916
111	36349403	122.5897	130	4299074	122	3312550
112	36350772	123.2089	131	4307091	123	3359795
113	36347530	123.4962	133	4350354	124	3359795
114	28945953	123.802	134	4366301	130	3405421
115	36348446	124.4452	135	4376246	131	3413437

116	36350281	125.0494	136	4407795	133	3456692
117	28947561	125.9424	137	4436378	134	3472627
118	36350592	127.1366	138	4439539	135	3482608
119	28947989	129.8666	139	4478154	136	3514299
120	28945577	130.8408	140	4517649	137	3542855
121	36347002	131.8118	141	4532179	138	3546018
122	36349059	132.4528	142	4533039	139	3582547
123	36350626	133.727	143	4543429	140	3622500
124	28946888	134.0465	144	4567073	141	3637071
125	36349467	134.3691	145	4610156	142	3637931
126	36345948	134.6845	147	4620139	143	3648316
127	36350012	135.3175	146	4633564	144	3671931
128	36346496	135.6142	148	4672269	145	3715021
129	28946281	136.7738	149	4685683	147	3724998
130	28946242	137.0645	150	4690365	146	3738435
131	28948060	138.2208	151	4708549	148	3777138
132	36346474	139.3805	152	4718953	149	3790560
133	36349845	140.5334	153	4729059	150	3795241
134	28948261	141.7458	154	4729835	151	3813428
135	36348402	143.5871	155	4783278	152	3823822
136	36350245	144.1753	156	4797615	153	3833919
137	28947330	144.4695	157	4832017	154	3834695
138	36348098	145.056	161	4884964	155	3858904
139	28946586	146.1958	160	4886503	156	3873242
140	28947120	146.4823	162	4942204	157	3907650
141	28947781	146.7705	163	4961644	161	3960674
142	28947882	147.6377	165	5001210	160	3962213
143	28948352	148.2277	166	5030026	162	4019407
144	36347555	152.5195	167	5040777	163	4038466
145	36348219	154.3167	168	5097911	165	4078092
146	28946928	154.9265	169	5107258	166	4106882
147	28947197	155.867	170	5161999	167	4117640
148	28948055	156.7988	171	5179014	168	4174958
149	36350067	157.6585	172	5198201	169	4184317
150	36348499	158.5281	176	5206490	170	4209412
151	28946506	160.6003	183	5537699	171	4226415
152	36345896	163.6039	182	5539275	172	4245443
153	28949425	165.9082	173	5569770	176	4253735
154	36350687	166.2058	174	5633733	183	4398631
155	28946917	166.5034	175	5698404	182	4400201
156	28947040	169.5685	177	6016595	178	4457355
157	36346096	170.2985	178	6045428	179	4476097
158	36349232	170.617	179	6064019	181	4493767
159	28949067	170.9173	181	6081848	#N/A	
160	28947296	171.2259	180	6087410	#N/A	
161	36346793	174.7574	#N/A	6203058	#N/A	
162	36348359	175.6584	#N/A		#N/A	
163	36349494	175.956	#N/A		#N/A	
164	28949083	176.2735	#N/A		#N/A	
165	36350923	177.5396	#N/A		#N/A	
166	36346807	178.8344	#N/A		#N/A	
167	36348904	182.4442	#N/A		#N/A	

168	28949572	183.0274	#N/A		#N/A	
169	28947030	185.2094	#N/A		#N/A	
170	36347736	185.8874	#N/A		#N/A	
171	36349801	187.2391	#N/A		#N/A	
172	36346475	188.1709	#N/A		#N/A	
173	36350166	189.0719	#N/A		#N/A	
174	36346514	189.3768	#N/A		#N/A	
175	28946201	190.2751	#N/A		#N/A	
176	100241067	197.3338	#N/A		#N/A	
177	28946922	197.9492	#N/A		#N/A	
178	36348282	199.119	#N/A		#N/A	
179	36351201	199.7055	#N/A		#N/A	
180	28946886	200.9838	#N/A		#N/A	
181	36346275	210.5131	#N/A		#N/A	
182	28946413	210.842	#N/A		#N/A	
183	28945787	210.843	#N/A		#N/A	

Supplementary Table S4. Candidate effector genes and predicted proteins for QTL identified in this study

QTL[a]	Reference genome	Peak marker position[b] (bp)	Start[c]	End[d]	Gene ID[e]	Effector/non[f] effector (EffectorP)	Expression[g] profile (Ismail and Able 2016, 2017)	Protein family (pfam)	Description (pfam)	Clan (pfam)	Protein (pfam)[h]
USQV2	*W1-1*	337069	317069	357069	NA	NA					NA
USQV5		3719823	3699823	3739823	PTTW11_06575	Effector	NA	NA	NA	NA	Hypothetical protein
					PTTW11_06576	Non effector					Hypothetical protein
					PTTW11_06577	Effector	Effector	Thioredoxin	Thioredoxin	CL0172	Thioredoxin
					PTTW11_06578	Non effector					Malate dehydrogenase
					PTTW11_06579	Non effector					Zeta-crystallin
					PTTW11_06580	Non effector					Hypothetical protein
					PTTW11_06581	Non effector					Hypothetical protein
					PTTW11_06582	Non effector					Nucleotid-trans domain containing protein
					PTTW11_06583	Non effector					APG6 domain containing protein
					PTTW11_06584	Non effector					Aldehyde dehydrogenase
					PTTW11_06585	Non effector	Effector	Peptidase A4 family	Peptidase A4 family	CL0004	Acid protease
					PTTW11_06586	Non effector					Integral membrane protein
					PTTW11_06587	Non effector					BCAS2 family protein
					PTTW11_06588	Non effector					Neutral ceramidase
					PTTW11_06589	Non effector					Dipeptidase domain containing protein
USQV8		6007171	5987171	6027171	NA						NA
USQNB5.1	*SG1*	313831	293831	333831	PTMSG1_05073	Effector	NA		CFEM domain		Hypothetical protein
					PTMSG1_05074	Non effector					Hypothetical protein
					PTMSG1_05075	Non effector					Hypothetical protein
					PTMSG1_05076	Non effector					Hypothetical protein
					PTMSG1_05077	Non effector					Hypothetical protein
					PTMSG1_05078	Non effector					Hypothetical protein
					PTMSG1_05079	Non effector					Hypothetical protein
					PTMSG1_05080	Non effector					Hypothetical protein

					PTMSG1_05081	Non effector					Hypothetical protein
					PTMSG1_05082	Non effector					Hypothetical protein
					PTMSG1_05083	Non effector					Hypothetical protein
					PTMSG1_05084	Non effector					Hypothetical protein
USQNB5.2		4362659	4342659	4382659	NA	NA					NA
		4413961	4393961	4433961							
USQV9		876223	856223	896223	PTMSG1_08700	Non effector					Hypothetical protein
					PTMSG1_08701	Non effector					Methyltransferase UbiE
					PTMSG1_08702	Non effector					Hypothetical protein
					PTMSG1_08703	Non effector					mfs multidrug transporter
					PTMSG1_08704	Non effector					Structural maintenance of chromosomes protein
					PTMSG1_08705	Non effector					DUF2838 domain containing protein
					PTMSG1_08706	Non effector					NAD-binding-8 multi-domain protein
					PTMSG1_08707	Non effector					Separin
					PTMSG1_08708	Non effector					Git3 multi-domain protein
USQNB11		318802	250568	394230	PTMSG1_09701	Non effector					Zuotin
					PTMSG1_09702	Non effector					Leucine zipper protein
					PTMSG1_09703	Non effector					Serine/threonine-protein kinase SRPK2
					PTMSG1_09704	Non effector					Acetolactate synthase
					PTMSG1_09705	Non effector					TPR-16 domain containing protein
					PTMSG1_09706	Non effector					Hypothetical protein
					PTMSG1_09707	Non effector					SWAP multi-domain protein
					PTMSG1_09708	Non effector					Hypothetical protein
					PTMSG1_09709	Non effector					Hypothetical protein
					PTMSG1_09710	Effector					Dolichol-phosphate mannosyltransferase
					PTMSG1_09711	Effector					Hypothetical protein
					PTMSG1_09712	Non effector					UbiH 2-polyprenyl-6-methoxyphenol hydroxylase
					PTMSG1_09713	Non effector					Hypothetical protein
					PTMSG1_09714	Effector					Hypothetical protein
					PTMSG1_09715	Non effector					Hypothetical protein

					PTMSG1_09716	Effector					Hypothetical protein
USQV12		128854	108854	148854	PTMSG1_10196	Non effector					Hypothetical protein
					PTMSG1_10197	Effector					Hypothetical protein
					PTMSG1_10198	Effector					Hypothetical protein
					PTMSG1_10199	Non effector					Hypothetical protein
					PTMSG1_10200	Non effector					zf-MIZ multi-domain protein
					PTMSG1_10201	Non effector					Polarized growth protein Boi2
					PTMSG1_10202	Effector					Hypothetical protein
					PTMSG1_10203	Non effector					Glycosyltransferase family 31 protein
					PTMSG1_10204	Effector				CL0199	Glycoside hydrolase family 45 protein
					PTMSG1_10205	Non effector					Sulfate permease
					PTMSG1_10206	Non effector					Hypothetical protein
					PTMSG1_10207	Non effector					Hypothetical protein
					PTMSG1_10208	Non effector					Hypothetical protein
					PTMSG1_10209	Non effector					Hypothetical protein
					PTMSG1_10210	Non effector					Maf Nucleotide-binding protein implicated in inhibition septummation
					PTMSG1_10211	Non effector					NADP-dependent leukotriene B4 12-hydroxydehydrogenase
USQNB12		1916192	1896192	1936192	NA	NA					NA

[a] QTL-Quantitative trait loci identified from this study

[b] Peak marker position-Peak marker position of the QTL corresponding reference genome

[c] Start- Stating position of the QTL

[d] End - Ending position of the QTL

[e] Gene ID- Candidate gene identity base on NCBI repository

[f] Effector/non effector- identification of the candidate gene as an effector by EffectorP

[g] Expression profile -identification of the candidate gene as an effector by Ismail and Able 2016, 2017

[h]pfam- The protein family database