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Pathogenic variation of *Pyrenophora teres* f. *teres* on *Hordeum vulgare* in Australia and identification of genomic regions for resistance and susceptibility to net form net blotch

Ryan Andrew Fowler

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Abstract

The research conducted in this thesis sought to fill knowledge gaps with regard to pathogenicity of *Pyrenophora teres* f. *teres* (*Ptt*) in Australia, knowledge of genomic regions in Australian differential cultivars that interact with prevalent isolates and identification of resistance and susceptibility QTL in Australian barley breeding germplasm. To successfully breed cultivars with resistance to pathogens within the target growing region, knowledge of the pathogen population is critical. Large shifts in the barley breeding structure in Australia over the last decade has meant that breeders often target broad adaptation of cultivars that allows them to be grown across the entire country, meaning that stable resistance to multiple pathotypes is relevant now more than ever.

A collection of *Ptt* isolates from five Australian states was assayed on differential genotypes at seedling stage. Hierarchical cluster analysis revealed that isolates belonged to four main groups that were each typified via differential virulence to four barley genotypes, Maritime, Prior, Skiff and Tallon. Further differentiation was observed within each of the four groups, suggesting that each group was not equivalent to a single pathotype. Different proportions of virulence were observed in each state and also between eastern, southern and Western Australia and adaption of isolates on locally grown cultivars was considered to be the driving force behind the state based diversity. Prior and Skiff were found to differentiate the greatest number of isolates and isolates from the widest geographic range. The genetics of resistance and susceptibility in these genotypes had not been previously studied.

Subsequently, a Prior x Skiff cross was used to develop a population of recombination inbred lines, which was phenotyped at seedling and adult growth stages with two *Ptt* isolates. Analysis discovered a total of five quantitative trait loci (QTL) on two chromosomes. All QTL in this Chapter co-located with that of previously published studies. Four QTL were located on 6H with two QTL closely linked in repulsion interacting with both isolates in a reciprocal manner, inspection of 256 diverse genotypes confirmed Skiff as the donor of susceptibility of one QTL and Prior was the donor of susceptibility to the other QTL. The undesirable allele for another QTL on 6H was omnipresent in Australian cultivars, while the undesirable allele or the fourth 6H QTL was only found in ancestors and selections of Prior. The QTL on 3H co-located with resistance from Tifang, however further research is needed to ascertain whether the resistance is the same.

Selection imposed on the northern region barley (NRB) breeding population has enriched the population for desirable alleles, however the genomic regions associated with resistance and susceptibility to *Ptt* are unknown. In order to identify QTL associated with desirable alleles, genome-wide association studies (GWAS) of 2012 and 2013 breeding population entries were conducted. Results discovered four QTL, one on 4H and three on 6H. The same reciprocal effect QTL from the previous chapter was re-identified, however the source of the undesirable allele was from the North Dakota (ND) germplasm pool, thus validating the effect of this QTL in unrelated germplasm. One of the other 6H QTL conditioned susceptibility to one isolate and was found to be derived from Moravian and English landraces, furthermore no genotype with this QTL is currently represented in any differential sets other than that detailed in previous research in this thesis. Tallon has been proposed as the representative genotype for this genomic region. The remaining QTL on 6H was contributed by CIho 5791 via the ND parents and is known to condition dominant resistance. The 4H QTL was contributed by PC 84 via the ND parents and is hypothesised to condition a resistance.

Utilisation of a diverse panel of genotypes in tandem with both mapping studies was able to uncover genotype lineages that harbour QTL associated with resistance or susceptibility to *Ptt*, further increasing the direct relevance of the mapping studies to Australian and international germplasm. The knowledge generated in this thesis is will enable Australian barley breeders and researchers to further their understanding of the complex interaction between barley and *Pyrenophora teres* f. *teres*.

Declaration by author

This thesis *is composed of my original work*, and *contains* no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, financial support and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my higher degree by research candidature and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

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Publications during candidature

Peer-reviewed journal articles

ElMor I, **Fowler RA**, Platz GJ, Sutherland MW, Martin A (2018) An improved detached leaf assay for phenotyping net blotch of barley caused by *Pyrenophora teres*. Plant Disease 102:760-763
DOI: 10.1094/PDIS-07-17-0980-RE

Fowler RA, Platz GJ, Bell KL, Fletcher SEH, Franckowiak JD, Hickey LT (2017) Pathogenic variation of *Pyrenophora teres* f. *teres* in Australia. Australasian Plant Pathology 46:115-128
DOI: 10.1007/s13313-017-0468-1

Hickey LT, Germán SE, Pereyra SA, Diaz JE, Ziems LA, **Fowler RA**, Platz GJ, Franckowiak JD, Dieters MJ (2016) Speed breeding for multiple disease resistance in barley. Euphytica 213:64
DOI: 10.1007/s10681-016-1803-2

Martin A, Platz GJ, de Klerk D, **Fowler RA**, Smit F, Potgieter FG, Prins R (2018) Identification and mapping of net form of net blotch resistance in South African barley. Molecular Breeding 38:53
DOI: 10.1007/s11032-018-0814-1

Singh D, Ziems LA, Dracatos PM, Pourkheirandish M, Tshewang S, Czembor P, German S, **Fowler RA**, Snyman L, Platz GJ, Park RF (2018) Genome-wide association studies provide insights on genetic architecture of resistance to leaf rust in a worldwide barley collection. Molecular Breeding 38:43
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Contributor	Statement of contribution
Fowler RA (Candidate)	Conception and design (50%) Analysis and interpretation (60%) Drafting and production (70%)
Platz GJ (Co-advisor)	Conception and design (30%) Analysis and interpretation (10%) Drafting and production (10%)
Bell KL	Conception and design (0%) Analysis and interpretation (20%) Drafting and production (5%)
Fletcher SEH	Conception and design (20%) Analysis and interpretation (10%) Drafting and production (2.5%)
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Research Involving Human or Animal Subjects

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Dedications

This thesis is dedicated to my wonderful family.

To our precious angel baby *Scarlette Rose Fowler*; you touched the hearts and lives of so many without ever having the chance to meet them.

Baby girl, you may be lost but you are forever loved and never forgotten.

Love you to the moon and back. 27.09.2016.

To our rainbow-baby *Jack Ryan Fowler*, your smile lights up our souls and fills our lives with overwhelming happiness.

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List of Abbreviations used in the thesis

bp	Base pair
CIho	Cereal Investigation *Hordeum*
CIM	Composite interval mapping
cM	Centimorgan
DArT™	Diversity Arrays Technology
DArTseq™	Diversity Arrays Technology genotyping-by-sequencing
DH	Double haploid
eBLUEs	Empirical best linear unbiased estimates
EMMA	Efficient mixed-model association
GAPIT	Genome Association and Prediction Integrated Tool
GWAS	Genome-wide association studies
LD	Linkage disequilibrium
LGs	Line groups
HSTs	Host specific toxins
HIR	High infection response
IGs	Isolate groups
IR	Infection response
LD	Linkage disequilibrium
LIR	Low infection response
LSD	Least significant difference
MAF	Minor allele frequency
MFA	Marker frequency analysis
MIM	Multiple interval mapping
MR	Moderately resistant
MS	Moderately susceptible
ND	North Dakota
NETS	Necrotrophic-effector induced susceptibility
NFNB	Net form net blotch
NRB	Northern region barley
NSW	New South Wales
QLD	Queensland

QTL	Quantitative trait loci
Ptt	*Pyrenophora teres* f. *teres*
PSR	Prior x Skiff RIL
REML	Residual maximum likelihood
RIL	Recombinant inbred line
S	Susceptible
SA	South Australia
SIM	Simple interval mapping
SNP	Single nucleotide polymorphism
Tas	Tasmania
WA	Western Australia
Vic	Victoria
VS	Very susceptible

Chapter 1

General introduction

1.1 Background

The necrotrophic fungus *Pyrenophora teres* f. *teres* (*Ptt*) that causes net form net blotch (NFNB) disease of barley (*Hordeum vulgare* L.) is a damaging pathogen of economic importance in Australia. This pathogen can cause severe yield loss in a very susceptible cultivar under favourable conditions. As a result, several practices are often used to reduce losses, which include crop rotation, fungicide application and sowing of resistant cultivars. While the use of fungicides to control *Ptt* can be effective, the preferred method of control is through the deployment of cultivars with genetic resistance. Although, achieving this goal is often complicated by host genotypes that are susceptible to specific pathotypes. In light of this, detailed knowledge of both the host and the pathogen are required to overcome these constraints.

Pathogenic diversity of *Ptt* in Western Australia has been document in many studies, which have shown a gradual reduction in number of unique virulences in the population over time (Gupta and Loughman 2001; Khan 1982; Khan and Boyd 1969b). Virulence to Prior was detected consistently across studies, while virulence to Beecher decreased over time. Two studies had assessed pathogenic diversity outside of Western Australia (Platz *et al.* 2000; Wallwork *et al.* 2016).

Resistance to pathogens has typically been described through a biotrophic gene-for-gene system (Flor 1955). However, necrotrophic pathogens including *Ptt*, often follow an inverse gene-for-gene model that is characteristic of dominant susceptibility genes (Abu Qamar *et al.* 2008). This nature on inheritance has practical breeding implications, which should be considered when incorporating parental material known to carry a dominant susceptibility. In any case, breeding for resistance should act to accumulate resistance genes whilst also removing susceptibility genes. Fortunately, these are not mutually exclusive and selection of low phenotype can theoretically achieve both outcomes simultaneously.

1.2 Rationale

In order for plant breeders to conduct resistance breeding against diverse pathogen populations, knowledge regarding the pathogenic variation of the pathogen population is necessary. Currently in Australia there is a knowledge gap surrounding the pathogenic variation of *Ptt* in the Australian population.

In addition, knowledge regarding the genetic resistance in differential genotypes is critical to allow meaningful interpretation of the results from pathogenicity studies and to make direct comparisons to commercial cultivars and breeding lines. Barley differential genotypes for *Ptt* avirulence/virulence are poorly understood at the genetic level, as most have not been characterised for key resistance and susceptibility regions.

Barley germplasm developed by the North Dakota State University (NDSU) is renowned for high resistance to *Ptt* (Adhikari 2017). The NRB breeding population is heavily derived from NDSU parents with strong resistance to multiple Australian *Ptt* isolates, however the genomic location of resistance is unknown in the NRB population.

1.3 Project objectives

1. Determine the pathogenicity of the Australian *Ptt* population in order to fill a knowledge gap the currently exists in this space.
2. Identify genomic regions conferring resistance and susceptibility in Prior and Skiff to Prior and Skiff virulent *Ptt* isolates.
3. Conduct genome-wide association studies (GWAS) on barley breeding populations to identify genomic regions conferring resistance and susceptibility to pathogenically diverse Australian isolates.

1.4 Thesis outline

This thesis consists of six chapters:

Chapter 1 – General introduction provides relevant background information
Chapter 2 – Literature review that summarises the current knowledge relevant to this thesis
Chapter 3 – 5 Three research chapters that address the project objectives of the thesis
Chapter 6 – General discussion that summaries the main findings and outcomes of the thesis

Chapter 2

Literature review

2.1 Introduction

Barley (*Hordeum vulgare* (Linnaeus 1753)) (hereafter *Hordeum vulgare* L.) is one of the most important grain crops in the world as it is highly adapted to different climatic conditions allowing it to be grown from arid regions to the Arctic Circle. Grain can be used raw as stock feed, malted, distilled to produce Shochu and pearled for food. Green crops are often grazed or used for fodder and young shoots are even juiced as a health drink. Over the last 40 years the average world-wide production has been steady at approximately 150 million metric tonnes, however grain yield has doubled in that time. In Australia, barley is second only to wheat in planted area, and is sown to approximately 4 million hectares, with a yield of approximately eight million metric tonnes (FAO 2018).

Net form net blotch (NFNB) disease caused by *Pyrenophora teres* f. *teres* (*Ptt*) is estimated to induce grain yield loss between $19 million and $117 million annually in Australia (Murray and Brennan 2010). These estimates are based on losses of 1.49% and 9% per hectare, respectively, and if applied to the global barley value, the annual economic loss caused by net form net blotch would be estimated to be between $400 million and $2.4 billion, respectively. Crop rotation, fungicide control and cultivation of disease resistant varieties are the most common practices to reduce the risk of yield penalties and reduced seed quality. The adoption of no-till farming practices has seen benefits with regard retained soil moisture, minimal disturbance to soil architecture and biota, though these benefits have come at a cost in the form of retained stubble harbouring pathogens into the subsequent growing season (Evans 1969). It is common for farmers to rely on fungicides for disease control, namely seed treatments, in-furrow and foliar applications. Genetic resistance is the preferred method of disease control and some breeding programs invest heavily in the development and selection of germplasm with improved resistance levels.

The success of a breeding program to deliver resistant cultivars relies knowledge of which resistance and susceptibility genes in barley are interacting with pathogen. This information can be attained through a comprehensive survey of the pathogen to identify relevant isolates and subsequent mapping of a segregating population to identify the underlying QTL. Breeding for

resistance to *Ptt* has been the focus of many breeding programs worldwide and will continue to be into the future in order to stay ahead of this dynamic and highly adaptive pathogen.

2.2 The host – barley (Hordeum vulgare L.*)*

Barley is classified in the *Hordeum* genus of the Triticeae tribe in the Poaceae family. Wheat, oats and rye also fall into the Triticeae tribe. Barley is thought to have undergone multiple separate domestication events in its long history with human cultivation over the last eight to ten thousand years (Badr *et al.* 2000; Morrell and Clegg 2007; Orabi *et al.* 2007). In such a model, barley domesticated in the Fertile Crescent became the major gene pool for western landraces, while barley domesticated east of the Zagros Mountains became the major gene pool for eastern landraces and barley domesticated in the Horn of Africa became the major gene pool for landraces from Ethiopia and Eritrea. Barley has been in written history for millennia with the oldest encryptions dating back to 3000 – 2400 BCE where Mesopotamian clay tablets were used to record barley rations of workers (Ellison 1981). The first detailed description of barley was compiled by Theophrastus in book eight of Historia Plantarum (Enquiry into Plants and Minor Works on Odour and Weather Signs) written between 350 and 287 BCE. Following the fall of the Byzantine empire in the 1400's, the books were translated into Latin by (Gaza 1483) and began to be used by renaissance botanists. The book was translated into English by Hort (1916). In the book Theophrastus described many aspects of barley including germination, plant architecture, spike row number, winter climate types, spring climate types, flowering, sowing rate and time, adaptation of barley to different geographic regions, soil tillage, lodging, storage pests, environmental factors influencing rust and observations of how some barleys were more susceptible to rust than others. The work by Theophrastus was overlooked for centuries due to novel ideas that were often considered heretical, however in admiration of is work, Carl Linnaeus described Theophrastus as 'the farther of botany' (Greene 1910).

Many names have been used to classify types of *Hordeum* over the ages, including; *cantherium*, *galaticum*, *hexastichum* and *mundum* (Columella 1745), *gymnocriton* (Galen), *ploystichum* (Fuchs 1542), *majus* and *minus* (Bock 1552), *nudum* (Cordus 1561), *nudum vulgo vocatum* and *polystichum vernum* (Lobel 1576), *vstum* or *Vestiligo hordei* (Burnt Barley) (Gerarde 1633), *distichum minus*, *hexastichum vernum* and *polystichum sive hybernum* (Parkinson 1640). Considering the large number of *Hordeum* species described, the re-classification by Linnaeus (1753) to a single species - *Hordeum vulgare* – greatly simplified classification. As such, five subspecies were used to group the formally described species; *coleste* (*polystichum vernum*),

hexastichum (*hexastichum*), *distichum* (*distichum*), *distichum nudum* (*nudum*) and *zeocriton* (*distichum minus*). This species description has not changed since, however many additional subspecies variants have been described (Beaven 1902; Körnicke 1895).

Barley is an inbreeding species and following removal of the anthers permits accurate cross-pollination, while self-fertility allows efficient generation advance to stabilise the genetic background when fixing breeding lines and segregating populations. Subsequently, the near absence of out-crossing has meant that linkage disequilibrium (LD) decays much slower than out-crossing species such as maize (Remington *et al.* 2001; Rostoks *et al.* 2006).

Barley is a diploid species with seven chromosome pairs (2n = 14). The initial chromosome designations followed Burnham and Hagberg (1956) according to Singh and Tsuchiya (1982), however the system of Triticeae genome symbols (Wang *et al.* 1994) was adopted shortly after (Linde-Laursen 1996), which has allowed direct comparison of chromosomes between cereals species. The barley genome was sequenced in 2012 (Consortium 2012) and the resource was a breakthrough for barley researchers, which has allowed for more efficient gene discovery. The order of the genome sequence recently refined by Mascher *et al.* (2017) is now even more accurate and will enable researchers to pinpoint precise regions of the barley physical map that are interacting with traits of interest.

2.3 The pathogen – Pyrenophora teres Drechslera f. *teres* Smedeg.

The fungal pathogen used in this study belongs to the *Pyrenophora* genus (syn. *Heminthosporium*) of the Pleosporaceae family, which is in the Pleosporales order of the Dothideomycetes class of the Ascomycota division. The *Helmisporium* genus was originally described by Link (1809), however Persoon (1822) illegitimately changed the spelling to *Heminthosporium* (Shoemaker 1959). The *teres* species was described by Saccardo (1882) and the teleomorphic stage as *Pyrenophora* by Diedicke (1902). Subsequent work by Drechsler (1923) sought to rationalise the increasing number of species described under *Heminthosporium*. In honour of this work Ito (1930) proposed that Drechsler be used to describe the anamorphic stage of the genus after a split was proposed (Nisikado 1929). The addition of two forms; *maculata* and *teres*, were proposed by Smedegård-Petersen (1971) to describe spot form net blotch and net form net blotch disease symptoms, respectively. As such the full name of the pathogen used in this thesis is *Pyrenophora* Diedicke *teres* Saccardo f. *teres* Smedegård-Petersen (anamorph *Drechslera* Drechsler *teres* Saccardo) (syn. *Helmisporium* Link *teres* Saccardo).

The fungus produces asexual conidia from infected plant tissue and pseudothecia, conidia usually have 4 to 6 septa, an inflated basal cell that is a diagnostic character and are typically 30 – 174 µm x 15 – 23 µm. The presence of the teleomorphic stage means the fungus is able to reproduce sexually via ascospores that are borne from beaked ascocarps. Acsospores have three transverse septa and one vertical septum and are typically 48 – 57 µm x 21 – 24 µm (Shoemaker 1962). Conidia are typically formed after leaf-wetness period of at least 16 hours and released during daylight hours when the humidity is lower (Martin and Clough 1984). However, due to their relatively large size conidia are typically limited in their dispersal, as a 95.7% reduction in windborne conidia trapped 10 metres from the crop edge when compared within the crop at equal heights was recorded by Martin and Clough (1984), suggesting that long distance dispersal of the pathogen is likely to occur via infected seed (Jordan 1981).

The life cycle of *Ptt* involves primary infection from infected seed or barley stubble (Piening 1968). Once infection is established on juvenile plants, lesions mature and become the source of secondary inoculum and in-crop infection cycles built up disease thought the season. Favourable conditions for spore release at flowering mean the caryopsis and embryo could become infected and facilitate the spread of the pathogen on grain (Youcef-Benkada *et al.* 1994). After the crop is harvested, *Ptt* may persist on the remaining stubble as pseudothecia. A diagram of the life of *Ptt* is shown in Figure 2.1.

Ptt infects barley under low light, high humidity conditions with temperatures ranging between 14°C and 25°C (Van den Berg and Rossnagel 1990). In order to penetrate the host epidermal cells, hyphae of germinating conidia form an appressorium and infection is usually complete after 24 hours, after which time primary and secondary vesicles and intracellular hyphae were observed (Keon and Hargreaves 1983). It was noted that cells within the necrotrophic lesion would undergo severe degeneration when in close contact with intracellular hyphae, while cells within the chlorotic margin would undergo degeneration that closely resembled general senescence. The authors suggested that two separate mechanisms could be involved; one involving diffusible toxins (indirect recognition) and the other centred on direct recognition of the pathogen. Abu Qamar *et al.* (2008) demonstrated a susceptible interaction with *Ptt* upon recognition by a dominant susceptibility gene in the host. One possible indirect recognition mechanism involves necrotrophic effectors (Liu *et al.* 2015), which are low molecular weight proteins that, if recognised, cause a cascade of incorrect defence responses for a necrotrophic pathogen, resulting in a favourable outcome for the pathogen. A recent study of a necrotrophic pathogen of wheat from the order

Pleosporales, *Parastagonospora nodorum*, showed that direct recognition of a fungal protein by a cell wall receptor, similar to those used to trigger immune responses to biotrophs, lead to a cascade of incorrect defence response for the necrotrophic pathogen, resulting in a favourable outcome for the pathogen (Shi *et al.* 2016). While no such interaction has been described in the barley-*Ptt* pathosystem, there maybe similar genetic controls shared across necrotrophic pathogens from the order Pleosporales.

Infectivity of *Pyrenophora teres* on barley is conferred by a single copy of a mitogen-activated protein kinase (MAPK) *PTK1* that controls appressorium formation and conidiation (Ruiz-Roldán *et al.* 2001). Independent genes encoding virulence to Rika (*VR1* and *VR2*) (Shjerve *et al.* 2014) and Kombar (*VK1* and *VK2*) (Shjerve *et al.* 2014) and avirulence genes to Harbin (*AvrHar*) (Lai *et al.* 2007; Weiland *et al.* 1999), Heartland ($Avr_{Heartland}$) (Beattie *et al.* 2007) and Prato (*AvrPra1* and *AvrPra2*) (Lai *et al.* 2007) have been described**.** A recent study identified multiple quantitative trait loci (QTL) conditioning virulence/avirulence to Beecher, Celebration, CIho 4922, Hector, Manchurian, Pinnacle, Stellar and Tifang (Koladia *et al.* 2017b).

Under controlled environment conditions, Khan (1969) showed that a more susceptible phenotype of Manchurian genotypes could be attained through the use of low light/no light during the incubation period directly following inoculation. This variable effect may be due to light induced regulation of some resistance/susceptibility transcription factors present in these genotypes. A recent study demonstrated the regulation of signalling genes was regulated by light in the presence of the pathogen (Shi *et al.* 2016). With this in mind, artificial inoculation experiments should be conducted in such a way to minimise disruption the normal biological process of the plant otherwise anomalous results that are not reproducible in nature may eventuate.

The genome of *Ptt* was first sequenced by Ellwood *et al.* (2010) and was recently updated using long sequence reads and linkage maps to increase resolution (Wyatt *et al.* 2018). The genome contains approximately 46.5 Mbp and approximately 11,500 predicted genes. Future research involving crosses between *Ptt* isolates to identify genomic loci involved in avirulence/virulence to barley, proteomics and gene cloning will benefit from this resource.

2.4 The plant defence system

Plants are equipped with innate defence systems that enable them to resist pathogen attack while they fulfil their lifecycle as sedentary organisms (Vidhyasekaran 2016). The outcome from the

interaction between barley and an invading pathogen depends on the plant deploying the correct transcriptional responses and associated biochemical pathways to mitigate the intruder. However, pathogens fall into three differing lifestyles; biotrophic, hemi-biotrophic and necrotrophic, which usually require different biochemical pathways for adequate suppression (Glazebrook 2005). The phytohormone salicylic acid (SA) and associated signalling is involved with suppression of biotrophs, while phytohormones jasmonic acid (JA) and ethylene (ET) and associated signalling pathways are involved with suppression of necrotrophs. These pathways are known to cross-communicate and results in regulation of the signalling network (Pieterse *et al.* 2009).

Pathogen-associated and microbe-associated molecular patters (PAMPs/MAMPs) are highly conserved, often structural molecules that are shared across pathogenic and non-pathogenic microorganisms (Lloyd *et al.* 2014). Well known PAMPs/MAMPs include flagella of bacteria and chitin of fungal cell walls. The defence system employs pattern recognition receptors (PRRs) that are usually receptor-like kinases (RLKs), e.g. wall-associated kinases (WAKs) (Li *et al.* 2009), to monitor the apoplastic space for PAMPs of invading pathogens and subsequent detection initiates the first line of defence, PAMP-triggered immunity (PTI). Pathogens have overcome this system through the use of small proteinaceous toxins or effectors that are able to suppress PTI through direct or indirect recognition by dominant host gene products, which leads to effector-triggered susceptibility (ETS) (Faris *et al.* 2010). The plant is able to mitigate ETS via the deployment of resistance (R) genes that serve to detect pathogen effectors and lead to effector-triggered immunity (ETI) (Staal *et al.* 2006). Plants also use specific leucine rich repeat (LRR) receptors to monitor for damage-associated molecular patterns (DAMPs), which are damaged host cells that serve as a trigger to identify the plant is under attack by pathogen effectors (De Lorenzo *et al.* 2011). Effector-triggered defence (ETD) has been proposed by Stotz *et al.* (2014) to capture the interaction where hemi-biotrophic pathogens secrete extracellular effectors that interact with R gene products, resulting in pathogen suppression. SA has been shown to be involved in induced resistance (IR), in which localised exposure to a pathogen 'primes' the entire host for subsequent attacks in what is known as systemic acquired resistance (SAR) (Ryals *et al.* 1996). In addition, SAR can be switched on via application of exogenous molecules e.g. β-aminobutyric acid (Cohen 2002). SAR has been shown to be inherited epigenetically from disease-exposed plants, providing resistance to the following generation (Luna *et al.* 2012). The interaction of dominant R gene products that result in the immune-like responses of PTI and ETI are usually found for biotrophic pathogens. Outright immunity in the interaction with necrotrophic pathogens has not yet been observed.

The first pathogen recognition receptor was recently discovered for the *Hv-Ptt* interaction. A dominant gene (*HvWRKY6* transcription factor) was found to underpin high resistance in CIho 5791 to *Ptt* isolate 0-1 through integrated advanced genetic approaches (Tamang 2017). Delayed and moderate expression of *HvWRKY6* was shown to positively influence resistance to 0-1 and was suggested that overexpression observed in Tifang was associated with down regulation of a crucial aspect of the signally pathway. Conversely, reduced expression of *WRKY6* in tobacco mutants resulted in reduced JA activity and increased vulnerability to herbivory to *Manduca sexta* larvae (Skibbe *et al.* 2008). Thus, it is possible that WRKY signally pathways involved in pathogen defence could also be involved in the response to other stimuli.

Phytohormones known to be involved in plant defence signalling system include; abscisic acid, gibberellic acid, jasmonic acid salicylic acid, auxin, brassinosteroids, cytokinin and ethylene (Vidhyasekaran 2016). Phytohormones and cellular compounds have been identified in the interaction with *Ptt* and barley. Accumulation of cytokinins at the infection site of *Pyrenophora teres* was associated with the susceptible response but not the resistant response (Angra-Sharma and Sharma 2000). Differential activity of reactive oxygen species (ROS) from two *Ptt* isolates, virulent and avirulent, has been shown to involved with the resistant and susceptible responses of one barley genotype (Able 2003). Comparative proteomics of two *Ptt* isolates, virulent and avirulent, identified three proteins that were shared across between isolates, suggesting that differences in pathogenicity could be due to different receptor targets of the isolates (Ismail *et al.* 2014). Secretome analysis of culture filtrates from 28 virulent *Ptt* isolates identified a plethora of proteins, thus giving insight into proteins involved in the *Ptt* host-pathogen interaction and potential knockout targets for gene expression studies (Ismail and Able 2016).

The identification of interactions between the *Ptt* and dominant gene products of barley shows that further recognition pathways are yet to be characterised. Avirulence products of *Ptt* have been shown to interact with barley in the classical gene-for-gene model (Beattie *et al.* 2007; Weiland *et al.* 1999). Dominant susceptibility genes have been shown to interact with *Ptt* in an inverse gene-for-gene model that is characteristic of necrotrophic ETS (NETS) (Abu Qamar *et al.* 2008; Liu *et al.* 2015). As such, the interaction between *Ptt* and barley is likely to involve the interaction of multiple pathways in a signalling network. A recent study by Shi *et al.* (2016) documented the interaction between SnTox1 toxin producing isolates of *Parastagonospora nodorum*, a necrotrophic pathogen of wheat and genotypes that carry the associated gene conferring susceptibility - *Snn1*. The susceptible response was found to be via direct recognition of SnTox1 protein by a wall associated kinase (WAK) receptor. Recognition at this receptor is usually involved

with biotrophic interactions leading to PTI, however the commandeering of signal transduction pathway for necrotrophic gain could be described as PAMP-triggered susceptibility (PTS) (Figure 2.2). The discovery of a necrotrophic pathogen exploiting a pathogen recognition receptor (PRR) to enhance necrosis is a world first and will revolutionise the way in which host-pathogen interactions are viewed. Furthermore, the utilisation of the same receptors by biotrophs and necrotrophs with opposing outcomes suggest that breeding for resistance to biotrophs may also breed susceptibility to the necrotrophs. Adequate characterisation of germplasm through selection would be recommended to avoid such an outcome.

The plant defence system constitutes a complex network of interactions, some antagonistic and others synergistic. To date, the interaction between barley and *Ptt* has been poorly studied at a biochemical level. The apparent lack of research in this area is unjustifiable considering the high commercial importance of the model crop species.

2.5 Pathogenic variation

Studies in Australia and other countries have documented pathogenic variability (Akhavan *et al.* 2016; Arabi *et al.* 2003; Bouajila *et al.* 2011; Boungab *et al.* 2012; Cromey and Parkes 2003; Douiyssi *et al.* 1998; Gupta and Loughman 2001; Jalli 2010; Jebbouj and El Yousfi 2010; Khan 1982; Khan and Boyd 1969b; Liu *et al.* 2012; Oğuz and Karakaya 2017; Platz *et al.* 2000; Robinson and Jalli 1996; Steffenson and Webster 1992a; Tekauz 1990; Tekauz and Mills 1974; Tuohy *et al.* 2006; Wallwork *et al.* 2016). Variation in the prevalence of different pathotypes is influenced predominately by the cultivars grown and their prevalence can change by geographic location and also fluctuate over time. This variation highlights the necessity for a broad screening approach while developing germplasm and the utilisation of multiple pathotypes to identify and remove susceptible breeding lines. This multiple pathotype screening technique would also aid in detecting lines that carry desirable combinations of resistances that are effective against multiple pathotypes. To successfully develop new cultivars with durable disease resistance a high level understanding of the target pathogen is required. As the genetics of both the host and pathogen are not static through time, scientists must employ a degree of foresight to maintain an adequate level of resistance in the host despite the presence of a highly adaptable pathogen.

2.5.1 Early studies

The earliest report of pathotypic variation of *Ptt* to was documented by Pon (1949) where author eluded that isolates caused different levels of pathogenicity on certain cultivars. Further support for the presence of pathotypic variation was evident in the results of subsequent publications (Buchannon and McDonald 1965; Dessouki *et al.* 1965; Kenneth *et al.* 1967; Khan *et al.* 1968; Shipton 1966; Singh 1956). Studies by McDonald and Buchannon (1962) and Gray (1966) made notes pertaining to the existence to specific physiological races.

2.5.2 Australasia

A study by Khan and Boyd (1969b) specifically set out to verify the existence of physiologic races of *Ptt* in Australia. A collection of 17 isolates from Western Australia was tested over a suite of 138 international barley lines and four locally grown cultivars. The published results of 59 lines identified 34 to be resistant as seedlings and adults. A further 15 lines gave intermediate seedling responses although remained resistant in the field. Four lines differentiated and six lines were fully susceptible. Two differentials; CIho 1179 (Algerian) susceptible to 47% of isolates and CIho 7584 (Tennessee Awnless D22-5) susceptible to 11.7% of isolates, were able to describe 3 physiologic races. CIho 2235 (Coast) and CIho 7996 (Rabat 071) also gave identical differential responses to CIho 7584. Local cultivars Beecher, Bussell, Dampier and Prior along with Atlas (USA) and Hazera 212 (Israel) were susceptible to all isolates tested. Bussell and Dampier both have Prior in their pedigree and are likely to carry similar resistance and susceptibility genes. Atlas is a direct parent of Beecher and is therefore also likely to carry similar genes. Khan and Boyd reported Hazera 212 to have Harbin in its pedigree. A search in the USDA GRIN database revealed three accessions, two of which (H-2127 and H-2141) have Harbin in the pedigree (Harbin/Arivat 3) and are 6-rowed barleys developed in Israel by Hazera Seeds Ltd. The pedigree of Arivat is identical to that of Beecher (Atlas/Vaughn). The third accession, BT Hazera 127/1 (CIho 12673) is also a 6-rowed barley developed in Israel but through the cross, Beecher/BMC/Tuniset. A *Ptt* diversity study published by Steffenson and Webster (1992a) also used an accession of Hazera (CIho 12673), which responded similarly to Beecher. Considering the pedigree of the accessions, it is possible that all three could be genetically similar to Beecher and therefore appear phenotypically similar with Beecher virulent isolates in Western Australia.

A study published by Khan (1973) tested the host specialisation of *Ptt* isolates collected from cultivated barley (*Hordeum vulgare*) and barley grass (*Hordeum leporinum*). The results demonstrated that the isolates from barley grass could not attack cultivated barley and vice versa.

These results confirmed that barley grass had no role as an alternative host in the lifecycle of *Ptt* specific to cultivated barleys.

Beecher had been one of the leading cultivars grown in Western Australia until the release of Dampier in 1967 and Clipper in 1968 (Sparrow 1984). Subsequently, the frequency of *Ptt* isolates with virulence to Beecher and Prior in Western Australian had been 100%. A pathogen survey of 52 *Ptt* isolates conducted by Khan (1982) between 1976 and 1980, recorded for the first time, a decline in the virulence frequency to a particular cultivar and the complete absence of virulence to another. Isolates with virulence to Beecher had declined to 20% over a period of 8 to 15 years and isolates with virulence to CIho 7584 were not detected. Despite the omittance of Prior in this study, subsequent studies by Platz *et al.* (2000) and Gupta and Loughman (2001) have demonstrated high similarity between Dampier and Prior infection responses. Isolates with virulence to Dampier/Prior remained steady at 100%, this may be attributed to the continued cultivation of Dampier.

A study published by Platz *et al.* (2000) tested 59 Australian *Ptt* isolates on 44 barley lines. 13 distinct pathotypes were identified using a concise set of 15 differentials. Analysis of similarity matrices was conducted and used to generate a hierarchical dendrogram of 25 genotypes. Lines with similar phenotypic profiles clustered together and lines that grouped above a fusion level threshold of 0.1 where hypothesised to have similar resistance or susceptibility genes. The genotypes formed five broad clusters at a fusion level threshold above 0.2877. The first line cluster included; Cameo, Gilbert, Golf, Grimmett and Tallon at a threshold above 0.13. The second line cluster included; Betzes, Cape, Clipper, Corvette, Dampier and Prior at a threshold above 0.238. The third line cluster included; Harbin, Kaputar, Kombar and Yerong at a threshold above 0.2877. The fourth line cluster included; Algerian, CIho 11458, Franklin, Herta, Patty, Rika and Skiff at a threshold above 0.25. The fifth line cluster included; Atlas, Beecher and Hazera at a threshold above 0.1. Genotypes used by Steffenson and Webster (1992a) of Ethiopian and Chinese origin were resistant to all isolates tested, whilst European genotypes; Rika and CIho 11458 and Californian - Beecher types responded with differential responses.

A study by Gupta and Loughman (2001) assessed 74 *Ptt* isolates from Western Australia and one isolate from Queensland using a differential set of 47 barley genotypes. The set of lines combined the full set of 22 differentials used by Steffenson and Webster (1992a), eight differentials used by Tekauz (1990), nine additional lines used by Platz *et al.* (2000) and four genotypes unique to the study. The results identified all isolates to have virulence to Prior. Two pathoypes were

identified in Western Australia and could be classified according to virulence to Atlas, Beecher, Hazera, Kombar, Prato and Yerong. These findings complemented the results of (Khan 1982; Khan and Boyd 1969b) and suggested the *Ptt* population had remained stable since 1980. The Queensland isolate; NB85, had a unique phenotype and combined virulence to Corvette, Dampier, Golf, Gilbert, Grimmett, Prior and Stirling.

A study by Cromey and Parkes (2003) phenotyped 29 *Ptt* isolates collected in New Zealand between 1999 and 2001. The authors used a set of 31 genotypes, combining those used by Khan (1982), Tekauz (1990), Steffenson and Webster (1992a) and Jonsson *et al.* (1997). The results detected 11 pathotypes and documented all isolates tested to have virulence to Herta and Rika. The isolates had differential virulence to Algerian and CIho 11458, some Californian types (Atlas, Cape and Prato) and some Chinese types (Harbin, Manchuria (CIho 2330), Manchurian and Ming). Virulence to the other nineteen differentials was not detected, this included the Ethiopian accessions; CIho 1243, CIho 5791, CIho 9819 and CIho 9820 as well as several of their descendants; Heartland, Norbert and TR473. Only pathotype 11-22 identified in this study was also recorded by (Steffenson and Webster 1992a).

A study by Wallwork *et al.* (2016) tested 37 *Ptt* isolates from South Australia on a set of 25 Australian barley cultivars as adult plants. Buloke, Clipper, Schooner, Scope, Sloop, Sloop SA, Sloop Vic and Vlamingh were identified as having a useful level of adult plant resistance. Most other genotypes displayed isolate specific phenotypic responses. An increase in virulence complexity was observed over time.

2.5.3 The Americas

A study published by Tekauz and Mills (1974) identified a previously unrecorded *Ptt* pathotype which combined virulence to the susceptible cultivar Betzes and the then previously moderately resistant cultivars Fergus and Herta. Betzes was developed in Germany by a cross between two landraces originating from Bohemia, Czech Republic. This cultivar responded with moderately susceptible and susceptible infection types to three isolates in the study. The pedigrees of Fergus and Herta can be traced back to Isaria; a cultivar developed in Germany from a cross between two landraces originating from lower Bavaria. These lines responded with moderately resistant, moderately susceptible and susceptible infection types to the three isolates studied.

A study by Tekauz (1990) employed a set of nine differential lines to phenotype 179 *Ptt* isolates collected over a distance of 2,000 kilometres from Central to Western Canada. The results identified 45 unique pathotypes. All nine differentials displayed susceptible infection types to at least one isolate. Isolates with virulence to CIho 5791, CIho 9820 and CIho 9214 were identified. Canadian breeding programs used these accessions as sources of resistance and several cultivars have been developed from CIho 5791; namely Norbert (1980) and Heartland (1985). *Ptt* isolates examined during the study displayed virulence to Norbert and Heartland, with frequencies of 37% and 58% on the cultivars, respectively. All but one isolate produced high infection types on the cultivar Herta. This increase in virulence frequency to almost endemic levels over such large geographical region demonstrates this pathogen's ability to rapidly disseminate and conserve virulence within a population.

A study by Steffenson and Webster (1992a) surveyed 91 *Ptt* isolates from California and used 22 differentials to describe the pathotypic variation in the population. A total of 13 pathotypes were identified, with 91.2% of isolates producing high infection types on combinations of the cultivars; Atlas, Beecher, Cape, Hazera, Kombar and Prato. One isolate had virulence to both CIho 11458 (reselection of Isaria) and Rika (Kenia/Isaria). One isolate displayed virulence to several lines of Chinese origin and included; CIho 4922, Harbin, Manchuria and Manchurian.

A study by Wu *et al.* (2003) phenotyped 23 geographically diverse *Ptt* isolates on a differential set of 25 barley genotypes, 22 of which were used by Steffenson and Webster (1992a), ND B112 and Hector were used by Douiyssi *et al.* (1998), Liu *et al.* (2012) and FR 926-77 was unique to the study. A total of 15 pathotypes were identified. Pathotypes 11-22-25 and 15-20-25 accounted for 34.8% of the isolates, while pathotypes 0, 22-25, 3-10-15-19-21-25, and 3-10-15-19-20-21-25 accounted for 34.8% of the isolates. Pathotype 1-2-3-6-7-10-13-16-18-25 was virulent on the greatest number of genotypes. Hector was susceptible to 13 of the 15 pathotypes, while CIho 5791 was resistant to all pathotypes.

The North Dakota *Ptt* population was studied by Liu *et al.* (2012) in 2012 using a set of 22 differentials; 17 in common with Steffenson and Webster (1992a) and three in common with Tekauz (1990). Phenotypic expression of 75 isolates differentiated into 49 pathotype groups. The greatest virulence frequencies were reported on lines of Chinese origin (Canadian Lake Shore, CIho 4922, Harbin, Manchuria, Manchurian, Ming and Tifang) and ranged between 62% and 91%. Isolates with virulence to lines of Californian origin ranged between 10% and 55% frequency. No isolate produced susceptible infection responses on CIho 5791 or Heartland despite sharing the

Canada/USA border with Manitoba and Saskatchewan where isolates with these virulences had been previously documented Tekauz (1990). The breeding program in North Dakota had used Chinese sources of resistance to *Ptt* in the past and this may contribute the high frequency of isolates with virulence to these genes (Franckowiak, personal communication).

A recent study by (Akhavan *et al.* 2016) phenotyped 39 *Ptt* isolates from western Canada on nine barley genotypes, eight of which were also used by Tekauz (1990), although Herta was substituted for OAC 21 in the study. A total of 16 pathotype groups were identified with two pathotypes comprising 43% of isolates and nine isolates were unique. BT 201 and OAC 21 were reported as the most susceptible genotypes, while CIho 5791 and CIho 9820 were resistant to all but one isolate. A shift in the population was observed since the study by Tekauz (1990).

2.5.4 Europe

A diversity study of the finish *Ptt* population conducted by Robinson and Jalli (1996) clustered 27 differentials into three groups; 1: resistant, 2: differentiating and 3: susceptible. Genotypes that clustered in the resistant group were: Algerian, Coast, CIho 4922, CIho 5791, CIho 7584, CIho 9819, Prato, Rojo and Tifang. Genotypes that clustered into the differentiating group were: Beecher, Canadian Lake Shore, Cape, CIho 5822, CIho 11458, Harbin, Kombar, Ming and Rika. Genotypes that clustered in the susceptible group were all Nordic 6-row spring barleys and included; Agneta, Artturi, Arve, H6221, Pohto and WW797. No analyses were conducted to group lines or isolates that responded similarly.

A study of 153 *Ptt* isolates from Slovakia identified 73 pathotypes (Jánošová and Kraic 1997). Isolates that induced virulent infection responses to the lines originating from Ethiopia: CIho 5791, CIho 9819 CIho 9820 and CIho 9825 were identified although only at a low frequency (data not published in study).

A study by Jonsson *et al.* (1997) identified 14 pathotypes from a collection of 25 Swedish and two Canadian Isolates examined using 18 differential genotypes. The three most common pathotypes comprised 59% of all isolates. Of a selection of 109 genotypes from diverse origins, 12 were resistant to a subset of seven isolates. These included; Abyssinia (CIho 5822), CDC Guardian, Cebada Capa (CIho 6193), CIho 4502, Heartland, Manchu (CIho 4795), SW 1114-93, Rabat 071 (CIho 9776) and Virden. The two reference Canadian isolates were phenotypically different from each other and to all Swedish isolates. The European lines; Alexis, Golide, Golf, Morocco (CIho

6311), Svani, SW1378-93 and SW 1471-93 all gave phenotypes that were relatively similar, although some variation was shown between Morocco and SW 1378-93 compared to the other lines. The differentials of Chinese origin; Canadian Lake Shore, CIho 4922, Harbin, Manchuria and Tifang responded with relatively similar infection responses across isolates.

A comprehensive study by Jalli (2010) phenotyped 239 Finnish *Ptt* isolates collected from 19 field locations. The results reported CIho 5971 and CIho 9819 to have the highest level of resistance and that all other lines displayed differential responses. Results are presented by way of virulence frequencies and regression lines of isolates are plotted. This method of analysis, while informative in determining the effectiveness of particular lines as sources of resistance, is unable to explain any detail as to the population structure of the isolates studied.

A study by Oğuz and Karakaya (2017) assessed 40 *Ptt* isolates that were collected from 23 provinces of Turkey on 25 barley differentials as used by Wu *et al.* (2003). A total of 24 pathotypes were identified. Pathotype 0 was most common, followed by pathotype 6-10-18. Pathotype 3-4-6-7-9-10-11-12-14-15-16-17-18-20-21-22-25 was virulent on the greatest number of barley genotypes.

2.5.5 Africa

A study by Douiyssi *et al.* (1998) tested a set of 38 barley genotypes of varied origin with 15 isolates of *Ptt* collected from Morocco. Every genotype gave seedling scores that were moderately susceptible or susceptible to one or more isolate. Heartland and CIho 9820 gave the most resistant seedling scores with a range of infection types of 1.0 – 6.3 and 1.0 – 7.0, respectively.

A study of Algerian *Ptt* isolates was conducted by Boungab *et al.* (2012). The authors employed the full set of 22 differential lines used by Steffenson and Webster (1992a) to determine the pathotypic variation of 48 isolates collected between 2008 and 2010. Twelve pathotypes were identified, two of which (3-10-15-19-21 and 3-10-15-19-20-21) were also detected by Steffenson and Webster (1992a) and one other (20-22) was also in common with published results by Cromey and Parkes (2003). The highest virulence frequencies were recorded on Rika (54%), Atlas (52%) and Kombar (52%). Eight of the lines did not display susceptible infection types, these were; Coast, CIho 5791, CIho 5822, CIho 7584, CIho 9819, Ming, Rojo and Tifang. Low virulence frequencies on lines of Chinese origin (Canadian Lake Shore, CIho 4922, Harbin, Manchuria and Manchurian) were also observed.

2.5.6 Asia

A comparative study of 18 Japanese and three Canadian *Ptt* isolates using 38 differential genotypes conducted by Sato and Takeda (1993) concluded that the Japanese isolates were pathogenically different from the Canadian isolates. No analyses were conducted to group lines or isolates that responded similarly.

2.6 Described resistance/susceptibility genes

(Moseman 1972) recommended the use of a three letter code to denote resistance genes, the first letter should be R signifying reaction and the following two should be consistent with the genus and species of the causal organism. Many genes conferring resistance or susceptibility to *Ptt* have been described and their *Rpt* designations are given below.

2.6.1 Rpt1 Complex (3HL)

A monofactorial incomplete dominant resistance gene was described from Tifang on chromosome 3H was designated *Pt* (Schaller 1955). Soon after, three dominantly inherited resistance genes were described by Mode and Schaller (1958). Pt_1 was present in Tifang and corroborated the previous result by Schaller (1955). Pt_2 was closely linked in repulsion with Pt_1 and was present CIho 4922, Canadian Lake Shore, Harbin, Manchurian and Ming. Pt_3 was unlinked was reported in CIho 4922 and Canadian Lake Shore. A dominant resistance gene described from Manchuria, Ming and Tifang was designated Pt_a, CIho 5791 and CIho 9819 were regarded as carrying alleles at this locus (Khan and Boyd 1969a). A recent study of a population of CIho 5791 x Tifang RILs identified two independently inherited dominant resistance genes on 3H, the gene in Tifang would likely be *Pt* /Pt_1/Pt_a (Koladia *et al.* 2017a). A dominant resistance gene was identified in CIho 5791 at a locus separate from Tifang (Appendix 1) and the gene was named *HvWRKY6* (MLOC_68299.2) (Tamang 2017). The resistance gene in Tifang has since been revised to *Rpt1.a* (BGN 2013).

2.6.2 Rpt2 (1H)

A monofactorial incomplete dominant resistance gene was described from CIho 9819 on chromosome 1H was designated *Rpt2c* (Bockelman *et al.* 1977). A study published by Manninen *et al.* (2006) also identified resistance on 1H from CIho 9819. The resistance gene in CIho 9819 has since been revised to *Rpt2.c* (BGN 2013).

2.6.3 *Rpt3* (2H)

A monofactorial incomplete dominant resistance gene was described from CIho 7584 (Tennessee Awnless D22-5) on chromosome 2H was designated *Rpt3d* (Bockelman *et al.* 1977). A recessive resistance was described from CIho 9831 (dominant susceptibility in Ledger) that was closely linked to *Vrs1* (two-row spike) (Appendix 1) (Ho *et al.* 1996). The resistance gene in CIho 7584 has since been revised to *Rpt3.d* (BGN 2013).

2.6.4 *Rpt5 Complex* (6H)

A monofactorial incomplete dominant resistance gene was described from CIho 9819 on chromosome 6H was designated *Rpt5* (Manninen *et al.* 2000; Manninen *et al.* 2006). A dominant resistance gene was identified from CIho 5791 and co-located with *Rpt5*, although no gene designation was given (Koladia *et al.* 2017a). A dominant resistance gene was described from CIho 5791 and CIho 9819 but was not given a gene designation (Khan and Boyd 1969a). Rika and Kombar carry dominant susceptibility genes closely linked in repulsion that co-located with *Rpt5* (Abu Qamar *et al.* 2008). A dominant susceptibility described from Hector was designated *SPN1* (sensitivity to *Ptt* necrotrophic effector 1) and did not co-locate with *Rpt5* (Appendix 1) (Liu *et al.* 2015). Chevron conferred resistance for a QTL that co-located with *Rpt5* and was designated *Rpt*, although inheritance studies were not conducted to determine the nature of resistance (Ma *et al.* 2004). Given the recurring detection of a dominant resistance gene in two Ethiopian landraces; CIho 5791 and CIho 9819, it is likely that many other studies have also detected this gene at the *Rpt5* locus. The resistance gene in CIho 5791 has since been revised to *Rpt5.f* (BGN 2013). BGN (2013) also proposed to revise *rpt.r* and *rpt.k* designations to *rpt5.r* and *rpt5.k*, respectively.

2.6.5 *Rpt7* (4H)

QTL identified on chromosome 4H for Halcyon (Raman *et al.* 2003) was proposed by to designate this gene *Rpt7.h*, however inheritance studies should be conducted to confirm inheritance of a dominant resistance gene before adoption of the gene designation (BGN 2013). This would also apply for the proposal to include Steptoe (Steffenson *et al.* 1996) and TR251 (Grewal *et al.* 2008) under the *Rpt7.h* designation.

2.7 Segregating populations for QTL analysis

To successfully identify QTL interacting with the trait of interest, it is necessary to phenotype a population that segregates for the trait at the molecular level, these populations usually consist of two parents (bi-parental) or multiple parents (multi-parental). Transient bi-populations can be quick to develop, as is the case for backcross (BC) populations, where one of the parents is backcrossed to the F_1; F_2 population where the F_1 is self-pollinated and F_2 families consisting of F_2 derived F_3 or F_4 families. The heterozygous transient nature of the populations mean they are not suited to traits that need to be phenotyped over many years. Immortal bi-parental populations include double haploid (DH), where there the pollen of an F_1 plant is treated induce doubling of the haploid chromosome to return diploidy and recombinant inbred lines (RILs), where F_2 selections are self-pollinated over six to eight generations. DHs are faster to produce than RILs but are also more expensive. Immortal multi-parental populations include nested association mapping (NAM) populations, where numerous donor lines are crossed to one or few recurrent parents and multi-parent advanced generation intercrosses (MAGIC), where eight parents are inter-crossed in all combinations. These populations require considerable resources to develop and phenotype due to their large size, however are extremely powerful in dissecting complex traits. Fixed populations represent an immortal resource that may be phenotype a limitless number of times, distributed to collaborators or deposited into gene banks for future use.

2.8 Marker platforms

Initial mapping studies used restriction fragment length polymorphisms (RFLP) were the first markers to be widely used for mapping applications, this was mainly owing to their low cost (Burr *et al.* 1983). However, as RFLPs did not utilise any form of amplification, they required large quantities of DNA. Amplified fragment length polymorphisms (AFLP) overcame the issue of DNA quantity as they were amplified via polymerase chain reaction (PCR) (Vos *et al.* 1995). Simple sequence repeats (SSRs) (Powell *et al.* 1996) were the most widely used marker platform to map QTL for *Ptt*. All the aforementioned marker technologies relied on gel electrophoresis to score maker polymorphisms thus bi-parental linkage maps were small, typically 10's of markers per chromosome. The number of individual genotypes and markers that could be used was limited by these technologies.

The introduction of low-cost high-throughput next-generation sequencing (NGS) Diversity Arrays Technology (DArT™) genetic markers saw a monumental increase in the number of polymorphic markers available for mapping studies. Bi-parental linkage maps of for DArT™

markers were between 40 and 80 markers per chromosome. Such an immense increase in marker density would allow much greater accuracy in positioning QTL onto linkage maps, thus enabling the dissection of complex genetic interactions and enhancing knowledge of critical genomic regions e.g. the *Ptt* interaction near the centromere of 6H. However, only two *Ptt* QTL mapping studies used DArT™ markers, neither of which was Australian. Thus, the continued use of SSR markers has been to the detriment of the Australian barley industry and wider *Ptt* research community.

Genetic markers that use single nucleotide polymorphisms (SNPs) is currently the platform of choice for genetic studies. SNPs occur at high density throughout the barley genome, can be accurately repeated across studies and can be positioned with high accuracy on the physical map (Mascher *et al.* 2017). Bi-parental linkage maps with current SNPs range from 120 to 150 markers per chromosome. The 9K Illumina iSelect SNP returns 7,842 SNPs while the 50K chip returns 43,461 SNPs and has shown promise for direct high resolution mapping of a bi-parental mapping populations (Bayer *et al.* 2017).

2.9 Linkage mapping

Linkage mapping with a segregating bi-parental population is considered the traditional method of QTL analysis. Once a population has been generated using an appropriate method, the progeny are genotyped and phenotyped for the trait of interest. A linkage map is produced based on recombination frequencies between marker loci to infer genetic distance (Kosambi 1944). Markers with less recombination between them are closer together while markers that are further apart have more recombination. QTL analysis should be conducted in an appropriate software package or alternatively in R.

Many different QTL analysis methods exist for QTL mapping. Single marker analysis (SMA) assumes one QTL and tests each marker as a locus for the presence of a QTL by using the difference between phenotype for genotypes at the marker. The accuracy of this method is quickly constrained in linkage maps of few markers as large voids are often present. Simple interval mapping (SIM) (Lander and Botstein 1989; Soller *et al.* 1976) assumes one QTL and uses regression (Haley and Knott 1992) to identify the most likely interval for the QTL location from evenly spaced positions along the linkage map. This method overcame the issues around accuracy of QTL detection between marker gaps. Composite interval mapping (CIM) (Jansen and Stam 1994; Zeng 1994) assumes two QTL and uses user inputted markers as covariates to improve the accuracy of detection of linked QTL by reducing the residual variation (Ahmadiyeh *et al.* 2003).

CIM cannot estimate the combined effect of the closely liked QTL as a single dimension scan is conducted. In the presence of multiple QTL per chromosome that may or may not be interacting, multiple interval mapping (MIM) (Kao *et al.* 1999) modelling is appropriate. MIM achieves result equal to CIM and SIM when one QTL is detected on a chromosome, while results are superior to CIM in the presence of closely linked QTL as the QTL model can estimate the effect of the interaction between the QTL.

2.10 Genome-wide association mapping (GWAS)

Genome-wide association studies (GWAS) employ linkage disequilibrium (LD) between genetic markers and the causal gene to identify marker trait associations (MTAs) in an unstructured population. A mixed linear model (MLM) is used and can be described as $Y = X\beta + Zu + e$, where Y is the phenotype, X is the genotype, β is a vector of fixed effect that includes genetic markers, population structure and the intercept, Z is the kinship matrix, u contains random additive genetic effects and e contains the residual (Zhang *et al.* 2010). GWAS of barley has been used to successfully identify MTAs for resistance and susceptibility to *Ptt* (Adhikari 2017; Vatter *et al.* 2017; Wonneberger *et al.* 2017a), spot blotch (*Bipolaris Sorokiniana*) (Kharub 2017), spot form net blotch (*Pyrenophora teres* f. *macualta*) (Wang *et al.* 2015) and leaf rust (*Puccinia hordei*) (Singh *et al.* 2018; Ziems *et al.* 2017; Ziems *et al.* 2014).

2.11 Projecting resistance genes/QTL onto the barley physical map

Mapping studies have identified genomic interactions with *Ptt* on all seven barley chromosomes, most of which have been positioned on the barley physical map in Appendix 1. The map was based the revised genome sequence order by Mascher *et al.* (2017). All QTL were positioned from the peak marker or the next closest marker based on the map published for the specific population or the barley consensus map (https://wheat.pw.usda.gov/GG3/).

Hundreds of QTL from 37 mapping studies were projected on the barley physical map in Appendix 1 (Abu Qamar *et al.* 2008; Adhikari 2017; Afanasenko *et al.* 2015; Cakir *et al.* 2003; Cakir *et al.* 2011; Friesen *et al.* 2006; Graner *et al.* 1996; Grewal *et al.* 2008; Grewal *et al.* 2012; Gupta *et al.* 2010; Gupta *et al.* 2011; Ho *et al.* 1996; Islamovic *et al.* 2017; König *et al.* 2013; Koladia *et al.* 2017a; Lehmensiek *et al.* 2007; Liu *et al.* 2010; Liu *et al.* 2015; Ma *et al.* 2004; Mace *et al.* 2007; Mannien *et al.* 2000; Mannien *et al.* 2006; O'Boyle *et al.* 2014; Raman *et al.* 2003; Richards *et al.* 2016; Richards *et al.* 2017; Richter *et al.* 1998; Spaner *et al.* 1998; St. Pierre *et al.*

2010; Steffenson *et al.* 1996; Tenhola-Roininen *et al.* 2011; Vatter *et al.* 2017; Wonnerberger *et al.* 2017a; Wonnerberger *et al.* 2017b; Yun *et al.* 2006).

In addition, markers linked to wheat sensitivity genes to *Parastagonospora nodorum* and *Pyrenophora tritici-repentis*; *Snn1*, *Snn2*, *Snn3*, *Snn4*, *Snn5*, *Snn6*, *Snn7*, *Tsc1*, *Tsc2* and *Tsn1* were also positioned on the barley physical map (Abeysekara *et al.* 2012; Abeysekara *et al.* 2010; Faris *et al.* 2010; Friesen *et al.* 2012; Gao *et al.* 2015; Liu *et al.* 2017; Shi *et al.* 2015; Shi *et al.* 2016; Zhang *et al.* 2009; Zhang *et al.* 2011).

Issues arose when attempting to position AFLP, RFLP and SSR based markers onto the barley physical map for comparison to highly accurate SNPs. Additionally, low marker resolution of older maps often resulted in the detection of identical QTL regions from material of unrelated genetic background, suggesting that genotypes carried similar resistance, which may not be correct in all cases. For example, BLAST searches in ENSEMBL database (http://plants.ensembl.org/Hordeum_vulgare/Info/Index) of the forward and reverse probe sequences of Bmag0173 from GrainGenes (https://wheat.pw.usda.gov/cgi-bin/GG3/report.cgi?class=probe;name=Bmag0173), identified possible locations on seven all chromosomes. Specifically, five on 1H, two on 2H, five on 3H, five on 4H, four on 5H, four on 6H and two on 7H for the forward probe and one on 1H, two on 2H, two on 3H, one 4H and one on 5H for the reverse probe. The 6H hits were positioned at 87,386,117 bp to 87,386,131 bp, 296,237,031 bp to 296,237,045 bp, 503,881,766 bp to 503,881,780 bp and 567,521,995 bp to 567,522,009 bp. The published location of Bmag0173 often varied in different studies and could be due to hybridisation to different locations on 6H. This inconsistency of positioning means comparison of Bmag0173 across studies is difficult and unreliable.

Many studies have shown CIho 5791 to carry a resistance gene near the centromere of 6H and because it was used as the original donor of resistance in Canadian cultivars; BT 201, Ellice, Heartland and Norbert, it is possible that some studies could be detecting the resistance from CIho 5791 (Emebiri *et al.* 2005; Friesen *et al.* 2006; Grewal *et al.* 2008; Grewal *et al.* 2012; Richter *et al.* 1998; Spaner *et al.* 1998; St. Pierre *et al.* 2010; Tenhola-Roininen *et al.* 2011; Yaniv *et al.* 2014).

QTL were often detected on chromosome 3H near the *Rpt1*.a locus of Tifang (Graner *et al.* 1996; Gupta *et al.* 2011; Koladia *et al.* 2017a). Three resistance genes on 3H were identified in genotypes of Manchurian origin and were originally described as Pt_1, Pt_2 and Pt_3 (Mode and

Schaller 1958; Schaller 1955) although (Bockelman *et al.* 1977) did not detect differentiation for the genes on 3H and described a single resistance factor, the designation has since been updated to *Rpt1.a* (BGN 2013). CIho 9819 was also shown to carry a resistance on 1H, 3H and 6H and were originally named *Rpt2*, Pt_a and *Rpt5*, respectively and were later updated to *Rpt2.c*, *Rpt1.b* and *Rpt5.f,* respectively (BGN 2013). Halcyon has been shown to carry a resistance on chromosome 4H (Raman *et al.* 2003; Read *et al.* 2003), which has the proposed designation - *Rpt7.h* (BGN 2013). QTL on 4H have been detected more frequently since 2017 (Adhikari 2017; Islamovic *et al.* 2017). Rika and Kombar were resistant/susceptible to reciprocal isolates and two close susceptibility genes linked in repulsion on 6H near *Rpt5* were able to explain the interaction (Abu Qamar *et al.* 2008).

2.12 Conclusion

Previous mapping studies using gel electrophoresis based markers often used the same markers and detected QTL in the same region, suggesting that studied genotypes carried the same QTL. This may not be true in all cases. High resolution maps of next-generation sequencing platforms will revolutionise QTL mapping studies as accurate projection and of QTL onto maps will allow comparison between QTL of separate studies. The projection of previously reported QTL onto the barley physical map (Appendix 1) is the first of its kind for *Ptt*. This serves as a starting point to build upon with continual addition and revision of catalogued QTL and genes for the advancement of collective knowledge for this damaging pathogen.

A population-wide marker selection methodology, genomic selection (GS), has shown the potential to achieve greater efficiency of genetic gain for complex traits when compared to marker-assisted selection (MAS) (Heffner *et al.* 2010). As such, GS will likely revolutionise population breeding due to higher accuracy of predictions on genotype performance and allowing the selection of un-phenotyped individuals for incorporation into future breeding cycles.

Ptt is an extremely complex and highly adaptive plant pathogen. This organism has been the focus of many studies over the past 60 years and given this length of time, large knowledge gaps still exist. Recent the advances in genetic analysis will bridge this this gap if they can be applied in a manner that efficiently integrates traditional plant pathology and molecular genetics.

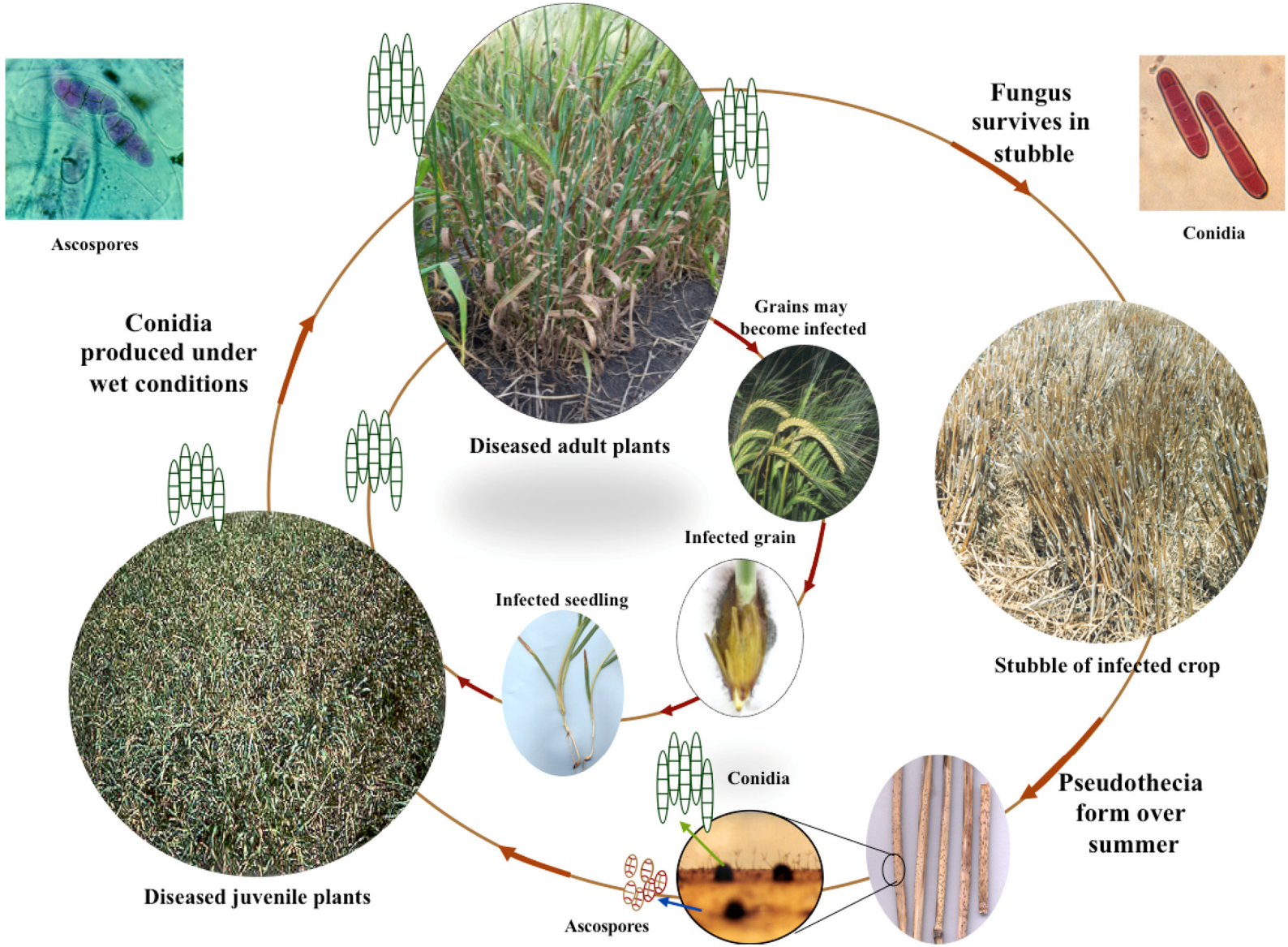
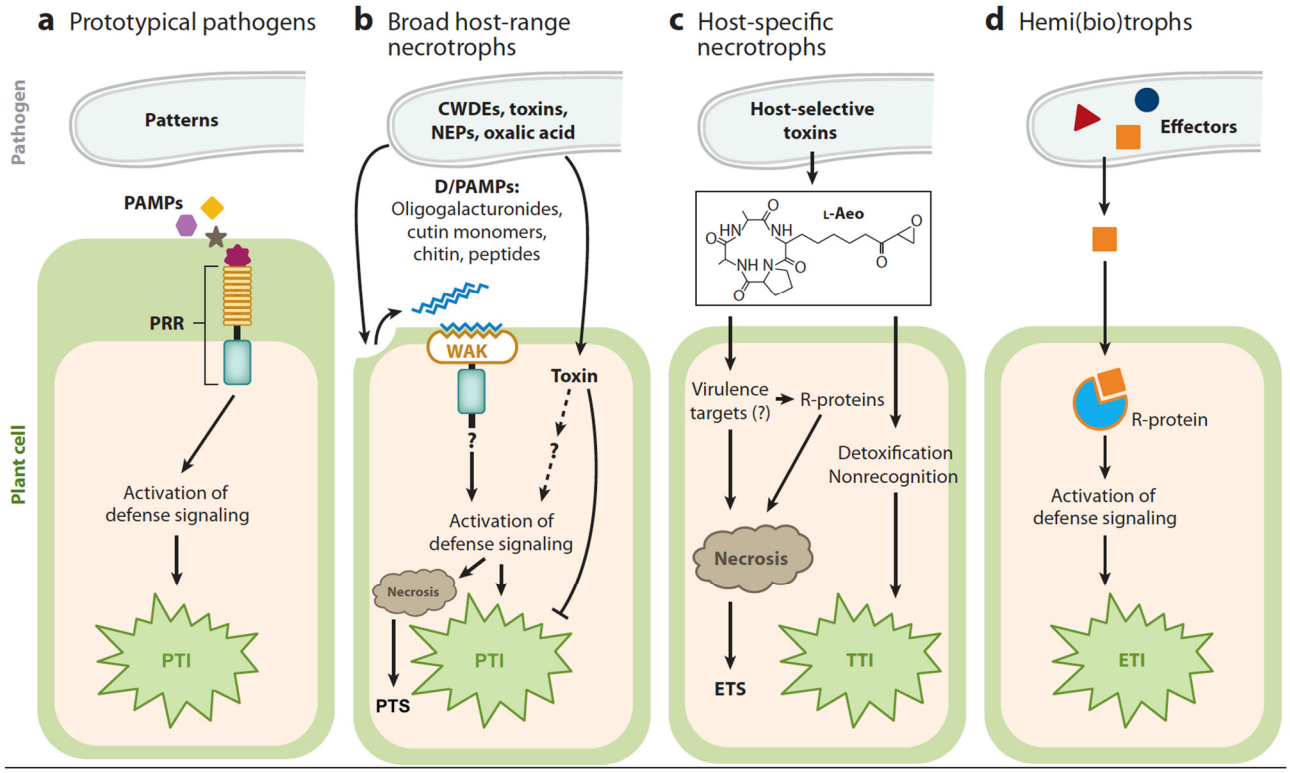


Figure 2.1. Life cycle of *Pyrenophora teres* f. *teres*.



Major D/PAMPs, virulence factors, and immune responses to pathogens of different lifestyles. (*a*) Simplified scheme showing recognition of D/PAMPs by PRRs and activation of basal immune responses. (*b*) Broad host-range necrotrophs produce diverse D/PAMPs that activate plant immune responses as well as virulence factors that suppress immune responses. PAMPs may also interact with wall associated kinases (WAKs) to induce a disease response that leads to necrosis and cell death. (*c*) Host-specific necrotrophs produce toxins that are major players for their virulence. Plants have immunity mechanisms, such as enzymes that detoxify the toxins or carry alleles encoding proteins impervious to the toxin or alleles that do not recognize the toxin. Toxins may also have direct host virulence targets whose manipulation is recognized by R-proteins, leading to ETS instead of the ETI in the guard hypothesis. The *Arabidopsis* LOV1, wheat TSN1, and sorghum PC genes encode R- or R-like proteins that mediate sensitivity to toxins but are not known to directly interact with the toxin itself. (*d*) Simplified scheme of ETI commonly associated with hemibiotrophic or biotrophic pathogens. Regardless of their specificity, toxins are predicted to target host proteins to promote susceptibility. In all cases, for simplicity only major factors are highlighted. Dashed arrows indicate limited or no experimental evidence. Abbreviations: CWDEs, cell wall–degrading enzymes; D/PAMPs, damage/pathogen-associated molecular patterns; ETI, effector-triggered immunity; ETS, effector-triggered susceptibility; NEPs, necrosis and ethylene-inducing proteins; PRRs, pattern recognition receptors; PTI, PAMP-triggered immunity; PTS, PAMP-triggered susceptibility; TTI, toxin-triggered immunity.

Figure 2.2. Stylised host-pathogen interactions. Adapted from Mengiste (2012).

Chapter 3

Pathogenic variation of *Pyrenophora teres* f. *teres* in Australia

3.1 Abstract

Pyrenophora teres f. *teres* (*Ptt*) is the causal agent of net form net blotch (NFNB) – a major foliar disease of barley (*Hordeum vulgare*) crops worldwide. Deployment of genetic resistance in cultivars is the preferred method of control but requires knowledge of the pathogenic variation of *Ptt* to be effective as spatial and temporal variation is common. In this study, 123 *Ptt* isolates collected from five states across Australia were examined for pathogenic variation using a set of 31 barley genotypes, composed of 11 international genotypes and 20 Australian cultivars. Barley seedlings were inoculated with spore suspensions from monoconidial isolate cultures and scored for infection response. Phenotypes were used to perform hierarchical cluster analysis for barley genotypes and *Ptt* isolates. Cluster analysis identified seven line groups, each containing barley genotypes that displayed similar responses to the *Ptt* isolates. Isolates clustered into four distinct isolate groups shown to harbour differential virulence to four key genotypes: Maritime, Prior, Skiff and Tallon. Isolates with virulence to any one of these genotypes accounted for 96.7% of the samples. Differential virulence was observed on a range of genotypes within each isolate group. The composition of isolate groups in eastern Australia was distinct from Western Australia, whereas all isolate groups were detected in southern Australia. Results suggest that cultivation of regionally adapted barley cultivars has led to regional evolution of *Ptt*, where the pathogen acquires virulence specific for genetic factors deployed in local cultivars. Detection of *Ptt* isolates that were highly virulent to historic cultivars indicates the long-term survival of virulence gene combinations in the pathogen population.

3.2 Introduction

The fungus *Pyrenophora teres* Drechslera (anamorph *Drechslera teres* (Sacc.) (Shoemaker 1959)) that causes net blotch disease of barley (*Hordeum vulgare* L.) has two morphologically identical forms that result in the expression of distinctly different disease symptoms. This study focused on net form net blotch (NFNB), which is caused by *Pyrenophora teres* Drechslera f. *teres* Smedeg. (*Ptt*) and typically induces longitudinal lesions that often display a distinct netting pattern. *Ptt* mainly infects leaves but can also infect leaf sheaths, stems, glumes and awns. The *Ptt* lifecycle includes asexual and sexual stages. The asexual stage involves the production of conidia, whereas

the sexual stage involves reproduction between isolates of compatible mating types and genetic recombination to produce ascospores. The adoption of management practices that retain crop stubble has increased the incidence of NFNB as the pathogen persists on plant residues. NFNB is a common disease in most barley growing regions of the world with yield losses of up to 35% (Jebbouj and El Yousfi 2009; Khan 1987; Piening and Kaufmann 1969; Smedegård-Petersen 1974; Steffenson *et al.* 1991; Sutton and Steele 1983). Yield losses can be caused by a reduction in kernel weight, number of kernels per spike and number of heads per plant (Deimel and Hoffmann 1991; Jordan 1981; Khan 1987). In Australia, barley production losses due to NFNB are estimated to cost the industry $19M annually with potential losses as high as $117M (Murray and Brennan 2010). These figures are based on conservative annual average yield loss estimates of 1.47% and 9.07%, respectively. However, for highly susceptible cultivars the economic losses due to NFNB would be much greater. The preferred method of reducing the economic impact of disease is deployment of cultivars incorporating genetic resistance as this reduces the cost to growers and are more environmentally friendly as they are not reliant on fungicides.

Qualitative resistance to *Ptt* is considered effective at all growth stages and is typically examined at the seedling stage and is usually underpinned by gene(s) with large effect that are often isolate specific (Abu Qamar *et al.* 2008). On the other hand, adult plant resistance (APR), is best expressed at adult growth stages and provides quantitative resistance to *Ptt* (Jonsson *et al.* 1998; Robinson and Jalli 1997; Steffenson and Webster 1992b). Genetic mapping studies performed at both seedling and adult growth stages have consistently reported a quantitative trait locus (QTL) in the centromeric region on chromosome 6H. This region appears to harbour multiple genetic factors that could be linked genes and/or multiple alleles that interact with isolates from geographically diverse regions (Cakir *et al.* 2011; Friesen *et al.* 2006; Lehmensiek *et al.* 2007; Steffenson *et al.* 1996). While this appears to be a key genomic region involved in the host pathogen interaction, QTL for seedling resistance have been identified across all seven chromosomes of barley (Ma *et al.* 2004; Manninen *et al.* 2006; Raman *et al.* 2003; Richter *et al.* 1998). Several QTL for APR have also been reported (Cakir *et al.* 2003; König *et al.* 2013; Lehmensiek *et al.* 2007; Steffenson *et al.* 1996). APR is common in Australian cultivars and provides protection at adult growth stages in the field, although isolate specificity has also been reported for this type of resistance (Usher *et al.* 2009; Wallwork *et al.* 2016).

Ptt is a necrotrophic pathogen that uses host-selective toxins (HSTs) as a means of stimulating host cell death to promote disease development (Friesen *et al.* 2007; Lamari and Bernier 1989; Smedegård-Petersen 1977; Yoder and Gracen 1975). HSTs follow a gene-for-gene model

termed necrotrophic-effector induced susceptibility (NETS), whereby dominant virulence genes that produce necrotrophic effectors are recognised by dominant susceptibility genes in the host resulting in a signal transduction pathway of programmed cell death (Liu *et al.* 2015). Several *Ptt* avirulence and virulence genes, which interact with dominant resistant and susceptibility genes in barley, respectively, have been described (Beattie *et al.* 2007; Liu *et al.* 2011; Shjerve *et al.* 2014; Weiland *et al.* 1999). A recent study by (Shjerve *et al.* 2014) mated two *Ptt* isolates with virulence to either Kombar or Rika and discovered two separate genes for virulence to Kombar (*VK1* and *VK2*) from one parent isolate and two separate genes for virulence to Rika (*VR1* and *VR2*) from the other parent isolate. Through QTL mapping, these virulence genes were found to interact with a genomic region on chromosome 6H that harboured the corresponding dominant susceptibility genes (Abu Qamar *et al.* 2008). These results confirm the model of necrotrophic effector-triggered susceptibility (NETS) in the pathogenicity of *Ptt* on barley. In a similar study, ElMor (2016) mated two *Ptt* isolates (NB29 and NB85) and the progeny displayed virulence to barley genotypes resistant to both parental isolates. This highlights that where such recombination in the field is possible, knowledge of the pathogenic diversity is critical to develop cultivars with effective resistance.

A high degree of *Ptt* pathogenic diversity has been documented in numerous studies worldwide (Akhavan *et al.* 2016; Arabi *et al.* 2003; Bouajila *et al.* 2011; Boungab *et al.* 2012; Cromey and Parkes 2003; Douiyssi *et al.* 1998; Gupta and Loughman 2001; Jalli 2010; Jebbouj and El Yousfi 2010; Khan 1982; Khan and Boyd 1969b; Liu *et al.* 2012; Platz *et al.* 2000; Robinson and Jalli 1996; Steffenson and Webster 1992a; Tekauz 1990; Tekauz and Mills 1974; Tuohy *et al.* 2006). Previous studies of *Ptt* in Western Australia by Khan and Boyd (1969b) documented differential virulence to Algerian and CIho 7584 and 100% virulence to Beecher, Dampier and Prior. A subsequent study by Khan (1982) did not detect virulence to CIho 7584, while differential virulence to Algerian and Beecher was detected and 100% of isolates displayed virulence to Dampier. The most recent study of *Ptt* in Western Australia by Gupta and Loughman (2001) did not detect virulence to Algerian, Beecher or CIho 7584 in isolates from commercial fields, yet detected 100% virulence to Dampier and Prior. Beecher was popular in Western Australia in the 1950's to 1970's, after which a decrease in Beecher virulence was observed. A study by Platz *et al.* (2000) examined *Ptt* isolates from Queensland and documented isolates with differential virulence to Betzes, CIho 11458, Cape, Clipper, Corvette, Dampier, Franklin, Gilbert, Grimmett, Herta, Prior, Skiff and Tallon. More recently, a study of South Australian *Ptt* isolates by Wallwork *et al.* (2016) documented differential virulence to Commander, Fleet, Franklin, Keel, Maritime, Skiff and other modern cultivars when tested at the adult plant stage. While pathogen diversity studies allow insight of the virulences present at a particular point in time, virulences within *Ptt* populations are dynamic

and fluctuate in response to available host genetics. This highlights the need to periodically monitor virulence of this important pathogen. An international set of differential barley genotypes was also proposed by Afanasenko *et al.* (2009) to enable worldwide comparisons of pathogenicity.

This study examined 123 isolates of *Ptt* collected from five Australian states between 1985 and 2012. *Ptt* isolates were inoculated onto 31 barley genotypes at the seeding stage and infection responses were analysed to determine pathogenic variation among isolates sampled across five states. Knowledge of current pathogenic variation will serve as a reference point for future Australian pathogenicity studies and will be used to identify relevant isolates for in mapping studies in later chapters of this thesis.

3.3 Materials and methods

3.3.1 Isolate collection

One hundred and twenty three single spore isolates of *Ptt* collected from the major barley growing regions of Australia between 1985 and 2012 were phenotyped at the Hermitage Research Facility in Warwick, Queensland. A summary of sampling information for isolates used in this study is presented in Table 3.2.

3.3.2 Isolate culture

Single conidial cultures were obtained from each isolate before phenotyping. Leaves showing NFNB symptoms were cut into 2 cm lengths and placed in a petri dish containing one filter paper disk overlying a water absorbent pad. Millipore-filtered water was added to each plate until free water was visible. Leaf tissue and plates were incubated at 19°C (± 1°C) with 12-hr diurnal fluorescent white and near UV light until sporulation was observed. Five single conidia were individually transferred to petri dishes containing V8 agar (150 mL Campbell's V8® vegetable juice, 850 mL water, 1.5 g $CaCO_3$ and 15 g agar) and incubated in the dark for 5 – 6 days at 25°C (± 1°C). Ten agar and mycelium plugs originating from one conidium were then transferred to two peanut oatmeal agar (POA) (50 g fresh peanut leaf filtrates in 500 mL water, 15 g oatmeal filtrates in 500 mL water and 20 g agar) plates (Speakman and Pommer 1986) and returned to 19°C (± 1°C) under diurnal light for 9 – 10 days for conidia production.

3.3.3 Barley genotypes

Three groups of seeds were sown into 10 cm diameter pots at three evenly spaced pot positions around the circumference of each pot. Five seeds of a single barley genotype from 31 available genotypes (Table 3.1) were sown to one pot position. Ten pots constituted one replicate and two replicates constituted one basket. Since there were more barley genotypes than the available 30 pot positions, partial replication (Smith *et al.* 2006) of genotypes was used so that all genotypes were exposed to all isolates. Each pot contained Searles® premium potting mix and plants were fertilised twice weekly with 1.3 g/L of Grow Force Flowfeed EX7 soluble fertilizer. Plants were top watered pre-inoculation and bottom watered post-inoculation. Differential lines were grown in a glasshouse at 20°C (± 5°C) under natural light for 14 days until they reached growth stage Z12 (Zadoks *et al.* 1974) when they were inoculated. Post inoculation, plants were transferred to a temperature controlled growth room maintained at 24/14°C (± 1°C) day/night temperature. A mixture of 2700K halogen, 2000K high pressure sodium and 4000K metal halide lights were used to provide a 12 hour diurnal photoperiod for plant growth and symptom development.

3.3.4 Experimental design

A series of screening experiments were conducted to evaluate all 123 isolates on the 31 barley genotypes. Each screening experiment was conducted across two benches, where each bench constituted a replicate block and contained 30 pots. The experimental design within a screening experiment was a split-plot design where barley genotypes were randomised to the 30 pot positions (subplots) per replicate within a basket; and three isolates were randomised to baskets (main plots) on each bench (replicate block). In total 64 screening experiments were completed. An incomplete blocking structure was used to allocate isolates to screening experiments so that isolates were replicated within and across screening experiments, ensuring valid comparisons could be made among isolates.

3.3.5 Inoculation

Conidia were washed from the POA plates into a beaker using 5ml of 18.2 MΩ-cm purified Tween®-water (two drops of Tween® 20 per 100 mL of purified water) and a fine paintbrush. The resultant spore suspensions were then filtered through a fine tea strainer and diluted with Tween®-water to give a standardised inoculum concentration of 10,000 conidia/mL. Inoculum was applied at 2.5 mL of suspension/pot using a Paasche® airbrush and immediately transferred to a humidity

chamber at 19°C (± 1°C) and 99% humidity for 24 hours with 14 hours dark followed by 10 hours of light.

3.3.6 Disease assessment

Infection responses (IR) of barley genotypes were determined according to a 1 – 10 rating scale (Tekauz 1985) 9 days post-inoculation based on the response observed within the central portion of the second leaf, where 1 was most resistant and 10 was most susceptible. Infection responses < 5 were considered a low infection response (LIR) and separated into two subclasses with scores 1 to < 2.5 considered moderately resistant (MR) and scores ≥ 2.5 to < 5 considered moderately susceptible (MS). Scores ≥ 5 were considered a high infection response (HIR) and separated into two subclasses with scores ≥ 5 to < 7.5 considered susceptible (S) and scores ≥ 7.5 considered very susceptible (VS). Phenotype scores ≥ 5 were used to identify susceptible responses and considered indicative of virulence in *Ptt* isolates.

3.3.7 Statistical analysis

Two separate linear mixed models were fitted to the phenotypic data. One model was used to determine the cluster groupings of the 31 barley lines based on their IRs to the 123 isolates. The other model was used to determine the cluster groupings of 123 isolates based on their ranking of the 31 lines. Both models had the same structural terms to account for blocking restrictions in the experimental design. Terms for Screening Experiment, Bench (replicate block), Basket (main plot), Pot and Pot Position (subplot) were fitted as a nested structure and considered random effects in the model. In addition, the model to determine cluster groups of the lines included isolate as a fixed effect and the line and line × isolate interaction as random effects. A factor analytic (FA) approach (Smith *et al.* 2001) was applied to the linear model to estimate the variance of lines within isolates and the covariance between isolates. Conversely, the model to determine cluster groups of the isolates included lines as a fixed effect and the isolate and line × isolate interaction as random effects, where the FA approach was used to estimate the variance of isolates within lines and the covariance between lines.

Using the correlation matrix estimated from each separate FA model, a dissimilarity matrix was calculated through a squared Euclidean distance. Ward's minimum variance method of clustering (Ward Jr 1963) was then applied to form the hierarchical clusters for each of the two models. The hierarchical clustering of the genotypes reached an agglomerative coefficient of 0.93.

The dendrogram of these clusters was intercepted at a height of 0.95 to identify groups of barley genotypes that responded similarly, termed line groups (LGs). The hierarchical clustering of the 123 isolates reached an agglomerative coefficient of 0.97. This dendrogram was intercepted at a height of 0.85 to identify isolate groups (IGs). The height on the dendrogram is a measure of the variance between cluster groups, as the height increases the variance within cluster groups increases. In conjunction with cluster groupings, which best described the virulence patterns, the interception point on the y-axis of the dendrogram was chosen at heights where longer (arms) distances between clusters first appear.

Least significant differences (LSD) for IGs and LGs were calculated using agricolae statistical package (De Mendiburu 2014) in RStudio software (RStudio 2015). To visualise the geographical distribution of the IGs, each isolate was plotted onto a map of Australia according to the state and region of origin and coloured according to IG (Figure 3.5).

3.4 Results

Isolates of *Ptt* exhibited differential virulence to barley genotypes. HIRs were observed for at least one barley genotype for 122 of the 123 isolates and HIRs were recorded for all genotypes except CIho 5791 (Table 3.2). The percentage of isolates that displayed HIRs to individual genotypes varied from 0% in CIho 5791 to 94% in Commander (Table 3.4). VS infection responses were not observed on Algerian, Buloke, CIho 5791, Kaputar or Vlamingh; conversely, MR infection responses were not observed for Betzes, Commander or Keel (Figure 3.4). More than 80% of isolates induced HIRs on Betzes, Commander, Harrington, Hindmarsh and Keel, while fewer than 20% of isolates induced HIRs on Algerian, Beecher, Buloke, CIho 11458, CIho 5791, Cape, Canadian Lake Shore, Fleet Australia, Harbin and Vlamingh (Table 3.4).

Isolates with virulence to Beecher, Buloke, Canadian Lake Shore, Cape, CIho 5791, Dampier, Harbin, Prior or Yerong were not detected in New South Wales. Isolates with virulence to Beecher or CIho 5791 were not detected in Queensland. Isolates with virulence to CIho 5791 or Vlamingh were not detected in South Australia. Isolates with virulence to CIho 11458, CIho 5791 or Vlamingh were not detected in Victoria. Isolates with virulence to Buloke, CIho 11458, CIho 5791, Herta, Patty, Skiff or Vlamingh were not detected in Western Australia.

3.4.1 Pathogenic variation between isolate groups (IGs)

On the *Ptt* isolate hierarchical cluster dendrogram, an interception height of 0.95 separated isolates into four distinct IGs (Figure 3.2). Four key genotypes; Maritime, Prior, Skiff and Tallon, displayed a high degree of isolate specificity between the four isolates groups (Table 3.3). Variation between IRs was observed between IGs for these key genotypes (Table 3.5) and as such, were used to describe the overall phenotype of isolates within groups. Virulence for Maritime, Prior, Skiff or Tallon was detected in 26%, 33%, 49% and 61% of isolates, respectively, while isolates with virulence to any one of these genotypes accounted for 96.7% of isolates. Disease symptoms on the key genotypes for each IG are displayed in Figure 3.1.

IG number 1 (IG1) contained 59 isolates that could be separated from other IGs by HIRs on key genotypes Skiff and Tallon and LIRs on key genotypes Maritime and Prior (Table 3.5). Isolates within this group displayed differential virulence to 21 barley genotypes and 100% virulence to four barley genotypes (Table 3.4).

IG number 2 (IG2) contained 15 isolates that could be separated from other IGs by HIRs on the key genotype Tallon and LIRs on key genotypes Maritime, Prior and Skiff (Table 3.5). Isolates within this group displayed differential virulence to 22 barley genotypes (Table 3.4).

IG number 3 (IG3) contained 35 isolates that could be separated from other IGs by HIRs on the key genotype Prior and LIRs on key genotypes Maritime, Skiff and Tallon (Table 3.5). Isolates within this group displayed differential virulence to 26 barley genotypes (Table 3.4).

IG number 4 (IG4) contained 14 isolates that could be separated from other IGs by HIRs on the key genotype Maritime and LIRs on key genotypes Prior, Skiff and Tallon (Table 3.5). Isolates within this group displayed differential virulence to 16 barley genotypes and 100% virulence to Kombar (Table 3.4).

3.4.2 Pathogenic variation within isolate groups

Isolates sampled from different states that clustered to the same IG displayed statistically significant variation in the mean score for some barley genotypes (Table 3.5). Isolates clustering to IG1 sampled from different states displayed mean scores that were statistically different for Betzes, Commander, Franklin, Clipper, Gilbert, Hindmarsh, Keel, Maritime, Prior and Tallon (Table 3.5). Differences between mean scores of some genotypes were observed for isolates in IG2 although the limited number of samples between states did not allow for statistical comparisons to be made. While the single IG2 isolate from WA displayed low aggressiveness overall, HIRs were recorded

for Dampier and Prior (Table 3.3). IG3 isolates sampled from different states displayed mean scores that were statistically different for Cape, Clipper, Corvette, Gilbert, Grout, Kaputar, Kombar, Maritime, Prior and Vlamingh (Table 3.5). Isolates clustering to IG4 from different states displayed mean scores that were statistically different for Cape, Clipper, Corvette, Gilbert, Grout, Kaputar, Kombar, Maritime, Prior and Vlamingh (Table 3.5).

3.4.3 Clustering of barley genotypes into line groups

On the barley genotype hierarchical cluster dendrogram, interception at a height of 0.85 separated genotypes into seven distinct LGs (Figure 3.3).

LG number 1 (LG1) consisted of Algerian and CIho 11458; both displayed significantly higher mean phenotypes to isolates in IG1 in comparison to isolates in IG2, IG3 and IG4 (Table 3.5).

LG number 2 (LG2) consisted of two sub-groups of genotypes that displayed differential responses to isolates in IG1 and IG2. The first sub-group was comprised of Franklin, Herta, Patty, Skiff and Vlamingh. Franklin, Herta, Patty and Skiff displayed significantly higher mean phenotypes to isolates in IG1 compared to isolates in IG2, IG3 and IG4. Mean phenotypes for Vlamingh were significantly higher to isolates in IG1 compared to isolates in IG2, IG3 and IG4 (Table 3.5). The second sub-group was comprised of Gilbert, Grimmett, Harrington and Tallon, which displayed significantly higher mean phenotypes to isolates in IG1 and IG2 compared to isolates in IG3 and IG4. Grimmett and Harrington responded with significantly lower mean phenotypes to isolates in IG4 compared to isolates in IG3, while phenotypes for Gilbert and Tallon did not differ significantly for isolates in IG3 and IG4 (Table 3.5).

LG number 3 (LG3) comprised Buloke, CIho 5791, Fleet Australia, Kaputar and Kombar. Mean phenotypes for CIho 5791 and Fleet Australia did not differ significantly for isolates from any IG. Buloke and Kaputar responded with significantly higher mean phenotypes to isolates in IG1 compared to isolates in IG3. Kombar displayed significantly higher phenotypes to isolates in IG4 compared to isolates in IG1, which were also higher than isolates in IG2 and IG3 (Table 3.5).

LG number 4 (LG4) comprised Betzes, Clipper, Commander, Hindmarsh and Keel. Both Betzes and Hindmarsh displayed significantly higher phenotypes to isolates in IG3 compared to isolates in IG1, IG2 and IG4. Clipper and Keel displayed significantly higher phenotypes to isolates

in IG1, IG2 and IG3 compared to isolates in IG4. Commander displayed significantly higher phenotypes to isolates in IG3 compared to isolates in IG4 (Table 3.5).

LG number 5 (LG5) comprised two genotypes; Corvette and Grout. Corvette displayed significantly higher phenotypes to isolates in IG3 compared to isolates in IG1. Mean phenotypes for Grout did not vary significantly across IGs (Table 3.5).

LG number 6 (LG6) comprised Beecher, Cape, Maritime and Yerong. These genotypes responded with significantly higher phenotypes to isolates in IG4 compared to IG1, IG2 and IG3. Beecher, Cape and Yerong also displayed significantly lower phenotypes to isolates in IG1 and IG2 compared to isolates in IG3 (Table 3.5).

LG number 7 (LG7) comprised Canadian Lake Shore, Dampier, Harbin and Prior. These genotypes responded with significantly higher phenotypes to isolates in IG3 compared to isolates in IG1, IG2 and IG4. Prior also responded with significantly lower phenotypes to isolates in IG1 and IG2 compared to isolates in IG4 (Table 3.5).

3.4.4 Geographical distribution of isolate groups in Australia

The composition of IGs varied across the different states of Australia (Figure 3.4). New South Wales (NSW) was mainly represented by isolates from IG1 (red) and two isolates from IG2 (orange). Notably, isolates from IG3 (green) and IG4 (blue) were not detected in NSW. Queensland (QLD) was represented by isolates from IG1, IG2 and IG3. Isolates from IG4 were not detected in QLD. On the other hand, South Australia (SA) and Victoria (Vic) were represented by isolates from all four IGs. Western Australia (WA) was mainly represented by isolates from IG3 and IG4 and one isolate from IG2.

3.5 Discussion

This is the most comprehensive study of the pathogenic variation of *Ptt* in Australia – reporting virulence of 123 isolates collected across Australia over 27 years. Analyses revealed four distinct groups of *Ptt* isolates that exhibited differential virulence to 31 barley genotypes, which varied across the five Australian states. Results from this study highlight the need for screening with diverse isolates of known virulence combinations to ensure the development of resistant barley cultivars in Australian breeding programs.

Virulence was detected to all genotypes except CIho 5791, indicating that this source of resistance is still effective in Australia and remains a useful donor for breeding programs. None of the sampled isolates induced a VS IR on Algerian, Buloke, Kaputar or Vlamingh suggesting that these genotypes may be useful donors for providing moderate levels of resistance. While Canadian Lake Shore and Harbin have never been grown commercially or used as resistance sources in Australia, the detection of isolates from IG3 that induced HIRs indicate that resistances from these sources would certainly be at risk if deployed. Clipper and Kaputar generally displayed MS LIRs and S HIRs. Clipper had significantly lower IRs to isolates in IG4 while Kaputar displayed significantly lower IRs to isolates in IG3. These genotypes may harbour minor resistance factors that are isolate specific. The remaining genotypes showed IRs that were isolate specific and these represent sources of resistance that have been defeated and are no longer effective in Australia.

Prevalence of virulence to each genotype varied in each state, indicating that the state-based *Ptt* populations were quite unique. Beecher virulence was not detected in Queensland or New South Wales, conversely Herta, Patty or Skiff virulence was not detected in Western Australia. Notably, this presence/absence of virulence reflects the historic cultivation of Beecher and Skiff within the respective states. Virulence to superseded cultivars was detected in all states, which suggests that accumulated virulence factors may remain within the *Ptt* population long after the cultivar that selected those virulence factors was grown. Prior was the dominant cultivar in Australia between 1910 and 1970 and this long history of interaction with *Ptt* is reflected in the pathogen population many years later as modern isolates with Prior virulence were common across Australia. A similar case was observed for Beecher, which was grown in South Australia and Western Australia between 1950 and 1980. Isolates collected from these states displayed Beecher virulence, indicating that virulence to Beecher is also conserved in the *Ptt* population. Another example is provided by Clipper – the dominant barley cultivar grown in South Australia between 1970 and 1990. Three of the four isolates that induced VS IRs on Clipper were sampled from South Australia. Notably, isolate nf27/12a, also induced a higher IR on adult plants of Clipper compared to other modern South Australian isolates in the study by Wallwork *et al.* (2016). This indicates that *Ptt* in South Australia accumulated virulence factors for Clipper, which can still be detected in the population almost 30 years later. Similarly, Corvette was grown widely in Queensland between 1976 and 1990, which likely increased virulence for this cultivar among isolates sampled from Queensland. Whilst very little Corvette is now grown in Queensland, virulence to this genotype was common in combination with virulence to Prior. These examples demonstrate that *Ptt* is highly responsive to the underlying genetics of cultivars to which the pathogen is exposed, exhibiting the ability to

accumulate and sustain virulence in the population over an extended period. A similar increase of virulence to widely grown cultivars and subsequent retention of virulence within the *Ptt* population was observed in Canada between 1974 and 2016 (Akhavan *et al.* 2016; Tekauz 1974; Tekauz 1990).

Diversity of virulence in the Australian *Ptt* population has implications for breeders seeking to develop resistant cultivars. Failure to screen with appropriate isolates in selection for resistance may result in susceptibility of newly developed cultivars to some isolates in the pathogen population prior to release. This scenario likely occurred with the cultivar Maritime, which was released in South Australia in 2004. Maritime was resistant when released and became popular in some areas of South Australia. It was responsible for an outbreak of NFNB in that state in 2007. Virulence to Maritime was present in combination with virulence to Beecher in the isolate NB29, which was collected from Western Australia in 1985. It is likely that isolates with virulence to Beecher, which also display virulence to Maritime, were not used to screen germplasm during the development of Maritime. This suggests that Beecher and Maritime carry similar resistance/susceptibility genes, highlighting the importance of screening breeding germplasm with diverse isolates with known virulences to identify potential weaknesses before variety release.

Annual *Ptt* assessment of Australian cultivars and advanced breeding lines through National Variety Trials has identified Vlamingh as one of the most resistant cultivars developed in Australia (www.nvtonline.com.au). Vlamingh has resistance derived from TR 118, a two-row Canadian breeding line of Harrington background (http://pgrc3.agr.ca/cgi-bin/npgs/html/acchtml.pl?49492). Vlamingh displayed LIRs to most IGs but displayed significantly higher IRs for isolates belonging to IG1. Notably, isolates from this group had not been exposed to broad scale cultivation of Vlamingh. However, some cultivars from LG2 that share common ancestors with Vlamingh also displayed HIRs to these isolates. This raises concern that the release of cultivars possessing only limited components of genetic resistance may erode the overall effectiveness of a stronger more complex resistance.

Isolates in each IG showed pathogenic variation on the chosen differentials in addition to virulence on the defining differential genotype. These minor variations could be explained by *Ptt* following the model of NETS (Friesen *et al.* 2007), as variation in the presence/absence of small effect virulence factors between isolates may result in small differences in IR in similar genotypes. Therefore, the number of pathotypes detected in a population may be a function of the number of

genotypes used to examine isolates. It is theoretically possible for each isolate to be a different pathotype in the model of NETS.

This study reports pathotypic variation of the Australian *Ptt* population determined by responses on barley seedlings. This is the standard protocol for similar work world-wide and is based on differences in genetic resistances of genotypes in the differential sets. The method is quick, clinical and requires little inoculum; however, it fails to identify the presence of APR in differential genotypes and the implications of such resistances in disease management. For instance, a recent study by Wallwork *et al.* (2016) reported significant changes in disease responses when *Ptt* isolates were evaluated on barley genotypes at the seedling stage in comparison to the adult stage. In our study, genotypes such as Commander, Hindmarsh and Keel exhibited HIRs to greater than 85% of isolates tested. However, these genotypes have been reported to carry moderate levels of resistance to some isolates at the adult stage in the field (www.nvtonline.com.au). Thus, isolates examined here may also interact with APR factors present in the barley genotypes and this aspect could be explored in future studies. Further work will also include phenotyping Australian *Ptt* isolates using the international set of barley differentials to better understand pathogen diversity world-wide.

3.6 Figures

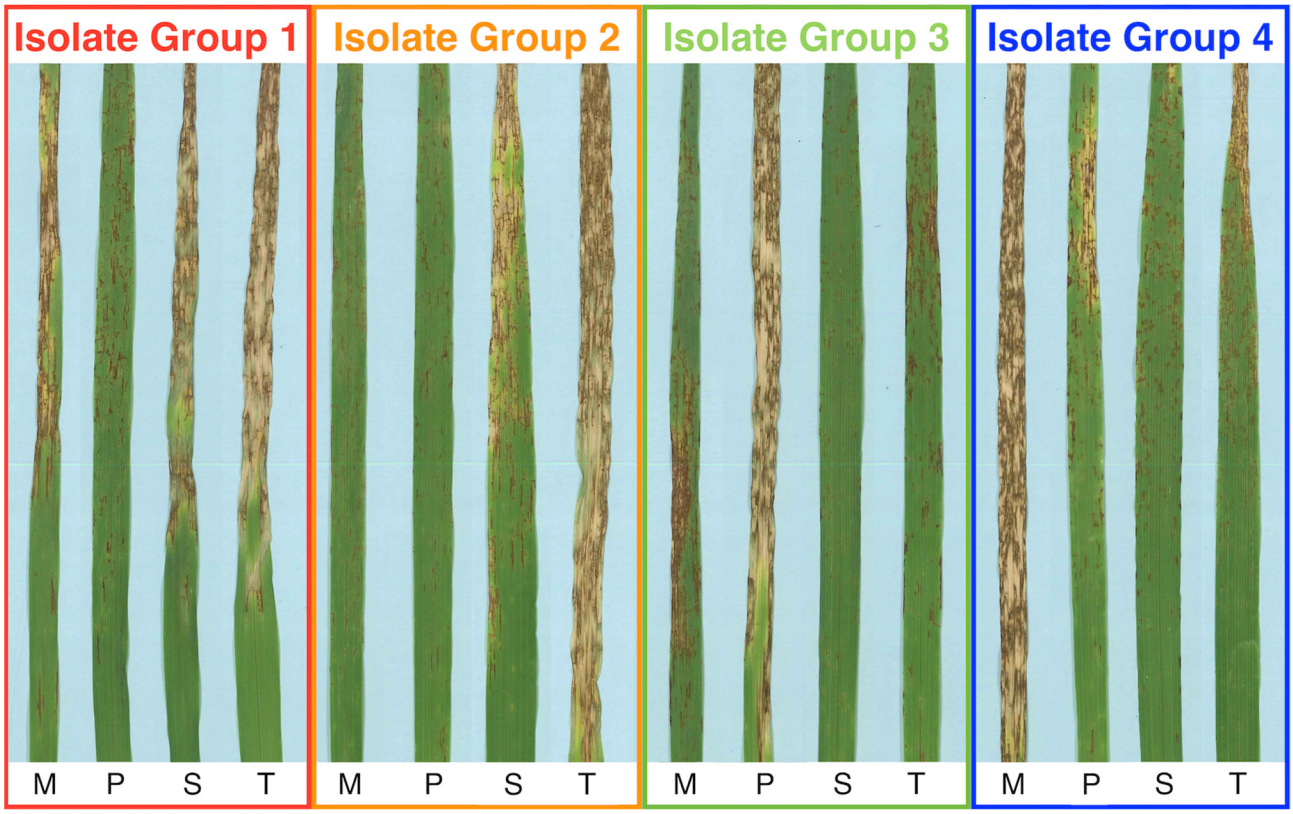


Figure 3.1. Disease symptoms of net form net blotch on barley seedling leaves. Differences in virulence profile between four isolate groups demonstrated by infected leaves of Maritime (M), Prior (P), Skiff (S) and Tallon (T).

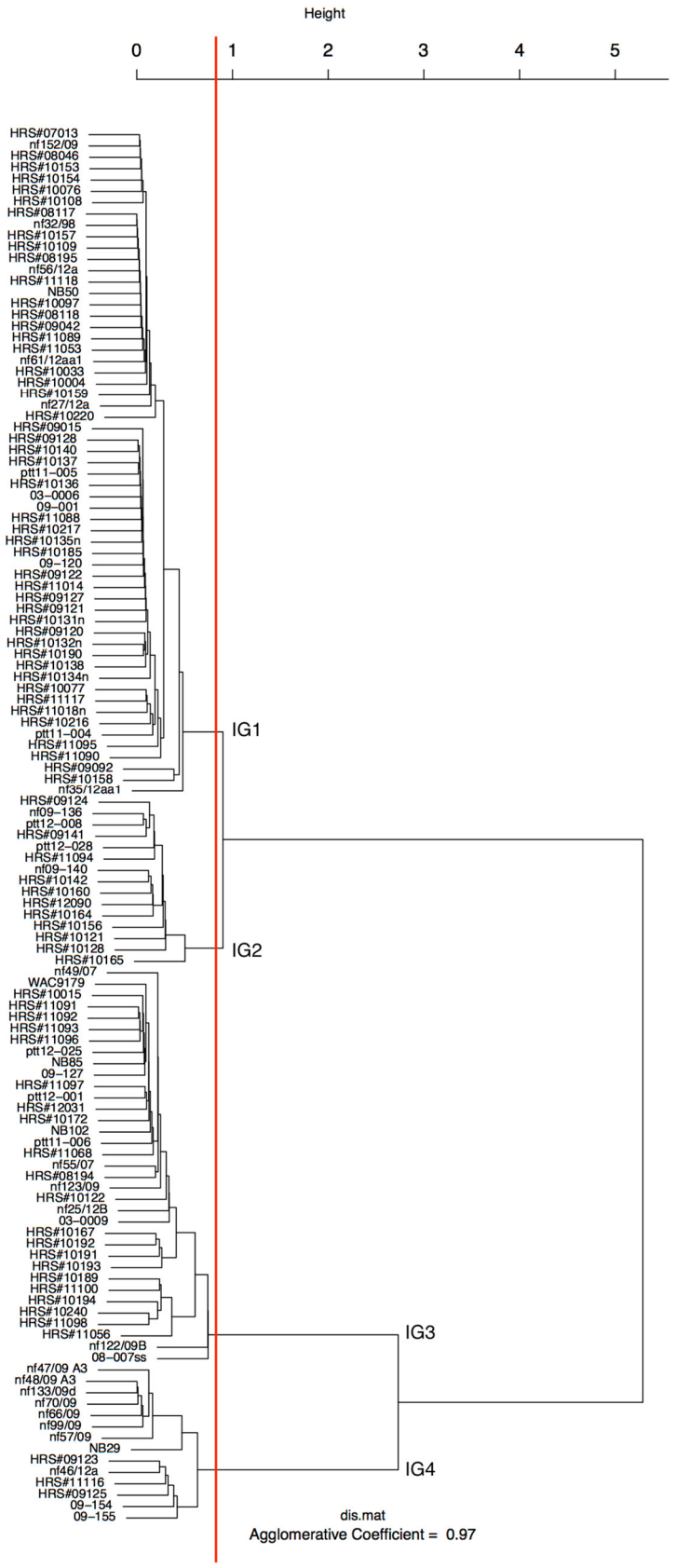


Figure 3.2. Hierarchical cluster dendrogram of 123 *Pyrenophora teres* f. *teres* isolates calculated using phenotypic data of 31 barley genotypes following seedling inoculation. Four groups of isolates clustered below a threshold of 0.85. Cluster branch points approaching 0 denote greater similarity in virulence profile of isolates.

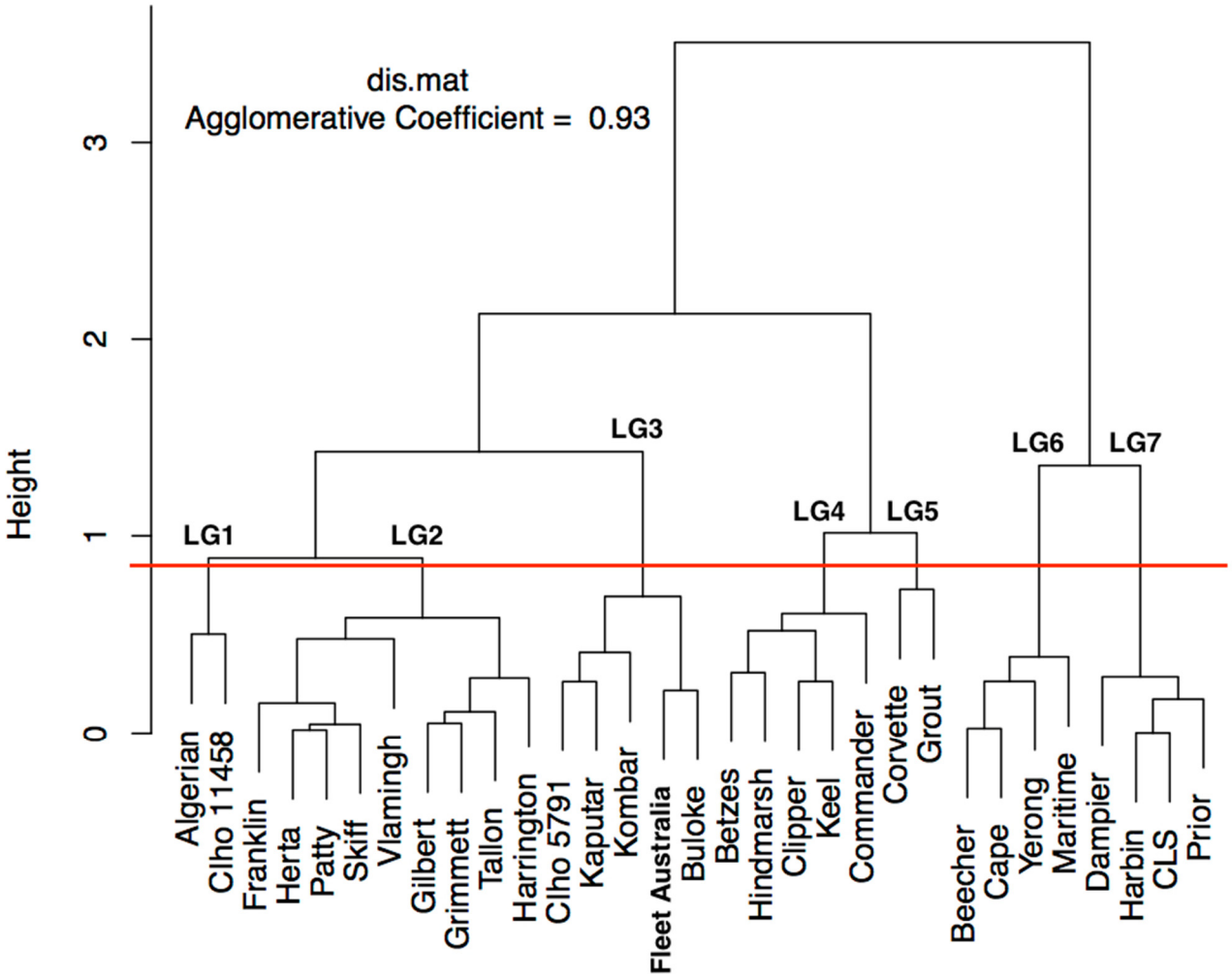


Figure 3.3. Hierarchical cluster dendrogram of 31 barley genotypes calculated using phenotypic data after seedling inoculation with 123 *Pyrenophora teres* f. *teres* isolates. Seven line groups clustered below a threshold of 0.85. Cluster branch points approaching 0 denote greater similarity of infection response between genotypes.

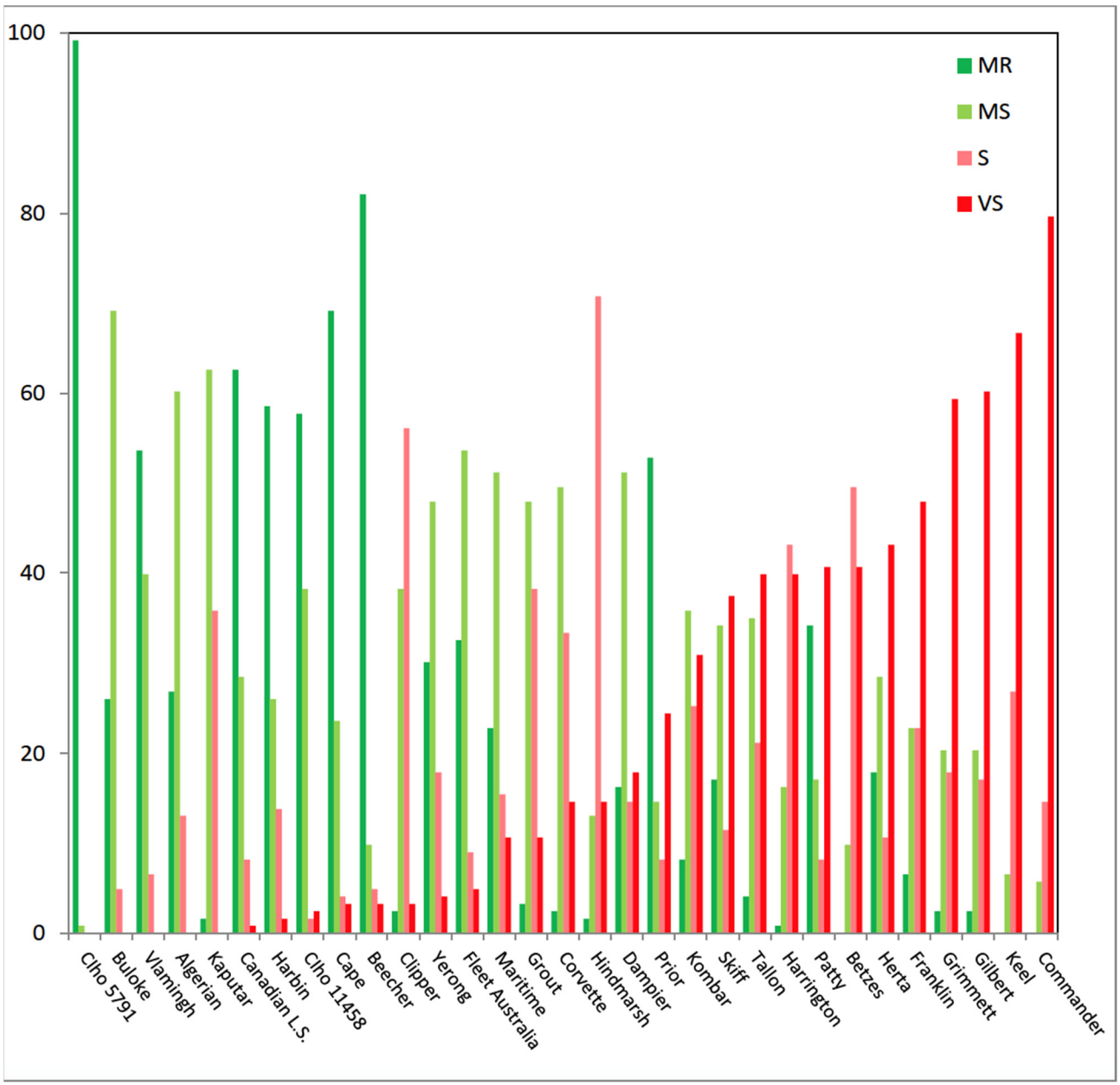


Figure 3.4. Infection response percentages of 31 barley genotypes after inoculation with 123 *Pyrenophora teres* f. *teres* isolates represented by four classes; MR (< IR 2.5) coloured dark green, MS (≥ IR 2.5 to < IR 5) coloured light green, S (≥ IR 5 to < IR 7.5) coloured pink and VS (≥ IR 7.5) coloured red.

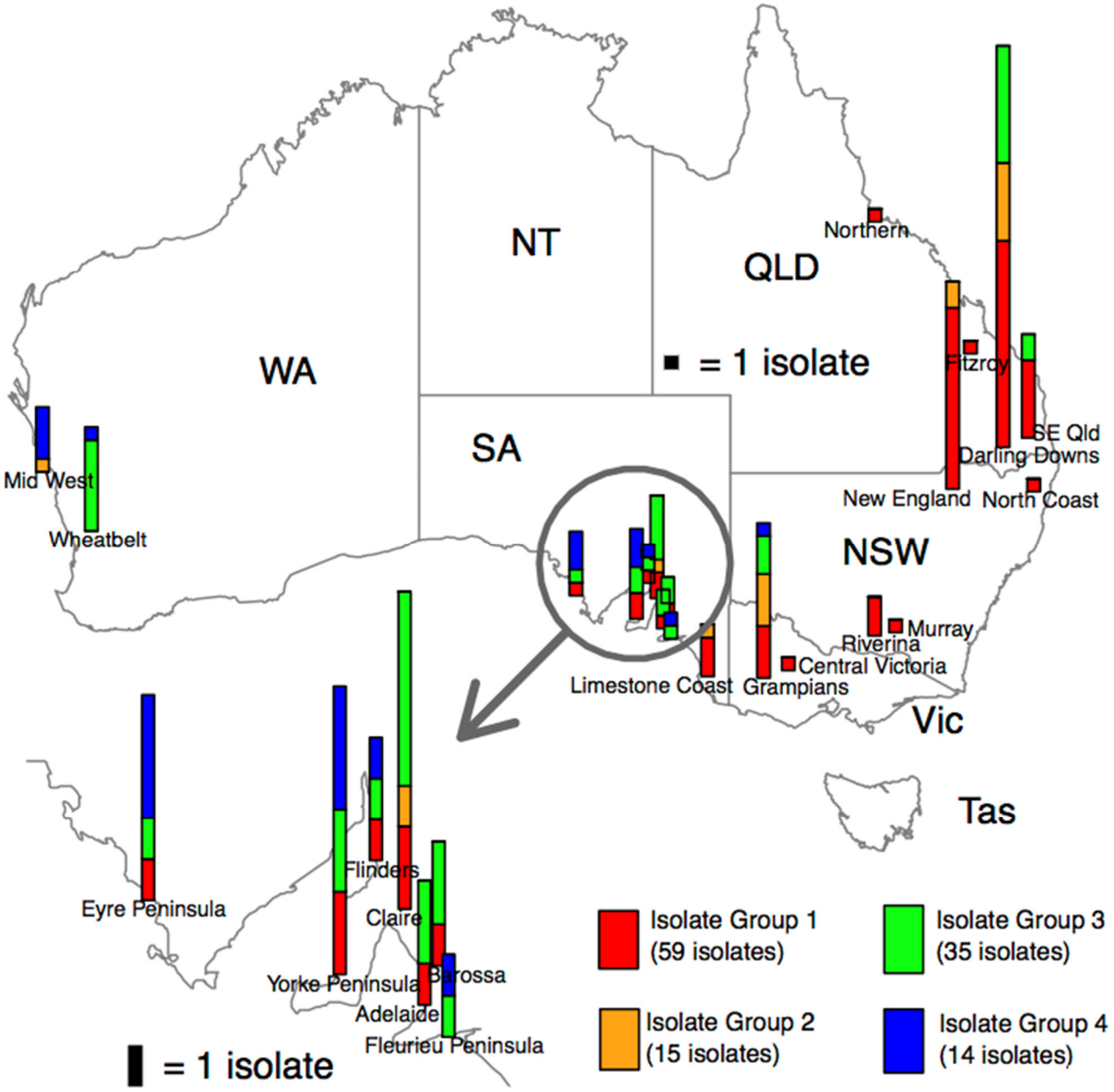


Figure 3.5. Geographical distribution of 123 *Pyrenophora teres* f. *teres* isolates represented by four isolate groups within five Australian States. Isolate group 1 (IG1) coloured red, isolate group 2 (IG2) coloured orange, isolate group 3 (IG3) coloured green and isolate group 4 (IG4) coloured blue.

3.7 Tables

Table 3.1 31 Barley genotypes used to determine pathogenic diversity of 123 Australian *Pyrenophora teres* f. *teres* isolates.

Genotype	Accession number	Origin	Year	Pedigree
Algerian[ab]	CIho 1179 / AGG495023	Algeria	1917	Landrace
Beecher[ab]	CIho 6566 / AGG495035	USA	1940	Atlas/Vaughn
Betzes[ab]	AGG400426	Germany	1938	Bethges II/Bethges III
Buloke[c]		Australia	2005	Franklin/2*VB9104 (Europa/IBON#7.148)
Canadian Lake Shore[ab]	CIho 2750 / AGG495016	USA	1907	Field selection from Manchurian genotype
Cape[ab]		South Africa	1900's	Unknown (is not CIho 1026 accession of Cape)
CIho 5791[ab]	CIho 5791 / AGG495026	Ethiopia	1927	Landrace
CIho 11458[ab]	CIho 11458 / AGG495025	Poland	1961	Selection from Isaria (Bavaria/Danubia)
Clipper[b]		Australia	1968	Proctor/PriorA
Commander[c]		Australia	2004	Keel/Sloop//Galaxy
Corvette[ab]		Australia	1976	Bonus/CIho 3576
Dampier[b]		Australia	1966	Olli selection (M98)/Research
Fleet Australia[c]		Australia	2006	Mundah/Keel//Barque
Franklin[b]		Australia	1989	Shannon/Triumph
Gilbert[b]		Australia	1992	Selection from Koru (Armelle//Lud/Luke)
Grimmett[b]		Australia	1983	Bussell/Zephyr
Grout[c]		Australia	2005	Cameo/Arupo
Harbin[ab]	CIho 4929 / AGG495027	China	1947	Landrace from Manchuria region
Harrington[ab]		Canada	1981	Klages/3/Gazelle/Betzes/Centennial
Herta[ab]		Sweden	1949	Kenia/Isaria
Hindmarsh[c]		Australia	2007	Dash/VB9409(O'Connor/WI2723)
Kaputar[b]		Australia	1993	Selection from Arupo (5604/1025/3/Emir/Shabet//CM67/4/F3 Bulk HIP)
-	-	-	-	
Keel[c]		Australia	1999	C.P.I.18197/Clipper//WI2645 (Mari/CM67)
Kombar[ab]	CIho 15694 / AGG495024	USA	1975	Minnesota 64-98 -8/2*Briggs

Table 3.1 Continued

Maritime[c]		Australia	2004	Dampier/A14//Kristina/3/Clipper/M11/Dampier/4/ Kristina/3/Dampier/A14/Union
-	-	-	-	
Patty[b]	AGG400167	France	1980	Volla/Athos
Prior[ab]		Australia	1903	Selection from Chevallier (English Landrace)
Skiff[ab]		Australia	1988	Abed Deba/3/Proctor/CIho 3576//C.P.I.18197/ Beka/4/Clipper/Diamant//Proctor/CIho 3576
-	-	-	-	
Tallon[b]		Australia	1991	Triumph/Grimmett
Vlamingh[c]		Australia	2006	WABAR0570 (72–0785/Tokak/5/Dampier/A14 //Kna/3/Sutter/4/Atlas57/A16//Clipper/ Delisa)/TR118
-	-	-	-	
Yerong[b]		Australia	1991	M22/Malebo

[a] Previously used in International pathogenic diversity study

[b] Previously used in Australian pathogenic diversity study

[c] First use in pathogenic diversity study

Table 3.2 Summary of sampling information of 123 *Ptt* isolates used to study pathogenic variation in Australia.

Isolate	Year	State[a]	Location	Host Genotype	Collector	Date Scored (Day/Month/Year)	IG
HRS#07013	2007	NSW	Grafton	Unknown	G. Platz	10/06/2010, 3/05/2013	1
nf152/09	2009	SA	Bordertown	Fairview	H. Wallwork	2/09/2010, 3/05/2013	1
HRS#08046	2008	Qld	Biloela	NRB07572	P. Keys	3/06/2010, 23/03/2013	1
HRS#10153	2010	Qld	Toowoomba	UWA intro.	R. Fowler	7/09/2011	1
HRS#10154	2010	Qld	Cleveland	Tallon	R. Fowler	7/09/2011	1
HRS#10076	2010	Qld	Cleveland	Gilbert	R. Fowler	22/09/2011	1
HRS#10108	2010	Qld	Gatton	Tallon	R. Fowler	22/09/2011	1
HRS#08117	2008	Qld	Tannymorel	Unknown	G. Platz	3/06/2010	1
nf32/98	1998	SA	Mallala	Unknown	H. Wallwork	16/09/2010	1
HRS#10157	2010	NSW	Tulloona	Henley	R. Fowler	1/09/2011	1
HRS#10109	2010	Qld	Clifton	Unknown	R. Fowler	22/09/2011	1
HRS#08195	2008	NSW	North Star	Unknown	G. Platz	10/06/2010, 12/06/2013	1
nf56/12a	2012	SA	Conmurra	Maritime	H. Wallwork	23/03/2013	1
HRS#11118	2011	Vic	Inverleigh	Fairview	M. McLean	4/04/2013	1
NB50	1994	Qld	Gatton	Unknown	G. Platz	3/06/2010, 12/06/2013	1
HRS#10097	2010	Qld	The Hermitage	NRB06059	R. Fowler	16/09/2010	1
nf25/08	2008	SA	Balaklava	Fleet Australia	A.W.Vater & CO	17/06/2010, 12/06/2013	1
HRS#09042	2009	Qld	Dalby	Skiff	J. Sturgess	27/05/2010, 12/06/2013	1
HRS#11089	2011	Qld	Toowoomba	VB0810	R. Fowler	11/05/2013	1
HRS#11053	2011	Qld	Jinghi	Binalong	R. Fowler	19/06/2013	1
nf61/12aa1	2012	SA	Conmurra	Oxford	H. Wallwork	16/03/2013, 29/05/2013	1
HRS#10033	2010	Qld	The Hermitage	Keel	R Fowler	22/07/2010	1
HRS#10004	2010	Qld	The Hermitage	Grimmett	R. Fowler	8/07/2010, 4/10/2013	1
HRS#10159	2010	NSW	Tulloona	Bass	R. Fowler	1/09/2011	1
nf27/12a	2012	SA	Brentwood	SYN8111-11A	H. Wallwork	12/04/2013, 19/06/2013	1
HRS#10220	2010	NSW	Bithramere	Commander	R. Fowler	19/06/2011	1

Table 3.2 Continued

HRS#09015	2009	Qld	The Hermitage	Barley Stubble	G. Platz	17/06/2010, 16/05/2013	1
HRS#09128	2009	NSW	Breeza	Skiff	G. Platz	29/05/2013	1
HRS#10140	2010	Qld	Allora	Tallon	I. Wallace	7/09/2011	1
HRS#10137	2010	NSW	Yallaroi	Shepherd	R. Fowler	3/05/2013	1
ptt11-005	2011	Vic	Logan	Fairview	M. McLean	16/05/2013	1
HRS#10136	2010	NSW	Yallaroi	Fleet Australia	R. Fowler	7/09/2011	1
03-0006	2003	Vic	Lake Bolac	Unknown	M. McLean	1/07/2010, 15/07/2010, 4/10/2013	1
09-001	2009	SA	Callington	Buloke	R. Prusa	29/07/2010, 15/09/2010	1
HRS#11088	2011	Qld	Fassifern	Unknown	R. Fowler	20/04/2013	1
HRS#10217	2010	NSW	Tamworth	Skiff	R. Fowler	19/06/2011	1
HRS#10135n	2010	NSW	Yallaroi	Mackay	R. Fowler	14/09/2011, 5/06/2013	1
HRS#10185	2010	Qld	Dalby	Hindmarsh	R. Evans	22/06/2011, 16/05/2013	1
09-120	2009	SA	Verran	Unknown	B. Purdie	15/07/2010, 4/10/2013	1
HRS#09122	2009	NSW	Yanco	TR129/Skiff	R. Graham	21/05/2010, 23/05/2013	1
HRS#11014	2011	NSW	Borambola	Volunteer	G. Platz	20/04/2013	1
HRS#09127	2009	NSW	Brocklesby	TR129/Skiff	R. Graham	29/05/2013	1
HRS#09121	2009	NSW	Wagga Wagga	TR129/Skiff	R. Graham	23/05/2013	1
HRS#10131n	2010	NSW	North Star	Unknown	R. Fowler	14/09/2011	1
HRS#09120	2009	Qld	The Hermitage	Shepherd	G. Platz	19/08/2010, 20/04/2013	1
HRS#10132n	2010	NSW	Mt Mitchell	Unknown	R. Fowler	3/05/2013	1
HRS#10190	2010	Qld	Wheatvale	Tallon	I. Wallace	22/06/2011	1
HRS#10138	2010	NSW	Yallaroi	Commander	R. Fowler	25/04/2013	1
HRS#10134n	2010	NSW	Yallaroi	Skiff	R. Fowler	14/09/2011	1
HRS#10077	2010	Qld	Cleveland	Unknown	R. Fowler	22/09/2011	1
HRS#11117	2011	Vic	Rupanyup	Commander	M. McLean	4/04/2013	1
HRS#11018n	2011	Qld	Mt Sturt	Grout	R. Fowler	20/04/2013	1
HRS#10216	2010	SA	Rosedale	Unknown	H. Wallwork	1/09/2011	1
ptt11-004	2011	Vic	Longerenong	SYN8111-11A	M. McLean	9/03/2013, 12/06/2013	1

Table 3.2 Continued

HRS#11095	2011	SA	Hart	Skiff	R. Fowler	19/06/2013	1
HRS#11090	2011	Qld	Toowoomba	NRB091090	R. Fowler	11/05/2013	1
HRS#09092	2009	Qld	Townsville	Shepherd	M. Hanks	24/06/2010, 5/06/2013	1
HRS#10158	2010	NSW	Tulloona	VB0432	R. Fowler	9/03/2013, 5/06/2013	1
nf35/12aa1	2012	SA	Pt Pirie	Fleet Aus.	H. Wallwork	16/03/2013	1
HRS#09124	2009	WA	Greenough	Buloke	C. Beard	27/05/2010, 23/03/2013	2
nf09-136	2009	Vic	Wonwondah	Barque	M. McLean	5/06/2013	2
ptt12-008	2012	Vic	Derrinallum	Unknown	M. McLean	3/05/2013	2
HRS#09141	2009	SA	Unknown	Unknown	M. McLean	23/05/2013	2
ptt12-028	2012	Vic	Marnoo	Buloke	M. McLean	11/05/2013	2
HRS#11094	2011	SA	Hart	Sloop	R. Fowler	15/03/2013	2
nf09-140	2009	Vic	Horsham	Barque	M. McLean	29/05/2013	2
HRS#10142	2010	NSW	Breeza	Grout	G. Platz	7/09/2011, 23/05/2013	2
HRS#10160	2010	Qld	Kurumbul	Grimmett	R. Fowler	1/09/2011	2
HRS#12090	2012	Qld	Junabee	Unknown	R. Fowler	25/04/2013	2
HRS#10164	2010	Qld	Allora	Grimmett	R. Fowler	1/09/2011, 23/05/2013	2
HRS#10156	2010	NSW	Tulloona	Grimmett	R. Fowler	7/09/2011, 23/05/2013	2
HRS#10121	2010	Qld	Yangan	Grout	B. Hempel	14/09/2011	2
HRS#10128	2010	Qld	Yelarbon	Barley Grass	R. Fowler	9/03/2013	2
HRS#10165	2010	Qld	Allora	Grout	R. Fowler	1/09/2011, 4/04/2013	2
nf49/07	2007	SA	Urrbrae	Keel	H. Wallwork	8/07/2010, 15/03/2013	3
WAC9179	1996	WA	Kalannie	Unknown	I. Goss	15/07/2010, 4/10/2013	3
HRS#10015	2010	Qld	The Hermitage	NRB06059	R. Fowler	27/05/2010, 23/03/2013	3
HRS#11091	2011	SA	Rosedale	Keel	R. Fowler	9/03/2013	3
HRS#11092	2011	SA	Hart	Prior	R. Fowler	16/05/2013	3
HRS#11093	2011	SA	Hart	Sloop SA	R. Fowler	15/03/2013	3
HRS#11096	2011	SA	Hart	Hindmarsh	R. Fowler	4/04/2013	3

Table 3.2 Continued

ptt12-025	2012	WA	Walebing	Baudin	G. Thomas	12/04/2013	3
NB85	1995	Qld	Gatton	Cape	G. Platz	20/04/2013	3
09-127	2009	SA	Rosedale	Unknown	H. Wallwork	29/07/2010, 20/04/2013, 3/05/2013	3
HRS#11097	2011	SA	Hart	Commander	R. Fowler	12/04/2013	3
ptt12-001	2012	WA	Northam	Bass	B. Paynter	19/06/2013	3
HRS#12031	2012	Qld	Kents Lagoon	Dictator	R. Fowler	19/06/2013	3
HRS#10172	2010	Qld	Junabee	Grimmett	R. Fowler	22/06/2011	3
NB102	1995	Qld	Brookstead	Gilbert	G. Platz	11/05/2013	3
ptt11-006	2011	Vic	Wonwondah	Commander	M. McLean	9/03/2013	3
HRS#11068	2011	Qld	Bringalilly	Mackay	G. Platz	22/09/2011, 5/06/2013	3
nf55/07	2007	SA	Urrbrae	Keel	H. Wallwork	24/06/2010, 23/03/2013	3
HRS#08194	2008	SA	Yorke Peninsula	NB diff. line	H. Wallwork	1/07/2010, 29/05/2013	3
nf123/09	2009	SA	Crystal Brook	Navigator	H. Wallwork	15/09/2010	3
HRS#10122	2010	Qld	Mt Sturt	Shepherd	B. Hempel	22/09/2011, 5/06/2013, 4/10/2013	3
nf25/12B	2012	SA	Urania	Fleet Aus.	H. Wallwork	12/04/2013	3
03-0009	2003	Vic	Horsham	Unknown	M. McLean	25/04/2013	3
HRS#10167	2010	Qld	Junabee	Grout	R. Fowler	22/06/2011	3
HRS#10192	2010	WA	Wongan Hills	Baudin	S. Cartlegde	19/06/2011	3
HRS#10191	2010	WA	Wongan Hills	Bass	S. Cartlegde	22/06/2011, 19/06/2011	3
HRS#10193	2010	WA	Muresk	Bass	S. Cartlegde	19/06/2011	3
HRS#10189	2010	Qld	Killarney	Mackay	G. Platz	22/06/2011, 14/09/2011	3
HRS#11100	2011	Qld	Mt Sturt	Shepherd	G. Platz	16/05/2013	3
HRS#10194	2010	WA	Muresk	Baudin	S. Cartlegde	19/06/2011, 19/06/2013	3
HRS#10240	2010	Vic	Lubeck	Commander	M. McLean	19/06/2011, 11/05/2013	3
HRS#11098	2011	SA	Hart	AC Metcalfe	R. Fowler	25/04/2013	3
HRS#11056	2011	Qld	Yandilla	Shepherd	R. Fowler	14/09/2011, 4/04/2013	3
nf122/09B	2009	SA	Ungarra	Fleet Aus.	H. Wallwork	17/06/2010, 12/04/2013	3
08-007ss	2008	SA	Meningie	Unknown	M. McLean	1/07/2010	3

Table 3.2 Continued

nf47/09 A3	2009	SA	Warooka	Maritime	H. Wallwork	10/06/2010, 12/04/2013	4
nf48/09 A3	2009	SA	Foul Bay	Maritime	H. Wallwork	24/06/2010, 16/03/2013	4
nf133/09d	2009	SA	Milang	Maritime	H. Wallwork	2/09/2010, 4/04/2013	4
nf70/09	2009	SA	Streaky Bay	Maritime	H. Wallwork	2/09/2010	4
nf66/09	2009	SA	Wandearah	Maritime	H. Wallwork	29/07/2010, 12/06/2013	4
nf99/09	2009	SA	Urania	Maritime	H. Wallwork	19/08/2010	4
nf57/09	2009	SA	SW Tumby Bay	Maritime	H. Wallwork	15/09/2010	4
NB29	1985	WA	Wongan Hills	Beecher	Unknown	19/08/2010	4
HRS#09123	2009	WA	Greenough	Vlamingh	C. Beard	21/05/2010, 16/09/2010, 29/05/2013	4
nf46/12a	2012	SA	Elliston	Fathom	H. Wallwork	23/03/2013	4
HRS#11116	2011	Vic	Horsham	Yagan	M. McLean	9/03/2013	4
HRS#09125	2009	WA	Greenough	Yagan	C. Beard	21/05/2010, 25/04/2013	4
09-154	2009	WA	Greenough	Baudin	M. McLean	22/07/2010, 11/05/2013	4
09-155	2009	WA	Greenough	Vlamingh	M. McLean	22/07/2010, 4/10/2013	4

[a] State codes: NSW = New South Wales, Qld = Queensland, SA = South Australia, Vic = Victoria and WA = Western Australia.

Table 3.3 Summary of isolate group and mean infection response for 123 *Ptt* isolates used to study pathogenic variation in Australia.

		Mean phenotype score[a]																													
		LG1		LG2									LG3					LG4					LG5		LG6				LG7			
Isolate[b]	IG	Algerian	Clho 11458	Franklin	Herta	Patty	Skiff	Vlamingh	Gilbert	Grimmett	Tallon	Harrington	Clho 5791	Kaputar	Kombar	Fleet Aus.	Buloke	Betzes	Hindmarsh	Clipper	Keel	Commander	Corvette	Grout	Beecher	Cape[c]	Yerong	Maritime	Dampier	Harbin	Canadian L.S.	Prior
#07013	1	2.5	1.5	9.3	9.0	9.3	9.0	3.3	9.0	9.0	6.5	8.3	1.0	5.0	7.8	3.5	2.8	8.3	6.8	6.0	9.3	9.0	5.0	4.3	1.0	1.0	2.0	3.5	3.3	1.5	1.3	2.0
nf152/09	1	2.5	3.0	9.3	9.0	9.0	9.3	3.0	9.0	9.3	8.8	9.0	1.0	4.8	8.8	4.3	3.5	8.5	6.0	6.3	9.0	9.3	5.5	3.8	1.3	1.3	2.8	3.3	3.3	2.5	2.7	2.3
#08046	1	5.5	3.3	10	9.8	10	8.5	3.5	10	9.8	10	9.8	1.0	5.8	7.8	4.5	5.3	8.3	7.5	5.8	9.8	9.8	6.8	9.0	1.3	1.3	3.8	4.0	4.0	2.0	1.8	2.0
#10153	1	2.0	1.5	9.0	7.5	7.5	7.0	2.0	9.0	9.0	9.0	9.0	1.0	5.0	9.0	2.5	3.0	7.0	7.0	5.5	9.0	9.0	5.5	7.0	1.0	1.0	1.5	3.0	2.0	1.0	1.0	1.5
#10154	1	3.5	4.0	6.0	9.0	9.0	9.0	3.0	9.0	9.5	10	9.5	1.0	5.0	7.5	3.5	3.5	9.0	6.0	7.0	9.5	9.0	5.5	7.0	1.0	1.0	2.5	2.0	2.0	1.5	1.0	1.0
#10076	1	3.0	2.5	8.0	9.0	8.5	8.5	1.0	9.5	9.0	8.5	9.0	1.0	6.0	5.5	4.5	2.0	9.0	5.0	7.0	8.5	9.0	6.0	6.5	1.0	1.0	2.5	3.0	3.0	1.5	1.0	1.5
#10108	1	3.5	2.0	7.0	9.0	9.0	8.0	1.5	9.0	9.0	9.0	8.5	1.0	4.0	7.5	3.0	4.0	6.0	5.0	5.0	9.0	9.0	3.5	5.0	1.0	1.0	2.0	3.0	7.5	1.0	1.5	1.0
#08117	1	4.5	3.0	9.0	9.5	8.0	9.0	4.5	9.0	9.0	9.0	9.0	1.0	4.0	9.5	5.0	4.5	7.0	5.0	5.5	8.5	9.0	4.5	4.5	1.0	1.5	2.5	3.0	4.0	1.5	1.5	1.0
nf32/98	1	3.0	2.0	9.0	9.0	8.0	8.0	3.0	9.0	9.0	9.0	7.0	1.0	5.0	9.0	3.5	3.0	6.0	6.0	5.0	7.0	9.0	3.0	3.0	1.5	1.5	2.0	4.0	2.5	1.5	1.5	1.5
#10157	1	4.0	2.5	9.0	9.0	9.0	9.0	3.0	9.0	9.0	9.0	6.0	1.0	4.5	7.0	4.0	3.0	6.0	7.0	5.0	9.0	9.0	3.5	6.0	1.0	1.0	3.5	3.0	2.0	1.0	1.0	1.0
#10109	1	3.5	3.0	9.0	9.0	9.0	9.0	2.0	9.0	9.0	9.0	8.5	1.0	5.5	7.5	4.5	2.5	7.5	6.0	7.0	9.0	9.0	4.0	7.0	1.5	1.0	3.5	2.0	2.0	1.0	1.5	1.0
#08195	1	4.3	2.0	9.0	9.0	9.8	9.5	4.3	9.5	9.5	9.5	9.0	1.0	6.0	8.8	4.3	3.0	7.3	5.5	5.3	9.3	7.3	3.8	5.8	1.3	1.0	2.0	4.0	3.5	1.0	1.0	1.5
nf56/12a	1	4.0	3.5	9.5	9.5	9.5	7.0	3.5	9.5	10	7.5	8.5	1.0	6.0	9.5	3.5	3.5	7.5	6.5	4.5	9.0	7.5	4.5	7.0	1.0	2.0	4.0	4.5	3.5	1.0	1.0	1.5
#11118	1	4.0	4.0	9.0	9.5	10	9.5	4.5	9.5	9.5	9.0	9.5	1.0	5.5	9.0	2.5	5.0	9.5	7.5	5.0	8.5	9.5	6.0	6.0	1.5	1.0	4.0	4.5	3.5	3.5	3.0	1.5
NB50	1	3.8	3.4	9.8	9.4	8.6	9.4	6.2	9.6	9.4	9.4	9.4	1.2	4.8	9.4	3.4	4.4	8.4	6.8	5.0	8.6	9.4	4.4	5.8	1.2	1.4	3.0	4.3	3.8	1.8	1.2	1.6
#10097	1	3.5	2.5	9.0	9.0	5.5	9.0	3.0	9.0	9.0	8.5	6.0	1.0	5.5	9.0	3.5	2.5	6.0	6.0	5.0	7.0	9.0	3.0	3.5	1.5	1.0	3.0	4.0	1.5	1.0	1.5	1.5
nf25/08	1	3.8	2.0	9.5	9.3	8.0	8.5	3.8	9.3	9.5	9.8	8.0	1.0	4.5	8.5	3.0	3.8	9.0	5.0	5.8	9.3	6.0	5.5	6.8	1.3	1.0	1.8	4.0	2.8	1.5	1.0	1.8
#09042	1	4.0	2.5	9.0	9.8	6.3	7.8	4.3	9.8	10	9.5	9.3	1.0	4.3	9.0	4.5	4.3	8.3	5.8	5.5	8.3	9.8	4.8	4.8	1.3	1.0	4.0	3.5	3.3	1.3	1.5	1.5
#11089	1	6.5	4.0	9.5	9.5	7.5	9.5	1.0	9.5	9.5	8.5	8.5	1.0	4.5	8.5	4.5	4.0	6.5	6.5	5.0	8.0	9.5	4.5	4.5	1.0	1.5	2.5	4.0	2.0	2.5	2.0	2.5
#11053	1	4.5	4.0	9.5	10	10	10	6.5	10	10	10	10	1.0	5.0	9.5	3.0	4.0	9.5	7.5	6.5	8.5	9.5	6.5	5.5	1.5	2.0	3.5	7.0	4.0	3.0	4.5	3.5
nf61/12aa1	1	3.0	2.8	9.3	8.8	8.3	8.5	1.3	7.8	9.0	7.8	7.8	1.0	5.3	5.0	2.8	3.3	7.8	6.0	4.5	9.3	5.3	4.5	5.3	1.0	1.5	1.8	5.7	3.3	1.0	1.5	1.8
#10033	1	3.0	1.0	9.0	6.0	5.5	6.5	3.0	8.0	8.0	8.5	6.0	1.0	3.5	9.0	3.0	2.5	5.5	5.5	5.0	7.0	6.5	4.5	5.0	1.0	1.5	1.5	3.0	2.5	1.5	1.0	1.0
#10004	1	3.0	2.0	9.0	8.5	9.0	6.0	4.5	9.0	9.0	6.5	5.5	1.0	3.0	9.0	2.5	2.5	5.0	5.0	3.5	5.5	9.0	3.5	3.0	1.5	1.5	2.0	3.0	1.5	2.0	1.5	1.5
#10159	1	4.0	2.0	9.0	6.5	5.0	5.0	5.0	9.0	9.5	9.0	9.0	1.0	5.5	9.0	4.5	4.5	5.0	7.0	5.0	9.0	9.0	3.5	4.0	1.0	1.5	3.0	4.0	4.0	2.5	1.5	1.0
nf27/12a	1	6.0	4.3	9.3	9.5	9.0	9.3	2.8	8.5	8.3	7.3	9.3	1.0	5.0	5.5	2.5	2.8	8.3	7.5	8.3	9.5	5.8	5.8	6.8	1.0	1.0	2.5	6.7	3.5	2.8	1.5	3.0
#10220	1	3.5	2.0	5.5	10	8.5	9.5	7.0	6.0	9.5	6.0	7.0	1.0	6.0	7.0	4.5	3.5	5.0	7.5	5.0	7.5	9.5	2.0	3.0	1.5	1.5	3.5	5.0	4.0	1.0	1.0	2.0
#09015	1	2.0	1.3	5.0	9.0	6.0	6.0	2.3	7.8	7.5	6.0	5.0	1.0	3.0	3.8	2.0	1.8	5.0	5.0	4.3	7.3	7.5	3.3	4.0	1.0	1.0	1.8	2.0	1.8	1.3	1.3	1.0
#09128	1	3.5	2.8	9.3	9.0	8.5	9.5	3.5	8.5	9.0	8.5	7.5	1.5	4.8	3.8	3.5	2.8	7.3	5.3	5.0	7.8	6.8	3.8	5.8	1.0	1.3	2.5	3.5	3.3	1.3	1.3	1.5

Table 3.3 Continued

#10140	1	3.5	2.5	9.0	9.0	8.5	9.0	1.5	9.0	9.0	9.0	8.5	1.0	4.5	6.0	1.5	3.0	6.0	5.0	4.5	6.5	7.0	2.5	4.0	1.0	1.0	2.0	2.0	4.0	1.0	1.0	1.0
#10137	1	4.0	3.5	8.5	9.5	9.0	9.5	2.5	9.5	10	9.0	7.5	1.0	4.5	5.0	4.0	2.5	8.0	4.5	5.0	9.0	8.5	6.5	4.5	1.0	1.0	2.5	4.0	3.0	2.0	2.5	3.0
ptt11-005	1	2.0	3.0	10	9.5	9.0	10	1.0	9.5	9.5	9.5	9.0	1.0	5.0	7.0	3.0	3.0	9.0	6.0	5.5	9.0	10	4.0	5.0	1.0	1.0	1.5	3.5	3.0	2.0	2.0	1.5
#10136	1	4.0	1.0	9.0	9.0	9.0	9.0	4.0	9.0	9.0	9.0	7.0	1.0	6.5	7.0	3.0	2.5	7.0	6.0	4.0	9.0	9.0	3.0	7.0	1.0	1.0	3.0	2.0	2.5	1.0	1.5	1.0
03-0006	1	2.5	1.8	9.0	9.3	9.0	9.3	2.5	9.0	9.0	6.8	6.8	1.0	4.3	8.0	3.3	2.5	5.0	4.8	4.5	7.8	9.3	2.8	3.0	1.0	1.0	2.3	3.0	3.0	1.0	1.0	1.5
09-001	1	4.0	3.3	9.8	9.5	9.8	9.8	4.0	9.5	9.8	9.5	9.5	1.3	6.5	9.0	4.7	3.8	8.5	7.0	4.5	9.5	9.5	3.5	4.5	2.3	2.3	3.0	4.0	3.0	2.8	3.0	2.5
#11088	1	4.0	2.5	9.5	8.5	7.5	9.0	2.0	10	10	7.0	5.5	1.0	4.0	3.0	2.0	3.5	6.0	4.0	3.5	7.0	8.5	4.0	4.5	1.0	1.0	1.5	2.5	2.5	1.0	1.0	1.0
#10217	1	3.5	2.0	8.5	9.5	8.5	10	5.0	9.5	9.5	8.5	6.5	1.0	5.5	6.0	4.5	2.5	4.5	7.0	5.5	7.5	9.0	3.0	4.0	1.5	1.0	2.5	5.0	3.5	1.5	1.0	2.0
#10135n	1	6.3	2.8	9.3	9.5	9.0	9.5	5.5	9.5	9.3	8.5	8.0	1.0	6.0	6.3	4.5	3.0	5.0	5.5	6.0	9.0	9.5	4.5	4.8	1.3	1.3	2.5	3.7	3.3	1.3	1.0	2.0
#10185	1	5.5	3.0	9.0	8.8	9.0	9.3	3.0	9.5	9.5	8.8	7.8	1.0	6.5	6.5	6.0	5.3	8.3	6.0	5.8	8.5	9.3	3.5	3.5	1.5	1.3	3.5	2.0	3.5	1.8	1.3	2.3
09-120	1	3.0	2.0	9.5	7.5	9.0	9.0	4.0	8.0	9.5	5.5	6.0	1.0	4.5	4.0	3.0	3.5	5.5	6.5	8.0	9.0	9.0	4.0	5.0	1.0	1.0	3.0	2.5	3.0	1.0	1.5	2.0
#09122	1	3.3	1.8	7.0	9.0	9.0	9.3	3.0	8.8	9.3	6.3	5.3	1.0	3.3	3.8	3.5	2.3	5.0	5.5	4.0	8.3	9.0	3.8	4.0	1.3	1.0	1.5	4.5	3.3	1.8	1.0	1.5
#11014	1	2.5	3.5	9.0	7.0	9.0	8.5	1.5	8.5	7.5	5.5	5.0	1.0	2.5	3.5	1.0	1.0	4.5	4.5	4.5	6.5	8.0	2.5	3.0	1.0	1.0	2.0	3.5	2.0	1.0	1.0	1.0
#09127	1	3.8	2.3	5.3	9.3	8.3	9.8	3.5	8.5	8.5	6.5	7.3	1.0	4.5	2.8	3.0	2.8	5.8	4.8	5.0	8.3	8.3	4.3	4.5	1.0	1.3	1.5	5.0	2.5	1.3	1.3	1.0
#09121	1	3.5	3.0	9.5	10	9.0	10	5.5	9.0	10	8.5	7.5	1.0	5.5	5.5	3.0	2.5	5.0	6.5	5.0	9.5	4.0	4.5	6.0	1.0	2.0	2.0	4.5	3.5	1.0	1.0	2.0
#10131n	1	3.0	4.5	9.0	9.0	8.0	9.0	3.0	9.0	9.0	7.0	5.5	1.0	5.5	4.5	3.5	3.0	6.5	6.5	5.5	8.5	7.0	6.0	9.0	2.0	2.0	2.0	3.0	3.5	2.0	1.5	1.5
#09120	1	6.4	8.0	7.6	9.2	8.0	4.9	2.2	9.8	9.6	8.6	7.6	1.0	3.6	3.4	7.0	5.4	5.4	4.0	5.0	5.2	7.8	6.8	5.0	2.0	2.5	4.4	2.3	4.2	4.6	4.4	2.8
#10132n	1	2.0	1.0	6.0	5.0	5.0	5.5	1.0	4.5	5.0	3.0	2.5	1.0	1.0	1.0	1.5	1.0	2.5	1.5	3.0	5.0	3.5	2.5	2.0	1.0	1.0	1.5	1.0	1.0	1.0	1.0	1.0
#10190	1	7.0	1.0	9.0	9.0	9.0	9.0	2.5	9.0	9.0	9.0	6.5	1.0	5.5	4.5	4.0	4.5	6.5	5.5	4.5	7.0	6.5	2.0	6.5	1.5	1.0	2.0	2.0	4.5	1.0	1.0	2.5
#10138	1	3.5	2.5	9.0	9.0	9.0	9.0	1.0	9.0	8.5	8.5	5.5	1.0	4.0	2.5	2.0	2.5	5.0	5.0	5.0	6.5	9.0	7.5	5.5	1.0	1.0	1.5	1.5	1.5	1.0	1.0	1.0
#10134n	1	2.0	4.0	9.0	9.5	9.5	10	5.0	9.5	9.5	7.0	7.0	1.0	6.0	5.5	1.5	3.5	5.0	6.0	5.0	7.5	7.5	7.0	9.5	1.0	2.5	3.0	3.0	3.5	1.0	1.0	1.0
#10077	1	3.0	1.5	9.0	9.0	9.0	7.0	1.5	9.0	9.0	9.0	7.0	1.0	5.5	3.5	3.5	2.5	6.5	6.0	5.5	9.0	9.0	4.5	7.0	1.0	1.0	1.5	1.0	1.0	1.0	2.0	1.0
#11117	1	4.0	4.0	9.5	10	8.0	9.0	1.5	7.5	9.5	4.5	9.5	1.0	5.5	4.0	3.0	4.5	9.5	7.0	6.0	9.5	10	6.0	5.5	1.5	1.5	3.5	4.0	4.5	2.0	2.0	2.5
#11018n	1	3.5	2.0	8.5	7.0	6.5	7.5	1.0	9.0	9.5	7.5	5.0	1.0	4.5	1.5	1.5	3.0	6.5	6.0	5.5	8.0	9.0	5.0	8.0	1.0	1.0	2.0	4.0	2.5	1.0	1.5	1.0
#10216	1	2.0	1.0	8.5	6.5	6.5	9.0	3.0	6.5	6.0	6.5	6.0	1.0	4.5	5.0	3.0	1.5	5.0	7.5	6.0	9.0	7.0	2.5	5.0	1.0	1.0	2.0	2.0	4.0	1.0	1.5	2.0
ptt11-004	1	6.5	1.5	9.5	9.3	9.0	7.3	2.8	5.3	7.8	5.3	6.3	1.0	3.3	5.0	2.3	2.3	5.8	5.8	7.3	9.3	9.3	4.3	4.5	1.0	1.0	1.3	3.3	3.5	1.0	1.3	2.5
#11095	1	4.0	3.0	8.5	7.5	8.5	9.0	3.5	6.0	8.5	7.0	9.0	1.0	5.5	5.0	2.5	4.0	7.0	9.0	9.0	9.0	3.5	4.0	6.0	1.0	1.0	1.5	4.5	4.5	3.0	1.5	3.0
#11090	1	3.5	3.0	8.0	9.5	9.5	9.0	1.0	9.0	8.0	6.5	10	1.0	4.5	1.5	8.0	4.0	8.0	6.0	6.0	6.0	8.5	9.5	6.5	1.5	1.0	2.5	1.0	2.0	2.0	1.5	1.5
#09092	1	6.5	7.5	7.0	9.3	7.5	3.5	1.5	9.5	9.5	7.8	6.8	1.0	3.3	3.8	5.5	4.5	4.0	4.8	4.3	6.0	8.3	5.0	3.8	2.5	2.0	4.3	3.0	3.5	4.5	4.0	2.3
#10158	1	7.3	8.8	8.3	9.5	8.3	4.5	1.0	10	10	8.5	8.3	1.0	3.3	3.3	5.8	4.3	4.5	5.3	5.0	5.8	9.0	7.0	4.3	1.5	1.3	4.5	2.3	4.5	4.5	4.3	2.8
nf35/12aa1	1	2.0	6.5	8.5	7.0	5.0	4.5	1.0	9.0	9.0	7.5	9.0	1.0	6.5	2.5	9.0	5.5	6.5	5.0	4.5	8.5	9.0	4.0	2.0	1.5	1.5	6.5	4.0	3.0	3.5	4.0	4.0
#09124	2	2.8	1.3	6.8	4.5	3.3	3.3	3.0	9.5	9.5	8.8	9.5	1.0	4.5	3.8	3.5	4.0	8.5	6.3	6.5	9.0	9.5	5.3	6.8	1.0	1.7	2.5	3.0	6.5	1.8	1.3	5.5
nf09-136	2	2.3	1.3	6.0	3.3	2.8	4.0	3.0	6.8	6.8	5.8	7.8	1.0	4.3	3.8	2.5	2.0	7.0	4.8	5.0	8.5	8.8	3.8	4.5	1.0	1.0	2.3	4.0	3.3	1.0	1.0	1.8
ptt12-008	2	1.0	1.0	1.5	1.5	2.5	2.0	1.0	1.5	1.0	1.0	2.0	1.0	1.0	1.0	1.0	1.0	3.5	2.0	1.0	2.5	2.5	2.5	1.0	1.0	1.0	1.5	1.5	1.5	1.0	1.0	1.0

Table 3.3 Continued

#09141	2	1.0	1.0	8.0	3.5	2.0	3.5	1.5	9.5	9.5	6.0	8.5	1.0	4.5	3.0	2.5	2.0	8.0	6.0	5.0	9.0	10	5.5	4.5	1.0	1.0	2.0	2.5	3.0	1.0	1.0	2.5
ptt12-028	2	7.0	3.0	7.0	9.0	7.0	7.0	1.0	9.0	9.5	7.5	6.0	1.0	4.0	3.5	2.0	4.0	9.0	6.0	7.5	9.5	10	6.0	7.5	1.0	1.0	4.5	5.0	8.5	4.0	4.0	7.0
#11094	2	3.0	2.0	8.0	4.5	4.5	6.0	1.5	5.5	6.5	4.5	6.0	1.0	4.0	2.0	1.0	3.5	5.5	6.0	7.0	7.0	7.0	5.0	4.5	1.0	1.0	2.0	5.0	4.0	1.0	3.0	2.0
nf09-140	2	2.5	1.8	8.3	4.0	4.3	4.5	2.8	7.5	8.0	7.0	8.0	2.0	4.3	4.0	2.0	3.0	8.0	7.0	5.0	7.3	8.3	5.5	4.8	1.0	1.3	1.8	2.5	3.3	1.3	1.0	1.5
#10142	2	2.3	1.3	5.8	5.5	3.8	4.8	2.8	8.8	9.0	9.3	7.8	1.3	5.5	5.3	3.7	3.0	6.8	5.8	6.0	8.3	9.3	3.5	5.5	1.3	1.0	2.3	4.0	3.8	1.5	1.5	1.8
#10160	2	3.0	2.0	9.0	5.5	4.5	4.0	4.0	9.0	9.0	9.0	9.0	1.5	5.0	9.0	5.5	4.0	8.5	6.0	5.5	9.0	9.0	3.5	4.0	1.0	1.0	3.0	2.0	3.5	1.5	1.5	2.0
#12090	2	2.5	1.0	7.5	3.5	3.5	3.0	3.5	9.0	9.0	8.5	8.0	1.0	4.5	4.5	2.5	3.0	5.5	4.0	5.5	9.0	9.0	4.5	4.5	1.0	1.0	2.5	4.0	2.5	1.5	1.5	1.0
#10164	2	3.3	1.5	8.5	7.0	4.8	5.3	1.8	9.5	9.5	8.8	8.3	1.0	4.5	3.8	3.5	3.5	6.3	7.5	5.8	8.8	9.5	8.8	8.0	1.0	1.0	2.0	2.0	2.5	1.0	1.0	1.0
#10156	2	1.0	1.0	5.0	3.8	1.0	1.5	1.3	9.3	9.5	8.3	7.0	1.0	4.0	3.0	3.3	3.3	5.0	5.0	5.3	8.5	9.5	2.8	4.0	1.0	1.0	1.3	2.3	2.0	1.0	1.0	1.0
#10121	2	1.5	1.0	9.0	3.5	4.0	4.0	2.5	9.0	9.0	9.0	5.0	1.0	5.0	5.0	2.5	3.0	6.0	5.5	5.5	7.5	8.5	7.5	8.5	1.5	1.5	2.5	2.0	4.0	2.0	2.5	1.0
#10128	2	3.5	1.0	7.5	2.0	2.5	3.5	2.5	10	10	7.5	7.0	1.0	4.0	4.5	3.0	3.0	4.5	5.5	5.0	7.5	9.0	9.0	7.5	1.0	1.0	2.5	4.0	3.5	1.0	1.0	1.0
#10165	2	3.3	1.0	7.0	1.5	1.8	2.3	1.3	9.5	9.3	6.3	8.5	1.0	4.5	3.0	6.5	4.0	6.0	5.3	4.0	5.5	7.5	7.8	5.8	1.0	1.0	6.3	3.0	2.0	1.5	1.3	1.3
nf49/07	3	3.0	4.5	4.0	4.3	3.5	4.0	1.0	4.0	4.3	3.5	5.5	1.0	3.3	2.8	1.5	2.0	8.3	6.0	5.3	9.5	7.0	5.0	4.0	2.0	2.3	3.8	9.0	9.5	5.8	4.3	9.3
WAC9179	3	2.0	1.5	2.0	2.5	2.0	3.0	2.0	4.0	5.0	3.0	4.0	1.0	3.0	5.0	2.0	2.5	7.0	6.5	4.5	9.0	9.0	3.5	4.0	1.5	2.5	3.5	2.5	6.5	4.5	2.5	9.0
#10015	3	3.3	1.3	4.5	2.8	1.8	2.5	1.3	5.3	4.5	3.3	6.8	1.3	3.5	4.0	2.0	2.3	7.8	6.8	4.8	9.5	10	9.8	7.3	1.8	2.7	4.5	4.5	8.0	4.8	4.0	10
#11091	3	3.5	2.0	5.5	3.0	2.0	2.5	2.5	8.5	7.0	4.5	8.0	1.0	3.5	5.0	2.5	3.5	9.0	7.5	7.0	10	10	6.5	5.5	2.5	4.0	5.0	5.0	10	7.0	6.5	10
#11092	3	2.0	1.0	5.0	1.0	1.0	2.5	1.0	4.5	5.5	3.5	7.0	1.0	3.0	2.0	2.0	2.0	9.5	7.0	5.0	9.5	9.5	4.5	4.5	2.0	2.0	4.0	2.5	7.5	4.0	2.5	10
#11093	3	1.5	1.0	5.5	2.5	2.0	1.5	1.5	4.5	4.5	4.5	5.5	1.0	5.0	2.0	2.0	3.0	9.0	7.0	5.5	10	10	7.0	5.5	2.0	3.5	4.0	6.0	10	6.0	4.5	10
#11096	3	3.0	1.0	5.0	1.5	1.5	1.5	1.0	4.5	6.5	3.5	6.0	1.0	3.5	3.5	1.5	2.0	6.5	5.0	4.0	9.0	9.0	4.0	4.0	2.0	2.5	4.0	2.5	9.0	5.0	4.5	9.5
ptt12-025	3	1.5	2.5	5.0	2.5	1.0	4.0	1.5	3.5	4.5	3.5	6.5	1.0	4.0	3.5	1.0	2.5	9.5	9.0	6.0	9.5	10	5.5	4.5	3.5	4.0	5.0	3.5	9.5	7.0	5.5	9.5
NB85	3	3.0	1.3	2.7	2.3	1.0	2.0	1.3	6.0	5.0	2.7	5.7	1.0	4.0	3.0	1.7	2.7	8.3	7.3	4.5	9.3	9.7	10	7.7	2.0	2.5	4.7	2.7	8.0	3.3	3.3	10
09-127	3	2.0	1.5	3.8	2.3	1.7	2.0	1.8	6.8	4.2	3.5	6.2	1.0	3.2	2.7	1.3	1.7	8.5	5.8	3.5	9.5	9.5	5.3	3.2	1.3	2.5	4.3	3.5	9.2	6.2	4.7	9.8
#11097	3	2.5	2.5	7.0	3.5	1.5	3.5	1.5	6.5	4.5	4.5	7.0	1.0	3.0	3.5	1.5	2.5	9.5	9.0	5.5	9.5	9.5	5.0	5.5	1.5	4.0	3.5	5.5	7.0	6.5	6.0	8.0
ptt12-001	3	3.5	3.5	6.0	3.5	3.0	3.0	2.5	6.0	7.0	4.5	7.5	1.0	4.5	6.5	2.5	3.0	8.5	7.0	6.5	10	10	5.0	4.5	1.5	3.5	5.0	6.5	10	4.5	5.0	9.5
#12031	3	5.0	3.5	6.0	4.0	3.0	3.5	2.5	9.5	9.5	7.0	8.5	1.0	4.5	5.5	2.5	3.5	10	9.0	6.0	10	10	10	9.5	2.5	5.0	5.5	6.5	10	7.5	6.0	10
#10172	3	2.0	2.5	3.0	2.5	1.0	3.0	1.0	7.0	6.0	3.0	7.0	1.0	4.0	3.0	1.0	2.0	9.0	5.5	4.0	7.0	9.0	9.0	7.0	2.0	2.5	4.0	1.0	5.5	4.0	3.5	9.0
NB102	3	1.0	1.0	3.0	1.0	1.0	2.0	1.0	8.0	5.5	3.0	6.5	1.0	2.5	1.5	2.0	1.0	5.5	5.5	3.5	7.5	9.0	10	4.5	1.5	1.5	4.5	1.0	7.5	4.0	3.5	10
ptt11-006	3	3.5	2.5	5.5	2.5	1.0	2.5	2.5	3.0	4.5	2.0	7.5	1.0	4.0	5.0	2.0	2.5	9.5	6.5	6.5	9.5	10	7.0	7.5	2.0	3.5	3.5	4.0	10	9.0	8.0	10
#11068	3	2.8	1.0	2.3	1.3	1.0	1.5	1.5	8.8	6.5	4.0	5.0	1.0	2.5	3.3	1.3	1.0	7.0	5.0	3.8	8.0	8.3	9.0	7.0	1.3	1.3	3.5	1.7	6.8	2.5	2.3	9.0
nf55/07	3	3.0	4.8	5.3	5.5	4.3	4.8	1.5	3.5	4.8	3.5	5.8	1.0	3.3	3.5	2.3	1.3	6.8	5.8	5.5	9.3	8.5	5.8	4.5	2.0	2.5	3.5	4.5	8.3	4.5	4.3	9.5
#08194	3	2.0	3.8	3.8	3.3	2.5	3.5	1.0	2.8	3.3	3.3	5.0	1.0	2.5	2.8	1.8	1.8	7.3	5.3	4.3	9.3	8.8	3.0	3.3	2.0	2.0	2.0	2.5	5.3	2.5	3.0	9.0
nf123/09	3	2.5	2.5	2.0	3.0	1.0	2.5	1.0	3.5	2.5	3.0	4.0	1.0	3.0	3.5	1.0	1.5	7.5	6.0	3.0	7.0	9.0	4.0	2.5	2.5	3.0	3.0	2.0	6.5	3.5	3.5	9.0
#10122	3	5.3	5.3	6.5	7.3	3.5	5.8	2.0	9.3	7.5	4.8	7.0	1.0	4.5	3.3	2.0	2.0	9.3	5.5	5.3	8.8	9.3	9.5	8.5	1.5	1.7	5.8	3.0	8.0	4.5	4.0	9.8
nf25/12B	3	4.0	2.5	6.5	3.5	3.0	4.5	3.0	5.5	7.0	5.5	6.5	2.5	7.0	4.0	9.0	6.0	9.5	8.5	5.5	9.5	9.5	5.0	5.0	4.5	5.0	9.0	5.0	9.0	7.0	6.0	8.0

Table 3.3 Continued

03-0009	3	2.4	1.2	2.8	1.4	1.2	1.4	4.2	4.4	6.0	3.6	6.2	1.6	5.4	1.6	7.5	4.2	7.8	8.2	4.2	9.0	9.4	3.0	3.0	2.0	3.0	6.0	2.3	7.2	6.0	5.4	7.0
#10167	3	3.5	1.0	2.0	2.5	1.0	1.5	1.0	6.5	4.0	3.0	7.0	1.0	3.5	5.0	2.5	2.0	7.5	4.5	3.0	7.5	9.0	9.0	7.0	2.0	2.0	3.0	2.0	7.0	3.0	2.5	9.0
#10192	3	1.0	1.5	4.5	2.0	1.0	2.5	1.5	2.5	3.5	2.5	3.5	1.0	2.5	7.5	1.5	1.5	9.0	7.0	5.0	9.0	9.0	3.0	3.0	2.5	2.5	5.5	5.0	6.5	3.0	1.5	9.0
#10191	3	2.0	2.0	2.5	2.5	1.5	2.5	2.5	3.0	4.5	3.0	4.5	1.0	4.5	7.5	2.5	3.0	7.0	6.5	7.0	9.0	9.0	2.5	4.0	2.0	2.5	6.0	4.0	8.0	6.0	4.5	9.5
#10193	3	2.5	2.5	4.0	1.5	2.0	2.5	3.0	3.5	5.0	3.5	5.0	1.0	5.0	7.0	3.0	1.5	5.5	6.5	3.5	4.5	9.0	3.0	4.5	3.0	3.0	5.5	6.0	9.0	5.0	3.5	7.5
#10189	3	5.0	1.3	3.8	6.0	2.5	6.0	1.5	7.0	3.8	3.0	6.5	1.0	4.8	5.5	1.5	2.5	8.8	8.3	4.5	7.0	8.8	9.5	7.3	1.5	1.8	4.8	2.5	5.3	1.8	1.0	8.3
#11100	3	1.5	1.0	3.5	1.0	1.0	1.0	1.0	9.5	6.5	4.0	6.0	1.0	2.5	3.0	1.0	2.0	6.0	7.0	3.5	7.0	9.5	9.5	5.0	1.0	2.0	3.5	2.0	3.0	3.0	2.5	5.0
#10194	3	3.0	3.5	4.5	4.0	3.0	4.0	2.5	4.5	7.5	4.0	7.5	1.0	3.5	7.0	2.5	3.0	7.0	7.5	4.5	7.5	10	5.5	4.5	2.0	4.0	5.0	5.5	10	4.5	4.0	5.5
#10240	3	3.5	1.3	5.8	3.3	2.3	3.8	2.0	8.5	9.0	6.8	7.3	1.3	7.0	6.3	3.3	3.0	9.5	8.5	6.5	9.8	10	6.0	6.5	1.8	2.7	6.3	6.3	5.5	2.8	2.3	5.0
#11098	3	1.5	1.0	2.0	2.0	1.0	2.0	1.5	5.5	4.5	4.5	7.5	1.0	4.0	3.5	1.5	2.0	8.5	8.0	5.0	8.5	9.0	6.5	3.5	1.5	1.5	3.0	3.0	3.5	1.0	1.0	3.5
#11056	3	2.0	1.5	5.0	2.0	1.3	1.8	1.3	7.0	7.3	6.0	5.0	1.0	3.0	3.5	1.8	1.8	4.8	4.3	3.0	5.5	7.8	5.3	5.3	1.8	1.3	2.3	2.0	3.5	5.0	4.0	2.3
nf122/09B	3	3.8	1.5	2.5	2.5	1.5	2.3	1.5	4.8	4.5	3.3	5.0	1.0	4.8	2.0	9.0	4.0	5.5	5.3	3.5	5.0	7.0	4.0	4.0	1.5	1.7	5.5	1.0	2.5	2.5	2.3	3.3
08-007ss	3	5.0	1.5	3.0	2.5	2.0	3.5	1.0	3.0	3.5	2.5	3.0	1.0	3.0	3.5	6.5	3.0	5.5	4.5	3.0	4.0	4.5	4.5	2.5	2.0	2.0	8.0	3.5	8.5	5.5	3.5	9.0
nf47/09 A3	4	3.2	1.2	3.6	2.8	1.8	3.8	2.8	7.2	7.2	4.4	4.8	1.6	4.8	9.6	2.2	2.8	6.6	6.6	4.6	6.2	8.2	8.2	7.4	5.8	5.0	6.2	9.7	2.8	1.2	1.2	3.0
nf48/09 A3	4	1.8	1.0	3.3	1.5	1.3	1.8	1.5	3.5	3.8	2.3	3.5	1.0	4.5	9.3	1.5	1.5	5.0	5.5	3.8	5.3	6.5	4.5	4.8	5.0	4.8	5.0	10	2.0	1.3	1.3	1.3
nf133/09d	4	2.0	1.0	3.0	2.5	1.3	2.8	2.0	4.5	3.5	3.5	5.0	1.3	4.8	9.3	2.8	2.8	6.3	6.0	3.8	5.8	6.5	6.3	5.8	5.3	4.0	5.3	9.0	2.5	1.3	1.0	2.0
nf70/09	4	2.0	1.5	2.5	3.0	2.0	3.0	2.5	3.0	3.5	3.5	4.0	1.0	6.0	9.5	2.5	2.5	5.5	6.0	4.0	3.5	5.5	5.5	4.5	4.0	3.0	4.0	9.0	3.0	1.0	1.0	1.5
nf66/09	4	2.0	1.0	4.3	2.8	1.0	2.5	2.0	3.3	3.8	2.8	3.8	1.0	4.8	9.5	1.5	2.3	7.3	5.8	4.5	6.3	9.5	7.0	5.8	4.8	4.8	5.0	10	2.3	1.3	1.5	1.3
nf99/09	4	3.0	1.0	3.0	3.5	2.5	3.5	3.0	2.5	3.0	3.0	4.0	1.0	6.5	10	3.5	3.0	9.0	7.0	3.5	6.0	7.0	7.0	5.5	6.5	4.5	5.0	10	3.5	1.0	1.5	2.5
nf57/09	4	3.5	1.0	2.0	2.5	1.5	2.0	2.0	2.0	1.5	3.0	3.5	1.0	3.0	9.0	1.5	3.0	3.5	4.0	2.0	3.5	4.0	3.5	3.0	6.0	3.5	4.0	9.0	2.0	2.5	1.5	2.0
NB29	4	5.7	2.3	2.7	2.0	1.0	2.0	1.3	4.3	2.7	2.3	2.7	1.0	3.3	9.0	3.7	1.7	3.3	4.3	2.7	3.7	3.7	4.0	3.0	9.3	9.3	6.0	7.5	2.3	2.0	2.3	3.0
#09123	4	1.8	1.0	2.5	1.2	1.2	1.3	1.0	4.0	3.8	2.0	3.2	1.0	3.5	5.3	3.3	1.8	4.5	3.2	1.8	3.5	6.5	2.3	2.0	2.7	4.2	4.2	3.7	5.3	2.0	1.8	5.0
nf46/12a	4	3.0	1.0	4.0	2.0	1.0	2.5	1.0	6.0	4.5	2.5	4.5	1.0	5.5	9.5	2.5	2.5	7.5	6.5	3.5	6.5	9.0	8.0	8.0	9.5	9.0	6.5	10	5.5	5.5	4.0	7.5
#11116	4	3.5	2.0	4.0	2.0	1.5	3.0	1.5	9.0	7.0	3.5	4.0	1.0	5.0	9.0	6.0	3.0	8.0	5.5	3.0	6.5	9.5	4.5	4.5	5.0	6.0	9.0	10	7.0	5.0	5.0	7.0
#09125	4	2.0	1.3	3.8	1.5	1.0	1.3	1.0	1.5	2.3	2.5	3.5	1.0	3.5	6.5	5.0	2.8	3.3	4.3	3.3	4.0	7.5	2.8	2.5	4.0	5.0	5.0	7.0	6.5	1.8	2.8	6.5
09-154	4	3.5	1.8	3.3	1.8	1.8	1.8	1.5	6.5	7.3	4.0	4.8	1.0	6.5	9.3	7.0	3.3	7.3	5.0	3.3	5.8	7.8	4.0	2.8	7.5	8.7	9.0	9.5	7.0	5.0	5.0	7.8
09-155	4	3.5	2.5	2.0	2.5	2.0	2.5	2.0	7.0	7.0	6.0	5.0	1.0	6.0	10	8.5	3.5	7.0	5.5	3.0	6.5	9.0	3.0	3.0	9.0	8.5	9.0	9.0	6.0	5.0	5.0	7.0

[a] Mean infection responses coloured as per four classes (MR, MS, S and VS) coloured dark green, light green, pink and red respectively.

[b] # indicates that isolate code has been condensed from HRS#

[c] Infection response of Cape not consistent with that of Cape (CIho 1026).

Table 3.4 Percentage of 123 *Pyrenophora teres* f. *teres* isolates that induced susceptible infection responses on barley genotypes according to state of origin and isolate group.

		State[a]						Isolate Group			
LG	Genotype	NSW	Qld	SA	Vic	WA	Overall	IG1	IG2	IG3	IG4
LG1	Algerian	10	22	6	15	8	13	17	7	11	7
	CIho 11458	5	7	3	0	0	4	7	0	3	0
LG2	Franklin	100	80	57	77	23	71	100	93	40	0
	Herta	95	68	34	46	0	54	100	27	9	0
	Patty	90	59	31	46	0	49	100	7	0	0
	Skiff	86	61	31	46	0	49	93	20	6	0
	Vlamingh	29	5	0	0	0	7	14	0	0	0
	Gilbert	95	100	57	77	31	77	98	93	51	36
	Grimmett	100	93	51	85	54	77	100	93	51	29
	Tallon	95	78	37	62	15	61	97	87	11	7
	Harrington	95	100	71	85	38	83	98	93	86	0
LG3	CIho 5791	0	0	0	0	0	0	0	0	0	0
	Kaputar	52	29	34	46	23	36	51	20	14	43
	Kombar	57	51	51	54	85	56	68	20	34	100
	Fleet Australia	5	17	11	15	23	14	12	13	11	29
	Buloke	0	7	6	8	0	5	8	0	3	0
LG4	Betzes	81	93	97	92	77	90	92	87	97	71
	Hindmarsh	81	85	94	77	77	85	86	80	91	71
	Clipper	81	61	49	69	38	59	75	87	46	0
	Keel	100	100	91	92	69	93	100	93	94	64
	Commander	90	100	91	92	92	94	95	93	97	86
LG5	Corvette	29	59	54	46	31	48	34	60	69	43
	Grout	43	68	46	46	8	49	54	47	46	36
LG6	Beecher	0	0	17	8	23	8	0	0	0	71
	Cape	0	2	9	8	31	7	0	0	6	50
	Yerong	0	7	31	23	77	22	2	7	40	79
	Maritime	14	5	46	23	62	26	10	13	31	93
LG7	Dampier	0	24	37	38	92	33	2	13	89	43
	Harbin	0	5	26	23	38	15	0	0	43	29
	Canadian L.S.	0	2	9	23	31	9	0	0	23	21
	Prior	0	24	37	38	92	33	0	13	91	43
IG	IG1	90	59	31	38	0	48				
	IG2	10	15	6	31	8	12				
	IG3	0	27	40	23	54	28				
	IG4	0	0	23	8	38	11				
	No. Isolates	21	41	35	13	13	123	59	15	35	14

[a]State codes: NSW = New South Wales, Qld = Queensland, SA = South Australia, Vic = Victoria and WA = Western Australia.

Table 3.5 Mean IR and SE according to isolate group and state of origin for 31 barley genotypes assayed with 123 *Ptt* isolates.

		Isolate group										State origin for isolates within IG1								State origin for isolates within IG2										State origin for isolates within IG3								State origin for isolates within IG4						
LG	Genotype	IG1		IG2		IG3		IG4		Overall		[c]NSW		Qld		SA		Vic		NSW		Qld		SA		Vic		WA		Qld		SA		Vic		WA		SA		Vic		WA	
LG1	Algerian	[a]3.8[a]	1.3	2.9[b]	1.5	2.8[b]	1.1	2.7[b]	1.1	3.3	1.4	3.7[a]	1.3	4.1[a]	1.4	3.4[a]	1.1	3.8[a]	1.6	1.6[a]	0.9	2.8[a]	0.7	2.0[a]	1.4	3.2[a]	2.6	2.8	NA	3.1[a]	1.5	2.8[a]	1.0	3.2[a]	0.6	2.2[a]	0.9	2.6[a]	0.7	3.5	NA	3.3[a]	1.6
	CIho 11458	[b]2.9[a]	1.6	1.4[b]	0.6	2.1[b]	1.2	1.4[b]	0.5	2.3	1.4	2.8[a]	1.7	2.9[a]	1.7	3.0[a]	1.5	2.9[a]	1.2	1.8[a]	0.2	1.2[a]	0.4	1.5[a]	0.7	1.8[a]	0.9	1.3	NA	1.8[a]	1.4	2.2[a]	1.3	1.6[a]	0.8	2.4[a]	0.8	1.1[b]	0.2	2.0	NA	1.7[a]	0.6
LG2	Franklin	8.6[a]	1.1	7.0[b]	1.9	4.2[c]	1.5	3.1[d]	0.7	6.4	2.6	8.3[b]	1.3	8.6[ab]	1.0	9.2[a]	0.5	9.4[a]	0.4	5.4[a]	0.6	8.1[a]	0.9	8.0[a]	0.0	5.7[a]	3.0	6.8	NA	3.8[a]	1.5	4.3[a]	1.6	4.7[a]	1.7	4.1[a]	1.4	3.2[a]	0.7	4.0	NA	2.9[a]	0.7
	Herta	8.8[a]	1.1	4.2[b]	2.0	2.8[c]	1.4	2.2[c]	0.7	5.8	3.2	8.8[a]	1.2	8.9[a]	0.9	8.5[a]	1.1	9.5[a]	0.3	4.6[a]	1.2	3.8[a]	2.1	4.0[a]	0.7	4.4[a]	3.2	4.5	NA	3.0[a]	2.0	2.9[a]	1.1	2.4[a]	1.0	2.6[a]	0.8	2.5[a]	0.6	2.0	NA	1.8[b]	0.5
	Patty	8.3[a]	1.3	3.5[b]	1.5	1.8[c]	0.9	1.5[c]	0.5	5.1	3.3	8.5[a]	1.3	8.1[a]	1.3	8.2[a]	1.4	9.0[a]	0.7	2.4[a]	2.0	3.5[a]	1.2	3.2[a]	1.8	4.1[a]	2.1	3.3	NA	1.6[a]	0.9	2.0[a]	1.0	1.5[a]	0.7	1.9[a]	0.8	1.5[a]	0.5	1.5	NA	1.4[a]	0.5
	Skiff	8.4[a]	1.6	3.9[b]	1.5	2.8[c]	1.2	2.4[c]	0.8	5.6	3.0	8.7[a]	1.7	8.0[a]	1.6	8.4[a]	1.5	9.0[a]	1.0	3.1[a]	2.3	3.7[a]	1.0	4.7[a]	1.8	4.4[a]	2.0	3.3	NA	2.8[a]	1.7	2.9[a]	1.1	2.5[a]	1.2	3.1[a]	0.7	2.7[a]	0.7	3.0	NA	1.8[b]	0.5
	Vlamingh	3.0[a]	1.5	2.2[b]	1.0	1.6[b]	0.6	1.8[b]	0.7	2.4	1.3	3.5[a]	1.7	2.7[a]	1.5	3.0[a]	1.0	2.5[a]	1.4	2.0[a]	1.1	2.6[a]	1.0	1.5[a]	0.0	1.9[a]	1.1	3.0	NA	1.4[b]	0.5	1.5[b]	0.6	1.9[ab]	0.8	2.2[a]	0.6	2.1[a]	0.7	1.5	NA	1.4[b]	0.4
	Gilbert	8.8[a]	1.1	8.2[a]	2.2	5.5[b]	2.1	4.6[b]	2.3	7.3	2.4	8.7[ab]	1.3	9.2[a]	0.6	8.4[b]	1.2	8.2[b]	1.8	9.0[ab]	0.3	9.3[a]	0.4	7.5[ab]	2.8	6.2[b]	3.3	9.5	NA	7.6[a]	1.5	4.8[b]	1.6	5.3[b]	2.9	3.8[b]	1.1	4.0[a]	1.8	9.0	NA	4.7[a]	2.2
	Grimmett	9.0[a]	0.9	8.3[a]	2.3	5.4[b]	1.6	4.3[c]	2.0	7.4	2.4	9.0[a]	1.1	9.2[a]	0.6	8.9[a]	1.1	9.1[a]	0.7	9.2[a]	0.4	9.3[a]	0.4	8.0[a]	2.1	6.3[a]	3.7	9.5	NA	6.0[a]	1.7	4.8[a]	1.3	6.5[a]	2.3	5.3[a]	1.4	3.9[a]	1.6	7.0	NA	4.6[a]	2.4
	Tallon	8.0[a]	1.5	7.1[a]	2.2	3.8[b]	1.1	3.2[b]	1.1	6.1	2.5	7.6[b]	1.6	8.5[a]	1.1	7.8[ab]	1.3	7.0[b]	2.2	8.8[a]	0.7	8.1[a]	1.1	5.2[ab]	1.0	5.3[ab]	3.0	8.8	NA	4.0[a]	1.4	3.8[a]	0.8	4.1[a]	2.4	3.4[a]	0.7	3.1[a]	0.7	3.5	NA	3.4[a]	1.7
	Harrington	7.6[a]	1.6	7.2[a]	1.9	6.0[b]	1.3	4.0[c]	0.8	6.7	1.9	6.8[ab]	1.6	7.8[a]	1.7	8.1[a]	1.3	8.2[a]	1.5	7.4[a]	0.6	7.6[a]	1.4	7.2[a]	1.8	5.9[a]	2.8	9.5	NA	6.4[a]	1.1	5.9[a]	1.3	7.0[a]	0.7	5.5[a]	1.7	4.1[a]	0.5	4.0	NA	3.8[a]	1.0
LG3	CIho 5791	1.1[a]	0.1	1.1[a]	0.3	1.1[a]	0.3	1.0[a]	0.2	1.1	0.2	1.0[a]	0.1	1.0[a]	0.0	1.0[a]	0.9	1.0[a]	0.0	1.2[a]	0.2	1.0[a]	0.2	1.0[a]	0.0	1.2[a]	0.5	1.0	NA	1.0[a]	0.1	1.1[a]	0.4	1.3[a]	0.3	1.0[a]	0.0	1.1[a]	0.3	1.0	NA	1.0[a]	0.0
	Kaputar	4.8[a]	1.1	4.2[ab]	1.0	3.8[b]	1.1	4.8[a]	1.2	4.4	1.2	4.8[a]	1.4	4.6[a]	1.0	5.3[a]	0.8	4.7[a]	0.9	4.7[a]	1.1	4.6[a]	0.4	4.2[a]	0.4	3.4[a]	1.6	4.5	NA	3.6[b]	0.9	3.7[b]	1.2	5.4[a]	1.5	3.9[b]	0.9	5.0[a]	1.1	5.0	NA	4.6[a]	1.6
	Kombar	6.1[b]	2.5	3.9[c]	1.8	4.0[c]	1.7	8.9[a]	1.3	5.6	2.6	5.3[a]	2.2	6.5[a]	2.7	6.5[a]	2.5	6.6[a]	2.1	4.1[a]	1.6	5.0[a]	2.1	2.5[a]	0.7	3.1[a]	1.4	3.8	NA	3.7[b]	1.2	3.2[b]	0.8	4.3[b]	2.4	6.3[a]	1.5	9.4[a]	0.3	9.0	NA	8.0[a]	2.0
	Fleet Australia	3.6[a]	1.5	3.0[ab]	1.5	2.6[ab]	2.1	3.7[a]	2.2	3.2	1.8	3.4[a]	1.2	3.9[a]	1.7	3.8[a]	1.9	2.8[a]	0.4	3.5[ab]	0.3	3.9[a]	1.7	1.8[b]	1.1	1.9[b]	0.6	3.5	NA	1.7[a]	0.5	3.1[a]	2.8	4.3[a]	2.9	2.1[a]	0.7	2.2[b]	0.7	6.0	NA	5.5[a]	2.2
	Buloke	3.3[a]	1.0	3.1[ab]	0.9	2.4[c]	1.0	2.6[bc]	0.6	2.9	1.0	2.8[b]	0.9	3.6[a]	1.1	3.5[ab]	1.0	3.5[ab]	1.2	3.1[a]	0.0	3.4[a]	0.5	2.7[a]	1.1	2.5[a]	1.3	4.0	NA	2.1[a]	0.7	2.6[a]	1.2	3.2[a]	0.9	2.4[a]	0.7	2.6[a]	0.5	3.0	NA	2.6[a]	0.8
LG4	Betzes	6.6[b]	1.6	6.5[b]	1.6	7.9[a]	1.5	6.0[b]	1.8	6.9	1.7	5.6[b]	1.4	6.9[a]	1.5	7.2[a]	1.3	7.8[a]	2.2	5.9[a]	1.3	6.1[a]	1.3	6.7[a]	1.8	6.8[a]	2.4	8.5	NA	7.6[a]	1.7	7.9[a]	1.4	9.0[a]	1.0	7.6[a]	1.4	6.3[a]	1.7	8.0	NA	5.1[a]	2.0
	Hindmarsh	5.9[b]	1.2	5.5[b]	1.3	6.6[a]	1.4	5.3[b]	1.1	6.0	1.3	5.6[b]	1.3	5.7[ab]	1.0	6.5[a]	1.2	6.2[ab]	1.0	5.4[a]	0.6	5.6[a]	1.1	6.0[a]	0.0	4.9[a]	2.2	6.3	NA	6.2[a]	1.5	6.5[a]	1.4	7.7[a]	1.1	7.1[a]	0.9	5.9[a]	0.9	5.5	NA	4.5[b]	0.9
	Clipper	5.4[a]	1.1	5.3[a]	1.5	4.7[a]	1.2	3.3[b]	0.8	4.9	1.3	4.9[b]	0.7	5.3[ab]	1.0	6.0[a]	1.7	5.7[ab]	1.1	5.6[a]	0.5	5.2[a]	0.6	6.0[a]	1.4	4.6[a]	2.7	6.5	NA	4.1[b]	0.9	4.7[ab]	1.2	5.7[a]	1.3	5.3[ab]	1.2	3.7[a]	0.8	3.0	NA	2.8[a]	0.6
	Keel	8.2[a]	1.2	7.8[a]	1.8	8.4[a]	1.6	5.2[b]	1.3	7.8	1.7	8.0[b]	1.3	7.8[b]	1.3	8.9[a]	0.7	8.8[ab]	0.7	8.4[a]	0.1	7.9[a]	1.4	8.0[a]	1.4	6.9[a]	3.1	9.0	NA	7.9[a]	1.4	8.5[a]	1.9	9.4[a]	0.4	8.4[a]	1.9	5.4[a]	1.2	6.5	NA	4.7[a]	1.4
	Commander	8.3[b]	1.5	8.5[ab]	1.9	9.0[a]	1.1	7.2[c]	1.9	8.4	1.6	8.0[bc]	1.7	8.7[ab]	0.9	7.4[c]	2.0	9.6[a]	0.4	9.4[a]	0.1	8.7[a]	0.7	8.5[a]	2.1	7.4[a]	3.3	9.5	NA	9.1[a]	0.7	8.6[a]	1.5	9.8[a]	0.3	9.4[a]	0.5	7.0[a]	1.8	9.5	NA	6.9[a]	2.0
LG5	Corvette	4.5[b]	1.5	5.4[ab]	2.1	6.1[a]	2.4	5.0[ab]	2.0	5.1	2.0	4.4[a]	1.7	4.7[a]	1.7	4.3[a]	1.1	4.6[a]	1.4	3.1[b]	0.5	6.8[a]	2.3	5.2[ab]	0.3	4.4[ab]	1.6	5.3	NA	9.1[a]	1.3	5.0[b]	1.1	5.3[b]	2.1	4.0[b]	1.3	6.2[a]	1.6	4.5	NA	3.2[b]	0.8
	Grout	5.2[a]	1.6	5.4[a]	2.0	5.1[a]	1.7	4.5[a]	1.9	5.1	1.7	5.1[a]	1.9	5.4[a]	1.6	5.0[a]	1.6	4.8[a]	1.2	4.7[a]	1.1	6.4[a]	1.9	4.5[a]	0.0	4.4[a]	2.7	6.8	NA	6.9[a]	1.5	4.1[b]	1.0	5.7[ab]	2.4	4.1[b]	0.5	5.6[a]	1.6	4.5	NA	2.6[b]	0.4
LG6	Beecher	1.2[c]	0.3	1.1[c]	0.1	2.0[b]	0.7	6.0[a]	2.1	2.0	1.7	1.2[a]	0.3	1.3[a]	0.4	1.3[a]	0.4	1.2[a]	0.3	1.1[a]	0.2	1.1[a]	0.2	1.0[a]	0.0	1.0[a]	0.0	1.0	NA	1.7[a]	0.4	2.1[a]	0.8	1.9[a]	0.1	2.3[a]	0.7	5.9[a]	1.6	5.0	NA	6.5[a]	3.0
	Cape	1.3[c]	0.4	1.1[c]	0.2	2.6[b]	1.0	5.7[a]	2.2	2.1	1.7	1.3[a]	0.4	1.3[a]	0.4	1.4[a]	0.5	1.1[a]	0.2	1.0[a]	0.0	1.1[a]	0.2	1.0[a]	0.0	1.1[a]	0.1	1.7	NA	2.2[b]	1.0	2.7[ab]	1.0	3.1[ab]	0.4	3.1[a]	0.7	4.8[a]	1.8	6.0	NA	7.1[a]	2.4
	Yerong	2.6[c]	1.0	2.6[c]	1.3	4.6[b]	1.5	6.0[a]	1.8	3.5	1.8	2.4[a]	0.8	2.6[a]	0.9	2.8[a]	1.4	2.5[a]	1.2	1.8[a]	0.7	3.1[a]	1.6	2.0[a]	0.0	2.5[a]	1.4	2.5	NA	4.2[a]	1.1	4.5[a]	2.0	5.3[a]	1.5	5.1[a]	0.8	5.1[a]	0.9	9.0	NA	6.6[a]	2.2
	Maritime	3.4[b]	1.2	3.1[b]	1.1	3.7[b]	1.9	8.8[a]	1.7	4.0	2.3	3.5[ab]	1.2	3.0[b]	1.2	4.1[a]	1.3	3.7[ab]	0.6	3.1[a]	1.2	2.8[a]	1.0	3.7[a]	1.8	3.2[a]	1.5	3.0	NA	2.6[b]	1.6	4.0[ab]	2.0	4.2[ab]	2.0	4.7[a]	1.4	9.6[a]	0.5	10.0	NA	7.3[b]	2.3
LG7	Dampier	3.1[c]	1.1	3.6[bc]	1.8	7.4[a]	2.1	4.1[b]	2.0	4.6	2.4	3.0[a]	0.9	3.0[a]	1.4	3.3[a]	0.6	3.5[a]	0.6	2.9[a]	1.3	3.0[a]	0.8	3.5[a]	0.7	4.1[a]	3.0	6.5	NA	6.6[a]	2.1	7.6[a]	2.4	7.6[a]	2.3	8.5[a]	1.5	3.0[b]	1.1	7.0	NA	5.4[a]	1.9
	Harbin	1.7[b]	1.0	1.5[b]	0.8	4.6[a]	1.8	2.5[b]	1.8	2.6	1.9	1.5[a]	0.8	1.7[a]	1.0	2.0[a]	1.0	1.9[a]	1.0	1.2[a]	0.3	1.4[a]	0.4	1.0[a]	0.0	1.8[a]	1.5	1.8	NA	3.9[a]	1.5	4.8[a]	1.8	6.0[a]	3.1	4.9[a]	1.3	1.9[a]	1.5	5.0	NA	3.2[a]	1.7
	Candaian L.S.	1.7[b]	0.9	1.6[b]	0.9	3.9[a]	1.6	2.5[b]	1.6	2.4	1.6	1.4[a]	0.8	1.7[a]	1.0	1.9[a]	0.9	1.9[a]	0.8	1.2[a]	0.3	1.5[a]	0.5	2.0[a]	1.4	1.7[a]	1.5	1.3	NA	3.3[a]	1.3	4.0[ab]	1.5	5.2[a]	2.9	3.8[a]	1.4	1.6[b]	1.0	5.0	NA	3.4[a]	1.5
	Prior	1.7[c]	0.7	2.1[c]	1.8	8.3[a]	2.1	4.1[b]	2.5	3.9	3.3	1.6[b]	0.6	1.6[b]	0.7	2.3[a]	0.8	1.9[ab]	0.5	1.4[a]	0.5	1.2[a]	0.4	2.2[a]	0.3	2.8[a]	2.8	5.5	NA	8.4[a]	2.5	8.4[a]	2.2	7.3[a]	2.5	8.5[a]	1.5	2.6[b]	2.0	7.0	NA	5.9[a]	1.9
	No. Isolates	59		15		35		14		123		19		24		11		5		2		6		2		4		1		11		14		3		7		8		1		5	

[a]Mean scores of genotypes between IGs or genotypes between States within IGs with same superscript letter are not statistically different (α = 0.05).
[b]Mean phenotype scores colour as per four infection response classes (MR, MS, S and VS) coloured dark green, light green, pink and red respectively.
[c]State codes: NSW = New South Wales, Qld = Queensland, SA = South Australia, Vic = Victoria and WA = Western Australia.

Chapter 4

Identification of genomic regions underpinning reciprocal infection response to Prior and Skiff virulent *Pyrenophora teres* f. *teres* isolates in Australia

4.1 Abstract

The necrotrophic fungus *Pyrenophora teres* f. *teres* (*Ptt*) that causes net form net blotch (NFNB) is a damaging pathogen of barley worldwide. A high degree of pathogenic variation has been documented in many Australian and international studies. To standardise genotypes used across such studies, an international differential set of 12 genotypes has been proposed to characterise NFNB isolates and includes two historic Australian cultivars; Prior and Skiff. Australian *Ptt* isolates fall into four isolate groups (IGs) based on virulence profiles (Chapter 3). Prior is susceptible and Skiff is resistant to isolates from the Prior group (IG3), alternatively Skiff is susceptible and Prior is resistant to isolates from the Skiff group (IG1). This study demonstrated the genomic regions involved with the inverse susceptibility of Prior and Skiff.

Five quantitative trait loci (QTL) regions were identified interacting with two Australian *Ptt* isolates - NB50 (Skiff susceptible / Prior resistant) and NB85 (Prior susceptible / Skiff resistant), following seedling and adult inoculations of a population of Prior x Skiff recombinant inbred lines (RILs). One QTL on chromosome 3H (*QRpt3H*) and one QTL on chromosome 6H (*QRpt6Hs*) were associated with infection response using NB50, while three regions on chromosome 6H (*QRpt6Ha*, *QRpt6Hp* and *QRpt6Hc*) were associated with infection response using NB85. No recombination was observed between two QTL closely linked in repulsion near the centromere of 6H, *QRpt6Hp* and *QRpt6Hs*. All QTL co-located with previously reported loci, for instance *QRpt3H* co-located with *Rpt1a*, *QRpt6Ha* co-located with *SPN1*, *QRpt6Hp* and *QRpt6Hs* co-located with *Spt1* and *QRpt6Hc* co-located with *AL_QRptt6-1*. Results presented here suggest that Prior harbours a unique gene at the *Rpt5/Spt1* locus that is closely linked to *rpt.r* although is likely to be different to *rpt.k*. These findings re-affirm the complexity of the barley-*Ptt* pathosystem, while providing critical information to understand varietal differences in response to Prior and Skiff virulent isolates in Australia.

4.2 Introduction

Net form net blotch (NFNB) caused by *Pyrenophora teres* f. *teres* (*Ptt*) is a damaging foliar disease of barley (*Hordeum vulgare* L.) (*Hv*) crops worldwide. Infection by the pathogen and subsequent

lesion development leads to a reduction in photosynthetic capability and loss in grain yield, which can be severe, as several studies have reported losses of up to 35% (Jebbouj and El Yousfi 2009; Khan 1987; Steffenson *et al.* 1991). A recent Australian study by Platz (2017) demonstrated yield reduction of almost 42% in a very susceptible cultivar in very favourable disease conditions. Murray and Brennan (2010) estimated this disease to cost the Australian barley industry between $19M and $117M annually. While control of NFNB with fungicides can be effective, deployment of genetic resistance is the favoured approach to minimising the impact of *Ptt* over the long term.

Prior, Australia's most successful barley cultivar, originated from a selection of Chevallier type barley in 1903 (Ullrich 2010). Prior was the predominant malting cultivar in most states until the release of Clipper in 1978 (Sparrow 1984). Prior was also used as a parent in many breeding programs. Consequently, most Australian-bred commercial cultivars have Prior in their pedigree. Skiff was developed from a cross between Abed Deba/WI2335 and CD-28/WI2231, where WI2335 is Proctor/CIho 3576//C.P.I. 18197/Beka, CD-28 is Clipper/Diamant progeny selection number 28 and WI2231 is Proctor/CIho 3576. Skiff was released as a feed grade cultivar in 1988 and was grown mostly in South Australia and New South Wales (NSW). Due to its success in NSW, it was used as a parent to develop the varieties Tantangara, Tulla and Yambla. Skiff derived lines were also used to develop Binalong, Cowabbie and Milby in NSW; Dhow, Finniss, Navigator and VT Admiral in South Australia (SA) and Lockyer and Rosalind in Western Australia (WA). Following an epiphytotic of NFNB in 2004 that severely affected Binalong crops, NSW farmers shifted to less susceptible cultivars. No commercial cultivar has been developed from direct crosses between Prior and Skiff.

As reported in Chapter 3, *Ptt* is extremely diverse for pathogenicity on barley grown in Australia. Prior was susceptible to isolates collected in Queensland (Qld), New South Wales (NSW), South Australia (SA), Victoria (Vic) and Western Australia (WA) and Skiff was susceptible to isolates collected in Qld, NSW and SA. Additionally, Skiff was susceptible while Prior was resistant to one isolate collected from Richmond, Tasmania in 2003 (data not shown) and two isolates collected from southern WA in 2014 (Dr S. Gupta, personal communications). Chapter 3 reported a high frequency of isolates able to induce a susceptible response on Prior or Skiff, whereby 82% of isolates induced a susceptible response on either cultivar. Specifically, 33% of isolates induced a susceptible response on Prior and 49% of isolates induced a susceptible response on Skiff, while only 3% of isolates induced a susceptible response on both cultivars. Population structure was defined by four isolate groups (IGs); IG1, IG2, IG3 and IG4, which primarily induced susceptible responses on Skiff, Tallon, Prior and Maritime, respectively. Collectively, Prior and

Skiff IGs comprised 76% of isolates sampled. Notably, isolates that induced a susceptible response on Prior also induced a resistant response on Skiff and alternatively, isolates that induced a susceptible response on Skiff induced a resistant response on Prior. Interestingly, a similar reciprocal susceptibility was observed in a Rika/Kombar population to two Californian isolates of *Ptt* (Abu Qamar *et al.* 2008).

International collaboration involving many researchers working on *Ptt* culminated in the proposal of a differential set of 12 genotypes to be used for characterising this pathogen worldwide (Afanasenko *et al.* 2009). Importantly, Prior and Skiff are included in this set because they effectively differentiate a large proportion of isolates from eastern Australia (Platz *et al.* 2000). Prior and Skiff could be differentiated using *Ptt* isolates from Europe (Afanasenko *et al.* 2009; Jalli 2010; Stefánsson 2009), yet these cultivars could not be differentiated using isolates from Canada (Tekauz *et al.* 2011).

Genetic mapping studies have identified quantitative trait loci (QTL) for resistance to *Ptt* on all seven barley chromosomes (Appendix 1), while resistance QTL on chromosome 3H are commonly reported (Chapter 2). Bockelman *et al.* (1977) identified a dominant resistance gene in Tifang on 3H that was designated *Rpt1a*. More recently, a study by Koladia *et al.* (2017a) mapped a dominant resistance gene in Tifang to a region of 3H and proposed that CIho 5791 carried either a dominant resistance gene that is closely linked in repulsion or an allele of the same gene in Tifang. Additionally, QTL that co-locate to the physical position of the locus on 3H have been detected in Igri (Graner *et al.* 1996), NDB 112 (Liu *et al.* 2015) and Pompadour (Gupta *et al.* 2010).

Chromosome 6H appears highly complex as numerous studies have reported QTL that interact in an isolate specific manner (Chapter 2). Abu Qamar *et al.* (2008) identified a region on 6H that either harboured two recessive resistance genes linked in repulsion or alleles of a single gene in Rika (*rpt.r*) and Kombar (*rpt.k*). Further refinement of this region was conducted by Liu *et al.* (2010) and later by Richards *et al.* (2016), which narrowed down the region to ~0.24 cM and was designated *Spt1*. Hypotheses regarding alleleism or closely linked genes were not resolved, however recessive genes; *rpt.r* and *rpt.k* were given dominant susceptibility designations, *Spt1.R* and *Spt1.K*, respectively. Furthermore, Liu *et al.* (2015) reported another region on 6H that interacted with multiple *Ptt* isolates from a diverse world-wide collection. F_2 analysis of a Hector/ND B112 population identified sensitivity to be dominant and was contributed by Hector. The region was designated *SPN1*. According to published QTL intervals, the genetic physical map positions of *Spt1* and *SPN1* are different (Appendix 1). Prior to 2015, QTL on the long arm of 6H

were rarely reported, although in the time since then, many QTL have been described that provide resistance to isolates from Australia, Germany, Japan, Norway, Russia and the USA (Adhikari 2017; Afanasenko *et al.* 2015; Liu *et al.* 2015; Read *et al.* 2003; Richards *et al.* 2017; Steffenson *et al.* 1996; Vatter *et al.* 2017; Wonneberger *et al.* 2017b). The exclusive use of SNP markers in the recent studies allowed QTL to be accurately positioned onto the physical map (Appendix 1). Further work to determine whether QTL impart dominant resistance or dominant susceptibility is necessary to assess the usefulness of this region as a breeding target.

Ptt isolate; NB50, has been used in several bi-parental QTL mapping studies (Cakir *et al.* 2011; Cakir *et al.* 2003; Gupta *et al.* 2010; Islamovic *et al.* 2017; Liu *et al.* 2015; Martin *et al.* 2018; Raman *et al.* 2003). Using this isolate, QTL have been detected on chromosome 1H (one study), 2H (three studies), 3H (six studies), 4H (three studies), 5H (one study) and 6H (five studies) and 7H (one study). Comparison of all QTL via accurate projection of marker physical position is difficult, as various marker platforms have been used. However, six QTL identified by studies that used markers based on single nucleotide polymorphisms (SNPs) (Islamovic *et al.* 2017; Liu *et al.* 2015) were unique and confirm that different genetic backgrounds interact with many virulence/avirulence products produced by NB50. NB85 has been used in one previous genetic mapping studies (Martin *et al.* 2018), which identified QTL on 1H and 7H. The QTL on 1H co-located with a QTL identified by Lehmensiek *et al.* (2007) and the QTL on 7H co-located with QTL identified by Mace *et al.* (2007) and Vatter *et al.* (2017) (Appendix 1). Prior is also susceptible to two other Queensland isolates that have been used in mapping studies. Two QTL (2H and 6H) were identified by Liu *et al.* (2015) using NB22; pathotype group 6 (Platz *et al.* 2000) and one 6H QTL was identified by Gupta *et al.* (2010) using NB81; pathotype group 5 (Platz *et al.* 2000).

In order to identify the underlying genomic regions interacting with two representative isolates from IG1 and IG3, Prior and Skiff were selected as parents to generate a bi-parental RIL population. This research was conducted to help understand the genetics controlling reciprocal responses of these varieties to the different isolates. Knowledge generated will allow researchers to connect pathotyping surveys to the underlying host genes and provide critical information to assist barley breeders develop resistant cultivars. Additional information summarising the presence/absence of markers associated with QTL in a diverse panel of barley genotypes will serve as a useful resource for barley researchers.

4.3 Materials and methods

4.3.1 Plant materials

Controlled pollination of Prior with pollen from Skiff was conducted in a glasshouse during the winter of 2011 at Hermitage Research Facility, Warwick, Queensland, Australia. Three F_1 plants were grown in the glasshouse during the summer of 2011/2012. F_2 seeds were space-planted during winter of 2012 and 311 single plants were harvested. F_3 and F_4 single plant generations were grown in a controlled environment chamber in 2013. Five F_5 seeds of each line were sown as a hill plot in the winter of 2014 and one head was harvested from each RIL. The final generation was grown in the glasshouse during the winter of 2015 and F_7 seed from 304 single F_6 plants was harvested. A total of 286 Prior x Skiff RILs (PSRs) were evaluated in all phenotyping experiments due to low seed quantity of some lines.

A diverse panel of 256 barley genotypes was assembled to determine proportion of QTL across five continents. Genotypes were from Africa (30), Australasia (103), Asia (13), Europe (55) and the Americas (55). Genotypes from Africa were from Algeria, Egypt, Ethiopia, Libya, Morocco South Africa, Tunisia and the International Centre for Agricultural Research in the Dry Areas (ICARDA). Genotypes from Asia were from China, Japan and Korea. Genotypes from Australasia were from Australia and New Zealand. Genotypes from Europe were from the Czech Republic, Denmark, France, Germany, Great Britain, Poland, Russia and Sweden. Genotypes from the Americas were from Canada, Mexico, Uruguay and the USA. Australasian genotypes represented cultivars released between 1903 and 2014. Information for name, accession number, origin, pedigree and alleles for eight QTL from this Chapter and Chapter 5 are presented in Appendix 2.

4.3.2 Pathogen isolates

Two single spore isolates of *Ptt* (NB50 and NB85) were used to phenotype the Prior x Skiff RIL population. Skiff was susceptible and Prior was resistant to NB50 (Figure 4.1). NB50 clustered to Isolate Group 1 (Fowler *et al.* 2017), pathotype group 12 (Platz *et al.* 2000) and is classified as pathotype 10-22 (Steffenson and Webster 1992a) and pathotype B1 (Tekauz 1990) (Table 4.1). NB50 was collected from an unknown barley genotype in a disease nursery at the Department of Agriculture and Fisheries Gatton Research Facility, Queensland on the 26th of July 1994. Prior was susceptible and Skiff was resistant to NB85 (Figure 4.2). NB85 clustered to Isolate Group 3 (Fowler *et al.* 2017), pathotype group 5 (Platz *et al.* 2000) and is classified as pathotype 20 (Steffenson and Webster 1992a) and pathotype A1 (Tekauz 1990) (Table 4.1). NB85 was collected from a

commercial crop of Cape barley near Lockyer creek, Gatton, Queensland on the 22nd of September 1995.

4.3.3 Pathogen cultures for inoculation

Cultures were stored in screw top tubes containing dried barley leaves infected with either NB50 or NB85 in a -80C freezer. The cultures were retrieved from long term storage and immediately heat shocked in a 45°C warm water bath for 3 minutes. Three leaf segments were placed into a 90 mm petri dish containing a filter paper disk on top of a make-up removal pad, which were saturated with 18.2 MΩ-cm Millipore-filtered water. Leaf segments were incubated in a culture cabinet that housed two 36 Watt fluorescent white and one 36 Watt blacklight blue UVA tubes situated 30 cm above a thermal plate. The culture cabinet was maintained at 19°C (± 1°C) and 12 hour photoperiod until sporulation was observed. An acupuncture needle was used to transfer five single conidia to five unique positions in a petri dish containing V8 agar (150 mL Campbell's V8® vegetable juice, 850 mL water, 1.5 g $CaCO_3$ and 15 g agar) that was subsequently incubated in the dark for five days at 25°C (± 1°C). A 4mm hole punch was used to excise plugs of agar containing mycelium from the perimeter of the chosen single conidial colony. Five plugs were transferred to each of two peanut oatmeal agar (POA) plates (50 g fresh peanut leaf filtrates in 500 mL water, 15 g oatmeal filtrates in 500 mL water and 20 g agar) (Speakman and Pommer 1986) and placed in the culture cabinet, at the same conditions as above, to induce conidiation and inoculum harvested after 9 – 10 days.

Pathogen cultures for adult experiments were generated independently from seedling experiments using the same protocol with the inclusion of additional steps following POA subculturing to generate mycelia balls. Five mycelial plugs were excised from eight day old POA cultures using the hole punch and transferred to one 250 mL TechnoPlas gamma sterile polypropylene jar (P10065SL) containing 100 mL Sigma-Aldrich® P6685 - 25 g/L potato dextrose broth (PDB). 70 PDB jars per isolate were produced and immediately placed on a Ratek platform mixer (OM8) set at 135 oscillations/min that was situated in a room with the air-conditioning set to 22°C (± 2°C). Mycelia balls were incubated for five days.

4.3.4 Seedling assays

Seedling experiments were sown into 10 cm diameter pots, where five seeds per genotype were sown at three evenly spaced pot positions around the outside of each pot. 216 pots were used which

provided a total of 648 pot positions. Pots were distributed across six growth room benches. Each bench held 36 pots, positioned in a 4 x 9 array and benches laid out in a 3 x 2 array. The experimental design considered each of the three pot positions within a pot to be a unique column and each pot to be a unique row. Latin square designs of 18 columns by 18 rows were utilised to assign RIL genotypes to 324 available pot positions per replicate. Replicates were arranged to give an experimental block of 36 columns by 18 rows, meaning half of the two centre benches aligned with each replicate. As not all pot positions were filled by two replicates of each genotype, genotypes were selected at random for the inclusion of a third replicate. The first seedling NB50 experiment (nb50s1) consisted of 2.11 replicates of 300 genotypes and the second NB50 seedling experiment (nb50s2) consisted of 2.19 replicates of 292 genotypes. The first NB85 seedling experiment (nb85s1) consisted of 2.16 replicates of 299 genotypes and the second NB85 seedling experiment (nb85s2) consisted of 2.19 replicates of 292 genotypes. Parents were included in all seedling experiments. Searles® premium potting mix was used and plants were fertilised with 1.3 g/L of Grow Force Flowfeed EX7 soluble fertilizer twice per week. Pots were top watered prior to inoculation and bottom watered following inoculation. Seedlings were grown in a growth room at 14°C (± 1°C) night temperature and 24°C (± 1°C) day temperature under 12 hour photoperiod. Light was provided by 2700K halogen, 2000K high pressure sodium and 4000K metal halide globes.

Seeding experiments were inoculated 14 days after sowing, at approximately growth stage Z12 (Zadoks *et al.* 1974). Four independent inoculations were conducted, two for NB50 and two for NB85. Conidia were washed from two POA plates into a 500 mL beaker using 50 mL of 18.2 MΩ-cm Millipore-filtered Tween®-water (two drops of Tween® 20 per 100 mL of Millipore-filtered water) and an 8mm wide paintbrush. Each spore suspension was filtered through a fine tea strainer, diluted with Tween®-water to 200 mL using a volumetric flask and placed onto a magnetic stirrer. A Reichert Bright-Line® haemocytometer was used conduct ten individual counts of 0.1 Microliter to calculate the absolute concentration of each spore suspension. Spore suspensions used to inoculate the first experiment of both isolates were standardised to a concentration of 4,320,216 conidia (± 1%) in 648 mL (6,667 conidia/mL at 3 mL/10cm seedling pot). The concentration of the spore suspensions for the second experiment of both isolates were standardised to 60% of that of the first, i.e. 2,592,000 conidia (± 1%) in 648 mL (4,000 conidia/mL at 3 mL/10cm seedling pot).

Inoculum was applied evenly from four sides using a Wallwick spray paint gun attached to a 240 L/min air compressor. Inoculated plants were immediately transferred to a clear acrylic chamber that was positioned within a growth room for incubation at 19°C (± 1°C) and 99%

humidity for 24 hours; 14 hours dark followed by 10 hours of light, supplied by 2000K high pressure sodium and 4000K metal halide lights. Following inoculation, the seedlings were transferred back to the initial growth room where the pots were spaced out according to the experimental design and subject to the same light and temperature parameters used to grow the seedlings.

Infection response (IR) of seedlings was scored based on the central portion of the second leaf nine days after inoculation according to a 1 – 9 rating scale adapted from Tekauz (1985), where 1 was most resistant and 9 was most susceptible. This scale usually includes a score of 10, though we considered phenotype scores 9 and 10 to be similar and thus combined them to allow direct comparison to the 1 – 9 adult scale. Phenotypes for scores 1 – 8 remained unchanged from the originally published scale. Seedling genotypes that displayed segregating phenotypes of greater than 3 IR units were excluded from QTL analyses.

4.3.5 Adult assays

Adult phenotyping experiments were conducted during the winter of 2016 in hill plot disease nurseries that were individually inoculated with NB50 and NB85. Disease nurseries were separated by at least 500 meters to minimise cross-contamination of isolates. 288 genotypes were sown as hill plots in randomised complete block designs of four columns and 144 rows. Each replicate block was composed of four columns and 72 rows. Both parents were included in all adult experiments. Approximately 5 seeds of each genotype were sown as a hill plot. Hill plots were sown as pairs that were not genetically identical. Hill plots were spaced 50 cm from neighbours along the row, 76 cm from neighbours across the row. A continuous five-row very susceptible disease spreader was sown 76 cm across from the plots and that ran the length of the nursery to facilitate localised dispersal of conidia. Five-row disease spreaders were sown on the two weeks prior to experimental hill plots, which were sown on the 19th and 20th of July for NB85 and NB50, respectively. Henley and breeding line, NRB06059 (Mackay*2/WI3214(Triumph/Galleon//Harrington)), were used as the spreader genotypes for NB50 and NB85, respectively. Genotypes were selected for high susceptibility to the target *Ptt* isolate and strong resistance to non-target *Ptt* pathotypes and other pathogens.

Inoculation of adult phenotyping experiments followed a two-stage process. In the first stage, mycelia balls and liquid contents of each PDB jar were poured into a high speed blender for 40 seconds to produce a mycelial broth. The blended mycelial broth was double-strained as it was

poured into a 14 litre battery powered backpack spray unit with a hand wand. Nufarm Bond® adjuvant and Uptima Tween® 20 detergent were added at a rate of 0.1% to increase efficiency of inoculation via improved leaf wettability and reduced droplet contact angle (Statler and Nordgaard 1980). The mycelial broth of each isolate was used to inoculate a specific 'pre-season disease increase block'; a 500m^2 field of a susceptible cultivar that was sown on the 29th of April. Mycelial broth inoculations took place after 5:00 pm following an irrigation period of at least 1 hour on the 18th and 19th of June for NB50 and NB85, respectively. Approximately 12 randomly selected patches of 3m^2 were inoculated and immediately covered with a tarpaulin that was pegged down in order to maintain high humidity for at least 15 hours. Tarpaulins were removed between 8:00am am and 9:00 am the following day. After infection was established, disease proliferation was promoted via frequent sprinkler irrigation events of 30 – 60 minutes after sunset.

In the second stage, infected plants from the 'pre-season disease increase blocks' were cut with a sickle bar mower and loaded onto a utility vehicle. Infected plants were immediately spread over the five-row spreaders in the respective disease nurseries at an approximate dry matter rate of 2,000 kg/Ha. Experimental nursery inoculations were conducted on the 29th and 31st of August for NB85 and NB50, respectively. Disease development was promoted via four weekly one hour sprinkler irrigation events after sunset during and September and October.

Adult IR was scored on a whole plot basis on a 1 – 9 scale similar to the technique proposed by Saari and Prescott (1975), where 1 was most resistant and 9 was most susceptible. Data for nb50a1 was collected on the 18th of October and data for nb50a2 was collected on the 9th of November. Data for nb85a1 was collected on the 20th of October and data for nb85a2 was collected on the 7th of November.

4.3.6 Analysis of phenotype datasets

All statistical analyses were conducted in the R statistical computing environment (Team 2013) using appropriate packages. The IR scores were analysed using a linear mixed model framework. A square root transformation was applied to seedling phenotype IR to ensure the homogeneity of variance across the fitted values, however no transformation was necessary for the adult phenotype data. Genotype was fitted as a fixed effect, while random effects were included to account for the structure of the experimental design. Using the methods of Gilmour (1997), the structure of the residual variance was extended to enable the modelling of local scale, smooth variation between neighbouring plants within and across pots/plots using an autocorrelation process

An example of the model fitted to the data is presented below:

```
" model.asr <- asreml (fixed = Response ~ Genotype,
                       random = ~ Replicate + Experimental.Design.Terms,
                           rcov = ~ id(Row) : id(Column),
                           data = dataframe.df) "
```

RIL phenotype predictions for subsequent QTL analysis were provided from the linear mixed model as empirical best linear unbiased estimates (eBLUEs). Variance components were estimated using residual maximum likelihood (REML) (Patterson and Thompson 1971), implemented through the 'asreml' package (Butler *et al.* 2009).

Correlation between phenotypes obtained across experiments was calculated using the Pearson algorithm in the 'PerformanceAnalytics' (v.1.4.3541) package (Peterson *et al.* 2014). The 'heritability' (v1.0) package (Kruijer *et al.* 2016) was used to estimate repeatability between replicates of raw data and narrow sense heritability based on eBLUE IR and a marker-based relatedness matrix that was generated in Genome Association and Prediction Integrated Tool (GAPIT) (Lipka *et al.* 2012) using EMMA kinship algorithm. A histogram of phenotype densities was plotted using ggplot2 (v2.2.1) package (Wickham 2009).

4.3.7 Genotyping and linkage map construction

Tissue was collected from young leaves of F_6 plants that were grown for the final single plant generation. For the diversity panel, samples from first and second leaves were bulked from three seeds per genotype. DNA was extracted using the CTAB protocol recommended by Diversity Arrays Technology (DArT™) (http://www.diversityarrays.com). The PSR population and diversity panel were genotyped by DArT™ using next generation sequencing platforms to generate marker data from DArTseq™ single nucleotide polymorphisms (SNPs). In order to align SNPs to the barley physical map of the masked pseudomolecule (Mascher *et al.* 2017), 55,585 nucleotide sequences of three DArTseq™ datasets were used in a command prompt local BLAST to search for significant alignments. SNPs that returned an alignment smaller than E value $8e^{-05}$ were accepted if they were unique or were positioned on the chromosome near original physical positions provided by DArT™. A total of 35,049 SNP markers were successfully aligned to the barley physical map.

To generate the linkage map, SNPs were manually curated in excel to select positioned markers with < 5% missing genotypes, < 7.5% heterozygous genotypes and SNPs that adhered to an approximate segregation ratio of 1:1. R/qtl package (Broman *et al.* 2003) was used to construct a linkage map of 2,153 SNPs using the "est.map" function with "map.function = "kosambi", maxit = 20000" arguments specified. In situations where two or more SNPs co-located to a single locus, the marker with the fewest missing values was selected for inclusion in the final linkage map of 1,079 unique recombination sites (Table 4.2). The final linkage map had a total length of 1,335 cM with individual chromosome lengths ranging between 138.7 cM and 228.9 cM and a genome-wide unique SNP density of 1.2 cM/locus with individual chromosomes ranging between 0.8 cM/locus and 2.9 cM/locus (Table 4.2).

4.3.8 QTL mapping

QTL mapping was conducted using the 'R/qtl' package (Broman *et al.* 2003). Missing marker genotypes were imputed using "fill.geno" function with "method = "argmax", map.function = "kosambi"" arguments specified. Significant logarithm of the odds (LOD) threshold for individual QTL was determined at $\alpha = 0.01$ via "scanone" function using Haley-Knott regression for three replicates of 100,000 permutations per dataset. LOD scores of individual QTL for nb50 datasets ranged from 3.77 to 3.82 and 3.81 to 3.85 for nb85 datasets. For consistency, the highest LOD score was used across all QTL scans, thus a LOD score that exceeded 3.85 was considered significant. Significant LOD threshold for interacting QTL was identified at $\alpha = 0.01$ via "scantwo" function using Haley-Knott regression for one replicate of 1,000 permutations per dataset. LOD scores of interacting QTL for nb50 datasets ranged from 4.8 to 5.02 and 4.6 to 4.98 for nb85 datasets. For consistency, the highest LOD score was used across all QTL scans, thus a LOD score that exceeded 5.0 was considered significant.

MIM was conducted using the "stepwiseqtl" function and associated workflow outlined by Broman and Sen (2009) with "max.qtl" argument incrementally increased from 2 through to 10 to identify the most frequently detected QTL across all datasets per isolate. A QTL object with QTL detected across all datasets per isolate was created using "makeqtl" function. Scans to detect additional QTL were conducted using "addqtl" function. Additional QTL that exceeded the significant LOD threshold for individual QTL in all datasets were included in the model. Interaction between QTL was estimated via "addint" function. Interactions that exceeded the LOD threshold for interacting QTL were included in the model one at a time from largest to smallest until no further significant interactions were detected. The "fitqtl" function was used to estimate LOD score

and estimated phenotypic variance explained by all terms in the model and marker effect and drop-one-QTL-at-a-time results were obtained using the "fitqtl" function.

Least significant difference (LSD) between mean IR phenotype of QTL allele combinations was calculated with 'agricolae' package (De Mendiburu 2014) using a significance threshold of $\alpha = 0.05$. The 'boxplot' function was used to generate box and whisker plots of QTL allele combinations for the mean of IR of PSRs for all NB50 datasets, all NB85 datasets and combined NB50 and NB85 datasets.

QTL names start with "*Q*" to designate QTL, followed by "*Rpt*" to designate reaction to *Pyrenophora teres*, followed by the chromosome of detection and lastly a letter to designate a sequential number if more than one QTL was detected on a single chromosome.

4.3.9 Diversity panel

Individual genotypes within the diversity panel were grouped by continent of origin in order to determine the proportion of genotypes that carry of the desirable allele. In order to determine the proportion of QTL in commercial Australasian cultivars, the group was divided according to the representative state from where the cultivars were developed and released. Two cultivars from New Zealand were also included. The analysis accounted for missing and heterozygous SNPs in the calculation. Introductory genotypes were grouped by as per the location of selection. For example; Prior was selected from Chevallier in Australia, thus Prior was grouped with the Australasian germplasm.

4.4 Results

4.4.1 Infection response to NB50

IR scores ranged from 1 to 9 in most nb50 datasets while IRs lower than the resistant parent (Prior) and higher than the susceptible parent (Skiff) were observed (Table 4.3). Repeatability between replicates within experiments was between 0.74 and 0.94 and narrow sense heritability was between 0.63 and 0.90 (Table 4.3). Correlation of nb50 datasets was high with 0.82 between adult assessments and 0.96 between seedling experiments while seedling to adult comparisons ranged from 0.78 to 0.86 (Figure 4.3). IR density distributions for all nb50 datasets followed a bimodal distribution that was strongly skewed in the direction of resistance (Figure 4.4). Analysis of $\chi 2$ for

segregation ratios between resistant and susceptible phenotypic classes was significantly different to 1:1 (single gene) and 3:1 (two genes) at $p = 0.05$ for all nb50 datasets (Table 4.4).

4.4.2 Infection response to NB85

IR scores ranged from 1 to 9 in most nb85 datasets while IRs lower than the resistant parent (Skiff) and higher than the susceptible parent (Prior) were observed (Table 4.3). Repeatability between replicates within experiments was between 0.88 and 0.97 and narrow sense heritability was between of 0.92 and 0.99 (Table 4.3). Correlation of nb85 datasets was extremely high with 0.92 between adult assessments and 0.98 between seedling experiments while seedling to adult comparisons ranged from 0.92 to 0.93 (Figure 4.3). IR density distributions for all nb85 datasets followed a bimodal distribution that was weakly skewed toward resistant phenotypes (Figure 4.5). Analysis of $\chi 2$ for segregation ratios between resistant and susceptible phenotypic classes was not significantly different to 1:1 at $p = 0.05$ for nb85a1, nb85s1 and nb85s2 datasets, while nb85a2 was significantly different (Table 4.4). All nb85 datasets were significantly different to 3:1 (two genes) at $p = 0.05$ (Table 4.4).

4.3.3 Reciprocal allele association for NB50 and NB85

MIM genome-wide scans for nb50 and nb85 datasets detected an association with SNP marker 3257446-28:G>T on 6H at 58.35 cM (368,527,587 bp on the physical map). The effect of this QTL was inverted for the isolates, where low IR for NB50 was associated with the 'G' allele and low IR for NB85 was associated with the 'T' allele. Inspection of the full set of markers for the PSR population identified a marker that was in complete linkage with 3257446-28:G>T and subsequently was not included in the linkage map. The maker, 3260813-56:A>T, was located at 364,757,662 bp on the physical map. Genotype specificity was observed for both markers as inspection of the diversity panel revealed that 3257446-28:G>T was present with Skiff and Isaria derived genotypes, while SNP marker 3260813-56:A>T was present with Prior and some of its descendants (Appendix 2).

4.4.4 Mapping response to NB50

MIM genome-wide scans successfully detected QTL associated with resistance to *Ptt* isolate NB50 in the PSRs. Two QTL were detected, one on chromosome 3H and the other on chromosome 6H (Figure 4.6). The QTL on 3H was designated *QRpt3H* and the QTL on 6H was designated

QRpt6Hs. Significant interaction between *QRpt3H* and *QRpt6Hs* was detected in all nb50 datasets. LOD scores for *QRpt3H* ranged from 58.77 to 105.32, explaining between 46.88 and 56.34 % of the phenotypic variation with an estimated allele effect between 1.10 and 1.85 units where the Skiff allele increased phenotype (Table 4.5). LOD scores for *QRpt6Hs* ranged from 48.43 to 88.08, explaining between 30.21 and 39.22 % of the phenotypic variation with an estimated allele effect between 0.82 and 1.45 units where the Skiff allele increased phenotype (Table 4.5). LOD scores for the interaction between *QRpt3H* and *QRpt6Hs* ranged from 15.24 to 26.22, explaining between 5.24 and 15.25 percent of the phenotypic variation. A positive interaction between QTL was observed (Table 4.5). All terms in the full model returned LOD scores between 76.61 and 131.14 and explained 73.45 and 88.80 % of the total phenotypic variation (Table 4.5)

4.4.5 Mapping response to NB85

MIM genome-wide scans successfully detected QTL associated with resistance to *Ptt* isolate NB85 in the PSRs. Three QTL were detected on chromosome 6H (Figure 4.7) and are identified as *QRpt6Ha*, *QRpt6Hp* and *QRpt6Hc*. Significant interaction between *QRpt6Ha* and *QRpt6Hp* was detected in both nb85 seedling datasets. LOD scores for *QRpt6Ha* ranged from 11.09 to 59.97, explaining between 3.09 and 6.00 percent of the phenotypic variation with an estimated allele effect between -0.62 and -1.08 units where the Skiff allele decreased phenotype (Table 4.5). LOD scores for *QRpt6Hp* ranged from 33.39 to 99.90, explaining between 11.98 and 14.97 % of the phenotypic variation with an estimated allele effect between -1.41 and -2.17 units where the Skiff allele decreased phenotype (Table 4.5). LOD scores for *QRpt6Hc* ranged from 7.70 to 12.86, explaining between 0.83 and 2.08 % of the phenotypic variation with an estimated allele effect between -0.36 and -0.42 units where the Skiff allele decreased phenotype (Table 4.5). LOD scores for the interaction between *QRpt6Ha* and *QRpt6Hp* in seedling datasets were 6.32 to 15.56, explaining between 0.70 and 1.03 % of the phenotypic variation and a positive interaction between QTL was observed (Table 4.5). All terms in the full model returned LOD scores between 108.50 and 201.24 and explained 84.61 and 96.48 % of the total phenotypic variation (Table 4.5)

4.4.6 Effect of QTL combinations on IR phenotype

Groups of PSRs carrying identical QTL allele combinations displayed significantly different mean IR phenotypes. Analysis of nb50a1, nb50s1 and nb50s2 identified SS, SP, PS and PP combinations as significantly different to each other (Table 4.6). Analysis of nb50a2 identified PS combination as not significantly different to either SP or SS combinations, while SS, SP and PP combinations were

significantly different to each other (Table 4.6). A box and whisker plot of the mean IR across all NB50 datasets for *QRpt3H* and *QRpt6Hs* QTL allele combinations is presented in Figure 4.8A. Analysis of nb85a1 identified no significant difference between PPS, SPP and SPS combinations and no significant difference between PSP, PSS and SSP combinations, while PPP and SSS combinations were significantly different to each other and all other combinations (Table 4.6) Analysis of nb85a2 identified no significant difference between SPP and SPS combinations and no significant between PSP, PSS, SSP and SSS combinations, while PPP and PPS combinations were significantly different to each other and all other combinations (Table 4.6). Analysis of nb85s1 and nb85s2 identified no significant difference between PSP, PSS and SSP combinations, while PPP, PPS, SPP, SPS and SSS combinations were significantly different to each other and all other combinations (Table 4.6). A box and whisker plot of the mean IR across all NB85 datasets for *QRpt6Ha*, *QRpt6Hp* and *QRpt6Hc* QTL allele combinations is presented in Figure 4.8B. Analysis of the overall mean IR phenotype of all datasets combined revealed significant statistical differences between groups, whereby QTL allele combination S-PPP was associated with the highest mean phenotype (6.18) and QTL allele combination P-SSS associated with the lowest mean phenotype (2.66) (Table 4.7). A box and whisker plot of the mean IRs across all NB50 and NB85 datasets for *QRpt3H* - *QRpt6Ha*, (P=*QRpt6Hp* or S=*QRpt6Hs)* and *QRpt6Hc* QTL allele combinations is presented in Figure 4.8C. As *QRpt6Hp* and *QRpt6Hs* were in complete linkage, the reciprocal allele for 325744-28:G>T was used for box and whisker plots. Specifically, P refers to the allele associated with resistance for *QRpt6Hp* and S refers to the allele associated with resistance for *QRpt6Hs*.

4.4.7 Positioning QTL on the barley physical map

Flanking markers of *QRpt3H* were positioned at 58.54 cM and 62.35 cM on the PSR linkage map and 415,363,466 bp to 490,257,835 bp on the barley physical map, while the peak marker was positioned at 61.99 cM on the PSR linkage map and 490,245,359 bp on the barley physical map (Table 4.8). Flanking markers of *QRpt6Ha* were positioned at 49.38 cM and 52.94 cM on the PSR linkage map and 44,234,721 bp and 80,019,061 bp on the barley physical map, while the peak marker was positioned at 49.65 cM on the PSR linkage map and 47,271,624 bp on the barley physical map (Table 4.8). Flanking markers of *QRpt6Hp* were positioned at 58.11 cM and 58.66 cM on the PSR linkage map and 357,490,943 bp and 375,529,371 bp on the barley physical map, while the peak marker was positioned at 58.35 cM on the PSR linkage map and 364,757,662 bp on the barley physical map (Table 4.8). Flanking markers of *QRpt6Hs* were positioned at 58.11 cM and 58.66 cM on the PSR linkage map and 357,490,943 bp and 375,529,371 bp on the barley physical

map, while the peak marker was positioned at 58.35 cM on the PSR linkage map and 368,527,587 bp on the barley physical map (Table 4.8). Flanking markers of *QRpt6Hc* were positioned at 80.53 cM and 81.38 cM on the PSR linkage map and 516,519,338 bp and 518,606,268 bp on the barley physical map, while the peak marker was positioned at 81.04 cM on the PSR linkage map and 518,256,321 bp on the barley physical map (Table 4.8). Comparison between physical positions of *QRpt3H*, *QRpt6Ha*, *QRpt6Hp*, *QRpt6Hs and QRpt6Hc* and all previously published QTL is summarised in Appendix 1.

4.4.8 Proportion of desirable alleles in diversity panel

The proportion of the desirable allele for *QRpt3H* ranged from 0.15 to 0.56 for the diversity panel groups, where Australasia was the lowest and Asia was the highest. The QTL was not observed in Tasmanian and New Zealand cultivars, while all other states recorded a low proportion of cultivars with the QTL. Victoria had the highest proportion of cultivars with the desirable allele (Appendix 3).

The proportion of the desirable allele for *QRpt6Ha* ranged from 0.29 to 0.75 for the diversity panel groups, where Africa was the lowest and Europe was the highest. Both cultivars from Tasmania carried carry the desirable allele while variation was observed in cultivars from all other states and ranged from 0.29 in Western Australia to 0.71 in Victoria (Appendix 3).

The proportion of the desirable allele for *QRpt6Hp* ranged from 0.74 to 1.00 for the diversity panel groups, where Africa was the lowest and Asia was 1.00. The desirable allele for *QRpt6Hp* was fixed in cultivars from New South Wales, New Zealand, Queensland, Tasmania and Victoria, while variation was observed in cultivars from South Australia (0.78) and Western Australia (0.76) (Appendix 3). The undesirable allele for *QRpt6Hp* was present in Prior and its descendants; Baudin, Dampier, Hamelin, Ketch, Noyep, Roe and Stirling. Other notable included Abed Deba (400701), Algerian, Beecher, Binder (411929), three Cape accessions, Canadian Lake Shore (495214), CIho 6311, Libya 221, Lion (412217), Torrens and Tunisia 344 (Appendix 2).

The proportion of the desirable allele for *QRpt6Hs* ranged from 0.64 to 1.00 for the diversity panel groups, where Europe was the lowest and Africa and Asia were 1.00. The desirable allele for *QRpt6Hs* was fixed in cultivars from New Zealand, Queensland, Tasmania and Victoria, while variation was observed in cultivars from New South Wales (0.67), South Australia (0.90) and Western Australia (0.95) (Appendix 3). Genotypes that carried the undesirable allele for *QRpt6Hs*

included; Binalong, Bowman, Ceres, Charger, CIho 11458, Cowabbie, Hanna (400973), Henley, Herta, three Isaria accessions, Moondyne, ND24260-3, Oxford, Patty, Perún, Pinnacle, Pompadour, Scarlett, Shakira, Skiff, Tantangara, Union, Volla, Wimmera and Yambla (Appendix 2).

The proportion of the desirable allele for *QRpt6Hc* ranged from 0.96 to 1.00 for the diversity panel groups, where Australasia and Europe were the lowest and the Americas and Asia were 1.00. The desirable allele was fixed in cultivars from all states except South Australia, where a proportion of 0.88 was observed (Appendix 3). Genotypes that carried the undesirable allele for *QRpt6Hc* included; Chevallier, CIho 1227, Prior and Volla (402217) (Appendix 2).

4.5 Discussion

Variability in both the host and pathogen has made disentangling the barley-*Ptt* interaction a difficult task. Typically, multiple QTL are identified in each bi-parental population per pathotype, however accurate projection of QTL onto the barley physical map has shown that some *Ptt* isolates interact with overlapping regions of the genome (Appendix 1).

Results presented here suggest that *Ptt* isolates NB50 and NB85 interact with closely linked QTL, *QRpt6Hp* and *QRpt6Hs*, at the *Spt1* locus (Richards *et al.* 2016). This conclusion is drawn from:-

1. The close proximity of peak markers to the *Spt1* locus (Richards *et al.* 2016) (Appendix 1),
2. Similarities in phenotypes observed for Prior and Dampier (Chapter 3, Gupta and Loughman 2001; Platz *et al.* 2000),
3. Prior and Dampier carry the allele associated with susceptibility to NB85 for *QRpt6Hp* (Appendix 2),
4. Similarities in phenotypes observed for Skiff, Patty, Herta and Rika (Platz *et al.* 2000) and Skiff, Herta and Patty (Chapter 3),
5. Skiff, Herta and Patty carry the allele associated with susceptibility to NB50 for *QRpt6Hs* (Appendix 2).

With regard to *rpt.k/Spt1.K*, Kombar was not genotyped thus a direct comparison could not be made, however Prior and Kombar respond differently to Prior virulent isolates from IG3 (Chapter 3; Gupta and Loughman 2001; Platz *et al.* 2000). This suggests that Prior may carry a different gene from Kombar at the *Spt1* locus, although further work is needed to ratify this hypothesis. Conservation of the undesirable allele for *QRpt6Hp* from Prior (selected in 1903) through to

Hamelin (released in 2001) demonstrates that without rigorous selection with appropriate pathotypes, undesirable alleles may persist in breeding populations over long periods of time. The low frequency of the undesirable allele in Australian cultivars suggests that this allele could be easily excluded from breeding programs.

The result of this study suggests that Skiff may carry *Spt1.R*, the dominant susceptibility from Rika at the *Spt1* locus. The allele associated with susceptibility was most frequent among European genotypes from the diversity panel, while it was also detected at a low level germplasm from the Americas and Australia and absent from Africa and Asia. This suggests that the alleles associated with susceptibility is likely of European origin and reinforces the hypothesis that Isaria is the origin of the susceptibility.

It should be noted that most Isaria and Hanna accessions that were genotyped in the diversity panel were not genetically similar between lines within each named cultivar. Isaria accessions were variable for *QRpt6Hs* alleles, as too were Hanna accessions (Appendix 2). The geographic origin of these genotypes is separated by approximately 300 kilometres, thus historic gene flow between populations may partly explain the variation at this locus. This observation provides a second possible source of *QRpt6Hs* susceptibility in Skiff, from Kneifel (correctly spelt Kneifl) via Beka. This is important to note, as the accession of Abed Deba (400701) that was genotyped was a six-row genotype, which is not correct. A second accession (400204) that was phenotyped but not genotyped, was resistant to NB50 at seedling stage (data not shown), suggesting that Isaria may not be the origin of susceptibility in Skiff. Genotyping more lines from this germplasm pool would be necessary in order to fully understand the origin of this QTL in Skiff.

Two additional regions on chromosome 6H interacted with NB85. The flanking markers identified for *QRpt6Ha* were positioned near the flanking markers of *SPN1* (Liu *et al.* 2015), which suggests that Prior may either carry *SPN1* or a susceptibility gene/allele in the same genetic region. The omnipresence of the undesirable allele for this QTL across genotypes diversity panel suggests that exclusion of the undesirable allele should be a breeding target for all programs in order to achieve improved resistance to *Ptt* isolates from Australia, Canada and the USA (Liu *et al.* 2015).

The third 6H QTL that interacted with NB85, *QRpt6Hc*, provided the smallest effect on disease phenotype of the three QTL on 6H. Very few genotypes from the diversity panel were identified carrying the allele associated with susceptibility and were mostly limited to direct relatives of Prior (Appendix 2). Volla (402217) also carried the allele associated with susceptibility,

however the origin could not be traced with the available pedigree and genotype information. A recent study of DH lines derived from a cross between two Norwegian cultivars (Arve and Lavrans) reported a QTL (*AL_QRptt6-1*) that co-located to same physical map position as *QRpt6Hc* (Wonneberger *et al.* 2017b) (Appendix 1). Even though *QRpt6Hc* and *AL_QRptt6-1* co-located to the same region, further work is necessary to determine if the gene is the same between cultivars. The absence of the allele conferring susceptibility in Australian germplasm, suggests there is no value in conducting marker assisted selection for *QRpt6Hc* in Australian varieties.

While many studies have reported interactions on chromosome 3H, some have reported QTL that co-locate with *QRpt3H*, specifically in the lines/varieties CIho 5791 and Tifang (Koladia *et al.* 2017a), Igri (Graner *et al.* 1996), NDB 112 (Liu *et al.* 2015), Pompadour (Gupta *et al.* 2011) and UVC8 (Martin *et al.* 2018). Notably, the 3H QTL carried by ND B112, Pompadour and UVC8 were detected using NB50. However, because all these genotypes are derived from distinct genetic backgrounds, fine mapping would need to be conducted to determine whether one gene, multiple genes or multiple alleles of a single gene underlie this important 3H QTL region.

Pathogenic variation of Australian *Ptt* isolates documented in Chapter 3 reported high frequency of susceptibility and strong population structure that was centred around Prior and Skiff. Conservation of virulence suggests that the underlying virulence/avirulence genes are highly heritable and/or highly advantageous in the pathogen population. Results presented here indicate that a strong genetic interaction with *QRpt6Hp* and *QRpt6Hs* is the major driver underpinning the inverse susceptibility of Prior and Skiff to NB50 and NB85. As previously mentioned, *QRpt6Hp* and *QRot6Hs* co-locate with *Spt1*; moreover, *Spt1* also displayed a similar genotype by isolate interaction that was associated with reciprocal susceptibility (Richards *et al.* 2016). Considering the high frequency of susceptibility of Prior and Skiff to isolates in the Australian *Ptt* population, further research is needed to determine if critical recombinants for resistance to both Prior and Skiff virulent isolates can be generated between *QRpt6Hp* and *QRpt6Hs*.

This is the first study to document genomic regions associated with reciprocal susceptible responses in the Australian and international net form net blotch differential genotypes Prior and Skiff. Knowledge of these genomic regions will assist pre-breeding researchers and breeding companies to develop germplasm with resistance to the two predominant groups of isolates in Australia and provide a better understanding of the genetics driving the barley-*Ptt* interaction, especially near the centromere of 6H.

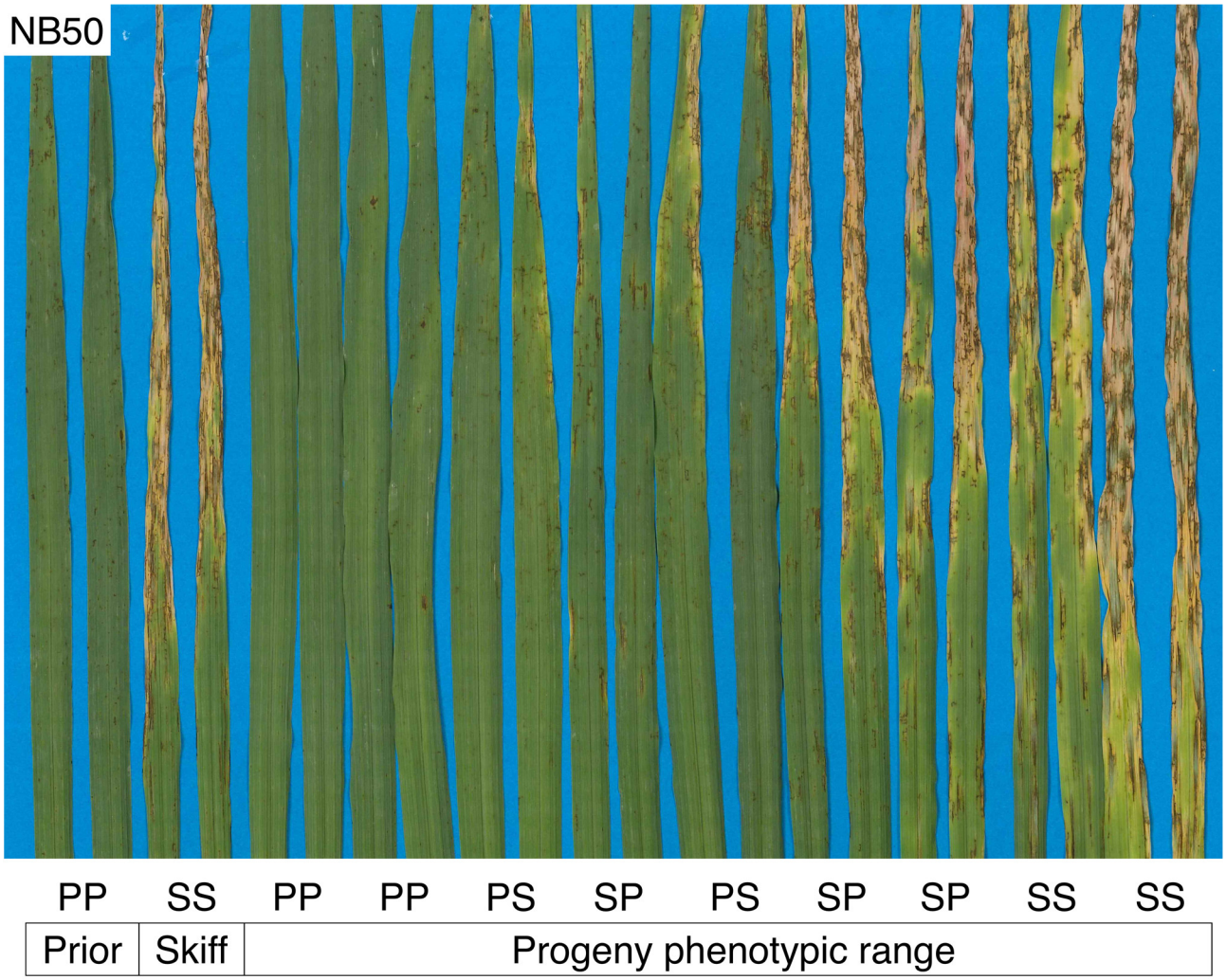


Figure 4.1. Infection response of Prior, Skiff and progeny lines nine days after inoculation with NB50. Paired leaves represent one genotype. Haplotype combinations are given for alleles of *QRpt3H* and *QRpt6Hs* QTLs respectively, where P = Prior and S = Skiff.

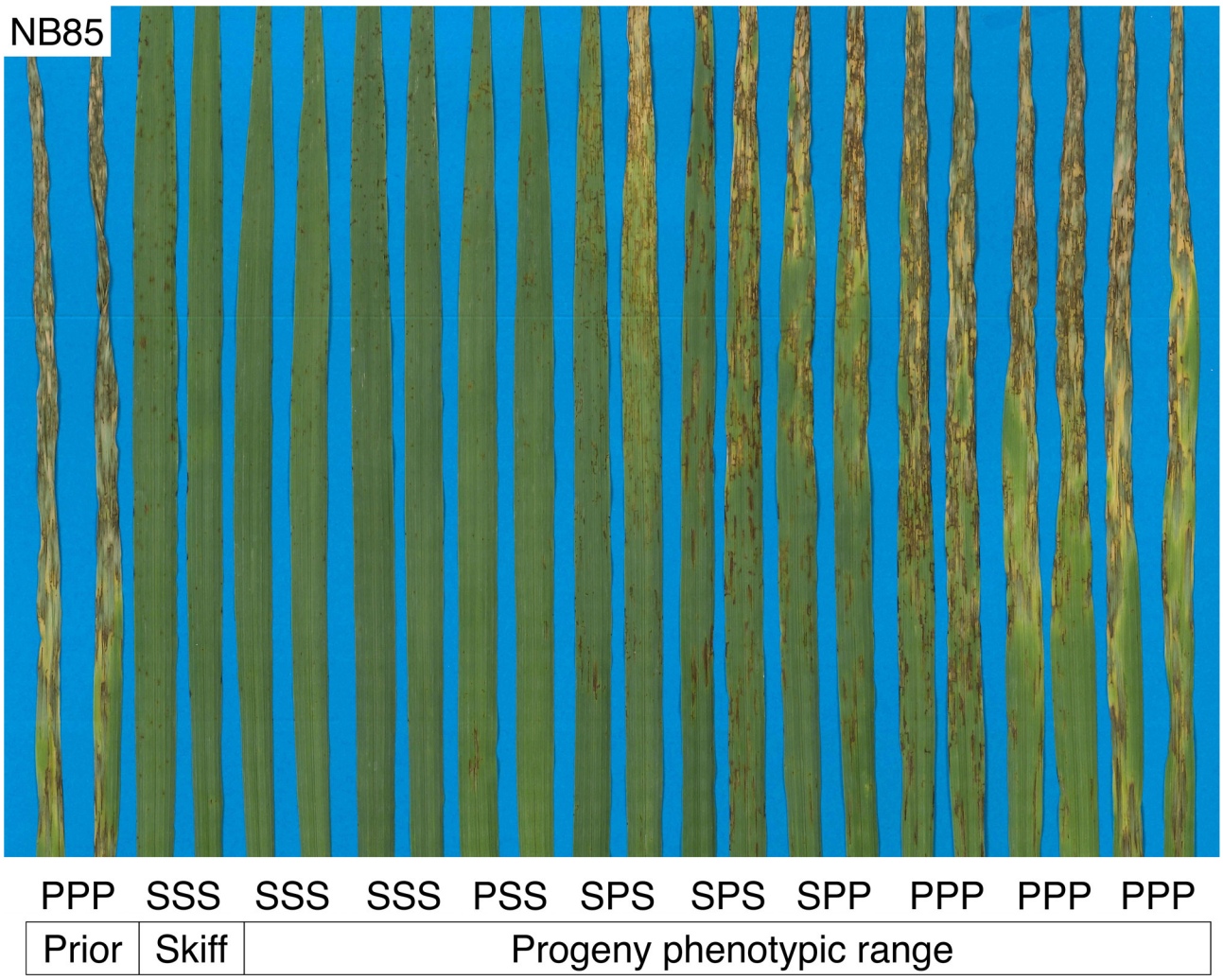


Figure 4.2. Infection response of Prior, Skiff and progeny lines nine days after inoculation with NB85. Paired leaves represent one genotype. Haplotype combinations are given for alleles of *QRpt6Ha*, *QRpt6Hp* and *QRpt6Hc* QTLs respectively, where P = Prior and S = Skiff.

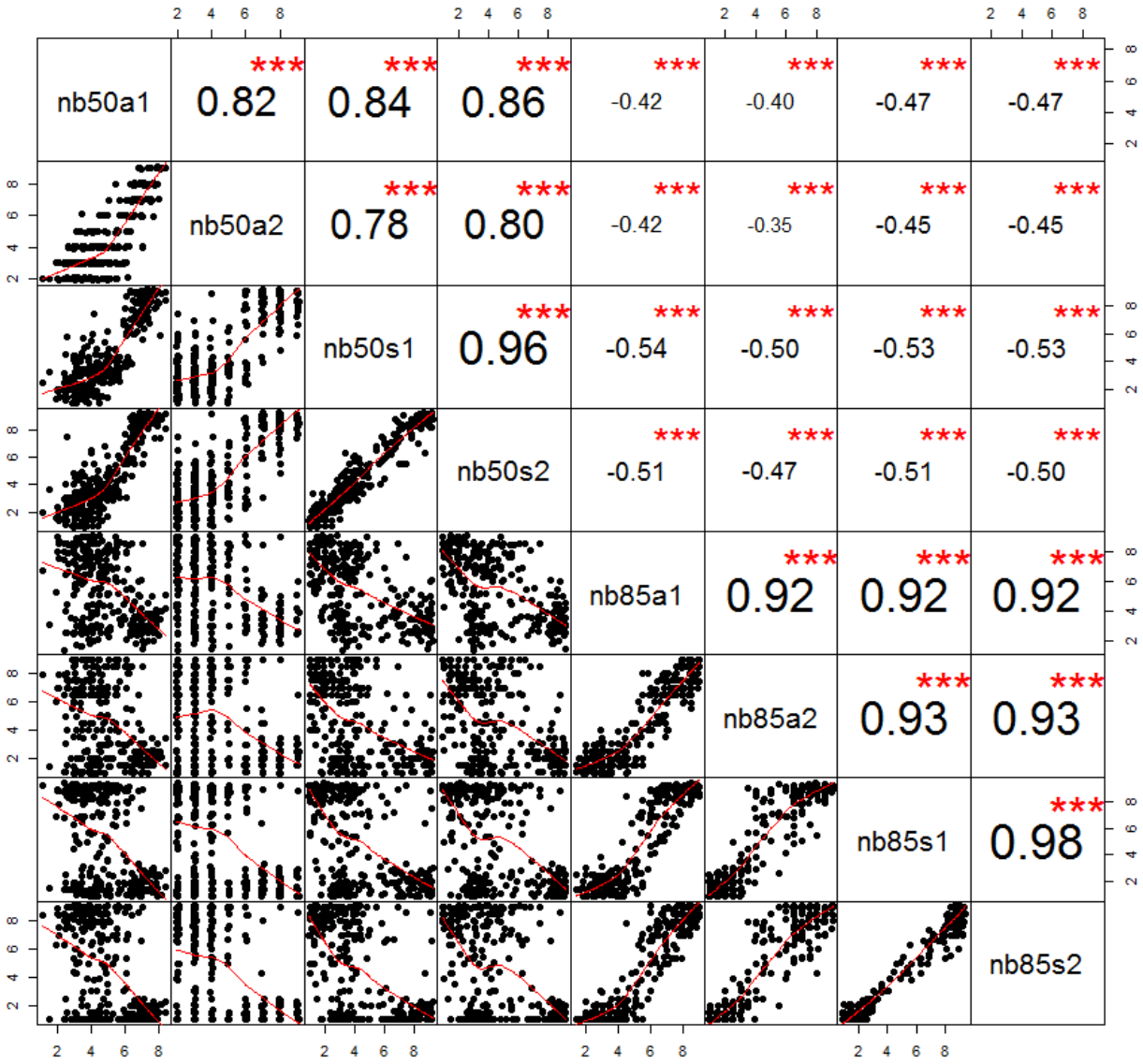


Figure 4.3. Pairwise correlation of infection responses between nb50 and nb85 datasets.

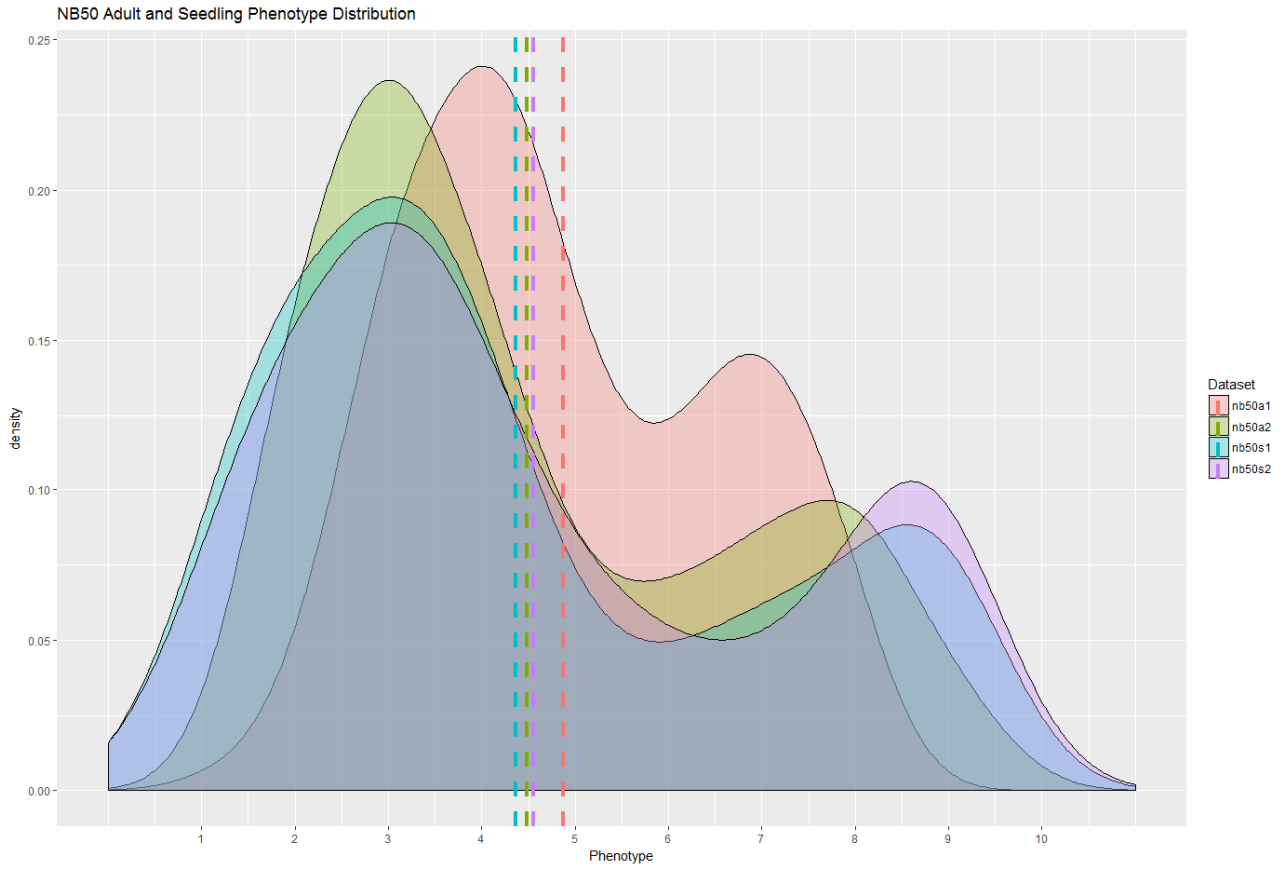


Figure 4.4. Density distribution of infection response (eBLUEs) for two adult assessments of NB50 (nb50a1 - red, nb50a2 - green) and two seedling experiments of NB50 (nb50s1 - blue, nb50s2 - purple). The dashed lines represent the mean infection response of each dataset.

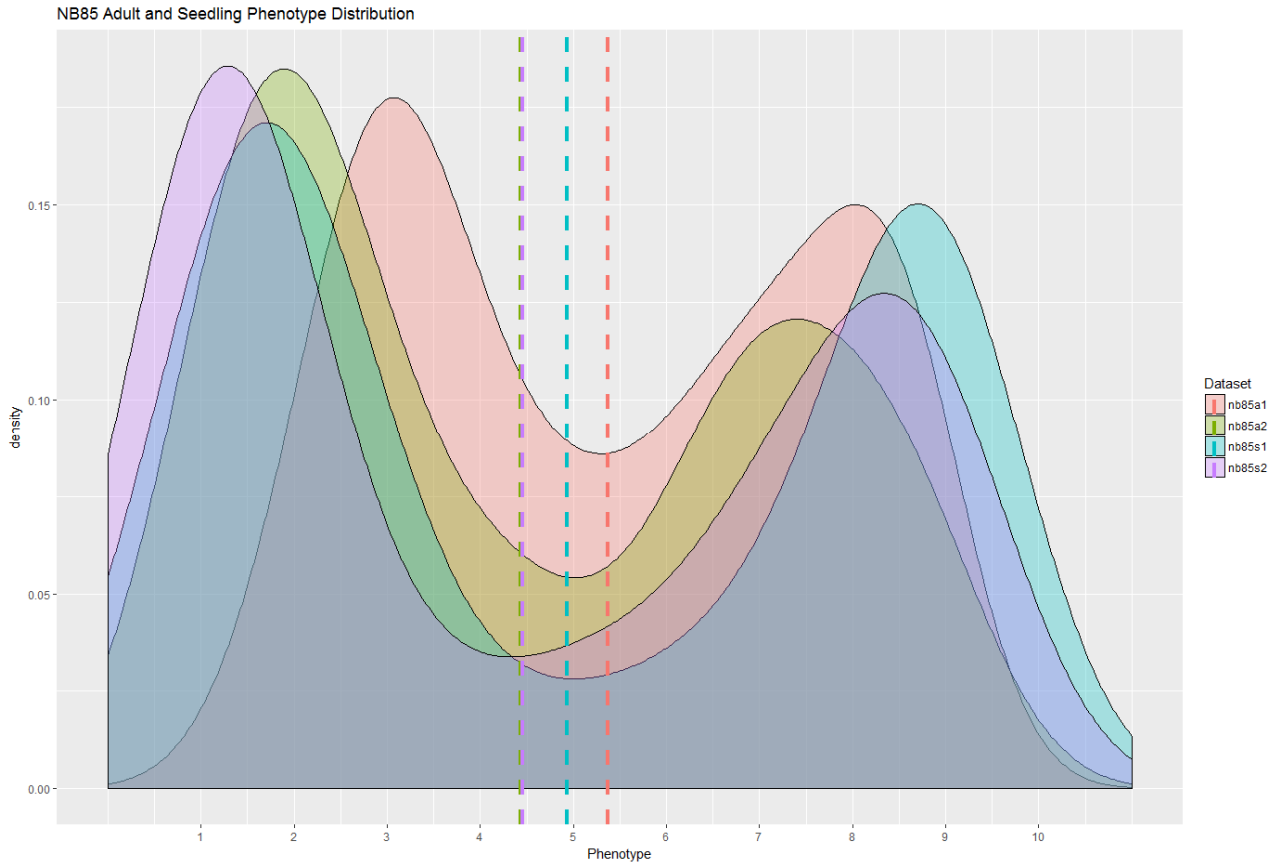


Figure 4.5. Density distribution of infection response (eBLUEs) for two adult assessments of NB85 (nb85a1 - red, nb85a2 - green) and two seedling experiments of NB85 (nb85s1 - blue, nb85s2 - purple). The dashed lines represent the mean infection response of each dataset.

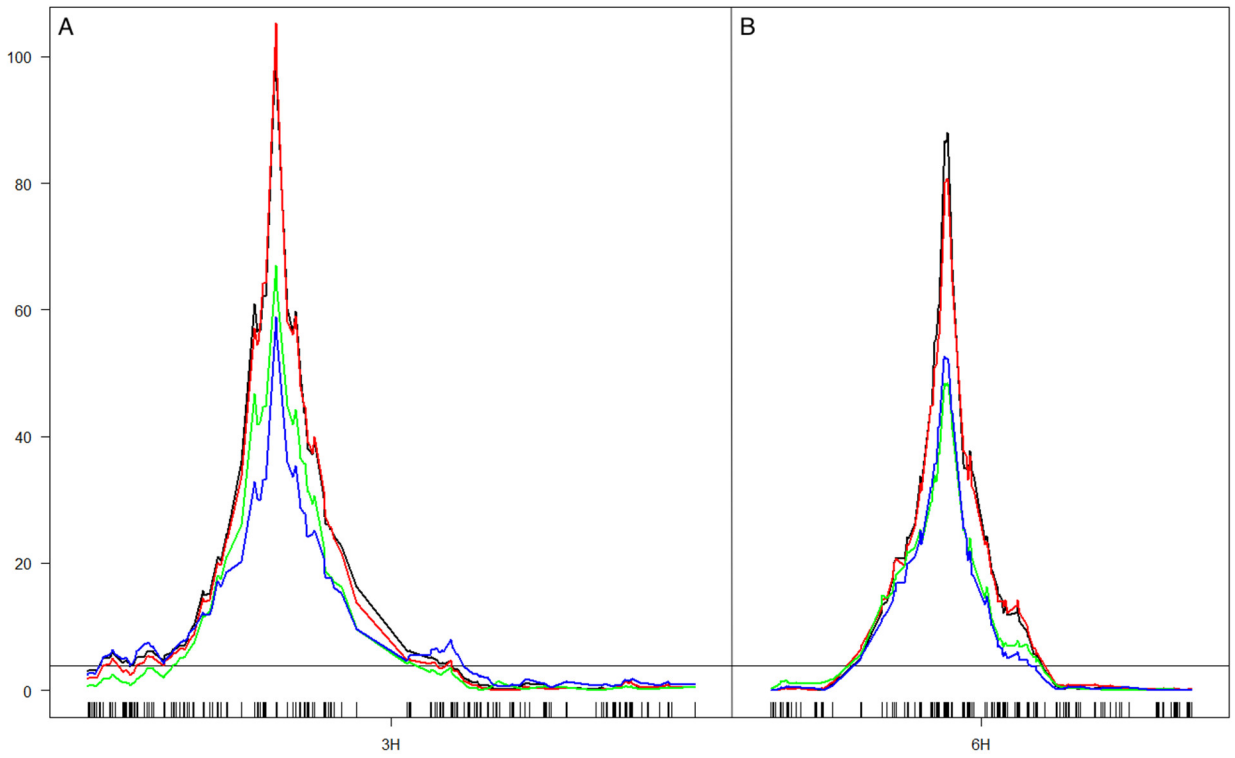


Figure 4.6. Multiple interval mapping QTL analysis of chromosomes 3H (A) and 6H (B) for resistance to *Pyrenophora teres* f. *teres* isolate NB50 in the Prior x Skiff RIL population for two adult assessments (nb50a1 - green, nb50a2 - blue) and two seedling experiments (nb50s1 - black, nb50s2 - red). Chromosome is plotted on the x-axis, LOD score is plotted on the y-axis and the horizontal line corresponds to critical LOD threshold of 3.85 ($\alpha = 0.01$).

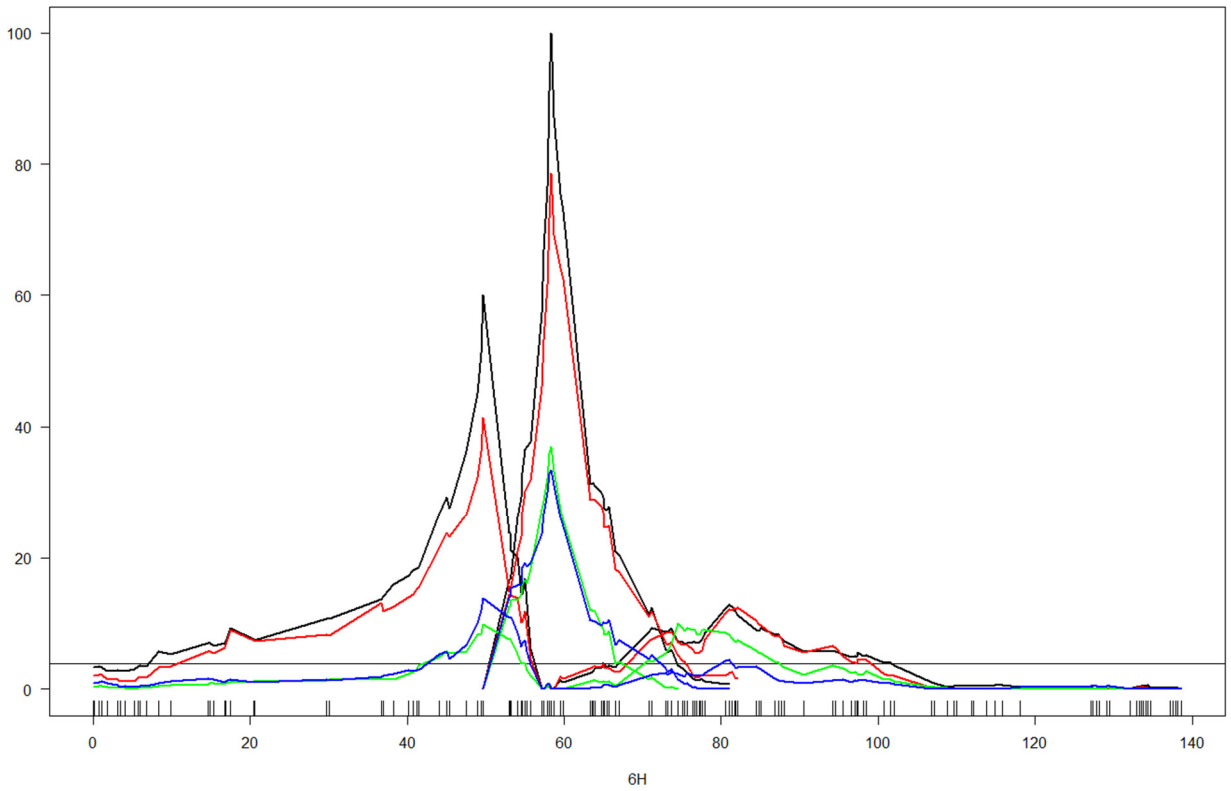


Figure 4.7. Multiple interval mapping QTL analysis of chromosome 6H for resistance to *Pyrenophora teres* f. *teres* isolate NB85 in the Prior x Skiff RIL population for two adult assessments (nb85a1 - green, nb85a2 - blue) and two seedling experiments (nb85s1 - black, nb85s2 - red). Chromosome is plotted on the x-axis, LOD score is plotted on the y-axis and the horizontal line corresponds to critical LOD threshold of 3.85 ($\alpha = 0.01$)

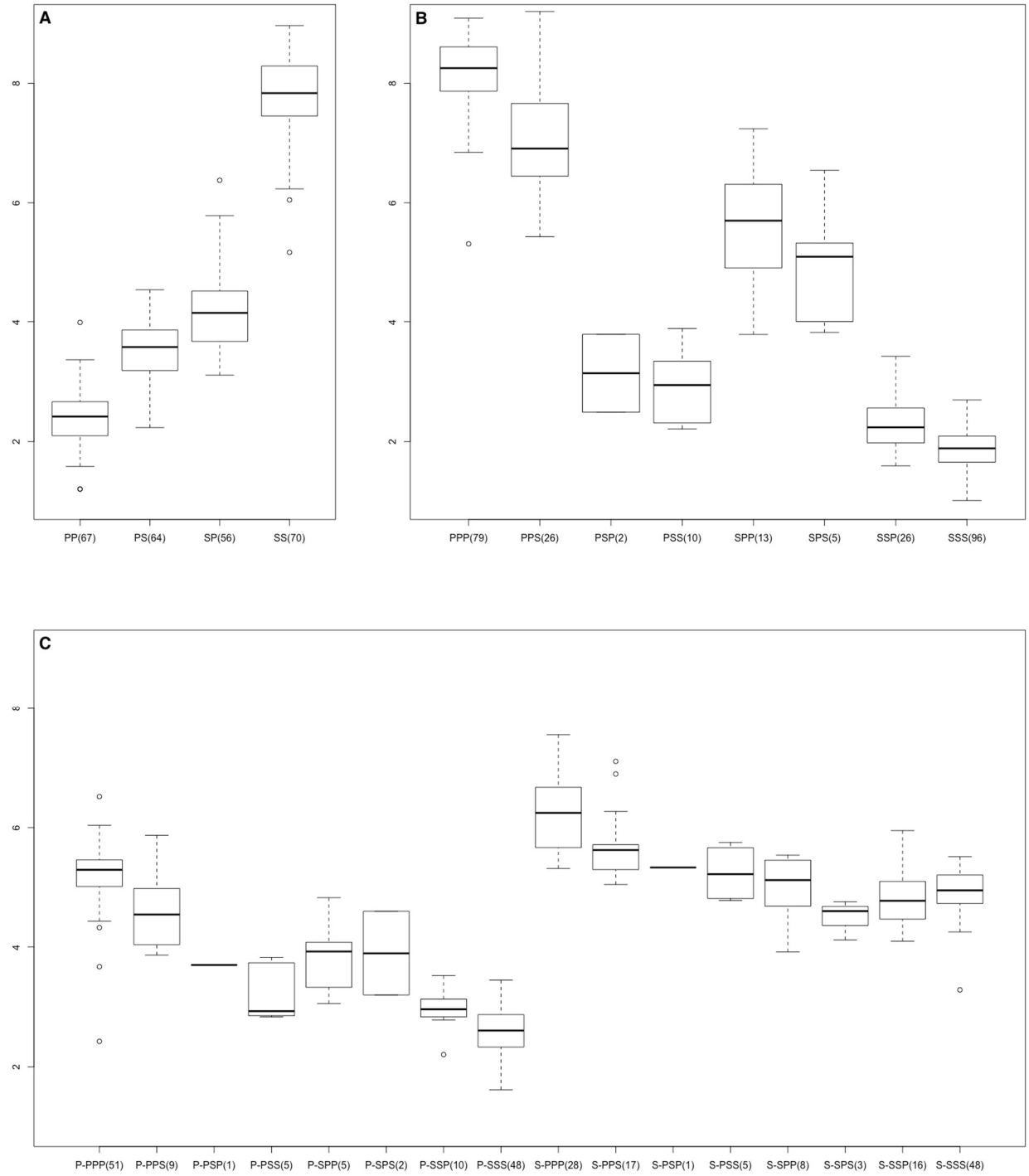


Figure 4.8. Box and whisker plots of QTL allele groups for mean infection response to *Pyrenophora teres* f. *teres* isolates following inoculation at seedling and adult growth stages. x-axis represents QTL allele group, where P = Prior and S = Skiff and bracketed value represents number of PSR progeny in each group. y-axis represents mean infection response. A: boxplot for response to NB50 for QTL allele groups; *QRpt3H* and *QRpt6Hs*. B: boxplot for response to NB85 for QTL allele groups; *QRpt6Ha*, *QRpt6Hp* and *QRpt6Hc*. C: boxplot for response to mean of NB50 and NB85 for QTL allele groups; *QRpt3H – QRpt6Ha*, (P=*QRpt6Hp* or S=*QRpt6Hs)* and *QRpt6Hc*.

4.7 Tables

Table 4.1 Mean infection response of differential barley genotypes from Steffenson *et al.* (1992) and Tekauz (1990) to classify *Ptt* isolates NB50 and NB85 to pathotypic groups.

No.	Genotype	Accession Number	Experiment[a]	NB50	NB85
1	Tifang	CIho 4407-1	A	1	3
2	Canadian Lake Shore	CIho 2750	ABD	1.3±0.5	3.5±0.6
3	Atlas	CIho 4118	A	2	2
4	Rojo	CIho 5401	A	1	2
5	Coast	CIho 2235	A	1	3
6	Manchurian	CIho 739	A	3	4
7	Ming	CIho 4797	A	1	3
8	CIho 9819	CIho 9819	A	2	1
9	Algerian	CIho 1179	ABD	3.7±0.5	4.0±2.2
10	Kombar	CIho 15694	ABCD	8.7±1.6	2.4±0.9
11	CIho 11458	CIho 11458	ABCD	2.6±1.6	1.5±0.8
12	CIho 5791	CIho 5791	ABCD	1.1±0.3	1.0±0.0
13	Harbin	CIho 4929	ABD	1.8±0.7	3.7±1.0
14	CIho 7584	CIho 7584	A	1	3
15	Prato	CIho 15815	AC	1.3±0.6	2.0±1.0
16	Manchuria	CIho 2330	AC	2.3±0.6	2.7±0.6
17	CIho 5822	CIho 5822	A	2	1
18	CIho 4922	CIho 4922	A	1	3
19	Hazera	CIho 12673	A	1	3
20	Cape	CIho 1026	A	4	9
21	Beecher	CIho 6566	ABCD	1.2±0.5	1.5±0.8
22	Rika	CIho 8096	AC	9.0±0.0	1.8±1.3
1	CIho 5791	CIho 5791	ABCD	1.1±0.3	1.0±0.0
2	CIho 9820	CIho 9820	F	1	3
3	TR473	CN 39420	E	3	2.5
4	Norbert	PI 452125	A	3	1
5	BT 201	CN 5	E	3	2.5
6	Heartland	PI 552963	A	1	1
7	Steptoe	CIho 15229	A	2	2
8	CIho 9214	CIho 9214	A	1	3
9	Herta	CIho 8097	ABD	9.3±0.5	2.5±0.6

[a] Phenotyping experiment. A = Data generated by Platz *et al.* (2000), B = Seedling differentials 2012, C = Seedling differentials 2014, D = This study, E = Adult phenotyping 2012, F = Adult phenotyping 2017.

Table 4.2 Summary of SNPs used in linkage map construction and unique SNPs used for multiple interval mapping in the Prior x Skiff RIL population.

Chromosome	Map[a]	Unique[b]	Length (cM)[c]	Density (cM/locus)[d]
1H	227	119	166.7	1.4
2H	453	226	189.1	0.8
3H	395	184	200.0	1.1
4H	140	75	220.1	2.9
5H	295	157	228.9	1.5
6H	294	140	138.7	1.0
7H	349	178	191.5	1.1
TOTAL	2153	1079	1335.0	1.2

[a] Number of SNPs used to create the Prior x Skiff linkage map.
[b] Number of SNPs at unique loci used in MIM.
[c] Length of each chromosome calculated via kosambi recombination frequency.
[d] Density of unique SNPs used in MIM.

Table 4.3 Summary of the phenotypic range, repeatability and heritability estimates for PSR progeny and parents to *Pyrenophora teres* f. *teres* isolates NB50 and NB85 for seedling and adult experiments.

Dataset	Prior	Skiff	Min IR	Mean IR	Max IR	Resistant[a]	Susceptible[b]	Repeat.[c]	h^2 [d]
nb50a1	3.31	6.30	1.11	4.87±1.67	8.42	3.68±0.81	6.61±0.90	0.74	0.84
nb50a2	2.99	6.93	1.89	4.48±2.17	9.12	3.13±0.88	7.27±1.12	- [e]	0.63
nb50s1	2.35	7.07	0.96	4.37±2.53	9.21	2.86±1.06	7.80±1.16	0.93	0.90
nb50s2	2.35	7.40	0.92	4.55±2.58	9.24	2.90±1.07	7.78±1.29	0.94	0.90
nb85a1	7.99	3.05	1.38	5.38±2.32	9.05	3.15±0.80	7.33±1.16	0.88	0.92
nb85a2	7.83	3.13	0.94	4.43±2.74	9.03	2.26±1.03	7.40±1.05	0.94	0.96
nb85s1	8.87	1.20	0.89	4.93±3.32	9.40	1.91±0.85	8.28±1.05	0.97	0.99
nb85s2	9.06	2.00	0.96	4.45±3.28	9.10	1.65±1.01	7.90±1.08	0.97	0.99

[a] Mean phenotype of PSRs < IR5.
[b] Mean phenotype of PSRs > IR5.
[c] Repeatability estimate of phenotype scores.
[d] Narrow sense heritability estimation.
[e] No estimate due to single replicate data.

Table 4.4 Segregation of Prior x Skiff RIL progeny to *Pyrenophora teres* f. *teres* isolates NB50 and NB85.

Dataset	Resistant	Susceptible	Total	χ^2 (1:1)	χ^2 (3:1)
nb50a1	169	117	286	9.45 [a]	38.61 [b]
nb50a2	192	93	285	34.39 [a]	8.85 [b]
nb50s1	207	91	298	45.15 [a]	4.87 [b]
nb50s2	191	98	289	29.93 [a]	12.24 [b]
nb85a1	134	152	286	1.13	120.84 [b]
nb85a2	165	121	286	6.77 [a]	45.69 [b]
nb85s1	156	141	297	0.76	80.01 [b]
nb85s2	160	130	290	3.10	60.8 [b]

[a] Significantly different from 1:1 at $p = 0.05$.
[b] Significantly different from 3:1 at $p = 0.05$.

Table 4.5 Quantitative trait loci associated with resistance to *Pyrenophora teres* f. *teres* isolates NB50 and NB85 in the Prior x Skiff RIL population.

QTL	*QRpt3H*	*QRpt6Ha*	Reciprocal 6H	*QRpt6Hc*	*QRpt3H*QRpt6Hs*	*QRpt6Ha*QRpt6Hp*	Full Model
Dataset	LOD[b] (%)[c] Effect[d]	LOD[b] (%)[c] Effect[d]	LOD[b] (%)[c] Effect[d]	LOD[b] (%)[c] Effect[d]	LOD (%) Int[e]	LOD (%) Int[e]	LOD[f] (%)[g]
nb50a1	67.00 (50.34) 1.10	–	48.43 (30.21) 0.82	–	15.24 (6.95) 0.44	–	84.86 (76.86)
nb50a2	58.77 (46.88) 1.19	–	52.41 (39.22) 1.06	–	26.22 (15.25) 0.60	–	76.61 (73.45)
nb50s1	103.58 (51.95) 1.70	–	88.08 (37.60) 1.45	–	24.60 (5.73) 0.60	–	131.14 (88.61)
nb50s2	105.32 (56.34) 1.85	–	80.85 (33.25) 1.40	–	22.58 (5.24) 0.60	–	128.31 (88.8)
nb85a1	–	11.09 (3.09) -0.62	36.83 (13.02) -1.41	7.70 (2.08) -0.40	–	–	111.25 (85.32)
nb85a2	–	13.82 (4.14) -0.87	33.39 (11.98) -1.60	4.50 (1.24) -0.36	–	–	108.50 (84.61)
nb85s1	–	59.97 (6.00) -1.08	99.90 (14.97) -2.17	12.86 (0.83) -0.36	–	15.56 (1.03) 0.53	201.24 (96.48)
nb85s2	–	38.30 (5.78) -1.08	68.22 (13.72) -2.06	10.06 (1.16) -042	–	6.32 (0.70) 0.43	162.79 (93.77)

[a] Reciprocal marker for *QRpt6Hp* and *QRpt6Hs*, where *QRpt6Hs* QTL associated with NB50 and *QRpt6Hp* QTL associated with NB85.
[b] LOD score of full model compared to model with single term removed.
[c] Estimation of the percentage phenotypic variance explained by model term.
[d] Estimated effect of QTL. Calculated from difference between mean phenotype of alleles. Reference is to Skiff allele.
[e] Interaction between QTL. Value < 0 indicates negative interaction and value > 0 indicates positive interaction.
[f] LOD score of full model relative to null QTL model.
[g] Estimation of the percentage phenotypic variance explained by all terms in model.

Table 4.6 Mean phenotype of Prior x Skiff RILs grouped by QTL allele combination for *Pyrenophora teres* f. *teres* isolates NB50 and NB85.

	NB50 QTL allele combinations[a]				NB85 QTL allele combinations[b]							
Dataset	SS[c]	SP	PS	PP	PPP	PPS	SPP	SPS	PSP	PSS	SSP	SSS
nb50a1	7.17 [A d]	4.62 [B]	4.02 [C]	3.29 [D]	-	-	-	-	-	-	-	-
nb50a2	7.49 [A]	3.64 [B]	3.33 [BC]	2.97 [C]	-	-	-	-	-	-	-	-
nb50s1	8.08 [A]	4.01 [B]	3.38 [C]	1.75 [D]	-	-	-	-	-	-	-	-
nb50s2	8.32 [A]	4.4 [B]	3.41 [C]	1.82 [D]	-	-	-	-	-	-	-	-
nb85a1	-	-	-	-	8.04 [A]	6.77 [B]	6.47 [B]	6.05 [B]	4.31 [C]	3.79 [C]	3.49 [C]	3.07 [D]
nb85a2	-	-	-	-	7.52 [A]	6.23 [B]	5.28 [C]	4.81 [C]	2.98 [D]	2.96 [D]	1.97 [D]	1.82 [D]
nb85s1	-	-	-	-	8.79 [A]	7.91 [B]	5.68 [C]	4.74 [D]	2.79 [E]	2.71 [E]	2.06 [E]	1.52 [F]
nb85s2	-	-	-	-	8.34 [A]	7.11 [B]	5.37 [C]	4.22 [D]	2.48 [E]	2.02 [E]	1.62 [E]	1.15 [F]
No. PSRs[e]	70	56	64	67	79	26	13	5	2	10	26	96

[a] QTL allele combinations for resistance to NB50. *QRpt3H* listed first and *QRpt6Hs* listed second.

[b] QTL allele combinations for resistance to NB85. *QRpt6Ha* listed first, *QRpt6Hp* listed second and *QRpt6Hc* listed third.

[c] Prior allele, gives resistance to NB50 and susceptibility to NB85. S = Skiff allele, gives resistance to NB85 and susceptibility to NB50.

[d] Capitalised superscript letters indicate statistical significance between groups within each dataset ($P = 0.05$).

[e] PSRs with missing data, heterozygous phenotype or heterozygous genotype were excluded.

Table 4.7 Mean phenotype of Prior x Skiff RILs grouped by four QTL allele combinations for *Pyrenophora teres* f. *teres* isolates NB50 and NB85.

QTL [a]	NB50 mean [b]	NB85 mean [c]	Overall mean [d]	No. PSRs [e]
P-PPP [f]	2.42 ^{D} [g]	8.15 ^{A}	5.28 ^{C}	51
P-PPS	2.58 ^{D}	6.74 ^{B}	4.66 ^{C}	9
P-SPP	2.61 ^{D}	5.31 ^{CD}	3.96 ^{D}	5
P-SPS	2.60 ^{D}	5.18 ^{CDE}	3.89 ^{DE}	2
P-PSP	3.58 ^{CD}	3.79 ^{EF}	3.69 ^{DEF}	1
P-PSS	3.62 ^{C}	2.83 ^{FG}	3.23 ^{EF}	5
P-SSP	3.64 ^{C}	2.26 ^{GH}	2.95 ^{FG}	10
P-SSS	3.05 ^{CD}	1.83 ^{H}	2.66 ^{G}	48
S-PPP	4.16 ^{BC}	8.21 ^{A}	6.18 ^{A}	28
S-PPS	4.24 ^{B}	7.15 ^{B}	5.69 ^{B}	17
S-SPP	4.05 ^{BC}	5.94 ^{C}	4.99 ^{C}	8
S-SPS	4.18 ^{BC}	4.79 ^{DE}	4.49 ^{CD}	3
S-PSP	8.17 ^{A}	2.49 ^{FGH}	5.33 ^{BC}	1
S-PSS	7.69 ^{A}	2.91 ^{F}	5.3 ^{BC}	5
S-SSP	7.45 ^{A}	2.30 ^{GH}	4.88 ^{C}	16
S-SSS	7.87 ^{A}	1.96 ^{H}	4.91 ^{C}	48

[a] QTL allele combination order, *QRpt3H* - *QRpt6Ha* (P=*QRpt6Hp* or S=*QRpt6Hs) QRpt6Hc*.

[b] Mean phenotype of four NB50 datasets.

[c] Mean phenotype of four NB85 datasets.

[d] Mean phenotype of all NB50 and NB85 datasets.

[e] PSRs with missing data, heterozygous phenotype or heterozygous genotype were excluded

[f] P = Prior allele, S = Skiff allele.

[g] Capitalised superscript letters indicate statistical significance between groups within each dataset (P = 0.05).

1

Table 4.8 Position intervals of four QTL detected in the Prior x Skiff RIL population for resistance to *Pyrenophora teres* f. *teres* isolates NB50 and NB85.

QTL	Isolate	R source	Type	Marker	Chr	Linkage (cM)	Physical (bp)
QRpt3H	NB50	Prior	Flanking	3257118-27:C>G	3H	58.54	415363466
			Peak	4170799-6:G>A	3H	61.99	490245359
			Flanking	3256655-65:T>C	3H	62.35	490257835
QRpt6Ha	NB85	Skiff	Flanking	3258496-13:G>A	6H	49.38	44234721
			Peak	3255277-6:T>C	6H	49.65	47271624
			Flanking	4016288-26:C>A	6H	52.94	80019061
QRpt6Hp	NB85	Skiff	Flanking	4170458-67:G>C	6H	58.11	357490943
			Peak	3260813-56:A>T	6H	58.35	364757662
			Flanking	3259255-10:C>T	6H	58.66	375529371
QRpt6Hs	NB50	Prior	Flanking	4170458-67:G>C	6H	58.11	357490943
			Peak	3257446-28:G>T	6H	58.35	368527587
			Flanking	3259255-10:C>T	6H	58.66	375529371
QRpt6Hc	NB85	Skiff	Flanking	4007559-36:C>G	6H	80.53	516519338
			Peak	3257602-33:G>C	6H	81.04	518256321
			Flanking	3257276-5:A>C	6H	81.38	518606268

Chapter 5

Unravelling the genetics of resistance and susceptibility to *Pyrenophora teres* f. *teres* in Australian barley breeding germplasm

5.1 Abstract

Two barley (*Hordeum vulgare* L.) breeding populations representative of the 2012 and 2013 Stage 2 entries from the Northern Region Barley (NRB) breeding program in Queensland, Australia were subjected to GWAS to identify genomic regions associated with resistance and susceptibility to *Pyrenophora teres* f. *teres* (*Ptt*), the causal agent of net form net blotch (NFNB). GWAS utilised 5,172 polymorphic DArTseq™ SNP markers and phenotypic data for 373 northern region breeding lines and 27 reference genotypes collected over two years for both seedling and adult growth stages for four *Ptt* isolates. A panel of diverse genotypes outlined in Chapter 4 served as a reference to trace the origin of alleles. GWAS performed in GAPIT detected one QTL on 4H and three QTL on 6H. The 4H QTL (*QRpt4H*) was associated with resistance to the *Ptt* isolates NB330 and NB85 at the seedling stage. The desirable allele for *QRpt4H* was contributed by the North Dakota (ND) parents and was postulated to originate from PC 84. *QRpt6Hm* on 6H was strongly associated with susceptibility to NB73 at seedling and adult stages and weakly associated with susceptibility to NB330 at the seedling stage. The undesirable allele for *QRpt6Hm* was contributed by the NRB parents and was postulated to originate from Moravian landraces and Archer via Carlsberg. The second QTL on 6H (*QRpt6Hs*) was associated with reciprocal resistance and susceptibility, where the 'G' allele was associated with resistance to isolates NB50 and NB73, while 'T' allele was associated with resistance to NB85. This reciprocal effect was also documented in Chapter 4, however the *QRpt6Hp* allele associated with susceptibility to NB85 was absent from the NRB population, suggesting a different genetic interaction could be involved. The allele associated with susceptibility to NB50 for *QRpt6Hs* was contributed by the ND parents and originated from Isaria via Fergus and Bowman. The third QTL on 6H (*Rpt5.f*) was associated with resistance to NB330 at the seedling stage and NB73 and NB85 at both growth stages. The desirable allele for *Rpt5.f* was contributed by the ND parents and was confirmed to originate from CIho 5791 via Norbert and Ellice. GWAS of the NRB breeding population successfully identified QTL associated with resistance and susceptibility to *Ptt*, however results were more meaningful with parallel analysis of a panel of diverse genotypes that revealed the origin of key alleles. Knowledge generated in this study is internationally relevant and will serve the wider barley community well in future research and breeding efforts.

5.2 Introduction

Net form net blotch (NFNB) of barley (*Hordeum vulgare* L.) caused by the necrotrophic fungal pathogen *Pyrenophora teres* f. *teres* (*Ptt*) is a damaging disease of economic significance worldwide. Severe infection in a very susceptible cultivar may result in yield losses of up to 35% during favourable environmental conditions. Currently in Australia, the sum cost of this pathogen from yield losses, chemical control and cultural control is estimated at AUD$19M annually, with the potential cost to the barley industry estimated to be as high AUD$117M if no disease control measures were used (Murray and Brennan 2010). Recent detection of demethylase inhibitor resistant *Ptt* in Australia has reinforced the need to utilise genetic resistance as the main control strategy for disease management (Mair *et al.* 2016).

Initial germplasm in the Northern Region Barley (NRB) breeding program was founded on Australian cultivars developed by the Southern and Western Australian breeding programs that were agronomically adapted to Australia, along with European cultivars with high malting quality (Greg Platz, personal communication). The first cultivars released by the program; Grimmett (1982), Tallon (1991) and Gilbert (1993) were susceptible to NFNB (Chapter 3) and large scale planting coupled with favourable weather conditions during 1998 resulted in a disease epiphytotic (Rees *et al.* 1999). As a direct result of this season, a higher priority was placed on breeding germplasm with resistance to NFNB (Ullrich 2010). Subsequently, resistant lines were identified and released; Mackay (2002) and Grout (2005), however these new cultivars were susceptible to Prior virulent isolates (Greg Platz, personal communication). Dr Jerome Franckowiak, the previous barley breeder from the North Dakota State University (NDSU), was appointed as the breeder of the NRB program in 2006. The appointment of Dr Franckowiak was coincided with the introduction of diverse North Dakota (ND) parents into the NRB program, many of which were resistant to multiple diseases, including NFNB (Franckowiak, personal communication). However, up till the late 2000's, susceptible cultivars such as Binalong, Cowabbie, Patty, Perún, Scarlett, Shepherd, Skiff and Tantangara were occasionally used as parents (Greg Platz, personal communication). The breeding lines examined in this study were developed from crosses between advanced NRB and ND parents.

Pathogenic variation of *Ptt* has been reported in many international studies, as reported in Chapter 2 and the most recent study of the Australian population is reported in Chapter 3. Results identified four broad groups of isolates that could be differentiated according to virulence profiles

on four Australian cultivars. Population stratification was observed from east to west with regard to isolates virulent on Skiff and Maritime. Isolates from Western Australia were virulent on Maritime, but not virulent on Skiff and isolates from eastern Australia were virulent on Skiff, but not Maritime. North-South stratification was less distinct, although differences in virulence were observed between Queensland and South Australian isolates. Three of the four isolate groups were detected in Queensland. Isolates from the Skiff group were most common, followed by isolates from the Prior group with isolates from the Tallon group less common. The study confirmed that the isolates used for screening breeding material at The Hermitage Research Facility were relevant to the current *Ptt* population in Queensland.

Specially constructed bi-parental mapping populations, usually doubled haploid (DH) or recombinant inbred lines (RILS), have been successful in detecting quantitative trait loci (QTL) that confer resistance and susceptibility to *Ptt*. QTL have been identified on all seven chromosomes of barley (Chapter 2), with most studies detecting significant QTL around the centromere of 6H. Closely linked recessive resistances identified in Rika and Kombar by Abu Qamar *et al.* (2008) were recently fine mapped to the newly described *Spt1* locus near the centromere of 6H (Richards *et al.* 2016). Major QTL in the resistant Ethiopian landraces CIho 5791, CIho 9819 and k-23874 were mapped to the centromeric region on 6H (Afanasenko *et al.* 2015; Koladia *et al.* 2017a; Manninen *et al.* 2000; Manninen *et al.* 2006). Additionally, QTL at the centromere have been mapped in numerous studies (Emebiri *et al.* 2005; Friesen *et al.* 2006; Graner *et al.* 1996; Grewal *et al.* 2008; Grewal *et al.* 2012; Ma *et al.* 2004; Mace *et al.* 2007; Martin *et al.* 2018; Raman *et al.* 2003; Richter *et al.* 1998; Spaner *et al.* 1998; St. Pierre *et al.* 2010; Steffenson *et al.* 1996; Tenhola-Roininen *et al.* 2011; Yaniv *et al.* 2014) and others (Appendix 1). Considering the large diversity of genetic backgrounds of genotypes studied, it suggests that *Ptt* could be interacting with multiple genes or alleles of a single gene near the centromere of 6H. In addition to 6H, QTL near the centromere of 4H have been reported (Afanasenko *et al.* 2015; Cakir *et al.* 2011; Grewal *et al.* 2008; Islamovic *et al.* 2017; Lehmensiek *et al.* 2007; Raman *et al.* 2003; Steffenson *et al.* 1996; Yun *et al.* 2005) suggesting that genes for resistance and susceptibility to *Ptt* may occur in two major clusters on two chromosomes.

An alternative mapping approach, genome-wide association studies (GWAS) exploits historic recombination events accumulated within study populations to increase accuracy of detected marker-trait associations (MTAs) (Zhu *et al.* 2008). However, this method is dependent on linkage disequilibrium (LD) and if population structure and genetic relatedness among individuals is not accounted for in the statistical model, false associations may be detected (Rafalski 2010). LD

should also be considered in order to determine the minimum number of genetic markers needed to accurately map traits and as barley is a self-pollinating species, LD is much higher than out-crossing species. Intra-chromosomal LD assessed from a panel of elite European cultivars was shown to extend long distances and as such Rostoks *et al.* (2006) concluded that GWAS could accurately detect MTAs by using one marker per cM. As GWAS does not necessarily require specially constructed populations, diversity panels from core collections, landrace accessions and breeding populations can be subject to analysis. GWAS has been used to successfully identify MTAs for resistance or susceptibility to *Ptt* in barley (Adhikari 2017; Richards *et al.* 2017; Vatter *et al.* 2017; Wonneberger *et al.* 2017a). The breeding populations used in this study were previously subjected to GWAS, which successfully detected MTAs for resistance to spot form of net blotch (*Pyrenophora teres* f. *maculata*) (Wang *et al.* 2015) and leaf rust (*Puccinia hordei*) (Ziems *et al.* 2017; Ziems *et al.* 2014) thus demonstrating the usefulness of the NRB germplasm for mapping disease resistance traits.

This study was conducted to:

1. Identify key QTL associated with resistance or susceptibility to *Ptt* through GWAS of barley breeding populations,
2. Report genotypes within a diversity panel that share desirable and undesirable alleles for QTL detected in GWAS of the NRB population,
3. Trace the origin of those QTL back to an original genotype through pedigree analysis of a panel of diverse genotypes.

The outcomes from this research will fill the current knowledge gap that exists between QTL identified in genetic studies and knowing which genotypes are likely to carry the reported resistance or susceptibility. This information is directly relevant to Australian and international barley breeders involved in pedigree breeding.

5.3 Materials and methods

5.3.1 Barley genotypes

A panel of 400 barley genotypes was assembled for GWAS, which comprised of 27 reference genotypes (Table 5.1) and 373 $F_{4:5}$ breeding lines from the Northern Region Barley (NRB) breeding program from 2012 and 2013. Reference genotypes included CIho 11458, NRB06059, Shakira, WPG8412-9-2-1, three Victoria Breeding (VB) breeding lines and 18 Australian cultivars. The

Australian cultivars Grimmett and Kaputar were included twice from two separate seed sources; breeding (B) and pathology (P). Reference genotype pedigree information and phenotypic responses to four *Ptt* isolates are summarised in Table 5.1. A total of 173 genotypes were phenotyped in 2012 and 273 genotypes were phenotyped in 2013. Across both years, a total of 46 genotypes were phenotyped.

The diverse panel of barley genotypes described in Chapter 4 was used in this study to determine the proportion of desirable alleles for QTL per geographic genotype group, compare the presence/absence of alleles among genotypes and trace the origin of alleles to original genotypes.

5.3.2 Pathogen isolates

Four *Ptt* isolates, NB50, NB73, NB85 and NB330 were used to phenotype the NRB breeding populations in 2012 and 2013. Details of the isolates are presented in Table 5.2. NB50 and NB330 are the same pathotype with NB50 being used for adult phenotyping and NB330 for seedling phenotyping. NB73 and NB85 were used for both seedling and adult phenotyping. Inoculum for each seedling experiment was conidia from the field and stored at -80°C. NB73 and NB85 were collected from heavily infected spreader rows of dedicated disease nurseries of those isolates in 2011 Conidia of NB330 were collected in 2003 from a heavily infected crop of Binalong grown on a property near Moree in northern New South Wales. Inoculum for the field nurseries was generated from single spore isolations of NB50, NB73 and NB85.

5.3.3 Seedling experiments

Seedling phenotyping experiments consisted of two complete replicates of barley genotypes that were carried out in an air conditioned glasshouse during the winter of 2012 and 2013 under natural light at approximately 19°C (± 4°C). Approximately five seeds per genotype were sown to three evenly spaced positions per 10 cm pot and 20 pots were held within a basket in a 5 x 4 arrangement during growing, inoculation and incubation. Searles® premium potting mix was used and plants were fertilised with 1.3 g/L of Grow Force Flowfeed EX7 soluble fertilizer twice weekly. Seedlings were grown in an air conditioned glasshouse compartment and inoculated 14 days after sowing at approximately growth stage Z12 (Zadoks *et al.* 1974). Field-collected conidial suspensions were adjusted to a concentration of 6,667 conidia/mL in 18.2 MΩ-cm Millipore-filtered Tween®-water (two drops of Tween®20 per 100 mL of Millipore-filtered water) and applied at a rate of 3 mL/pot. Baskets containing pots were grouped to form one block on a bench within an ultrasonically

humidified clear vinyl tent housed in an air conditioned glasshouse compartment with movable blackout curtains. A Wallwick spray paint gun attached to a 240 L/min air compressor was used to apply inoculum evenly from all four sides. Immediately following inoculation, the vinyl tent was sealed, the ultrasonic humidifier was turned on and the blackout curtains were closed. Seedlings were incubated in darkness at 19°C (± 4°C) in high humidity for 20 hours after which they were transferred back to the previous air conditioned compartment, spaced out on trays and held under the same light and temperature regime used pre-inoculation. Pots were bottom watered post-inoculation and fertilised under the same pre-inoculation regime. Phenotypic infection responses (IR) were recorded 10 days post inoculation adhering to the 1 – 10 seedling scale proposed by Tekauz (1985), where 1 is resistant and 10 is very susceptible. Seedling datasets were uniquely coded as follows; 2012 data for NB330 was coded nb330s12; 2013 data for NB330 was coded nb330s13; 2012 data for NB73 was coded nb73s12; 2013 data for NB73 was coded nb73s13; 2012 data for experiment 1 of NB85 was coded nb85s12_1; 2012 data for experiment 2 of NB85 was coded nb85s12_2 and 2013 data for NB85 was coded nb85s13.

5.3.4 Adult experiments

Six adult phenotyping experiments were conducted in dedicated disease nurseries during the winter/spring of 2012 and 2013 at the Hermitage Research Facility, Warwick, Queensland, Australia. Each year individual nurseries were inoculated with either NB50, NB73 or NB85. Nurseries were separated by a minimum of 180 m to minimise airborne cross-contamination of isolates. Approximately 15 seeds of each genotype were sown as hill plots in rows in a randomised design of two replicates. Materials and methods used to the conduct adult phenotyping were similar to those described in Chapter 4. Key dates for the field experiments are displayed in Table 5.3. Infection responses (IRs) was scored on a whole plot basis using a 1 – 9 scale that was adapted from Saari and Prescott (1975). Adult datasets were uniquely coded as follows; 2012 data for NB50 was coded nb50a12; 2013 data for NB50 was coded nb50a13; 2012 data for NB73 was coded nb73a12; 2013 data for NB73 was coded nb73a13; 2012 data for NB85 was coded nb85a12 and 2013 data for NB85 was coded nb85a13.

5.3.5 Analysis of phenotype data

Analysis of the infection responses was conducted using a linear mixed model, whereby genotype was fitted as a fixed effect and terms to account for the experimental structure, such as replicate, were fit as random effects. A spatial correlation process was applied to model potential local scale

variation between genotypes in each experiment, where genotypes were indexed by their column and row position. Predictions of genotype performance were provided as empirical best linear unbiased estimates (eBLUEs), as the predictions were to be used for a subsequent stage of analysis for genome wide association scans (GWAS). The mixed model was fit using ASReml-R (Butler *et al.* 2009) in the R statistical software environment (Team 2013), whereby all variance components were estimated using residual maximum likelihood (REML) (Patterson and Thompson 1971) as per the model detailed in Chapter 4. Histograms of phenotype densities were plotted using 'ggplot2' (v2.2.1) package (Wickham 2009). Correlation of phenotype datasets across growth stages and years was calculated using the Pearson algorithm in the 'PerformanceAnalytics' (v.1.4.3541) package (Peterson *et al.* 2014). The 'heritability' (v1.0) R package (Kruijer *et al.* 2016) was used to estimate narrow sense heritability based on eBLUE IR and the efficient mixed-model association (EMMA) (Kang *et al.* 2008) kinship matrix that was also used for GWAS.

5.3.6 Genotyping

Genomic DNA was extracted from bulked first and second leaves of three plants per genotype using the CTAB protocol recommended by Diversity Arrays Technology (DArT™) (http://www.diversityarrays.com/sites/default/files/resources/DArT_DNA_isolation.pdf). Barley genotypes from the NRB breeding populations and diversity panel were genotyped using next generation sequencing platforms to generate marker data from DArTseq™ single nucleotide polymorphisms (SNPs). DArTseq™ SNP markers were aligned to the masked pseudomolecule (Mascher *et al.* 2017) using the procedure detailed in Chapter 4. Genotypic data was manually curated in Microsoft Excel to exclude markers > 20 % missing, > 30 % heterozygous and minor allele frequency (MAF) < 5%. A total 5,172 markers were used for GWAS of 2012 and 2013 breeding populations.

5.3.7 Genome-wide association studies

GWAS using a mixed linear model (MLM) was conducted in Genome Association and Prediction Tool (GAPIT) (Lipka *et al.* 2012) R package. To reduce inflation of false positives, population structure was inferred through principal component analysis (PCA) and genetic relatedness of individuals was corrected for with a genetic kinship (K) variance-covariance matrix that was estimated using EMMA algorithm. A MLM can be described as $Y = X\beta + Zu + e$, where Y is the phenotype, X is the genotype, β is a vector of fixed effect that includes genetic markers, population structure and the intercept, Z is the kinship matrix, u contains random additive genetic effects and e

contains the residual. In order to determine the optimal number of principal components to select for 2012 and 2013 analyses, preliminary GWAS was conducted in GAPIT with ten principal components (PCs) specified. Visual inspection of the scree plot was conducted to identify the inflection point for 2012 and 2013 analyses separately.

GWAS was conducted using 173 genotypes for 2012 datasets and 273 genotypes for 2013 datasets. The inflection point of the scree plot for 2012 was at five PCs (Figure 5.4A). The inflection point of the scree plot for 2013 was at seven PCs (Figure 5.4C). GWAS models specified PCA=5 for 2012 analyses and PCA=7 for 2013 analyses.

In order to ascertain if linkage disequilibrium between strongly resistant genotypes was causing inflation of *p*-values, a comparative analysis was conducted and with a subset of resistant genotypes removed. Reduced genotype GWAS was conducted with 152 genotypes for 2012 datasets and 259 genotypes for 2013 datasets. 31 genotypes (four shared across both years), which carried the allele associated with resistance for 3256608-45:C>G, were excluded from phenotype data (Table 5.5). The inflection point of the scree plot for 2012 was at five PCs (Figure 5.4B). The inflection point of the scree plot for 2013 was at seven PCs (Figure 5.4D). GWAS models specified PCA=5 for 2012 analyses and PCA=7 for 2013 analyses.

Bonferroni correction threshold originally described by Holm (1979) was applied to call a marker-trait association significant ($-\log_{10}(p)$ value > 5.01). Quantile-Quantile (Q-Q) plots of full genotype GWAS and reduced genotype GWAS results were generated using 'qqman' (v.0.1.4) package (Turner 2014). Manhattan plots of reduced genotype GWAS results were generated with 'ggplot2' R package.

To tease apart a highly significant interaction positioned in the centromeric region of 6H, LD was estimated between highly significant markers. Specifically, pairwise LD between 38 significant markers was estimated across 400 barley genotypes. To determine the degree of LD across 6H among highly resistant genotypes, LD of 94 genotypes was compared to CIho 5791. LD was estimated across the full length of 6H using 1,008 markers and across a 98.5Mb window between 361,531,190 bp and 460,088,004 bp on 6H using 123 markers. Estimates of Linkage disequilibrium (LD) were analysed using 'LDcorSV' (v.1.3.2) R package (Desrousseaux *et al.* 2016) and reported as r^2 values.

To allow comparison of seedling and adult phenotype data for box and whisker plots, seedling phenotype data was rescaled from 1 – 10 to 1 – 9 via an indexing calculation ((seedling phenotype / 10) x 9). Phenotype data was averaged across years and growth stages for each isolate to produce one dataset per isolate. NB50 and NB330 data was combined into one dataset. Box and whisker plots of mean phenotype data and allele combinations of QTL from representative SNP markers were generated using 'qboxplot' R package (v.0.2) for each isolate (Turner 2017). Least significant differences between mean phenotype of combined 2012 and 2013 seedling and adult data for NB50/NB330, NB73 and NB85 were determined using 'agricolae' R package (De Mendiburu 2014). BLAST searches were conducted using BARLEX (Colmsee *et al.* 2015), ensemble (http://plants.ensembl.org/Hordeum_vulgare) and GrainGenes (https://wheat.pw.usda.gov/GG3/) databases.

5.3.8 Pedigree and marker frequency analyses

A pedigree file of NRB breeding lines, North Dakota State University lines used as parents and genotypes from the diversity panel was generated via online searches of the following databases: U.S. National Plant Germplasm System (https://npgsweb.ars-grin.gov/gringlobal/search.aspx?), GRIN Czech 1.9.1 (www.grin-global.org), CIMMYT-Wheat Germplasm Bank 1.9.4 (http://wgb.cimmyt.org/gringlobal/search.aspx), Plant Gene Resources of Canada (http://pgrc3.agr.gc.ca/acc/search-recherche_e.html), T3 Barley Sandbox (https://t3sandbox.org/t3/sandbox/barley/about.php), Genesys (https://www.genesys-pgr.org/welcome), Plant Variety Journals Australia (https://www.ipaustralia.gov.au/tools-resources/pbr-journals) and the Czech barley pedigree catalogue (http://genbank.vurv.cz/barley/pedigree/default.htm). Helium (Shaw *et al.* 2014) was used to visualise pedigree relationships and track desirable and undesirable alleles between generations. Individual genotypes within the diversity panel were grouped by continent of origin in order to determine the proportion of genotypes that carry the desirable allele. Missing and heterozygous SNPs were not included in the calculation. Introductory genotypes were grouped as per the original location of development/selection. For example; Prior was selected from Chevallier and Chevallier was originally from England, thus Prior was grouped with the European germplasm.

5.4 Results

5.4.1 Infection response to NB50 and NB330

Adult NB50 IRs for 2012 ranged from 1.6 to 7.4 and had a mean of 4.08 and 2013 IRs ranged from 1.7 to 8.0 and had a mean of 3.80 (Table 5.4). Seedling NB330 IRs for 2012 ranged from 1.0 to 10.5 and had a mean of 4.77 and 2013 IRs ranged from 1.5 to 9.5 and had a mean of 5.26 (Table 5.4). Narrow sense heritability (h^2) was estimated at 0.92 to 0.99 for the datasets (Table 5.4). The phenotype density distributions of nb50a12 and nb50a13 were similar, as were the distributions for nb330s12 and nb330s13. A high proportion of adult phenotype scores were distributed around IR3, while seedling phenotypes were more evenly distributed across the entire phenotypic range. (Figure 5.1A). Pairwise correlation between seedling and adult datasets from 2012 was 0.60 (Figure 5.2A), seedling and adult datasets from 2013 was 0.64 (Figure 5.2B) and correlation between IRs of reference genotypes across both seedling and adult datasets for both years ranged from 0.60 to 0.88 (Figure 5.3). Correlation within growth stage across years was higher than correlation within year across growth stages (Figure 5.3).

5.4.2 Infection response to NB73

Adult NB73 IRs for 2012 ranged from 1.4 to 9.1 and had a mean of 4.69 and 2013 IRs ranged from 1.8 to 9.6 and had a mean of 5.43 (Table 5.4). Seedling NB73 IRs for 2012 ranged from 1.0 to 10.0 and had a mean of 5.12 and 2013 IRs ranged from 1.0 to 10.0 and had a mean of 4.65 (Table 5.4). Narrow sense heritability (h^2) was estimated at 0.99 for each dataset (Table 5.4). The phenotype density distributions of nb73a12 and nb73s12 were similar, as were the distributions for nb73a13 and nb73s13. A higher proportion of seedling and adult phenotypic data from 2013 were distributed between IR4 to IR5, while seedling and adult data from 2012 was more evenly distributed across the entire phenotypic range. (Figure 5.1B). Pairwise correlation between seedling and adult datasets from 2012 was 0.81 (Figure 5.2A), seedling and adult datasets from 2013 was 0.77 (Figure 5.2B) and correlation between IRs of reference genotypes across both seedling and adult datasets for both years ranged from 0.78 to 0.93 (Figure 5.3). Correlation within growth stage across years was higher than correlation within year across growth stages (Figure 5.3).

5.4.3 Infection response to NB85

Adult NB85 IRs for 2012 ranged from 1.3 to 9.0 and had a mean of 5.05 and 2013 IRs ranged from 1.5 to 8.7 with a mean of 4.96 (Table 5.4). Seedling NB85 IRs for the first 2012 dataset ranged from 1.5 to 10.0 and had a mean of 6.64. The second 2012 dataset ranged from 1.0 to 10.0 and had a mean of 4.46. The 2013 IRs ranged from 1.0 to 10.0 and had a mean of 5.82 (Table 5.4). Narrow sense heritability (h^2) was estimated at 0.99 for each dataset (Table 5.4). The phenotype density

distributions of nb85a12, nb85a13, nb85s12_1 and nb85s12_2 were evenly distributed across the entire phenotypic range and were more similar to each other than to nb85s13, which had a high proportion of phenotypes distributed around IR5 (Figure 5.1). Pairwise correlation between nb85a12 and nb85s12_1 was 0.80; nb85a12 and nb85s12_2 was 0.78 and between nb85s12_1 and nb85s12_2 was 0.85 (Figure 5.2A). Correlation between seedling and adult datasets from 2013 was 0.72 (Figure 5.2B) and between IRs of reference genotypes across both seedling and adult datasets for both years ranged from 0.70 to 0.87 (Figure 5.3). Correlation within growth stage across years was higher than correlation within year across growth stages (Figure 5.3).

5.4.4 Inspection of quantile-quantile plots

Visual inspection of Q-Q plots revealed improvements to the deviation between expected $-\log_{10}(p)$ values and observed $-\log_{10}(p)$ values for full genotype GWAS compared to reduced genotype GWAS in several datasets. These datasets included nb330s13 (Figure 5.5G and 5.5H), all NB73 datasets (Figure 5.6), nb85a13 (Figure 5.7C and 5.7D), nb85s12_1 (Figure 5.7E and 5.7F), nb85s12_2 (Figure 5.7G and 5.7H) and nb85s13 (Figure 5.7I and 5.7J). Full genotype GWAS Q-Q plots that exhibited strong deviation from expected $-\log_{10}(p)$ values suggested that a large number of markers were significantly associated with resistance, whereas the reciprocal reduced genotype GWAS Q-Q plot suggested that fewer markers were significantly associated with resistance.

5.4.5 Significant markers identified by GWAS

A combined total of 38 SNP markers were identified as significantly associated with resistance to *Ptt* from full and reduced genotype GWAS analyses (Table 5.6). Full genotype GWAS identified a total of 37 markers and reduced genotype GWAS identified a total of 10 markers. A total of nine markers were identified as significant in both full and reduced genotype GWAS analyses (Table 5.6). A stronger signal was generally observed for 2013 datasets compared to 2012 datasets and also for seedling datasets compared to adult datasets. Summary of the significant markers with physical map location, nucleotide sequence, gene at SNP and gene description is given in Appendix 4.

5.4.6 GWAS of response to NB50 and NB330

GWAS of NB50 and NB330 revealed significant MTAs on 4H and 6H (Figure 5.8). The NB50 and NB330 datasets identified two significant markers on 4H at 53,032,932 bp and 69,382,105 bp, respectively. They identified 12 significant markers on 6H between 340,307,078 bp and

459,335,236 bp (Table 5.6). The two markers (3255709-40:A>G and 3257855-10:A>G) on 4H were significant in both full and reduced GWAS analyses of nb330s13. The marker (3256608-45:C>G) on 6H located at 378,772,740 bp that was used to select genotypes to exclude from phenotype data for reduced genotype GWAS, was significant in full genotype GWAS of nb330s13 only. Three markers (3254817-15:C>A, 3257446-28:G>T and 3262096-64:C>T) on 6H located between 340,307,078 bp and 378,974,018 bp were significant in both full and reduced GWAS analyses of nb50a13 and nb330s13, while 3262096-64:C>T was also significant in full genotype GWAS of nb50a12. The remaining eight markers (3257608-6:A>G, 4175123-58:C>A, 3256765-18:T>C, 3262659-31:C>G, 3434193-36:T>G, 3255255-56:T>A, 3261554-30:C>T and 3259228-14:G>C) located on 6H between 361,531,190 bp and 459,335,236 bp were significant in full genotype GWAS of nb330s13 only (Table 5.6).

5.4.7 GWAS of response to NB73

GWAS of NB73 revealed significant MTAs on 6H (Figure 5.9). Specifically, NB73 datasets identified 35 significant markers on 6H between 193,444,571 bp and 461,514,241 bp (Table 5.6). The marker (3256608-45:C>G) on 6H located at 378,772,740 bp that was used to select genotypes to exclude from phenotype data for reduced genotype GWAS, was significant in full genotype GWAS analyses of nb73a13, nb73s12 and nb73s13. Seven markers (3257954-50:G>A, 3434214-43:A>T, 3256458-52:T>C, 3255777-67:T>G, 3254817-15:C>A, 3257446-28:G>T and 3262096-64:C>T) on 6H located between 193,444,571 bp and 378,974,018 bp were significant in both full and reduced GWAS analyses. Six of these markers (3257954-50:G>A, 3256458-52:T>C, 3255777-67:T>G, 3254817-15:C>A, 3257446-28:G>T and 3262096-64:C>T) were significant in both full and reduced GWAS analyses of nb73a13 and nb73s13. The remaining 23 markers (3259111-21:A>C, 3398663-60:C>T, 3254735-54:A>C, 3257608-6:A>G, 3259058-41:G>A, 3259255-17:C>T, 4175123-58:C>A, 3256765-18:T>C, 3262659-31:C>G, 3255625-14:C>T, 3434176-13:T>C, 3432738-29:G>A, 3432352-13:G>T, 3254663-15:T>A, 3255134-29:C>A, 3434193-36:T>G, 3255255-56:T>A, 4171893-67:C>T, 3921095-18:T>C, 3257464-10:T>A, 3261554-30:C>T, 3263983-33:G>T and 3262437-68:C>T) located on 6H between 210,766,011 bp and 461,514,241 bp were significant in full genotype GWAS analyses only (Table 5.6).

5.4.8 GWAS of response to NB85

GWAS of NB85 revealed significant MTAs on 4H and 6H (Figure 5.10). Specifically, NB85 datasets identified three significant markers on 4H between 53,032,932 bp and 70,434,783 bp and

16 significant markers on 6H between 368,527,587 bp and 460,084,925 bp (Table 5.6). One marker (3257855-10:A>G) on 4H located at 69,382,105 bp was significant in both full and reduced GWAS analyses of nb85s13, while two markers (3255709-40:A>G and 3256237-67:A>G) located at 53,032,932 bp and 70,434,783 bp, respectively, were significant in reduced genotype GWAS of nb85s13. The marker (3256608-45:C>G) on 6H located at 378,772,740 bp that was used to select genotypes to exclude from phenotype data for reduced genotype GWAS, was significant in full genotype GWAS analyses of nb85a13, nb85s12_1, nb85s12_1 and nb85s13. One marker (3257446-28:G>T) on 6H located at 368,527,587 bp was significant in reduced genotype GWAS of nb85s12_2. The remaining 14 markers (3257608-6:A>G, 3259255-17:C>T, 4175123-58:C>A, 3256765-18:T>C, 3262659-31:C>G, 3432738-29:G>A, 3432352-13:G>T, 3254978-54:G>A, 3258749-25:G>C, 3434193-36:T>G, 3255255-56:T>A, 3921095-18:T>C, 3259228-14:G>C, 3258275-14:G>C) on 6H located between 36,153,1190 bp and 460,084,925 bp were significant in full genotype GWAS analyses only (Table 5.6).

5.4.9 Markers associated with resistance to multiple *Ptt* isolates

The marker (3256608-45:C>G) on 6H located at 378,772,740 bp, used to select genotypes to exclude from phenotype data for reduced genotype GWAS, was significantly associated with resistance to NB330, NB73 and NB85 (Table 5.6).

Four markers were significantly associated with resistance to more than one *Ptt* isolate in both full and reduced genotype GWAS analyses (Table 5.6). Marker 3257855-10:A>G located on 4H, was significantly associated with resistance to NB330 and NB85. SNP marker 3254817-15:C>A located on 6H, was significantly associated with resistance to NB330 and NB73 and 3262096-64:C>T was significantly associated with resistance to NB50 and NB73. SNP marker 3257446-28:G>T located on 6H, was significantly associated with resistance to NB50, NB73 and NB85. SNP effect inversion was observed for 3257446-28:G>T, where the 'G' allele was associated with resistance to NB50 and NB73, while the 'T' allele was associated with resistance to NB85. Marker 3255709-40:A>G located on 4H, was significantly associated with resistance to NB330 and NB85 in reduced genotype GWAS analyses (Table 5.6).

A total of 12 markers were identified as significantly associated with resistance to more than one *Ptt* isolate solely from full genotype GWAS analyses (Table 5.6). Six markers; - 3257608-6:A>G, 4175123-58:C>A, 3256765-18:T>C, 3262659-31:C>G, 3434193-36:T>G and 3255255-56:T>A - located on 6H were significantly associated with resistance to NB330, NB73 and NB85.

Marker 3261554-30:C>T located on 6H, was significantly associated with resistance to NB330 and NB73. While marker 3259228-14:G>C located on 6H, was significantly associated with resistance to NB330 and NB85. SNP markers; 3259255-17:C>T, 3432738-29:G>A, 3432352-13:G>T and 3921095-18:T>C located on 6H, were significantly associated with resistance to NB73 and NB85 (Table 5.6).

5.4.10 Linkage disequilibrium among associated markers

Pairwise LD was estimated between 38 markers identified as significantly associated with resistance to *Ptt* (Table 5.7). Pairwise LD was high between markers that were identified solely from full genotype GWAS analyses. Specifically, pairwise LD estimates were high between 3256608-45:C>G, the marker used to select genotypes to exclude from phenotype data for reduced genotype GWAS, and SNP markers that were identified solely from full genotype GWAS. LD estimates between 3256608-45:C>G and the 27 SNP markers identified solely from full genotype GWAS, showed that 20 markers had r^2 estimates ≥ 0.3, 14 markers had r^2 estimates ≥ 0.5, eight markers had r^2 estimates ≥ 0.7 and thee markers had r^2 estimates ≥ 0.9. The desirable allele of 3256608-45:C>G and markers in high LD, also occurred at low frequency in the breeding population (Table 5.7). SNP markers in high LD ($r^2 \geq 0.7$) with 3256608-45:C>G were located on 6H between 361,531,190 bp and 460,084,925 bp. Three SNP markers identified solely from full genotype GWAS showed high LD ($r^2 = 0.7 – 0.9$) with five SNP markers located on 6H between 193,444,571 bp and 340,307,078 bp that were identified from both full and reduced genotype GWAS (Table 5.7).

5.4.11 Linkage disequilibrium between genotypes for 6H

LD across the full length of 6H between CIho 5791 and genotypes that carried the desirable allele for 3256608-45:C>G was strongest among Ethiopian landraces ($r^2 = 0.970$ to $r^2 = 0.623$), while developed germplasm ranged from $r^2 = 0.275$ to $r^2 = 0.035$. LD across the 98.5Mb window was relatively strong for Ethiopian landraces and most developed germplasm ($r^2 = 0.979$ to $r^2 = 0.458$), while LD was weak for WI2291 ($r^2 = 0.050$), three ND lines ($r^2 = 0.370$ to $r^2 = 0.209$) and five NRB lines ($r^2 = 0.327$ to $r^2 = 0.065$) (Appendix 5).

LD between CIho 5791 and genotypes that carried the undesirable allele for 3256608-45:C>G was weak except for four Ethiopian landrace accessions (CIho 1227, K8755 (495220),

K20019 (495213) and K20019 (495218)), which ranged between $r^2 = 0.584$ and $r^2 = 0.621$ for the full length of 6H and $r^2 = 0.781$ and $r^2 = 0.822$ for the 98.5Mb window (Appendix 5).

5.4.12 QTL designation and pedigree analysis

Nomenclature used to designate QTL followed the principle described in Chapter 4. Four distinct groups of markers, one group on 4H and three groups on 6H, that displayed moderate to high LD ($r^2 = 0.5 – 0.9$) were observed among SNP markers that were identified from combined GWAS and were postulated to constitute four independent QTL (Table 5.7).

The QTL on 4H consisted of three markers (3255709-40:A>G, 3257855-10:A>G and 3256237-67:A>G) that displayed moderate to high LD between markers ($r^2 = 0.5 – 0.7$) and were located between 53,032,932 bp and 70,434 783 bp (Table 5.7). The 4H QTL was designated *QRpt4H* and the most significant marker, 3257855-10:A>G, was used to represent the QTL. Pedigree visualisation traced desirable alleles in NRB breeding lines to ND parental lines, where the original donor of the alleles appeared to be PC 84, a line from the International Maize and Wheat Improvement Centre (CIMMYT). The desirable alleles were also observed in Australian cultivars that were derived from CIMMYT lines, VB9104 and Yagan and Malebo; a selection from an Algerian landrace. The desirable alleles were also observed in germplasm of African origin (Appendix 2). Associated markers were positioned on the barley physical map presented in Appendix 1.

The first group on 6H consisted of five SNP markers (3257954-50:G>A, 3434214-43:A>T, 3256458-52:T>C, 3255777-67:T>G and 3254817-15:C>A) that were located on 6H between 193,444,571 bp and 340,307,078 bp and displayed high LD between markers ($r^2 = 0.8 – 1.0$) (Table 5.7). Pedigree visualisation traced the undesirable alleles to genotypes that originated from the Moravia region of the Czech Republic and the English landrace; Archer (Appendix 2). This QTL was designated *QRpt6Hm*. The most significant marker, 3254817-15:C>A, was used to represent the QTL. Associated markers were positioned on the barley physical map presented in Appendix 1.

The second group on 6H consisted of two SNP markers (3257446-28:G>T and 3262096-64:C>T) that were located on 6H between 368,527,587 bp and 378,974,018 bp and displayed moderate LD between markers ($r^2 = 0.5$) (Table 5.7). Pedigree visualisation traced the alleles associated with susceptibility to NB50 and NB73 to Isaria, which was developed from a cross between two landraces from the Bavaria region of Germany (Appendix 2 and 6). Alternate alleles,

'G' and 'T', of the most significant marker, 3257446-28:G>T, were associated with resistance to NB50 and NB85, respectively. The same effect of resistance reversal of alleles for this marker was observed in Chapter 4. This QTL was designated *QRpt6Hs* in Chapter 4, thus the designation was also adopted for this Chapter. Associated markers were positioned on the barley physical map presented in Appendix 1.

The fourth group on 6H consisted of 3256608-45:C>G and six SNP markers (4175123-58:C>A, 3256765-18:T>C, 3262659-31:C>G, 3255625-14:C>T, 3432738-29:G>A and 3254663-15:T>A) that were located on 6H between 378772740 bp and 396127146 bp and displayed high LD between markers (r^2 = 0.8 – 0.9) (Table 5.7). Pedigree visualisation traced the desirable alleles to Ethiopian landrace, CIho 5791, which was introduced into NRB breeding lines via ND germplasm via Norbert and Ellice (Appendix 7). The resistance gene from CIho 5791 was designated *Rpt5.f* in BGN (2013), which was adopted for this QTL. Associated markers were positioned on the barley physical map presented in Appendix 1.

5.4.13 Proportion of desirable alleles in diversity panel

The proportion of genotypes with the desirable allele for *QRpt4H* ranged from 0.08 to 0.60 for reference and NRB genotypes, respectively and 0.00 to 0.33 for the diversity panel groups, where Asia was 0.00 and Africa was the highest. The desirable allele was absent from cultivars from New Zealand, Queensland and Tasmania, while variation was observed in all other states where South Australia was 0.04, Victoria was 013, Western Australia was 0.15 and New South Wales was 0.33 (Appendix 3).

The proportion of genotypes with the desirable allele for *QRpt6Hm* ranged from 0.88 to 0.98 for 2012 and 2013 NRB genotypes, respectively and 0.89 for reference genotypes. The diversity panel genotypes ranged from and 0.63 to 1.00 for the diversity panel groups, where Europe was the lowest and the Africa, the Americas and Asia were 1.00. The desirable allele was fixed in cultivars from New South Wales, New Zealand, South Australia, Tasmania and Western Australia, while the Queensland had the lowest proportion (0.33) and Victoria was 0.82 (Appendix 3).

The proportion of genotypes with the desirable allele for *QRpt6Hs* ranged from 0.89 to 0.95 for reference and NRB genotypes, respectively and 0.64 to 1.00 for the diversity panel groups, where Europe was the lowest and Africa and Asia were 1.00. The desirable allele was fixed in cultivars from New Zealand, Queensland, Tasmania and Victoria, while New South Wales was the

lowest (0.67) and South Australia and Western Australia were 0.90 and 0.95, respectively (Appendix 3). The NRB population was fixed for the desirable allele at *QRpt6Hp*, the QTL closely linked to *QRpt6Hs* (appendix 3).

The proportion of genotypes with the desirable allele for *Rpt5.f* ranged from 0.08 to 0.15 for reference and NRB genotypes, respectively and 0.00 to 0.31 for the diversity panel groups, where Asia was 0.00 and the Americas was highest. The desirable allele was absent from all cultivars from every state except Western Australia (Appendix 3).

Notably, the ND germplasm had a consistently high proportion of genotypes with desirable alleles for each QTL when compared to all other groups (Appendix 3).

5.4.14 QTL allele effect on disease phenotype

Analysis of the QTL allele combinations for mean phenotype of combined NB50-NB330 seedling and adult datasets revealed significant statistical differences between means of QTL combinations for alleles of; *QRpt4H – QRpt6Hm QRpt6Hs Rpt5.f* (Figure 5.11). R-RSS, S-SRS and S-RSS combinations were not significantly different from each other, while S-SRS and S-RSS were not significantly different from each other but were significantly different to all other combinations. R-RSS, R-SRS and S-RRS combinations were not significantly different from each other, while R-RSS and S-RRS were significantly different to all other combinations. R-RRS, R-SRS, S-RRR and S-SRR were not significantly different from each other, while R-RRS and R-SRS were significantly different to all other combinations. R-RRR, S-RRR and S-SRR were not significantly different from each other. Change in mean phenotype through *QRpt4H* allele substitution was significantly different for the R-RRS and S-RRS combination and R-SRS and S-SRS combination. Change in mean phenotype through *QRpt6Hm* allele substitution was only significantly different for the S-RRS, S-SRS combination. Change in mean phenotype through *QRpt6Hs* allele substitution was significantly different for the S-RRS and S-RSS combination and R-RRS and S-RSR combination. Change in mean phenotype through *Rpt5.f* allele substitution was significantly different for the R-RRR and R-RRS combination, S-RRR and S-RRS combination and S-SRR and S-SRS combination (Figure 5.11).

Analysis of the QTL allele combinations for mean phenotype of combined NB73 seedling and adult datasets revealed significant statistical differences between means of QTL combinations for alleles of; *QRpt6Hm QRpt6Hs Rpt5.f* (Figure 5.12). S-SS had the highest mean phenotype and

R-RR had the lowest mean phenotype. SRR combination was not significantly different from with RRR or RRS, though RRR and RRS were significantly different from each other. All other combinations were significantly different from each other. Change in mean phenotype through *QRpt6Hm* allele substitution was only significantly different for the SRS and RRS combination. Change in mean phenotype through *QRpt6Hs* allele substitution was only significantly different for the RSS and RRS combination. Change in mean phenotype through *Rpt5.f* allele substitution was significantly different for the SRS and SRR combination and the RRS and RRR combination. The SSS combination was not observed (Figure 5.12).

Analysis of the QTL allele combinations for mean phenotype of combined NB85 seedling and adult datasets revealed significant statistical differences between means of QTL combinations for alleles of; *QRpt4H* - *QRpt6Hs Rpt5.f* (Figure 5.13). S-SS had the highest mean phenotype and R-SR has the lowest mean phenotype. S-SS and R-SS combinations were significantly different from each other and all other combinations. S-RS and R-RS combinations were not significantly different from each other, although they were significantly different from all other combinations. S-SR and R-SR combinations were not significantly different from each other, but they were significantly different from all other combinations. Change in mean phenotype through *QRpt4H* allele substitution was only significantly different for the S-SS and R-SS combinations only. Change in mean phenotype through *QRpt6Hs* allele substitution was significantly different for the S-SS and S-RS combination and R-SS and R-RS combination. Change in mean phenotype through *Rpt5.f* allele substitution was significantly different for the S-SS and S-SR combination and the R-SS and R-SR combination. R-RR combination was not observed (Figure 5.13).

5.5 Discussion

These genome-wide association studies (GWAS) successfully identified genomic regions associated with resistance and susceptibility to *Ptt* in barley breeding populations. GWAS used seedling and adult phenotype data of two Northern Region Barley breeding populations for four *Ptt* isolates. A total of four QTL were detected, one QTL on 4H and three QTL in the centromeric region of 6H. *QRpt4H* and *QRpt6Hm* were associated with resistance to two isolates, while *QRpt6Hs* and *Rpt5.f* were associated with resistance to three isolates. The origin of resistance/susceptibility alleles was investigated using a panel of diverse genotypes and putative sources were identified.

This study identified a genomic region on 4H, *QRpt4H*, which was associated with resistance to NB330 and NB85 at seedling stage in the NRB barley breeding population. A

significant reduction in IR was observed when the undesirable allele was substituted for the desirable allele. The desirable allele was present in more than half of the elite breeding lines, which was considerably higher than any group of germplasm from the diversity panel. This observation suggests that the allele was under selection in the breeding program and could be identified phenotypically.

Previous studies have reported QTL near the centromere of 4H from diverse genetic backgrounds that include AC Metcalfe, Halcyon, OUH602, Sloop, Steptoe, TR251, Zernogradsky 813 and GWAS of the Halle Exotic Barley 25 (HEB-25) nested association mapping (NAM) population, Nordic barley accessions and accessions from the National Small Grains Collection (Afanasenko *et al.* 2015; Cakir *et al.* 2011; Grewal *et al.* 2008; Lehmensiek *et al.* 2007; Raman *et al.* 2003; Richards *et al.* 2017; Steffenson *et al.* 1996; Vatter *et al.* 2017; Wonneberger *et al.* 2017a; Yun *et al.* 2005). Projection of reported SNPs and peak QTL intervals onto the physical map did not indicate that these QTL co-locate with *QRpt4H* (Appendix 1). However, two recent studies reported QTL from a genomic region similar to *QRpt4H*. The first was from a Falcon/Azhul RIL and the second was from a GWAS of two-row North Dakota State University (N2) breeding lines (Adhikari 2017; Islamovic *et al.* 2017). Projection of significant SNPs onto the physical map revealed that the QTL intervals of both studies co-located to the same 17.4 Mb region that was detected via GWAS of the NRB populations. Thus, this genomic region was associated with resistance to ten geographically diverse *Ptt* isolates. Five originated from the USA, three from Australia and one each from Canada and Japan. One Australian isolate, NB50, was used by Islamovic *et al.* (2017) at the seedling stage and in this study for adult experiments, although an association was not detected at the adult stage. The isolate used in seedling experiments, NB330, has been shown to have a similar virulence profile as NB50 (Greg Platz, personal communication) and was significantly associated with resistance at the seedling stage. Collectively, the results of these three studies have independently validated the effectiveness of the *QRpt4H* QTL to multiple *Ptt* isolates from different continents.

Falcon, the resistant parent used by Islamovic *et al.* (2017) was developed by CIMMYT and selected in Canada while the N2 population would likely share some common genetic background to the NRB population. With this in mind, pedigree analysis of the origin of the 4H resistance included all ancestral ND pedigrees and a large proportion of historic North American cultivars in order to investigate all available linkages to founding genotypes. ND derivatives of crosses to PC 84 were frequently seen to donate the desirable allele in NRB genotypes. PC 84 (PI 584764) was developed by Dr Hugo E. Vivar at CIMMYT and was shown to carry resistance to at least two

diseases (Jin *et al.* 1994; St. Pierre *et al.* 2010). Subsequently, Dr Jerome Franckowiak developed germplasm from crosses made to PC 84 and released Rawson (ND19119-2) in 2006 (Franckowiak *et al.* 2007). Dr Franckowiak used a multiple sister lines of ND19119 through successive crossing cycles to develop the advanced parents that were ultimately introduced to the NRB program. The QTL profiles of some ND19119 derived parental lines are shown in Appendix 2. Considering the shared CIMMYT ancestry of germplasm used across all three mapping studies, it is possible that these three studies independently detected a similar genomic region for resistance of CIMMYT origin.

Inspection of the diversity panel revealed that the desirable allele was also observed in Australian cultivars from three independent sources. Yagan, a line of unknown CIMMYT origin, was the source of resistance in Fleet Australia, Mundah and Urambie. VB9104, a line from ICARDA, was the source of resistance in Buloke, Lockyer and Scope CL. Malebo, an Algerian landrace, was the source of resistance in Yerong. African landraces and cultivars derived from Cape and Coast types also carried the desirable allele. Notably, Algerian, Beecher, Cape, CIho 9776 and Prato have all been used as differential genotypes for pathogenicity studies (Chapter 2), although none has been used in mapping studies. Further work is needed in order to confirm whether these genotypes carry the same resistance.

Previous mapping studies, along with the mapping performed in Chapter 2, have documented the centromere of chromosome 6H as a major genomic region for resistance. GWAS performed in this study also detected multiple QTL in the centromeric region of 6H. Three closely positioned QTL, *QRpt6Hm*, *QRpt6Hs* and *Rpt5.f,* were significantly associated with shifts in IR to *Ptt* in the NRB breeding populations. Tightly linked genes in repulsion along with high LD across the centromere might explain the strong heritability of resistance/susceptibility to the isolates used in phenotyping experiments.

The *QRpt6Hm* QTL on 6H identified in this study was strongly associated with resistance to NB73 at seedling and adult stages, while the peak marker was also associated with resistance to NB330 at the seedling stage. A significant reduction in IR for NB50/NB330 and NB73 was observed where the undesirable allele was substituted for the desirable allele. The undesirable allele was found to be present in genotypes of specific origin and suggests high heritability of the allele associated with susceptibility (Appendix 2). Several mapping populations have been developed from genotypes that carry the undesirable allele, specifically Tallon/Kaputar (Cakir *et al.* 2003), Mackay/Baronesse and Mackay/Tallon (Mace *et al.* 2007) (Appendix 2) and UVC8/SABBIErica

(Martin *et al.* 2018). These studies mapped QTL to a similar genomic region as *QRpt6Hm* and in each case the genotype that carried the undesirable allele for *QRpt6Hm* gave the higher phenotype of the parents. It is likely the undesirable allele for *QRpt6Hm* was detected in these populations.

Recent GWAS studies of two diverse populations and a collection of breeding populations have reported significant associations that co-located to *QRpt6Hm* (Adhikari 2017; Richards *et al.* 2017; Wonneberger *et al.* 2017a). The closest reported SNPs to the peak of marker of *QRpt6Hm* were associated with germplasm from the Busch Agricultural Resources Inc. (BARI), two-row genotypes from the Barley Coordinated Agricultural Project (CAP) and diverse genotypes from the National Small Grains Collection (NSGC). However, no conclusions can be drawn across studies, as information regarding which genotypes carried either desirable or undesirable alleles was not included.

GWAS for the HEB-25 NAM, which is based on Barke backcrosses, did not detect an association within the *QRpt6Hm* region (Appendix 1) (Vatter *et al.* 2017). This is interesting as Westminster (Barke/NSL 97-5547) and NRB breeding lines derived from Barke, both carry the undesirable allele for *QRpt6Hm*. These results suggest the field isolate(s) used for screening the HEB-25 in Germany did not have virulence to *QRpt6Hm.* Numerous other mapping studies have also identified a QTL close to *QRpt6Hm* (Appendix 1). However, diversity of genetic backgrounds and the presence of other resistance or susceptibility genes around the centromere of 6H suggest that these populations likely detected a genetic interaction other than *QRpt6Hm*.

Pedigree analysis of genotypes that carried the undesirable allele for *QRpt6Hm* revealed that the allele may have originated from two sources; landraces in the Moravia region of the Czech Republic and Archer, an English landrace. Two successful cultivars descended from these landraces are Carlsberg and Diamant, which were used to develop many cultivars and effectively disseminate the undesirable allele for *QRpt6Hm*. The undesirable allele for *QRp6Hm* is present in Australian cultivars; Gilbert, Grimmett, Lindwall, Research, Resibee, Shepherd, Tallon, Weeah and Westminster (Appendix 2), while phenotypic results suggest that the undesirable allele may also be present in RGT Planet (derived from Westminster) and also Granger. High susceptibility of genotypes that carry the undesirable allele (Rees *et al.* 1999) and widespread presence of the associated virulence (Chapter 3), suggest that the release of germplasm with the undesirable allele for *QRpt6Hm* should be avoided in Australia.

Considering that the undesirable allele for *QRpt6Hm* originated from Europe, it is likely that isolates may differentiate for this virulence in Europe. However, there is currently no internationally recognised differential genotype to identify this susceptibility. The pathogenicity study conducted in Chapter 2 identified Tallon as the most suitable genotype to represent this group. Thus, it is recommended that Tallon be considered for future *Ptt* pathogenicity studies.

GWAS of the NRB population revealed that the peak marker (3257446-28:G>T) for *QRpt6Hs* QTL was significantly associated with resistance and susceptibility in a reciprocal manner. Specifically, the 'G' allele was associated with resistance to NB50 and NB73 and susceptibility to NB85 and vice versa for the 'T' allele. A significant reduction in IR was observed for all three isolates when the undesirable allele was substituted for the desirable allele. A reciprocal allele effect for resistance and susceptibility to NB50 and NB85 was also documented in Chapter 4 at this locus. Two closely linked markers were identified, which lead to the description of two QTL, *QRpt6Hp* and *QRpt6Hs*. The undesirable allele for *QRpt6Hp* was specifically in Prior and some of its descendants and explained high phenotypic response to NB85. The undesirable allele for *QRpt6Hs* was specifically in Skiff and Isaria descendants and explained high phenotypic response to NB50. The undesirable allele for *QRpt6Hp* was absent from the NRB population (Appendix 3), this suggests that a different genetic interaction could be involved at this locus. Further work is necessary to determine if multiple genes or multiple alleles of *QRpt6Hp* are interacting with NB85 at this locus.

Significant MTAs in the interval between the two markers for *QRpt6Hs* were detected from GWAS of NGSC, *NBP_QRptt6-1* of the Nordic Barley Panel (NBP), six-row NDSU breeding lines (N6), and *QPt.6H-1 and QPt.6H-2* from the HEB-25 (Adhikari 2017; Richards *et al.* 2017; Vatter *et al.* 2017; Wonneberger *et al.* 2017a). The dominant susceptibility region described from Rika/Kombar immortal recombinants, *Spt1*, also co-located with the two markers for *QRpt6Hs* (Richards *et al.* 2016). *QPt.6H-1 and QPt.6H-2* QTL from the HEB-25 and *NBP_QRptt6-1* from the NBP were associated with SCRI_RS_186193. In the HEB-25 NAM, the Barke allele conditioned a lower phenotype more often than the *Hordeum vulgare* spp. *spontaneum* alleles. A second marker for *NBP_QRptt6-1* and the N6 QTL were both associated with 11_10513. All of the associated markers for the NSGC were positioned between the rpt-M20 flanking marker and *Spt1*. The detection of multiple MTAs from independent studies within the *Spt1* region further reinforces this locus as a critical region that requires further investigation.

As cultivars that carried the allele for *QRpt6Hs* that was associated with susceptibility to NB50/NB330 were used as parents in the NRB program up till the late 2000's, it could be assumed that the source of the undesirable allele in the breeding lines used in this study would likely be from Skiff. However, this was not true. Pedigree analysis of the 29 breeding lines that carried the undesirable allele revealed that only one breeding line was descended from Skiff, suggesting that effective early generation disease screening had almost completely removed the undesirable allele from advanced NRB parental lines. Thus, the effective re-introduction of the undesirable allele was hypothesised to originate from ND germplasm. Further analyses confirmed that the susceptibility in the remaining 28 genotypes was derived from several ND parents. The source of the undesirable allele was traced back to Bowman via Fergus and ultimately to Isaria (Appendix 6). While this study could not confirm the presence of the undesirable allele for *QRpt6Hs* in Fergus, pathotypes described from Canada suggest that Fergus and Herta share a susceptibility (Tekauz and Mills 1974). Furthermore, the pedigree of Herta can be traced back to Isaria and the undesirable allele for *QRpt6Hs* was also observed in Herta (Appendix 2). In light of these results, it was concluded that Fergus would likely carry the undesirable allele for *QRpt6Hs*. Considering the results of this study as well as the frequent detection of isolates with virulence to genotypes that are likely to carry *Spt.R* (Richards *et al.* 2016) e.g. Herta, Patty, Rika and Skiff, it is likely that isolates around the world carry *VR1* and/or *VR2* (Shjerve *et al.* 2014).

One of the key results from Chapter 4 was cross-validated in this study, as the *QRpt6Hs* allele associated with susceptibility to NB50 was confirmed in an unrelated population where the origin of susceptibility was independent from Australian cultivars. Further work to fine map the location of the *QRpt6Hs* allele associated with susceptibility to NB85 should be conducted in order to better understand the *Spt1* locus.

The QTL that gave the strongest association across GWAS analyses of multiple isolates was *Rpt5.f.* The QTL was detected as highly significant in eight out of 13 data sets. A significant reduction in IR was observed in all cases where the undesirable allele was substituted for the desirable allele. The strongest association was detected between 378,772,740 bp and 396,127,146 bp on the physical map. Pedigree analysis confirmed that ND parents were the origin of the desirable allele in the NRB population and the allele was traced back to CIho 5791. CIho 5791 was shown to highly resistant to all Australian isolates that were phenotyped in Chapter 3. Vlamingh, an Australian cultivar that was used as a differential genotype in Chapter 3, displayed a resistant to moderately resistant phenotype to the majority of isolates tested. Vlamingh was also confirmed to carry the allele for *Rpt5.f* (Appendix 2).

Recent GWAS identified MTAs from the NSGC and germplasm from breeding populations from the University of Minnesota (MN), Montana State University (MSU), N2, United States Department of Agriculture (USDA), Barley CAP I (2006), Barley CAP II (2007), Barley CAP III (2008), Barley CAP IV (2009) and the complete Barley CAP (Adhikari 2017; Richards *et al.* 2017). SNP markers 11_10377 and 12_30857 were significantly associated in the both NSGC and multiple USA breeding programs. Whilst this region co-located to *Rpt5.f*, no conclusions can be drawn across studies, as information regarding which genotypes carried either desirable or undesirable alleles were not included.

Many studies have documented QTL near *Rpt5.f* - specifically, the studies that used CIho 5791, M120, ND11231-12, SM89010, TR251 and WPG8412 (Cakir *et al.* 2003; Friesen *et al.* 2006; Grewal *et al.* 2008; Gupta *et al.* 2011; Koladia *et al.* 2017a; St. Pierre *et al.* 2010). The pedigrees of the genotypes used in these studies could be traced back to CIho 5791 via Heartland, Norbert or Ellice (Appendix 7). While the presence of the desirable allele for *Rpt5.f* was also confirmed in BT 201, CIho 5791, CIho 9819, CIho 9825, Heartland, Norbert, TR215, WPG9412-9-2-1 and other Canadian and ND lines (Appendix 2). In addition, strong LD was observed between CIho 5791 and genotypes that carry the desirable allele across a 98.5Mb region around the centromere of 6H (Appendix 5). In light of these results, it is highly likely that the genotypes used in the previously mentioned studies carry the same resistance gene from CIho 5791, *Rpt5.f* (BGN 2013).

While *Rpt5.f* in CIho 5791 has been shown to condition effective resistance to all Australian *Ptt* isolates, it should be noted that all genotypes in the diversity panel that carried the desirable allele for *Rpt5.f* also carried the desirable alleles for *QRpt6Hm*, *QRpt6Hp* and *QRpt6Hs*. This is likely due to strong LD that was observed via analyses conducted in this study. This situation is ideal from a breeding perspective as the introgression of one chromosomal segment conditions strong resistance whilst excluding two closely linked factors conferring susceptibility. Furthermore, two advanced ND parents from the diversity panel, ND24168 and 2ND25389 and one NRB breeding line from the 2013 population, NRB120543, carried the desirable alleles to all eight QTL that were identified in Chapters 4 and 5. Germplasm with multiple stacked resistances is very valuable genetic resource. It should enable efficient resistance breeding delivering more durable resistance and should be exploited to provide farmers with cultivars that are not dependent on chemical control of net form net blotch.

While it is known that resistance to *Ptt* is commonly conferred by dominant resistance and susceptibility genes, it has been suggested that resistance to *Ptt* in Australian cultivars Clipper, Schooner and Sloop, is conditioned by multiple minor genes that impart a level of adult plant resistance (Wallwork *et al.* 2016). While this may be possible, results presented in this thesis suggest an alternative hypothesis; that these genotypes exhibit stable phenotype across multiple pathotypes because they do not harbour any QTL associated with resistance or susceptibility. These cultivars are highly related to each other and share a common ancestor, Proctor. All four genotypes likely share the same allele combination for the seven QTL identified through this research. The shared allele combination is absent for resistance for *QRpt3H*, *QRpt4H* and *Rpt5.f*, but is also absent for the remaining pathotype specific susceptibilities; *QRpt6Ha*, *QRpt6Hm*, *QRpt6Hp*, *QRpt6Hs* and *QRpt6Hc*. Theoretically, the resultant phenotype of this genotype would not be susceptible to any of the pathotypes used in these studies and would most likely display only a moderate level of resistance or susceptibility. The described phenotype was consistently observed for Clipper in Chapter 3 and for Schooner in annual NVT testing (www.nvtonline.com.au). Considering the long reported history of the durable resistance in Clipper (Wallwork *et al.* 2016), perhaps the simple exclusion of pathotype specific susceptibility/sensitivity genes may be adequate to confer a suitable level of resistance to a broad spectrum of pathotypes.

In addition to the detection of several QTL, GWAS of the breeding population revealed that high linkage disequilibrium present near the centromeric region of 6H caused inflation of *p*-values in direct association with a low frequency QTL of large effect, *Rpt5.f*. Marker inflation and potential spurious detection of false positives was improved through a simple comparative analysis technique, whereby genotypes positive for the peak marker of *Rpt5.f* were excluded from the phenotype dataset prior to secondary GWAS. This highlights the potential risk of false association when performing GWAS where strong LD and large effect traits are present. The method developed here could be explored should a similar situation arise in other GWAS.

The study conducted here successfully utilised GWAS of a barley breeding population to identify four QTL, three of which were positioned close together on 6H. In addition, a panel of diverse genotypes was used to determine the origin of alleles and identify genotypes that carry combinations of desirable QTL. These discoveries will be useful to barley breeders to further their understanding of the barley-*Ptt* relationship in a context that will allow efficient breeding of resistant cultivars.

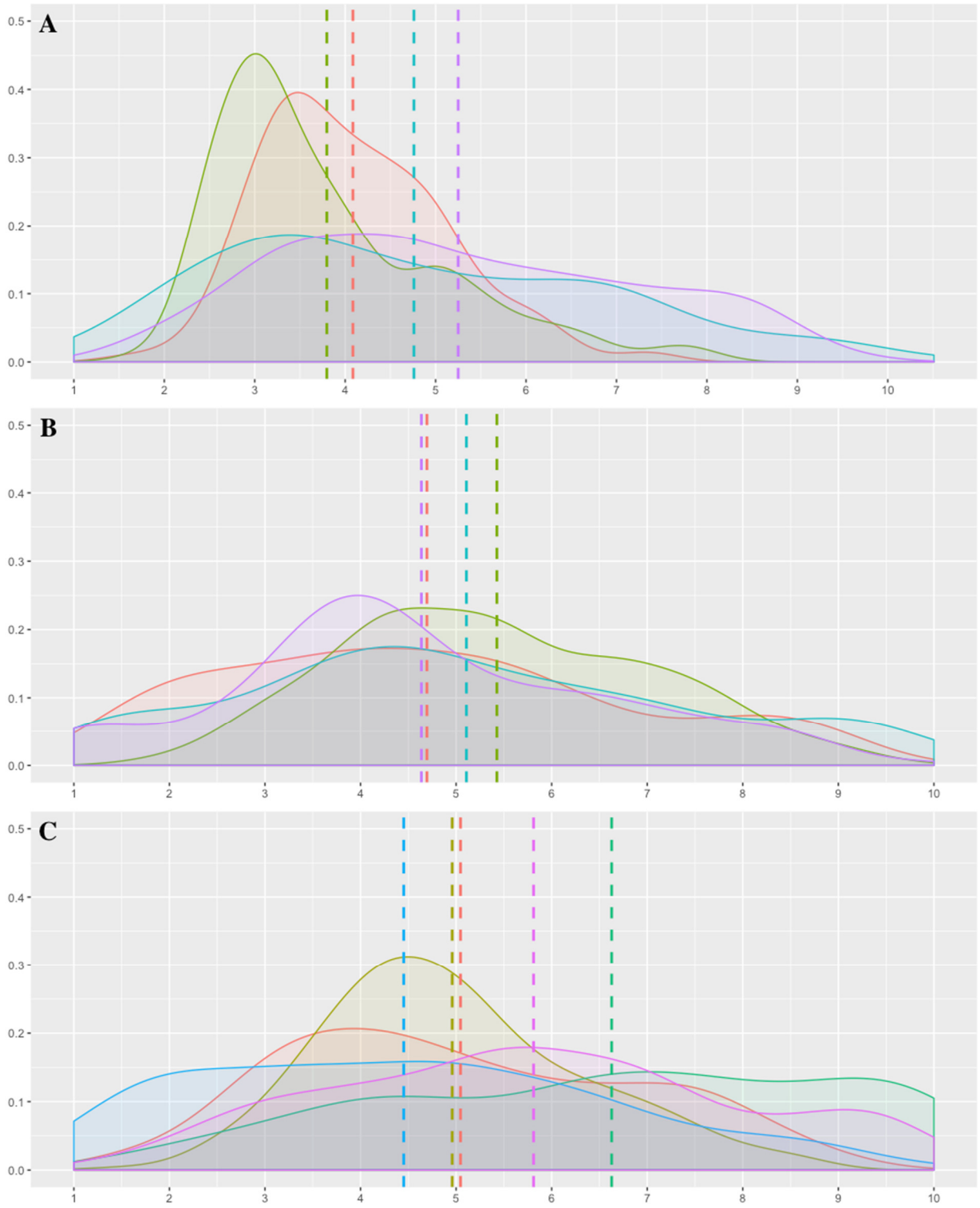


Figure 5.1. Density distribution of infection responses (IRs) for four *Ptt* isolates at two growth stages and two years. IR represented on x-axis, density represented on y-axis and mean represented by vertical line. A: Phenotype plot for NB330 and NB50. nb50a12=red, nb50a13=green, nb330s12=blue, nb330s13=purple. B: Phenotype plot for NB73. nb73a12=red, nb73a13=green, nb73s12=blue, nb73s13=purple. C: Phenotype plot for NB85. nb85a12=red, nb85a13=olive, nb85s12_1=green, nb85s12_2=blue, nb85s13=pink.

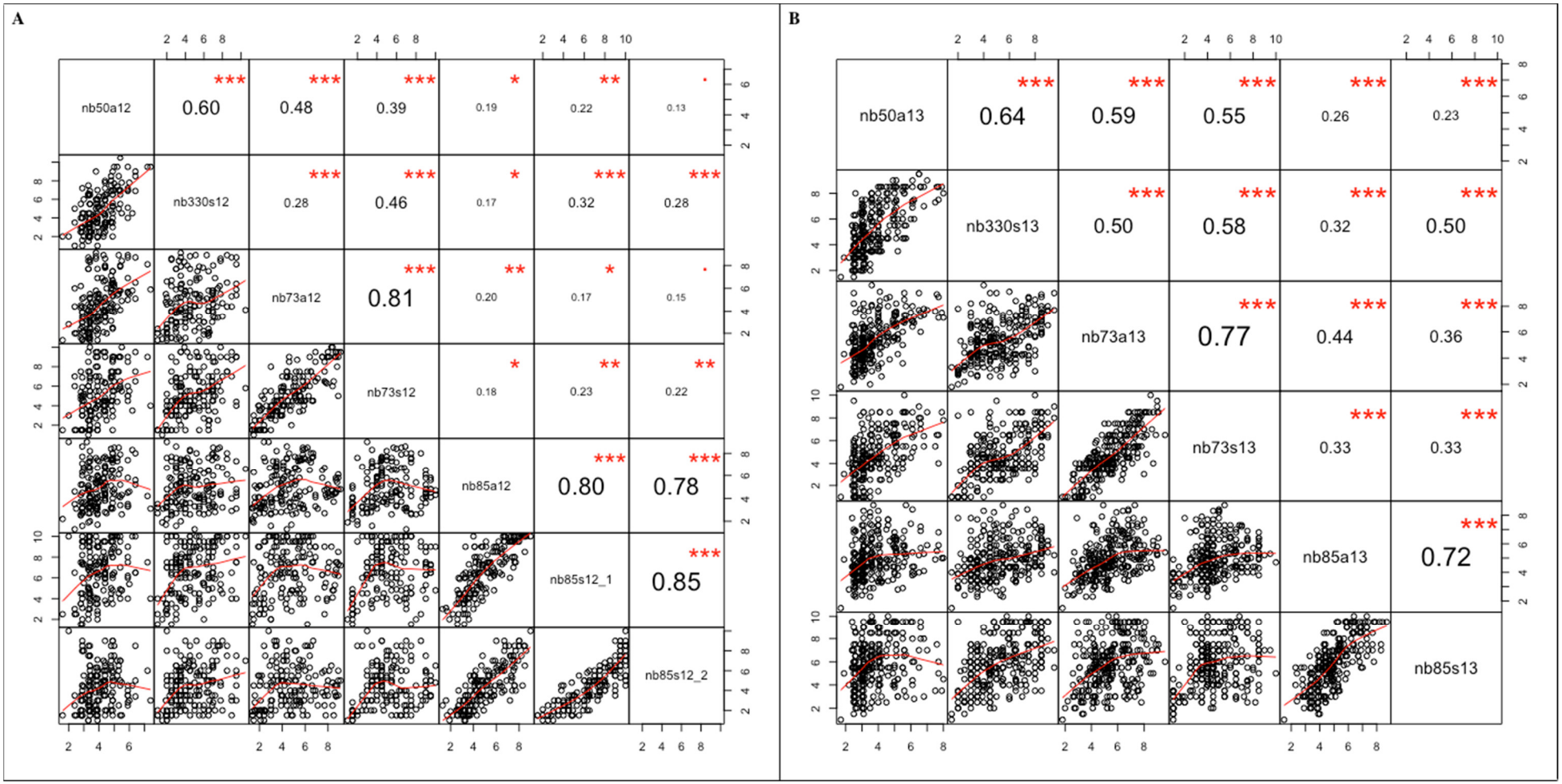


Figure 5.2. Pairwise correlation of infection responses to four *Pyrenophora teres* f. *teres* isolates across seedling and adult datasets. A: Correlation matrix of seven datasets from 2012 for phenotypic response of 173 genotypes. B: Correlation matrix of six datasets from 2013 for phenotypic response of 273 genotypes.

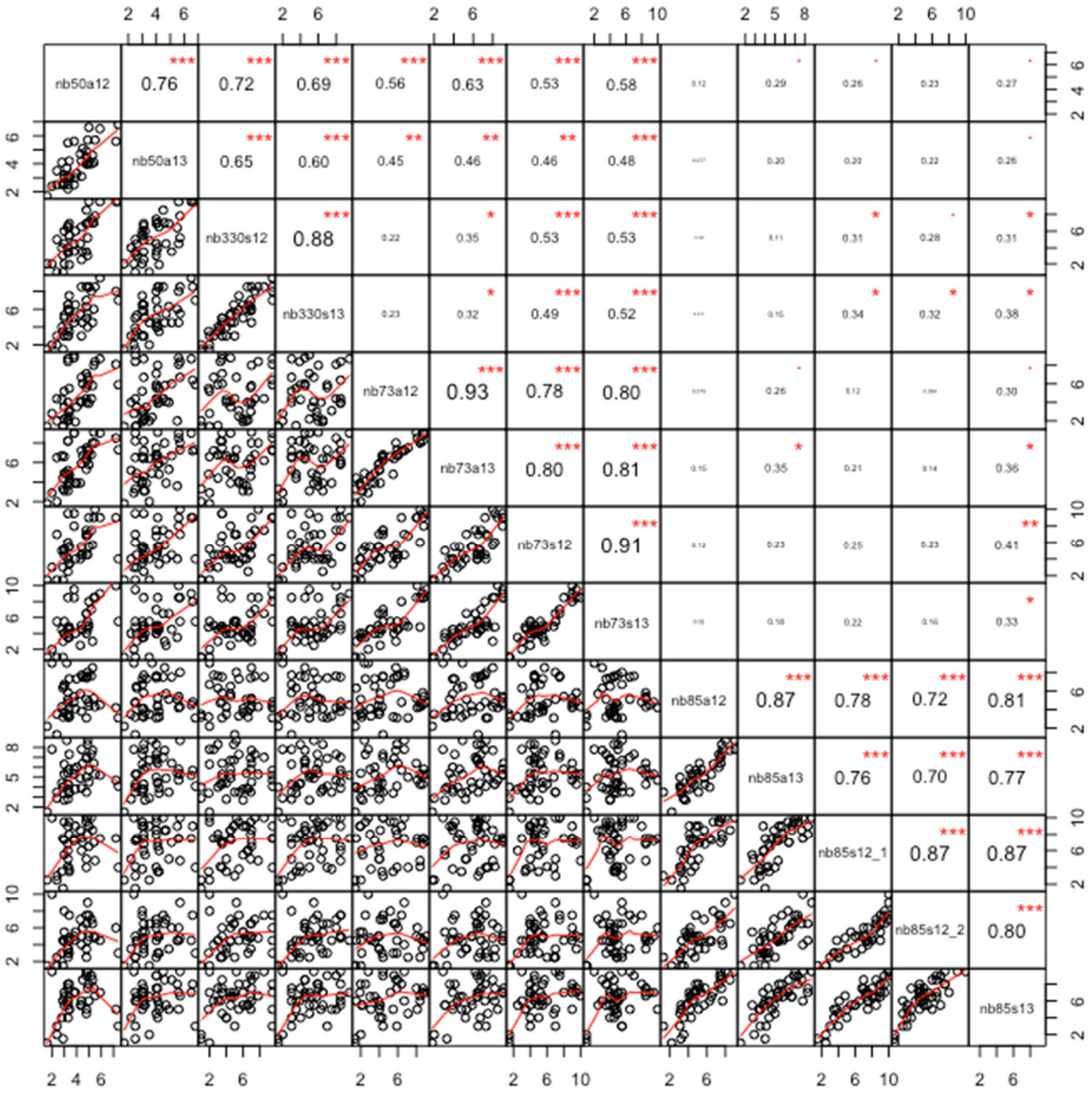


Figure 5.3. Pairwise correlation of infection responses to four *Pyrenophora teres* f. *teres* isolates for 27 reference genotypes across 13 datasets at seedling and adult growth stages for 2012 and 2013.

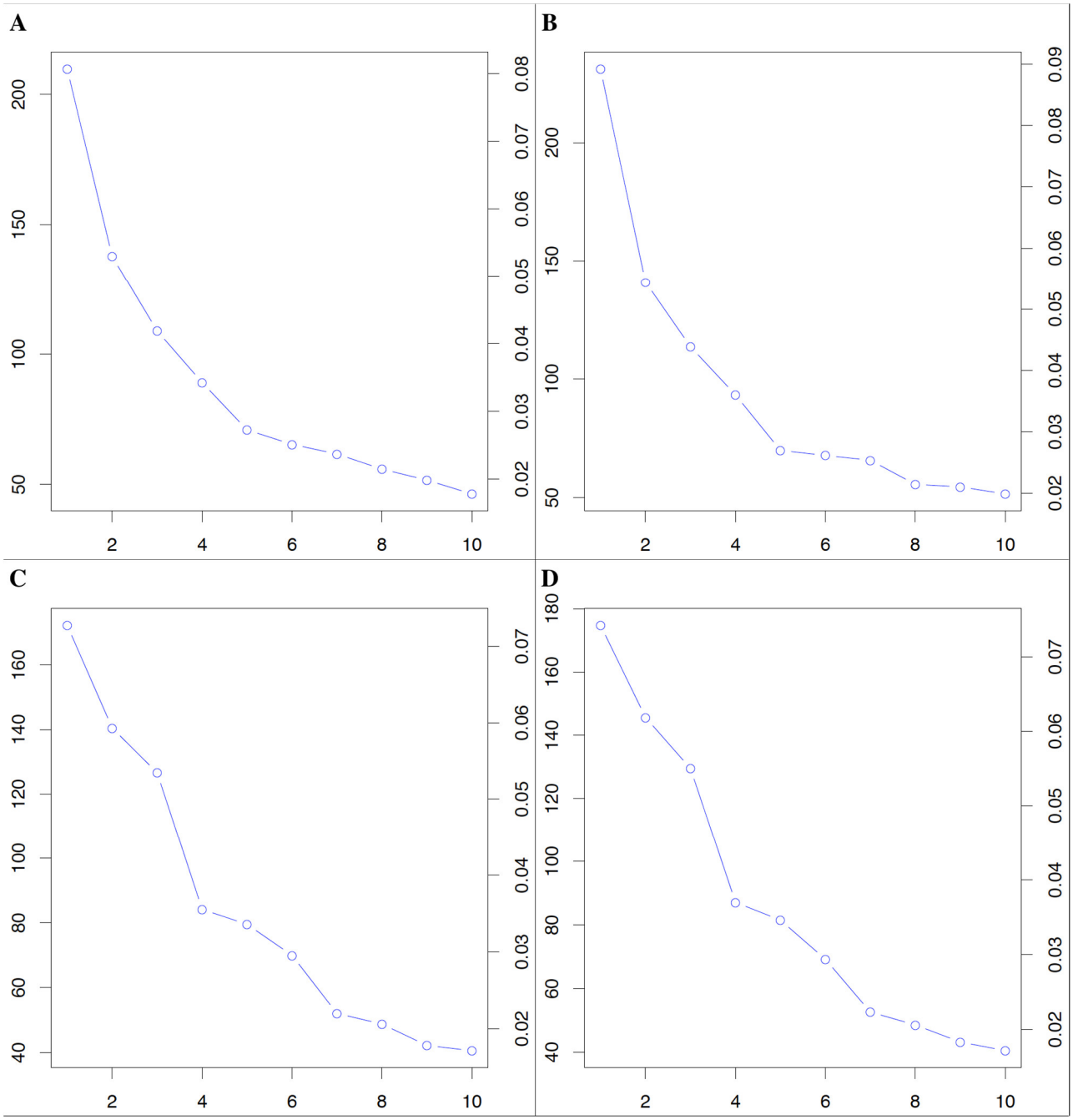


Figure 5.4. Scree plot of eigenvalue variance on left side of y-axis and percentage on right side for ten principal components (x-axis). A: 2012 full genotype GWAS. B: 2012 reduced genotype GWAS. C: 2013 full genotype GWAS. D: 2013 reduced genotype GWAS.

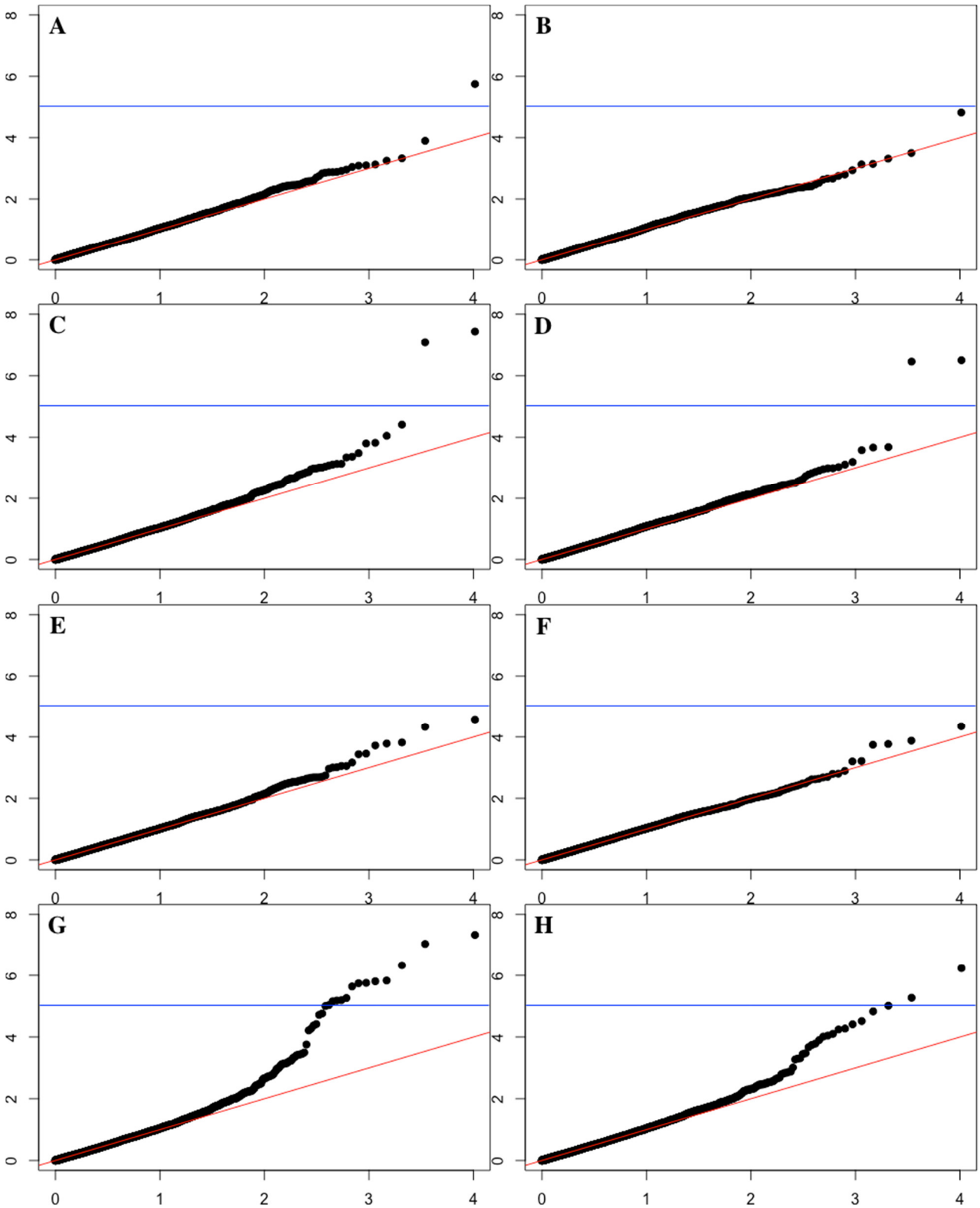


Figure 5.5. Q-Q plots of expected $-\log_{10}(p)$ value (x-axis) and observed $-\log_{10}(p)$ value (y-axis) for GWAS results of *Pyrenophora teres* f. *teres* isolates; NB50 and NB330. Blue horizontal line represents Bonferroni correction threshold. A: nb50a12 full genotype GWAS. B: nb50a12 reduced genotype GWAS. C: nb50a13 full genotype GWAS. D: nb50a13 reduced genotype GWAS. E: nb330s12 full genotype GWAS. F: nb330s12 reduced genotype GWAS. G: nb330s13 full genotype GWAS. H: nb330s13 reduced genotype GWAS.

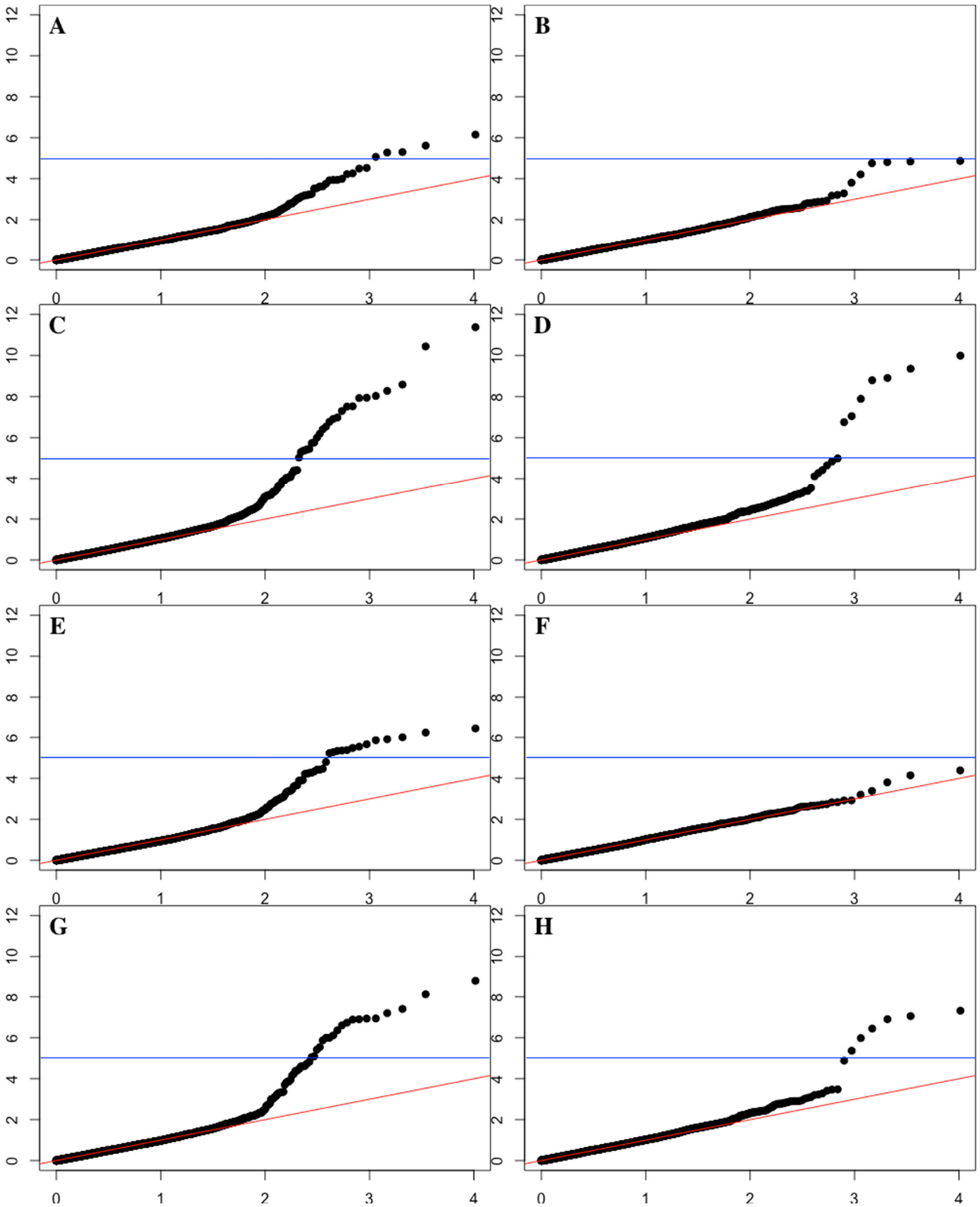


Figure 5.6. Q-Q plots of expected $-\log_{10}(p)$ value (x-axis) and observed $-\log_{10}(p)$ value (y-axis) for GWAS results of *Pyrenophora teres* f. *teres* isolate; NB73. Blue horizontal line represents Bonferroni correction threshold. A: nb73a12 full genotype GWAS. B: nb73a12 reduced genotype GWAS. C: nb73a13 full genotype GWAS. D: nb73a13 reduced genotype GWAS. E: nb73s12 full genotype GWAS. F: nb73s12 reduced genotype GWAS. G: nb73s13 full genotype GWAS. H: nb73s13 reduced genotype GWAS.

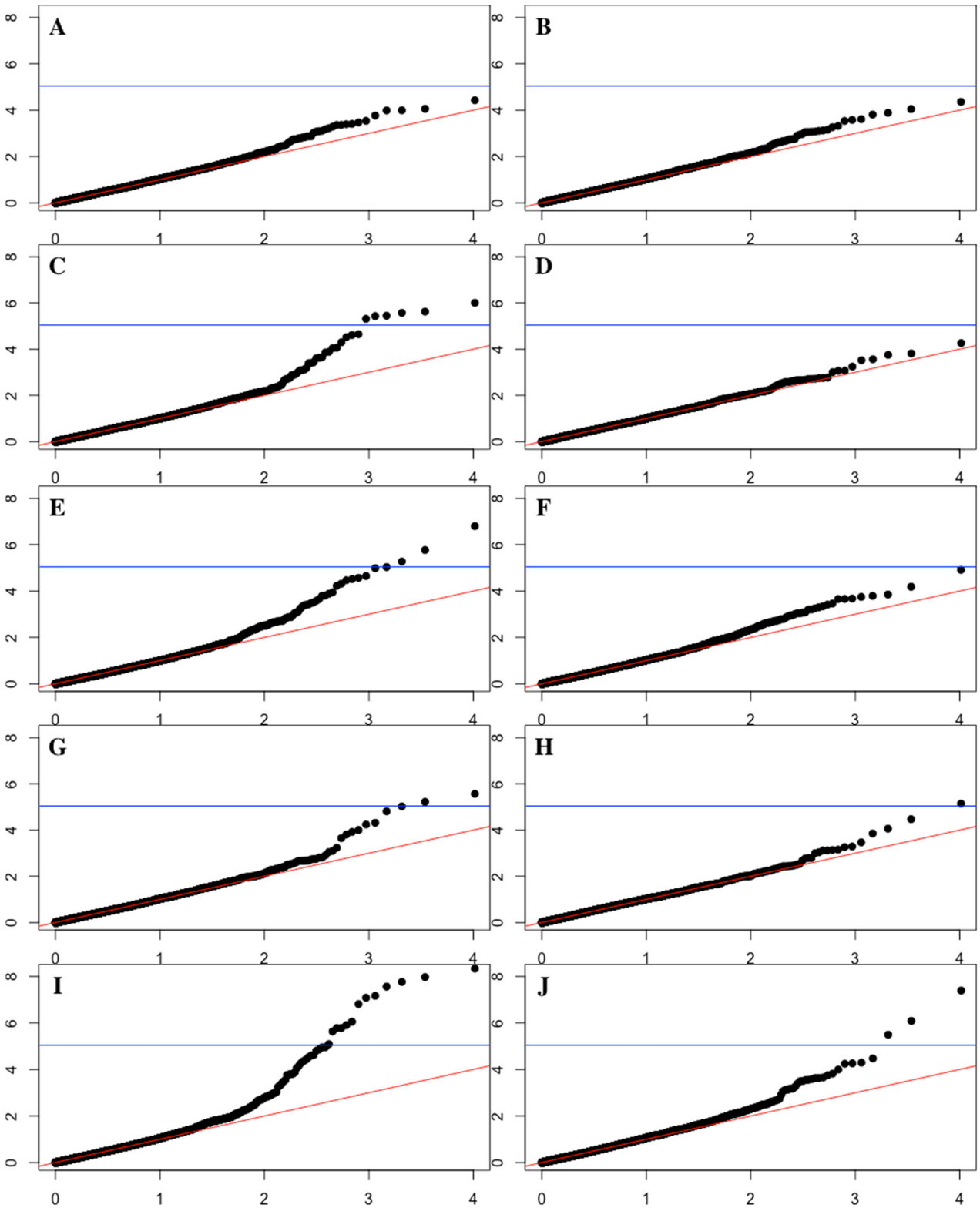


Figure 5.7. Q-Q plots of expected $-\log_{10}(p)$ value (x-axis) and observed $-\log_{10}(p)$ value (y-axis) for GWAS results of *Pyrenophora teres* f. *teres* isolate; NB85. Blue horizontal line represents Bonferroni correction threshold. A: nb85a12 full genotype GWAS. B: nb85a12 reduced genotype GWAS. C: nb85a13 full genotype GWAS. D: nb85a13 reduced genotype GWAS. E: nb85s12_1 full genotype GWAS. F: nb85s12_1 reduced genotype GWAS. G: nb85s12_2 full genotype GWAS. H: nb85s12_2 reduced genotype GWAS. I: nb85s13 full genotype GWAS. J: nb85s13 reduced genotype GWAS.

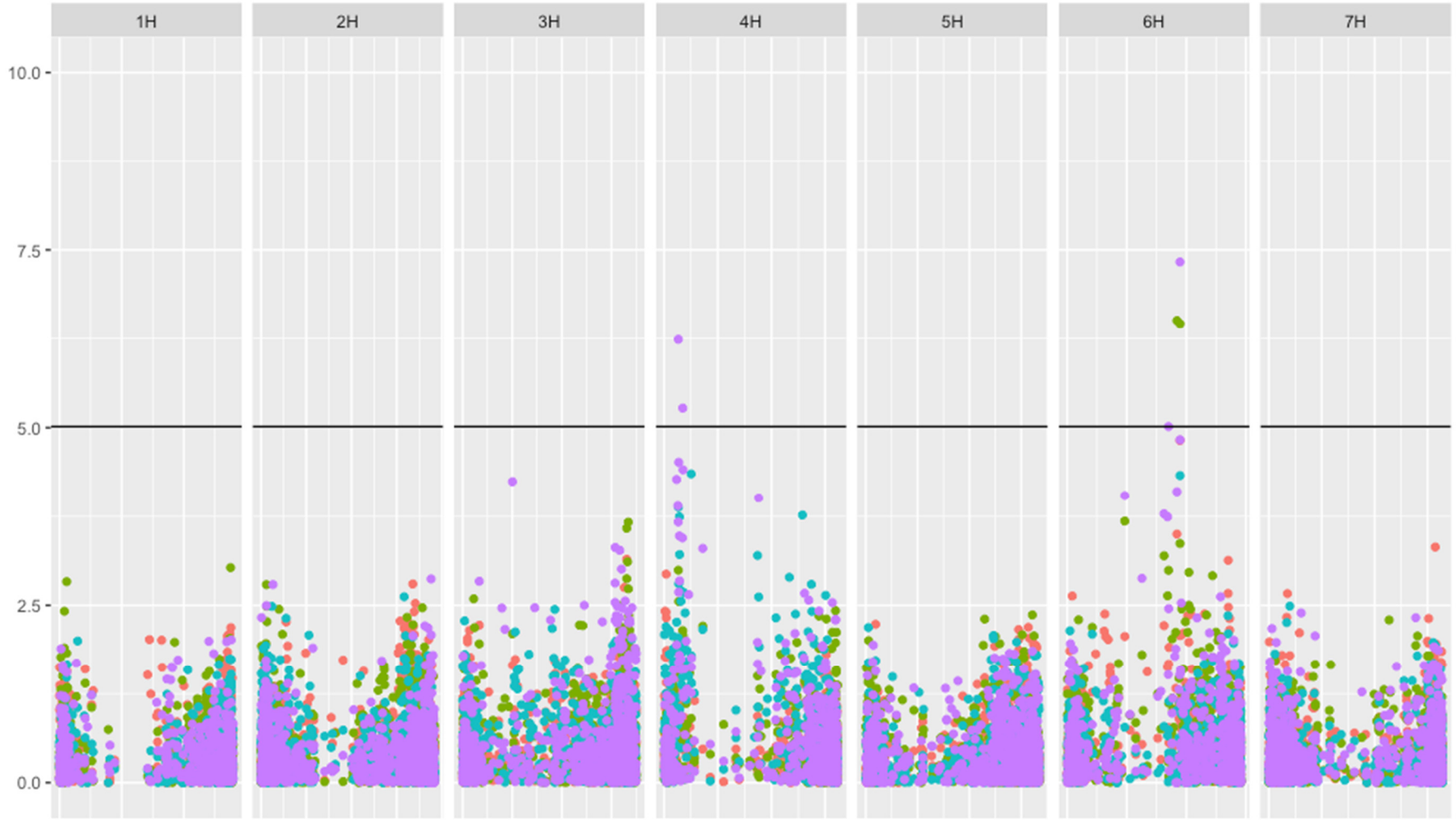


Figure 5.8. Manhattan plot of four reduced genotype GWAS results of *Pyrenophora teres* f. *teres* isolates NB50 and NB330 at two growth stages over two years. Chromosome physical position represented on x-axis and $-\log_{10}(p)$ value represented on y-axis. Bonferroni correction threshold represented by black horizontal line. Full genotype GWAS $-\log_{10}(p)$ values used for 3256608-45-C>G to represent genomic location on 6H. nb50a12 coloured red, nb50a13 coloured green, nb330s12 coloured blue, nb330s13 coloured purple.

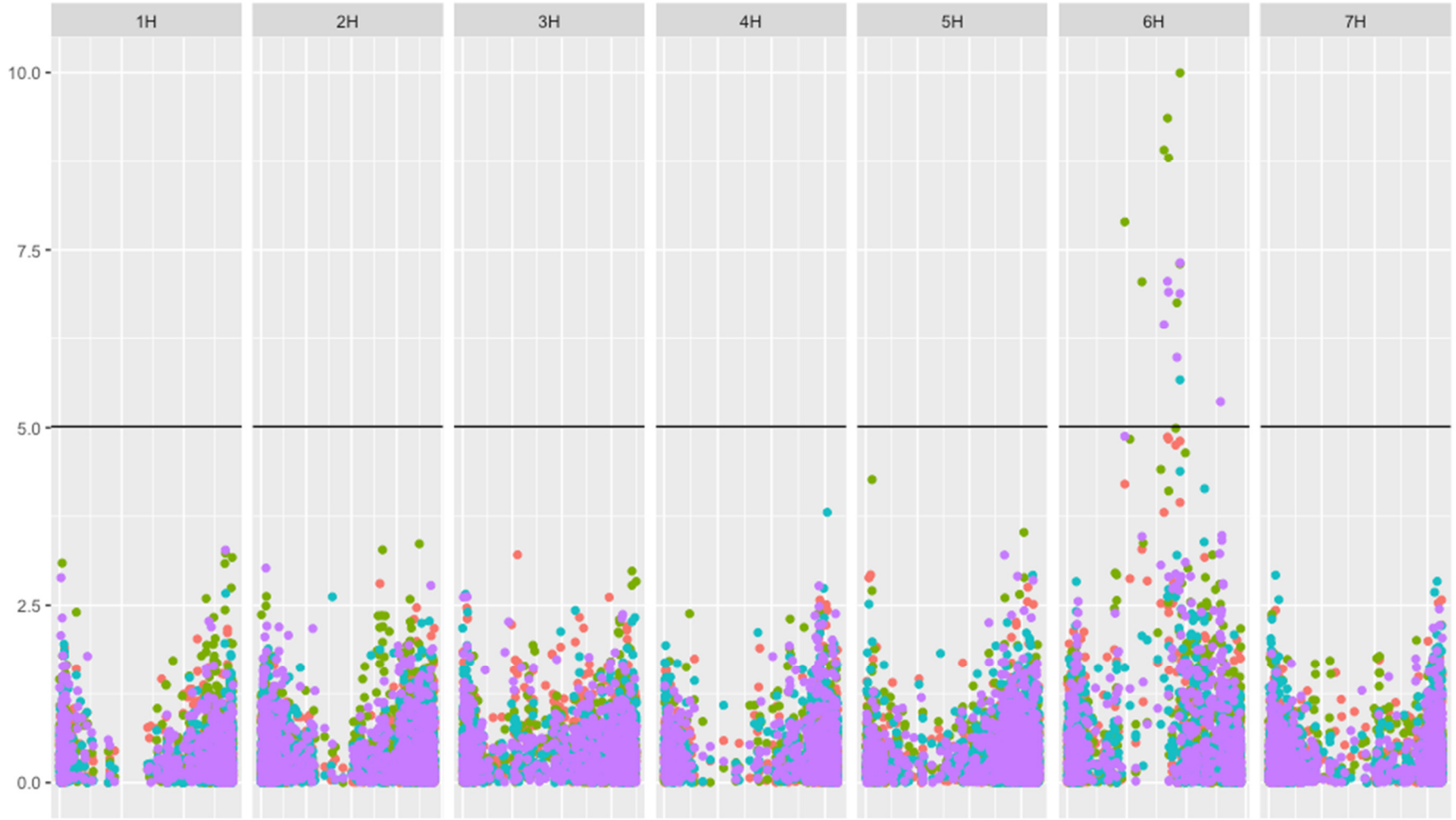


Figure 5.9. Manhattan plot of four reduced genotype GWAS results of *Pyrenophora teres* f. *teres* isolate NB73 at two growth stages over two years. Chromosome physical position represented on x-axis and $-\log_{10}(p)$ value represented on y-axis. Bonferroni correction threshold represented by black horizontal line. Full genotype GWAS $-\log_{10}(p)$ values used for 3256608-45-C>G to represent genomic location on 6H. nb73a12 coloured red, nb73a13 coloured green, nb73s12 coloured blue, nb73s13 coloured purple.

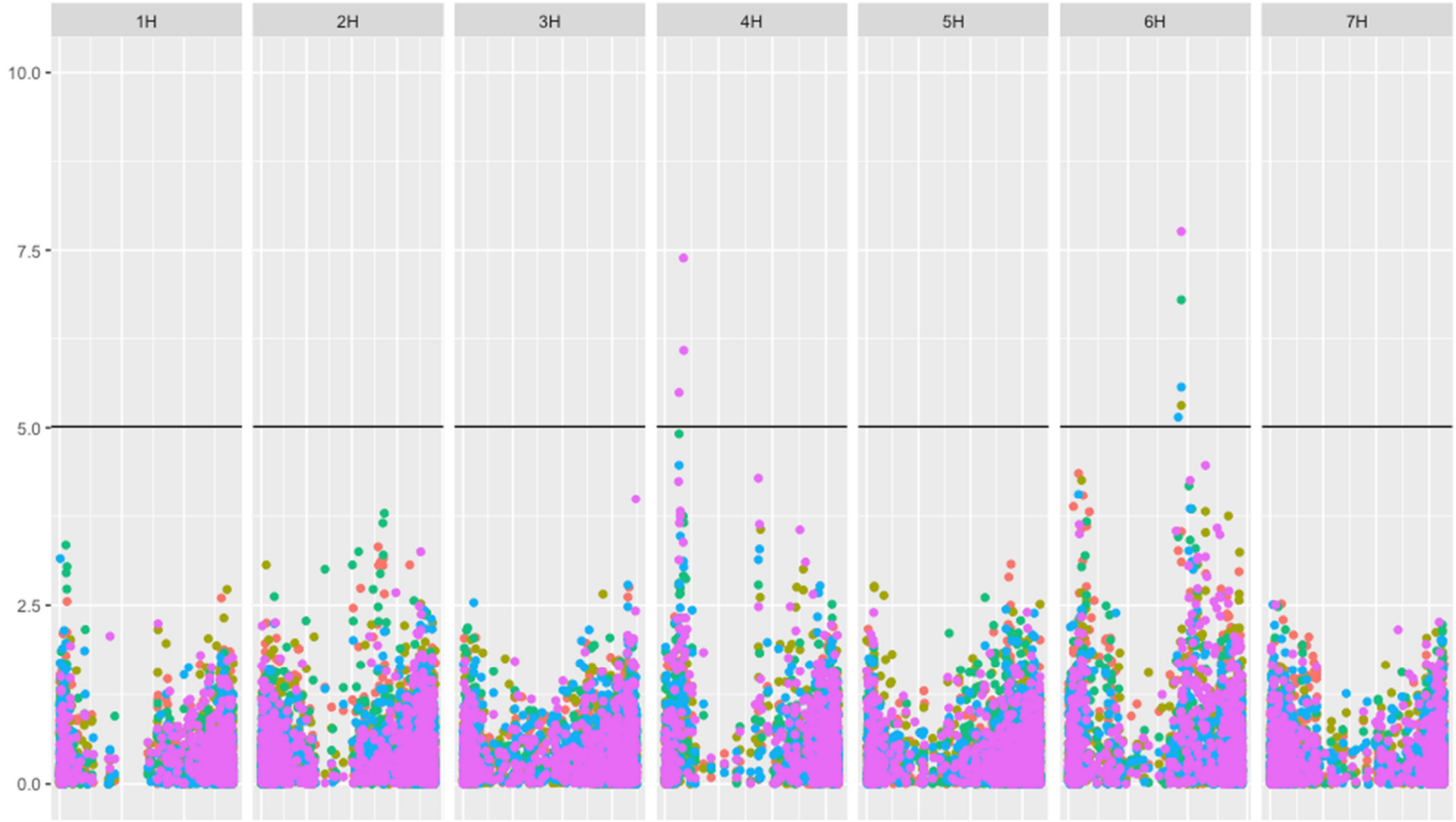


Figure 5.10. Manhattan plot of five reduced genotype GWAS results of *Pyrenophora teres* f. *teres* isolate NB85 at two growth stages over two years. Chromosome physical position represented on x-axis and $-\log_{10}(p)$ value represented on y-axis. Bonferroni correction threshold represented by black horizontal line. Full genotype GWAS $-\log_{10}(p)$ values used for 3256608-45-C>G to represent genomic location on 6H. nb85a12 coloured red, nb85a13 coloured olive, nb85s12_1 coloured green, nb85s12_2 coloured blue, nb85s13 coloured pink.

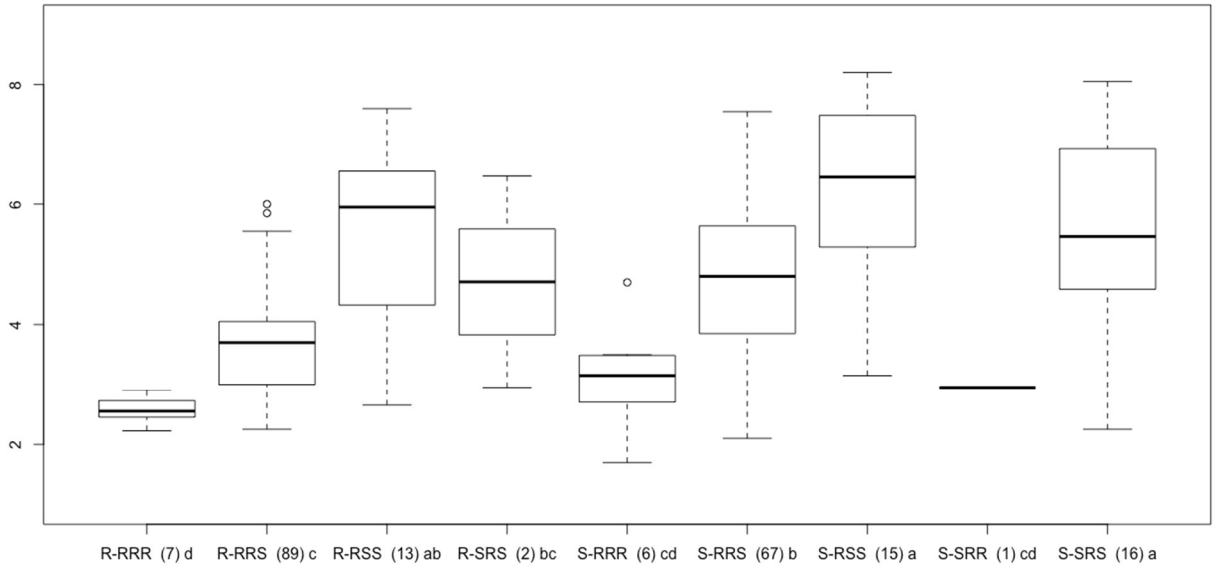


Figure 5.11. Box plot for combinations of SNP alleles for QTL significantly associated with infection response to *Pyrenophora teres* f. *teres* isolates NB50 and NB330. Box plot shows mean infection response across 2012 and 2013 seedling and adult datasets for 216 barley genotypes. SNP allele combination of QTL represented on x-axis and mean infection response represented on y-axis. Desirable allele is denoted by R, undesirable allele is denoted by S, number of genotypes for each combination in brackets and lower case letter indicates statistical significance between means. QTL order is *QRpt4H – QRpt6Hm QRpt6Hs Rpt5.f*. Desirable allele for *QRpt6Hs* is 'G'.

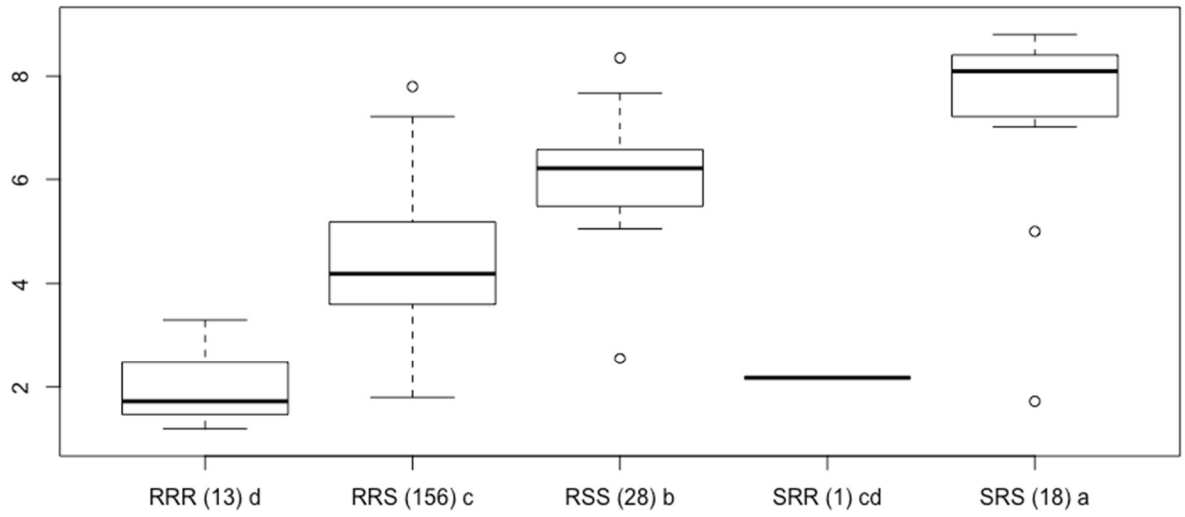


Figure 5.12. Box plot for combinations of SNP alleles for QTL significantly associated with infection response to *Pyrenophora teres* f. *teres* isolate NB73. Box plot shows mean infection response across 2012 and 2013 seedling and adult datasets for 216 barley genotypes. SNP allele combination of QTL represented on x-axis and mean infection response represented on y-axis. Desirable allele is denoted by R, undesirable allele is denoted by S, number of genotypes for each combination in brackets and lower case letter indicates statistical significance between means. QTL order is *QRpt6Hm QRpt6Hs Rpt5.f*. Desirable allele for *QRpt6Hs* is 'G'.

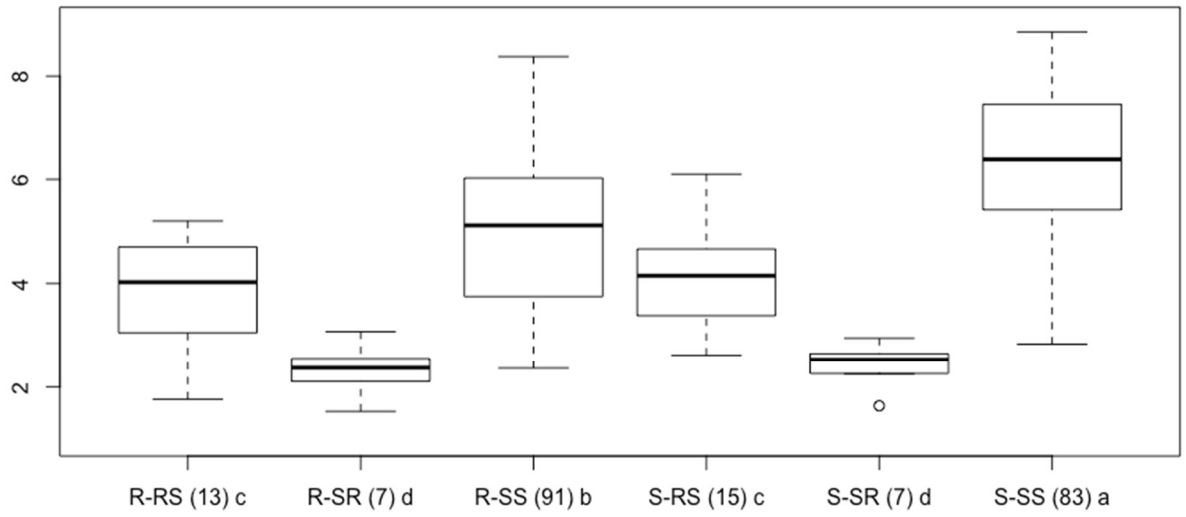


Figure 5.13. Box plot for combinations of SNP alleles for QTL significantly associated with infection response to *Pyrenophora teres* f. *teres* isolate NB85. Box plot shows mean infection response across 2012 and 2013 seedling and adult datasets for 216 barley genotypes. SNP allele combination of QTL represented on x-axis and mean infection response represented on y-axis. Desirable allele is denoted by R, undesirable allele is denoted by S, number of genotypes for each combination in brackets and lower case letter indicates statistical significance between means. QTL order is *QRpt4H – QRpt6Hs Rpt5.f*. Desirable allele for *QRpt6Hs* is 'T'.

Table 5.1 Mean 2012 and 2013 seedling and adult phenotype for 27 reference genotypes for four isolates of *Pyrenophora teres* f. *teres*.

Genotype	Pedigree	nb330s	nb50a	nb73s	nb73a	nb85s	nb85a
Baudin	Franklin/Stirling	7.5	3.3	2.5	3.4	9.2 ± 1.1	6.8
Buloke	Franklin/2*VB9104	4.0 ± 0.0	3.5 ± 0.9	4.7 ± 0.4	5.5 ± 1.7	3.5 ± 0.5	4.3 ± 0.1
CIho 11458	Isaria selection	4.0 ± 1.4	2.5 ± 0.0	6.2 ± 1.1	5.5 ± 1.5	2.8 ± 0.3	2.4 ± 1.5
Commander	Keel/Sloop//Galaxy	8.5 ± 0.0	4.7 ± 0.1	7.0 ± 0.7	5.3 ± 1.3	9.0 ± 1.3	7.2 ± 0.4
Corvette	Bonus/CIho 3576	NA	3.4 ± 0.3	NA	3.7 ± 1.7	NA	8.8 ± 0.2
Fitzroy	WI2808/Alexis	6.2 ± 0.3	3.4 ± 0.7	4.0 ± 0.0	2.9 ± 1.4	7.2 ± 1.8	4.0 ± 0.2
Gairdner	Onslow/TAS83-587	6.2 ± 0.3	4.4 ± 1.5	3.7 ± 0.4	2.8 ± 1.1	7.2 ± 2.4	5.2 ± 0.2
Grimmett (B)	Bussell/Zephyr	9.5 ± 0.0	6.2 ± 0.4	9.0 ± 0.0	8.9 ± 0.2	6.3 ± 1.6	4.9 ± 0.5
Grimmett (P)	Bussell/Zephyr	8.7 ± 1.1	6.4 ± 1.2	9.5 ± 0.7	8.3 ± 0.3	6.2 ± 1.3	4.5 ± 0.4
Grout	Cameo/Arupo	5.7 ± 0.3	3.2 ± 0.3	4.5 ± 1.4	3.3 ± 1.2	8.3 ± 1.0	7.3 ± 0.0
Hindmarsh	Dash/VB9409	5.0 ± 0.7	4.5 ± 0.3	3.2 ± 1.1	4.0 ± 0.2	7.0 ± 1.5	5.3 ± 0.6
Kaputar (B)	Arupo selection	6.5 ± 1.4	3.2 ± 0.3	4.0 ± 0.7	3.5 ± 0.6	4.4 ± 0.6	3.4 ± 0.1
Kaputar (P)	Arupo selection	5.5 ± 0.7	3.1 ± 0.1	4.5 ± 0.7	2.6 ± 0.6	5.5 ± 0.0	3.2 ± 0.7
Mackay	Cameo/Koru	5.7 ± 1.8	5.0 ± 0.4	4.4 ± 1.9	6.9 ± 1.5	7.8 ± 1.2	8.1 ± 0.6
Navigator	WI3788/WI3847	6.0	6.0	4.5	7.5	9.5	7.4
NRB06059	Mackay*2/WI3214	5.3 ± 0.5	5.3 ± 0.2	6.2 ± 1.1	7.0 ± 0.4	9.5 ± 0.5	8.4 ± 0.5
Prior	Chevallier selection	2.0 ± 0.0	2.2 ± 0.2	2.5 ± 0.7	2.8 ± 0.1	9.8 ± 0.3	8.4 ± 0.8
Shakira	Pewter/Prestige	9.5 ± 0.0	6.5 ± 0.0	8.0 ± 0.0	7.7 ± 0.0	5.5 ± 0.0	4.0 ± 0.0
Shepherd	Baronesse selection	4.5 ± 0.0	4.7 ± 1.3	8.2 ± 0.3	8.9 ± 0.1	6.3 ± 1.6	5.6 ± 0.3
Skiff	Abed Deba/WI2335//CD-28/WI2231	8.2 ± 1.8	7.1 ± 0.5	4.7 ± 1.1	6.6 ± 1.0	3.3 ± 0.6	3.6 ± 0.7
Skipper	Buloke/Commander//WI3786	6.6 ± 0.1	3.2 ± 0.2	3.0 ± 0.7	2.6 ± 0.8	4.8 ± 1.6	3.5 ± 0.4
Stirling	Dampier/A14//Piroline	NA	3.7 ± 0.5	NA	3.7 ± 0.3	NA	6.6 ± 1.0
VB0810	Gleam/WI3586//Yarra	9.0 ± 0.7	5.8 ± 1.1	8.5 ± 0.7	6.8 ± 0.5	8.2 ± 0.8	5.2 ± 0.7
VB0931	Hindmarsh sib/Fleet	3.0	3.4	3.0	3.8	5.1 ± 1.6	4.2
VB0933	Hindmarsh sib/Fleet	3.2 ± 0.3	2.7 ± 0.3	3.0 ± 0.0	3.0 ± 2.1	6.0 ± 1.5	5.2 ± 1.6
Vlamingh	WABAR0570/TR118	2.0	3.3	1.5	2.1	2.0 ± 1.4	3.2
WPG8412-9-2-1	BowmanTR473//Ellice/TR451	1.7 ± 0.4	1.6 ± 0.1	1.2 ± 0.3	1.6 ± 0.3	1.6 ± 0.8	1.8 ± 0.5

Table 5.2 *Pyrenophora teres* f. *teres* isolates used to phenotype Northern Region Barley breeding populations in 2012 and 2013.

Isolate	Cultivar	Location	State	Date Collected	Defining Virulence
NB50	Unknown	Gatton	Qld	26/07/1994	Grimmett, Skiff
NB73	Gilbert	Tansey	Qld	18/07/1995	Grimmett, CIho 11458, Shepherd
NB85	Cape	Gatton	Qld	22/09/1995	Cape, Corvette, Navigator, Prior
NB330	Binalong	Moree	NSW	9/10/2003	Grimmett, Skiff

Table 5.3 Timeline of field experiments for phenotyping of Northern Region Barley breeding populations in 2012 and 2013 for three isolates of *Pyrenophora teres* f. *teres.*

Activity	Isolate	2012	2013
Sow pre-season increase block	NB50	3/05/2012	12/04/2013
	NB73	23/04/2012	12/04/2013
	NB85	23/04/2012	12/04/2013
Inoculate pre-season increase block	NB50	2/06/2012	28/05/2013
	NB73	4/06/2012	31/05/2013
	NB85	3/06/2012	30/05/2013
Sow disease nursery spreader rows	NB50	15/06/2012	6/06/2013
	NB73	18/06/2012	6/06/2013
	NB85	15/06/2012	6/06/2013
Sow experimental plots	NB50	9/07/2012	27/06/2013
	NB73	8/07/2012	8/07/2013
	NB85	9/07/2012	26/06/2013
Inoculate disease nursery spreader rows	NB50	13/08/2012	8/08/2013
	NB73	14/08/2012	8/08/2013
	NB85	14/08/2012	8/08/2013
Score experimental plots	NB50	31/10/2012	16/10/2013
	NB73	19/10/2012	25/10/2013
	NB85	24/10/2012	21/10/2013

Table 5.4 Summary of phenotypic range and heritability for 2012 and 2013 Northern Region Barley populations assayed with four *Pyrenophora teres* f. *teres* isolates at seedling and adult stage.

Dataset	Minimum	Mean ± StDev	Maximum	h^2 [a]
nb50a12	1.6	4.08 ± 1.03	7.4	0.92
nb50a13	1.7	3.80 ± 1.25	8.0	0.93
nb330s12	1.0	4.77 ± 2.13	10.5	0.99
nb330s13	1.5	5.26 ± 1.94	9.5	0.99
nb73a12	1.4	4.69 ± 2.09	9.1	0.99
nb73a13	1.8	5.43 ± 1.61	9.6	0.99
nb73s12	1.0	5.12 ± 2.32	10	0.99
nb73s13	1.0	4.65 ± 1.95	10	0.99
nb85a12	1.3	5.05 ± 1.81	9.0	0.99
nb85a13	1.5	4.96 ± 1.35	8.7	0.99
nb85s12_1	1.5	6.64 ± 2.35	10	0.99
nb85s12_2	1.0	4.46 ± 2.12	10	0.99
nb85s13	1.0	5.82 ± 2.11	10	0.99

[a] Narrow sense heritability estimated from EMMA kinship matrix and phenotype data

Table 5.5 Mean infection response and standard deviation of 13 phenotyping experiments for 31 genotypes that carried desirable allele for 3256608-45:C>G (*Rpt5.f*), which was associated with resistance to multiple isolates *Pyrenophora teres* f. *teres* at seedling and adult growth stages.

Dataset	Mean ± StDev
nb50a12	3.35 ± 0.69
nb330s12	2.44 ± 1.23
nb73a12	2.28 ± 1.09
nb73s12	1.79 ± 0.69
nb85a12	3.17 ± 0.81
nb85s12_1	3.41 ± 1.31
nb85s12_2	1.79 ± 0.58
nb50a13	2.84 ± 0.6
nb330s13	2.68 ± 0.82
nb73a13	3.16 ± 0.79
nb73s13	1.52 ± 1.01
nb85a13	3.06 ± 0.71
nb85s13	2.72 ± 1.09

Table 5.6 Comparative summary of –log10(*p*) value and SNP effect from full and reduced genotype GWAS of 13 datasets of four *Pyrenophora teres* f. *teres* isolates over two years.

		Reduced genotype GWAS significant markers											Full genotype GWAS significant markers																											
	SNP marker [a]	3255709-40:A>G	3257855-10:A>G	3256237-67:A>G	3257954-50:G>A	3434214-43:A>T	3256458-52:T>C	3255777-67:T>G	3254817-15:C>A	3257446-28:G>T	3262096-64:C>T	3256608-45:C>G	3259111-21:A>C	3398663-60:C>T	3254735-54:A>C	3257608-6:A>G	3259058-41:G>A	3259255-17:C>T	4175123-58:C>A	3256765-18:T>C	3262659-31:C>G	3255625-14:C>T	3434176-13:T>C	3432738-29:G>A	3432352-13:G>T	3254663-15:T>A	3255134-29:C>A	3254978-54:G>A	4171893-67:C>T	3921095-18:T>C	3257464-10:T>A	3261554-30:C>T	3259228-14:G>C	3258275-14:G>C	3263983-33:G>T	3262437-68:C>T	4615142 41
	Position (bp)	53032932	69382105	70434783	193444571	251009458	325194805	337179867	340307078	368527587	378974018	378772740	210766011	268997406	314450784	361531190	364356525	375529364	380193974	382482733	383141804	384803137	384884765	386021835	388486267	396127146	397034107	404316342	408391789	417070659	417821936	422773531	424801489	449601223	450717343	459335236	460084925	460088004	461514241
	Chr	4H	4H	4H	6H	6H	6H	6H	6H	6H	6H	6H	6H	6H	6H	6H	6H	6H	6H	6H	6H	6H	6H	6H	6H	6H	6H	6H	6H	6H	6H	6H	6H	6H	6H	6H	6H	6H	6H
	Ref. SNP [b]	G	A	A	G	A	T	T	C	G	C	G	A	T	C	G	G	T	A	C	C	C	T	A	T	A	C	A	C	T	A	T	C	T	C	C	C	T	T
Full genotype GWAS	−log10(*p*) value																																						
	nb50a12	1.26	0.65	0.78	2.40	0.82	2.46	2.07	2.12	3.90	5.75^ac	2.07	1.10	3.25	0.85	0.77	1.57	2.39	1.84	2.30	2.22	2.41	3.05	1.39	1.57	2.32	2.75	1.21	0.99	0.72	0.92	0.48	0.44	1.20	1.21	0.97	0.22	1.10	2.61
	nb50a13	2.37	2.15	1.33	3.82	1.99	2.46	2.86	3.01	7.44^	7.09^	3.37	1.25	0.15	0.95	1.75	1.79	3.14	2.78	2.96	1.99	4.41	1.66	1.48	2.30	2.19	3.49	0.90	1.03	2.43	2.71	1.28	1.83	2.45	4.05	3.05	0.04	1.11	1.55
	nb330s12	3.82	1.76	1.87	1.10	0.30	0.60	1.05	1.16	1.63	1.79	4.33	0.40	3.05	0.09	2.96	0.17	3.05	3.72	3.78	2.64	3.01	2.43	2.27	2.40	4.58	2.13	0.16	0.02	2.70	2.53	2.74	1.62	1.67	2.26	2.68	0.16	2.60	1.13
	nb330s13	5.19^	5.16^	3.42	4.42	3.50	3.20	4.37	5.26^	5.00	5.76^	7.33^	1.68	1.14	1.78	5.64^	2.19	4.27	7.04^	5.03^	5.83^	4.72	2.45	2.13	3.76	3.46	3.15	0.03	0.38	5.74^	6.33^	2.27	4.21	3.44	5.18^	5.81^	0.68	2.97	1.19
	nb73a12	0.20	0.86	0.70	4.22	3.52	3.19	5.30^	5.28^	2.14	6.16^	3.94	3.62	5.62^	3.21	2.77	5.07^	3.99	3.23	3.76	4.53	2.93	1.22	4.26	3.54	3.93	3.15	0.03	0.03	1.79	1.97	1.33	1.47	1.75	2.07	1.36	0.22	1.79	4.49
	nb73a13	0.32	0.36	0.02	8.59^	8.28^	6.98^	10.45^	7.95^	7.52^	11.38^	7.30^	6.41^	1.93	5.45^	6.92^	5.30^	6.19^	8.04^	6.00^	3.87	6.78^	5.03^	5.36^	4.05	5.43^	7.93^	0.16	0.01	5.77^	7.53^	3.18	6.54^	5.38^	5.74^	4.25	0.02	2.05	3.11
	nb73s12	0.05	0.71	0.23	1.96	2.28	1.24	3.06	3.18	4.28	5.91^	5.67^	1.54	5.28^	1.45	4.42	3.35	5.34^	4.80	6.44^	6.24^	4.47	1.03	6.01^	5.86^	4.43	4.23	0.05	0.29	5.24^	5.49^	5.36^	4.34	3.09	3.90	3.89	0.01	5.38^	5.55^
	nb73s13	0.54	0.84	0.02	5.54^	4.62	5.42^	8.12^	6.12^	6.94^	8.78^	6.89^	2.45	1.99	4.16	7.20^	3.26	6.74^	7.41^	6.90^	6.36^	6.60^	2.75	4.39	5.06^	4.60	6.00^	0.10	0.07	6.00^	6.93^	3.31	5.87^	4.74	5.09^	4.47	0.03	2.68	1.46
	nb85a12	2.25	1.21	1.51	0.17	0.05	0.19	0.16	0.27	2.82	2.74	3.11	0.42	0.82	0.28	1.64	0.53	1.96	2.31	2.00	1.50	0.77	0.46	1.96	1.86	2.60	0.07	2.75	2.83	0.64	0.80	1.35	1.20	0.09	0.89	1.56	0.70	0.42	0.34
	nb85a13	1.78	1.97	0.71	0.46	1.26	0.49	0.40	0.47	0.10	0.20	5.31^	0.95	2.70	1.77	4.52	0.04	5.63^	5.43^	3.88	2.87	3.09	0.66	5.45^	6.01^	3.08	0.63	2.08	1.86	2.88	3.11	3.86	4.61	0.04	2.00	5.57^	4.04	0.07	1.08
	nb85s12_1	4.32	2.48	3.39	0.19	0.03	0.09	0.08	0.01	2.27	0.16	6.80^	0.02	2.07	0.41	5.03^	0.07	4.52	4.65	5.77^	3.88	3.33	0.58	4.47	4.22	4.98	0.18	5.27^	4.57	2.51	2.49	3.06	3.24	0.60	1.85	3.51	1.37	0.88	0.85
	nb85s12_2	3.92	2.73	2.68	0.40	0.46	0.20	0.38	0.32	4.00	0.79	5.57^	0.03	1.52	0.48	2.75	0.19	3.05	4.82	5.22^	3.10	2.26	0.19	4.31	3.65	2.81	0.25	4.24	5.03^	1.60	1.53	2.01	2.36	0.33	2.10	2.67	1.13	0.59	1.23
	nb85s13	4.62	7.17^	4.96	0.17	1.20	0.29	0.45	0.51	0.83	0.37	7.76^	0.70	1.88	1.91	8.33^	0.40	4.44	7.97^	5.90^	5.09^	2.69	0.27	5.78^	4.80	2.64	0.57	4.86	6.81^	5.63^	5.78^	4.95	7.08^	0.43	3.01	7.56^	6.05^	1.67	0.85
	Phenotypic effect of SNP																																						
	nb50a12	-0.24	-0.14	-0.18	-0.49	-0.21	-0.45	-0.45	-0.47	-0.51	-0.58	-0.44	-0.28	-0.42	-0.22	-0.20	-0.31	-0.41	-0.39	-0.44	-0.38	-0.40	-0.36	-0.31	-0.32	-0.41	-0.33	0.23	0.19	-0.20	-0.24	-0.15	-0.14	-0.25	-0.29	-0.24	0.08	-0.26	-0.35
	nb50a13	-0.35	-0.31	-0.25	-0.66	-0.46	-0.49	-0.61	-0.65	-0.91	-0.64	-0.71	-0.30	-0.04	-0.27	-0.35	-0.33	-0.48	-0.61	-0.58	-0.41	-0.66	-0.26	-0.42	-0.40	-0.41	-0.43	0.19	0.22	-0.56	-0.60	-0.34	-0.45	-0.46	-0.71	-0.60	-0.02	-0.29	-0.28
	nb330s12	-0.94	-0.55	-0.62	-0.58	-0.19	-0.34	-0.58	-0.63	-0.58	-0.55	-1.39	-0.26	-0.81	-0.07	-0.96	-0.12	-0.97	-1.22	-1.21	-0.82	-0.89	-0.60	-0.85	-0.84	-1.21	-0.58	0.10	-0.01	-0.98	-0.95	-0.98	-0.73	-0.63	-0.87	-0.96	0.11	-0.93	-0.39
	nb330s13	-0.79	-0.73	-0.63	-1.01	-0.93	-0.80	-1.11	-1.28	-1.02	-0.80	-1.60	-0.52	-0.30	-0.57	-1.01	-0.52	-0.82	-1.52	-1.13	-1.10	-0.97	-0.47	-0.75	-0.77	-0.76	-0.57	-0.01	-0.15	-1.33	-1.44	-0.70	-1.07	-0.81	-1.17	-1.25	-0.27	-0.77	-0.33
	nb73a12	0.10	0.30	0.28	-1.16	-0.91	-0.88	-1.37	-1.39	-0.60	-1.00	-1.12	-1.00	-0.99	-0.89	-0.80	-1.09	-0.97	-0.95	-1.03	-1.01	-0.77	-0.33	-1.07	-0.91	-0.96	-0.62	0.02	-0.02	-0.63	-0.68	-0.52	-0.58	-0.55	-0.70	-0.53	0.13	-0.62	-0.81
	nb73a13	-0.10	-0.10	-0.01	-1.19	-1.21	-1.02	-1.48	-1.30	-1.03	-0.95	-1.28	-0.92	-0.33	-0.89	-0.91	-0.71	-0.82	-1.31	-1.00	-0.69	-0.96	-0.58	-1.05	-0.65	-0.80	-0.78	0.06	0.00	-1.06	-1.26	-0.69	-1.11	-0.85	-0.99	-0.84	-0.01	-0.49	-0.48
	nb73s12	-0.03	0.31	0.14	-0.88	-0.85	-0.59	-1.20	-1.24	-1.10	-1.18	-1.69	-0.69	-1.16	-0.65	-1.26	-1.03	-1.40	-1.47	-1.72	-1.42	-1.17	-0.36	-1.59	-1.49	-1.23	-0.90	-0.03	-0.15	-1.49	-1.55	-1.51	-1.37	-0.96	-1.25	-1.23	-0.01	-1.49	-1.07
	nb73s13	-0.19	-0.24	-0.01	-1.17	-1.10	-1.11	-1.61	-1.41	-1.25	-1.03	-1.56	-0.66	-0.43	-0.96	-1.18	-0.67	-1.08	-1.58	-1.37	-1.17	-1.20	-0.51	-1.18	-0.93	-0.91	-0.84	0.05	-0.03	-1.38	-1.53	-0.89	-1.31	-0.99	-1.17	-1.08	-0.02	-0.73	-0.38
	nb85a12	-0.45	-0.28	-0.36	0.09	-0.03	0.09	0.09	0.14	0.54	0.46	-0.74	0.18	-0.22	-0.12	-0.44	0.19	-0.48	-0.59	-0.53	-0.38	-0.24	-0.13	-0.51	-0.47	-0.57	0.03	-0.51	-0.48	-0.24	-0.28	-0.40	-0.39	-0.04	-0.31	-0.44	-0.24	-0.17	-0.11
	nb85a13	-0.26	-0.26	-0.14	-0.14	-0.30	-0.14	-0.14	-0.17	-0.04	-0.05	-0.83	-0.22	-0.32	-0.36	-0.56	0.01	-0.60	-0.82	-0.61	-0.46	-0.48	-0.12	-0.83	-0.64	-0.45	-0.12	-0.30	-0.29	-0.55	-0.59	-0.61	-0.71	0.01	-0.41	-0.77	-0.53	-0.03	-0.19
	nb85s12_1	-0.96	-0.66	-0.86	-0.15	0.02	-0.07	-0.07	-0.01	0.69	0.08	-1.75	0.01	-0.60	-0.25	-1.26	0.05	-1.18	-1.33	-1.49	-0.99	-0.91	-0.22	-1.24	-1.14	-1.22	-0.09	-1.12	-0.94	-0.89	-0.89	-1.00	-1.07	-0.30	-0.74	-1.08	-0.57	-0.44	-0.31
	nb85s12_2	-0.82	-0.63	-0.67	-0.24	-0.23	-0.12	-0.24	-0.21	0.88	0.27	-1.39	0.02	-0.45	-0.25	-0.79	0.11	-0.83	-1.22	-1.27	-0.78	-0.65	-0.08	-1.09	-0.94	-0.78	-0.11	-0.88	-0.90	-0.60	-0.59	-0.69	-0.79	-0.17	-0.71	-0.82	-0.45	-0.29	-0.35
	nb85s13	-0.68	-0.81	-0.72	-0.09	-0.44	-0.14	-0.23	-0.26	0.30	0.12	-1.52	-0.26	-0.38	-0.55	-1.16	0.15	-0.77	-1.50	-1.14	-0.93	-0.64	-0.09	-1.25	-0.82	-0.60	-0.17	-0.73	-0.91	-1.21	-1.25	-1.03	-1.33	-0.18	-0.78	-1.34	-0.99	-0.49	-0.24
Reduced genotype GWAS	−log10(*p*) value																																						
	nb50a12	1.23	0.85	0.72	2.06	0.54	2.21	1.63	1.67	3.50	4.82	0.64	0.74	1.56	0.33	0.14	1.04	0.90	0.38	0.83	0.75	0.92	2.09	0.13	0.42	0.89	1.41	2.35	1.98	0.06	0.02	0.32	0.42	0.54	0.36	0.04	0.58	0.31	2.38
	nb50a13	2.99	2.15	2.07	3.68	1.80	3.20	2.63	2.99	6.51^	6.46^	0.62	0.95	0.23	0.75	0.24	2.12	1.47	0.39	1.05	0.57	2.45	0.47	0.08	0.80	0.60	2.27	2.12	2.50	0.41	0.50	0.09	0.07	0.93	1.85	0.97	0.16	0.27	1.09
	nb330s12	3.88	1.96	1.60	0.78	0.12	0.79	0.66	0.74	1.01	1.12	0.04	0.05	0.39	0.20	0.08	0.02	0.24	0.11	0.05	0.30	0.18	0.96	0.42	0.01	1.03	0.60	0.88	0.64	0.59	0.50	0.45	0.00	0.52	0.40	0.66	1.05	1.37	0.37
	nb330s13	6.24^	5.27^	4.41	4.05	2.88	3.79	3.74	5.02^	4.10	4.83	1.81	0.96	0.15	1.28	1.78	1.88	1.31	1.55	0.57	2.53	1.14	0.49	0.20	1.00	0.58	1.15	1.09	0.55	1.33	1.42	0.19	0.44	0.92	1.39	1.79	0.12	1.01	0.48
	nb73a12	0.27	0.62	0.81	4.21	3.29	3.80	4.87	4.84	1.55	4.81	0.50	2.87	2.84	2.53	0.43	4.76	1.04	0.15	0.59	1.71	0.35	0.15	1.22	0.82	1.02	1.42	0.65	0.62	0.10	0.14	0.13	0.15	0.44	0.51	0.17	0.43	0.40	3.17
	nb73a13	0.25	0.12	0.05	7.89^	7.05^	8.91^	9.36^	8.80^	6.76^	9.99^	2.15	4.84	0.85	4.42	2.67	4.99	2.14	2.08	1.26	1.15	2.81	2.38	0.45	0.75	1.77	4.65	1.85	1.67	0.85	1.96	0.06	1.30	2.48	1.81	0.39	0.70	0.94	1.60
	nb73s12	0.13	0.69	0.56	1.62	2.07	1.47	2.62	2.73	3.20	4.39	0.67	1.08	2.01	0.82	0.81	2.62	1.27	0.42	1.64	2.45	0.77	0.01	1.55	1.89	0.67	1.74	0.58	0.28	2.10	2.38	2.29	1.40	1.06	1.22	1.05	0.25	3.39	4.15
	nb73s13	0.64	0.65	0.09	4.88	3.47	6.45^	7.06^	6.91^	5.98^	7.32^	1.73	1.32	0.82	3.06	2.76	2.94	2.71	1.60	2.08	2.91	2.44	0.63	0.21	1.58	1.17	3.10	1.69	1.25	1.28	1.59	0.01	1.01	1.88	1.50	0.56	0.57	1.36	0.49
	nb85a12	2.19	1.23	1.56	0.29	0.08	0.38	0.35	0.47	3.27	3.53	0.76	0.94	0.27	0.06	0.46	0.64	0.39	0.21	0.18	0.27	0.33	0.09	0.31	0.29	0.76	0.77	1.68	1.80	0.30	0.18	0.08	0.02	0.37	0.00	0.23	0.29	0.22	0.08
	nb85a13	1.89	1.87	1.10	0.07	0.54	0.41	0.04	0.28	0.19	0.16	0.97	0.28	1.55	1.06	1.00	0.38	1.99	0.68	0.25	0.72	0.48	0.43	0.42	1.77	0.41	0.42	0.63	0.32	0.62	0.30	0.09	0.14	2.10	0.42	1.00	2.71	1.06	0.06
	nb85s12_1	4.91	2.92	3.66	0.01	0.22	0.00	0.15	0.25	3.46	0.81	2.57	0.33	0.02	0.05	1.72	0.45	1.01	0.71	1.66	1.08	0.38	0.22	1.00	0.93	1.43	0.91	4.18	3.41	0.41	0.21	0.37	0.67	0.35	0.11	0.82	0.76	0.23	0.04
	nb85s12_2	4.47	3.12	3.04	0.20	0.33	0.18	0.17	0.12	5.15^	1.63	1.01	0.30	0.22	0.16	0.23	0.51	0.25	0.83	1.13	0.64	0.04	0.73	0.72	0.57	0.13	0.56	3.26	3.85	0.09	0.03	0.29	0.50	0.35	0.36	0.68	0.49	0.12	0.31
	nb85s13	5.49^	7.39^	6.08^	0.00	0.64	0.49	0.10	0.30	1.68	1.00	2.28	0.15	0.53	1.24	3.54	0.66	1.00	1.98	1.30	1.90	0.03	1.17	0.87	0.88	0.03	0.45	2.61	4.26	0.70	0.66	0.53	1.44	0.96	0.06	2.29	4.47	0.14	0.10
	Phenotypic effect of SNP																																						
	nb50a12	-0.25	-0.19	-0.19	-0.47	-0.16	-0.46	-0.41	-0.43	-0.51	-0.54	-0.20	-0.22	-0.33	-0.12	0.07	-0.25	-0.29	-0.25	-0.37	-0.23	-0.28	-0.31	-0.07	-0.17	-0.30	-0.24	0.40	0.33	0.03	-0.01	0.15	0.20	-0.17	-0.15	-0.02	0.18	-0.13	-0.39
	nb50a13	-0.41	-0.31	-0.33	-0.65	-0.43	-0.59	-0.58	-0.65	-0.85	-0.62	-0.19	-0.25	0.07	-0.23	-0.10	-0.37	-0.34	-0.26	0.22	-0.20	-0.56	-0.12	-0.03	-0.25	-0.20	-0.35	0.36	0.41	-0.23	-0.27	0.05	-0.05	-0.27	-0.54	-0.37	0.06	-0.11	-0.23
	nb330s12	-0.95	-0.60	-0.57	-0.46	-0.09	-0.43	-0.41	-0.45	-0.42	-0.40	-0.03	-0.04	-0.23	0.14	-0.08	-0.01	-0.20	0.17	-0.07	-0.20	-0.14	-0.34	0.36	-0.01	-0.60	-0.25	0.39	0.28	-0.44	-0.39	-0.36	0.00	-0.30	-0.30	-0.47	0.50	-0.74	-0.19
	nb330s13	-0.87	-0.74	-0.72	-0.95	-0.81	-0.91	-1.00	-1.22	-0.90	-0.71	-0.56	-0.35	-0.06	-0.45	-0.58	-0.47	-0.44	-0.95	0.19	-0.75	-0.47	-0.17	-0.10	-0.40	-0.27	-0.31	0.32	0.20	-0.73	-0.78	0.15	-0.32	-0.38	-0.62	-0.76	-0.07	-0.41	-0.18
	nb73a12	0.13	0.24	0.32	-1.17	-0.87	-1.02	-1.29	-1.31	-0.48	-0.86	-0.27	-0.87	-0.76	-0.78	-0.29	-1.05	-0.52	-0.18	-0.46	-0.64	-0.22	-0.07	-0.68	-0.43	-0.53	-0.39	0.27	0.24	-0.08	-0.12	0.11	0.14	-0.23	-0.32	0.14	0.23	-0.26	-0.74
	nb73a13	-0.08	-0.04	-0.02	-1.08	-1.05	-1.15	-1.32	-1.31	-0.92	-0.84	-0.48	-0.75	-0.19	-0.74	-0.58	-0.65	-0.46	-0.89	0.26	-0.35	-0.64	-0.38	0.14	-0.25	-0.44	-0.57	0.35	0.34	-0.41	-0.73	0.04	-0.53	-0.55	-0.57	-0.20	0.21	-0.30	-0.32
	nb73s12	0.08	0.31	0.29	-0.77	-0.79	-0.67	-1.06	-1.11	-0.90	-0.98	-0.39	-0.55	-0.74	-0.45	-0.53	-0.87	-0.71	-0.52	-1.11	-0.92	-0.46	0.01	-0.94	-0.90	-0.46	-0.53	0.29	0.16	-1.07	-1.18	-1.15	-0.88	-0.52	-0.70	-0.67	0.18	-1.34	-0.99
	nb73s13	-0.20	-0.19	-0.04	-1.04	-0.89	-1.22	-1.43	-1.45	-1.10	-0.89	-0.53	-0.43	-0.24	-0.77	-0.75	-0.61	-0.68	-0.95	0.46	-0.80	-0.75	-0.20	0.10	-0.53	-0.43	-0.58	0.42	0.36	-0.70	-0.82	-0.01	-0.57	-0.59	-0.64	-0.34	0.23	-0.49	-0.18
	nb85a12	-0.47	-0.31	-0.40	0.15	0.04	0.17	0.18	0.22	0.62	0.57	-0.29	0.34	0.12	-0.03	-0.24	0.23	-0.20	-0.20	-0.14	-0.14	0.17	0.03	-0.20	-0.16	-0.34	0.20	-0.41	-0.40	0.18	0.12	-0.06	-0.02	0.16	0.00	-0.14	-0.13	0.13	-0.04
	nb85a13	-0.26	-0.24	-0.18	-0.03	-0.16	-0.12	0.02	-0.11	0.06	0.04	-0.22	-0.08	-0.22	-0.24	-0.24	0.09	-0.35	-0.33	0.06	-0.20	-0.15	0.09	0.11	-0.35	-0.13	0.09	-0.13	-0.08	0.26	0.15	-0.04	-0.08	0.39	0.16	-0.32	-0.40	0.26	-0.02
	nb85s12_1	-1.05	-0.74	-0.92	-0.01	0.14	0.00	0.12	0.19	0.87	0.31	-0.90	0.21	0.01	0.04	-0.82	0.24	-0.56	-0.71	-1.04	-0.50	-0.25	0.11	-0.65	-0.52	-0.71	0.32	-1.00	-0.82	-0.32	-0.19	-0.30	-0.49	0.21	-0.10	-0.52	-0.39	0.19	-0.02
	nb85s12_2	-0.92	-0.71	-0.76	-0.14	-0.19	-0.12	-0.12	-0.09	1.03	0.46	-0.46	0.18	0.13	-0.11	-0.17	0.25	-0.18	-0.74	-0.75	-0.32	0.03	0.25	-0.48	-0.34	-0.10	0.21	-0.80	-0.82	-0.08	-0.03	-0.23	-0.37	0.20	-0.25	-0.43	-0.26	0.10	-0.14
	nb85s13	-0.73	-0.81	-0.79	0.00	-0.27	-0.21	-0.06	-0.17	0.47	0.24	-0.58	-0.08	-0.16	-0.40	-0.80	0.21	-0.33	-1.01	0.31	-0.56	-0.02	0.29	0.27	-0.33	-0.02	0.14	-0.51	-0.70	-0.43	-0.42	-0.29	-0.67	0.35	-0.05	-0.80	-0.81	-0.08	-0.04

[a] Purple = marker used to select genotypes to exclude from phenotype data for reduced genotype GWAS, green = significant in both GWAS, orange = significant in reduced genotype GWAS only and yellow = significant in full genotype GWAS only. [b] Reference SNP used to estimate phenotypic effect. [c] Capitalised superscript A used to indicate SNP markers exceeded Bonferroni correction threshold.

Table 5.7 Pairwise LD estimates (r^2) for SNPs significantly associated with resistance to *Pyrenophora teres* f. *teres* from full and reduced genotype GWAS of NRB genotypes.

			Reduced genotype GWAS significant markers											Full genotype GWAS significant markers																										
SNP marker[a]	Chr	Position (bp)	3255709-40:A>G	3257855-10:A>G	3256237-67:A>G	3257954-50:G>A	3434214-43:A>T	3256458-52:T>C	3255777-67:T>G	3254817-15:C>A	3257446-28:G>T	3262096-64:C>T	**3256608-45:C>G**	3259111-21:A>C	3398663-60:C>T	3254735-54:A>C	3259058-41:G>A	3257608-6:A>G	3259255-17:C>T	4175123-58:C>A	3256765-18:T>C	3262659-31:C>G	3255625-14:C>T	3434176-13:T>C	3432738-29:G>A	3432352-13:G>T	3254663-15:T>A	3255134-29:C>A	3254978-54:G>A	3258749-25:G>C	3434193-36:T>G	3255255-56:T>A	4171893-67:C>T	3921095-18:T>C	3257464-10:T>A	3258275-14:G>C	3259228-14:G>C	3261554-30:C>T	3263983-33:G>T	3262437-68:C>T
3255709-40:A>G	4H	53032932	1	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3257855-10:A>G	4H	69382105	0.5	1	0.7	0.1	0.1	0.1	0.1	0.1	0.0	0.0	**0.0**	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3256237-67:A>G	4H	70434783	0.5	0.7	1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	**0.0**	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3257954-50:G>A	6H	193444571	0.0	0.1	0.1	1	0.9	0.8	0.9	0.9	0.0	0.2	**0.0**	0.8	0.0	0.7	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3434214-43:A>T	6H	251009458	0.0	0.1	0.1	0.9	1	0.9	0.9	0.9	0.0	0.2	**0.0**	0.9	0.0	0.8	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3256458-52:T>C	6H	325194805	0.0	0.1	0.1	0.8	0.9	1	0.9	0.9	0.0	0.2	**0.0**	0.9	0.0	0.7	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3255777-67:T>G	6H	337179867	0.0	0.1	0.1	0.9	0.9	0.9	1	1.0	0.0	0.2	**0.0**	0.9	0.0	0.8	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3254817-15:C>A	6H	340307078	0.0	0.1	0.1	0.9	0.9	0.9	1.0	1	0.0	0.2	**0.0**	0.9	0.0	0.8	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3257446-28:G>T	6H	368527587	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1	0.5	**0.0**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.3	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3262096-64:C>T	6H	378974018	0.0	0.0	0.0	0.2	0.2	0.2	0.2	0.2	0.5	1	**0.0**	0.2	0.0	0.1	0.2	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
3256608-45:C>G	**6H**	**378772740**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**1**	**0.0**	**0.3**	**0.0**	**0.0**	**0.7**	**0.4**	**0.9**	**0.9**	**0.9**	**0.8**	**0.4**	**0.8**	**0.4**	**0.8**	**0.1**	**0.3**	**0.3**	**0.6**	**0.6**	**0.5**	**0.6**	**0.2**	**0.7**	**0.5**	**0.0**	**0.5**	**0.1**
3259111-21:A>C	6H	210766011	0.0	0.1	0.1	0.8	0.9	0.9	0.9	0.9	0.0	0.2	**0.0**	1	0.0	0.8	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3398663-60:C>T	6H	268997406	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.3**	0.0	1	0.0	0.0	0.4	0.1	0.3	0.3	0.3	0.3	0.1	0.3	0.1	0.3	0.3	0.1	0.1	0.2	0.2	0.2	0.2	0.0	0.2	0.2	0.1	0.1	0.0
3254735-54:A>C	6H	314450784	0.0	0.0	0.1	0.7	0.8	0.7	0.8	0.8	0.0	0.1	**0.0**	0.8	0.0	1	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3257608-6:A>G	6H	361531190	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.7**	0.0	0.4	0.0	0.0	1	0.2	0.7	0.6	0.6	0.5	0.2	0.6	0.2	0.6	0.1	0.2	0.2	0.5	0.5	0.4	0.5	0.1	0.5	0.4	0.0	0.4	0.1
3259058-41:G>A	6H	364356525	0.0	0.1	0.1	0.8	0.8	0.7	0.8	0.8	0.0	0.2	**0.0**	0.8	0.0	0.6	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3259255-17:C>T	6H	375529364	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	**0.4**	0.0	0.1	0.0	0.0	0.2	1	0.4	0.4	0.8	0.4	0.1	0.4	0.9	0.3	0.0	0.1	0.1	0.2	0.2	0.2	0.2	0.0	0.3	0.3	0.1	0.2	0.0
4175123-58:C>A	6H	380193974	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.9**	0.0	0.3	0.0	0.0	0.7	0.4	1	0.9	0.9	0.8	0.4	0.8	0.4	0.8	0.1	0.3	0.3	0.6	0.6	0.5	0.6	0.2	0.7	0.5	0.0	0.5	0.1
3256765-18:T>C	6H	382482733	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.9**	0.0	0.3	0.0	0.0	0.6	0.4	0.9	1	0.9	0.8	0.4	0.8	0.4	0.8	0.1	0.3	0.3	0.6	0.6	0.5	0.6	0.2	0.7	0.5	0.0	0.5	0.1
3262659-31:C>G	6H	383141804	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.9**	0.0	0.3	0.0	0.0	0.6	0.8	0.9	0.9	1	0.8	0.3	0.8	0.8	0.8	0.1	0.3	0.3	0.6	0.6	0.5	0.6	0.2	0.6	0.6	0.0	0.5	0.1
3255625-14:C>T	6H	384803137	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.8**	0.0	0.3	0.0	0.0	0.5	0.4	0.8	0.8	0.8	1	0.4	0.7	0.3	0.7	0.1	0.3	0.2	0.5	0.5	0.4	0.5	0.1	0.6	0.4	0.0	0.5	0.1
3434176-13:T>C	6H	384884765	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	**0.4**	0.0	0.1	0.0	0.0	0.2	0.1	0.4	0.4	0.3	0.4	1	0.3	0.1	0.5	0.2	0.1	0.1	0.2	0.2	0.2	0.2	0.0	0.3	0.2	0.0	0.2	0.0
3432738-29:G>A	6H	386021835	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.8**	0.0	0.3	0.0	0.0	0.6	0.4	0.8	0.8	0.8	0.7	0.3	1	0.4	0.8	0.1	0.3	0.2	0.6	0.6	0.6	0.6	0.2	0.7	0.6	0.0	0.5	0.1
3432352-13:G>T	6H	388486267	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	**0.4**	0.0	0.1	0.0	0.0	0.2	0.9	0.4	0.4	0.8	0.3	0.1	0.4	1	0.4	0.0	0.1	0.1	0.3	0.3	0.2	0.3	0.0	0.3	0.3	0.1	0.2	0.0
3254663-15:T>A	6H	396127146	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.8**	0.0	0.3	0.0	0.0	0.6	0.3	0.8	0.8	0.8	0.7	0.5	0.8	0.4	1	0.1	0.3	0.2	0.6	0.6	0.5	0.6	0.1	0.7	0.5	0.0	0.5	0.1
3255134-29:C>A	6H	397034107	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	**0.1**	0.1	0.3	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.2	0.1	0.0	0.1	1	0.0	0.0	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.0
3254978-54:G>A	6H	404316342	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.1	**0.3**	0.0	0.1	0.0	0.0	0.2	0.1	0.3	0.3	0.3	0.3	0.1	0.3	0.1	0.3	0.0	1	0.9	0.4	0.4	0.3	0.4	0.0	0.3	0.3	0.1	0.3	0.0
3258749-25:G>C	6H	408391789	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.1	**0.3**	0.0	0.1	0.0	0.0	0.2	0.1	0.3	0.3	0.3	0.2	0.1	0.2	0.1	0.2	0.0	0.9	1	0.4	0.4	0.3	0.4	0.0	0.3	0.3	0.1	0.3	0.0
3434193-36:T>G	6H	417070659	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.6**	0.0	0.2	0.0	0.0	0.5	0.2	0.6	0.6	0.6	0.5	0.2	0.6	0.3	0.6	0.1	0.4	0.4	1	0.9	0.8	0.9	0.3	0.8	0.8	0.0	0.8	0.1
3255255-56:T>A	6H	417821936	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.6**	0.0	0.2	0.0	0.0	0.5	0.2	0.6	0.6	0.6	0.5	0.2	0.6	0.3	0.6	0.1	0.4	0.4	0.9	1	0.8	0.9	0.3	0.8	0.8	0.0	0.9	0.1
4171893-67:C>T	6H	422773531	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.5**	0.0	0.2	0.0	0.0	0.4	0.2	0.5	0.5	0.5	0.4	0.2	0.6	0.2	0.5	0.1	0.3	0.3	0.8	0.8	1	0.8	0.2	0.7	0.8	0.0	0.8	0.1
3921095-18:T>C	6H	424801489	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.6**	0.0	0.2	0.0	0.0	0.5	0.2	0.6	0.6	0.6	0.5	0.2	0.6	0.3	0.6	0.1	0.4	0.4	0.9	0.9	0.8	1	0.3	0.8	0.8	0.0	0.8	0.1
3257464-10:T>A	6H	449601223	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	**0.2**	0.0	0.0	0.0	0.0	0.1	0.0	0.2	0.2	0.2	0.1	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.3	0.3	0.2	0.3	1	0.7	0.3	0.4	0.6	0.3
3261554-30:C>T	6H	450717343	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.7**	0.0	0.2	0.0	0.0	0.5	0.3	0.7	0.7	0.6	0.6	0.3	0.7	0.3	0.7	0.1	0.3	0.3	0.8	0.8	0.7	0.8	0.7	1	0.8	0.0	0.7	0.3
3259228-14:G>C	6H	459335236	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.5**	0.0	0.2	0.0	0.0	0.4	0.3	0.5	0.5	0.6	0.4	0.2	0.6	0.3	0.5	0.1	0.3	0.3	0.8	0.8	0.8	0.8	0.3	0.8	1	0.0	0.8	0.1
3258275-14:G>C	6H	460084925	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.4	0.0	0.0	1	0.0	0.1
3263983-33:G>T	6H	460088004	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.5**	0.0	0.1	0.0	0.0	0.4	0.2	0.5	0.5	0.5	0.5	0.2	0.5	0.2	0.5	0.1	0.3	0.3	0.8	0.9	0.8	0.8	0.6	0.7	0.8	0.0	1	0.2
3262437-68:C>T	6H	461514241	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.1**	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.3	0.3	0.1	0.1	0.2	1

[a] Purple = used to select genotypes to exclude for reduced genotype GWAS, green = significant in both GWAS, orange = significant in reduced genotype GWAS only, yellow = significant in full genotype GWAS only.

Chapter 6

General Discussion

The research undertaken in this thesis was conducted to help fill knowledge gaps that exist around the occurrence and origin of resistance and susceptibility factors for *Pyrenophora teres* f. *teres* (*Ptt*) in Australian barley germplasm. While extensive work has been conducted to identify quantitative trait loci (QTL) associated with resistance and susceptibility to *Ptt* (Liu *et al.* 2011), there is very little information that directly relates the findings of these studies to barley cultivars and variants of the pathogen population. The studies presented here utilised germplasm from Australia and the US to identify QTL conferring resistance and susceptibility to nationally relevant *Ptt* isolates and document the origin of the QTL. The genomic regions from the studies were positioned on the barley physical map and directly compared to previously identified QTL to infer whether genotypes from similar genetic backgrounds were implied. The studies identified QTL that were shared across genotypes of distinct lineages and could ultimately be traced back to founding landraces. This information is highly relevant to researchers working with *Ptt* to understand the origin of susceptibility in modern cultivars.

Firstly, to fill the knowledge gap surrounding pathogenicity of *Ptt* on a national level, isolates were collected from the major barley growing regions of Australia and assessed on a set of relevant barley differentials. This study identified similar isolates to those detected in a previous study of Western Australian isolates (Gupta and Loughman 2001) and eastern Australian isolates (Platz *et al.* 2000). Result suggested that the population had been relatively stable in the time between studies in regard to generation of new pathotypes. However, a shift in the proportion of isolate groups was observed in Queensland across the studies, where Skiff virulent isolates appeared to be more prevalent in the current study. Isolates from southern Australia were poorly represented in the previous studies; therefore, comparisons cannot be made to the current study. Although the current study discovered that the southern Australian population had higher diversity of virulent isolates compared to the east and west. The present study did not look to identify individual pathotypes, but rather took a population based analytical approach to cluster isolates with similar virulence profile. This cluster analysis identified four main groups of isolates, however it should not be assumed that this equates to four pathotypes, as differences in virulence profile between isolates was observed within each isolate group that the cluster analysis could not capture. Differential genotypes used in the current study included a subset originally proposed by Steffenson and Webster (1992a) and also Herta from Tekauz (1990). This was due to non-differentiating infection

responses documented during the previous Australian studies. However, this initial oversight of differential genotype selection has meant that comparisons to studies that used the full set of genotypes cannot be made. Thus, it is recommended that future Australian pathogenicity studies include genotypes from the Steffenson and Webster (1992a). Detailed knowledge stemming from this study allowed the selection of highly relevant isolates for further investigation in the subsequent studies in this thesis. Prior and Skiff differential genotypes were recognised as relevant to the highest proportion of Australian *Ptt* isolates, however the underlying genetic factors conferring susceptibility to Prior and Skiff virulent isolates were unknown. Subsequently, Prior and Skiff were selected for detailed genetic analysis.

A Prior × Skiff segregating population was generated and phenotyped with Prior and Skiff virulent isolates to identify and catalogue genomic regions conferring resistance and susceptibility. All five QTL identified co-located to QTL reported in previous studies (Abu Qamar *et al.* 2008; Adhikari 2017; Graner *et al.* 1996; Gupta *et al.* 2010; Koladia *et al.* 2017a; Liu *et al.* 2015; O'Boyle *et al.* 2014; Richards *et al.* 2016; Richards *et al.* 2017; Vatter *et al.* 2017; Wonneberger *et al.* 2017a; Wonneberger *et al.* 2017b). Results suggested that Prior and Skiff virulent isolates from Australian share common avirulence and/or virulence factors to isolates used in other studies and conversely, that Prior and Skiff harbour similar resistance and/or susceptibility QTL to genotypes used in other studies. This hypothesis has implications for resistance breeding world-wide. As such, if the QTL effect from the genotypes used in the other studies is validated with the Australian isolates, resistance and/or susceptibility could be selected on an international basis for deployment in a world-wide context. In addition, the frequent detection of the same QTL in multiple world-wide studies suggests that the genomic regions in question are likely to be highly influential on disease phenotype in a range of diverse backgrounds. With regard to the overarching aim of this chapter, which also sought to determine the origin of QTL, additional information provided by a diversity panel coupled with detailed pedigree analysis was critical to the success of this aspect. As such, QTL could be traced back to Chevallier, Isaria and Prior and the omnipresence of one QTL in landraces and modern cultivars was also documented. Moreover, this information is extremely useful to barley breeders and other researchers as efficient decisions can be made directly from these results, thus circumventing the need to conduct costly and time-consuming research to reach the same conclusion.

Finally, elite breeding material from the Northern Region Barley (NRB) breeding program was subject to genome-wide association studies (GWAS) to identify QTL associated with resistance and susceptibility to *Ptt*. Results re-discovered one QTL from Chapter 4, although the origin of the

undesirable allele was derived from parents from the North Dakota State University breeding program. Furthermore, the deleterious effect of the allele was validated in an unrelated genetic background and different environment. Thus, breeding to exclude the allele associated with susceptibility to NB50 is recommended, however inspection of the diversity panel revealed that this was rare in Australian cultivars and as such active selection at this locus would be irrelevant. Although, the genetic marker for this QTL was associated with resistance and susceptibility in a reciprocal manner, suggesting the presence of two genes closely linked in repulsion or alternately, alleles of a single gene. A similar reciprocal effect at this locus was observed for Rika and Kombar (Abu Qamar *et al.* 2008) and was recently fine mapped (Richards *et al.* 2016). Another detected QTL on 6H was associated with susceptibility to *Ptt* that was derived from Moravian and English landraces. Breeding to exclude the undesirable allele is recommended, however inspection of the diversity panel highlighted that the allele was rare in modern Australian cultivars and in most cases would be irrelevant. Another QTL detected on 6H is of particular importance, as the allele associated with resistance was derived from the CIho 5791, which is known to be highly resistant in many parts of the world (Afanasenko *et al.* 2009; Akhavan *et al.* 2016; Boungab *et al.* 2012; Liu *et al.* 2012). This QTL was identified as the most effective source of resistance to *Ptt*. In addition, high LD associated with this QTL also had the added advantage of excluding closely linked QTL associated with isolate specific susceptibility. A QTL on 4H was also detected, which co-located to a previously QTL identified from germplasm that could be traced back to the International Maize and Wheat Improvement Centre (CIMMYT) this population and two others (Adhikari 2017; Islamovic *et al.* 2017). The QTL was detected using world-wide isolates, thus suggesting that the QTL could confer a level of resistance to *Ptt* on multiple continents. While Prior and Skiff were relevant to most of the isolates examined in this thesis, additional genetic diversity exists for susceptibility in Australian germplasm and corresponding isolates, as such further research is necessary to capture the and exploit this knowledge for the betterment of the Australian barley industry.

To attain effective resistance to multiple *Ptt* pathotypes, accumulating resistance and excluding susceptibility genes is necessary. As heritability of resistance is high and phenotyping is simple, phenotypic selection using appropriate isolates has successfully accumulated desirable alleles within the NRB breeding population within few breeding cycles (Appendix 2). However, genetic breeding methodologies such as marker-assisted backcrossing (MABC), have been an efficient method of introgressing complex traits into advanced germplasm while recovering a high proportion of the recurrent parent (Collard and Mackill 2008). In the absence of phenotyping facilities, MABC could be employed to introgress QTL. Deployment of genomic selection (GS) within breeding programs would not only allow for the accurate selection and accumulation of

desirable alleles for resistance to *Ptt*, but would also facilitate the accurate selection of desirable alleles for other pathogens and traits through a multi-trait index (Wolc *et al.* 2015).

The next generation of genetic tools are set to revolutionise genetics research and plant breeding. Genome editing in the form of transcription activator-like effector nucleases (TALENs) (Zhang *et al.* 2013), zinc finger nucleases (ZFNs) (Townsend *et al.* 2009) and clustered regularly interspaced short palindromic repeats (CRISPR) Cas9n (Ran *et al.* 2013) technology will enable gene specific targeting. Following the identification of genes conditioning dominant susceptibility, CRISPR/Cas9n could be deployed to silence the deleterious gene, effectively generating a mutant resistant plant. Gene silencing of a functional resistance gene has been successful in barley (Lawrenson *et al.* 2015). Likewise, dominant resistance genes could be stacked and inserted within a single locus, thus enabling heritability of all resistances simultaneously (Luo *et al.* 2016). Additionally, this approach would circumvent genetic/haplotype bottlenecks in breeding programs, as the introgression of resistance would not impart linkage drag of the donor genetics. Moreover, the insertion of gene stacks would not reshuffle current genetic structure and as such, diversity would be conserved. Common genetic loci could be fixed for resistance, which would allow recombination to take place without the need for reselection or of resistant plants, thus enabling the exploration of previously unavailable genetic combinations. The deployment of this technology in Australian and international barley cultivars may spell the end of fungicide application as a disease control strategy, the benefits of which are enormous for the environment as a whole.

Knowledge generated from this thesis will enable Australian barley breeders to more efficiently identify and recombine desirable alleles in advanced germplasm, ultimately leading to cultivars with improved disease resistance.

List of References

Abeysekara NS, Faris JD, Chao S, McClean PE, Friesen TL (2012) Whole-Genome QTL Analysis of *Stagonospora nodorum* Blotch Resistance and Validation of the *SnTox4–Snn4* Interaction in Hexaploid Wheat Phytopathology 102:94-104

Abeysekara NS, Friesen TL, Liu Z, McClean PE, Faris JD (2010) Marker development and saturation mapping of the tan spot *Ptr ToxB* sensitivity locus *Tsc2* in hexaploid wheat The Plant Genome 3:179-189

Able AJ (2003) Role of reactive oxygen species in the response of barley to necrotrophic pathogens Protoplasma 221:137-143

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

Adhikari A (2017) Association Mapping for Net Blotch Resistance in Barley and a Study of Barley/Cereal Yellow Dwarf Virus in Minnesota. Masters Thesis, University of Minnesota

Afanasenko O, Koziakov A, Hedlay P, Lashina N, Anisimova A, Manninen O, Jalli M, Potokina E (2015) Mapping of the loci controlling the resistance to *Pyrenophora teres* f. *teres* and *Cochliobolus sativus* in two double haploid barley populations Russian Journal of Genetics: Applied Research 5:242-253

Afanasenko OS, Jalli M, Pinnschmidt HO, Filatova O, Platz GJ (2009) Development of an international standard set of barley differential genotypes for *Pyrenophora teres* f. *teres*. Plant Pathology 58:665-676

Ahmadiyeh N, Churchill GA, Shimomura K, Solberg LC, Takahashi JS, Redei EE (2003) X-linked and lineage-dependent inheritance of coping responses to stress Mammalian genome 14:748-757

Akhavan A, Turkington TK, Askarian H, Tekauz A, Xi K, Tucker JR, Kutcher HR, Strelkov SE (2016) Virulence of *Pyrenophora teres* populations in western Canada. Canadian Journal of Plant Pathology:1-14

Angra-Sharma R, Sharma D (2000) Cytokinins in pathogenesis and disease resistance of P*yrenophora teres*-barley and *Dreschslera maydis*-maize interactions during early stages of infection Mycopathologia 148:87-95

Arabi MIE, Al-Safadi B, Charbaji T (2003) Pathogenic Variation among Isolates of *Pyrenophora teres*, the Causal Agent of Barley Net Blotch. Journal of Phytopathology 151:376-382

Badr A, Sch R, Rabey HE, Effgen S, Ibrahim H, Pozzi C, Rohde W, Salamini F (2000) On the origin and domestication history of barley (*Hordeum vulgare*) Molecular Biology and Evolution 17:499-510

Bayer MM, Rapazote-Flores P, Ganal M, Hedley PE, Macaulay M, Plieske J, Ramsay L, Russell J, Shaw PD, Thomas W (2017) Development and Evaluation of a Barley 50k iSelect SNP Array Frontiers in plant science 8:1792

Beattie AD, Scoles GJ, Rossnagel BG (2007) Identification of molecular markers linked to a *Pyrenophora teres* avirulence gene. Phytopathology 97:842-849

Beaven E (1902) Varieties of barley Journal of the Institute of Brewing 8:542-600

BGN (2013) Barley Genetics Newsletter Volume 43 Barley Genetics Newsletter 43:1-249

Bock H (1552) Hieronymi Tragi De stirpium, maxime earum, quae in Germania nostra nascuntur, usitatis nomenclaturis, proprijsque differentijs, neque non temperaturis ac … https://books.google.com.au/books?id=JbICRqjDgGgC

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

Bouajila A, Zoghlami N, Al Ahmed M, Baum M, Ghorbel A, Nazari K (2011) Comparative virulence of *Pyrenophora teres* f. *teres* from Syria and Tunisia and screening for resistance sources in barley: implications for breeding. Letters in Applied Microbiology 53:489-502

Boungab K, Belabid L, Fortas Z, Bayaa B (2012) Pathotype diversity among Algerian isolates of *Pyrenophora teres* f. *teres*. Phytopathologia Mediterranea 51

Broman KW, Sen S (2009) A Guide to QTL Mapping with R/qtl vol 46. Springer

Broman KW, Wu H, Sen Ś, Churchill GA (2003) R/qtl: QTL mapping in experimental crosses Bioinformatics 19:889-890

Buchannon KW, McDonald WC (1965) Sources of resistance in barley to *Pyrenophora teres*. Canadian journal of plant science 45:189-193

Burnham CR, Hagberg A (1956) Cytogenetic notes on chromosomal interchanges in barley Hereditas 42:467-482

Burr B, Evola S, Burr F, Beckmann J (1983) The application of restriction fragment length polymorphism to plant breeding. In: Genetic engineering. Springer, pp 45-59

Butler DG, Cullis BR, Gilmour AR, Gogel BJ (2009) Mixed models for S language environments: ASReml-R reference manual. Brisbane: Queenland Department of Primary Industries and Fisheries

Cakir M, Gupta S, Li C, Hayden M, Mather DE, Ablett GA, Platz GJ, Broughton S, Chalmers KJ, Loughman R (2011) Genetic mapping and QTL analysis of disease resistance traits in the barley population Baudin × AC Metcalfe. Crop and Pasture Science 62:152-161

Cakir M, Gupta S, Platz GJ, Ablett GA, Loughman R, Emebiri L, Poulsen D, Li C, Lance RCM, Galwey NW (2003) Mapping and validation of the genes for resistance to *Pyrenophora teres* f. *teres* in barley (*Hordeum vulgare* L.). Crop and Pasture Science 54:1369-1377

Cohen YR (2002) β-aminobutyric acid-induced resistance against plant pathogens Plant disease 86:448-457

Collard BC, Mackill DJ (2008) Marker-assisted selection: an approach for precision plant breeding in the twenty-first century Philosophical Transactions of the Royal Society B: Biological Sciences 363:557-572

Colmsee C, Beier S, Himmelbach A, Schmutzer T, Stein N, Scholz U, Mascher M (2015) BARLEX–the barley draft genome explorer Molecular plant 8:964-966

Columella LJM (1745) L. Junius Moderatus Columella Of Husbandry: In Twelve Books: and His Book Concerning Trees. A. Millar. https://books.google.com.au/books?id=qcNbAAAAMAAJ

Consortium IBGS (2012) A physical, genetic and functional sequence assembly of the barley genome. Nature 491:711-716

Cordus V (1561) Annotationes in Pedacii Dioscoridis Anazarbei De medica materia libros. Excudebat Iosias Rihelius. https://www.biodiversitylibrary.org/bibliography/8036#/summary

Cromey M, Parkes R (2003) Pathogenic variation in *Drechslera teres* in New Zealand. New Zealand Plant Protection 56:251-256

De Lorenzo G, Brutus A, Savatin DV, Sicilia F, Cervone F (2011) Engineering plant resistance by constructing chimeric receptors that recognize damage-associated molecular patterns (DAMPs) FEBS letters 585:1521-1528

De Mendiburu F (2014) Agricolae: statistical procedures for agricultural research. R package version 1:1-6

Deimel H, Hoffmann G (1991) Detrimental effects of net blotch disease to barley plants caused by *Drechslera teres* (Sacc.) Shoemaker. Zeitschrift fuer Pflanzenkrankheiten und Pflanzenschutz 98:137-161

Desrousseaux D, Sandron F, Siberchicot A, Cierco-Ayrolles C, Mangin B (2016) LDcorSV: Linkage disequilibrium corrected by the structure and the relatedness. R package version 1.3. 1.

Dessouki S, Mansour A, Khalifa M (1965) Genetic sources of resistance to net blotch of barley. Agric Res Rev, Cairo 43:47-52

Diedicke H (1902) Ueber den Zusammenhang zwischen Pleospora-und Helminthosporium-Arten vol 9. Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten, Erste Abteilung: .

Douiyssi A, Rasmusson D, Roelfs A (1998) Responses of barley cultivars and lines to isolates of *Pyrenophora teres.* Plant disease 82:316-321

Drechsler C (1923) Some Graminicolous Species of Helminthosporium: I Journal of Agricultural Research 24:641-740

Ellison R (1981) Diet in Mesopotamia: the evidence of the barley ration texts (c. 3000–1400 BC) Iraq 43:35-45

Ellwood S, Liu Z, Syme R, Lai Z, Hane J, Keiper F, Moffat C, Oliver R, Friesen T (2010) A first genome assembly of the barley fungal pathogen *Pyrenophora teres* f. *teres*. Genome Biol 11:1-14 doi:10.1186/gb-2010-11-11-r109

ElMor IM (2016) Investigating the virulence of isolates produced by sexual recombination between different *Pyrenophora teres* isolates. Masters Thesis, School of Agricultural, Computational and Environmental Science, University of Southern Queensland

Emebiri L, Platz G, Moody D (2005) Disease resistance genes in a doubled haploid population of two-rowed barley segregating for malting quality attributes. Crop and Pasture Science 56:49-56

Evans S (1969) Observations on the development of leaf blotch and net blotch of barley from barley debris, 1968. Plant pathology 18:116-118

FAO (2018) Barley Production. Referenced: 10042018 Available at: http://www.fao.org/faostat/en/ - data/QC

Faris JD, Zhang Z, Lu H, Lu S, Reddy L, Cloutier S, Fellers JP, Meinhardt SW, Rasmussen JB, Xu SS (2010) A unique wheat disease resistance-like gene governs effector-triggered susceptibility to necrotrophic pathogens Proceedings of the National Academy of Sciences 107:13544-13549

Flor HH (1955) Host-parasite interactions in flax rust-its genetics and other implications. Phytopathology 45:680-685.

Fowler R, Platz G, Bell K, Fletcher S, Franckowiak J, Hickey L (2017) Pathogenic variation of *Pyrenophora teres* f. *teres* in Australia Australasian Plant Pathology 46:115-128

Franckowiak J, Horsley R, Neate S, Schwarz P (2007) Registration of 'Rawson'barley Journal of plant registrations 1:37-38

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

Friesen TL, Chu C, Xu SS, Faris JD (2012) SnTox5–Snn5: a novel *Stagonospora nodorum* effector–wheat gene interaction and its relationship with the *SnToxA–Tsn1* and *SnTox3–Snn3–B1* interactions Molecular plant pathology 13:1101-1109

Friesen TL, Meinhardt SW, Faris JD (2007) The *Stagonospora nodorum*-wheat pathosystem involves multiple proteinaceous host-selective toxins and corresponding host sensitivity genes that interact in an inverse gene-for-gene manner. The Plant Journal 51:681-692

Fuchs L (1542) De historia stirpium commentarii insignes officina Isingriniana, Basileae. https://www.biodiversitylibrary.org/bibliography/75249#/summary

Gao Y, Faris J, Liu Z, Kim Y, Syme R, Oliver R, Xu S, Friesen T (2015) Identification and characterization of the *SnTox6-Snn6* interaction in the *Parastagonospora nodorum*–wheat pathosystem Molecular Plant-Microbe Interactions 28:615-625

Gaza T (1483) Theophrasti Historia plantarum. https://www.biodiversitylibrary.org/bibliography/62512#/summary

Gerarde J (1633) The herball or generall historie of plantes. Adam Islip, Joice Norton and Richard Whitakers, London, UK. https://www.biodiversitylibrary.org/bibliography/121658#/summary

Gilmour A ASREML for testing fixed effects and estimating multiple trait variance components. In: Proceedings of the Association for the Advancement of Animal Breeding and Genetics, 1997. pp 386-390

Glazebrook J (2005) Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens Annu Rev Phytopathol 43:205-227

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

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

Greene EL (1910) Landmarks of botanical history: a study of certain epochs in the development of the science of botany vol 54. vol 1. Smithsonian institution

Grewal T, Rossnagel B, Pozniak C, Scoles G (2008) Mapping quantitative trait loci associated with barley net blotch resistance. Theoretical and Applied Genetics 116:529-539

Grewal TS, Rossnagel BG, Scoles GJ (2012) Mapping quantitative trait loci associated with spot blotch and net blotch resistance in a doubled-haploid barley population Molecular breeding 30:267-279

Gupta S, Li C, Loughman R, Cakir M, Platz G, Westcott S, Bradley J, Broughton S, Lance R (2010) Quantitative trait loci and epistatic interactions in barley conferring resistance to net type net blotch (Pyrenophora teres f. teres) isolates. Plant breeding 129:362-368

Gupta S, Li C, Loughman R, Cakir M, Westcott S, Lance R (2011) Identifying genetic complexity of 6H locus in barley conferring resistance to *Pyrenophora teres* f. *teres*. Plant Breeding 130:423-429 doi:10.1111/j.1439-0523.2011.01854.x

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

Haley CS, Knott SA (1992) A simple regression method for mapping quantitative trait loci in line crosses using flanking markers Heredity 69:315

Heffner EL, Lorenz AJ, Jannink J-L, Sorrells ME (2010) Plant breeding with genomic selection: gain per unit time and cost Crop science 50:1681-1690

Ho K, Choo T, Tekauz A, Martin R (1996) Genetic studies on net blotch resistance in a barley cross. Canadian journal of plant science 76:715-719

Holm S (1979) A simple sequentially rejective multiple test procedure Scandinavian journal of statistics:65-70

Hort A (1916) Theophrastus enquiry into plants and minor works on odour and weather signs. With an English translation. London: William Heinemann.
Volume I, Book I-V: https://www.biodiversitylibrary.org/item/58434#page/9/mode/1up.
Volume II, Book VI-IX: https://archive.org/details/enquiryintoplant02theouoft.

Islamovic E, Bregitzer P, Friesen TL (2017) Barley 4H QTL confers NFNB resistance to a global set of *P. teres* f. *teres* isolates Molecular Breeding 37:29

Ismail I, Godfrey D, Able A (2014) Proteomic analysis reveals the potential involvement of xylanase from *Pyrenophora teres* f. *teres* in net form net blotch disease of barley Australasian Plant Pathology 43:715-726

Ismail IA, Able AJ (2016) Secretome analysis of virulent *Pyrenophora teres* f. *teres* isolates Proteomics 16:2625-2636

Ito S (1930) On some new ascigerous stages of the species of *Helminthosporium* parasitic on cereals Proceedings of the Imperial Academy 6:352-355

Jalli M (2010) The virulence of Finnish *Pyrenophora teres* f. *teres* isolates and its implications for resistance breeding. PhD Thesis, Faculty of Agriculture and Forestry, University of Helsinki

Jánošová M, Kraic J (1997) Analysis of *Pyrenophora teres* (Died.) Drechs. Population from Slovakia. In: Dehne HW, Adam G, Diekmann M, Frahm J, Mauler-Machnik A, Halteren P (eds) Diagnosis and Identification of Plant Pathogens, vol 11. Developments in Plant Pathology. Springer Netherlands, pp 315-316. doi:10.1007/978-94-009-0043-1_66

Jansen RC, Stam P (1994) High resolution of quantitative traits into multiple loci via interval mapping Genetics 136:1447-1455

Jebbouj R, El Yousfi B (2009) Barley yield losses due to defoliation of upper three leaves either healthy or infected at boot stage by *Pyrenophora teres* f. *teres*. European Journal of Plant Pathology 125:303-315

Jebbouj R, El Yousfi B (2010) An integrated multivariate approach to net blotch of barley: Virulence quantification, pathotyping and a breeding strategy for disease resistance. European Journal of Plant Pathology 127:521-544

Jin Y, Steffenson BJ, Fetch TG (1994) Sources of resistance to pathotype QCC of *Puccinia graminis* f. sp. *tritici* in barley Crop science 34:285-288

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

Jonsson R, Bryngelsson T, Jalli M, Gustafsson M (1998) Effect of growth stage on resistance to *Drechslera teres* f. *teres* in barley. Journal of Phytopathology 146:261-265

Jordan VWL (1981) Aetiology of barley net blotch caused by *Pyrenophora teres* and some effects on yield. Plant Pathology 30:77-87

Kang HM, Zaitlen NA, Wade CM, Kirby A, Heckerman D, Daly MJ, Eskin E (2008) Efficient control of population structure in model organism association mapping Genetics 178:1709-1723

Kao C-H, Zeng Z-B, Teasdale RD (1999) Multiple interval mapping for quantitative trait loci Genetics 152:1203-1216

Kenneth R, Atzmon Y, Khair J, Koltin Y, Wahl I (1967) Problems in breeding barley resistant to net blotch disease in Israel. In: Summaries of Lectures. 1st Israel Congress of Plant Pathology, 16-17 January 1967. pp 46-47.

Keon J, Hargreaves J (1983) A cytological study of the net blotch disease of barley caused by *Pyrenophora teres* Physiological Plant Pathology 22:321IN325-329IN314

Khan TN (1969) Inheritance of resistance to net blotch in barley. I. Factors affecting the penetrance and expressivity of gene (s) conditioning host resistance. Canadian journal of genetics and cytology 11:587-591

Khan TN (1973) Host specialization by Western Australian isolates causing net blotch symptoms on Hordeum. Transactions of the British Mycological Society 61:215-220

Khan TN (1982) Changes in pathogenicity of *Drechslera teres* relating to changes in barley cultivars grown in Western Australia. Plant Diseases 66:655-656

Khan TN (1987) Relationship between net blotch (*Drechslera teres*) and losses in grain yield of barley in Western Australia. Crop and Pasture Science 38:671-679

Khan TN, Boyd WJR (1969a) Inheritance of resistance to net blotch in barley. II. Genes conditioning resistance against race WA-2. Canadian journal of genetics and cytology 11:592-597

Khan TN, Boyd WJR (1969b) Physiologic specialization in *Drechslera teres.* Australian Journal of Biological Sciences 22:1229-1236

Khan TN, Boyd WJR, Shipton WA (1968) Barley diseases in Western Australia: their distribution and pathogenic characteristics. Journal of the Royal Society of Western Australia 51:123-128

Kharub AS (2017) Genome wide association study GWAS of resistance to barley spot blotch in South Asia. http://hdl.handle.net/20.500.11766/5799

Koladia V, Faris J, Richards J, Brueggeman R, Chao S, Friesen T (2017a) Genetic analysis of net form net blotch resistance in barley lines CIho 5791 and Tifang against a global collection of *P. teres* f. *teres* isolates Theoretical and Applied Genetics 130:163-173

Koladia VM, Richards JK, Wyatt NA, Faris JD, Brueggeman RS, Friesen TL (2017b) Genetic analysis of virulence in the *Pyrenophora teres* f. *teres* population BB25× FGOH04Ptt-21 Fungal Genetics and Biology 107:12-19

König J, Perovic D, Kopahnke D, Ordon F (2013) Development of an efficient method for assessing resistance to the net type of net blotch (*Pyrenophora teres* f. *teres*) in winter barley and mapping of quantitative trait loci for resistance. Molecular Breeding 32:641-650 doi:10.1007/s11032-013-9897-x

Körnicke F (1895) Die hauptsächlichsten Formen der Saatgerste Bonn, Universitats-Buch-druckerei von Carl Georgi

Kosambi D (1944) The estimation of map distances from recombination values Annals of Eugenics 12:172–175

Kruijer W, Flood P, Kooke R (2016) Heritability: Marker-Based Estimation of Heritability Using Individual Plant or Plot Data URL https://cranr-projectorg/package=heritability, r package version 1

Lai Z, Faris JD, Weiland JJ, Steffenson BJ, Friesen TL (2007) Genetic mapping of *Pyrenophora teres* f. *teres* genes conferring avirulence on barley. Fungal Genetics and Biology 44:323-329

Lamari L, Bernier CC (1989) Toxin of *Pyrenophora tritici-repentis*: host-specificity, significance in disease, and inheritance of host reaction. Phytopathology 79:740-744

Lander ES, Botstein D (1989) Mapping mendelian factors underlying quantitative traits using RFLP linkage maps Genetics 121:185-199

Lawrenson T, Shorinola O, Stacey N, Li C, Østergaard L, Patron N, Uauy C, Harwood W (2015) Induction of targeted, heritable mutations in barley and *Brassica oleracea* using RNA-guided Cas9 nuclease Genome Biol 16:258

Lehmensiek A, Platz GJ, Mace E, Poulsen D, Sutherland MW (2007) Mapping of adult plant resistance to net form of net blotch in three Australian barley populations. Crop and Pasture Science 58:1191-1197

Li H, Zhou S-Y, Zhao W-S, Su S-C, Peng Y-L (2009) A novel wall-associated receptor-like protein kinase gene, *OsWAK1*, plays important roles in rice blast disease resistance Plant molecular biology 69:337-346

Linde-Laursen I (1996) Recommendations for the designation of the barley chromosomes and their arms Barley Genet Newsl 26:1-3

Link HF (1809) Observationes in ordines plantarum naturales: Dissertatio Ima complectens anandrarum ordines epiphytas, mucedines gastromycos et fungos. 3:3-42. https://www.deutsche-digitale-bibliothek.de/item/3JPFZZELIUQ26TR2QK3L2W7LYZJF6M2U

Linnaeus C (1753) Species plantarum, Tomus 1 Impensis Laurentii Salvii, Holmiae. https://books.google.com.au/books?id=Kd2Cam5c-KEC

Lipka AE, Tian F, Wang Q, Peiffer J, Li M, Bradbury PJ, Gore MA, Buckler ES, Zhang Z (2012) GAPIT: genome association and prediction integrated tool Bioinformatics 28:2397-2399

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

Liu Z, Faris JD, Edwards MC, Friesen TL (2010) Development of Expressed Sequence Tag (EST)–based markers for genomic analysis of a barley 6H Region harboring multiple net form net blotch resistance genes. The Plant Genome 3:41-52

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

Liu Z, Zurn JD, Kariyawasam G, Faris JD, Shi G, Hansen J, Rasmussen JB, Acevedo M (2017) Inverse gene-for-gene interactions contribute additively to tan spot susceptibility in wheat Theoretical and Applied Genetics 130:1267-1276

Liu ZH, Zhong S, Stasko AK, Edwards MC, Friesen TL (2012) Virulence profile and genetic structure of a North Dakota population of *Pyrenophora teres* f. *teres*, the causal agent of net form net blotch of barley. Phytopathology 102:539-546

Lloyd SR, Schoonbeek H-j, Trick M, Zipfel C, Ridout CJ (2014) Methods to study PAMP-triggered immunity in Brassica species Molecular Plant-Microbe Interactions 27:286-295

Lobel Md (1576) Plantarum seu stirpium historia Plantini Antuerpia. https://www.biodiversitylibrary.org/bibliography/7094#/summary

Luna E, Bruce TJ, Roberts MR, Flors V, Ton J (2012) Next-generation systemic acquired resistance Plant physiology 158:844-853

Luo M, Gilbert B, Ayliffe M (2016) Applications of CRISPR/Cas9 technology for targeted mutagenesis, gene replacement and stacking of genes in higher plants Plant cell reports 35:1439-1450

Ma ZQ, Lapitan NLV, Steffenson BJ (2004) QTL mapping of net blotch resistance genes in a doubled-haploid population of six-rowed barley. Euphytica 137:291-296 doi:10.1023/B:EUPH.0000040441.36990.58

Mace E, Franckowiak J, Platz G, Poulsen D, Collard B, McPhail M, McKavanagh J, Christopher M, Fox G, Rodgers D (2007) Application of pedigree-based genome mapping in barley breeding Australia (BBA)-North. In: Proc. 13 th Australian Barley Technical Symposium, 2007. pp 151-168

Mair WJ, Deng W, Mullins JG, West S, Wang P, Besharat N, Ellwood SR, Oliver RP, Lopez-Ruiz FJ (2016) Demethylase inhibitor fungicide resistance in *Pyrenophora teres* f. sp. *teres* associated with target site modification and inducible overexpression of *Cyp51* Frontiers in microbiology 7:1279

Manninen O, Kalendar R, Robinson J, Schulman AH (2000) Application of BARE-1 retrotransposon markers to the mapping of a major resistance gene for net blotch in barley. Molecular and General Genetics MGG 264:325-334 doi:10.1007/s004380000326

Manninen OM, Jalli M, Kalendar R, Schulman A, Afanasenko O, Robinson J (2006) Mapping of major spot-type and net-type net-blotch resistance genes in the Ethiopian barley line CI 9819. Genome 49:1564-1571

Martin A, Platz GJ, de Klerk D, Fowler RA, Smit F, Potgieter FG, Prins R (2018) Identification and mapping of net form of net blotch resistance in South African barley. Molecular Breeding 38:53 DOI: 10.1007/s11032-018-0814-1

Martin RA, Clough KS (1984) Relationship of airborne spore load of *Pyrenophora teres* and weather variables to net blotch development on barley. Canadian Journal of Plant Pathology 6:105-110

Mascher M, Gundlach H, Himmelbach A, Beier S, Twardziok SO, Wicker T, Radchuk V, Dockter C, Hedley PE, Russell J (2017) A chromosome conformation capture ordered sequence of the barley genome Nature 544:427-433

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

Mengiste T (2012) Plant immunity to necrotrophs. Annual review of phytopathology 50:267-294

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

Morrell PL, Clegg MT (2007) Genetic evidence for a second domestication of barley (*Hordeum vulgare*) east of the Fertile Crescent Proceedings of the national academy of sciences 104:3289-3294

Moseman JG (1972) Report on genes for resistance to pests. Barley Genet Newsl 2:145-147

Murray GM, Brennan JP (2010) Estimating disease losses to the Australian barley industry. Australasian Plant Pathology 39:85-96

Nisikado Y (1929) Studies on the Helminthosporium diseases of Gramineae in Japan Berichte des Ohara Instituts für landwirtschaftliche Forschungen 4:111-126

O'Boyle P, Brooks W, Barnett M, Berger G, Steffenson B, Stromberg E, Maroof M, Liu S, Griffey C (2014) Mapping net blotch resistance in 'Nomini'and CIho 2291 barley Crop Science 54:2596-2602

Oğuz AÇ, Karakaya A (2017) Pathotypes of *Pyrenophora teres* on barley in Turkey Phytopathologia Mediterranea 56:224-234

Orabi J, Backes G, Wolday A, Yahyaoui A, Jahoor A (2007) The Horn of Africa as a centre of barley diversification and a potential domestication site Theoretical and Applied Genetics 114:1117-1127

Parkinson J (1640) Theatrum botanicum: The theater of plants; or, An herball of a large extent. London, UK. https://books.google.com.au/books?id=xM28oyCftJwC

Patterson HD, Thompson R (1971) Recovery of inter-block information when block sizes are unequal. Biometrika 58:545-554

Persoon CH (1822) Mycologia europaea vol 1. https://books.google.com.au/books?id=8lY-AAAAcAAJ

Peterson BG, Carl P, Boudt K, Bennett R, Ulrich J, Zivot E, Lesstel M, Balkissoon K, Wuertz D (2014) PerformanceAnalytics: Econometric tools for performance and risk analysis. R package version 1.4. 3541.

Piening L (1968) Development of barley net blotch from infested straw and seed. Canadian Journal of Plant Science 48:623-625

Piening L, Kaufmann ML (1969) Comparison of the effects of net blotch and leaf removal on yield in barley. Canadian Journal of Plant Science 49:731-735

Pieterse CM, Leon-Reyes A, Van der Ent S, Van Wees SC (2009) Networking by small-molecule hormones in plant immunity Nature chemical biology 5:308-316

Platz GJ, Bell KL, Rees RG, Galea VJ (2000) Pathotype variation of the Australian net blotch population. In: 8th International Barley Genetics Symposium, 2000. Waite Campus, Adelaide University, pp 160-162

Platz GJS, L; Forknall, C (2017) Barley disease yield loss response curves. https://grdc.com.au/resources-and-publications/grdc-update-papers/tab-content/grdc-update-papers/2017/02/barley-disease-yield-loss-response-curves.

Pon DS Physiologic specialization and variation in *Helminthosporium teres*. . In: Phytopathology, 1949. vol 1. American Phytopathological Society 3340 Pilot Knob Road, ST Paul, MN 55121, pp 18-18

Powell W, Machray GC, Provan J (1996) Polymorphism revealed by simple sequence repeats Trends in plant science 1:215-222

Rafalski JA (2010) Association genetics in crop improvement Current opinion in plant biology 13:174-180

Raman H, Platz GJ, Chalmers KJ, Raman R, Read BJ, Barr AR, Moody DB (2003) Mapping of genomic regions associated with net form of net blotch resistance in barley. Crop and Pasture Science 54:1359-1367

Ran FA, Hsu PD, Lin C-Y, Gootenberg JS, Konermann S, Trevino AE, Scott DA, Inoue A, Matoba S, Zhang Y (2013) Double nicking by RNA-guided CRISPR Cas9 for enhanced genome editing specificity Cell 154:1380-1389

Read BJ, Raman H, McMichael G, Chalmers KJ, Ablett GA, Platz GJ, Raman R, Genger RK, Boyd WJR, Li C-D (2003) Mapping and QTL analysis of the barley population Sloop × Halcyon. Crop and Pasture Science 54:1145-1153

Rees RG, Strong WM, Neale TJ (1999) The effects of foliar diseases on production of wheat and barley in the northern region in 1998. A report prepared for the GRDC Northern Panel

Remington DL, Thornsberry JM, Matsuoka Y, Wilson LM, Whitt SR, Doebley J, Kresovich S, Goodman MM, Buckler ES (2001) Structure of linkage disequilibrium and phenotypic associations in the maize genome Proceedings of the National Academy of Sciences 98:11479-11484

Richards J, Chao S, Friesen T, Brueggeman R (2016) Fine Mapping of the Barley Chromosome 6H Net Form Net Blotch Susceptibility Locus. G3: Genes| Genomes| Genetics:g3. 116.028902

Richards JK, Friesen TL, Brueggeman RS (2017) Association mapping utilizing diverse barley lines reveals net form net blotch seedling resistance/susceptibility loci Theoretical and Applied Genetics 130:915-927

Richter K, Schondelmaier J, Jung C (1998) Mapping of quantitative trait loci affecting *Drechslera teres* resistance in barley with molecular markers. Theoretical and applied genetics 97:1225-1234

Robinson J, Jalli M (1996) Diversity among Finnish net blotch isolates and resistance in barley. Euphytica 92:81-87

Robinson J, Jalli M (1997) Quantitative resistance to *Pyrenophora teres* in six Nordic spring barley accessions. Euphytica 94:201-208 doi:10.1023/A:1002996722383

Rostoks N, Ramsay L, MacKenzie K, Cardle L, Bhat PR, Roose ML, Svensson JT, Stein N, Varshney RK, Marshall DF (2006) Recent history of artificial outcrossing facilitates whole-

genome association mapping in elite inbred crop varieties Proceedings of the National Academy of Sciences 103:18656-18661

RStudio (2015) RStudio: integrated development for R. Version 0.98.507. Boston, MA: RStudio. Available at: http://www.rstudio.com

Ruiz-Roldán MC, Maier FJ, Schäfer W (2001) *PTK1*, a mitogen-activated-protein kinase gene, is required for conidiation, appressorium formation, and pathogenicity of *Pyrenophora teres* on barley Molecular Plant-Microbe Interactions 14:116-125

Ryals JA, Neuenschwander UH, Willits MG, Molina A, Steiner H-Y, Hunt MD (1996) Systemic acquired resistance The plant cell 8:1809

Saari E, Prescott J (1975) Scale for appraising the foliar intensity of wheat diseases Plant Disease Reporter

Saccardo PA (1882) Michelia: commentarium mycologicum fungos in primas italicos illustrans vol 2. typis Seminarii

Sato K, Takeda K (1993) Pathogenic variation of *Pyrenophora teres* isolates collected from Japanese and Canadian spring barley. Bull Res Inst Bioresour Okayama Univ 1:147-158

Schaller CW (1955) Inheritance of resistance to net blotch of barley. Phytopathology 45:6

Shaw PD, Graham M, Kennedy J, Milne I, Marshall DF (2014) Helium: visualization of large scale plant pedigrees BMC bioinformatics 15:259

Shi G, Friesen TL, Saini J, Xu SS, Rasmussen JB, Faris JD (2015) The wheat gene confers susceptibility on recognition of the necrotrophic effector *SnTox7* The Plant Genome 8

Shi G, Zhang Z, Friesen TL, Raats D, Fahima T, Brueggeman RS, Lu S, Trick HN, Liu Z, Chao W (2016) The hijacking of a receptor kinase–driven pathway by a wheat fungal pathogen leads to disease Science advances 2:e1600822

Shipton WA (1966) Effect of net blotch infection of barley on grain yield and quality. Animal Production Science 6:437-440

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

Shoemaker R (1959) Nomenclature of *Drechslera* and *Bipolaris*, grass parasites segregated from *'Helminthosporium'* Canadian Journal of Botany 37:879-887

Shoemaker RA (1962) Drechslera Ito. Canadian Journal of Botany 40:809-836

Singh D, Ziems L, Dracatos P, Pourkheirandish M, Tshewang S, Czembor P, German S, Fowler R, Snyman L, Platz G (2018) Genome-wide association studies provide insights on genetic architecture of resistance to leaf rust in a worldwide barley collection Molecular Breeding 38:43

Singh R, Tsuchiya T (1982) Identification and designation of telocentric chromosomes in barley by means of Giemsa N-banding technique Theoretical and Applied Genetics 64:13-24

Singh S (1956) Physiology and epidemiology of *Helminthosporium teres.*, University of Minnesota

Skibbe M, Qu N, Galis I, Baldwin IT (2008) Induced plant defenses in the natural environment: Nicotiana attenuata *WRKY3* and *WRKY6* coordinate responses to herbivory The Plant Cell 20:1984-2000

Smedegård-Petersen V (1971) *Pyrenophora teres* f. *maculata* f. nov. and *Pyrenophora teres* f. *teres* on barley in Denmark Yearbook of the Royal Veterinary and Agricultural University (Copenhagen) 1971:124-144

Smedegård-Petersen V (1974) Reduction in yield and grain size of barley due to attack by the net blotch fungus *Pyrenophora teres.* Royal Veterinary and Agricultural University, Copenhagen, Denmark, Yearbook 1974:108-117

Smedegård-Petersen V (1977) Isolation of two toxins produced by *Pyrenophora teres* and their significance in disease development of net-spot blotch of barley. Physiological Plant Pathology 10:203-211

Smith A, Cullis B, Thompson R (2001) Analyzing variety by environment data using multiplicative mixed models and adjustments for spatial field trend. Biometrics 57:1138-1147

Smith AB, Lim P, Cullis BR (2006) The design and analysis of multi-phase plant breeding experiments. The Journal of Agricultural Science 144:393-409

Soller M, Brody T, Genizi A (1976) On the power of experimental designs for the detection of linkage between marker loci and quantitative loci in crosses between inbred lines Theoretical and applied genetics 47:35-39

Spaner D, Shugar LP, Choo TM, Falak I, Briggs KG, Legge WG, Falk DE, Ullrich SE, Tinker NA, Steffenson BJ (1998) Mapping of disease resistance loci in barley on the basis of visual assessment of naturally occurring symptoms. Crop science 38:843-850

Sparrow DHB (1984) Barley improvement at the Waite Agricultural Research Institute. Waite Agricultural Research Institute Biennial Report 1982-1983:12-28

Speakman JB, Pommer EH (1986) A simple method for producing large volumes of *Pyrenophora teres* spore suspension. Bulletin of the British Mycological Society 20:129-130

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

Staal J, Kaliff M, Bohman S, Dixelius C (2006) Transgressive segregation reveals two Arabidopsis TIR-NB-LRR resistance genes effective against *Leptosphaeria maculans*, causal agent of blackleg disease The Plant Journal 46:218-230

Statler GD, Nordgaard JT (1980) Leaf wettability of wheat in relation to infection by *Puccinia recondita* f. sp. *tritici*. Phytopathology 70:641-643

Stefánsson TS (2009) Barley pathogens in Iceland: Identification, virulence and genetic structure of major barley pathogens in Iceland. Masters Thesis, Faculry of Land and Animal Resources, Agricultural University of Iceland

Steffenson BJ, Hayes PM, Kleinhofs A (1996) Genetics of seedling and adult plant resistance to net blotch (*Pyrenophora teres* f. *teres*) and spot blotch (*Cochliobolus sativus*) in barley. Theoretical and Applied Genetics 92:552-558

Steffenson BJ, Webster RK (1992a) Pathotype diversity of *Pyrenophora teres* f. *teres* on barley. Phytopathology 82:170-177

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

Steffenson BJ, Webster RK, Jackson LF (1991) Reduction in yield loss using incomplete resistance to *Pyrenophora teres* f. *teres* in barley. Plant disease 75:96-100

Stotz HU, Mitrousia GK, de Wit PJ, Fitt BD (2014) Effector-triggered defence against apoplastic fungal pathogens Trends in plant science 19:491-500

Sutton JC, Steele P (1983) Effects of seed and foliar fungicides on progress of net blotch and yield in barley. Canadian Journal of Plant Science 63:631-639

Tamang P (2017) Genetic Mapping and Characterization of Net Blotch Dominant Resistance and Dominant Susceptibility Loci in Barley. PhD Thesis, Agriculture and Applied Science, North Dakota State University

Team RC (2013) R: A language and environment for statistical computing

Tekauz A (1985) A numerical scale to classify reactions of barley to *Pyrenophora tere*s. Canadian Journal of Plant Pathology 7:181-183

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

Tekauz A, Desjardins M, Kleiber F Evaluating the *Pyrenophora teres* international standard barley differential set with Canadian isolates of the pathogen. In: 4th International Workshop on Barley Leaf Blights, June, 2011. pp 27-29

Tekauz A, Mills JT (1974) New types of virulence in *Pyrenophora teres* in Canada. Canadian Journal of Plant Science 54:731-734

Tenhola-Roininen T, Jalli M, Erkkilä M, Afanasenko O, Manninen OM (2011) Mapping of net blotch resistance locus in barley line c-8755. In: 4th international workshop on barley leaf blights, Dundee, Scotland, June 27-29, 2011. The James Hutton Institute

Townsend JA, Wright DA, Winfrey RJ, Fu F, Maeder ML, Joung JK, Voytas DF (2009) High-frequency modification of plant genes using engineered zinc-finger nucleases Nature 459:442

Tuohy JM, Jalli M, Cooke BM, O' Sullivan E (2006) Pathogenic variation in populations of *Drechslera teres* f. *teres* and *D. teres* f. *maculata* and differences in host cultivar responses. European Journal of Plant Pathology 116:177-185

Turner SD (2014) qqman: an R package for visualizing GWAS results using QQ and manhattan plots BioRxiv:005165

Turner SD (2017) qboxplot: Quantile-Based Boxplot R Package CRAN

Ullrich SE (2010) Barley: Production, improvement, and uses vol 12. John Wiley & Sons

Usher T, Delacy I, Barsby J, Platz G (2009) Rapid detection of adult plant resistance to net form net blotch. In: 14th Australian Barley Technical Symposium, Sunshine Coast, Australia, 13-16 September 2009.

Van den Berg CGJ, Rossnagel BG (1990) Effects of temperature and leaf wetness period on conidium germination and infection of barley by *Pyr*enophora *teres*. Canadian Journal of Plant Pathology 12:263-266

Vatter T, Maurer A, Kopahnke D, Perovic D, Ordon F, Pillen K (2017) A nested association mapping population identifies multiple small effect QTL conferring resistance against net blotch (*Pyrenophora teres* f. *teres*) in wild barley PloS one 12:e0186803

Vidhyasekaran P (2016) Role of Plant Immune Signals and Signaling Systems in Plant Pathogenesis. In: Switching on Plant Innate Immunity Signaling Systems. Springer, pp 27-90

Vos P, Hogers R, Bleeker M, Reijans M, van De Lee T, Hornes M, Friters A, Pot J, Paleman J, Kuiper M (1995) AFLP: a new technique for DNA fingerprinting Nucleic acids research 23:4407-4414

Wallwork H, Butt M, Capio E (2016) Pathogen diversity and screening for minor gene resistance to *Pyrenophora teres* f. *teres* in barley and its use for plant breeding. Australasian Plant Pathology:1-5 doi:10.1007/s13313-016-0433-4

Wang R, Von Bothmer R, Dvorak J, Fedak G, Linde-Laursen I, Muramatsu M (1994) Genome symbols in the Triticeae (Poaceae)

Wang X, Mace ES, Platz GJ, Hunt CH, Hickey LT, Franckowiak JD, Jordan DR (2015) Spot form of net blotch resistance in barley is under complex genetic control. Theoretical and Applied Genetics 128:489-499

Ward Jr JH (1963) Hierarchical grouping to optimize an objective function. Journal of the American statistical association 58:236-244

Weiland JJ, Steffenson BJ, Cartwright RD, Webster RK (1999) Identification of molecular genetic markers in *Pyrenophora teres* f. *teres* associated with low virulence on 'Harbin' barley. Phytopathology 89:176-181

Wickham H (2009) ggplot2: Elegant Graphics for Data Analysis Springer-Verlag New York

Wolc A, Zhao HH, Arango J, Settar P, Fulton JE, O'sullivan NP, Preisinger R, Stricker C, Habier D, Fernando RL (2015) Response and inbreeding from a genomic selection experiment in layer chickens Genetics Selection Evolution 47:59

Wonneberger R, Ficke A, Lillemo M (2017a) Identification of quantitative trait loci associated with resistance to net form net blotch in a collection of Nordic barley germplasm Theoretical and Applied Genetics 130:2025-2043

Wonneberger R, Ficke A, Lillemo M (2017b) Mapping of quantitative trait loci associated with resistance to net form net blotch (*Pyrenophora teres* f. *teres*) in a doubled haploid Norwegian barley population PloS one 12:e0175773

Wu HL, Steffenson BJ, Li Y, Oleson AE, Zhong S (2003) Genetic variation for virulence and RFLP markers in *Pyrenophora teres*. Canadian Journal of Plant Pathology 25:82-90

Wyatt NA, Richards JK, Brueggeman RS, Friesen TL (2018) Reference Assembly and Annotation of the *Pyrenophora teres* f. *teres* Isolate 0-1 G3: Genes, Genomes, Genetics 8:1-8

Yaniv E, Tanskanen J, Törönen P, Kalendar R, Mishra P, Kiviharju E, Erkkilä M, Tenhola-Roininen T, Jalli M, Doležel J (2014) Fine-mapping of the Rpt5 net blotch resistance gene region in barley COST Action SUSTAIN - FA1208 - Pathogen-informed strategies for sustainable broad-spectrum crop resistance - 2nd Annual Conference, Zakopane, Poland, 15-17 October 2014

Yoder OC, Gracen VE (1975) Segregation of pathogenicity types and host-specific toxin production in progenies of crosses between races T and O of *Helminthosporium maydis* (*Cochliobolus heterostrophus*). Phytopathology 65:273-276

Youcef-Benkada M, Bendahmane B, Sy A, Barrault G, Albertini L (1994) Effects of inoculation of barley inflorescences with Drechslera teres upon the location of seed-borne inoculum and its transmission to seedlings as modified by temperature and soil moisture Plant pathology 43:350-355

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

Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. Weed research 14:415-421

Zeng Z-B (1994) Precision mapping of quantitative trait loci Genetics 136:1457-1468

Zhang Y, Zhang F, Li X, Baller JA, Qi Y, Starker CG, Bogdanove AJ, Voytas DF (2013) Transcription activator-like effector nucleases enable efficient plant genome engineering Plant physiology 161:20-27

Zhang Z, Ersoz E, Lai C-Q, Todhunter RJ, Tiwari HK, Gore MA, Bradbury PJ, Yu J, Arnett DK, Ordovas JM (2010) Mixed linear model approach adapted for genome-wide association studies Nature genetics 42:355

Zhang Z, Friesen TL, Simons KJ, Xu SS, Faris JD (2009) Development, identification, and validation of markers for marker-assisted selection against the *Stagonospora nodorum* toxin sensitivity genes Tsn1 and Snn2 in wheat Molecular breeding 23:35-49

Zhang Z, Friesen TL, Xu SS, Shi G, Liu Z, Rasmussen JB, Faris JD (2011) Two putatively homoeologous wheat genes mediate recognition of *SnTox3* to confer effector-triggered susceptibility to *Stagonospora nodorum* The Plant Journal 65:27-38

Zhu C, Gore M, Buckler ES, Yu J (2008) Status and prospects of association mapping in plants. The plant genome 1:5-20

Ziems L, Franckowiak J, Platz G, Mace E, Park R, Singh D, Jordan D, Hickey L (2017) Investigating successive Australian barley breeding populations for stable resistance to leaf rust Theoretical and Applied Genetics 130:2463-2477

Ziems LA, Hickey LT, Hunt CH, Mace ES, Platz GJ, Franckowiak JD, Jordan DR (2014) Association mapping of resistance to *Puccinia hordei* in Australian barley breeding germplasm. Theoretical and Applied Genetics:1-14

Appendices

Appendix 1. Summary of all currently published QTL for *Pyrenophora teres* f. *teres* anchored to the 2016 barley physical map. Adjacent markers from author's map or integrated consensus map used to represent QTL where peak or flanking markers could not be accurately positioned.

QTL or gene	Marker	Chr[a]	Position[b]	Desirable[c]|Undesirable[d]	GS[e]	Isolate used	Origin[f]	Pop[g]-Method[h]	Study
Snn4	BG262267	1A	2926803	Wheat	-	SnTox4	-	-	Abeysekara *et al.* 2012
Tsc1	IWA8622	1A	4313520	Wheat	-	ToxC	-	-	Liu *et al.* 2017
1H flanking	3257690	1H	11373977	SABBIErica|UVC8	A	NB50, NB73, NB85	Aus	DH-CIM	Martin *et al.* 2018
1H flanking	Bmag0213	1H	11374045	SABBIErica|UVC8	A	NB50, NB73, NB85	Aus	DH-CIM	Martin *et al.* 2018
QNFNBAPR.Ar/F-1H	Bmac0213	1H	13077934	Arapiles|Franklin	A	NB329, NB330	Aus	DH-CIM	Lehmensiek *et al.* 2007
QNFNBAPR.Ar/F-1H	GBM1007	1H	13518189	Arapiles|Franklin	A	NB329, NB330	Aus	DH-CIM	Lehmensiek *et al.* 2007
Rpt-1H-5-6	Bmag0872	1H	23943123	Harrington|OUH602	S	30199013	USA	RIL-CIM	Yun *et al.* 2006
Snn1	HORVU1Hr1G011860	1B	28888726	Wheat	-	SnTox1	-	-	Shi *et al.* 2016
NBP_QRptt1-1	SCRI_RS_153785	1H	33444893	Nordic Barley Panel	A	Field 2015, 2016	Nor	DP-GWAS	Wonnerberger *et al.* 2017a
NBP_QRptt1-1	SCRI_RS_170869	1H	33846694	Nordic Barley Panel	A	Field 2015, 2016	Nor	DP-GWAS	Wonnerberger *et al.* 2017a
NBP_QRptt1-1	SCRI_RS_170878	1H	33847082	Nordic Barley Panel	A	Field 2015, 2016	Nor	DP-GWAS	Wonnerberger *et al.* 2017a
NBP_QRptt1-1	11_10764	1H	34087686	Nordic Barley Panel	A	Field 2015, 2016	Nor	DP-GWAS	Wonnerberger *et al.* 2017a
NBP_QRptt1-1	SCRI_RS_189483	1H	35725028	Nordic Barley Panel	A	Field 2015, 2016	Nor	DP-GWAS	Wonnerberger *et al.* 2017a
-	SCRI_RS_199178	1H	39314012	Ethiopian & Eritrean Panel	S	30112002	USA	DP-GWAS	Adhikari 2017
Rpt-1H-5-6	HVM43	1H	83228119	Harrington|OUH602	S	30199013	USA	RIL-CIM	Yun *et al.* 2006
-	12_30672	(1H)	271801990	2-row	S	See footnote.	USA	BP-GWAS	Adhikari 2017
-	BMS90	1H	343934827	CIho 9819|Rolfi	S	84-28-1, 92-46/15, UK 80-12	Global	DH-SIM/CIM	Mannien *et al.* 2006
NBP_QRptt1-1	11_21333	1H	407009083	Nordic Barley Panel	A	Field 2013	Nor	DP-GWAS	Wonnerberger *et al.* 2017a
-	3087-1763	1H	412142539	Hector|NDB 112	S	15A	USA	RIL-CIM	Liu *et al.* 2015
-	SCRI_RS_231735	1H	443922320	(CIho 5791 or Tifang)	S	6A	USA	RIL-CIM	Koladia *et al.* 2017a
1H-TRAIT 1/9	MWG943	1H	480291969	Hor 9088|Arena	S	04/6T	-	F2-CIM	Richter *et al.* 1998
-	11_11189	1H	503413212	Zernogradsky 813|Ranniy 1	S	PK4, PP7, PN18,PP5, PP6, PK5	Rus	DH-CIM	Afanasenko *et al.* 2015
-	MWG518	1H	508385617	CIho 9819|Rolfi	S	84-28-1, 92-46/15, UK 80-12	Global	DH-SIM/CIM	Mannien *et al.* 2006
QPt.1H-1 (RT)	11_10357	1H	517540377	HEB-25 (-0.2)|-0.27 / -0.01	A	JKI Field	DEU	NAM-GWAS	Vatter *et al.* 2017
-	12_30191	1H	522448103	6-row	S	See footnote.	USA	BP-GWAS	Adhikari 2017
-	11_20844	1H	525478648	Zernogradsky 813|Ranniy 1	S	PP5	Rus	DH-CIM	Afanasenko *et al.* 2015
NBP_QRptt1-2	SCRI_RS_4928	1H	554563783	Nordic Barley Panel	A	Field 2013 UI	Nor	DP-GWAS	Wonnerberger *et al.* 2017a

Appendix 1. Continued.

QRptts1.1	E32M48.4	1H	17.8–22.9	CDC Bold|TR251	S	WRS858	CN	DH-MQM	Grewal *et al.* 2012
-	ISSR-D6	1H	22 cM	CIho 9819|Rolfi	S	84-28-1, 92-46/15, UK 80-12	Global	DH-SIM/CIM	Mannien *et al.* 2006
QRptts1.2	E38M59.8	1H	52.4–56.8	CDC Bold|TR251	S	WRS1607	CN	DH-MQM	Grewal *et al.* 2012
Snn2	XTC253803	2D	6420977	Wheat	-	SnTox2	-	-	Zhang *et al.* 2009
2HS-TRAIT 1/9	MWG878	2H	11119350	Hor 9088|Arena	S	04/6T	-	F2-CIM	Richter *et al.* 1998
Tsc2	BE444541	2B	11634497	Wheat	-	ToxB	-	-	Abeysekara *et al.* 2010
NBP_QRptt2-1	SCRI_RS_167465	2H	11898430	Nordic Barley Panel	S	6949B	Nor	DP-GWAS	Wonnerberger *et al.* 2017a
NBP_QRptt2-1	SCRI_RS_103515	2H	12239912	Nordic Barley Panel	S	6949B	Nor	DP-GWAS	Wonnerberger *et al.* 2017a
-	9490-843	2H	14591926	NDB 112|Hector	S	NB022	Aus	RIL-CIM	Liu *et al.* 2015
-	SCRI_RS_605	2H	15541232	Ethiopian & Eritrean Panel	S	30107004, Comb.	USA	DP-GWAS	Adhikari 2017
-	SCRI_RS_157480	2H	16189787	Ethiopian & Eritrean Panel	S	30107004	USA	DP-GWAS	Adhikari 2017
-	12_30155	2H	16221898	Ethiopian & Eritrean Panel	S	30107004	USA	DP-GWAS	Adhikari 2017
-	791–1113	2H	21582729	NDB 112|Hector	S	15A	USA	RIL-CIM	Liu *et al.* 2015
QNFNBAPR.Ar/F-2H	HVM36	2H	21930381	Arapiles|Franklin	A	NB329, NB330	Aus	DH-CIM	Lehmensiek *et al.* 2007
QNb.StMo-2H	ABG2	2H	29040972	Morex|Steptoe	A	ND89-19	USA	DH-IM	Steffenson *et al.* 1996
QPt.2H-2 (AO)	BK_12	2H	29125791	Barke (6.79)|3.05 / 10.2	A	JKI Field	DEU	NAM-GWAS	Vatter *et al.* 2017
QPt.2H-2 (AO)	BK_13	2H	29126530	-	A	JKI Field	DEU	NAM-GWAS	Vatter *et al.* 2017
QPt.2H-1 (RT)	BK_15	2H	29127449	Barke (0.68)|0.36 / 0.82	A	JKI Field	DEU	NAM-GWAS	Vatter *et al.* 2017
-	Bmag0740	2H	47210672	Baudin|AC Metcalfe	S	NB50	Aus	DH-IM	Cakir *et al.* 2011
QNFNBAPR.Ar/F-2H	psr131	2H	54631937	Arapiles|Franklin	A	NB329, NB330	Aus	DH-CIM	Lehmensiek *et al.* 2007
-	SCRI_RS_221843	2H	78370511	Ethiopian & Eritrean Panel	S	30112002, Combined	USA	DP-GWAS	Adhikari 2017
-	SCRI_RS_8366	2H	78371876	Ethiopian & Eritrean Panel	S	30112002, Combined	USA	DP-GWAS	Adhikari 2017
-	12_30691	2H	92831355	Ethiopian & Eritrean Panel	S	30107003	USA	DP-GWAS	Adhikari 2017
-	11_20674	2H	93146188	Ethiopian & Eritrean Panel	S	30107003	USA	DP-GWAS	Adhikari 2017
QNb.StMo-2H	ABG459	2H	106592953	Morex|Steptoe	A	ND89-19	USA	DH-IM	Steffenson *et al.* 1996
-	EBmac0607	2H	128355360	Kaputar|Tallon	S	NB52B, NB54, NB81, NB97	Aus	DH-IM	Cakir *et al.* 2003
-	HVHOTR1	2H	150906289	AC Metcalfe|Baudin	S	NB50	Aus	DH-IM	Cakir *et al.* 2011
QNFNBAPR.W/Al-2H	EBmac0640	2H	292774470	W2875-1|Alexis	A	NB329, NB330	Aus	RIL-CIM	Lehmensiek *et al.* 2007
QPt.2H-3 (AO)	SCRI_RS_13639	2H	339062444	Barke or HEB-25 (0.80)|-0.28 / 4.03	A	JKI Field	DEU	NAM-GWAS	Vatter *et al.* 2017
QNFNBAPR.Al/S-2H	Bmag0114	2H	505375523	Sloop|Alexis	A	NB329, NB330	Aus	DH-CIM	Lehmensiek *et al.* 2007
QNFNBAPR.W/Al-2H	Bmag0114	2H	505375523	W2875-1|Alexis	A	NB329, NB330	Aus	RIL-CIM	Lehmensiek *et al.* 2007
-	Bmag0114	2H	505375523	Kaputar|Tallon	S	NB52B, NB54, NB81, NB97	Aus	DH-IM	Cakir *et al.* 2003
-	11_10909	2H	545242939	Zernogradsky 813|Ranniy 1	S	PN10	Rus	DH-CIM	Afanasenko *et al.* 2015
-	11_10651	2H	606128866	CAP-III	S	See footnote.	USA	BP-GWAS	Adhikari 2017

Appendix 1. Continued.

QNb.StMo-7H	BCD129	2H	611545760	Morex|Steptoe	A	ND89-19	USA	DH-IM	Steffenson *et al.* 1996
Vrs1	HORVU2Hr1G092300	2H	652094642	CIho 9831|Ledger	S	WRS102	Canada	F2	Ho *et al.* 1996
-	MWG865	2H	654782195	CIho 9819|Rolfi	S	84-28-1, 92-46/15, UK 80-12, 27-36	Global	DH-SIM/CIM	Mannien *et al.* 2006
-	SCRI_RS_10670	2H	655815347	Ethiopian & Eritrean Panel	S	30107003, 30199012, Combined	USA	DP-GWAS	Adhikari 2017
-	SCRI_RS_128449	2H	655877532	Ethiopian & Eritrean Panel	S	30199012	USA	DP-GWAS	Adhikari 2017
-	SCRI_RS_138463	2H	663877590	Ethiopian & Eritrean Panel	S	30199012	USA	DP-GWAS	Adhikari 2017
QNFNBAPR.Al/S-2H	Bmag0125	2H	666290083	Sloop|Alexis	A	NB329, NB330	Aus	DH-CIM	Lehmensiek *et al.* 2007
Snn7	xcdf44	2D	674403984	Wheat	-	SnTox7	-	-	Shi *et al.* 2015
QRpta2S	bPb-3870	2H	698224671	Baronesse|Tallon	S	NB329, NB330	Aus	F5-CIM	Mace *et al.* 2007
QRpta2S	bPb-2680	2H	704373632	Baronesse|Mackay	S	NB329, NB330	Aus	F5-CIM	Mace *et al.* 2007
-	SCRI_RS_7392	2H	713895540	Ethiopian & Eritrean Panel	S	30199012	USA	DP-GWAS	Adhikari 2017
-	12_30690	2H	720339994	Nat. Small Grain Coll.	S	15A	USA	DP-GWAS	Richards *et al.* 2017
QRptta2	bPb-3858	2H	723107770	TR251|CDC Bold	S	WRS1607	CN	DH-MQM	Grewal *et al.* 2012
-	285-2932	2H	723653192	Falcon|Azhul	S	NB50	Aus	RIL-CIM	Islamovic *et al.* 2017
-	12_10579	2H	727570263	Nat. Small Grain Coll.	S	15A	USA	DP-GWAS	Richards *et al.* 2017
-	678-310	2H	729751285	Falcon|Azhul	S	6A	USA	RIL-CIM	Islamovic *et al.* 2017
QRptts2	bPb-4877	2H	739059336	TR251|CDC Dolly	S	WRS858	CN	DH-MQM	Grewal *et al.* 2008
QTL_{UH}-2H	GBM1036	2H	763960844	HHOR3073|Uschi	A	Stubble	DEU	DH-MQM	König *et al.* 2013
Snn7	xcfd50	2D	766101128	Wheat	-	SnTox7	-	-	Shi *et al.* 2015
2HL-TRAIT 1/9	I4133_7-E4449_D	2H	not listed	Hor 9088|Arena	S	04/6T	-	F2-CIM	Richter *et al.* 1998
NBP_QRptt3-1	12_31448	3H	2471227	Nordic Barley Panel	A	Field 2013	Nor	DP-GWAS	Wonnerberger *et al.* 2017a
-	ConsensusGBS0194-1	3H	3691178	NDB 112|Hector	S	LDN07Pt5	USA	RIL-CIM	Liu *et al.* 2015
-	12_10173	3H	3865263	BARI, N6, 6-row, CAP-I, II, III, B CAP	S	See footnote.	USA	BP-GWAS	Adhikari 2017
-	11_20252	3H	3868857	BARI, N6, USU, 6-row, CAP-II, B CAP	S	See footnote.	USA	BP-GWAS	Adhikari 2017
-	11_20252	3H	3868857	Ethiopian & Eritrean Panel	S	30107004	USA	DP-GWAS	Adhikari 2017
-	11_20159	3H	3981157	BARI, N6, USU, 6-row, CAP-II, III, B CAP	S	See footnote.	USA	BP-GWAS	Adhikari 2017
-	11_20159	3H	3981157	Nat. Small Grain Coll.	S	LDNH04Ptt19	USA	DP-GWAS	Richards *et al.* 2017
-	SCRI_RS_180343	3H	4180617	Nat. Small Grain Coll.	S	LDNH04Ptt19	USA	DP-GWAS	Richards *et al.* 2017
-	12_31409	3H	4184471	USU, B CAP	S	See footnote.	USA	BP-GWAS	Adhikari 2017
-	12_31409	3H	4184471	Nat. Small Grain Coll.	S	LDNH04Ptt19	USA	DP-GWAS	Richards *et al.* 2017
-	SCRI_RS_172351	3H	4918152	Nat. Small Grain Coll.	S	LDNH04Ptt19	USA	DP-GWAS	Richards *et al.* 2017
-	SCRI_RS_119379	3H	5000348	Nat. Small Grain Coll.	S	LDNH04Ptt19	USA	DP-GWAS	Richards *et al.* 2017
-	12_31230	3H	7368193	BARI, N6, USDA, 6-row, CAP-I, II, III, B CAP	S	See footnote.	USA	BP-GWAS	Adhikari 2017

Appendix 1. Continued.

-	bPb-3689	3H	7431908	Baronesse|Mackay	S	NB329, NB330	Aus	F5-CIM	Mace *et al.* 2007
-	11_21398	3H	7767159	Barley CAP	S	See footnote.	USA	BP-GWAS	Adhikari 2017
-	11_20976	3H	8913941	6-row	S	See footnote.	USA	BP-GWAS	Adhikari 2017
QPt.3H-1 (RT)	11_10112	3H	11039299	HEB-25 (-0.18)|-0.38 / -0.02	A	JKI Field	DEU	NAM-GWAS	Vatter *et al.* 2017
QPt.3H-2 (AO)	11_10112	3H	11039299	Barke or HEB-25 (-0.86)|-1.55 / 0.36	A	JKI Field	DEU	NAM-GWAS	Vatter *et al.* 2017
-	bPb-7199	3H	12322851	Baronesse|Mackay	S	NB329, NB330	Aus	F5-CIM	Mace *et al.* 2007
QNb.StMo-3H.1	ABA303	3H	22062644	Morex|Steptoe	A	ND89-19	USA	DH-IM	Steffenson *et al.* 1996
3H-TRAIT 2/9	MWG584	3H	28895565	Hor 9088|Arena	S	04/6T	-	F2-CIM	Richter *et al.* 1998
QNb.StMo-3H.1	ABG460	3H	28895668	Morex|Steptoe	A	ND89-19	USA	DH-IM	Steffenson *et al.* 1996
3H-TRAIT 2/9	MWG595	3H	42514452	Hor 9088|Arena	S	04/6T	-	F2-CIM	Richter *et al.* 1998
-	11_20356	3H	55590274	Nat. Small Grain Coll.	S	LDNH04Ptt19	USA	DP-GWAS	Richards *et al.* 2017
Rpt-3H-4	Bmag0828	3H	67629366	OUH602|Harrington	S	30199013	USA	RIL-CIM	Yun *et al.* 2006
-	12_30721	3H	111849845	Nat. Small Grain Coll.	S	15A	USA	DP-GWAS	Richards *et al.* 2017
NBP_QRptt3-2	11_21109	3H	160752469	Nordic Barley Panel	A	Field 2013	Nor	DP-GWAS	Wonnerberger *et al.* 2017a
Rpt-3H-4	Bmac0067	3H	174418521	OUH602|Harrington	S	30199013	USA	RIL-CIM	Yun *et al.* 2006
HvWRKY6 (MLOC_68299.2)	178,816,923-178,819,972	3H	178819117	Heartland|CI5791-γ8	S	0-1	USA	MECS	Tamang 2017
-	ConsensusGBS0508-1	3H	186950158	Azhul|Falcon	S	NB50	Aus	RIL-CIM	Islamovic *et al.* 2017
QRpt3H flanking	3257118-27:C>G	3H	415363466	Prior|Skiff	S+A	NB50	Aus	RIL-MIM	Thesis Chapter 4
QPt.3H-3 (RT)	11_10966	3H	416613563	Barke or HEB-25 (-0.65)|-1.08 / 0.39	A	JKI Field	DEU	NAM-GWAS	Vatter *et al.* 2017
-	Bmag0603	3H	417108407	Pompadour|Stirling	S	NB50, NB52B	Aus	DH-IM	Gupta *et al.* 2010
Pt,,a	BCD828	3H	485716120	Igri|Franka	S	WRS1240	CN	DH-SMA	Graner *et al.* 1996
NBP_QRptt3-2	SCRI_RS_221644	3H	490226429	Nordic Barley Panel	A	Field 2015	Nor	DP-GWAS	Wonnerberger *et al.* 2017a
$3H_{Tifang}$	SCRI_RS_221644	3H	490226429	Tifang|CIho 5791	S	15A, 6A, Br.Pteres, BB06	Global	RIL-CIM	Koladia *et al.* 2017a
$3H_{CI5791}$	SCRI_RS_221644	3H	490226429	CIho 5791|Tifang	S	JPT0101, JPT9901	JPN	RIL-CIM	Koladia *et al.* 2017a
QRpt3H peak	4170799-6:G>A	3H	490245359	Prior|Skiff	S+A	NB50	Aus	RIL-MIM	Thesis Chapter 4
QRpt3H flanking	490257835	3H	490257835	Prior|Skiff	S+A	NB50	Aus	RIL-MIM	Thesis Chapter 4
NBP_QRptt3-2	SCRI_RS_152172	3H	491376968	Nordic Barley Panel	S	LR9, 5050B, 6949B	Nor	DP-GWAS	Wonnerberger *et al.* 2017a
NBP_QRptt3-2	SCRI_RS_186102	3H	491850614	Nordic Barley Panel	S	LR9, 5050B, 6949B	Nor	DP-GWAS	Wonnerberger *et al.* 2017a
NBP_QRptt3-2	11_10728	3H	491895585	Nordic Barley Panel	S	LR9, 5050B, 6949B	Nor	DP-GWAS	Wonnerberger *et al.* 2017a
-	2804-1832	3H	496167125	NDB 112|Hector	S	BB06, NB50, Br.Pteres	DK, Aus, Br	RIL-CIM	Liu *et al.* 2015
Pt,,a	MWG680	3H	499363893	Igri|Franka	S	WRS1240	CN	DH-SMA	Graner *et al.* 1996

Appendix 1. Continued.

3Ha flanking	Bmag0122	3H	538150332	UVC8|SABBIErica	A	NB50	Aus	DH-CIM	Martin *et al.* 2018
3Ha flanking	Bmag0006	3H	538162697	UVC8|SABBIErica	A	NB50	Aus	DH-CIM	Martin *et al.* 2018
QTL$_{UH}$-3H	HVM33	3H	544865879	HHOR3073|Uschi	A	Stubble	DEU	DH-MQM	König *et al.* 2013
-	HVM0060	3H	576629522	AC Metcalfe|Baudin	S	NB50	Aus	DH-IM	Cakir *et al.* 2011
-	HVM0060	3H	576629522	AC Metcalfe|Baudin	A	NB324	Aus	DH-IM	Cakir *et al.* 2011
-	HVM0060	3H	576629522	CIho 9819|Rolfi	S	84-28-1, 92-46/15	Global	DH-SIM/CIM	Mannien *et al.* 2006
QNFNBAPR.Al/S-3H	Bmag0225	3H	582616593	Sloop|Alexis	A	NB329, NB330	Aus	DH-CIM	Lehmensiek *et al.* 2007
QNFNBAPR.W/Al-3H	Bmag0225	3H	582616593	W2875-1|Alexis	A	NB329, NB330	Aus	RIL-CIM	Lehmensiek *et al.* 2007
QPt.3H-4 (AO)	12_10583	3H	589722805	HEB-25 (-0.86)|-2.59 / -0.12	A	JKI Field	DEU	NAM-GWAS	Vatter *et al.* 2017
-	MWG2132	3H	596599508	TR306|Harringon	A	Natural Field	N.Am	DH -SIM	Spaner *et al.* 1998
-	SCRI_RS_235849	3H	596708949	Nat. Small Grain Coll.	S	LDNH04Ptt19	USA	DP-GWAS	Richards *et al.* 2017
-	1898-580	3H	606945369	NDB 112|Hector	S	JPT9901	JPN	RIL-CIM	Liu *et al.* 2015
3Hb flanking	USQ3_1329	3H	622814735	UVC8|SABBIErica	A	SA2013, SA2014	SthAf	DH-CIM	Martin *et al.* 2018
3Hb flanking	USQ3_0927	3H	622817031	UVC8|SABBIErica	A	SA2013, SA2014	SthAf	DH-CIM	Martin *et al.* 2018
QNb.StMo-3H.2	His4B	3H	624022781	Morex|Steptoe	A	ND89-19	USA	DH-IM	Steffenson *et al.* 1996
-	SCRI_RS_5194	3H	625627809	Ethiopian & Eritrean Panel	S	30107003	USA	DP-GWAS	Adhikari 2017
-	BOPA1_5488-1097	3H	629156883	-	S	6A	USA	RIL-CIM	Koladia *et al.* 2017a
-	MWG847	3H	632310643	TR306|Harringon	A	Natural Field	N.Am	DH -SIM	Spaner *et al.* 1998
-	11_10821	3H	633085996	c-8755|Harrington	S	V278 (aka Pt87)	Fin	DH-IM	Tenhola-Roininen *et al.* 2011
-	6716-823	3H	633641840	NDB 112|Hector	S	LDN07Pt5, ND89-19, BrPteres	USA, Br	RIL-CIM	Liu *et al.* 2015
QNb.StMo-3H.2	ABG4	3H	637917437	Morex|Steptoe	A	ND89-19	USA	DH-IM	Steffenson *et al.* 1996
-	2335-1614	3H	638623189	NDB 112|Hector	S	0–1, BB06, 6A	USA, DK	RIL-CIM	Liu *et al.* 2015
-	3718-1026	3H	642592656	NDB 112|Hector	S	NB50	Aus	RIL-CIM	Liu *et al.* 2015
QPt.3H-5 (AO)	SCRI_RS_146197	3H	643146457	Barke or HEB-25 (0.12)|-0.42 / 4.67	A	JKI Field	DEU	NAM-GWAS	Vatter *et al.* 2017
3Hc flanking	3259968	3H	645461774	SABBIErica|UVC8	A	SA2016	SthAf	DH-CIM	Martin *et al.* 2018
3Hc flanking	Bmag0013	3H	645461842	SABBIErica|UVC8	A	SA2016	SthAf	DH-CIM	Martin *et al.* 2018
QNFNBAPR.Al/S-3H	Bmag0013	3H	646313368	Sloop|Alexis	A	NB329, NB330	Aus	DH-CIM	Lehmensiek *et al.* 2007
QNFNBAPR.W/Al-3H	Bmag0013	3H	646313368	W2875-1|Alexis	A	NB329, NB330	Aus	RIL-CIM	Lehmensiek *et al.* 2007
-	11_20920	3H	654767726	Zernogradsky 813|Ranniy 1	S	PL9	Rus	DH-CIM	Afanasenko *et al.* 2015
AL_QRPtt3-1	SCRI_RS_10016	3H	656381638	Lavrans|Arve	A	NB15_1	Nor	DH-MQM	Wonnerberger *et al.* 2017b
QRptta3	bPb-2888	3H	665418361	TR251|CDC Dolly	A	Field 05	CN	DH-MQM	Grewal *et al.* 2008
-	HVM62	3H	673602497	CIho 9819|Rolfi	S	84-28-1, 92-46/15	Global	DH-SIM/CIM	Mannien *et al.* 2006
-	SCRI_RS_238412	3H	680226077	Ethiopian & Eritrean Panel	S	30112002	USA	DP-GWAS	Adhikari 2017
-	SCRI_RS_188420	3H	681788954	Nat. Small Grain Coll.	S	15A	USA	DP-GWAS	Richards *et al.* 2017
-	11_10343	3H	694855059	MN	S	See footnote.	USA	BP-GWAS	Adhikari 2017
3HL-TRAIT 2/7	E4547_13-E4047_4	3H	not listed	Hor 9088|Arena	S	04/6T	-	F2-CIM	Richter *et al.* 1998

Appendix 1. Continued.

-	p13m47KT191-p11m47TK118	3H	not listed	Kaputar|Tallon	S	NB97	Aus	DH-IM	Cakir *et al.* 2003
-	SCRI_RS_154517	4H	2259618	Nat. Small Grain Coll.	S	15A	USA	DP-GWAS	Richards *et al.* 2017
NBP_QRptt4-1	SCRI_RS_154517	4H	2259618	Nordic Barley Panel	S	LR9	Nor	DP-GWAS	Wonnerberger *et al.* 2017a
QPt.4H-1 (RT)	SCRI_RS_206744	4H	3580547	Barke (0.12)|-0.01 / 0.21	A	JKI Field	DEU	NAM-GWAS	Vatter *et al.* 2017
-	11_11345	4H	4314841	Zernogradsky 813|Ranniy 1	S	PK5	Rus	DH-CIM	Afanasenko *et al.* 2015
QPt.4H-2 (AO)	12_30150	4H	9579405	Barke or HEB-25 (1.00)|-0.57 / 1.65	A	JKI Field	DEU	NAM-GWAS	Vatter *et al.* 2017
-	4544-461	4H	46140224	Falcon|Azhul	S	6A, NB50	USA, Aus	RIL-CIM	Islamovic *et al.* 2017
QRpt4H flanking	3255709-40:A>G	4H	53032932	Nothern Region Barley	S	NB330, NB85	Aus	BP-GWAS	Thesis Chapter 5
-	11_10756	4H	63865143	N2	S	See footnote.	USA	BP-GWAS	Adhikari 2017
-	11_21073	4H	66861565	N2	S	See footnote.	USA	BP-GWAS	Adhikari 2017
-	11_10577	4H	69380591	N2	S	See footnote.	USA	BP-GWAS	Adhikari 2017
QRpt4H	3257855-10:A>G	4H	69382105	Nothern Region Barley	S	NB330, NB85	Aus	BP-GWAS	Thesis Chapter 5
QRpt4H flanking	3256237-67:A>G	4H	70434783	Nothern Region Barley	S	NB330, NB85	Aus	BP-GWAS	Thesis Chapter 5
-	11_20269	4H	72688992	N2, 2-row, B CAP	S	See footnote.	USA	BP-GWAS	Adhikari 2017
-	1944-1901	4H	72688992	Falcon|Azhul	S	0-1, JPT0101	CN, JPN	RIL-CIM	Islamovic *et al.* 2017
QRpts4	EBmac0906	4H	92756642	Halcyon|Sloop	S	NB50	Aus	DH-SIM	Raman *et al.* 2003
-	11_10942	4H	94604607	N2	S	See footnote.	USA	BP-GWAS	Adhikari 2017
QNFNBAPR.Al/S-4Ha	GMS089	4H	100740137	Sloop|Alexis	A	NB329, NB330	Aus	DH-CIM	Lehmensiek *et al.* 2007
-	GMS089	4H	100740137	AC Metcalfe|Baudin	S	NB50	Aus	DH-IM	Cakir *et al.* 2011
Rpt-4H-5-7	GMS089	4H	100740137	OUH602|Harrington	S	30199013	USA	RIL-CIM	Yun *et al.* 2006
QRpts4	GMS089	4H	100740137	Halcyon|Sloop	S	NB50	Aus	DH-SIM	Raman *et al.* 2003
QNFNBAPR.Al/S-4Ha	Bmac0181	4H	125536752	Sloop|Alexis	A	NB329, NB330	Aus	DH-CIM	Lehmensiek *et al.* 2007
QRpts4	Bmac0181	4H	125536752	Halcyon|Sloop	S	NB50	Aus	DH-SIM	Raman *et al.* 2003
QRpts4	HVM03	4H	166878463	TR251|CDC Dolly	S	WRW858	CN	DH-MQM	Grewal *et al.* 2008
NBP_QRptt4-2	SCRI_RS_135637	4H	350047931	Nordic Barley Panel	S	LR9	Nor	DP-GWAS	Wonnerberger *et al.* 2017a
QNb.StMo-4H	ABG484	4H	428986135	Steptoe|Morex	S+A	ND89-19	USA	DH-IM	Steffenson *et al.* 1996
QNb.StMo-4H	ABA3	4H	433572226	Steptoe|Morex	S+A	ND89-19	USA	DH-IM	Steffenson *et al.* 1996
-	11_10480	4H	437167992	CAP-III	S	See footnote.	USA	BP-GWAS	Adhikari 2017
-	12_30450	4H	440029216	CAP-III	S	See footnote.	USA	BP-GWAS	Adhikari 2017
-	SCRI_RS_170494	4H	469807342	Nat. Small Grain Coll.	S	6A	USA	DP-GWAS	Richards *et al.* 2017
-	SCRI_RS_181886	4H	470947123	Nat. Small Grain Coll.	S	6A, LDNH04Ptt19	USA	DP-GWAS	Richards *et al.* 2017
4HS-TRIAT 2/9	MWG58	4H	471263513	Arena|Hor 9088	S	04/6T	-	F2-CIM	Richter *et al.* 1998
AL_QRptt4-1	11_10262	4H	484881273	Arve|Lavrans	S	6949B	Nor	DH-MQM	Wonnerberger *et al.* 2017b
-	11_11207	4H	529292786	Zernogradsky 813|Ranniy 1	S	PN19	Rus	DH-CIM	Afanasenko *et al.* 2015
AL_QRptt4-1	SCRI_RS_147712	4H	548294745	Arve|Lavrans	S	5050B	Nor	DH-MQM	Wonnerberger *et al.* 2017b
-	12_30620	4H	550661796	N2	S	See footnote.	USA	BP-GWAS	Adhikari 2017

Appendix 1. Continued.

Rpt-4H-5-7	Bmac0310_4H	4H	578898073	OUH602|Harrington	S	30199013	USA	RIL-CIM	Yun *et al.* 2006
QPt.4H-3 (RT)	SCRI_RS_175327	4H	580329876	Barke (0.17)|0.06 / 0.51	A	JKI Field	DEU	NAM-GWAS	Vatter *et al.* 2017
4Ha flanking	Bmac0310	4H	583400485	UVC8|SABBIErica	A	NB73	Aus	DH-CIM	Martin *et al.* 2018
4Ha flanking	3268978	4H	583402082	UVC8|SABBIErica	A	NB73	Aus	DH-CIM	Martin *et al.* 2018
-	ABG472	4H	594540727	TR306|Harrington	A	Natural Field	N.Am	DH -SIM	Spaner *et al.* 1998
QNFNBAPR.Al/S-4Hb	wg719	4H	604747747	Sloop|Alexis	A	NB329, NB330	Aus	DH-CIM	Lehmensiek *et al.* 2007
QNFNBAPR.W/Al-4H	wg719	4H	604747747	W2875-1|Alexis	A	NB329, NB330	Aus	RIL-CIM	Lehmensiek *et al.* 2007
-	ABG618	4H	607801395	TR306|Harrington	A	Natural Field	N.Am	DH -SIM	Spaner *et al.* 1998
QPt.4H-4 (RT)	SCRI_RS_167808	4H	623326233	HEB-25 (-0.74)|-0.86 / -0.59	A	JKI Field	DEU	NAM-GWAS	Vatter *et al.* 2017
QPt.4H-5 (AO)	SCRI_RS_167808	4H	623326233	HEB-25 (-5.49)|-6.18 / -4.48	A	JKI Field	DEU	NAM-GWAS	Vatter *et al.* 2017
QNFNBAPR.Al/S-4Hb	cdo63	4H	625146296	Sloop|Alexis	A	NB329, NB330	Aus	DH-CIM	Lehmensiek *et al.* 2007
QNFNBAPR.W/Al-4H	cdo63	4H	625146296	W2875-1|Alexis	A	NB329, NB330	Aus	RIL-CIM	Lehmensiek *et al.* 2007
4Hb flanking	3261363	4H	626845555	UVC8|SABBIErica	A	SA2013, SA2014	SthAf	DH-CIM	Martin *et al.* 2018
4Hb flanking	4015794	4H	626845623	UVC8|SABBIErica	A	SA2013, SA2014	SthAf	DH-CIM	Martin *et al.* 2018
Snn5	mag3652	4B	627002331	Wheat	-	SnTox5	-	-	Friesen *et al.* 2012
Snn5	wmc349	4B	630180480	Wheat	-	SnTox5	-	-	Friesen *et al.* 2012
4HL-TRAIT 1/7	MWG616	4H	640343605	Hor 9088|Arena	S	04/6T	-	F2-CIM	Richter *et al.* 1998
Snn3	BE606637	5B	2897125	Wheat	-	SnTox3	-	-	Zhang *et al.* 2011
Tsn1	HORVU5Hr1G001020	5B	3550774	Wheat	-	ToxA	-	-	Faris *et al.* 2010
QNFNBAPR.W/Al-5H	abg705a	5H	21724258	W2875-1|Alexis	A	NB329, NB330	Aus	RIL-CIM	Lehmensiek *et al.* 2007
-	4977-567	5H	24737422	NDB 112|Hector	S	BrPteres	Br	RIL-CIM	Liu *et al.* 2015
-	4334-482	5H	26848667	NDB 112|Hector	S	JPT9901	JPN	RIL-CIM	Liu *et al.* 2015
QNb.StMo-5H	ABG395	5H	28955156	Steptoe|Morex	A	ND89-19	USA	DH-IM	Steffenson *et al.* 1996
-	4570-591	5H	34998568	NDB 112|Hector	S	ND89-19	USA	RIL-CIM	Liu *et al.* 2015
QNb.StMo-5H	CDO749	5H	39815282	Steptoe|Morex	A	ND89-19	USA	DH-IM	Steffenson *et al.* 1996
-	11_21480	5H	75877481	Zernogradsky 813|Ranniy 1	S	PN3	Rus	DH-CIM	Afanasenko *et al.* 2015
Snn3	BF200555	5B	98325994	Wheat	-	SnTox3	-	-	Zhang *et al.* 2011
QNFNBAPR.W/Al-5H	Bmag0387	5H	111693326	W2875-1|Alexis	A	NB329, NB330	Aus	RIL-CIM	Lehmensiek *et al.* 2007
-	1861–2382	5H	214882696	NDB 112|Hector	S	NB50	Aus	RIL-CIM	Liu *et al.* 2015
-	2664-314	5H	369237514	NDB 112|Hector	S	6A	USA	RIL-CIM	Liu *et al.* 2015
-	Bmac0096	5H	397599073	Baudin|AC Metcalfe	A	NB324	Aus	DH-IM	Cakir *et al.* 2011
NBP_QRptt5-1	SCRI_RS_221999	(5H)	399797033	Nordic Barley Panel	A	Field 2013 UI, Field 2013	Nor	DP-GWAS	Wonnerberger *et al.* 2017a
QRpta5S	bPb-6260	5H	460605134	Mackay|Baronesse	S	NB329, NB330	Aus	F5-CIM	Mace *et al.* 2007
-	HVLEU	5H	481700637	Rolfi|CIho 9819	S	P7, P8,P40, P58	Fin	DH-SIM/CIM	Mannien *et al.* 2000
NBP_QRptt5-1	SCRI_RS_205235	5H	491233708	Nordic Barley Panel	A	Field 2013 UI, Field 2013	Nor	DP-GWAS	Wonnerberger *et al.* 2017a
NBP_QRptt5-1	12_20350	5H	493783822	Nordic Barley Panel	A	Field 2013	Nor	DP-GWAS	Wonnerberger *et al.* 2017a

Appendix 1. Continued.

QTL$_{PH}$-5H-2	bPb-7852	5H	506965044	Post/Viresa|HHOR9484	A	Stubble	DEU	DH-MQM	König *et al.* 2013
-	SCRI_RS_152347	5H	522541240	Nat. Small Grain Coll.	S	LDNH04Ptt19	USA	DP-GWAS	Richards *et al.* 2017
QTLPH-5H-2	bPb-1485	5H	527111455	Post/Viresa|HHOR9484	A	Stubble	DEU	DH-MQM	König *et al.* 2013
QRpta5S	bPb-6288	5H	542990217	Mackay|Baronesse	S	NB329, NB330	Aus	F5-CIM	Mace *et al.* 2007
-	11_21314	5H	558194881	Nat. Small Grain Coll.	S	6A	USA	DP-GWAS	Richards *et al.* 2017
QPt.5H-1 (RT)	11_10834	5H	559204073	HEB-25 (-0.11)|-0.30 / 0.06	A	JKI Field	DEU	NAM-GWAS	Vatter *et al.* 2017
-	12_30848	5H	560570414	2-row	S	See footnote.	USA	BP-GWAS	Adhikari 2017
QTL$_{UH}$-5H-1	bPb-9476	5H	563974938	HHOR3073|Uschi	A	Stubble	DEU	DH-MQM	König *et al.* 2013
QTL$_{UH}$-5H-2	bPb-6643	5H	563974938	HHOR3073|Uschi	A	Stubble	DEU	DH-MQM	König *et al.* 2013
5H flanking	3398320	5H	569309660	SABBIErica|UVC8	A	SA2014, SA2016	SthAf	DH-CIM	Martin *et al.* 2018
5H flanking	3810891	5H	569309728	SABBIErica|UVC8	A	SA2014, SA2016	SthAf	DH-CIM	Martin *et al.* 2018
-	MWG914	5H	572516926	Harrington|TR306	A	Natural Field	N.Am	DH-SIM	Spaner *et al.* 1998
QRptta5	bPb-6126	5H	575222503	TR251|CDC Dolly	A	Field 05	CN	DH-MQM	Grewal *et al.* 2008
-	MWG894	5H	579732179	Harrington|TR306	A	Natural Field	N.Am	DH -SIM	Spaner *et al.* 1998
-	SCRI_RS_154288	5H	580511056	Ethiopian & Eritrean Panel	S	30199012	USA	DP-GWAS	Adhikari 2017
QRpta5S	bPb-0710	5H	585706736	Baronesse|Mackay	S	NB329, NB330	Aus	F5-CIM	Mace *et al.* 2007
QRpta5S	bPb-2325	5H	589736571	Baronesse|Mackay	S	NB329, NB330	Aus	F5-CIM	Mace *et al.* 2007
QPt.5H-2 (AO)	SCRI_RS_228463	5H	603537537	HEB-25 (-2.28)|-2.91 / 0.01	A	JKI Field	DEU	NAM-GWAS	Vatter *et al.* 2017
AL_QRptt5-1	SCRI_RS_128407	5H	605366696	Lavrans|Arve	S	LR9, 6949B, 5050B	Nor	DH-MQM	Wonnerberger *et al.* 2017b
-	11_10845	5H	605405791	Zernogradsky 813|Ranniy 1	S	PP7	Rus	DH-CIM	Afanasenko *et al.* 2015
QRptts5	bPb-2960	5H	606031409	CDC Dolly|TR251	S	WRS858	CN	DH-MQM	Grewal *et al.* 2008
QTL$_{PH}$-5H-1	bPb-3887	5H	614000000	HHOR9484|Post x Viresa	A	Stubble	DEU	DH-MQM	König *et al.* 2013
QTL$_{PH}$-5H-3	bPb-2006	5H	615132222	HHOR9484|Post x Viresa	A	Stubble	DEU	DH-MQM	König *et al.* 2013
NBP_QRptt5-2	SCRI_RS_165290	5H	648412051	Nordic Barley Panel	A	Field 2014	Nor	DP-GWAS	Wonnerberger *et al.* 2017a
NBP_QRptt5-2	12_20867	5H	648513686	Nordic Barley Panel	A	Field 2014	Nor	DP-GWAS	Wonnerberger *et al.* 2017a
NBP_QRptt5-2	SCRI_RS_179841	5H	648555743	Nordic Barley Panel	A	Field 2014	Nor	DP-GWAS	Wonnerberger *et al.* 2017a
AL_QRptt5-2	SCRI_RS_140499	5H	650977156	Lavrans|Arve	A	NB14, LR9, 5050B	Nor	DH-MQM	Wonnerberger *et al.* 2017b
AL_QRptt5-2	SCRI_RS_235652	5H	652929697	Lavrans|Arve	S+A	NB15_2, NB15_2, NB15, NB16_1, NB16_2, NB16, 6949B	Nor	DH-MQM	Wonnerberger *et al.* 2017b
QPt.5H-3 (AO)	11_21138	5H	653914929	Barke (0.99)|-0.03 / 1.59	A	JKI Field	DEU	NAM-GWAS	Vatter *et al.* 2017
-	11_10405	5H	657266491	Ethiopian & Eritrean Panel	S	30107003, 30107004	USA	DP-GWAS	Adhikari 2017
-	SCRI_RS_194337	5H	660623582	Ethiopian & Eritrean Panel	S	30112002, Combined	USA	DP-GWAS	Adhikari 2017
QRptta5.1	E35M49.5	5H	112.1–120.5	TR251|CDC Bold	S+A	WRS1607, Field 08	CN	DH-MQM	Grewal *et al.* 2012

Appendix 1. Continued.

-	ISSR-C2	5H	2 cM	CIho 9819|Rolfi	S	92-46/15	Global	DH-SIM/CIM	Mannien *et al.* 2006
QRptta5.2	E33M47.7	5H	200.4–206.8	CDC Bold|TR251	S+A	WRS1607, Field 07	CN	DH-MQM	Grewal *et al.* 2012
-	REMAP-M10	5H	44 cM	CIho 9819|Rolfi	S	27-36	Global	DH-SIM/CIM	Mannien *et al.* 2006
AL_QRptt7-1	12_31350	6H	6314541	Lavrans|Arve	A	NB14	Nor	DH-MQM	Wonnerberger *et al.* 2017b
6H-bin2	bPb-2751	6H	8098629	Sep2-72|M120	S	See footnote.	USA	RIL-CIM	St. Pierre *et al.* 2010
-	11_10165	6H	14306449	Ethiopian & Eritrean Panel	S	30112002	USA	DP-GWAS	Adhikari 2017
6H-bin2	bPb-8836	6H	15772149	Sep2-72|M120	S	See footnote.	USA	RIL-CIM	St. Pierre *et al.* 2010
Rpt-CIho 2291	GBM1215	6H	24621085	CIho 2291|Hector	S	ND89-19	USA	F2-DPM	O'Boyle *et al.* 2014
-	MWG916	6H	29107216	TR306|Harrington	A	Natural Field	N.Am	DH -SIM	Spaner *et al.* 1998
AL_QRptt7-2	SCRI_RS_179005	6H	31911992	Lavrans|Arve	A	NB16_2, NB16	Nor	DH-MQM	Wonnerberger *et al.* 2017b
-	11_20936	6H	34020654	Nat. Small Grain Coll.	S	15A	USA	DP-GWAS	Richards *et al.* 2017
-	SCRI_RS_213547	6H	37707648	Nat. Small Grain Coll.	S	15A	USA	DP-GWAS	Richards *et al.* 2017
AL_QRptt7-2	SCRI_RS_220780	6H	38050520	Lavrans|Arve	S	LR9	Nor	DH-MQM	Wonnerberger *et al.* 2017b
-	11_21281	6H	38242974	MN	S	See footnote.	USA	BP-GWAS	Adhikari 2017
-	SCRI_RS_162581	6H	42572271	Nat. Small Grain Coll.	S	15A	USA	DP-GWAS	Richards *et al.* 2017
QRpt6Ha flanking	3258496-13:G>A	6H	44234721	Skiff|Prior	S+A	NB85	Aus	RIL-MIM	Thesis Chapter 4
NBP_QRptt6-1	SCRI_RS_210025	6H	46298970	Nordic Barley Panel	A	Field 2016	Nor	DP-GWAS	Wonnerberger *et al.* 2017a
-	11_10013	6H	46541638	Nat. Small Grain Coll.	S	15A	USA	DP-GWAS	Richards *et al.* 2017
QPt.6H-1 (RT)	11_10013	6H	46541683	see SCRI_RS_186193	A	JKI Field	DEU	NAM-GWAS	Vatter *et al.* 2017
QPt.6H-2 (AO)	11_10013	6H	46541683	-	A	JKI Field	DEU	NAM-GWAS	Vatter *et al.* 2017
SPN1 flanking	4191-268	6H	47261864	ND B112|Hector	S	0–1, 15A, LDN07Pt5, ND89-19, NB022	Global	RIL-CIM	Liu *et al.* 2015
QRpt6Ha peak	3255277-6:T>C	6H	47271624	Skiff|Prior	S+A	NB85	Aus	RIL-MIM	Thesis Chapter 4
-	SCRI_RS_142506	6H	47363401	Nat. Small Grain Coll.	S	15A	USA	DP-GWAS	Richards *et al.* 2017
-	SCRI_RS_151282	6H	47377128	Nat. Small Grain Coll.	S	15A	USA	DP-GWAS	Richards *et al.* 2017
-	11_10539	6H	48979786	Nat. Small Grain Coll.	S	15A	USA	DP-GWAS	Richards *et al.* 2017
-	SCRI_RS_196458	6H	50169169	Nat. Small Grain Coll.	S	15A	USA	DP-GWAS	Richards *et al.* 2017
-	12_30658	6H	50346904	Nat. Small Grain Coll.	S	15A	USA	DP-GWAS	Richards *et al.* 2017
-	12_30316	6H	50801220	Nat. Small Grain Coll.	S	15A	USA	DP-GWAS	Richards *et al.* 2017
-	SCRI_RS_168111	6H	50943882	Nat. Small Grain Coll.	S	15A	USA	DP-GWAS	Richards *et al.* 2017
-	SCRI_RS_119674	6H	51410692	Nat. Small Grain Coll.	S	15A	USA	DP-GWAS	Richards *et al.* 2017
-	SCRI_RS_120783	6H	51817157	Nat. Small Grain Coll.	S	15A	USA	DP-GWAS	Richards *et al.* 2017
-	SCRI_RS_140158	6H	60466498	Nat. Small Grain Coll.	S	LDNH04Ptt19	USA	DP-GWAS	Richards *et al.* 2017
-	SCRI_RS_211299	6H	60836029	Nat. Small Grain Coll.	S	LDNH04Ptt19	USA	DP-GWAS	Richards *et al.* 2017
-	SCRI_RS_152174	6H	61217160	Nat. Small Grain Coll.	S	LDNH04Ptt19	USA	DP-GWAS	Richards *et al.* 2017
-	12_10199	6H	66485252	Nat. Small Grain Coll.	S	6A	USA	DP-GWAS	Richards *et al.* 2017
QRpt6Ha flanking	4016288-26:C>A	6H	80019061	Skiff|Prior	S+A	NB85	Aus	RIL-MIM	Thesis Chapter 4

Appendix 1. Continued.

SPN1 flanking	ABC08769-1-1-205	6H	91401417	ND B112|Hector	S	0–1, 15A, LDN07Pt5, ND89-19, NB022	Global	RIL-CIM	Liu *et al.* 2015
Rpt-CIho 2291	Bmag0500	6H	111884984	CIho 2291|Hector	S	ND89-19	USA	F2-DPM	O'Boyle *et al.* 2014
Rpt-CIho 2291	GMS006	6H	113675049	CIho 2291|Hector	S	ND89-19	USA	F2-DPM	O'Boyle *et al.* 2014
-	12_30569	6H	115112608	N2, N6, 2-row, CAP-I, III, B CAP	S	See footnote.	USA	BP-GWAS	Adhikari 2017
-	12_30473	6H	115445291	N2, 2-row, B CAP	S	See footnote.	USA	BP-GWAS	Adhikari 2017
NBP_QRptt6-1	SCRI_RS_182195	6H	120065893	Nordic Barley Panel	S	5050B	Nor	DP-GWAS	Wonnerberger *et al.* 2017a
NBP_QRptt6-1	12_30441	6H	123871545	Nordic Barley Panel	S	5050B	Nor	DP-GWAS	Wonnerberger *et al.* 2017a
NBP_QRptt6-1	12_31005	6H	129177918	Nordic Barley Panel	S	5050B	Nor	DP-GWAS	Wonnerberger *et al.* 2017a
NBP_QRptt6-1	SCRI_RS_219810	6H	158189215	Nordic Barley Panel	A	Field 2014	Nor	DP-GWAS	Wonnerberger *et al.* 2017a
NBP_QRptt6-1	12_30120	6H	164749119	Nordic Barley Panel	A	Field 2014	Nor	DP-GWAS	Wonnerberger *et al.* 2017a
-	SCRI_RS_148652	6H	187823870	Nat. Small Grain Coll.	S	15A	USA	DP-GWAS	Richards *et al.* 2017
-	12_30749	6H	187979020	Nat. Small Grain Coll.	S	15A	USA	DP-GWAS	Richards *et al.* 2017
-	SCRI_RS_162760	6H	192855349	Nat. Small Grain Coll.	S	15A	USA	DP-GWAS	Richards *et al.* 2017
QRpt6Hm flanking	3257954-50:G>A	6H	193444571	-|Moravian LV	S+A	NB73	Aus	BP-GWAS	Thesis Chapter 5
-	SCRI_RS_118255	6H	195457853	Nat. Small Grain Coll.	S	15A	USA	DP-GWAS	Richards *et al.* 2017
-	SCRI_RS_144162	6H	197871410	Nat. Small Grain Coll.	S	15A	USA	DP-GWAS	Richards *et al.* 2017
-	HVM11	6H	208945443	SM89010|Q21861	S	0-1, 15A, ND89-19	CN, USA	DH-SIM/CIM	Friesen *et al.* 2006
-	SCRI_RS_144579	6H	210767018	Nat. Small Grain Coll.	S	15A	USA	DP-GWAS	Richards *et al.* 2017
-	SCRI_RS_153797	6H	214741965	Nat. Small Grain Coll.	S	15A	USA	DP-GWAS	Richards *et al.* 2017
-	12_31006	6H	233293369	Nat. Small Grain Coll.	S	15A	USA	DP-GWAS	Richards *et al.* 2017
-	SCRI_RS_239917	6H	238807820	Nat. Small Grain Coll.	S	15A	USA	DP-GWAS	Richards *et al.* 2017
QRpt6Hm flanking	3434214-43:A>T	6H	251009458	-|Moravian LV	A	NB73	Aus	BP-GWAS	Thesis Chapter 5
QNb.StMo-6H.1	ABG387B	6H	259699042	Steptoe|Morex	S+A	ND89-19	USA	DH-IM	Steffenson *et al.* 1996
-	SCRI_RS_162504	6H	261292336	Nat. Small Grain Coll.	S	15A	USA	DP-GWAS	Richards *et al.* 2017
-	11_21124	6H	288957373	Nat. Small Grain Coll.	S	LDNH04Ptt19	USA	DP-GWAS	Richards *et al.* 2017
QNb.StMo-6H.1	ABG458	6H	298368767	Steptoe|Morex	S+A	ND89-19	USA	DH-IM	Steffenson *et al.* 1996
Rpt	ksuA3B	6H	298439127	Chevron|Stander	S	ND89-19	USA	DH -SIM	Ma *et al.* 2004
Rpt-Nomini	Bmag0103a	6H	313089636	Nomini|Hector	S	ND89-19	USA	F2-DPM	O'Boyle *et al.* 2014
-	Bmac0018 (BMS18)	6H	319224172	Kaputar|Tallon	S	NB50,NB52B, NB54,NB81,NB97	Aus	DH-IM	Cakir *et al.* 2003
-	BMS18	6H	319224172	CIho 9819|Rolfi	S	P7, P8,P40, P58	Fin	DH-SIM/CIM	Mannien *et al.* 2000
Rpt5	BMS18	6H	319224172	CIho 9819|Rolfi	S	84-28-1, 92-46/15, UK 80-12, 27-36	Global	DH-SIM/CIM	Mannien *et al.* 2006
-	11_10749	6H	322885901	BARI	S	See footnote.	USA	BP-GWAS	Adhikari 2017
QRpt6Hm flanking	3256458-52:T>C	6H	325194805	-|Moravian LV	S+A	NB73	Aus	BP-GWAS	Thesis Chapter 5

Appendix 1. Continued.

6H flanking	USQ2_0799, Bmag0173, HVM74, Bmag0009, USQ1_1140	6H	335741625	UVC8|SABBIErica	A	NB50, NB73, SA2013, SA2014, SA2016	Aus, SthAf	DH-CIM	Martin *et al.* 2018
6H flanking	USQ3_0144	6H	335741856	UVC8|SABBIErica	A	NB50, NB73, SA2013, SA2014, SA2016	Aus, SthAf	DH-CIM	Martin *et al.* 2018
-	11_20329	6H	336379278	2-row	S	See footnote.	USA	BP-GWAS	Adhikari 2017
QRpt6Hm flanking	3255777-67:T>G	6H	337179867	-|Moravian LV	S+A	NB73	Aus	BP-GWAS	Thesis Chapter 5
-	5497-661	6H	340035749	Falcon|Azhul	S	0-1, JPT0101	CN, JPN	RIL-CIM	Islamovic *et al.* 2017
QRpt6Hm	3254817-15:C>A	6H	340307078	-|Moravian LV	S+A	NB73, NB330	Aus	BP-GWAS	Thesis Chapter 5
-	12_30254	6H	345288532	Nat. Small Grain Coll.	S	6A	USA	DP-GWAS	Richards *et al.* 2017
-	12_31479	6H	348843035	Nat. Small Grain Coll.	S	15A	USA	DP-GWAS	Richards *et al.* 2017
-	12_30305	6H	350303742	6-row	S	See footnote.	USA	BP-GWAS	Adhikari 2017
QRpts6C	HVM74	6H	350438998	Mackay|Baronesse	S	NB329, NB330	Aus	F5-CIM	Mace *et al.* 2007
QRpt6	HVM74	6H	350438998	TR251|CDC Dolly	S+A	WRS858, WRS1607, Field 05, Field 06	CN	DH-MQM	Grewal *et al.* 2008
-	HVM74	6H	350438998	Stirling|Pompadour	S	NB50, NB52B	Aus	DH-IM	Gupta *et al.* 2010
-	HVM74	6H	350438998	Stirling|Pompadour	S	NB73	Aus	DH-MTA	Gupta *et al.* 2011
-	HVM74	6H	350438998	Pompadour|Stirling	S	97NB1, 95NB100, NB81	Aus	DH-IM	Gupta *et al.* 2010
-	HVM74	6H	350438998	Pompadour|Stirling	S	97NB1	Aus	DH-MTA	Gupta *et al.* 2011
-	HVM74	6H	350438998	WPG8412|Stirling	S	97NB1, NB73	Aus	DH-MTA	Gupta *et al.* 2011
QRpts6C	HVM74	6H	350438998	Mackay|Tallon	S	NB329, NB330	Aus	F5-CIM	Mace *et al.* 2007
-	11_11153	6H	351737563	6-row	S	See footnote.	USA	BP-GWAS	Adhikari 2017
-	12_21482	6H	351737595	6-row	S	See footnote.	USA	BP-GWAS	Adhikari 2017
rpt.r/rpt.k	BE636841	6H	352574089	Kombar/Rika|Rika/Kombar	S	6A, 15A, 15A x 6A #4	USA, Lab	DH-IM	Abu Qamar *et al.* 2008
rpt.r/rpt.k	BE636841	6H	352574089	Kombar/Rika|Rika/Kombar	S	6A, 15A	USA	RIL-IM	Liu *et al.* 2010
QRpt6Hp/b flanking	4170458-67:G>C	6H	357490943	Prior - Skiff reciprocal	S+A	NB50, NB85	Aus	RIL-MIM	Thesis Chapter 4
QPt.6H-1 (RT)	SCRI_RS_239642	6H	357492292	see SCRI_RS_186193	A	JKI Field	DEU	NAM-GWAS	Vatter *et al.* 2017
-	SCRI_RS_136604	6H	357929989	Nat. Small Grain Coll.	S	15A, 6A	USA	DP-GWAS	Richards *et al.* 2017
-	WG223	6H	359588181	TR306|Harrington	A	Natural Field	N.Am	DH -SIM	Spaner *et al.* 1998
Rpt	WG223	6H	359588181	Chevron|Stander	S	ND89-19	USA	DH -SIM	Ma *et al.* 2004
-	11_10227	6H	359588787	MSU, N2, USDA, 2-row, CAP-II, III, IV, B CAP	S	See footnote.	USA	BP-GWAS	Adhikari 2017
-	11_10227	6H	359588787	Nat. Small Grain Coll.	S	6A	USA	DP-GWAS	Richards *et al.* 2017
-	SCRI_RS_224389	6H	360336441	Nat. Small Grain Coll.	S	15A	USA	DP-GWAS	Richards *et al.* 2017
-	12_30681	6H	360471468	BARI, CAP-III, B CAP	S	See footnote.	USA	BP-GWAS	Adhikari 2017

Appendix 1. Continued.

-	11_20835	6H	361066555	MSU, N2, USDA, 2-row, CAP-II, III, IV, B CAP	S	See footnote.	USA	BP-GWAS	Adhikari 2017
-	11_20835	6H	361066555	Nat. Small Grain Coll.	S	6A	USA	DP-GWAS	Richards *et al.* 2017
-	SCRI_RS_213566	6H	361531305	Nat. Small Grain Coll.	S	6A	USA	DP-GWAS	Richards *et al.* 2017
-	SCRI_RS_142541	6H	364361882	Ethiopian & Eritrean Panel	S	30199012	USA	DP-GWAS	Adhikari 2017
-	SCRI_RS_142541	6H	364361882	Nat. Small Grain Coll.	S	6A	USA	DP-GWAS	Richards *et al.* 2017
QRpt6Hp	3260813-56:A>T	6H	364757662	Skiff|Prior	S+A	NB85	Aus	RIL-MIM	Thesis Chapter 4
6H-bin6	bPb-9051	6H	365882030	M120|Sep2-72	S	See footnote.	USA	RIL-CIM	St. Pierre *et al.* 2010
-	SCRI_RS_138001	6H	366210041	Nat. Small Grain Coll.	S	6A, LDNH04Ptt19	USA	DP-GWAS	Richards *et al.* 2017
-	SCRI_RS_188305	6H	366400745	Nat. Small Grain Coll.	S	15A, 6A	USA	DP-GWAS	Richards *et al.* 2017
QRpt6Hs	3257446-28:G>T	6H	368527587	Prior|Skiff	S+A	NB50,	Aus	RIL-MIM	Thesis Chapter 4
QRpt6Hs	3257446-28:G>T	6H	368527587	ND parents via Bowman	S+A	NB50, NB73, NB85	Aus	BP-GWAS	Thesis Chapter 5
Spt1 flanking	rpt-M8	6H	370428695	-	S	6A, 15A	USA	ICR-HRM	Richards *et al.* 2016
NBP_QRptt6-1	SCRI_RS_186193	6H	370429082	Nordic Barley Panel	A	Field 2014	Nor	DP-GWAS	Wonnerberger *et al.* 2017a
QPt.6H-1 (RT)	SCRI_RS_186193	6H	370429082	Barke or HEB-25 (0.40)|-0.22 / 1.03	A	JKI Field	DEU	NAM-GWAS	Vatter *et al.* 2017
QPt.6H-2 (AO)	SCRI_RS_186193	6H	370429082	Barke or HEB-25 (3.59)|-1.84 / 10.86	A	JKI Field	DEU	NAM-GWAS	Vatter *et al.* 2017
Spt1	rpt-M12.k	6H	373416587	Rika/Kombar	S	15A, 15A × 6A #20, 15A × 6A #63	USA	ICR-HRM	Richards *et al.* 2016
Spt1	rpt-M12.r	6H	373416607	Kombar/Rika	S	6A, 15A × 6A #72	USA	ICR-HRM	Richards *et al.* 2016
rpt.r/rpt.k	ABC04320	6H	373417144	Kombar/Rika|Rika/Kombar	S	6A, 15A	USA	RIL-IM	Liu *et al.* 2010
Spt1	rpt-M13	6H	373420409	-	S	6A, 15A	USA	ICR-HRM	Richards *et al.* 2016
-	SCRI_RS_188243	6H	373423645	Nat. Small Grain Coll.	S	15A, 6A	USA	DP-GWAS	Richards *et al.* 2017
-	SCRI_RS_176650	6H	373424916	Nat. Small Grain Coll.	S	15A	USA	DP-GWAS	Richards *et al.* 2017
-	SCRI_RS_13935	6H	373616190	Nat. Small Grain Coll.	S	LDNH04Ptt19	USA	DP-GWAS	Richards *et al.* 2017
-	SCRI_RS_195914	6H	373617031	Nat. Small Grain Coll.	S	15A	USA	DP-GWAS	Richards *et al.* 2017
-	SCRI_RS_7104	6H	374525968	Nat. Small Grain Coll.	S	6A, LDNH04Ptt19	USA	DP-GWAS	Richards *et al.* 2017
-	SCRI_RS_137464	6H	374867096	Nat. Small Grain Coll.	S	LDNH04Ptt19	USA	DP-GWAS	Richards *et al.* 2017
Spt1 flanking	rpt-M20	6H	374869142	-	S	6A, 15A	USA	ICR-HRM	Richards *et al.* 2016
-	11_10513	6H	374876068	N6	S	See footnote.	USA	BP-GWAS	Adhikari 2017
NBP_QRptt6-1	11_10513	6H	374876068	Nordic Barley Panel	S	6949B	Nor	DP-GWAS	Wonnerberger *et al.* 2017a
QRpt6Hp/s flanking	3259255-10:C>T	6H	375529371	Prior - Skiff reciprocal	S+A	NB50, NB85	Aus	RIL-MIM	Thesis Chapter 4
-	12_31178	6H	378210444	Nat. Small Grain Coll.	S	15A, LDNH04Ptt19	USA	DP-GWAS	Richards *et al.* 2017
-	12_31178	6H	378210479	MSU, N2, USDA, 2-row, CAP-II, III, IV, B CAP	S	See footnote.	USA	BP-GWAS	Adhikari 2017
Rpt5.f peak	3256608-45:C>G	6H	378772740	CIho 5791	S+A	NB330, NB73, NB85	Aus	BP-GWAS	Thesis Chapter 5

Appendix 1. Continued.

QRpt6Hb	3262096-64:C>T	6H	378974018	Prior - Skiff reciprocal	S+A	NB50, NB73	Aus	BP-GWAS	Thesis Chapter 5
Rpt5.f	4175123-58:C>A	6H	380193974	CIho 5791	S+A	NB330, NB73, NB85	Aus	BP-GWAS	Thesis Chapter 5
Rpt5.f	3256765-18:T>C	6H	382482733	CIho 5791	S+A	NB330, NB73, NB85	Aus	BP-GWAS	Thesis Chapter 5
Rpt5.f	3262659-31:C>G	6H	383141804	CIho 5791	S+A	NB330, NB73, NB85	Aus	BP-GWAS	Thesis Chapter 5
-	11_10377	6H	383275592	Nat. Small Grain Coll.	S	LDNH04Ptt19	USA	DP-GWAS	Richards *et al.* 2017
-	11_10377	6H	383275596	MN, MSU, 2-row, CAP-I, II, III, IV, B CAP	S	See footnote.	USA	BP-GWAS	Adhikari 2017
Rpt5.f	3255625-14:C>T	6H	384803137	CIho 5791	S+A	NB330, NB73, NB85	Aus	BP-GWAS	Thesis Chapter 5
-	SCRI_RS_165041	6H	384412630	Nat. Small Grain Coll.	S	6A	USA	DP-GWAS	Richards *et al.* 2017
-	12_30857	6H	384634322	N2, USDA, 2-row, CAP-II, CAP-III	S	See footnote.	USA	BP-GWAS	Adhikari 2017
-	12_30857	6H	384634322	Nat. Small Grain Coll.	S	6A	USA	DP-GWAS	Richards *et al.* 2017
-	12_30144	6H	384884951	MSU, N2, USDA, 2-row, CAP-I, II, IV	S	See footnote.	USA	BP-GWAS	Adhikari 2017
rpt.r/rpt.k	ABC01797	6H	386021619	Kombar/Rika|Rika/Kombar	S	6A, 15A, 15A x 6A #4	USA, Lab	DH-IM	Abu Qamar *et al.* 2008
	3432738-29:G>A	6H	386021835	CIho 5791	S+A	NB330, NB73, NB85	Aus	BP-GWAS	Thesis Chapter 5
6H$_{CI5791}$	SCRI_RS_140091	6H	390761574	CIho 5791|Tifang	S	15A, 6A, Br.Pteres, BB06, LDNH04Ptt-19, Tra-A5, FGOH04Ptt-21, JPT0101, JPT9901	Global	RIL-CIM	Koladia *et al.* 2017a
QRpts6C	bPb-0019	6H	391122395	Mackay|Tallon	S	NB329, NB330	Aus	F5-CIM	Mace *et al.* 2007
-	EBmac0874	6H	393569274	WPG8412|Pompadour	A	97NB1, NB73	Aus	DH-MTA	Gupta *et al.* 2011
-	EBmac0874	6H	393569274	SM89010|Q21861	S	0-1, 15A, ND89-19	CN, USA	DH-SIM/CIM	Friesen *et al.* 2006
-	EBmac0874	6H	393569274	Kaputar|Tallon	S	NB50, NB52B, NB54, NB81, NB97	Aus	DH-IM	Cakir *et al.* 2003
-	EBmac0874	6H	393569274	ND11231-12|VB9524	S	NB50, NB52B, NB54, NB81, NB97	Aus	DH-MTA	Cakir *et al.* 2003
Rpt5.f	3254663-15:T>A	6H	396127146	CIho 5791	S+A	NB330, NB73, NB85	Aus	BP-GWAS	Thesis Chapter 5
-	SCRI_RS_158011	6H	400155528	Nat. Small Grain Coll.	S	6A	USA	DP-GWAS	Richards *et al.* 2017

Appendix 1. Continued.

-	11_11067	6H	404560107	MSU	S	See footnote.	USA	BP-GWAS	Adhikari 2017
-	11_11067	6H	404560107	k-23874|Pirkka	S	V-278, PL5, PN10	Fin, Rus	DH-CIM	Afanasenko *et al.* 2015
rpt.r/rpt.k	ABC02895	6H	404560119	Kombar/Rika|Rika/Kombar	S	6A, 15A, 15A x 6A #4	USA, Lab	DH-IM	Abu Qamar *et al.* 2008
-	12_30346	(6H)	405535548	MSU	S	See footnote.	USA	BP-GWAS	Adhikari 2017
-	11_21339	(6H)	406409688	MSU, WSU	S	See footnote.	USA	BP-GWAS	Adhikari 2017
-	11_10270	6H	407357254	MSU, N2, 2-row, CAP-IV	S	See footnote.	USA	BP-GWAS	Adhikari 2017
-	SCRI_RS_106581	6H	418524543	Nat. Small Grain Coll.	S	6A	USA	DP-GWAS	Richards *et al.* 2017
-	11_10964	6H	428615244	MSU, WSU	S	See footnote.	USA	BP-GWAS	Adhikari 2017
QRpts6C	bPb-3230	6H	430342068	Mackay|Baronesse	S	NB329, NB330	Aus	F5-CIM	Mace *et al.* 2007
6H-bin6	bPb-3068	6H	431201708	M120|Sep2-72	S	See footnote.	USA	RIL-CIM	St. Pierre *et al.* 2010
Rpt-Nomini	Bmgtttttt0001	6H	436041156	Nomini|Hector	S	ND89-19	USA	F2-DPM	O'Boyle *et al.* 2014
-	11_10189	6H	439399099	MSU, 2-row, CAP-I	S	See footnote.	USA	BP-GWAS	Adhikari 2017
-	11_21310	6H	441812706	MSU, 2-row, CAP-I	S	See footnote.	USA	BP-GWAS	Adhikari 2017
-	11_21310	6H	441812706	Nat. Small Grain Coll.	S	6A	USA	DP-GWAS	Richards *et al.* 2017
-	11_20058	6H	443897804	MSU, 2-row, CAP-I	S	See footnote.	USA	BP-GWAS	Adhikari 2017
QNb.StMo-6H.2	ksuD17	6H	449472551	Steptoe|Morex	S	ND89-19	USA	DH-IM	Steffenson *et al.* 1996
QNb.StMo-6H.2	ksuA3D	6H	466282343	Steptoe|Morex	S	ND89-19	USA	DH-IM	Steffenson *et al.* 1996
-	SCRI_RS_139937	6H	478129350	Nat. Small Grain Coll.	S	LDNH04Ptt19	USA	DP-GWAS	Richards *et al.* 2017
QPt.6H-3 (RT)	SCRI_RS_157316	6H	499930243	Barke or HEB-25 (0.40)|-0.22 / 1.03	A	JKI Field	DEU	NAM-GWAS	Vatter *et al.* 2017
-	5187-752	6H	503880223	NDB 112|Hector	S	JPT9901	JPN	RIL-CIM	Liu *et al.* 2015
-	12_10393	6H	504512152	CAP-IV	S	See footnote.	USA	BP-GWAS	Adhikari 2017
QRpt6Hc flanking	4007559-36:C>G	6H	516519338	Skiff|Prior	S+A	NB85	Aus	RIL-MIM	Thesis Chapter 4
AL_QRptt6-1	SCRI_RS_137215	6H	517272740	Lavrans|Arve	S	LR9, 5050B	Nor	DH-MQM	Wonnerberger *et al.* 2017b
QRpt6Hc	3257602-33:G>C	6H	518256321	Skiff|Prior	S+A	NB85	Aus	RIL-MIM	Thesis Chapter 4
QRpt6Hc flanking	3257276-5:A>C	6H	518606268	Skiff|Prior	S+A	NB85	Aus	RIL-MIM	Thesis Chapter 4
AL_QRptt6-1	SCRI_RS_13815	6H	526490502	Lavrans|Arve	S	5050B	Nor	DH-MQM	Wonnerberger *et al.* 2017b
Snn6	BE403326	6A	534458635	Wheat	-	SnTox6	-	-	Gao *et al.* 2015
QPt.6H-4 (AO)	SCRI_RS_7640	6H	545554978	HEB-25 (-0.96)|-2.98 / 0.00	A	JKI Field	DEU	NAM-GWAS	Vatter *et al.* 2017
AL_QRptt6-2	SCRI_RS_222802	6H	545740702	Lavrans|Arve	A	NB15_1, NB15_2, NB15	Nor	DH-MQM	Wonnerberger *et al.* 2017b
-	11_10635	6H	546608851	MSU	S	See footnote.	USA	BP-GWAS	Adhikari 2017
-	11_20531	6H	552807238	Zernogradsky 813|Ranniy 1	S	PP1, PP6	Rus	DH-CIM	Afanasenko *et al.* 2015
AL_Qrptt6-3	SCRI_RS_6720	6H	562233252	Lavrans|Arve	A	NB15	Nor	DH-MQM	Wonnerberger *et al.* 2017b
AL_Qrptt6-3	11_20355	6H	562812969	Lavrans|Arve	A	NB15_2	Nor	DH-MQM	Wonnerberger *et al.* 2017b
Snn6	BE424987	6A	574799111	Wheat	-	SnTox6	-	-	Gao *et al.* 2015
QRpts6L	WG0622-2	6H	576815342	Halcyon/Sloop	S	NB50	Aus	DH-SIM	Raman *et al.* 2003

Appendix 1. Continued.

QRpt6	HVM62b	6H	63.4–63.5	TR251|CDC Bold	S+A	WRS858, WRS1607, Field 06, 07, 08	CN	DH-MQM	Grewal *et al.* 2012
-	Bmag0173	6H	multiple	Baudin|AC Metcalfe	S+A	NB50, NB324, NB329	Aus	DH-IM	Cakir *et al.* 2011
6H-TRAIT 2/9	E4548_17-E3551_4	6H	not listed	Hor 9088|Arena	S	04/6T	-	F2-CIM	Richter *et al.* 1998
6H-TRAIT 1/7	E4048_1-E3847_9	6H	not listed	Hor 9088|Arena	S	04/6T	-	F2-CIM	Richter *et al.* 1998
-	p11M48_160-p11m53_88	6H	not listed	ND11231-12|VB9524	A	Stubble	Aus	DH-MTA	Cakir *et al.* 2003
-	11_10244	(6H)	52.2 cM	MN	S	See footnote.	USA	BP-GWAS	Adhikari 2017
-	11_20266	(6H)	65.08 cM	MSU, WSU	S	See footnote.	USA	BP-GWAS	Adhikari 2017
QNFNBAPR.Al/S-7Ha	abg704	7H	229818	Alexis|Sloop	A	NB329, NB330	Aus	DH-CIM	Lehmensiek *et al.* 2007
QPt.7H-1 (AO)	SCRI_RS_200895	7H	2351162	Barke or HEB-25 (0.48)|-3.69 / 9.16	A	JKI Field	DEU	NAM-GWAS	Vatter *et al.* 2017
QTL$_{PH}$-7H	bPb-4064	7H	4417638	HHOR9484|Post x Viresa	A	Stubble	DEU	DH-MQM	König *et al.* 2013
QPt.7H-1 (AO)	SCRI_RS_156237	7H	6823655	-	A	JKI Field	DEU	NAM-GWAS	Vatter *et al.* 2017
QNFNBAPR.Ar/F-7H	Bmag0206	7H	13838115	Arapiles|Franklin	A	NB329, NB330	Aus	DH-CIM	Lehmensiek *et al.* 2007
QNFNBAPR.Al/S-7Ha	Bmag0206	7H	13838115	Alexis|Sloop	A	NB329, NB330	Aus	DH-CIM	Lehmensiek *et al.* 2007
QNFNBAPR.W/Al-7Ha	Bmag0206	7H	13838115	Alexis|W2875-1	A	NB329, NB330	Aus	RIL-CIM	Lehmensiek *et al.* 2007
NBP_QRptt7-1	SCRI_RS_150517	7H	32895825	Nordic Barley Panel	A	Field 2013 UI	Nor	DP-GWAS	Wonnerberger *et al.* 2017a
NBP_QRptt7-1	11_20993	7H	34778348	Nordic Barley Panel	A	Field 2013 UI	Nor	DP-GWAS	Wonnerberger *et al.* 2017a
QPt.7H-2 (AO)	SCRI_RS_179937	7H	41835096	HEB-25 (-1.86)|-2.20 / -0.21	A	JKI Field	DEU	NAM-GWAS	Vatter *et al.* 2017
QNFNBAPR.Ar/F-7H	cdo665b	7H	67579222	Arapiles|Franklin	A	NB329, NB330	Aus	DH-CIM	Lehmensiek *et al.* 2007
QNFNBAPR.W/Al-7Ha	cdo665b	7H	67579222	Alexis|W2875-1	A	NB329, NB330	Aus	RIL-CIM	Lehmensiek *et al.* 2007
-	11_11098	7H	98043395	Zernogradsky 813|Ranniy 1	S	PL9	Rus	DH-CIM	Afanasenko *et al.* 2015
-	12_31055	7H	428681744	Nat. Small Grain Coll.	S	6A	USA	DP-GWAS	Richards *et al.* 2017
-	11_10700	7H	428682740	Nat. Small Grain Coll.	S	6A	USA	DP-GWAS	Richards *et al.* 2017
-	Vatp57A	7H	525216830	TR306|Harrington	A	Natural Field	N.Am	DH -SIM	Spaner *et al.* 1998
-	BMS64	7H	581053435	CIho 9819|Rolfi	S	84-28-1, 92-46/15, UK 80-12	Global	DH-SIM/CIM	Mannien *et al.* 2006
-	MWG571D	7H	582419364	TR306|Harrington	A	Natural Field	N.Am	DH -SIM	Spaner *et al.* 1998
NBP_QRptt7-2	SCRI_RS_161285	7H	616908316	Nordic Barley Panel	A	Field 2013 UI	Nor	DP-GWAS	Wonnerberger *et al.* 2017a
QNFNBAPR.Al/S-7Hb	EBmac0755	7H	637006308	Sloop|Alexis	A	NB329, NB330	Aus	DH-CIM	Lehmensiek *et al.* 2007
QNFNBAPR.W/Al-7Hb	EBmac0755	7H	637006308	W2875-1|Alexis	A	NB329, NB330	Aus	RIL-CIM	Lehmensiek *et al.* 2007
QRpta7L	bPb-7983	7H	641170931	Baronesse|Tallon	S	NB329, NB330	Aus	F5-CIM	Mace *et al.* 2007
NBP_QRptt7-3	SCRI_RS_16316	7H	641203161	Nordic Barley Panel	S	LR9	Nor	DP-GWAS	Wonnerberger *et al.* 2017a
-	SCRI_RS_183593	7H	643580704	Nat. Small Grain Coll.	S	15A, 6A, LDNH04Ptt19	USA	DP-GWAS	Richards *et al.* 2017
QNFNBAPR.Al/S-7Hb	Bmac0156	7H	644923959	Sloop|Alexis	A	NB329, NB330	Aus	DH-CIM	Lehmensiek *et al.* 2007

QNFNBAPR.W/Al-7Hb	Bmac0156	7H	644923959	W2875-1|Alexis	A	NB329, NB330	Aus	RIL-CIM	Lehmensiek *et al.* 2007
QRpta7L	bPb-5556	7H	651729568	Baronesse|Tallon	S	NB329, NB330	Aus	F5-CIM	Mace *et al.* 2007
7H flanking	4006892	7H	655050176	UVC8|SABBIErica	A	NB50, NB85	Aus	DH-CIM	Martin *et al.* 2018
7H flanking	3261638	7H	655054691	UVC8|SABBIErica	A	NB50, NB85	Aus	DH-CIM	Martin *et al.* 2018
QPt.7H-3 (RT)	SCRI_RS_123211	7H	656871198	HEB-25 (-0.12)|-0.20 / 0.00	A	JKI Field	DEU	NAM-GWAS	Vatter *et al.* 2017
QRpt7 [i]	222163	7H	116-134	TR251|CDC Dolly	A	Field 05	CN	DH-MQM	Grewal *et al.* 2008
-	REMAP-G8	7H	26 cM	CIho 9819|Rolfi	S	84-28-1, 92-46/15, UK 80-12	Global	DH-SIM/CIM	Mannien *et al.* 2006

[a] Year in parentheses indicates that Morex_2012 physical position used where Morex_2016 physical position was unknown.
[b] Morex_2016 physical position listed in base pairs as per (Mascher *et al.* 2017)
[c] Value in parentheses for Vatter *et al.* QTL from Supplementary file 7 footnote b, where value indicates effect of wild type allele compared to Barke allele.
[d] Value in parentheses for Vatter *et al.* QTL from Supplementary file 7 footnote c, where value indicates maximum and minimum effect of wild type allele compared to Barke allele.
[e] Growth stage where QTL was detected. Seedling experiment denoted by S and Adult experiments denoted by A.
[f] Country codes: Aus = Australia, Br = Brazil, CN = Canada, DEU = Germany, Global = refer to isolates used as per study, DK = Denmark, Fin = Finland, JPN = Japan, Lab = Laboratory cross, N.Am = North America, Nor = Norway, Rus = Russia, SthAf = South Africa, USA = United States of America.
[g] Population codes: BP = Breeding Population, DH = Double Haploid, DP = Diversity Panel, F2 = F2 segregating, F5 = F_5 breeding line, ICR = Immortal Critical Recombinant, NAM = Nested Association Mapping, RIL = Recombinant Inbred Line.
[h] Method codes: IM = Interval Mapping, CIM = Composite IM, DPM = Discrete Phenotype Mapping, GWAS = Genome-Wide Association Study, HRM = High Resolution Mapping, MTA = Marker-Trait Analysis, MIM = Multiple IM, MQM = Multiple QTL Mapping, MECS = Mutagenesis and Exome Capture Sequencing, SIM = Simple IM, SMA = Single Marker Analysis
[i] Placement of *QRpt7* unsuccessful. Current information positioned closest DArT™ marker (bPb-1813) at chrUn:249194373-249194740 (%ID = 100, E-val = 97.6) and chr5H:632991432-632991782 (%ID = 96.6, E-val = 1.9E-172), further investigation needed.
Adhikari 2017 used isolates: 30199002-1, 30199003-1, 30100003, 30100004
St. Pierre *et al.* 2010 used isolates: 3010001, 30190005-2, 30199019-1, 30199012-2, 30199010-3
Wonnerberger *et al.* 2017a used isolates: LR9, 5050B, 6949B, 6744A, 6744C for Field 2013 and LR9, 5050B, 6949B for Field 2014, Field 2015 and Field 2016. Field 2013 un-inoculated shortened to UI.
Wonnerberger *et al.* 2017b used isolates: LR9, 5050B, 6949B for NB14, NB14_1, NB14_2, NB15, NB15_1, NB15_2, NB16, NB16_1 and NB16_2.

Appendix 2. Summary of desirable and undesirable SNP alleles for eight QTL associated with resistance to *Pyrenophora teres* f. *teres* from Chapter 4 and Chapter 5 for a worldwide collection of 255 diverse barley genotypes.

				QTL	*QRpt3H*	*QRpt4H*	*QRpt6Ha*	*QRpt6Hm*	*QRpt6Hp*	*QRpt6Hs*	*Rpt5.f*	*QRpt6Hc*
				Desirable SNP	A	A	C	C	A	G	G	G
				Undesirable SNP	G	G	T	A	T	T	C	C
				Heterozygous	R	R	Y	M	W	K	S	S
Genotype	Acc'n No.	Origin[a]	Pedigree									
179S8/28		Aus.	Emir/'2920/4'		G	G	T	C	A	G	C	G
212Y1		Aus.	Emir/A17		N	G	T	C	A	G	C	G
251V64/M2/M1		Aus.	Morex/HB2032		G	G	T	C	A	G	C	G
266G4		Aus.	Emir/A17-1		G	G	T	C	A	G	C	G
Abed Deba (6-row)	400701	Eu.	Denso/Weihenstephan Mehltauresistente II		A	N	T	C	T	G	C	G
Algerian	495023	Af.	Landrace Algeria		G	A	C	C	T	G	N	G
Alinghi	411577	Eu.	LP 6-460/1665-24//Lomerit		N	A	T	C	A	G	C	G
Annabell	411578	Eu.	ST-900-14-DH/KRONA		N	N	C	A	A	G	C	G
Arapiles	406994	Aus.	Noyep/Proctor//CIho 3576/Union/4/ Kenia/3/Research/2/Noyep/Proctor/5/Domen		G	G	C	C	A	G	C	G
Arimont	400298	Am.	Mutant selection from composite cross XXX-C		N	G	T	C	A	G	C	G
Athos	400318	Eu.	Lignee 207/Emir		G	G	C	A	A	G	C	G
Babette	411579	Eu.	NORD 95540-32/Carreo		N	A	T	C	A	G	C	G
Barque	406368	Aus.	Triumph/Galleon		G	G	T	C	A	G	C	G
Bass	412296	Aus.	B28719/Alexis		G	G	C	C	A	G	C	G
Baudin	409483	Aus.	Franklin/Stirling		G	G	T	C	T	G	C	G
Beecher	400396	Am.	Atlas/Vaughn		A	A	T	C	T	G	C	G
Beecher	495035	Am.	Atlas/Vaughn		A	A	T	C	A	G	C	G
Betzes		Eu.	Bethges II/Bethges III		N	N	Y	C	A	G	C	G

Appendix 2. Continued.

Binalong	409322	Aus.	Blenheim/Skiff//O'Connor	G	G	C	C	A	T	C	G
Binder	400434	Eu.	selection from Hanna	G	N	C	C	A	G	C	G
Binder	411292	Eu.	selection from Hanna	A	G	C	A	A	G	C	G
Bowman	WA10371	Am.	Klages//Fergus/Nordic/3/ND1156/4/Hector	G	G	C	C	A	T	C	G
Brindabella	406059	Aus.	Weeah/CIho 7115//HCB27(Hiproly Clipper Backcross)/3/Jadar II/4/Cantala	G	G	C	C	A	G	C	G
BT 201	407187	Am.	CIho 5791/2*Parkland	G	G	T	C	A	G	G	G
Buloke	411103	Aus.	Franklin/2*VB9104	G	A	C	C	A	G	C	G
Bussell	400521	Aus.	Prior/Ymer	G	G	T	C	A	G	C	G
C2-05-337-2		Am.	ND19119-5/'200A12/8/2/M2'// Bowman*8/Mult. Dom.	A	G	T	C	A	G	C	G
Canadian Lake Shore	495016	Am.	Selection from Manchurian landrace	A	G	T	C	A	G	C	G
Canadian Lake Shore	495214	Am.	Not correct seed	G	G	T	C	T	G	C	G
Canadian Lake Shore	495217	Am.	Selection from Manchurian landrace	G	A	T	C	A	G	C	G
Canela	WA11505	Am.	Maris Canon/Laurel//Aleli	G	G	Y	C	A	G	C	G
Cantala	400185	Aus.	Kenia/Erectoides16 (X-ray mutant in Maja)	G	G	T	C	A	G	C	G
Cape	400554	Af.	Landrace Southern Africa	G	A	T	C	T	G	C	G
Cape	400555	Af.	Landrace Southern Africa	A	A	T	C	T	G	C	G
Cape	400556	Af.	Landrace Southern Africa	A	A	T	C	T	K	C	G
Capstan	410947	Aus.	Waveney/Sloop sibling//Chariot/Chebec	G	G	T	C	A	G	C	G
CBSS95M00804T-F-1M-3Y-4M-4Y-0M	ZBJ00-41	Am.	Gobernadora/Humai10/3/MPYT169.1Y/Laural//Olmo/4/Canela	N	N	T	C	A	G	C	G
Ceres	400583	Eu.	Bordia/Kenia//Piroline	G	G	T	C	A	T	C	G
Ceres	411243	Eu.	Bordia/Kenia//Piroline	G	G	T	C	A	T	C	G
Charger	413282	Aus.	Barabas//Charmay/Gairdner	G	G	C	C	A	T	C	G
Chebec	406877	Aus.	Orge Martin/2*Clipper(86)//Schooner	G	G	C	C	A	G	C	G
Chevallier	400603	Eu.	Landrace England	A	G	T	A	A	G	C	C
CIho 11458	495025	Eu.	Selection from Isaria (Danubia/Bavaria)	A	G	Y	C	A	T	C	G
CIho 1227		Af.	Landrace Ethiopia	G	G	T	C	A	G	C	C
CIho 16150	403710	Am.	Durani(CIho 6316)/4*Manchuria(CIho 2330)	N	G	T	C	A	G	C	G

Appendix 2. Continued.

CIho 3576	401952 or 403055	Af.	Landrace Egypt	N	G	T	C	A	G	G	G
CIho 4502	490067	Asia	Landrace China	A	G	T	C	A	G	C	G
CIho 4922	495032	Asia	Landrace Heilongjiang. China	A	N	T	C	A	G	C	G
CIho 5286		Am.	Selection from composite cross. CIho 4116	G	G	Y	C	A	G	C	N
CIho 5791	495210	Af.	Landrace Ethiopia	G	G	T	C	A	G	G	G
CIho 5791	495216	Af.	Landrace Ethiopia	G	G	T	C	A	G	G	G
CIho 6311	490064	Af.	Landrace Morocco	N	N	Y	C	T	G	C	G
CIho 9214	490079	Asia	Landrace Korea	N	G	T	M	A	G	C	G
CIho 9647	490068	Af.	Landrace Shewa. Ethiopia	G	A	C	C	A	G	C	G
CIho 9776	490069	Af.	Rabat 071 (unknown pedigree)	N	A	C	C	A	G	C	G
CIho 9819	490055	Af.	Landrace Welo. Ethiopia	G	G	T	C	A	G	G	G
CIho 9825	495211	Af.	Landrace Ethiopia	G	G	T	C	A	G	S	G
CIho 9825	495222	Af.	Landrace Ethiopia	G	G	T	C	A	G	G	G
CLE 245	411056	Am.	(INIA Uruguay) Otis/Canela	N	G	T	C	A	G	C	G
Clipper	400190	Aus.	Proctor/PriorA	G	G	C	C	A	G	C	G
CMB87-634-C-1Y-1B-1Y-1M-0B-1M-0Y	406984	Am.	Gloria'S'/Come'S'//Orge Fichedrett 3270/Row 906.73 (BYDV-018)	N	N	Y	C	A	G	C	G
Coast	495019	Af.	Landrace Africa	N	G	C	C	N	G	C	G
Commander	400641	Aus.	Selection from Coast (CIho 6011)	G	A	T	C	A	G	C	G
Commander	411763	Aus.	Keel/Sloop//Galaxy	G	G	T	C	A	G	C	G
Compass	413281	Aus.	Commander//County/Commander	G	G	T	C	A	G	C	G
Conlon	PI 597789	Am.	Bowman*2/DWS1008//ND10232	N	G	C	C	N	G	G	G
Corvette	400660	Aus.	Bonus/CIho 3576	G	G	T	C	A	G	C	G
Cowabbie	411127	Aus.	AB6/2*Franklin//Rubin/Skiff	G	G	C	C	A	T	C	G
Cutter	400179	Aus.	Proctor/PriorA	G	G	C	C	A	G	C	G
Dairokkaku	407907	Asia	Landrace Japan (Sel.'Dairokkaku' (1916))	G	G	C	N	A	G	C	G
Dampier	400681	Aus.	Olli/Research	A	G	T	C	T	G	C	G

Appendix 2. Continued.

Dash	409484	Aus.	Chad/Joline//Cask	A	G	T	C	A	G	C	G
Dhow	410912	Aus.	WI2808(Clipper/C.P.I.18197(14)/2/2EBYT-23)//Skiff/Haruna Nijo 9	G	G	T	C	A	G	C	G
Diamant	400713	Eu.	Mutant of Valticky (Starnovsky Kneifel/Moravian landvariety)	A	G	C	A	A	G	C	G
Diamant	411245	Eu.	Mutant of Valticky (Starnovsky Kneifel/Moravian landvariety)	G	G	C	A	A	G	C	G
Diamant	412209	Eu.	Mutant of Valticky (Starnovsky Kneifel/Moravian landvariety)	R	G	Y	A	A	G	C	G
Diamant	412251	Eu.	Mutant of Valticky (Starnovsky Kneifel/Moravian landvariety)	G	G	C	A	A	G	C	G
Diamant	412280	Eu.	Mutant of Valticky (Starnovsky Kneifel/Moravian landvariety)	G	G	C	A	A	G	C	G
Dictator	411581	Aus.	Virginia Hooded/Jet	G	N	T	C	A	G	C	G
Dictator	411851	Aus.	Virginia Hooded/Jet	G	N	T	C	A	G	C	G
Doolup	409481	Aus.	XBVT210/3/Prior/Lenta//Noyep/Lenta/5/ Dampier//A14(Prior/Ymer)/3/Kristina/4/Clipper/Volbar	G	G	Y	C	W	G	C	G
Egypt 70		Af.	Landrace Egypt	A	G	T	C	A	G	C	G
Fairview	411856	Aus.	Alexis/H86004-37 (IMC breeder's line)	G	G	C	C	A	G	C	G
Fathom	412301	Aus.	C.P.I.71284-48/3*Barque// Mundah/Keel//Barque	G	N	T	C	A	G	C	G
Feebar	400829	Am.	Peatland/Vaughn	A	N	T	C	A	G	C	G
Finesse	400152	Eu.	Igri/Maris Otter	G	N	T	A	A	G	C	G
Finniss	411800	Aus.	CIMMYT 42002/Galleon//Skiff	N	N	T	C	A	G	C	G
Fitzgerald	408174	Aus.	Onslow//Shannon/Triumph	G	G	T	C	A	G	C	G
Fitzroy	411104	Aus.	WI2808(Clipper/C.P.I.18197(14)/2/2EBYT-23)//Alexis	G	G	C	C	A	G	C	G
Flagship	411762	Aus.	Chieftain/Barque//Manley/VB9104	G	G	C	C	A	G	C	G
Fleet Australia	411798	Aus.	Mundah/Keel//Barque	G	A	T	C	A	G	C	G
Forrest (AUS)	400180	Aus.	Atlas57//Prior/Ymer	G	G	T	C	A	G	C	G
Franklin	405994	Aus.	Shannon/Triumph	G	G	C	C	A	G	C	G
GA-28	407035	Am.	Volbar/Atlas 66	G	G	T	C	A	G	C	G
Gairdner	408175	Aus.	Onslow/TAS83-587 (Shannon/Triumph)	N	G	C	C	A	G	C	G
Galleon	400182	Aus.	Clipper/Hiproly//3*Proctor/CIho 3576	G	G	T	C	A	G	C	G
Gilbert	406923	Eu.	Selection from Koru (Armelle//Lud/Luke)	A	G	C	A	A	G	C	G
Golden Promise	WA00774	Eu.	Maja/Irish Goldthorpe	G	G	Y	C	N	G	C	G
Grimmett	400186	Aus.	Bussell/Zephyr	G	G	C	A	A	G	C	G

Appendix 2. Continued.

Grout	411106	Aus.	Cameo/Arupo	G	G	T	C	A	G	C	G
Gus	400953	Am.	Selection from composite cross XXXII-76	G	A	T	C	A	G	C	G
Hamelin	409482	Aus.	Stirling/Harrington	G	G	T	C	T	G	C	G
Hanna	400973	Eu.	Landrace Hanna	A	G	C	C	A	T	C	G
Hanna	400974	Eu.	Landrace Moravia. CIho 2217	A	G	C	A	A	G	C	G
Harbin	495215	Asia	Landrace Manchuria	G	G	C	C	A	G	C	G
Harbin	495224	Asia	Landrace Manchuria	N	G	T	C	A	G	C	G
Harrington	495219	Am.	Klages/3/Gazelle/Betzes//Centennial	N	G	C	C	A	G	C	G
Haruna Nijo		Asia	Satsuko Nijo//K-3/G-65	N	G	T	C	A	G	C	G
Heartland	495039	Am.	Klondike/BT 416	R	N	Y	C	A	G	G	G
Henley		Eu.	99-24/NLS 97-5547	G	G	C	C	A	T	C	G
Herta	401011	Eu.	Kenia/Isaria	N	G	C	C	A	T	C	G
Hindmarsh	411107	Aus.	Dash/VB9409(O'Connor/WI2723)	A	G	T	C	A	G	C	G
IBON-05-6	ZBA04-257	Am.	Limon/Bichy2000//MSEL	N	G	Y	C	N	G	C	G
ICARDA SN326		Af.	Unknown	N	A	T	C	A	G	C	G
ICB77-0187-1AP-2AP-3AP-0AP	490276	Af.	Roho//Alger/Ceres 362-1-1	G	G	C	C	A	G	C	G
ICB88-1292-4AP-2AP-3APH-0AP-0AP		Af.	H.spont.41-/Unknown	N	G	T	C	N	G	C	G
ICB88-1295-1AP-1AP-3APH-0AP-0AP		Af.	H.spont.41-3/Unknown	N	G	T	C	A	G	N	G
Isaria	401102	Eu.	Bavaria/Danubia	A	N	T	C	A	G	C	G
Isaria	401103	Eu.	Bavaria/Danubia	A	G	T	C	A	G	C	G
Isaria	402132	Eu.	Bavaria/Danubia	G	G	C	C	A	T	C	G
Isaria	403591	Eu.	Bavaria/Danubia	A	G	C	C	A	T	C	G
Isaria	406079	Eu.	Bavaria/Danubia	A	G	C	C	A	T	C	G
Jet		Af.	Landrace Ethiopia	N	G	T	C	A	G	G	G
K20019	495213	Af.	Landrace Ethiopia	G	G	C	C	A	G	C	G
K20019	495218	Af.	Landrace Ethiopia	G	N	C	C	A	G	C	G

Appendix 2. Continued.

K8755	495212	Af.	Landrace Ethiopia	G	G	T	C	A	G	G	G
K8755	495220	Af.	Landrace Ethiopia	G	N	C	C	A	G	C	G
Kaputar	406996	Aus.	Arupo"S" (5604/1025/3/Emir/Shabet//CM67/4/F3 Bulk HIP)	G	G	T	C	A	G	C	G
Keel	408179	Aus.	C.P.I.18197/Clipper//Mari/CM67	G	G	T	C	A	G	C	G
Kenia	403551	Eu.	Binder/Gull	G	G	C	C	N	G	C	G
Kenia	411258	Eu.	Binder/Gull	A	G	T	C	A	G	C	G
Ketch	401195	Aus.	Noyep/Lenta	G	G	T	C	T	G	C	G
Klages	401215	Am.	Betzes/Domen	G	G	T	C	A	G	C	G
Kristina	411790	Eu.	Domen/Mari	G	G	T	C	A	G	C	G
La Trobe		Aus.	Hindmarsh sibling (Dash/VB9409(O'Connor/WI2723))	A	G	T	C	A	G	C	G
Lara	401259	Aus.	Research/Lenta	G	G	C	C	A	G	C	G
Libya 221		Af.	Landrace Libya	A	A	T	C	T	G	C	G
Libya 241		Af.	Landrace Libya. Crown rot resistant Herde	N	G	T	C	N	G	C	G
Lindwall	408178	Aus.	Triumph/Grimmett	G	G	C	A	A	G	C	G
Lion	412217	Eu.	Landrace Russia (white seeded)	A	N	T	C	T	G	C	S
Lion	495044	Eu.	Landrace Russia (black seeded)	A	G	C	A	A	G	C	G
Lockyer	411467	Aus.	Tantangara/VB9104	G	A	C	C	A	G	C	G
Mackay	410819	Aus.	Cameo/Koru	G	G	T	C	A	G	C	G
Macquarie	411825	Aus.	Gairdner//Alexis/Gairdner	G	G	C	C	A	G	C	G
Malebo	400181	Aus.	Outcross derivative of C.P.I.11083. WWB18 (Algeria)	G	A	T	C	N	G	C	G
Maritime	410948	Aus.	Dampier/A14//Kristina/3/ Clipper/M11(Cree)/4/Dampier/A14// Kristina/3/Dampier/A14//Union	G	G	T	C	W	G	C	G
Ming	495021	Asia	Landrace Heilongjiang. China.	A	G	T	C	N	G	C	G
Moby	411852	Aus.	White Hooded Selection from Dictator	N	N	T	C	A	G	C	G
Molloy	407599	Aus.	Golden Promise/WI2395(WARI2-38)/4/ XBVT210(72S:267)/3/Atlas57(66S08-4)//(A14)Prior/Ymer(82S837)/O'Connor	G	G	T	C	A	G	C	G
Moondyne	402713	Aus.	Dampier/2/(A14)Prior/Ymer/3/Kristina/4/ Clipper/Volbar	G	G	T	C	A	T	C	G
Morex	401476	Am.	Cree/Bonanza	A	G	C	C	A	G	C	G

Appendix 2. Continued.

Morrell	406995	Aus.	WUM221/P23822 (81S806)/5/Forrest (81S719)/4/Psaknon (80S564)/Dampier//M19 (76T111)/3/Zephyr	G	G	C	C	A	G	C	G
Mundah	407598	Aus.	O'Connor/Yagan	G	A	T	C	A	G	C	G
MXB.468 (F4 SEL.)	405701	Am.	CIMMYT Breeding line	N	G	T	C	A	G	C	G
Namoi	400533	Am.	Sultan/Nackta//RM1508/Godiva	G	G	C	C	A	G	C	G
Navigator	412297	Aus.	Chieftain/VB9624/4/Keel/3/ Sahara/WI2723//Chebec/5/ Dhow/Keel//Fitzgerald	A	G	T	C	A	G	C	G
NC80-1	407202	Am.	Boone/Clayton	G	G	C	C	A	G	C	G
ND B112	495037	Am.	Selection from (Kindred CI 6969/CI 7117–77)	A	G	T	C	A	G	C	G
ND17293-1	495244	Am.	ND14651/ND15062	G	G	C	C	A	G	C	G
ND19119-5	PI 643330	Am.	ND15403-3/ND15368//ND16453	A	A	C	C	A	G	C	G
ND22996-1	PI 643368	Am.	ND19922/ND18172-1	G	N	C	C	N	G	G	G
ND23146-1	PI 643370	Am.	ND18187//ND18370/ND19119-1	N	G	C	C	A	G	C	G
ND23164	PI 643371	Am.	ND19012/ND19929	G	N	C	C	N	G	G	G
ND23203	PI 643372	Am.	ND19957/ND18380-1	G	G	C	C	A	G	S	G
ND24168	2ND24168	Am.	Logan/ND19119-5(Rawson sibling)	A	A	C	C	A	G	G	G
ND24181	2ND24181	Am.	ND19119-5//ND18380-1/ND19929	R	A	C	C	A	G	G	G
ND24260-3	2ND24260	Am.	ND19869-1//ND17274/ND19119	N	A	C	C	A	T	C	G
ND24379	PI 643376	Am.	ND20824//ND20028/ND19119-1	A	G	T	C	A	G	C	G
ND24388	2ND24388	Am.	ND17274/ND19119//ND19854	N	A	C	C	A	G	C	G
ND24502	2ND24502	Am.	ND19119-5//ND21059/ND19929-7	G	A	C	C	A	G	G	G
ND25389	2ND25389	Am.	ND19119-1/Lacey/3/ ND19922//ND19974/ND19119	A	A	C	C	A	G	G	G
ND25459	2ND25459	Am.	ND19119*2//ZAU 7/Bowman	A	A	C	C	N	G	C	G
ND5883	495008	Am.	Clipper/6/Betzes//CIho 5791/2*Parkland/3/ Betzes/Piroline/4/Akka/5/Centennial	N	G	C	C	A	G	G	G
Nepal 81	411584	Asia	Landrace Nepal	N	G	C	N	A	G	C	G
Norbert	495007	Am.	Betzes//CIho 5791/2*Parkland/3/Betzes/Piroline/4/ Akka/5/Centennial/6/Klages	G	G	C	C	A	G	G	G
Nordic	402540	Am.	Dickson/3/CIho 4738//Traill/UM 570	N	G	T	C	A	G	C	G

Appendix 2. Continued.

Norteña Daymán	495251	Am.	(ND11993) ND8968/ND9163	N	N	C	C	A	G	C	G
Noyep	401584	Aus.	Prior Selection	A	N	T	C	T	G	C	S
NRB06059		Aus.	Mackay*2/WI3214 (Triumph/Galleon//Harrington	G	G	T	C	A	G	C	G
O'Connor	401600	Aus.	Proctor/CIho 3576/3/Atlas57//A14(Prior/Ymer)	G	G	T	C	A	G	C	G
Onslow	406008	Aus.	Forrest/Aapo	G	G	T	C	A	G	C	G
Orge289(Esperance)	401653	Af.	unknown pedigree	N	G	Y	M	A	G	S	G
Oxford	411857	Eu.	Tavern/Chime	G	N	T	C	A	T	C	G
Parwan	400177	Aus.	Plumage Archer/Prior//Lenta/3/ Research/Lenta	G	G	C	C	A	G	C	G
Patty	400167	Eu.	Volla/Athos	G	G	C	C	A	T	C	G
Perún		Eu.	HE-1728/Karat	N	G	C	C	A	T	C	G
Pinnacle	PI 643354	Am.	ND18172/ND19130	N	N	C	C	A	T	C	G
Plumage	411308	Eu.	Landrace Denmark	A	G	C	A	A	G	C	N
Pompadour	406438	Eu.	FD-0192/Patty	A	G	C	C	A	T	C	S
Prato	495029	Am.	CM 67/3*Briggs/4/Briggs*4/3/ California Mariout*4/CIho 1179//2*California Mariout*6/Club Mariout	A	A	Y	C	A	G	C	G
Prior	401778	Aus.	Chevallier selection	A	G	T	C	T	G	C	C
Prior	495208	Aus.	Chevallier selection	A	G	T	C	T	G	C	C
Proctor	401781	Eu.	Kenia/Plumage Archer	G	N	C	C	A	G	C	G
Research	401833	Aus.	Prior/Plumage Archer	A	G	C	A	A	G	C	G
Resibee	401834	Aus.	Research Selection	A	G	C	A	A	G	C	G
Roe	411466	Aus.	Doolup//Windich/Morex	G	N	T	C	T	G	C	G
Rojo	495018	Am.	Composite Cross I Selection (CIho 4116)	A	N	N	C	N	G	C	G
SB03702	WA11117	Am.	Canadian breeding line	G	G	C	C	A	G	G	G
Scarlett	407505	Eu.	Amazona/Breun ST 2730 E//Kym	G	G	C	C	A	T	C	G
Schooner	400187	Aus.	Proctor/PriorA//Proctor/CIho 3576	G	G	C	C	A	G	C	G
Scope	411824	Aus.	EMS Buloke Mutant (Franklin/2*VB9104)	G	A	C	C	A	G	C	G
Scrabble	413278	Eu.	Quench/Massilia	A	G	C	C	A	G	G	G
Shakira		Eu.	Pewter/Prestige	G	G	C	C	A	T	C	G
Shannon	400178	Aus.	Proctor*4/CIho 3208-1	G	G	C	C	A	G	C	G

Appendix 2. Continued.

Shepherd	411782	Aus.	Selection from Baronesse (Mentor/Minerva//Vada mutant/4/ Carslberg/Union//Opavsky/Salla/3/ Ricardo/5/Oriol/6153 P40)	A	G	C	A	A	G	C	G
Skiff	403001	Aus.	Abed Deba/3/Proctor/CIho 3576//C.P.I. 18197/ Beka/4/Clipper/Diamant// Proctor/CIho 3576	G	G	C	C	A	T	C	G
Skipper	412300	Aus.	Buloke/Commander/3/Chieftain/VB9623//Manley/VB9104	N	G	C	C	A	G	C	G
Sloop	408180	Aus.	Schooner/Norbert//Golden Promise/WI2395/3/Schooner	G	N	C	C	A	G	C	G
Sloop SA	499061	Aus.	Chebec/3*Sloop	G	N	C	C	A	G	C	G
Sloop Vic	499062	Aus.	Sahara/WI2723//Chebec/3*Sloop	G	N	C	C	A	G	C	G
SM01645	WA9691	Am.	Unknown	G	N	C	C	A	G	G	G
Stirling	400183	Aus.	Dampier//Prior/Ymer/3/Piroline	G	G	T	C	A	G	C	G
Stirling		Aus.	Dampier//Prior/Ymer/3/Piroline	N	G	T	C	T	G	C	G
Summitt	402022	Eu.	HP-1203//Zephyr/Tern	N	G	C	M	A	G	C	G
Sunshine	413277	Eu.	Br6770a6/Braemar	G	G	C	A	A	G	C	G
Taixing 9425	411518	Asia	Chinese Landrace via CIMMYT	G	G	T	C	A	G	C	G
Tallon	406324	Aus.	Triumph/Grimmett	G	G	C	A	A	G	C	G
Tantangara	407092	Aus.	AB6/Skiff	G	G	C	C	A	T	C	G
Tifang	495015	Asia	Landrace Manchuria	A	G	T	C	A	G	C	G
Tilga	407651	Aus.	Forrest/Cantala	G	G	T	C	A	G	C	G
Tolar	411831	Eu.	HE-4710/HWS-78267-83	N	G	C	A	A	G	C	G
Torrens	411855	Aus.	Galleon/CIMMYT42002	G	N	T	C	T	G	C	G
TR03189		Am.	Unknown	A	G	C	C	A	G	G	G
TR251		Am.	TR229//AC Oxbow/ND7556 (Norbert//ND4856/M37)	G	G	C	C	A	G	G	G
TR473	400192	Am.	S75285/WM751-2	G	G	C	C	N	G	G	G
Triumph	400189	Eu.	Diamant/'Hadm. Stamm 14029/64/6'	G	G	C	A	A	G	C	G
Triumph	495094	Eu.	Diamant/'Hadm. Stamm 14029/64/6'	G	G	C	A	A	G	C	G
Triumph	499013	Eu.	Diamant/'Hadm. Stamm 14029/64/6'	G	G	C	A	A	G	C	G
Tulla	411128	Aus.	Skiff/FM 437(PI 467849)	G	G	T	C	A	G	C	G
Tunisia 344		Af.	Unknown	N	A	T	C	T	G	C	G
Tunisia 352		Af.	Unknown	A	G	T	C	A	G	C	G

Appendix 2. Continued.

Ulandra	402729	Aus.	Warboys/Alpha	A	G	T	C	A	G	C	G
Union	411285	Eu.	Weihenstephaner Mehltauresistente I/Donaria//Firlbecks III	A	G	C	C	A	T	C	G
Urambie	411126	Aus.	Yagan/2*Ulandra	A	A	T	C	A	G	C	G
VB0810		Aus.	Gleam/3/Keel/Gairdner//Gairdner/4/Yarra	N	G	C	C	N	G	C	G
VB0931		Aus.	Hindmarsh sibling/Fleet Australia	A	A	T	C	A	G	C	G
VB0933		Aus.	Hindmarsh sibling/Fleet Australia	G	A	T	C	A	G	C	G
VB9104		Aus.	Europa/IBON#7.148	G	A	C	C	A	G	C	G
Vlamingh	411465	Aus.	WABAR0570(72–0785/Tokak/5/Dampier/A14//Kna/3/Sutter/4/ Atlas57/A16//Clipper/ Delisa)/6/TR118	G	N	C	C	A	G	G	G
Volla	402186	Eu.	Breuns Wisa/Heines Haisa I	A	G	C	C	A	T	C	G
Volla	402216	Eu.	Breuns Wisa/Heines Haisa I	A	G	C	C	A	T	C	G
Volla	402217	Eu.	Breuns Wisa/Heines Haisa I	A	G	C	C	A	T	C	C
Volla	411290	Eu.	Breuns Wisa/Heines Haisa I	A	G	C	C	A	T	C	G
VT Admiral	412298	Aus.	SH302/Keel/2/Chieftain/3/Torrent/4/Dhow/Keel//Fitzgerald	G	G	T	C	A	G	C	G
Waranga	402772	Aus.	Plumage Archer/3/Prior/Lenta/2/ Research/Lenta/4/Clipper	G	G	C	C	A	G	C	G
Weeah	402239	Aus.	Prior/Research	R	G	C	A	A	G	C	G
Westminster	413256	Eu.	NSL 97-5547/Barke	A	G	C	A	A	G	C	G
WI2291	410835	Aus.	CIho 3576/Union//Union	N	G	T	C	N	G	G	G
Wimmera	412299	Aus.	Scarlett/Gairdner	G	G	C	C	A	T	C	G
WPG8412-9-2-1	406303	Am.	Bowman/TR473//Ellice/TR451	A	G	C	C	N	G	G	G
Yagan	402996	Aus.	Unknown CIMMYT	G	A	T	C	A	G	C	G
Yambla	408141	Aus.	Skiff/FM 437(PI 467849)	G	G	C	C	A	T	C	G
Yangsimai 3	411530	Asia	Chinese Cultivar	A	G	C	C	A	G	C	G
Yarra	411105	Aus.	Clipper/Galleon//Alexis/3/VB9104	G	G	C	C	A	G	C	G
Yerong	406299	Aus.	M22/Malebo	G	A	C	C	A	G	C	G
Zhhlaoluomang	411586	Asia	Landrace Zhejiang. China. (ZDM2689)	G	N	C	N	A	G	C	G

[a] Genotype origin code where selection originated or where cultivar was developed: Af. Africa, Am. = Americas, Aus. = Australasia, Eu. = Europe. Desirable SNPs coloured green, undesirable SNPs coloured red, heterozygous SNPs coloured yellow, missing SNP call or insertion/deletion not coloured.

Appendix 3. Proportion of desirable SNP allele for eight QTL associated with resistance to *Pyrenophora teres* f. *teres* from Chapter 4 and Chapter 5 for 373 NRB breeding lines, 27 reference cultivars and an international panel of 256 diverse barley genotypes.

	QTL	*QRpt3H*	*QRpt4H*	*QRpt6Ha*	*QRpt6Hm*	*QRpt6Hp*	*QRtp6Hs*	*Rpt5.f*	*QRpt6Hc*
	Desirable SNP	A	A	C	C	A	G	G	G
	Undesirable SNP	G	G	T	A	T	T	C	C
Group	No. Genotypes								
Reference	27	0.25	0.08	0.50	0.89	0.86	0.89	0.08	0.96
North Dakota [a]	22	0.54	0.44	0.82	1.00	1.00	0.86	0.38	1.00
NRB 2012	142	0.34	0.53	0.85	0.88	1.00	0.84	0.15	1.00
NRB 2013	231	0.39	0.60	0.74	0.98	1.00	0.95	0.04	1.00
Africa	30	0.26	0.33	0.29	1.00	0.74	1.00	0.27	0.97
Americas	55	0.43	0.29	0.60	1.00	0.96	0.95	0.31	1.00
Asia	13	0.56	0.00	0.38	1.00	1.00	1.00	0.00	1.00
Australia	103	0.15	0.14	0.42	0.93	0.86	0.92	0.02	0.96
Europe	55	0.52	0.04	0.75	0.63	0.96	0.64	0.02	0.96
New South Wales	12	0.17	0.33	0.50	1.00	1.00	0.67	0.00	1.00
New Zealand	2	0.00	0.00	0.50	1.00	1.00	1.00	0.00	1.00
Queensland	6	0.17	0.00	0.67	0.33	1.00	1.00	0.00	1.00
South Australia	31	0.14	0.04	0.35	1.00	0.78	0.90	0.00	0.88
Tasmania	2	0.00	0.00	1.00	1.00	1.00	1.00	0.00	1.00
Victoria	17	0.31	0.13	0.71	0.82	1.00	1.00	0.00	1.00
Western Australia	22	0.05	0.15	0.29	1.00	0.76	0.95	0.05	1.00
Global [b]	256	0.31	0.17	0.51	0.89	0.91	0.88	0.11	0.98

[a] Includes breeding lines and released cultivars.

[b] Global population includes all genotypes from the diverse panel and excludes NRB breeding lines.

Appendix 4. Summary of base pair sequences and corresponding candidate genes for 38 SNP markers significantly associated with resistance to four *Pyrenophora teres* f. *teres* isolates at adult and seedling growth stage following GWAS of the 2012 and 2013 NRB populations.

SNP marker[a]	Chr	Position (bp)	Reference sequence	Gene ID	Description
3255709-40:A>G	4H	53032932	TGCAGTGGGCACTGATCATAGCA GATCCACGAGACCATACAGTGGA CAGAACCCAAGCCCACTTATATA	HORVU4Hr1G014440	BSD domain containing protein, expressed
3257855-10:A>G	4H	69382105	TGCAGTTGCCAGTATCCTTCACA ATAAATTCGCTTGATGCAGCCTA CAATCAGCAGCAATGCTGTTAGA	HORVU4Hr1G016640	Protein transport protein Sec24-like
3256237-67:A>G	4H	70434783	TGCAGGTGCAAACCAACACCCGC TTGCCGCACAGCTCGGCCTCACC TTACCGATGCCTGCCTTGCAGAT	HORVU4Hr1G016730	Carboxypeptidase Y
3257954-50:G>A	6H	193444571	TGCAGTACATAACGCACCATTGC AGCAGACACTACACGAGTCATCA CAAGGTGCCACTTGTGTATTTGT	No hits found	
3434214-43:A>T	6H	251009458	TGCAGCAGCACCAGCAACGGCTG TCGCGGCCACCAGCGACCCCAGC TACAGCACCGCGCCGCTCGGCAC	No hits found	
3256458-52:T>C	6H	325194805	TGCAGGCAACCAAGAACCTGCCT GACTTCAAAAAGGATGACCAGAA GGCCATTGATGCTGAGTTGATCA	HORVU6Hr1G052600	60S ribosomal protein L6
3255777-67:T>G	6H	337179867	TGCAGTTGCACTCCCCTTGGCCA ATTGCAGAGCCGAGTACGAGATG GGGATAAAGCTACGGCTAAAATC	HORVU6Hr1G053730	Protein GLE1
3254817-15:C>A	6H	340307078	TGCAGGGGTTTCGTTCAACAAAC AAAACCCTAGTACTACTTCTGAG AGCAAAGTATTAATCTGATAATT	HORVU6Hr1G054050	B3 domain-containing protein
3257446-28:G>T	6H	368527587	TGCAGGACCTCCAGCAGCTGCAC CTCGGGACCGCCGCTCACAACTA TTTCCAGGCATCGTCGAGCTCCA	HORVU6Hr1G057060	AP2-like ethylene-responsive transcription factor

Appendix 4. Continued

3262096-64:C>T	6H	378974018	TGCAGATTCGATTGGCGAGCGTTT TCGGTGGAGAGGAGGGAAACTGA AATTTCGATTGGCGAGCCTGTC	HORVU6Hr1G058090	Pre-mRNA-processing factor 40-like protein A
3256608-45:C>G	6H	378772740	TGCAGCGTGGAGCGGTTCCAGAG GGCGGTTGACGCGGCGAGGGCCC AAGAACGGCACCATCATCATCAC	HORVU6Hr1G058060	SAUR-like auxin-responsive protein family
3259111-21:A>C	6H	210766011	TGCAGCAATCGGTCTCTCTCTATA TATATATGCTTATTATCATCTTACA AAGAGTCCATGTGGTCTTGA	HORVU6Hr1G039940	Protein phosphatase 2C family protein
3398663-60:C>T	6H	268997406	TGCAGGCCACGTCAACCCCCCCTA TCCCACGATAGTTGGTCGCTGTCT CTGTGCTCGAGCCGTGCAATG	No hits found	
3254735-54:A>C	6H	314450784	TGCAGCTTCAGTGGAGCACGAAG AGCACGATCTCGGTATGAACGTTG CAGCATCAACTTGACATGAACG	No hits found	
3257608-6:A>G	6H	361531190	TGCAGCATATGAATCTTTGCTTCA TCTCGTAAACCAGAATGCACTCGG TGAAATGGTTGGCATCAGAAT	HORVU6Hr1G056280	Protein GrpE
3259058-41:G>A	6H	364356525	TGCAGAATCAGATCATCCAACTCA AGGTCTAACATGACCACGCACGA CCGAGATCGGAAGAGCGGTTCA	HORVU6Hr1G056490	Sister chromatid cohesion protein PDS5 homolog B
3259255-17:C>T	6H	375529364	TGCAGCGCTGCACGAGGCATCCTG ATTGATTGATTTCCGCGCACACCG CTAAAAGCCCACACCCAAATC	No hits found	
4175123-58:C>A	6H	380193974	TGCAGAGAGCCCCCTCTCCCTCCT CCCCCTCTTTTTCCACCAAAATCTC CGTTTCGTTCGGTGTCTTCT	No hits found	
3256765-18:T>C	6H	382482733	TGCAGATTCTCGAGCCAGTTTCTT CACCTCCCGCCGACGCTCCCTCGA AAAAGGGATCAGCCGCGCCCC	HORVU6Hr1G058340	Protein LIGHT-DEPENDENT SHORT HYPOCOTYLS 3

Appendix 4. Continued

3262659-31:C>G	6H	383141804	TGCAGGCATTTGGCACAGATCAGT TAAATGCCCACCGTTACCTCAGAA AAAAAGGAAGTTAAATGAGCA	HORVU6Hr1G058450	RNA-binding protein 42
3255625-14:C>T	6H	384803137	TGCAGAAACAACAGCCTGATTTGA AATTTGGATTGTAGGTTTCAGTTA AGATTTTCCGAGATCGGAAGA	HORVU6Hr1G058750	adenosine kinase 1
3434176-13:T>C	6H	384884765	TGCAGAGCCGGGGTCCCACGGGC GGCACGCTCTAAATCTGCCTCGAT CTGCTCTGGCGAAGTCTCGGAT	HORVU6Hr1G058780	Protein kinase superfamily protein
3432738-29:G>A	6H	386021835	TGCAGCTCCGAGCAGTAAGAGGC CATGGCGATCTCGGCGCCTTTGAA GCCGTAGTCCAAGCTTGGGTTG	HORVU6Hr1G058840	phenylalanine ammonia-lyase 2
3432352-13:G>T	6H	388486267	TGCAGCATTCCTTGTACTGATACA GTGATGACATGACGGTTGGGCCG AGATCGGAAGAGCGGTTCAGCA	No hits found	
3254663-15:T>A	6H	396127146	TGCAGAGTAAGTTCCTCTAGGTTG GGAGCATTGTTGAGAAACAACTCT AGCATGTTGTACACTTCGCCG	HORVU6Hr1G059780	F-box/RNI-like superfamily protein
3255134-29:C>A	6H	397034107	TGCAGGGCGAGGACTCGCAGATT GCAGAACCCCCCTGCAATGACGTT CAGATCGTCGTCGATAACACCG	HORVU6Hr1G059950	F-box/LRR-repeat protein 2
3254978-54:G>A	6H	404316342	TGCAGCTTTGGGACCCTTGTTTCC ATTCCATGTAAGCCCACGCGGTTT TACGCAGGATATCCTACTGTT	No hits found	
3258749-25:G>C	6H	408391789	TGCAGGCTTGCAGTCAGTTAAAAT AGGTGATGGCATACTTTTCTACTC GTTTATCACTTTCAGGGACCT	No hits found	
3434193-36:T>G	6H	417070659	TGCAGTCCTACCCTAGTTCCCGAG CACACCCGAGCGTACCAGAACCG CCGCCGCCGCCGTCACCACAAG	HORVU6Hr1G062230	Nucleic acid-binding, OB-fold-like protein

Appendix 4. Continued

3255255-56:T>A	6H	417821936	TGCAGTCTGCACTCGAGCCATGGC AACATGCTACACGCACATTTCGAC CGTCTACGTACACACACTACT	No hits found	
4171893-67:C>T	6H	422773531	TGCAGGCGTCGGTGATCCGCGACC TGGTCCTCCTCTCCTGCGTCGGCC TGCGCCCCGTGCTCGTGCACG	HORVU6Hr1G062960	Acetylglutamate kinase
3921095-18:T>C	6H	424801489	TGCAGCTAAAGCTGCATGTCGATG TACCCAAGTTGTGTGTTTTTTTACC ACGCAATCCTTGAGATAAAT	HORVU0Hr1G018320	expansin B4
3257464-10:T>A	6H	449601223	TGCAGCGCCGTATAGGAGTCACTG GATTCACCATCGTTTGGTGAACGC GCGGGCCATCAAGCATGCTGG	No hits found	
3261554-30:C>T	6H	450717343	TGCAGTAGGTGCGCTAACAGCTAA ATGGACCCGGCTCACCGAGCTCTT CACGTTGGTTGCCTCGGAAAT	No hits found	
3259228-14:G>C	6H	459335236	TGCAGATGATCGATGAACCCGCG AGACGAGGGATTGTGATTGTGCGT CGTTGGCGATGGATGAATGAAG	No hits found	
3258275-14:G>C	6H	460084925	TGCAGCGCACCCAAGAACAATCT GATGACATGGACCGAACCAGGTC CGCATCGACGCGCGGCACGACGC	No hits found	
3263983-33:G>T	6H	460088004	TGCAGAAAACAGAAGGTGAACAG ATCATGTTAGGCAAATCTTCACAG GGAGGATATCTGGAGTTTGTTT	HORVU6Hr1G066460	Regulator of chromosome condensation (RCC1) family with FYVE zinc finger domain
3262437-68:C>T	6H	461514241	TGCAGGACGGGACCCCGCGCTGTC TGTGGTAGCGTCCGAGCTTTGGCA CCGCAGGTCGGAGACAAAGCC	No hits found	

[a] Purple = marker used to select genotypes to exclude from phenotype data for reduced genotype GWAS, green = significant in both GWAS, orange = significant in reduced genotype GWAS only and yellow = significant in full genotype GWAS only.

Appendix 5. Linkage disequilibrium comparisons between CIho 5791 (495210) and 60 genotypes that carry *Rpt5.f* and 35 genotypes the do not carry *Rpt5.f* across entire length of chromosome 6H and chromosome 6H between 361,531,190 bp and 460,088,004 bp.

Genotype	AGG No.	3256608-45:C>G	6H	361531190 - 460088004
CIho 5791	495210	Yes	1.000	1.000
CIho 5791	495210	Yes	0.952	0.979
Conlon		Yes	0.114	0.976
NRB11346		Yes	0.169	0.974
CIho 5791	495216	Yes	0.970	0.969
NRB090290		Yes	0.090	0.937
Scrabble	413278	Yes	0.142	0.936
SB03702		Yes	0.107	0.936
NRB11682		Yes	0.090	0.933
CIho 9819	490055	Yes	0.640	0.933
NRB091043		Yes	0.115	0.932
TR251		Yes	0.137	0.930
NRB11570		Yes	0.137	0.929
Norbert	495007	Yes	0.153	0.928
TR251		Yes	0.156	0.926
NRB120121		Yes	0.107	0.926
BT 201	407187	Yes	0.275	0.925
ND5883		Yes	0.258	0.925
TR03189		Yes	0.120	0.925
SM01645		Yes	0.129	0.917
NRB120132		Yes	0.215	0.905
NRB120131		Yes	0.167	0.905
TR473	400192	Yes	0.168	0.905
NRB121200		Yes	0.102	0.904
NRB100285		Yes	0.157	0.897
NRB101125-10		Yes	0.152	0.896
NRB11060		Yes	0.074	0.895
NRB11150		Yes	0.247	0.895
WPG8412-9-2-1	406303	Yes	0.106	0.894
Vlamingh	411465	Yes	0.160	0.893
NRB11334		Yes	0.102	0.892
NRB11061		Yes	0.092	0.892
2ND25389		Yes	0.097	0.891
CIho 3576		Yes	0.627	0.891
NRB121137		Yes	0.164	0.884
NRB11313		Yes	0.083	0.883
NRB11337		Yes	0.092	0.881
NRB120777		Yes	0.083	0.876
CIho 9825	495222	Yes	0.631	0.871
CIho 9825	495211	Yes	0.627	0.870

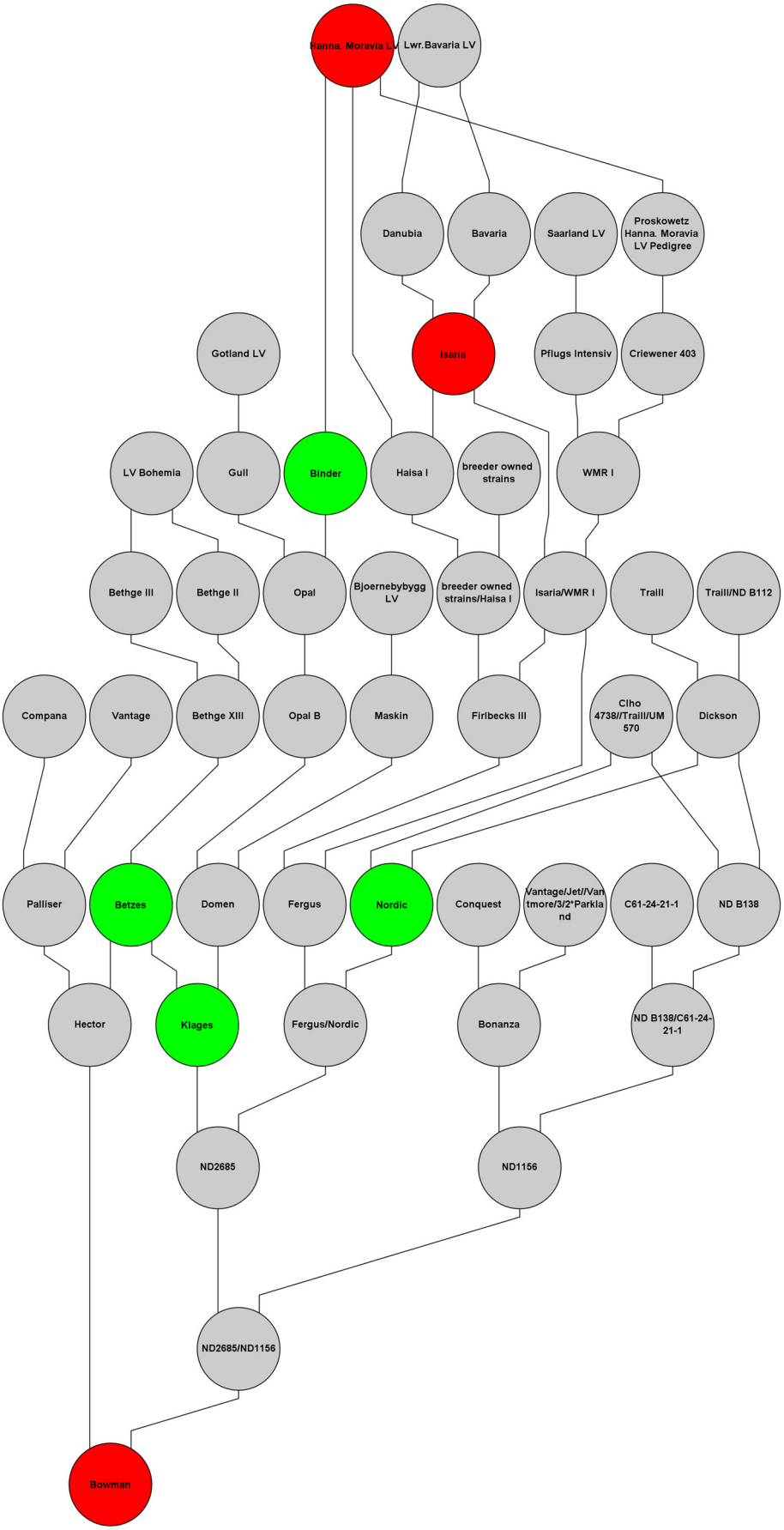
Appendix 5. Continued.

ND22996-1		Yes	0.113	0.868
NRB11093		Yes	0.099	0.864
NRB120008		Yes	0.077	0.862
NRB120005		Yes	0.084	0.855
NRB11713		Yes	0.071	0.845
K20019	495213	No	0.593	0.822
NRB121175		Yes	0.139	0.821
K8755	495220	No	0.600	0.812
CIho 9825		Yes	0.623	0.805
CIho 1227		No	0.621	0.800
K20019	495218	No	0.584	0.781
ND24181		Yes	0.169	0.751
Heartland	495039	Yes	0.180	0.733
ND23203		Yes	0.067	0.531
K8755	495212	Yes	0.420	0.496
Jet		Yes	0.366	0.485
ND24502		Yes	0.065	0.370
NRB11335		Yes	0.067	0.327
ND24168		Yes	0.035	0.271
ND23164		Yes	0.050	0.209
Dampier	400681	No	0.023	0.134
Algerian	495023	No	0.051	0.134
CIho 9776	490069	No	0.026	0.127
NRB11622		Yes	0.065	0.120
NRB11626		Yes	0.094	0.114
Ming	495021	No	0.015	0.113
Prior	495208	No	0.036	0.108
Coast		No	0.033	0.103
NRB120543		Yes	0.049	0.102
CIho 9647	490068	No	0.029	0.099
Cape	400555	No	0.041	0.090
Harbin	495224	No	0.001	0.085
Canadian Lake Shore	495217	No	0.017	0.083
Morex	401476	No	0.007	0.077
CIho 4922	495032	No	0.001	0.077
Tifang	495015	No	0.011	0.070
WI2291		Yes	0.050	0.065
NRB11627		Yes	0.073	0.065
Rojo	495018	No	0.038	0.055
ND B112	495037	No	0.003	0.036
Beecher	400396	No	0.009	0.031
Fleet Australia		No	0.076	0.022
Betzes		No	0.026	0.018
Sloop	408180	No	0.032	0.009
Corvette	400660	No	0.015	0.008

Appendix 5. Continued.

Clipper	400190	No	0.023	0.007
Franklin	405994	No	0.053	0.006
Harrington	495219	No	0.007	0.004
CIho 9214	490079	No	0.017	0.003
Maritime	410948	No	0.009	0.002
Skiff	403001	No	0.072	0.002
Gilbert		No	0.017	0.002
Patty	400167	No	0.024	0.001
Herta		No	0.027	0.000
CIho 11458	495025	No	0.014	0.000
Union	411285	No	0.025	0.000

Appendix 6. Pedigree of Bowman showing familial linkage to Isaria. Red circles represent undesirable allele for *QRpt6Hb* and green circles represent desirable allele for *QRpt6Hb*.



Appendix 7. Pedigree map of germplasm derived from CIho 5791. Green circles represent desirable allele for *Rpt5.f* and red circles represent undesirable allele for *Rpt5.f*.