First reported sexual recombination between *Pyrenophora teres* isolates from barley and barley grass

Buddhika Amarasinghe Dahanayaka[1*], Alexandros G. Sotiropoulos[1], Niloofar Vaghefi[1,2], Barsha Poudel[1], Anke Martin[1*]

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

[2]School of Agriculture and Food, University of Melbourne, Parkville, VIC 3010, Australia.

[*]Corresponding authors: Anke Martin; anke.martin@unisq.edu.au , Buddhika Amarasinghe Dahanayaka; BuddhikaAmarasinghe.Dahanayaka@unisq.edu.au

ABSTRACT

Barley grass (*Hordeum leporinum*), which often occurs in proximity to commercial barley (*Hordeum vulgare*) cultivars, is an alternative host to *Pyrenophora teres*, an economically important pathogen causing net blotch in barley. This study is the first to report the sexual recombination of *P. teres* isolates collected from barley with those collected from barley grass. The sexual recombination between *P. teres* isolates from barley and barley grass was confirmed using a neighbour-net network and haploblock plots based on whole genome sequencing of seven progeny isolates. Pathogenicity assays revealed that *P. teres* isolates from barley grass were not host specific and could infect both barley and barley grass and the progeny isolates were virulent on commercially grown barley cultivars. Our results contradict previous population and pathogenicity studies of *P. teres* isolates obtained from barley and barley grass which have reported that the two populations are genetically distinct and host specific, suggesting that isolates collected from barley or barley grass could be two different entities. Despite the genetic divergence of *P. teres* isolates from barley and barley grass revealed through our phylogenomic analysis, there seems to be no complete host or reproductive

separation between these populations. Therefore, there is a potential for generation of novel pathotypes through sexual recombination between *P. tere*s isolates associated with barley and barley grass, with a risk of increased impacts on commercial barley cultivars that do not carry resistance to these pathotypes.

Keyword: Genetic divergence, *Hordeum leporinum*, *Hordeum vulgare,* Hybrids, Net blotch, Novel pathotypes

Introduction

Pyrenophora teres (syn. *Drechslera teres*) is the causal agent of net blotch on barley. Yield loss due to net blotch in susceptible barley cultivars can range from 10 to 70% (Jayasena et al. 2007; Wallwork et al. 2016) making *P. teres* one of the most important fungal pathogens of barley industries globally. Net blotch in barley appears as two types: net-form net blotch and spot-form net blotch, caused by the two forms *P. teres* f. *teres* (*Ptt*) and *P. teres* f. *maculata* (*Ptm*), respectively. Symptoms caused by *Ptt* appear as dark-brown, net-like transverse and longitudinal necrotic striations while symptoms caused by *Ptm* develop as dark-brown circular to elliptic lesions on susceptible barley cultivars (Smedegård-Petersen 1971).

The *P. teres* life cycle constitutes both asexual and sexual reproduction. Asexual reproduction of the fungus occurs by producing genetically identical conidia under favourable environmental conditions. For successful sexual reproduction, *P. teres* requires two opposite mating type thalli. The sexual reproduction is controlled by a single mating type locus (*MAT1*), which exists as two idiomorphs, i.e., *MAT1-1* and *MAT1-2* (McDonald, 1963). *Pyrenophora teres* has been reported on several grass species worldwide. The first report of a *P. teres* pandemic in Australia dates back to 1953 on cultivated barley (MacNish 1967). In addition to barley (*Hordeum vulgare*), *P. teres* can infect barley grass (*Hordeum leporinum*), wheat (*Triticum aestivum*) and oats (*Avena sativa*) (Khan and Boyd 1969; Linde and Smith 2019; McLean et al. 2009; Shipton 1966). Barley grass is an annual winter weed that grows alongside commercial barley

fields, which has also been reported as a source of inoculum for net blotch infection (MacNish 1967; Shipton 1966).

Host specificity of *P. teres* isolates has been disputed in the past. Kenneth (1962) reported net-form net blotch symptoms on a large number of *Hordeum* spp., including wild species, such as *H. spontaneum* and *H. leporinum*, and reported that *Ptt* isolates from wild barley caused net-form net blotch symptoms on cultivated barley and *vice versa* (Kenneth 1962). A later study by Kenneth et al. (1967) concluded that *H. spontaneum*, an ancestral progenitor of cultivated barley, is a host for *P. teres* and, therefore, has a role in the epidemiology of net bloch in Israel. A study conducted with randomly collected *Ptt* and *Ptm* isolates from *Hordeum murinum* (wild barley) and *Bromus diandrus* (ripgut brome grass) growing close to cultivated barley fields in South Africa showed that *Ptt* isolates caused disease on cultivated barley genotypes Stirling and B87/14, while *Ptm* isolates collected only from *Bromus diandrus* also caused disease on Stirling and B87/14 (Louw 1996). This study also reported that isolates collected from spot-form-type lesions on *Hordeum murinum* were found to be *Ptt* isolates based on disease symptoms on barley cultivars Stirling and B87/14 (Louw 1996). Another grass species *Bromus diandrus* (great brome) (Khan and Boyd 1968) from Western Australia was also reported to be a host for *P. teres.* Western Australian *P. teres* isolates collected from *Bromus diandrus* were able to cause symptoms on cultivated barley genotypes (Khan and Boyd 1968). A later study using five isolates of *Ptt* from barley and one from barley grass reported net blotch symptom development on *B. diandrus*, but no disease expression on *A. sativa* (Brown et al. 1993). The same study assessed pathogenicity of *Ptt* isolates obtained from barley and barley grass on 43 gramineous species in growth room experiments, which showed that 28 out of 43 tested species developed symptoms after inoculation with at least one of the five *Ptt* isolates from barley (Brown et al. 1993). The isolate collected from barley grass caused no infection responses on

two barley cultivars, Prato and Kombar, suggesting that the isolate was host specific (Brown et al. 1993).

A pathogenicity trial conducted with Australian *Ptt* isolates from barley grass ($n = 7$) and barley ($n = 15$) showed that isolates from barley grass were unable to cause symptoms on barley and isolates from barley were unable to cause disease on barley grass (Khan 1973). A recent study conducted with 10 *Ptt* isolates from barley grass, concluded that none of the barley grass isolates were virulent on barley cultivars. Hence, *P. teres* isolates from barley were host specific (Linde and Smith 2019). In contrast, a pathogenic variation study conducted with Australian *Ptt* isolates from barley and one isolate from barley grass, HRS10128, revealed that the barley grass isolate was moderately to highly virulent on 11 out of 31 barley genotypes (Fowler et al. 2017). From the above studies we conclude that the host specificity of *P. teres* isolates from barley grass and barley has not been confirmed.

Population genetic studies reveal that *P. teres* isolates from wild grass species including barley grass and barley show a distinct genetic separation from each other. A recent study found that a *P. teres* population from USA (California) collected from wild barley represented a separate lineage or species of *Pyrenophora* that was more closely related to *P. graminea* than *P. teres* (Taliadoros et al. 2023). A study based on amplified fragment length polymorphic markers (AFLP) revealed that *P. teres* isolates collected from barley grass contained many AFLP DNA bands corresponding to *Ptt* and *Ptm* isolates collected from barley (Poudel et al. 2017). However, *P. teres* isolates collected from barley grass also had unique AFLP bands which were not detected in *Ptt* or *Ptm* isolates collected from barley, and *P. teres* isolates from barley grass were genetically distinct from *P. teres* barley isolates (Poudel et al. 2017). A large and significant population genetic differentiation ($PhiPT = 0.306$, $P = 0.001$) between *P. teres* isolates from barley grass ($n = 298$) and barley ($n = 567$) was also reported by Linde and Smith (2019) based on 17 SSR markers. The study also reported that *P. teres* isolates from barley

grass and barley showed different evolutionary history even though all isolates had been collected from similar geographic areas and *Ptt* isolates were strictly host specific. Hence, the study suggested that *Ptt* isolates from barley grass and barley should be treated as non-interacting populations (Linde and Smith 2019). Even though two recent studies have investigated the genetic distance between *P. teres* isolates from barley grass and barley, phylogenetic studies using whole genome sequences have not been conducted before to understand the phylogenetic divergence between these populations to confirm whether these two populations are two separate entities as suggested in the previous study (Linde and Smith 2019).

The possibility of sexual recombination between *P. teres* isolates from barley grass and barley was unknown. Sexual reproduction of a pathogen plays a major role in their life cycle and is important for evolution and adaptation of the pathogen to the changing environment. Sexual recombination between *P. teres* isolates from barley grass and barley may lead to novel pathotypes which could infect commercial barley varieties and survive on barley grass. Hence, this study aimed to determine the host specificity of *P. teres* isolates from barley grass and barley through pathogenicity trials and determine whether barley grass *P. teres* can undergo sexual recombination with barley *P. teres.*

Results

Ascospore collection

Pseudothecia from the crosses, developed to check the sexual recombination between *P. teres* from barley and barley grass isolates, started to emerge on barley straws of crosses after eight to 10 weeks. Only one cross, NB81 (*Ptt*)/SNB172 (*BGPt*) was successful and produced conidia. Ascospores were continuously collected at 120 to 162 days after establishing the cross, resulting in a total of 31 single-ascospore cultures.

Gene annotation of *Pyrenophora teres* isolates from barley and barley grass

Whole genome sequences of *P. teres* from barley, barley grass and progeny isolates were used to identify how recombination occurs in the progeny genomes. llumina paired end (150 bp) sequencing of the 10 isolates (NB81, *n* = 1; SNB172, *n* = 1; SNB172i, *n = 1*; progeny isolates of NB81 and SNB172, *n* = 7) generated around 50 million reads each, with ~ 100× coverage of the whole genome. Draft genome assemblies ranged from 37.78 to 43.88 Mb (Supplementary Table 2). The number of contigs/scaffolds (≥ 1000 bp) of the genome assemblies ranged from 3,157 to 3,703 (Supplementary Table 2). The largest contig size in the final assemblies ranged from 267.31 to 446.0 kbp. N_{50} values and CG contents of the genomes ranged from 34.89 to 45.22 kbp and 46.54 to 48.61%, respectively. Completeness of the genome assemblies based on BUSCOs in dothideomycetes ranged from 95.5 to 95.8% (Supplementary Table 2). Out of 3,786 total BUSCOs searched, 3,604 to 3,626 complete single copy BUSCOs were found (Supplementary Table 2), confirming the high quality of the generated draft assemblies and suitability for additional analyses.

The composition of the genomes was compared to check significant differences among the genomes. The compositions of the DNA transposons of the progeny isolates ranged from 2.78 to 3.87% of the total genome (Table 1). The long interspersed nuclear element (LINE) composition of the progeny genomes ranged from 0.49 to 0.81% and the long terminal repeat (LTR) retrotransposon ranged from 12.59 to 17.24% of the whole genome. Gene annotation was conducted to detect signal peptides. The total number of annotated protein-coding genes generated using BRAKER2 for the progeny isolates ranged from 11,241 to 11,447 among the progeny isolates (Table 1). The total number of signal peptides candidates detected for progeny isolates ranged from 1095 to 1112 (Table 1).

Phylogenomic analysis and neighbour-net network of *Pyrenophora* species

A phylogram was developed by *P. teres* isolates from barley and barley grass along with other *Pyrenophora* species available in NCBI to indicate the distance of *P. teres* from barley and

barley grass compared to other *Pyrenophora* species. According to the results of the phylogram developed from ~ 3600 orthologous gene sequences of *Pyrenophora* species, *BGPt* isolates clustered within the *P. teres* group (Supplementary Figure 2). However, within this group, *Ptt* and *Ptm* from barley and *BGPt* from barley grass formed three distinct clusters with maximum bootstrap support. The genetic distance between *Pyrenophora teres* isolates collected from barley and barley grass was lower than the genetic distance between *P. teres* and *P. tritici-repentis*. *P. teres* isolates collected from barley grass showed a closer relationship to *Ptt* than to *Ptm* isolates collected from barley.

A neighbour-net network was also used to visualise the genetic distance among the isolates within the *Pyrenophora* genus. A close genetic relationship was observed among *P. teres* isolates from barley and barley grass. The neighbour-net network also showed that *P. teres* isolates collected from barley grass were closer to *Ptt* than they were to *Ptm* isolates collected from barley (Figure 1).

Sexual recombination between *Pyrenophora teres* isolates from barley and barley grass

A different neighbour-net phylogenetic network was used to visualise the relationship between *P. teres* isolates from barley and barley grass. The neighbour-net network of orthologous genes of *P. teres* isolates collected from barley (*Ptt; n* = 7*, Ptm; n* = 5, *Ptt/Ptm* hybrid; *n* = 1), barley grass (*BGPt; n* = 5) and progeny isolates of the NB81/SNB172 cross (*n* = 7) exhibited three distinct groups, one each for *Ptt*, *Ptm* and *BGPt* (Figure 2). The *Ptt/Ptm* hybrid isolate positioned between the *Ptt* and *Ptm* groups of the network. The progeny isolates of the NB81/SNB172 cross were positioned between *P. teres* from barley (*Ptt* and *Ptm*) and barley grass (*BGPt*), supporting the *Ptt*/*BGPt* hybrid nature of these seven isolates.

A haploblock plot was created to identify the parental genomic regions/blocks shared by the progeny isolates. The Haploblocks that resulted for the progeny isolates were mosaic because

of genomic regions contributed by both parents (Figure 3). The total number of SNPs involved in block formation was 593,218. The number of SNPs that resulted for the 12 chromosomes varied between 19,938 to 76,671 (Supplementary Table 3), the highest number and the lowest of SNPs were observed on chromosome 3 and 12, respectively.

Fifteen randomly selected shared proteins identified by BUSCO from the parental isolates NB81 and SNB172, five *Ptt*, three *BGPt* and seven progeny isolates, eight protein sequences showed amino acid polymorphisms (Figure 4). Segregation for these amino acid polymorphisms was detected in the progeny isolates. The protein Ubiquitin-activating enzyme E1-like and ATP-dependent DNA helicase II subunit 1 showed amino acid polymorphisms at two locations while the remaining six proteins showed polymorphisms at a single location.

Pathogenicity assay of barley grass isolates and progeny isolates

A pathogenicity assay was conducted with a previously reported *BGPt* isolates to observe the different virulence levels of these isolates. Disease response scores of *Ptt, Ptm* and *BGPt* isolates ranged from 2 to 6 when inoculated on barley grass (Table 2). *Ptt* isolate HRS16083 and *Ptm* isolate SNB74 were avirulent (disease response score of 2) on barley grass (Table 2). *BGPt* isolates ranged from being avirulent (1) to virulent (6) on the barley cultivars Commander, Compass, Kombar and TR250 (Table 2, Figure 5).

Another pathogenicity assay was conducted to assess the ability of the progeny isolates of the *Ptt* and *BGPt* cross to cause disease. Out of 31 progeny isolates of the NB81/SNB172 cross, 10 isolates produced conidia, and these were used in the pathogenicity assay. Average disease response scores of these 10 progeny isolates ranged from 0 to 4 on barley cultivar Clho 5791, 0 to 5 on Corvette, 0 to 6 on Navigator and 0 to 4 on Prior (Table 3). Parental isolate NB81 was virulent (6) on barley grass, Corvette, Navigator and Prior, and avirulent (1) on Clho 5791. The parental isolate collected from barley grass, SNB172, had disease reaction scores between 2 and 4 on the four tested barley genotypes and a 6 on barley grass.

Discussion

In this study we present the first successful sexual recombination event between *P. teres* isolates from barley grass and barley. Even though both barley grass and barley are considered hosts of *P. teres* the host specificity of *P. teres* has been controversial (Kenneth 1962; Khan 1973; Linde and Smith 2019). Results derived from this study highlight the importance of management of weeds such as barley grass in commercial barley fields for better management of net blotch disease in barley.

The barley grass parent of this cross, SNB172, was described in two previous studies (Lehmensiek et al. 2010; Poudel et al. 2017). A genetic characterisation study conducted with AFLP analysis reported SNB172 as a *Ptm* but noted that it needed to be further investigated as the isolate clustered separately from other *Ptt* and *Ptm* isolates (Lehmensiek et al. 2010). A subsequent study using form-specific molecular markers for *Ptt* and *Ptm* along with AFLP analysis confirmed that SNB172 was a barley grass isolate (Poudel et al. 2017). It was also subsequently confirmed that SNB172 was originally collected from barley grass (G. Platz, Queensland Department of Agriculture and Fisheries, *personal communication*; Poudel et al., 2017). However, in the aforementioned study, some AFLP bands distinctive of *P. teres* isolates from barley were also identified in SNB172 and absent in other *BGPt* isolates (Poudel et al. 2017). Thus, SNB172 contained some genomic regions distinctive to *P. teres* from barley and this may be the reason why this was the only isolate that successfully recombined with *P. teres* isolate from barley. The phylogram and the neighbour-net network of the current study also indicates a low level of genetic separation of SNB172 from the rest of the *BGPt* isolates. Considering the current and previous studies involving SNB172, we think that this isolate may be a predating lineage of *BGPt* and *P. teres* from barley or unknown intermediary lineages of *P. teres* from barley grass and barley. With the exception of this cross, the other crosses of the current study failed to produce ascospores. Sexual recombination within the two forms of *P.*

teres is rare in nature due to sexual incompatibility (Dahanayaka et al. 2021). Sexual recombination between *P. teres* isolates from barley and barley grass may also be limited due to sexual incompatibility between the two groups due to genetic barriers like differences in mating type loci or lack of sexual structure formation at the same time.

Genetic exchange between *BGPt* and *P. teres* from barley may lead to novel pathotypes and accelerate evolution of the pathogen to overcome disease resistance. Evaluation of the pathogenicity of the progeny isolates confirmed potential virulence on barley cultivars, however the interactions were isolate and cultivar dependent. Furthermore, pathogenicity tests conducted in the current study showed that *BGPt* isolates could also be pathogenic on barley cultivars. Sexual recombination between *P. teres* from barley grass and barley could lead to intermediary lineages possessing genomic regions responsible for the virulence of both hosts. Previously, pathogenicity studies with *BGPt* including HRS10128 (Fowler et al. 2017; Kenneth 1962) along with a study showing spot-form symptoms of SNB172 on barley cultivars (Lehmensiek et al. 2010) also provide evidence for the virulence of *BGPt* isolates on barley. Our study highlights the importance of managing weeds like barley grass in commercial barley fields, not only in terms of weed management but also to control pathogens like *P. teres* and limit their evolution.

Ellwood et al. (2012) suggested that the divergence of *Ptt* and *Ptm* occurred in the middle Pleistocene of the Quaternary Period based on intergenic divergence estimates of ten intergenic genomic regions from *Ptt* and *Ptm* genomes. As the genetic isolation of *Ptt* and *Ptm* occurred before the domestication of barley, it was suggested that *Ptt* and *Ptm* from barley could be considered as two different species (Ellwood et al. 2012). In contrast, a recent study suggested that *P. teres* f. *teres* emerged and co-evolved during barley domestication as depicted in other cereal pathogens (Taliadoros, et al. 2023) like *Zymoseptoria tritici* and rice blast disease in rice (Thierry et al. 2005), respectively. The close genetic relationship observed in the neighbour-

net network between *P. teres* isolates from barley grass and barley observed in the current study suggests that the *P. teres* isolates from barley grass and barley are closely related to each other and *P. teres* isolates from barley grass could also be relatively ancient. Based on the phylogram and the neighbour-net network for *Pyrenophora* genus, we suggest two hypotheses for the evolution of *P. teres* from barley and barley grass; 1. if *P. teres* from barley grass had diverged from *P. teres* from barley, the divergence had occurred at relatively close timepoints 2.various lineages of *P. teres* may have evolved on different lineages of wild barley like barley grass and these lineages may have recently migrated or jump to cultivated barley. However, in order to confirm the evolution of *P. teres* from barley and barley grass, further studies are needed.

Host jump or host-driven specialization is a keystone in the evolutionary process and the widespread form of ecological diversification of plant-associated fungi (Restrepo et al. 2014; Schirrmann et al. 2018; Vialle et al. 2013). Host jumping is more frequently observed in necrotrophic pathogens like *P. teres* than biotrophic pathogen as they survive and continue their life cycle in dead tissues (Thines 2019). Establishment of fungal pathogens on new hosts coupled with speciation is suggested to be a rapid process (Gladieux et al. 2011; Giraud et al. 2006; Giraud et al. 2010; Thines 2019). We favour the hypothesis that different lineages of *BGPt* might have jumped to barley and adapted to its new host and diverged to current *P. teres* isolates from barley. Barley grass land prevalence was diminished by the introduction of domesticated barley in most of the barley grass environment. This might have caused a reduction in the effective population size of the barley grass *P. teres* population through a bottleneck effect. Since the close relative of domesticated barley would be found in most fields where barley grass used to be, a host jump could be possible, where the lineages of *BGPt* might have jumped to barley and adapted to its new host and diverged to current *P. teres* isolates from barley. In natural ecosystems, population dynamics of the pathogen and the host is maintained by co-evolution (Möller and Stukenbrock 2017). However, in cropping systems, where

artificial selection is carried out based on agriculturally important traits such as disease resistance, the pathogens may be more likely to replace/alter its genes to overcome the host resistance genes (Möller and Stukenbrock 2017) and/or invade new hosts (Menardo et al. 2016). Barley has been one of the most widely grown crops globally since its domestication and has since been under selection pressure by breeders in the production of elite barley cultivars. This might have led to some of the *P. teres* isolates jumping across to barley grass growing alongside barley. However, in order to confirm the host jump and ongoing evolutionary divergence of *P. teres* isolates from barley and barley grass, a comprehensive study with co-phylogenomic analyses of the pathogen with its hosts is needed.

Sexual recombination/interbreeding among diverged lineages as seen in *P. teres* is plausible and might include lineages predating divergence and unknown intermediary lineages (Dahanayaka et al. 2021; Ellwood et al. 2012; Stukenbrock 2013). If *P. teres* isolates from barley grass and barley have undergone genetic divergence, there could be intermediate natural progeny which are able to infect both hosts. The sexual recombination between *P. teres* isolates from barley grass and barley detected in the current study confirms the possibility of exchange of genetic material between *P. teres* isolates from barley grass and barley.

In conclusion, this study reports the first sexual recombination event between *P. teres* isolates from barley grass and barley. The phylogram in this study suggests that *P. teres* isolates from barley and barley grass are closely related to each other. Based on the results of the current study, we support the hypothesis that isolates of *P. teres* from wild barley, such as barley grass, may have undergone a change in host range that led to the establishment of the population now observed on domesticated barley.. However, to confirm the genetic divergence and evolution of *P. teres* isolates from barley grass and barley, a comprehensive evolutionary study with co-phylogenomic analyses of the pathogen with its hosts is needed. Although speciation and host specificity of diverged groups occur through evolution, this might take a few centuries or

millennia to happen and progenies arising from these different lineages may cause threats to the barley industry. Results derived from pathogenicity tests of *P. teres* isolates from barley and progeny isolates also confirmed that *BGPt* isolates can be virulent on commercially cultivated barley; hence, management of ancillary hosts like barley grass is important to limit the evolution of the pathogen as well as to control disease.

Materials and Methods

Fungal isolates and establishment of crosses

For this study, four *Ptt* (NB81, HRS16083, HRS11093, HRS18043) (Fowler et al. 2017; Lehmensiek et al. 2010; Martin et al. 2019), four *Ptm* (SNB74, 16FRG073, SNB171 and U7) (Ellwood et al. 2018; Lehmensiek et al. 2010; Mair et al. 2019; McLean et al. 2014), nine barley grass *P. teres* (*BGPt)* isolates (SNB172, Ptt12013, CLG741, CLG947, HRS10128, BG14-011, CLG781, CLG759 and *Ptm*14015) (Fowler et al. 2017; Lehmensiek et al. 2010; Poudel et al. 2017) and progeny isolates from a *Ptt* x *BGPt* cross were used. All the isolates used in this study were either collected from Australia or produced by crossing isolates collected from Australia.

Two copies of SNB172 were used to confirm that this was one of the parents of the population developed for this study. SNB172 is the same sample that was used in the Poudel et al. (2017) study and was used as the parent to produce the population for this study. SNB172i is the original isolate collected from leaf material in 2008. Fungal isolates were either isolated from infected barley leaf samples following the method described by Dahanayaka et al. (2021) or were retrieved from 15% glycerol tubes stored at -80 °C at the Centre for Crop Health, University of Southern Queensland, and were grown on Potato Dextrose Agar (PDA). All the isolates used in this study were single spore isolates.

Crosses were made and ascospores collected according to the method described by Dahanayaka et al. (2021). For example, *BGPt* isolate SNB172 (*MAT*-2) was crossed with the *Ptt* isolates

NB81 (*MAT*-1) and HRS11093 (*MAT*-1), and the *Ptm* isolates 16FRG073 (*MAT*-1), SNB171 (*MAT*-1) and U7 (*MAT*-1). *BGPt* isolates whose mating types were unknown were crossed with both *MAT*-1 and *MAT*-2 barley *P. teres* isolates. Mating type of SNB172 was determined by PCR amplifications with mating type specific markers (Supplementary Fig. 1).

DNA extraction and whole genome sequencing

Ascospores of seven progeny isolates (BG2, BG6, BG8, BG12, BG20, BG25 and BG29) of the NB81/SNB172 cross and single conidia of NB81, SNB172 and SNB172i were grown on half-strength PDA medium (20 g/litre PDA; Biolab Merck, Darmstadt, Germany) at 22°C for 10 to 20 days depending on the growth rate of isolates. The DNA of the 10 isolates from mycelia was extracted using the Wizard® Genomic DNA Purification kit following the protocol of the supplier (Promega Corporation, Wisconsin, USA). DNA quality and quantity were measured with a NanoDropTC 2000/2000c (IMPLEN, Munich, Germany) and a Qubit 4 Fluorometer using a Qubit™ dsDNA BR assay kit (Thermo Fisher Scientific, Massachusetts, USA). DNA samples were submitted to Macrogen (Seoul, Republic of Korea) for whole genome sequencing. Genome libraries for each isolate were generated with TruSeq sample preparation kits (Illumina, California, USA) and paired-end, 150 bp, indexed reads were generated on an Illumina NovaSeq 6000 (Illumina, California, USA).

Genome assembly and annotation

The sequence quality of the paired-end reads for all isolates was examined using FastQC v0.11.8 (Andrews 2010). The adapter sequences of paired-end reads were trimmed by Trimmomatic v0.39 (Bolger et al. 2014) using 6 bp as the set head crop parameter. Sequences shorter than 70 bp and sequences with average phred quality scores lower than 33 were removed. The quality of trimmed sequences was examined using FastQC v0.11.8. Trimmed sequences were used to perform *de novo* whole genome assembly using SPAdes v3.15.2 (Bankevich et al. 2012) by adjusting kmer size from 40 to 70 bp. Kraken2 v2.1.1 (Wood and

Salzberg 2014) was run with default fungal databases to identify possible contamination of the assembled genomes with bacterial sequences. Contiguity and completeness of the 10 draft genomes were evaluated by QUAST v5.0.2 (Gurevich et al. 2013) and Benchmarking Universal Single-Copy Orthologs (BUSCO) v.4.1.2 (Simão et al. 2015) using the dothideomycetes fungi_odb10 as the reference database.

Repeat element compositions in the 10 genomes were detected by RepeatModeler v1.0.11 (Smit and Hubley 2008), using the Repbase v2.0.4 library (Bao et al. 2015) and repeat regions were masked using RepeatMasker v4.0.9 (Nishimura 2000). Gene prediction was performed by BRAKER2 v.2.1.6 (Hoff et al. 2019) genome annotation pipeline, using the protein sequences of *Ptt* 0–1 (Ellwood et al. 2010) as evidence to train AUGUSTUS (Stanke et al. 2006). The total number of predicted genes from BRAKER2 v.2.1.6 for each genome were used for prediction of signal peptides in the secretory pathway using SignalP v5.0.0 (Petersen et al. 2019) to detect potential effector.

Phylogenomic analysis and neighbour-net network of *Pyrenophora* genus

A maximum likelihood phylogenomic tree (ML tree) based on probabilistic method was used for the phylogenomic analysis of *BGPt*, *Ptt* and *Ptm*. A phylogram of the *Pyrenophora* genus was constructed using single copy orthologues extracted from *Ptt* (*n* = 7)*, Ptm* (*n* = 5), *BGPt* (*n* = 5), *P. tritici-repentis* (*n* = 5)*, P. graminea* (*n* = 1) and *P. seminiperda* (*n* = 1) genomes (Supplementary Table 1). Genomes other than the five *BGPt* isolates and *Ptt* NB81 were obtained from the NBCI data base (Supplementary Table 1). Benchmarking Universal Single-Copy Orthologs from each isolate were extracted by BUSCO using dothideomycetes fungi_odb10 as the reference genome and used as the input file for OrthoFinder v2.5.4 (Emms and Kelly 2019) to detect shared single copy orthologues. The resultant protein sequences were aligned with MAFFT v7.453 (Katoh et al. 2002). The best-fit nucleotide substitution model for the aligned sequences was determined by Modeltest v3.7 (Posada and Crandall 1998). The ML

tree was generated by RAxML v8.2.12 (Stamatakis 2006) using *Pyrenophora seminiperda* (CCB06) (Biosample SAMN02981545 under the Bioproject PRJNA192591 available at NCBI) as the outgroup genome with 1000 bootstraps. Figtree v.1.3.1 was used to visualise the ML tree (Rambaut 2009).

Recombination can have an impact on the phylogram. As sexual recombination has occurred among individuals used in this study, this should be taken into consideration to avoid overinterpretation of the results. A neighbour-net network was also created to understand and visualise the genetic relationship among *P. teres* from barley and barley grass with other isolates from *Pyrenophora* genus. The same sequence alignments used to draw the phylogram was also used to create the neighbour-net network. The neighbour-net network was drawn by Splitstree4 v4.17.1 (Moulton 2003) with 1000 bootstaps.

Sexual recombination between *Ptt* and *BGPt*

Sexual recombination of *BGPt* with *Ptt* was investigated by a neighbour-net network developed with 1000 bootstraps using single copy orthologues and comparing amino acid sequences extracted from the genome sequences of parental isolates NB81 and SNB172 and seven progeny isolates. Single copy orthologous genes of the parental isolates and seven progeny isolates were extracted from Orthofinder v2.5.4 (Emms and Kelly 2019) and aligned with each other to generate a multiple sequence alignment (MSA) using MAFFT. The MSA was used in Splitstree4 v4.17.1 to develop the neighbour-net network to identify the genetic relationship between the progeny isolates and other *P. teres* isolates. In addition to the progeny and parental isolates of the NB81/SNB172 cross, six *Ptt* (used in phylogram), five *Ptm* (used in phylogram), a field hybrid (*Ptt* x *Ptm*) (Supplementary Table 1), and three *BGPt* isolates (used in phylogram) were used in the neighbour-net network to confirm that the progeny isolates originated from a cross between NB81 and SNB172.

Haploblock plots were produced from a merged variant calling file (vcf) of seven progeny and parental isolates using the haplobloc package available at https://github.com/wicker314/haplobloc. The vcf files of the progeny and parental isolates were created using trimmed paired-end reads mentioned above. Variant calling for the isolates was performed by GATK v.4.1.2.0 (DePristo et al. 2011; McKenna et al. 2010; Van der Auwera et al. 2013) using *Ptt* isolate W1-1 as the reference genome following the steps described by Sotiropoulos et al. (2022).

For amino acid sequence comparison, the complete orthologous sequences of seven *Ptt*, four *BGPt* and seven progeny isolates used in the neighbour-net network, identified by OrthoFinder v2.5.4 were used. Fifteen randomly selected proteins from parental isolates NB81 and SNB172, progeny and *Ptt* isolates were aligned using Muscle v5.0.0 (Edgar 2004) and sequences were compared for amino acid differences among *BGPt* and *Ptt* isolates. The segregation of these amino acids was examined in the progeny isolates to confirm that the progeny isolates were hybrids of the barley and barley grass *P. teres* isolates.

Pathogenicity assays

A pathogenicity assay using a set of *P. teres* isolates collected from barley grass was conducted to assess their host specificity (Table 2). A completely randomized design was used in a glasshouse at the University of Southern Queensland, Australia, for eight *BGPt*, with one *Ptt* and one *Ptm* isolate originating from barley as controls, on four barley cultivars (Commander, Compass, Kombar and TR250) (Table 2). These barley cultivars were selected based on susceptibility to *Ptt* (Fowler et al. 2017) and *Ptm* (McLean et al. 2014). Each isolate was replicated three times and the experiment was also repeated three times.

Another pathogenicity assay was conducted for 10 progeny isolates (Table 3) of the NB81/SNB172 cross with four barley cultivars (Clho 5791, Corvette, Navigator and Prior) and barley grass to determine the pathogenicity of progeny isolates using parental isolates as

controls. Barley cultivars Corvette, Navigator and Prior were chosen based on susceptibility to parental isolate NB81, while Clho 5791 was reported to be resistant to NB81 (Fowler et al. 2017). The same experimental design with replicates was used as mentioned in the previous pathogenic assay.

The barley and barley grass plants were grown in pots with 5 cm diameter and 14 cm height at 20 ± 5°C. Each pot contained four plants each of four barley cultivars. Barley cultivars were grown for 14 days prior to inoculation. Barley grass seeds were sown two weeks prior to the barley cultivars due to the slow growth of barley grass (28 days).

The conidial suspension for the pathogenicity assays was prepared as described in Dahanayaka et al. (2022). Fourteen days after planting barley plants in each pot were sprayed with 3 mL of a 10^4 conidia/mL suspension. Inoculated plants were incubated in the dark for 24 hours at 95% humidity with a temperature of 20 ± 1°C. After 24 hours, plants were transferred to the glasshouse for nine days with diurnal light and a temperature of 20 ± 5°C. Nine days after inoculation, disease severity on the second leaf of barley plants was scored using a 1 to 10 range, with 1 being highly resistant and 10 being highly susceptible (Tekauz 1985). For barley grass, a whole plant score based on overall disease symptoms was given as the leaves were narrow and difficult to score as single leaves. Average disease reaction scores were taken into account for further analyses.

Acknowledgement

We thank Lisle Snyman, Judy Mcllroy and Janet Barsby, Hermitage Research Facility, Warwick, Australia, Celeste Linde from The Australian National University, Simon Ellwood and Fran Lopez-Ruiz from Curtin University and Mark S. McLean from Agriculture Victoria, Horsham, Australia for providing us with *P. teres* isolates.

Data availability

The raw data generated from this study for whole-genome sequencing were deposited in the NCBI GenBank database under PRJNA838266.

Literature Cited

Andrews, S. 2010. FastQC: a quality control tool for high throughput sequence data. Retrieved May 17:2018.

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., Lesin, V. M., Nikolenko, S. I., Pham, S., and Prjibelski, A. D. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J. Comput. Biol. 19:455-477.

Bao, W., Kojima, K. K., and Kohany, O. 2015. Repbase Update, a database of repetitive elements in eukaryotic genomes. Mobile DNA 6:1-6.

Bolger, A. M., Lohse, M., and Usadel, B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114-2120.

Brown, M., Steffenson, B., and Webster, R. 1993. Host range of *Pyrenophra teres* f. *teres* isolates from California. Plant Dis.

Dahanayaka, B. A., Vaghefi, N., Snyman, L., and Martin, A. 2021. Investigating in vitro mating preference between or within the two forms of *Pyrenophora teres* and its hybrids. Phytopathology 111:2278-2286.

Dahanayaka, B. A., Snyman, L., Vaghefi, N., and Martin, A. 2022. Using a hybrid mapping population to identify genomic regions of *Pyrenophora teres* associated with virulence. Front Plant Sci:2059.

DePristo, M. A., Banks, E., Poplin, R., Garimella, K. V., Maguire, J. R., Hartl, C., Philippakis, A. A., Del Angel, G., Rivas, M. A., and Hanna, M. 2011. A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat. Genet. 43:491-498.

Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32:1792-1797.

Ellwood, S. R., Syme, R. A., Moffat, C. S., and Oliver, R. P. 2012. Evolution of three Pyrenophora cereal pathogens: recent divergence, speciation and evolution of non-coding DNA. Fungal Genet Biol 49:825-829.

Ellwood, S. R., Piscetek, V., Mair, W. J., Lawrence, J. A., Lopez-Ruiz, F. J., and Rawlinson, C. 2018. Genetic variation of *Pyrenophora teres* f. *teres* isolates in Western Australia and emergence of a Cyp51A fungicide resistance mutation. Plant Pathology.

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

Emms, D. M., and Kelly, S. 2019. OrthoFinder: phylogenetic orthology inference for comparative genomics. Genome Biol. 20:1-14.

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

Gladieux, P., Guérin, F., Giraud, T., Caffier, V., Lemaire, C., Parisi, L., Didelot, F. and Le Cam, B., 2011. Emergence of novel fungal pathogens by ecological speciation: importance of the reduced viability of immigrants. Mol/ Ecol. 20:4521-4532.

Giraud, T., Villaréal, L.M., Austerlitz, F., Le Gac, M. and Lavigne, C., 2006. Importance of the life cycle in sympatric host race formation and speciation of pathogens. Phytopathology 96:280-287.

Giraud, T., Gladieux, P., Gavrilets, S., 2010. Linking the emergence of fungal plant diseases with ecological speciation. Trends Ecol Evol. 25:387-95.

Gurevich, A., Saveliev, V., Vyahhi, N., and Tesler, G. 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29:1072-1075.

Hoff, K. J., Lomsadze, A., Borodovsky, M., and Stanke, M. 2019. Whole-genome annotation with BRAKER. Pages 65-95 in: Gene Prediction. Springer.

Jayasena, K., Van Burgel, A., Tanaka, K., Majewski, J., and Loughman, R. 2007. Yield reduction in barley in relation to spot-type net blotch. Australasian Plant Pathology 36:429-433.

Katoh, K., Misawa, K., Kuma, K. i., and Miyata, T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. 30:3059-3066.

Kenneth, R. 1962. On the taxonomy, morphology and geographic origins of *Pyrenophora teres Drechsler* and allied species. Bulletin of the Research Council of Israel 11:55-82.

Kenneth, R., Atzmon, Y., Khair, J., Koltin, Y., and Whal, I. 1967. Problems of breeding barley resistant to net blotch in Israel. The First Israel Congress of Plant Pathology:46-47.

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

Khan, T., and Boyd, W. 1968. *Gramineae* species harbouring *Drechslera teres* in Western Australia. Aust. J. Biol. Sci. 30:373.

Khan, T., and Boyd, W. 1969. Physiologic specialization in *Drechslera teres*. Australian Journal of Biological Sciences 22:1229-1236.

Lehmensiek, A., Bester-van der Merwe, A., Sutherland, M., Platz, G., Kriel, W., Potgieter, G., and Prins, R. 2010. Population structure of South African and Australian *Pyrenophora teres* isolates. Plant Pathology 59:504-515.

Linde, C. C., and Smith, L. M. 2019. Host specialisation and disparate evolution of Pyrenophora teres f. teres on barley and barley grass. BMC evolutionary biology 19:139.

Louw, J. 1996. Relative importance of the barley net blotch pathogens *Pyrenophora teres* f. *teres* and *P. teres* f. *maculata*. African Plant Protection 2.

MacNish, G. 1967. Supplementary list of disease recorded on various hosts in Western Australia. Journal of the Department of Agriculture, Western Australia, Series 4 5:991-995.

Mair, W. J., Thomas, G. J., Dodhia, K., Hills, A. L., Jayasena, K. W., Ellwood, S. R., Oliver, R. P., and Lopez-Ruiz, F. J. 2019. Parallel evolution of multiple mechanisms for demethylase inhibitor fungicide resistance in the barley pathogen *Pyrenophora teres* f. sp. *maculata*. bioRxiv. [Preprint] Available at: https://doi.org/10.1101/798991 [Accessed 19 May 2020]. .

Martin, A., Paula, M., Yongfu, Y., Mcilroy, J., Ellwood, S., Fowler, R. A., Platz, G. J., Kilian, A., and Snyman, L. 2019. Genomic Regions Associated with Virulence in Pyrenophora teres f. teres Identified by Genome-Wide Association Analysis and Bi-parental Mapping. Phytopathology.

McDonald, W. 1963. Heterothalism in *Pyrenophora teres*. Phytopathology. 53:771

McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., Garimella, K., Altshuler, D., Gabriel, S., and Daly, M. 2010. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 20:1297-1303.

McLean, M., Martin, A., Gupta, S., Sutherland, M., Hollaway, G., and Platz, G. 2014. Validation of a new spot form of net blotch differential set and evidence for hybridisation between the spot and net forms of net blotch in Australia. Australasian Plant Pathology 43:223-233.

McLean, M. S., Howlett, B. J., and Hollaway, G. J. 2009. Epidemiology and control of spot form of net blotch (*Pyrenophora teres f. maculata*) of barley: a review. Crop and Pasture Science 60:303-315.

Menardo, F., Praz, C. R., Wyder, S., Ben-David, R., Bourras, S., Matsumae, H., McNally, K. E., Parlange, F., Riba, A., and Roffler, S. 2016. Hybridization of powdery mildew strains gives rise to pathogens on novel agricultural crop species. Nat. Genet. 48:201-205.

Möller, M., and Stukenbrock, E. H. 2017. Evolution and genome architecture in fungal plant pathogens. Nat. Rev. Microbiol. 15:756.

93 Moulton, V. 2003. SplitsTree: A network-based tool for exploring evolutionary relationships. The
94 phylogenetic handbook: a practical approach to DNA and protein phylogeny:312.
95 Nishimura, D. 2000. RepeatMasker. Biotech Softw. Internet Rep. 1:36-39.
96 Petersen, T. N., Brunak, S., Heijne, G. V., and Nielsen, H. 2019. SignalP 5.0 improves signal peptide
97 predictions using deep neural networks. Nat. Biotechnol. 37:420-423.
98 Posada, D., and Crandall, K. A. 1998. MODELTEST: testing the model of DNA substitution.
99 Bioinformatics 14:817-818.
100 Poudel, B., Ellwood, S. R., Testa, A. C., McLean, M., Sutherland, M. W., and Martin, A. 2017. Rare
101 Pyrenophora teres hybridization events revealed by development of sequence-specific PCR
102 markers. Phytopathology 107:878-884.
103 Rambaut, A. 2009. FigTree v1. 3.1. in: http://tree. bio. ed. ac. uk/software/figtree/.
104 Restrepo, S., Tabima, J. F., Mideros, M. F., Grünwald, N. J., and Matute, D. R. 2014. Speciation in fungal
105 and oomycete plant pathogens. Annu. Rev. Phytopathol. 52:289-316.
106 Schirrmann, M. K., Zoller, S., Croll, D., Stukenbrock, E. H., Leuchtmann, A., and Fior, S. 2018.
107 Genomewide signatures of selection in Epichloë reveal candidate genes for host
108 specialization. Mol. Ecol. 27:3070-3086.
109 Shipton, W. 1966. Net blotch of barley. Journal of the Department of Agriculture, Western Australia,
110 Series 4 7:132-133.
111 Simão, F. A., Waterhouse, R. M., Ioannidis, P., Kriventseva, E. V., and Zdobnov, E. M. 2015. BUSCO:
112 assessing genome assembly and annotation completeness with single-copy orthologs.
113 Bioinformatics 31:3210-3212.
114 Smedegård-Petersen, V. 1971. *Pyrenophora teres* f. *maculata* f. nov. and *Pyrenophora teres* f. *teres*on
115 barley in Denmark. Yearbook of the Royal Veterinary and Agricultural University (Copenhagen)
116 1971:124-144.
117 Smit, A. F., and Hubley, R. 2008. RepeatModeler Open-1.0.
118 Sotiropoulos, A. G., Arango-Isaza, E., Ban, T., Barbieri, C., Bourras, S., Cowger, C., Czembor, P. C., Ben-
119 David, R., Dinoor, A., and Ellwood, S. R. 2022. Global genomic analyses of wheat powdery
120 mildew reveal association of pathogen spread with historical human migration and trade. Nat.
121 Commun. 13:4315.
122 Stamatakis, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands
123 of taxa and mixed models. Bioinformatics 22:2688-2690.
124 Stanke, M., Schöffmann, O., Morgenstern, B., and Waack, S. 2006. Gene prediction in eukaryotes with
125 a generalized hidden Markov model that uses hints from external sources. BMC Bioinformatics
126 7:1-11.
127 Stukenbrock, E.H., Banke, S., Javan-Nikkhah, M., and McDonald B.A. 2007. Origin and domestication
128 of the fungal wheat pathogen *Mycosphaerella graminicola* via sympatric speciation. Mol. Biol. Evol.
129 24:398–411.
130 Stukenbrock, E.H., 2013. Evolution, selection and isolation: a genomic view of speciation in fungal plant
131 pathogens. New Phytologist, 199. 4:895-907.
132 Syme, R., Martin, A., Wyatt, N., Lawrence, J., Muria-Gonzalez, M., Friesen, T., and Ellwood, S. 2018.
133 Transposable element genomic fissuring in Pyrenophora teres is associated with genome
134 expansion and dynamics of host-pathogen genetic interactions. Frontiers in genetics 9:130.
135 Taliadoros, D., Feurtey, A., Wyatt, N., Gladieux, P., Friesen, T., and Stukenbrock, E. H. 2023. Emergence
136 and spread of the barley net blotch pathogen coincided with crop domestication and
137 cultivation history. bioRxiv:2023.2007. 2028.550921.
138 Talvia, P. 1966. The occurrence of plant diseases in 1965. Maatalous ja Koetoiminta 20:19-20.

139 Tekauz, A. 1985. A numerical scale to classify reactions of barley to *Pyrenophora teres*. Can. J. Plant
140 Pathol. 7:181-183.
141 Thierry, M., Charriat, F., Milazzo, J., Adreit, H., Ravel, S., Cros-Arteil, S., Borron, S., Sella, V., Kroj, T.,
142 Ioos, R. and Fournier, E., 2022. Maintenance of divergent lineages of the Rice Blast Fungus
143 Pyricularia oryzae through niche separation, loss of sex and post-mating genetic
144 incompatibilities. PLoS pathogens 18:1010687.
145 Thines, M. 2019. An evolutionary framework for host shifts–jumping ships for survival. New Phytol.
146 224:605-617.
147 Van der Auwera, G. A., Carneiro, M. O., Hartl, C., Poplin, R., Del Angel, G., Levy-Moonshine, A., Jordan,
148 T., Shakir, K., Roazen, D., and Thibault, J. 2013. From FastQ data to high-confidence variant
149 calls: the genome analysis toolkit best practices pipeline. Curr. Protoc. 43:11.10. 11-11.10. 33.
150 Vialle, A., Feau, N., Frey, P., Bernier, L., and Hamelin, R. C. 2013. Phylogenetic species recognition
151 reveals host-specific lineages among poplar rust fungi. Mol. Phylogenet. Evol. 66:628-644.
152 Wallwork, H., Butt, M., and Capio, E. 2016. Pathogen diversity and screening for minor gene resistance
153 to *Pyrenophora teres* f. *teres* in barley and its use for plant breeding. Australas. Plant Pathol.
154 45:527-531.
155 Wood, D., and Salzberg, S. 2014. Kraken: ultrafast metagenomic sequence classification using exact
156 alignments. Genome Biol. 10.

157

158

Table 1. Genome annotation statistics of the progeny and parental isolates of the NB81/SNB172 cross compared to the reference genomes W1-1 and SG1

	NB81[a]	SNB172[a]	BG2	BG6	BG8	BG12	BG20	BG25	BG29	W1-1[b]	SG1[c]
OrthoFinder analysis											
Complete single copy genes	3607	3604	3602	3602	3603	3605	3600	3611	3602	3620	3611
SignalP analysis											
Predicted effectors	1109	1098	1095	1103	1098	1112	1095	1102	1092	NA	NA
Repeat annotation											
DNA Transposons (%)	3.85	2.66	3.56	3.87	3.53	3.83	2.78	3.61	3.59	2.60	6.00
LINEs (%)	0.17	1.16	0.72	0.68	0.73	0.73	0.49	0.81	0.73	0.70	0.90
LTR (%)	11.83	15.87	13.69	17.24	12.59	16.63	15.43	16.12	14.47	28.90	17.70
Unclassified (%)	7.90	6.88	8.31	9.07	8.05	7.57	8.77	8.65	8.54	NA	NA
Simple repeats (%)	0.68	0.66	0.66	0.62	0.68	0.64	0.65	0.64	0.69	NA	NA
Total gene annotation	11319	11314	11447	11406	11362	11241	11350	11257	11384	NA	NA

[a] Parental isolates

[b] Reference genome *Pyrenophora teres* f. *teres* (Syme et al. 2018) (Biosample SAMN02981545 under the Bioproject PRJNA342572 available at NCBI) GenBank Bioproject: PRJNA342572 and SAMN05762406

[c] Reference genome *Pyrenophora teres* f. *maculata* (Syme et al. 2018) GenBank PRJNA342572

NA not available

167 Table 2. Average disease severity (1-10) on barley grass and barley cultivars inoculated with
168 *Pyrenophora teres* isolates obtained from barley and barley grass

Isolate	Original host	Barley grass	Commander	Compass	Kombar	TR250	Symptoms
Ptt12013	Barley grass	4	3	4	2	1	Net-form
CLG741	Barley grass	3	6	6	6	6	Net-form
CLG947	Barley grass	3	2	3	2	1	Net-form
HRS10128	Barley grass	6	2	3	2	1	Net-form
BG14-011	Barley grass	4	2	5	2	1	Spot-form
CLG781	Barley grass	6	3	5	3	2	Net-form
CLG759	Barley grass	6	3	5	2	1	Net-form
Ptm14015	Barley grass	2	6	6	6	1	Spot-form
SNB74	Barley	2	7	7	4	7	Spot-form
HRS16083	Barley	2	7	4	6	3	Net-form

169

170

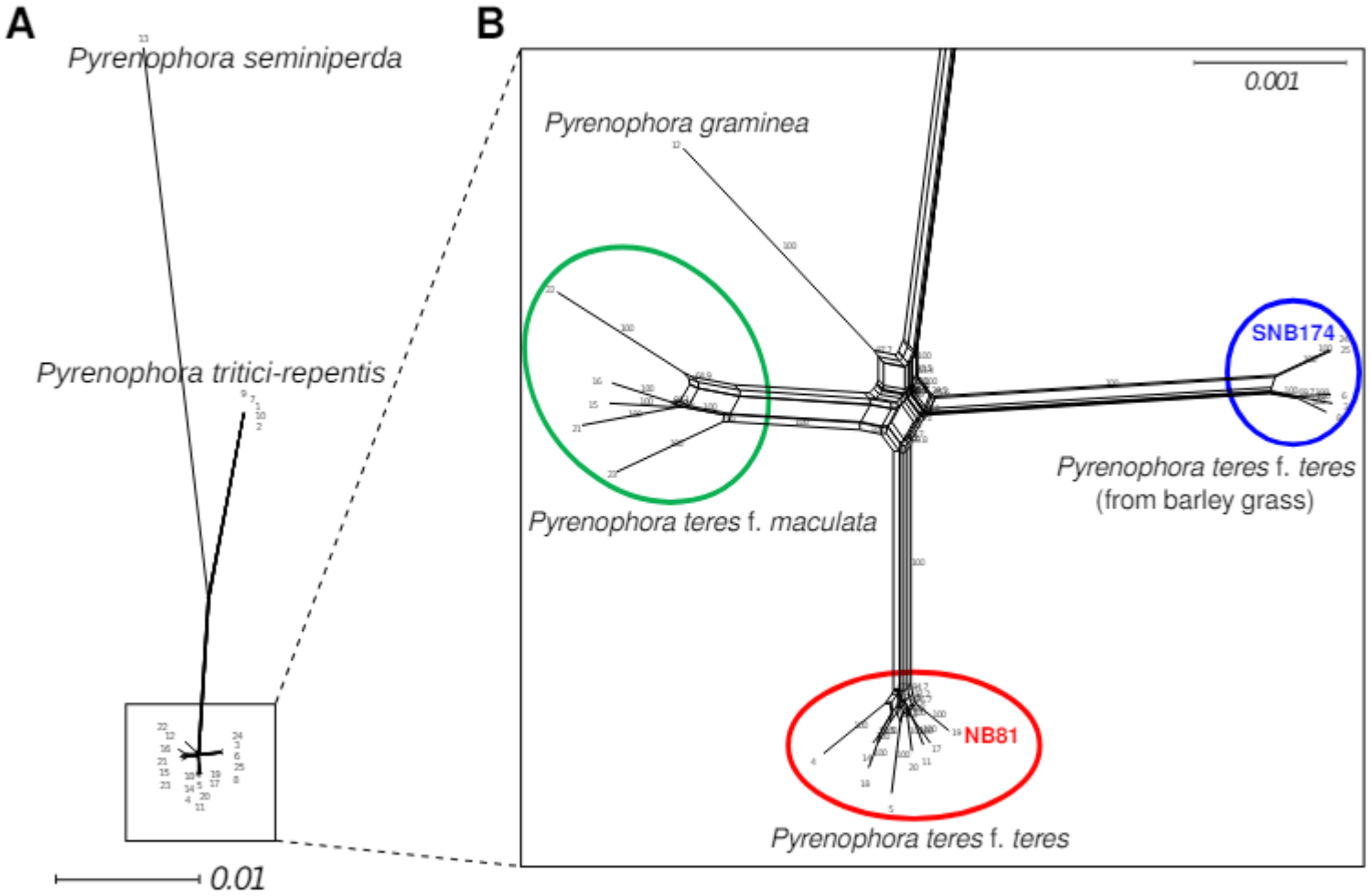
171 Table 3. Average disease severity on barley grass and barley cultivars inoculated with
172 recombinant progeny isolates obtained after crossing barley and barley grass *P. teres* isolates
173 (NB81/ SNB172)

Isolate	Barley grass	Clho 5791	Covette	Navigator	Prior
BG1	6	1	0	0	2
BG2	3	0	0	0	0
BG3	4	2	5	6	3
BG6	3	0	1	1	2
BG9	4	1	1	1	0
BG12	4	0	0	4	1
BG20	5	2	3	0	0
BG23	6	0	0	0	0
BG25	5	0	0	0	1
BG29	5	4	5	5	4
NB81[a]	6	1	6	6	6
SNB172[a]	6	4	2	4	4

174

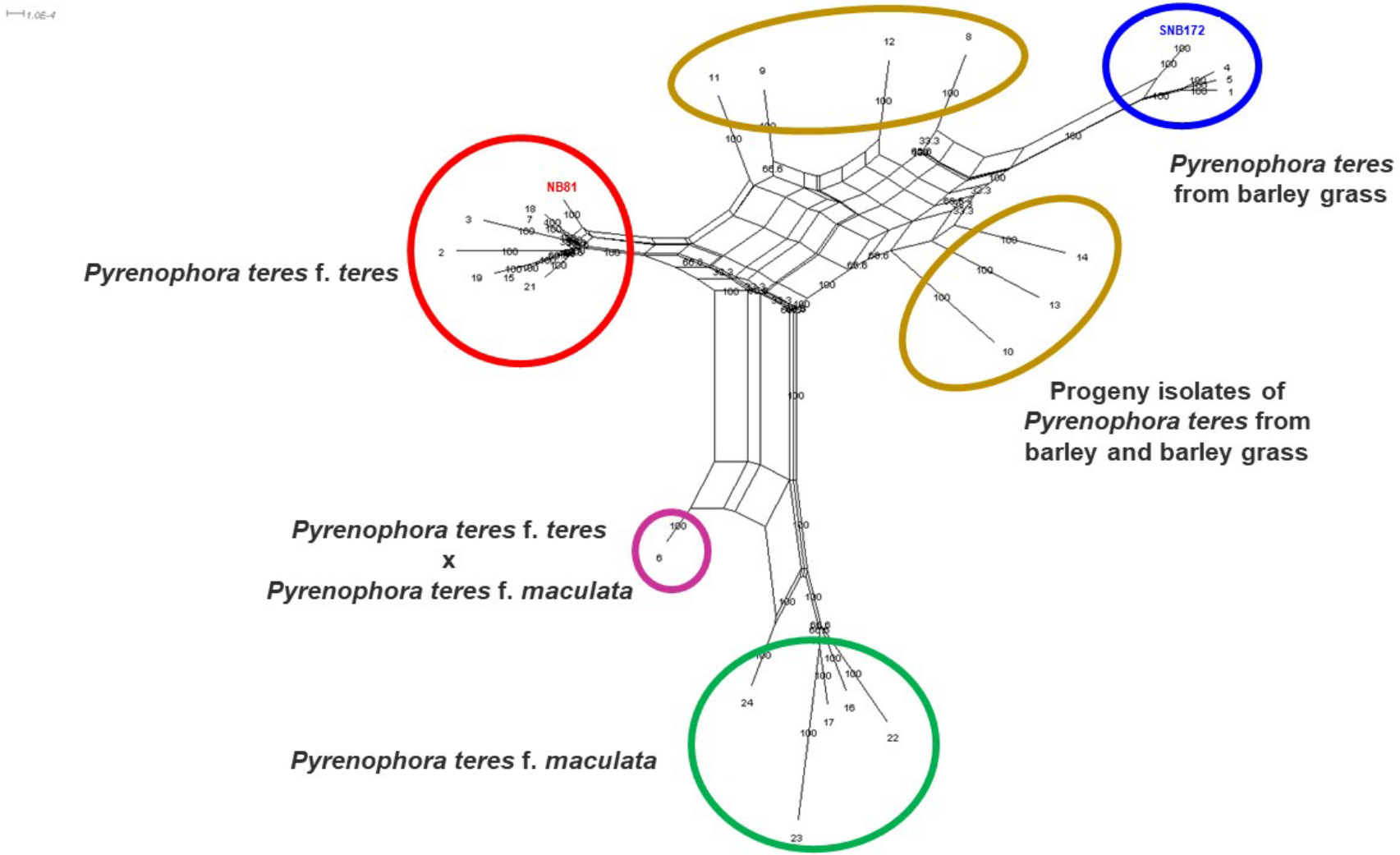
175 [a] Parental isolates

176


A
Pyrenophora seminiperda
Pyrenophora tritici-repentis
0.01
B
0.001
Pyrenophora graminea
SNB174
Pyrenophora teres f. teres
(from barley grass)
Pyrenophora teres f. maculata
NB81
Pyrenophora teres f. teres

178 Figure 1. A neighbour-net network (1000 bootstraps) constructed by Splitstree4 (A) includes
179 all isolates and is showing three main clusters of the *Pyrenophora* genus, *Pyrenophora tritici-*
180 *repentis* from wheat, *P. seminiperda* from wild grasses, and *P. teres* and *P. graminea* clustering
181 together at (B) expanded view of the *P. teres* and *P. graminea* cluster, including *P. teres* f.
182 *teres* (red circle) and *P. teres* f. *maculata* from barley (green circle), and *Pyrenophora* teres
183 from barley grass (blue circle), and one *P. graminea* isolate from barley. The location of the
184 parental *P. teres* isolates (NB81 and SNB172) used in this study are also indicated.

185


1.0E-4
NB81
SNB172
Pyrenophora teres f. teres
Pyrenophora teres
from barley grass
Progeny isolates of
Pyrenophora teres from
barley and barley grass
Pyrenophora teres f. teres
x
Pyrenophora teres f. maculata
Pyrenophora teres f. maculata

186

Figure 2. Neighbour-net network constructed by Splitstree4 for the progeny isolates (brown circles) of the NB81/SNB172 cross and parental isolates, *Pyrenophora teres* from barley (*P. teres* f. *teres*; red circle and *P. teres* f. *maculata*; green circle) and barley grass (blue circle), with a field collected hybrid isolate of *P. teres* f. teres and *P. teres* f. *maculata* with 1000 bootstraps replicates from the concatenated alignment of complete orthologous genes using dothideomycetes_fungi_odb10 as the reference genome.

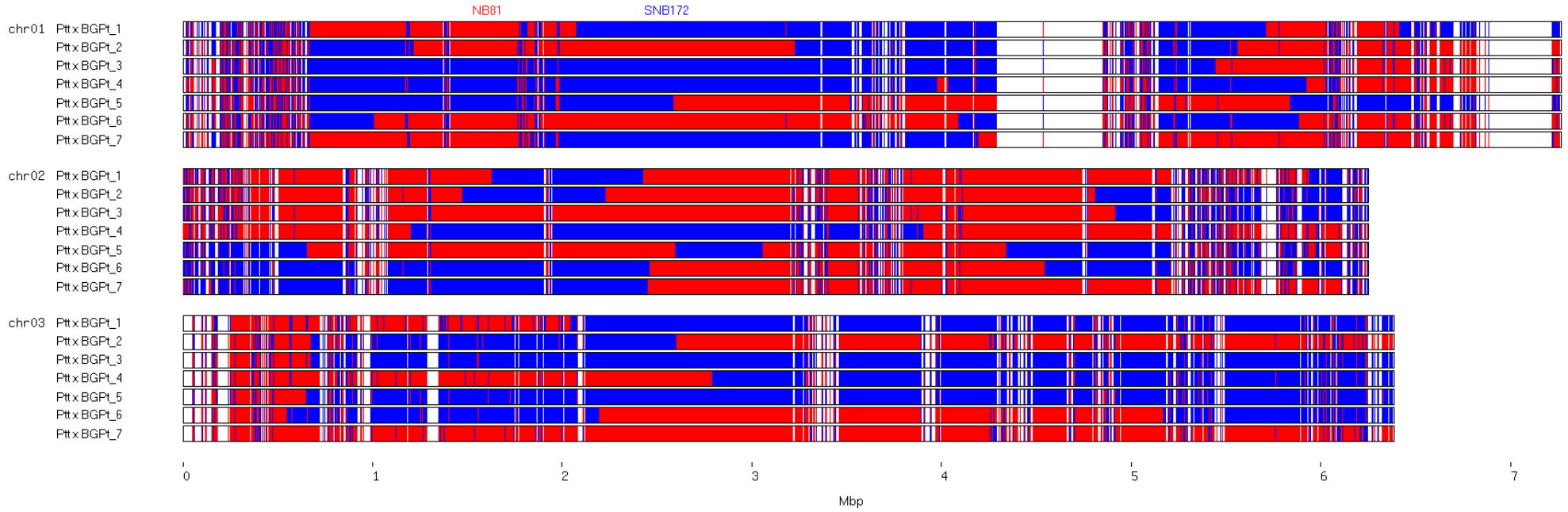
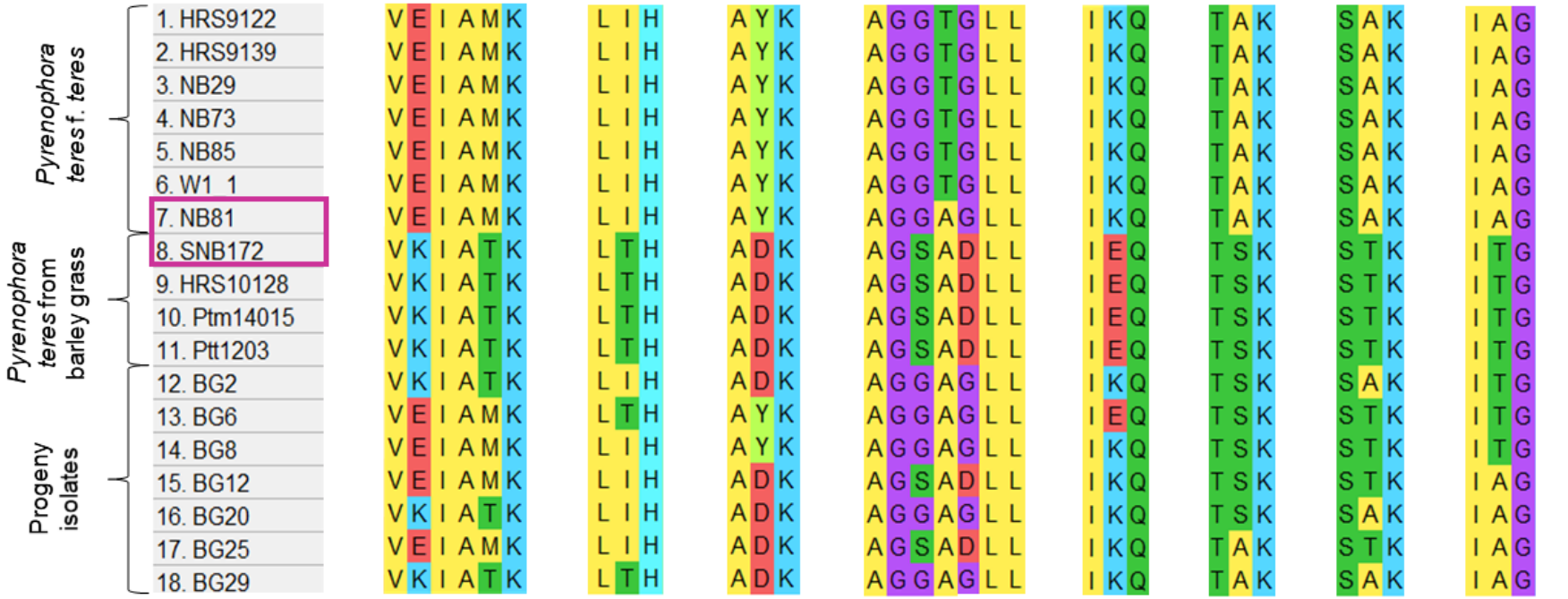


Figure 3. Haploblock plots of chromosomes 1, 2 and 3 of the progeny isolates of NB81/SNB172. Plots show genomic mosaics caused by genomic regions contributed from parental isolates NB81 (red) and SNB172 (blue). Nucleotide variants indicated by vertical bars and regions in white are not distributed to either parent because there is not sufficient data. This could be because these are repetitive regions for example the centromere or transposable elements.



PTT_15364-Ubiquitin-activating enzyme E1-like
PTT_17935-Asparagine_tRNA ligase
PTT_13426-AA_TRNA_LIGASE_II domain-containing protein
PTT_12162-ATP-dependent DNA helicase II subunit 1

PTT_07463- Uncharacterised protein
PTT_20301-RNA helicase
PTT_17566-Rieske domain-containing protein
PTT_12669-BING4CT domain-containing protein

Parental isolates

199

200 Figure 4. Amino acid differences in eight randomly selected polymorphic proteins of
201 *Pyrenophora teres* f. *teres* and *Pyrenophora teres* isolates from barley grass and the
202 segregation of the amino acid differences in the progeny isolates.

203

204

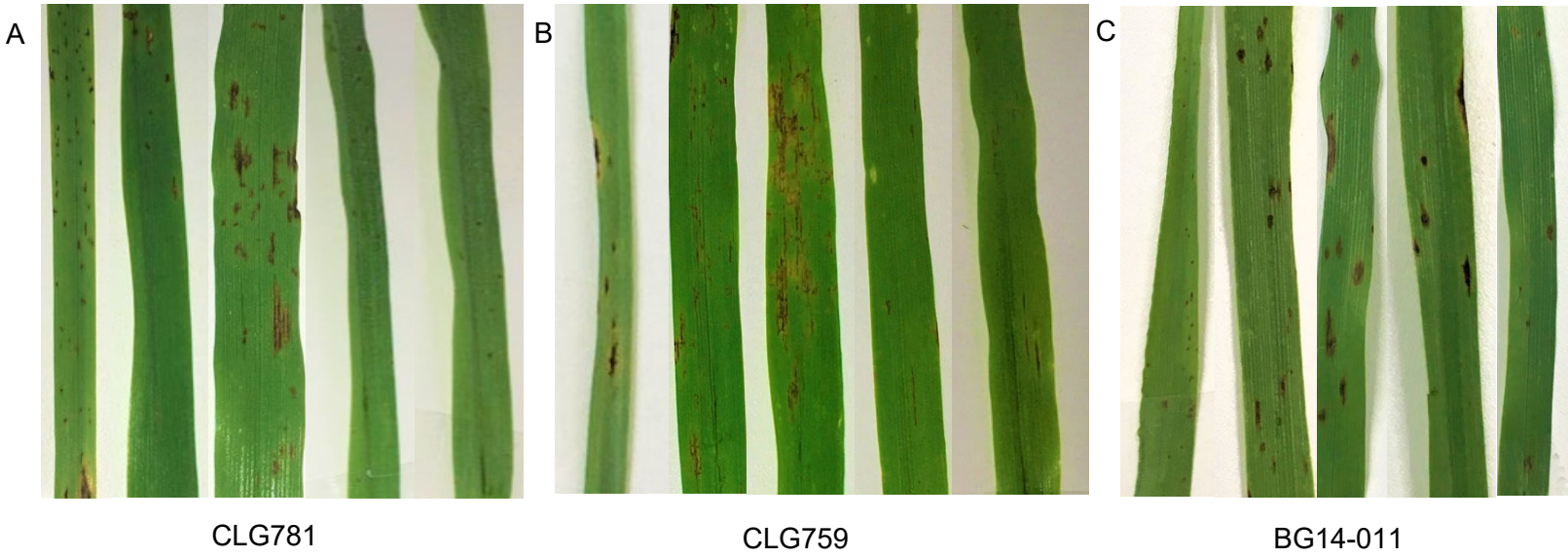


Figure 5. Disease symptoms caused by three *P. teres* isolates collected from barley grass (*BGPt*) (A) CLG781, (B) CLG759 and (C) BG14-011 on barley grass, Commander, Compass, Kombar and TR250 genotypes, respectively.