Genomic Regions Associated with Virulence in *Pyrenophora teres* f. *teres* Identified by Genome-Wide Association Analysis and Bi-parental Mapping

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Abstract

Net form net blotch (NFNB), caused by the fungal pathogen *Pyrenophora teres* f. *teres* (*Ptt*), is an important foliar disease present in all barley producing regions of the world. This fungus is a hemibiotrophic and heterothallic ascomycete where sexual recombination can lead to changes in disease expression in the host. Knowledge of the genetic architecture and genes involved in virulence is vital to increase the durability of NFNB resistance in barley cultivars. We used a genome-wide association mapping approach to characterise *Ptt* genomic regions associated with virulence in Australian barley cultivars. One-hundred and eighty-eight *Ptt* isolates collected across five Australian states were genotyped using DArTseq™ markers and phenotyped across twenty different barley genotypes. Association mapping identified fourteen different genomic regions associated with virulence with the majority located on *Ptt* chromosomes 3 and 5 and one each present on chromosomes 1, 6 and 9. Four of the regions identified were confirmed by quantitative trait loci (QTL) mapping. The QTL regions are

discussed in the context of their genomic architecture, together with examination of their gene contents which identified 20 predicted effectors. The number of QTL shown in this study at the population level clearly illustrates a complex genetic basis of *Ptt* virulence compared to pure necrotrophs such as the wheat pathogens *Parastagonospora nodorum* and *P. tritici-repentis*.

Keywords: *Pyrenophora teres* f. *teres*, genome-wide association mapping, QTL analysis, virulence, candidate genes

INTRODUCTION

Net blotch, caused by the fungal pathogen, *Pyrenophora teres*, was first recorded in Australia as an important foliar disease of barley (*Hordeum vulgare*) in the 1960s. In other countries, such as the United States of America, yield losses due to this disease were recorded as early as 1922 (McLean et al. 2009; Shipton 1966). Net blotch is now found in all barley producing regions of the world (Liu et al. 2011). In 1971, net blotch was classified as two forms, namely those having net-like symptoms *P. teres* f. *teres* (*Ptt*) and others having spot-like symptoms *P. teres* f. *maculata* (*Ptm*) (Smedegård-Petersen 1971). Smedegård-Petersen (1971) successfully produced progeny from crosses between *Ptt* and *Ptm* which confirmed the belief that the two pathogens are of different forms rather than two different species, although phylogenetic studies indicate they are discrete populations and natural field hybrids appear to be rare events (Ellwood et al. 2012; Poudel et al. 2017; Poudel et al. 2019b). *Pyrenophora teres* is a heterothallic haploid ascomycete, requiring two opposite mating genotypes for sexual reproduction (Rau et al. 2007). The fruiting bodies are pseudothecia which are produced on barley straw during sexual reproduction. They are dark brown globoid bodies with spiky setae. The pseudothecia contain club-shaped asci with eight ascospores (Liu et al.

2011). Under wet conditions ascospores can be distributed by rain splash or discharged into still air (Jordan 1981). Mature pseudothecia can cause primary infection of barley grown a number of years after an infected barley crop was produced (Jordan 1981; Liu et al. 2011; McLean et al. 2009; Piening 1961). Asexually produced conidia are a secondary source of inoculum and these are produced under favourable conditions throughout the growing season (Lai et al. 2007).

Sexual recombination can lead to changes in both disease expression in the host and also to fungicide sensitivity in the pathogen (Campbell et al. 1999; Jalli 2011). Changes in virulence can be devastating to the barley industry especially if a limited number of barley cultivars with common resistances are grown. In a recent study the composition of isolate groups determined by hierarchical clustering of barley genotypes and *Ptt* isolate phenotypes was found to be different between the eastern and western Australia, with all isolate groups detected in southern Australia (Fowler et al. 2017). This suggested that *Ptt* pathotypes had evolved regionally depending on the barley cultivars prevalent in a certain region. The authors identified four distinct isolate groups by phenotyping *Ptt* isolates across 31 different barley genotypes (Fowler et al. 2017). These isolate groups harboured differential virulence to the four barley genotypes Maritime, Prior, Skiff and Tallon. Isolates with virulences to any of these four genotypes made up 97% of the total 123 isolates tested. Knowledge of the genetic structure and range of genes involved in virulence is vital to researchers and breeders to increase the durability of *Ptt* resistance in barley cultivars.

Different approaches can be used to characterise genomic regions associated with virulence, these include association mapping and QTL analysis using bi-parental populations. The effective use of association mapping in fungi has been confirmed in recent fungal studies (Broberg et al. 2018; Castiblanco et al. 2017; Gao et al. 2016; Hartmann et al. 2017; Korinsak et al. 2019; Talas et al. 2016). Association mapping requires high-throughput

genotyping and sequencing methods to provide large numbers of markers. One such method, Diversity Arrays Technology sequence™ (DArTseq™) has been developed for a number of fungal pathogens including *P. teres* (Poudel et al. 2019a; Sharma et al. 2014; Syme et al. 2018; Wittenberg et al. 2009). DArTseq™ involves a combination of genomic complexity reduction methods and next generation sequencing where the markers detect single nucleotide polymorphisms (SNPs) and the presence/absence of fragments (Courtois et al. 2013; Cruz et al. 2013; Kilian et al. 2012; Raman et al. 2014).

The first report on the identification of genetic markers associated with virulence in a *Ptt*/*Ptt* population used a cross between isolates 0-1 and 15A (Weiland et al. 1999). Random amplified polymorphic DNA (RAPD) markers were used to identify five markers that were associated with the virulence phenotype. Lai et al. (2007) used the same population to identify a gene conferring avirulence to cultivars Tifang and Canadian Lake Shores and two avirulence genes on Prato. Six AFLPs located close to an avirulence gene to cultivar Heartland were identified by Beattie et al. (2007) who employed a bulked segregant analysis approach using 67 progeny from a *Ptt*/*Ptt* cross between isolates WRS 1906 and WRS 1607. Shjerve et al. (2014) used 118 progeny from a cross between isolates 15A and 6A to identify two major QTL associated with virulence on Kombar and Rika. Three progeny isolates harbouring different single virulence genes were screened across a Kombar/Rika barley population and the susceptibility induced by each of these isolates was mapped to the same region on chromosome 6H. This study demonstrated that isolate/host interactions can in part be explained by pathogen-produced necrotrophic effectors that interact with dominant susceptibility genes of barley resulting in necrotrophic effector triggered susceptibility. Recently avirulence/virulence gene regions were mapped in 109 progeny of a *Ptt*/*Ptt* cross using isolates BB25 from Denmark and FGOH04*Ptt*-21 from the USA as parents (Koladia et

al. 2017). Nine unique QTL, of which three had major effects, were identified on eight linkage groups.

To gain a better understanding of the number of virulence genes present in Australian *Ptt* isolates and to identify genomic regions associated with virulence we have used a genome-wide association mapping approach (GWAS) with DArTseq™ markers genotyped on 188 *Ptt* isolates collected across five different Australian states. We confirmed the major regions identified through GWAS by QTL mapping using two different *Ptt*/*Ptt* populations. To provide gene candidates for further functional studies, QTL regions were explored for predicted effector-like proteins (Syme et al. 2018) and secreted proteins known to be expressed *in planta* during net blotch disease of barley (Ismail and Able 2016, 2017). Knowledge of the virulence genes present in the population will provide barley breeding programs with valuable information for future breeding of *Ptt* resistant barley cultivars.

MATERIALS AND METHODS

Pyrenophora teres f. *teres* isolates

Two different types of populations were used to investigate the genetic basis underlying virulence in *Ptt*. A panel of isolates capturing the Australian diversity was used in genome-wide association analysis and bi-parental populations were used in QTL analysis. The panel consisted of 188 *Ptt* isolates, including 61 collected from Queensland, 30 from New South Wales, 49 and 11 from South Australia and Victoria, respectively and 37 from Western Australia (Supplementary Table 1). Single conidial cultures were obtained as indicated in Fowler et al. (2017).

The two bi-parental populations were produced similar to the method described in Smedegård-Petersen (1971). Isolate NB029 was separately crossed with HRS09122 and NB085. NB029 was originally sampled in Western Australia and is virulent on barley cultivar

Beecher and avirulent on Skiff and Prior, whereas HRS09122 was sampled in New South Wales and is virulent on Skiff and avirulent on Beecher and Prior. Isolate NB085 originated from Queensland and is virulent on Prior and avirulent on Beecher and Skiff. Plates for crossings were prepared by placing five to six 50 mm long autoclaved barley straw pieces on Sach's agar (Hebert, 1971) before the agar had set. Isolates were grown on Potato Dextrose Agar (PDA) to obtain enough mycelial growth for the crossing plates. An approximately 25 mm^2 mycelial plug from each of the two isolates was taken from the PDA plates and placed on opposite sides of the barley straw. Plates were placed into plastic bags to prevent desiccation of the media and incubated at 15°C with a 12-hour light/12-hour dark photoperiod. Plates were checked each week for the formation of mature pseudothecia. Once mature pseudothecia were observed, i.e. pseudothecia were forming a short cylindrical beak or neck, 1% water agar plates were placed on top of the crossing plate with the agar facing the pseudothecia. Plates were sealed with PARAFILM® M (Merck Pty Ltd) and returned to the incubator. Plates were checked each day for ascospores. Ascospores were obtained within three and two months of setting up the NB029/HRS09122 and NB029/NB085 population, respectively. Ascospores which had been ejected onto the water agar were removed with a sealed glass needle and single ascospores were transferred onto PDA plates. These were incubated at 22°C until enough fungal mycelium had been produced for DNA extraction. All ascospores were collected and DArTseq™ markers were used to identify identical ascospores. One ascospore from each identical pair was removed from further analyses to avoid clones from the same ascus. Thus of the 107 ascospores obtained for the NB029/HRS09122 population 78 were unique and used in subsequent analyses. Similarly, 72 of the 89 ascospores collected for the NB029/NB085 population were used in further analyses.

Phenotypic evaluation of association mapping isolates

Production of conidia for phenotypic assessment was conducted as described in Fowler et al. (2017). The 188 isolates used in association mapping were phenotyped at the seedling stage across twenty different barley genotypes (Algerian, Beecher, Buloke, CIho 5791, CIho 11458, Commander, Corvette, Fleet, Gilbert, Harbin, Harrington, Herta, Hindmarsh, Kombar, Maritime, Prior, Skiff, Tallon, Vlamingh and Yerong). These barley genotypes were chosen as they exhibited different resistance profiles (Fowler et al., 2017). The experimental design was similar to that used in Fowler et al. (2017) using a split-plot design with two replicates and randomised barley genotypes. Five seeds of each differential line were sown into a 10 cm pot at three evenly spaced positions around the circumference of the pot. Seedlings were grown in pots containing Searles® premium potting mix and fertilized twice a week after emergence with Grow Force Flowfeed soluble fertilizer at 1.5 grams per litre applied via a watering can. Seedlings were top watered until inoculation. Plants were grown and maintained in a growth room at 24/14°C day/night temperature and a 12-hour light/12-hour dark photoperiod as indicated in Fowler et al. (2017). Plants were inoculated 13 days after potting when most genotypes were at GS12 (Zadoks et al., 1974). Inoculations were conducted as indicated in Fowler et al. (2017) using a KC-625CG gravity feed spray gun. Post inoculation, plants were immediately transferred to a dew chamber and maintained at 99% relative humidity and 19°C under 14-hour dark and 10-hour light. Pots were then returned to the growth room and bottom watered until development of disease infection.

Phenotypic evaluation of *Pyrenophora teres* f. *teres* populations

Four different barley genotypes (Beecher, Skiff, Prior and Kombar) were used to examine virulences of individuals of the NB029/HRS09122 and NB029/NB085 cross. Variety Kombar was used as the susceptible control. Four seeds each of four different genotypes were

sown into one 15 cm diameter pot with clusters of seeds of each genotype evenly spaced around the circumference of the pot. Two replicates were grown for each isolate. Pots were placed in a glass house for 14 days prior to inoculation.

Conidia were produced by placing five plugs of a mycelial culture on the margins of sterilised sorghum or maize leaf segments on 1% water agar medium (Lightfoot and Able, 2010). Plates were incubated in the laboratory at approximately 22°C under natural daylight conditions for six days. They were then incubated at 15°C for another four days with a 12-hour light/12-hour dark photoperiod. To remove conidia, distilled water containing two drops of Tween®20/100 mL water was added to the fungal plate and the conidia dislodged with a fine paintbrush into a beaker. Conidia were counted using a haemocytometer and the conidial suspension adjusted to 10 000 conidia/mL. This inoculum suspension was applied at 2.5 mL/pot using a Preval sprayer (SEDL Agencies, Australia). Pots were randomly placed in an Aralab Dew Chamber (Bioline Australia) at 19°C for 24 hours in the dark. Pots were then transferred into a glass house with natural day/night light.

Disease assessment

The Tekauz (1985) 1-10 scale was used to rate the infection response of the second leaf of at least three plants per genotype nine days after inoculation. Disease ratings did not differ by more than two units across replications.

DArTseq™ analysis

Single-spore isolates were grown on PDA at 22°C for about one week until enough mycelial growth was available for DNA extraction. The DNA of the isolates and ascospores was extracted with a Wizard® Genomic DNA Purification kit following the protocol of the supplier (Promega Corporation, Australia). DNA of the 188 isolates for use in association

mapping, 107 progeny of the NB029/HRS09122 and 89 progeny of the NB029/NB085 population plus the parents of the populations were sent to Diversity Arrays Technology (DArT) Pty Ltd, Canberra (http://www.diversityarrays.com) for DArTseq™ analysis. DNA samples were processed in digestion/ligation reactions principally as described in Kilian et al. (2012) but replacing a single PstI-compatible adaptor with two different adaptors corresponding to two different Restriction overhangs. The PstI-compatible adapter was designed to include the Illumina flowcell attachment sequence, sequencing primer sequence and "staggered", varying length barcode regions, similar to the sequence reported by Elshire et al. (2011). Reverse adapters contained the flowcell attachment region and MseI-compatible overhang sequence. Only "mixed fragments" (PstI-MseI) were effectively amplified in 30 rounds of PCR using the following reaction conditions: 94°C for 1 min, 30 cycles of 94° C for 20 s, 58 °C for 30 s and 72 °C for 45 s and a final step of 72 °C for 7 min. After amplification, equimolar amounts of the products from each sample of the 96-well microtiter plate were bulked and applied to a c-Bot (Illumina) bridge PCR followed by sequencing on Illumina Hiseq2500 platform. The sequencing (single read) was run for 77 cycles. Sequences generated from each lane were processed using proprietary DArT analytical pipelines. In the primary pipeline the fastq files were first processed to filter away poor-quality sequences, applying more stringent selection criteria to the barcode region compared to the rest of the sequence so that the assignments of the sequences to specific samples carried in the "barcode split" step were very reliable. Approximately 2,000,000 (+/- 7%) sequences per barcode or sample were used in marker calling. Identical sequences were collapsed into "fastqcall files". These files were used in the secondary pipeline for DArT Pty Ltd's proprietary SNP and SilicoDArT (presence/absence of restriction fragments in representation) calling algorithms (DArTsoft14).

Only markers of known chromosomal location and minor allele frequency (MAF) > 0.01 were used in the population structure and GWAS analyses. Individual SNP markers with > 50% missing data were removed from further analysis.

Population structure analysis and linkage disequilibrium (LD) analysis

STRUCTURE version 2.3.4 (Pritchard et al., 2000) was used to infer the genetic structure and the number of clusters (K) in the dataset. Ten independent runs were performed for each value of K, K varying from one to six. The default settings of the program were used, i.e. admixture ancestry model, uncorrelated allele frequencies between populations and the degree of admixture alpha inferred from the data (Falush et al., 2007, Pritchard and Wen, 2003). Each run was set to a burn-in period of 10,000 iterations followed by 100,000 Monte Carlo Markov Chain (MCMC) iterations. The most likely number of clusters was chosen by computing Evanno's ΔK (Evanno et al., 2005) across multiple values of K through the web-based program STRUCTURE HARVESTER v.0.6.94 (Earl and Vonholdt, 2012). CLUMPP version 1.1.1 (Jakobsson and Rosenberg, 2007) was used to permute one output from the ten independent cluster outputs produced by STRUCTURE and the graph was constructed in Microsoft Excel 2013. Pairwise LD (r2) was calculated using TASSEL version 5.2.58 with MAF > 0.01. The LD decay graph was drawn by fitting a smooth spline of average r2 over physical distance in R version 3.3.1.

Genome-wide association analysis

Genome wide association analysis (GWAS) was performed using the mixed linear model (MLM) in TASSEL version 5.2.59 (Bradbury et al., 2007). Q-matrix and K-matrix were calculated using built-in functions in TASSEL version 5.2.59 to control population structure and genetic relatedness, respectively. Thresholds for defining significant associations were

set using the Bonferroni correction. This P-value (P) was used to estimate the significant LOD score by using the equation LOD = -LOG10(P). Manhattan plots were drawn using the Memory-efficient, Visualization-enhanced, and Parallel-accelerated Tool (MVP) in R version 3.6.1 (https://github.com/xiaolei-lab/rMVP).

QTL analysis

Genetic linkage maps were produced for populations NB029/HRS09122 and NB029/NB085. MapManager QTXb20 (Manly et al., 2001) was used to partition DArTseq ™ markers into linkage groups and RECORD (Van Os et al., 2005) was used to order markers within linkage groups. The Kosambi function was used to calculate map distances. Map figures were produced with MapChart version 2.1 (Voorrips, 2002). Composite interval mapping was conducted using Windows QTL Cartographer version 2.5 (Wang et al., 2007). A permutation test run 1000 times at a significance level of 0.05 was used to determine the LOD threshold.

***Pyrenophora teres* f. *teres* net form net blotch QTL gene analysis**

The *Ptt* isolate W1-1 genome (Syme et al., 2018) was used as the reference throughout this analysis. The genomic positions of the association mapping markers from the QTL peaks were identified in W1-1 and based on locus disequilibrium calculations 20 kb either side of the markers were searched for candidate effector genes (Supplementary Table 2). A flanking region of 40 kb was estimated to be the same QTL/GWAS region.

W1-1 predicted gene annotation that overlapped QTL regions was then identified using bedtools intersect v2.26.0 (Quinlan, 2014) and potential biosynthetic gene clusters identified using antiSmash fungal version 5.0.0 (Blin et al., 2019) with options for KnownClusterBlast, ActiveSiteFinder, ClusterBlast, Cluster Pfam analysis, and SubClusterBlast. In addition,

secreted proteins during net blotch disease of barley (Ismail and Able, 2017, Ismail and Able, 2016) were retrieved for predicted effector gene support.

Other available *Ptt* isolates NB029, NB073, NB085, HRS09122, and HRS09139 (Syme et al., 2018) that show specific virulence on Beecher, Shepherd, Prior, Skiff and Fleet barley cultivars respectively, were selected to identify genomic regions of presence/absence for the 14 QTL regions. High quality PacBio genome assembly sequences for *Ptt* isolates NB029, NB085, NB073, HRS09122, and HRS09139 were aligned to W1-1 using MUMmer NUCmer version 3.1 (Delcher et al., 2003) and W1-1 genome coordinates were used to identify regions of presence and absence for each isolate. All W1-1 genes, isolate alignments and BGC regions were then visualised with the Integrated Genome Viewer (IGV) version 2.5.0 (Thorvaldsdottir et al., 2013). The genomes for NB029 (WJSO00000000), NB073 (WJSN00000000), NB085 (WJSM00000000), HRS09122 (WJSL00000000) and HRS09139 (WJSK00000000) are available under NCBI BioProject PRJNA577527 and W1-1 (OCTH00000000) is available under PRJEB18107.

RESULTS

DArTseq™ genotyping and LD decay

Genotyping by DArTseq™ analysis of the 188 *Ptt* isolates used in population structure and association mapping analysis provided 14,286 SilicoDArT (dominant markers) and 6,662 SNP markers. Individual SNP markers with >50% missing data were removed from further analysis. This resulted in 2,277 SilicoDArT and 1,113 SNP markers or 3,390 markers in total used in the analyses. The distribution and density of markers present on each of the 12 *Ptt* chromosomes is indicated in Supplementary Figure 1. Rapid LD decay was observed in this population to within half of the maximum at approximately 20 kb and to background level at 125 kb (Figure 1).

Population structure

Model-based structuring analysis of the *Ptt* isolates using the 3,390 markers in STRUCTURE indicated that the most likely number of clusters was K = 2 (Figure 2; Supplementary Table 3) based on the rate of variation in likelihood values between successive K values (Evanno et al. 2005). Isolates were assigned into the specific groups based on the highest percentage of membership with isolates having less than 70% similarity considered to be of mixed origin. Eighty and 89 isolates clustered within groups 1 and 2 respectively and 19 isolates were considered to be of mixed origin (Figure 3a, Supplementary Table 4). Of the isolates used in the bi-parental QTL mapping populations, HRS09122 was placed in group 1 and isolates NB029 and NB085 were placed in group2. No geographical patterns were observed within the groups with isolates from the five Australian states occurring in similar numbers within both groups.

Phenotypic assessments of *Ptt* isolates

Phenotyping for the GWAS analysis involved the assessment of the *Ptt* isolates across 20 different genotypes (Supplementary Table 1, Supplementary Figure 2). Isolates HRS15059b, NB270 and HRS18021a had the highest average infection response (≥ 7) across all cultivars. A high proportion of isolates were virulent on barley genotypes Commander, Gilbert and Harrington with average infection responses greater than 7. Genotype CIho 5791 was resistant to all 188 isolates with an average infection response of 1.2 and CIho 11458 and Vlamingh were resistant to most of the isolates tested with an average infection response of 2.8. For some barley genotypes a clear difference in infection responses was observed between the clusters determined by STRUCTURE analysis, i.e. isolates virulent on Beecher,

Maritime and Prior clustered mainly in group 1 and isolates virulent on Skiff and Herta clustered mainly in group 2 (Figure 3b).

Infection responses of the 78 ascospores of the NB029/HRS09122 population ranged from avirulent to virulent on both Beecher and Skiff (Figure 4) with an average infection response of 4.603 (standard error = 0.227) and 6.026 (standard error = 0.278) for Beecher and Skiff, respectively. Eight individuals were virulent (infection responses ≥7) on both Beecher and Skiff. The 72 ascospores of the NB029/NB085 population screened across barley genotypes Beecher and Prior had infection responses ranging from avirulent scores of 1 to virulent scores of 10 with most of the isolates having intermediate or virulent infection responses on Prior (Figure 5). An average infection response of 6.127 (standard error = 0.321) and 7.556 (standard error = 0.269) was observed for Beecher and Prior, respectively. Twenty-five individuals of the NB029/NB085 population were virulent (infection responses ≥7) on both Beecher and Prior.

Genome-wide association analysis of virulence genes

Genome-wide association analysis was conducted using the infection response of 188 *Ptt* isolates on 20 different barley genotypes to identify genomic regions associated with virulence. For a marker to be significantly associated with a trait, a Bonferroni significance threshold of greater than 4.8 LOD (P = 1.475E-5) was used. Thirty-nine significant marker-trait associations were identified for 13 of the 20 barley genotypes (Table 1, Figure 6). Markers associated with *Ptt* infections were further clustered into QTL using a window of 20 kb based on an approximate LD decay to half the maximum, resulting in 14 different QTL regions. Five different QTL were identified for Beecher, with three of these located on chromosome 3 (QTL3, QTL4 and QTL7) and one each on chromosome 1 (QTL1) and 9 (QTL14) with the proportion of phenotypic variances explained between 10.2 and 23.6%.

Two of the QTL on chromosome 3 (QTL3 and QTL4) was also observed with Maritime and explained between 12.7 and 18.7% of the phenotypic variance. Four more QTL were identified at different positions on chromosome 3 (QTL2, QTL5, QTL6 and QTL8) two of these were present with the genotypes Herta and Skiff. Four different QTL locations were identified on chromosome 5, two of these were identified with genotypes Fleet and Yerong (QTL9 and 10) and explained between 17.6 and 23.0% of the phenotypic variance. Harbin and Prior virulences were also associated with chromosome 5 (QTL11 and QTL12). A QTL on chromosomes 6 (QTL13) explaining 10.9% of the phenotypic variance was associated with the Tallon virulence. The locations of the 14 association mapping QTL on the W1-1 chromosomes are summarised in Supplementary Figure 3.

Linkage mapping and bi-parental QTL analysis

Over 1800 SilicoDArT and SNP markers were obtained for the bi-parental *Ptt* populations through DArTseq™ analysis. Of these 733 and 524 high quality markers for populations NB029/HRS09122 and NB029/NB085, respectively were polymorphic and were allocated to linkage groups. The genetic map of population NB029/HRS09122 consisted of 12 linkage groups ranging in size from 89.49 to 290.85 cM with a total map distance of 1998 cM (Supplementary Figure 4). Chromosomes are numbered relative to the reference genome *Ptt* W1-1 (Syme et al. 2018). Population NB029/NB085 also had a genetic map consisting of 12 linkage groups ranging in size from 49.29 to 227.39 cM and a total map distance of 1548 cM (Supplementary Figure 5). The average distance between markers was 2.73 and 2.95 cM for the NB029/HRS09122 and NB029/NB085 map, respectively. Three hundred and forty-eight and 255 markers were non-redundant in the NB029/HRS09122 and NB029/NB085 population respectively and were used in the QTL analysis. Based on the size of the *Ptt* W1-1 genome estimated at 49 Mb (Syme et al. 2018) the physical to genetic distance ratio for the

NB029/HRS09122 and NB029/NB085 populations is approximately 25 and 32 kb/cM respectively. Most of the markers in common between the populations were in the same order on both maps (Supplementary Figure 6).

A LOD value threshold of 3 was used to identify significant QTL after performing 1000 permutations on each of the data sets. Four significant QTL were identified in each population. In the NB029/HRS09122 population, a major virulence QTL on chromosome 3 was identified against barley cultivar Skiff explaining 24% of the phenotypic variation (LOD = 6.6, Table 2). This QTL was in the same position as QTL2 identified with the Skiff and Herta phenotypes in the GWAS analysis (Table 1). A major QTL was also identified on chromosome 3 on Beecher in the NB029/NB085 population explaining 36% of the phenotypic variation (LOD = 12.0, Table 2). This QTL was in a different location to the one identified on Skiff but was in the same location as the GWAS QTL3 identified with the Beecher, Maritime and Kombar-virulent phenotype. Three minor QTL were identified in the NB029/HRS09122 population with two located on chromosome 5 and one on chromosome 9 (Table 2). The chromosome 5 QTL were in a similar location to QTL10 and QTL11 identified in the GWAS analysis, however they were identified with different virulence phenotypes, against Fleet and Yerong for QTL10 and Harbin and Prior for QTL11. Minor QTL identified in the NB029/NB085 population were located on chromosomes 7 and 8 (Table 2).

Analysis of QTL regions to identify candidate effector genes

The association mapping QTL regions in W1-1 were investigated for predicted effector genes, secondary metabolite biosynthetic gene clusters, GC content and presence/absence in a number of *Ptt* isolates with different barley cultivar virulence, to identify candidate genes. While most QTL were in GC-equilibrated regions, chromosome 5 QTL 10 and 11, chromosome 6 QTL 13

and chromosome 9 QTL14 were clearly found in the AT-rich regions of the genome with sparse gene content (Supplementary Figure 7).

The gene content in the 14 QTL regions identified from the association mapping was then examined against the reference isolate W1-1 and a total of 85 genes were present (Supplementary Table 2). Of these, 20 were predicted effectors according to EffectorP (Sperschneider et al. 2018; Sperschneider et al. 2016), with a score greater than 0.5 (Table 1). A single predicted effector (*Ptt*_W11.g06727) was also identified as a known secreted protein associated with virulence, G7 (XP_003297558.1) (Ismail and Able 2017) within QTL11 on chromosome 5 (Table 3). Of the 20 predicted effectors, 12 were hypothetical proteins and lacked any known protein domains. Only four predicted effector genes had known protein domains annotated by the protein family database, Pfam (Finn et al. 2016). These domains were related to an unknown function DUF1168(PF06658)(Ptt_W11.g00010); a membrane translocon-associated protein (TRAP) (PF03896)(Ptt_W11.g03157); an ORMDL family protein believed to be involved in protein folding in the endoplasmic reticulum (PF04061) (Ptt_W11.g06660) and a glycosyl transferase, family 28 C-terminal domain (PF04101)(Ptt_W11.g06663)(Supplementary Table 2).

For the W1-1 genome, a total of 57 biosynthetic gene clusters were identified and, although many biosynthetic gene clusters flanked the QTL regions, only two overlapped QTL2 and QTL4 by a few genes on chromosome 3. These two gene clusters were represented by a single hybrid T1PKS-NRPS (polyketide synthase - non-ribosomal peptide synthetase) cluster and a NRPS, respectively (Supplementary Figure 7).

To identify QTL regions containing potential effector genes with virulence specificity to particular barley cultivars, the genomes of six isolates were aligned and summarised against the QTL regions in W1-1 (Supplementary Table 5 and Supplementary Figure 7), a virulent

isolate on barley cultivar Beecher and located close to NB029 in the model-based structuring analysis (Supplementary Table 4). A single QTL region (QTL3) on chromosome 3 was conserved in the two Beecher infecting isolates (W1-1 and NB029) and lacked sequence conservation for the remaining isolates, which have virulences to different barley cultivars. This region contained a single small predicted effector protein (55 aa) identified in W1-1 (*Ptt*_W11.g03125) that had 100% sequence identity in NB029 and no homology in the remaining genomes (NB73, HRS9122, HRS9139 and NB085).

DISCUSSION

Barley is grown across a distance of over 3000 km in Australia. To gain an overview of the composition of virulence genes present in the *Ptt* population, a combination of genetic structuring, association mapping and bi-parental mapping was undertaken. Model-based genetic structuring analysis of 188 *Ptt* isolates indicated that the most likely number of clusters is two. This is the same number of K groups as identified by Ellwood et al. (2019), using isolates from WA only. An association between the clusters and some of the phenotypic assessments of the isolates was observed with most of the isolates virulent on Beecher, Maritime and Prior clustering in one group and those showing greater infection rates on Herta and Skiff clustering in group 2. By producing a hierarchical cluster dendrogram of 31 barley genotypes using phenotypic data from 123 *Ptt* isolates Fowler et al. (2017) identified seven groups of barley genotypes that responded similarly, called line groups (LGs). Interestingly Herta and Skiff were also closely clustered in the same line group (LG2) whereas Beecher, Maritime and Prior were clustered in LG6 and LG7 which showed similar infection responses. Although some previous studies have investigated possible correlations between distinct populations and virulence spectra none found a clear association between phenotypes and molecular clusters (Lehmensiek et al. 2010; Liu et al. 2012). Liu et al.

(2012) identified 40 genotypes in a North Dakota *Ptt* population using microsatellite markers and suggested that this was similar to the number of identified pathotypes (49), based on a differential set of 22 barley lines. They found that isolates with a high level of genetic similarity also had similar virulence patterns. Liu et al. (2012) explained isolates which were genotypically similar but having phenotypic differences may result from single genetic mutations that lead to changes in virulence without affecting the neighbouring microsatellite markers or that the markers were not closely linked to genes controlling virulence. Our study indicated an association between some virulences and genotypes suggesting that DArTseq™ markers may be more useful in genetic diversity studies.

Association mapping in this study provided an approximate LD half-decay value of 20 kb for Australian *Ptt* isolates. This value seems to vary in ascomycetes with a rapid decay of less than 0.1 kb in some species such as *Schizophyllum commune* and up to 162 kb in *Candida albicans* (Nieuwenhuis and James 2016). The relatively low LD value of the Australian *Ptt* isolates suggests frequent sexual reproduction (Nieuwenhuis and James 2016).

Marker associations using GWAS analysis were observed for 13 of the 20 barley genotypes. Genotypes without any marker associations were mainly those with a poor distribution of infection responses either being mostly resistant (CIho 5791 and Vlamingh; Supplementary Figure 2) or mostly susceptible (Gilbert, Harrington and Hindmarsh). However, GWAS analysis across 13 genotypes allowed 14 different QTL regions to be identified and these were mainly located on chromosomes 3 and 5. The number of virulence QTL in *Ptt* corresponds to the complexity of the host resistance genetics (Liu et al. 2011; Richards et al. 2017) and an expanded genome with evidence of relatively rapid transposon-driven changes (Dong et al. 2015; Syme et al. 2018).

Interestingly, isolates virulent on Herta and Skiff which clustered within one group of the STRUCTURE analysis also showed association with the same markers on chromosome 3 in

the GWAS analysis. Similarly, isolates virulent on Beecher and Maritime, which were clustered within the other group identified by STRUCTURE analysis, also had QTL in common on chromosome 3. It is known that some isolates with virulence to Beecher also show virulence to Maritime (Fowler et al. 2017), including the isolate NB029 used in our bi-parental mapping populations. This suggests that the QTL identified on chromosome 3 are the ones responsible for both the Beecher and Maritime virulences. In Australia 97% of the isolates of the *Ptt* population are virulent on either Maritime, Prior, Skiff or Tallon (Fowler et al. 2017). We identified different genomic regions significantly associated with all of these virulences.

We collected as many ejected ascospores as we could from the two *Ptt* populations used in this study as opposed to collecting single ascospores from groups of ascospores that avoids clones originating from the same ascus (Shjerve et al. 2014), as after meiosis four pairs of identical ascospores are present in an ascus (Fincham 1971). DArTseq™ markers were used to identify genetically identical individuals. Marker data indicated that 29 out of 107 (27%) ascospores sampled for the NB029/HRS09122 population and 17 out of 89 (19%) of the NB029/NB085 population were genetically identical. Thus, collecting all of the ascospores resulted in a higher number of useful individuals per population.

Isolate NB029 which was used as one of the parents in both populations is virulent on barley variety Beecher, whereas the other parental isolates, HRS09122 and NB085 are virulent on Skiff and Prior, respectively. Transgressive segregation for both populations was observed when phenotyping individual ascospores across the genotypes on which the parental isolates were virulent. This suggested the involvement of more than one gene and that progeny possessed different gene combinations to the parental isolates. Similar to our results Weiland et al. (1999) found that many of the progeny produce infection responses different from those of the parents in a cross between *Ptt* isolate 0-1 possessing high virulence on barley cultivar

Harbin and *Ptt* 15A possessing low virulence on Harbin. Transgressive segregation was also observed in a cross between isolate BB25 from Denmark and FGOH04*Ptt*-21 from North Dakota, USA indicating that these isolates had different sets of virulence and/or avirulence genes (Koladia et al. 2017).

Genetic maps constructed of the two *Ptt* populations consisted of 12 chromosomes which is in agreement with the findings of Syme et al. (2018) who constructed comprehensive genome assemblies of *Ptt*. The total map distance for the two populations used in our study were 1548 and 1998 cM. These map distance are similar to the total map size of 1906 cM reported by Koladia et al. (2017) for the *Ptt* population BB25/FGOHo4*Ptt*-21. Their largest linkage group was 231 cM in length compared to 290.85 cM and 227.39 cM in this study.

Two major QTL were identified on chromosome 3 in both populations NB029/HRS09122 and NB029/NB085, albeit in different locations. The QTL associated with phenotyping on Skiff in the NB029/HRS09122 population was contributed by HRS09122 and NB029 contributed the virulence allele on Beecher in the NB029/NB085 population. The Beecher QTL was also associated with Beecher and Maritime virulence using GWAS. This QTL was in a similar location to a virulence locus VK1 reported by Shjerve et al. (2014). They are separate, however, with a distance of approximately 58 kb between their flanking markers (Friesen Timothy, USDA-ARS, personal communication). In a recent study *Ptt* QTL explaining up to 56% of the phenotypic variation were identified using cultivar Beecher on linkage groups 1.1 and 2.1 (chromosomes were numbered in that study to correspond with *P. tritici-repentis* chromosomes numbers) with the virulence allele contributed by a North Dakota, USA isolate FGOH04*Ptt*-21 (Koladia et al. 2017). Neither of these QTL seem to be in the same region as the Beecher QTL identified in our study, suggesting QTL governing the same cultivar virulence may not be conserved between geographically distant isolates.

AT-rich regions in filamentous fungal pathogens are considered to be important in generating pathogenesis effectors (Dong et al. 2015; Raffaele and Kamoun 2012; Wyatt et al. 2019). Sequence analysis of the QTL regions indicated that the majority of chromosome 5 and the chromosome 9 QTL were in or flanked by AT-rich regions, but the chromosome 3 QTL were in GC equilibrated genomic regions. Thus, *Ptt* presents a more complex scenario in which such regions may be a result of recent transposition events since they typically include MITEs and LTRs (unpublished data). Chromosome 3 QTL also appeared co-located with a region of biosynthetic gene cluster expansion (Syme et al. 2018) as compared to *P. teres* f. *maculata* and *P. tritici-repentis*, although these only partly overlapped the QTL. Closer examination of the *Ptt* isolate W1-1 QTL regions and the genomes of other *Ptt* isolates from Syme et al. (2018) identified a genomic region that was specific to Beecher virulent isolates W1-1 and NB029 on chromosome 3 (QTL3). This region may therefore contain possible candidates for Beecher specific virulence, one candidate for which is a predicted effector gene.

In conclusion, this is the first GWAS study undertaken to identify loci associated with virulence in *P. teres*. The confirmation of some of these loci by bi-parental QTL mapping demonstrates the effective use of this approach with this pathogen. The identification of predicted pathogenicity effector genes in the QTL regions and a potential role of NRPS biosynthetic clusters flanking QTL on chromosome 3 both require further characterisation. The number of genomic regions found to be associated with different virulences among Australian pathotypes demonstrates the need for phenotyping and breeding strategies to introgress multiple resistance genes into future barley cultivars.

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Supporting Information legends

Supplementary Table 1. Details of isolates used in genome-wide association mapping and infection response of each isolates across different genotypes. NSW = New South Wales, Qld = Queensland, SA = South Australia, VIC = Victoria and WA = Western Australia

Supplementary Table 2. *Pyrenophora teres* f. *teres* isolate W1-1 QTL gene content. *Ptt* W1-1 association mapping QTL chromosome gene position and annotations are tabled with predicted effectors, secretome and biosynthetic gene clusters.

Supplementary Table 3. Mean log (ln) probability values (L(K) estimated over 10 independent runs for each value of K).

Supplementary Table 4. Details of estimates of population structure of 188 *Pyrenophora teres* f. *teres* isolates.

Supplementary Table 5. Alignment summary of barley virulent *Pyrenophora teres* f. *teres* isolate genomes to major QTL regions for isolate W1-1. Barley virulent *Pyrenophora teres* f. *teres* isolates (labelled *Ptt* isolate-barley cultivar) conserved with W1-1 association mapping QTL are highlighted in grey, while QTL regions specific to Beecher virulent isolates (W1-1 and NB029) are highlighted in orange.

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

Supplementary Figure 2. Distribution of infection responses of 188 *Pyrenophora teres* f. *teres* isolates across 20 different barley genotypes

Supplementary Figure 3. *Pyrenophora teres* f. *teres* isolate W1-1 chromosome overview of QTL regions. Chromosomes 1, 3, 5, 6 and 9 are shown with histograms of gene counts in a 10 kb window (red histogram), QTL regions (black) on the left of each chromosome, and biosynthetic gene clusters on the right of each chromosome.

Supplementary Figure 4. Genetic map of population NB029/HRS09122

Supplementary Figure 5. Genetic map of population NB029/NB085

Supplementary Figure 6. Comparison between marker positions of genetic maps of population NB029/HRS09122 and NB029/NB085. Markers present in both genetic maps are indicated in red.

Supplementary Figure 7. *Pyrenophora teres* f. *teres* isolate W1-1 overview of QTL, genes and isolate genome alignments for chromosomes 1, 3, 5, 6 and 9. Panels A-E represent the chromosomes of *Ptt* W1-1 with association mapping QTL for chromosomes 1, 3, 5, 6 and 9 respectively. The tracks for each panel are in order from top to bottom, W1-1 chromosome length, percent GC bar plot in 10 kb window, Ptt isolate genome alignments for NB029 Beecher virulent (yellow), NB073, HRS09122, HRS09139, NB085 association mapping QTL regions (red), predicted biosynthetic gene clusters (green), predicted effector genes for EffectorP version 1 and version 2, and the gene annotations for W1-1.

Table legends

Table 1. Genome-wide association mapping results indicating markers significantly associated with virulence in *Pyrenophora teres* f. *teres*

Table 2. Summary of quantitative trait loci identified in *Pyrenophora teres* f. *teres* populations NB029/HRS09122 and NB029/NB085

Table 3. *Pyrenophora teres* f. *teres* isolate W1-1 summary of predicted effector genes associated with QTL genomic regions.

Figure legends

Figure 1. Linkage disequilibrium decay based on 3390 DArT marker positions located on 12 chromosomes of *Pyrenophora teres* f. *teres*.

Figure 2. STRUCTURE analysis of 188 *Pyrenophora teres* f. *teres* isolates indicating estimated delta K values for ten independent runs from K1 to K6.

Figure 3. (a) Estimates population structure of 188 *Pyrenophora teres* f. *teres* isolates. Each individual isolate is represented by a bar, group 1 is indicated in orange and group 2 in blue, isolates of mixed origin are white. (b) Infection response scores (1 = resistant to 10 = susceptible) of the isolates on five different genotypes, Beecher, Maritime, Prior, Herta and Skiff. Individuals have been ordered according to the order on the population structure bar plot.

Figure 4. Infection responses of 78 individuals of the NB029/HRS09122 population on barley genotypes Beecher (a) and Skiff (b). Infection responses of the parental isolates NB029 and HRS09122 are indicated.

Figure 5. Infection responses of 72 individuals of the NB029/NB085 population on barley genotypes Beecher (a) and Prior (b). Infection responses of the parental isolates NB029 and NB085 are indicated.

Figure 6. Manhattan plots of the genome-wide analysis conducted to identify markers associated with virulence in *Pyrenophora teres* f. *teres*. The Bonferroni significance threshold is indicated by the solid red line.

Table 1. Genome-wide association mapping results indicating markers significantly associated with virulence in *Pyrenophora teres* f. *teres*

QTL-ID	Trait	Marker	[a]Chr	[a]Position (bp)	LOD score	[b]PVE	[c]Bi-parental mapping
1	Beecher	28947393	1	679123	6.07	14.58	
2	Fleet	41806492|F|0-7:A>G-7:A>G	3	102867	5.02	18.93	Skiff, NB029/HRS09122
2	Herta	28949576	3	114611	6.70	16.33	Skiff, NB029/HRS09122
2	Skiff	28949576	3	114611	6.32	15.15	Skiff, NB029/HRS09122
3	Beecher	28946135	3	788584	8.94	21.62	Beecher, NB029/NB085
3	Beecher	41805542	3	795925	9.15	21.92	Beecher, NB029/NB085
3	Kombar	41805542	3	795925	5.26	12.07	Beecher, NB029/NB085
3	Maritime	41805542	3	795925	6.83	16.38	Beecher, NB029/NB085
3	Beecher	28945535	3	796216	7.60	17.50	Beecher, NB029/NB085
3	Beecher	100130307	3	796281	9.77	23.58	Beecher, NB029/NB085
3	Kombar	100130307	3	796281	5.54	12.97	Beecher, NB029/NB085
3	Maritime	100130307	3	796281	7.47	18.65	Beecher, NB029/NB085
4	Beecher	36352701	3	1028769	7.46	17.48	
4	Maritime	36352701	3	1028769	5.34	12.71	
5	CIho11458	100133310	3	1480380	5.41	12.46	
6	Herta	28945761	3	1905727	6.95	16.93	
6	Skiff	28945761	3	1905727	6.71	16.12	
7	Beecher	28949829|F|0-28:C>T-28:C>T	3	3985143	5.15	11.03	
8	Commander	28945998	3	5556417	5.05	12.69	
9	Fleet	28948035	5	4110850	8.14	21.47	
9	Yerong	28948035	5	4110850	7.59	18.72	
10	Fleet	36347132	5	4456299	8.89	22.98	Skiff, NB029/HRS09122
10	Yerong	36347132	5	4456299	7.27	17.55	Skiff, NB029/HRS09122
11	Harbin	28950148|F|0-5:A>C-5:A>C	5	5195933	7.59	21.31	Beecher, NB029/HRS09122
11	Prior	28950148|F|0-5:A>C-5:A>C	5	5195933	5.11	13.50	Beecher, NB029/HRS09122
11	Harbin	36347644	5	5196299	8.39	21.10	Beecher, NB029/HRS09122
11	Prior	36347644	5	5196299	6.08	13.98	Beecher, NB029/HRS09122
11	Harbin	28945265	5	5196364	8.51	21.51	Beecher, NB029/HRS09122
11	Prior	28945265	5	5196364	5.78	13.10	Beecher, NB029/HRS09122
11	Harbin	28949624	5	5205990	8.55	21.66	Beecher, NB029/HRS09122
11	Prior	28949624	5	5205990	5.74	13.15	Beecher, NB029/HRS09122
11	Harbin	36350488	5	5209123	7.66	20.65	Beecher, NB029/HRS09122
11	Prior	36350488	5	5209123	5.48	12.72	Beecher, NB029/HRS09122
12	Corvette	36352140|F|0-22:T>C-22:T>C	5	5243799	4.79	14.19	
12	Harbin	36352141	5	5243799	8.34	21.61	
12	Harbin	36352140|F|0-22:T>C-22:T>C	5	5243799	5.71	19.00	
12	Prior	36352141	5	5243799	5.58	12.95	
13	Tallon	100139818	6	410017	5.04	10.90	
14	Beecher	28949038	9	839959	4.82	10.19	

[a]Chromosome on which marker is located on and position according to W1-1 reference genome (Syme *et al*. 2018)
[b]Percentage of phenotypic variance explained
[c]Indicates QTL that were also identified in bi-parental mapping populations, showing trait associated with virulence QTL and population

Table 2. Summary of quantitative trait loci identified in *Pyrenophora teres* f. *teres* populations NB029/HRS09122 and NB029/NB085

Chr no	Position of peak of QTL (cM)	[a]Genotype	LOD score	[b]R^2	Parent contributing the QTL	[c]Position on W1-1 reference genome
NB29/HRS09122						
3	258	Skiff	6.6	24	HRS09122	114611
5	11	Beecher	4.0	15	NB029	5183980-5208563
5	39	Skiff	4.8	19	HRS09122	3980200-4457075
9	39	Beecher	3.0	11	NB029	1073073-1122817
NB29/NB85						
3	21	Beecher	12.0	36	NB029	796216-970812
7	49	Beecher	3.9	11	NB085	2166986-2928737
7	49	Prior	3.6	18	NB085	2166986-2928737
8	22	Beecher	3.0	7	NB029	1883966-1972858

[a]Genotype on which QTL was identified

[b] Percentage of phenotypic variation explained

[c] Position of QTL on W1-1 reference genome (Syme et al. 2018)

Table 3. *Pyrenophora teres* f. *teres* isolate W1-1 summary of predicted effector genes associated with QTL regions

W1-1 chromosome	QTL-ID	QTL start	QTL end	Number of predicted effectors	Biosynthetic cluster type	Secreted proteins
1	QTL1	659123	699123	3	-	-
3	QTL2	94611	134611	2	-	
3	QTL3	768584	816281	1	-	
3	QTL4	1008769	1048769	2	NRPS	
3	QTL5	1460380	1500380	1	NRPS	
3	QTL6	1885727	1925727	2	-	
3	QTL7	3965143	4005143	2		
3	QTL8	5536417	5576417	2		
5	QTL9	4090850	4130850	3		
5	QTL10	4436299	4476299	0	-	
5	QTL11	5175933	5229123	2	-	G7[ab]
5	QTL12	5223799	5263799	0	-	
6	QTL13	390017	430017	0	-	
9	QTL14	819959	859959	0	-	

[a] Secreted proteins identified by Ismail and Able (2016), [b]Virulent RNA expression for *Ptt* proteins (Ismail and Able, 2017)

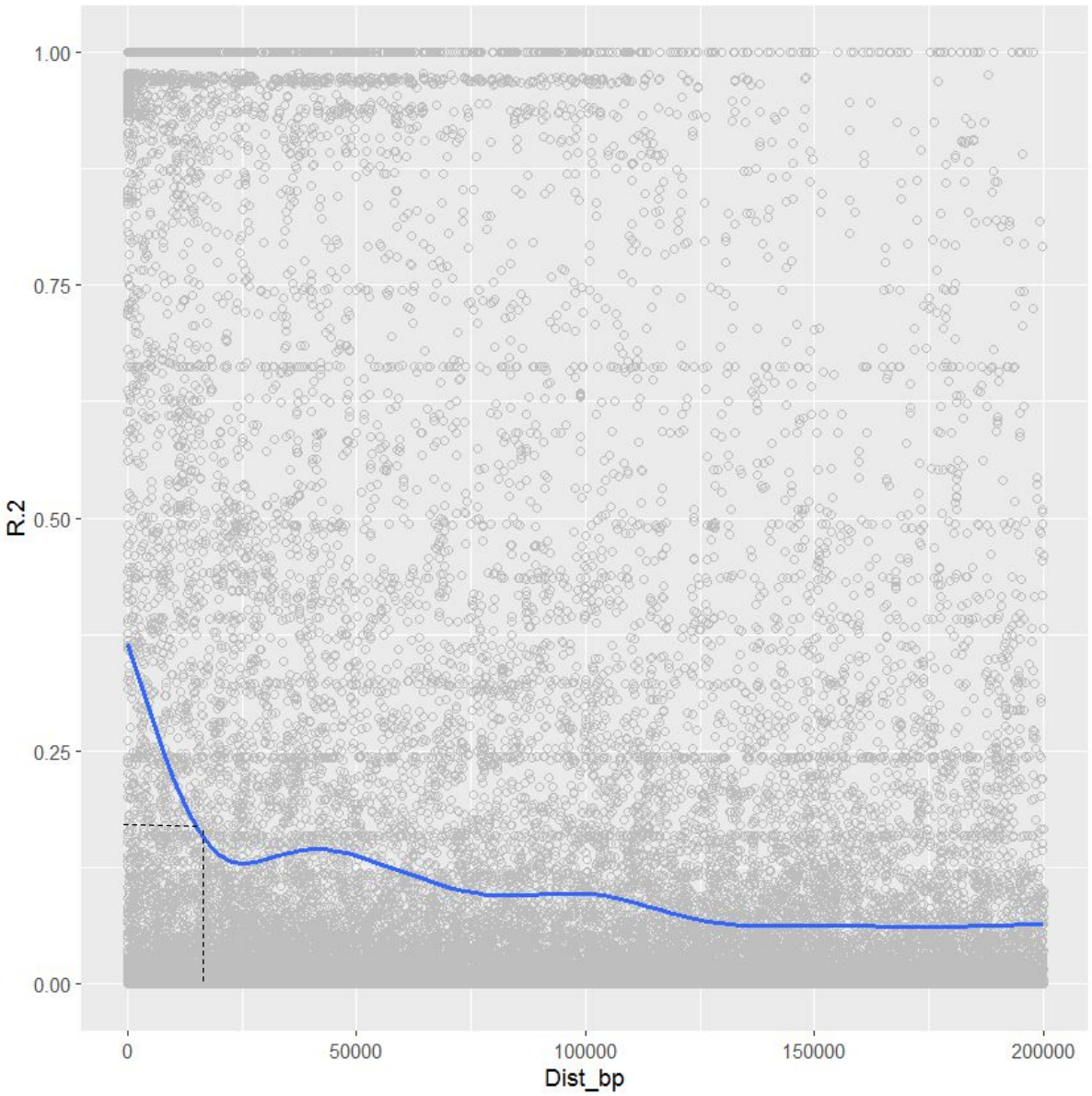


Figure 1. Linkage disequilibrium decay based on 3390 DArTseq™ marker positions located on 12 chromosomes of *Pyrenophora teres* f. *teres*. Arrow indicates half decay position.

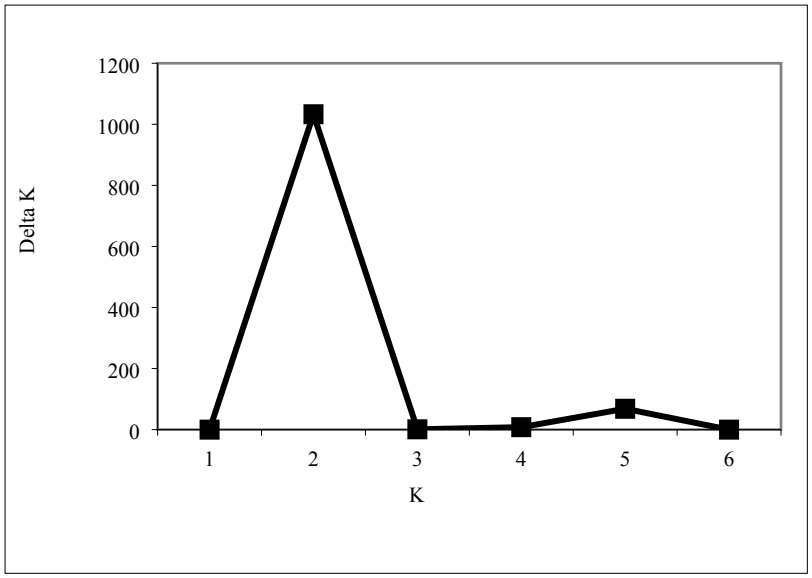


Figure 2. STRUCTURE analysis of 187 *Pyrenophora teres* f. *teres* isolates indicating estimated delta K values for ten independent runs from K1 to K6

a

b

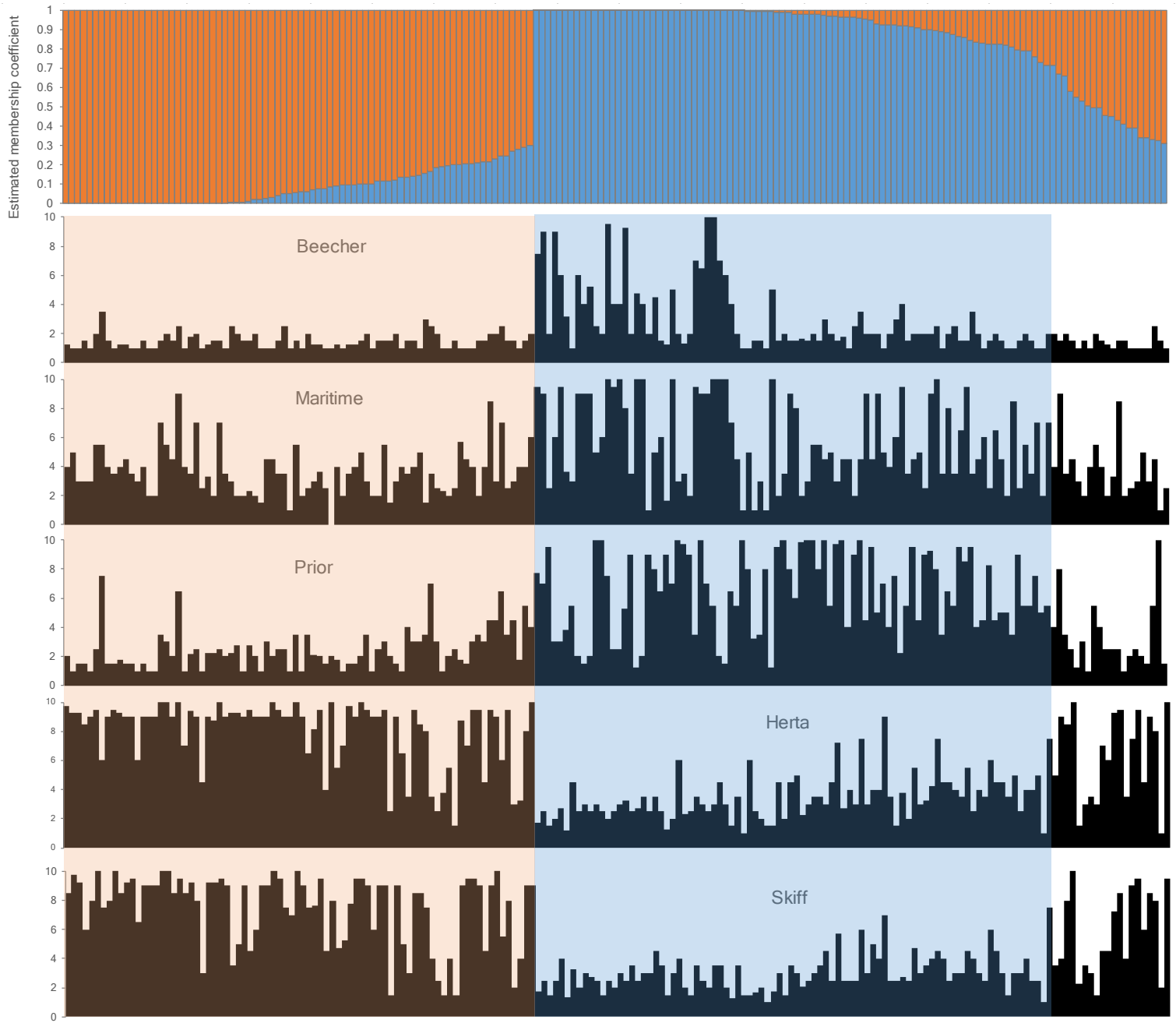

Estimated membership coefficient
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Beecher
Maritime
Prior
Herta
Skiff
10
8
6
4
2
0

Figure 3. (a) Estimates of population structure of 187 *Pyrenophora teres* f. *teres* isolates. Each isolate is represented by a bar, group 1 is indicated in orange and group 2 in blue, isolates of mixed origin are white. (b) Infection response scores (1 = resistant to 10 = susceptible) of the isolates on five different genotypes, Beecher, Maritime, Prior, Herta and Skiff. Individuals have been ordered according to the order on the population structure bar plot.

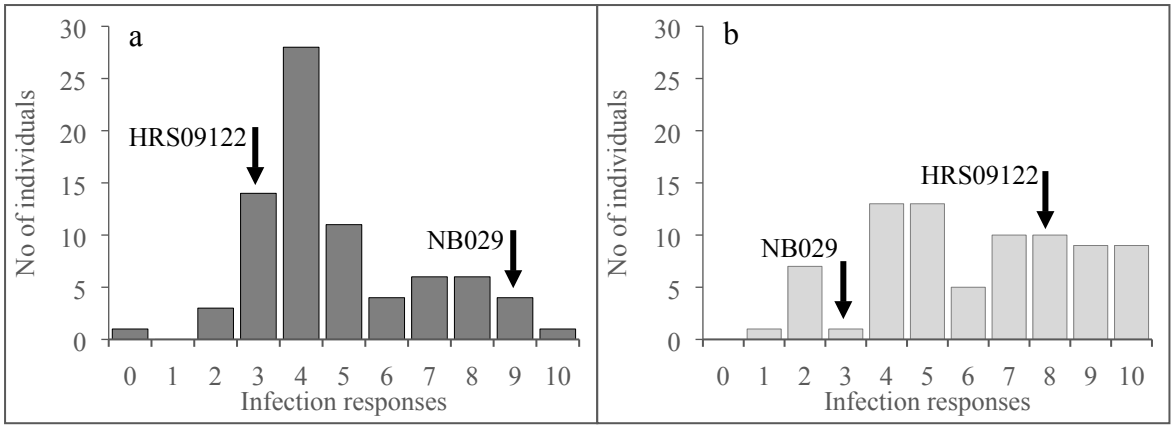


Figure 4. Infection responses of 78 individuals of the NB029/HRS09122 population on barley genotypes Beecher (a) and Skiff (b). Infection responses of the parental isolates NB029 and HRS09122 are indicated.

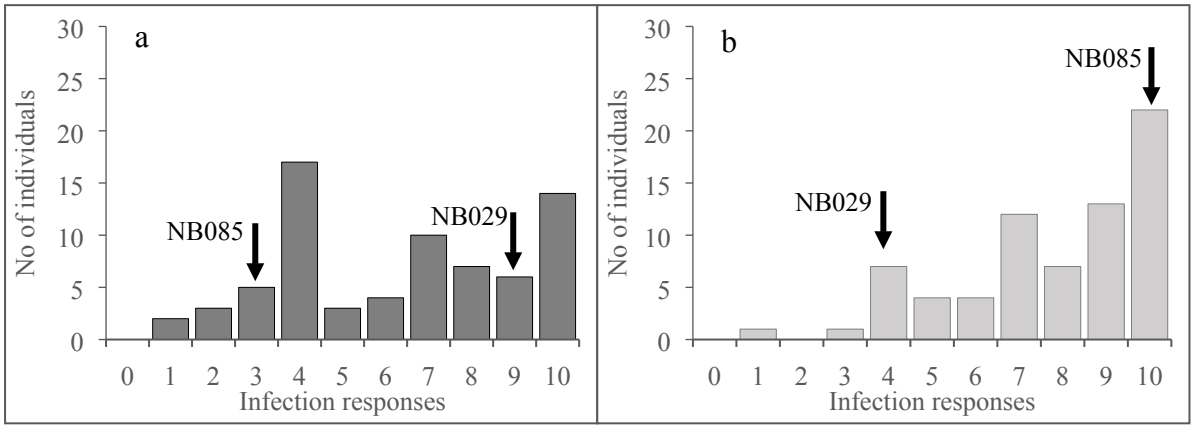
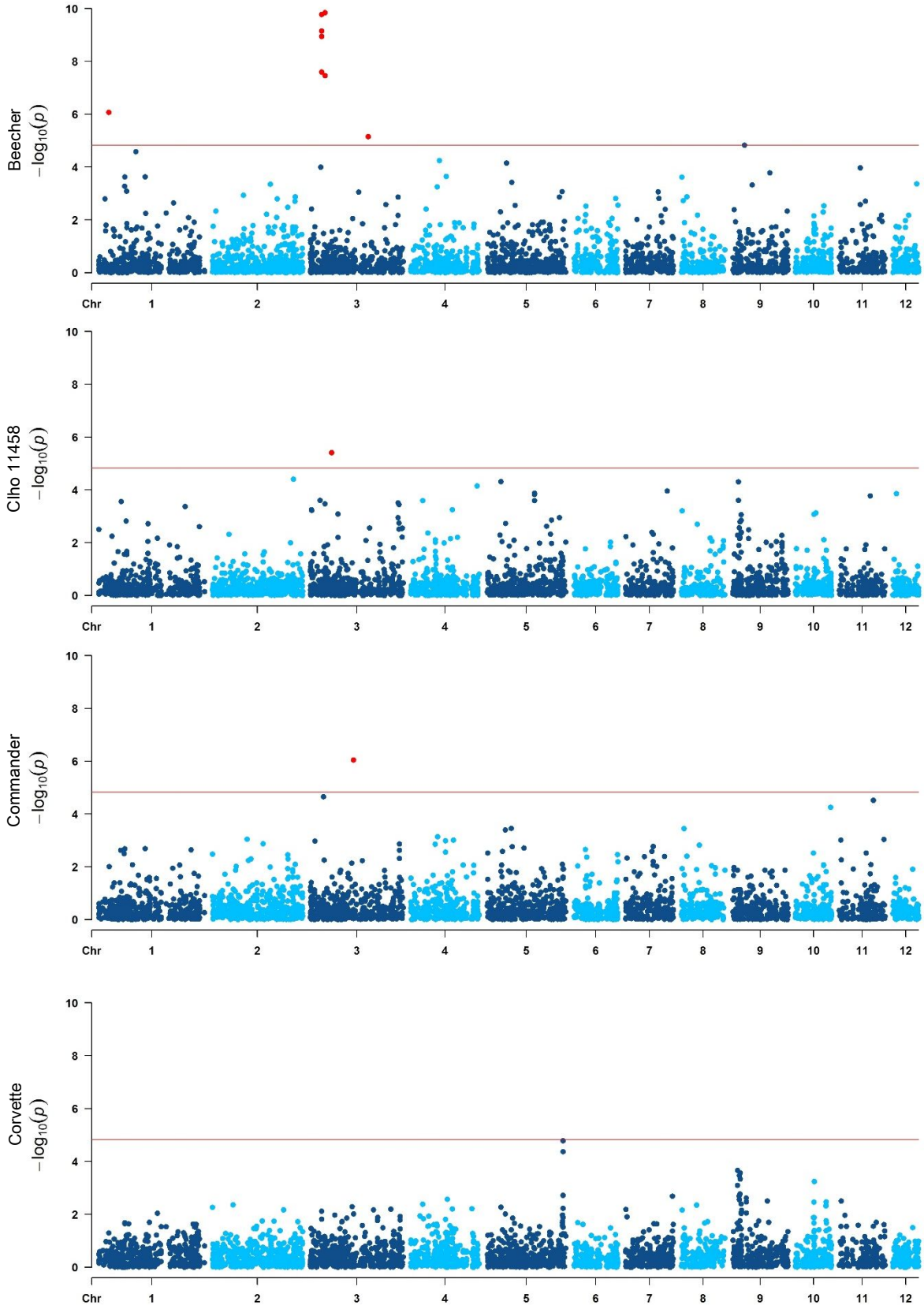
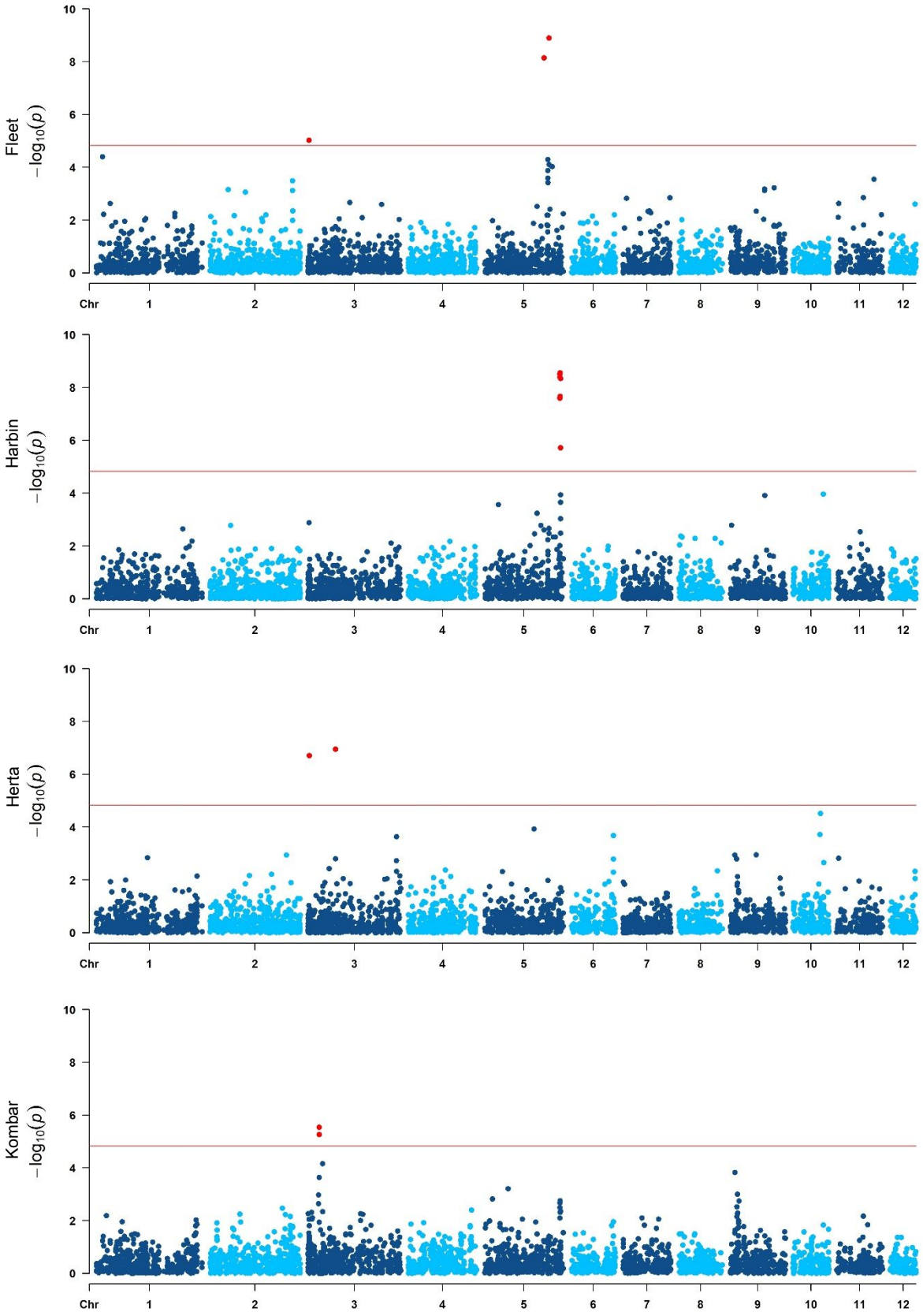
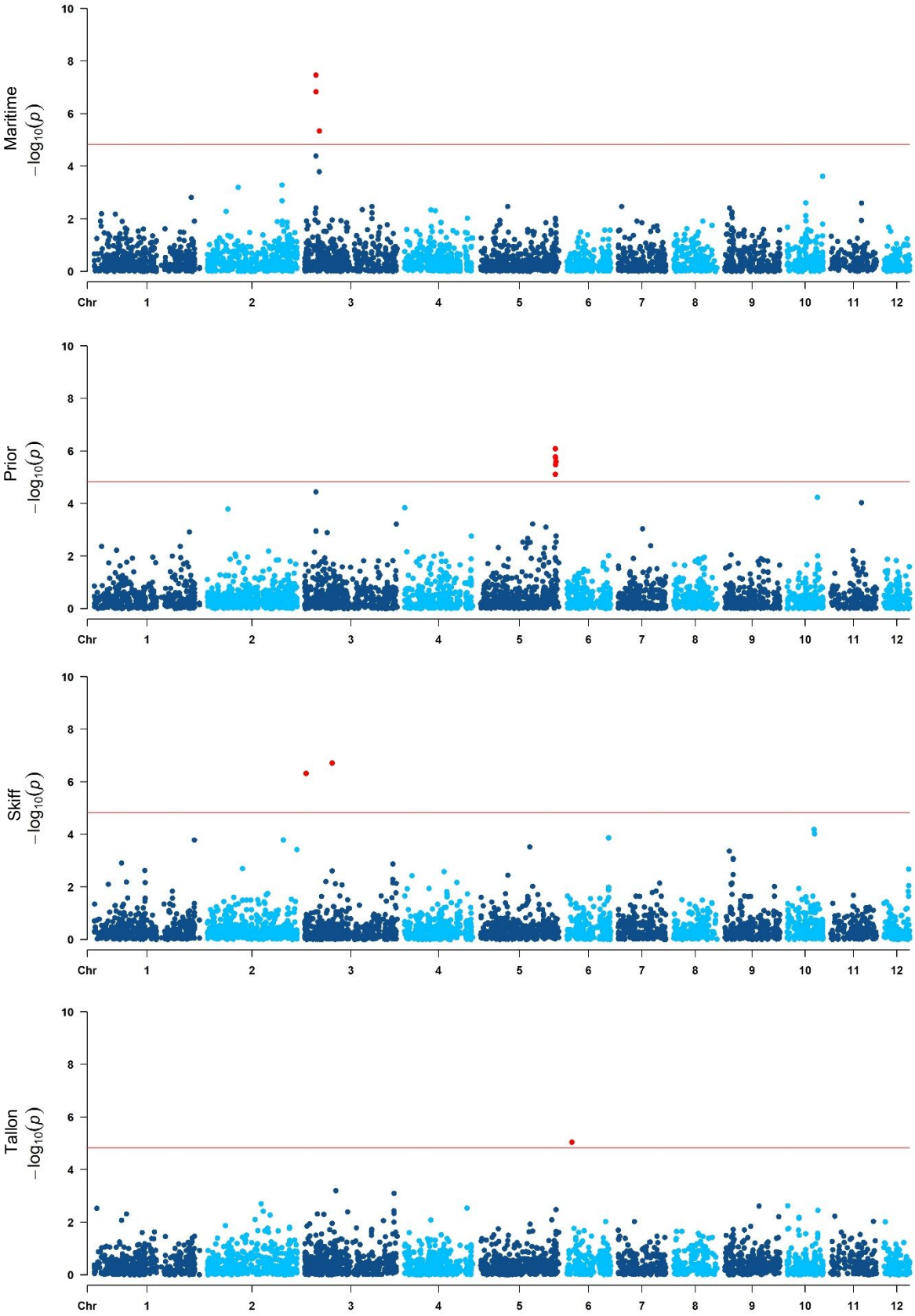


Figure 5. Infection responses of 72 individuals of the NB029/NB085 population on barley genotypes Beecher (a) and Prior (b). Infection responses of the parental isolates NB029 and NB085 are indicated.


Beecher
−log10(p)
Clho 11458
−log10(p)
Commander
−log10(p)
Corvette
−log10(p)
Chr
1
2
3
4
5
6
7
8
9
10
11
12
0
2
4
6
8
10


Fleet
$-\log_{10}(p)$
Harbin
$-\log_{10}(p)$
Herta
$-\log_{10}(p)$
Kombar
$-\log_{10}(p)$
0
2
4
6
8
10
Chr
1
2
3
4
5
6
7
8
9
10
11
12


Maritime
$-\log_{10}(p)$
Prior
$-\log_{10}(p)$
Skiff
$-\log_{10}(p)$
Tallon
$-\log_{10}(p)$
0
2
4
6
8
10
Chr
1
2
3
4
5
6
7
8
9
10
11
12

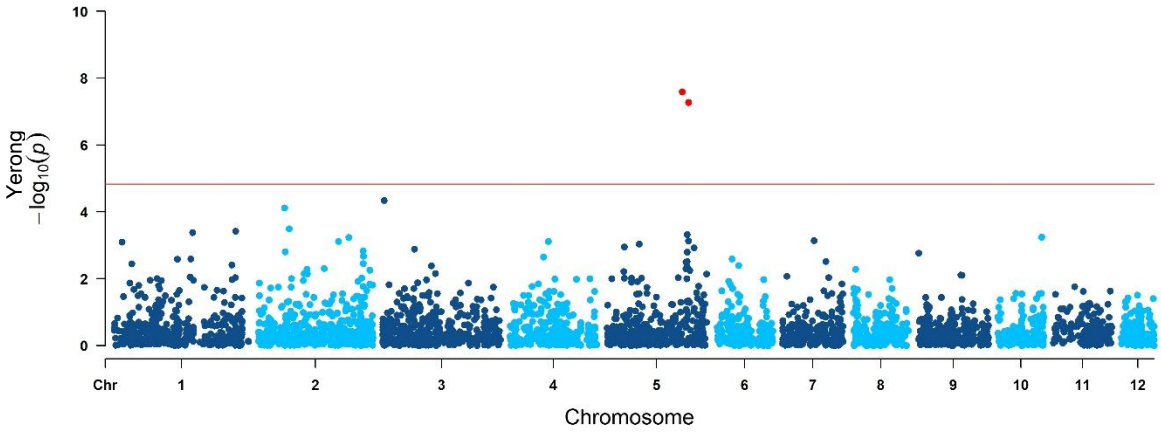
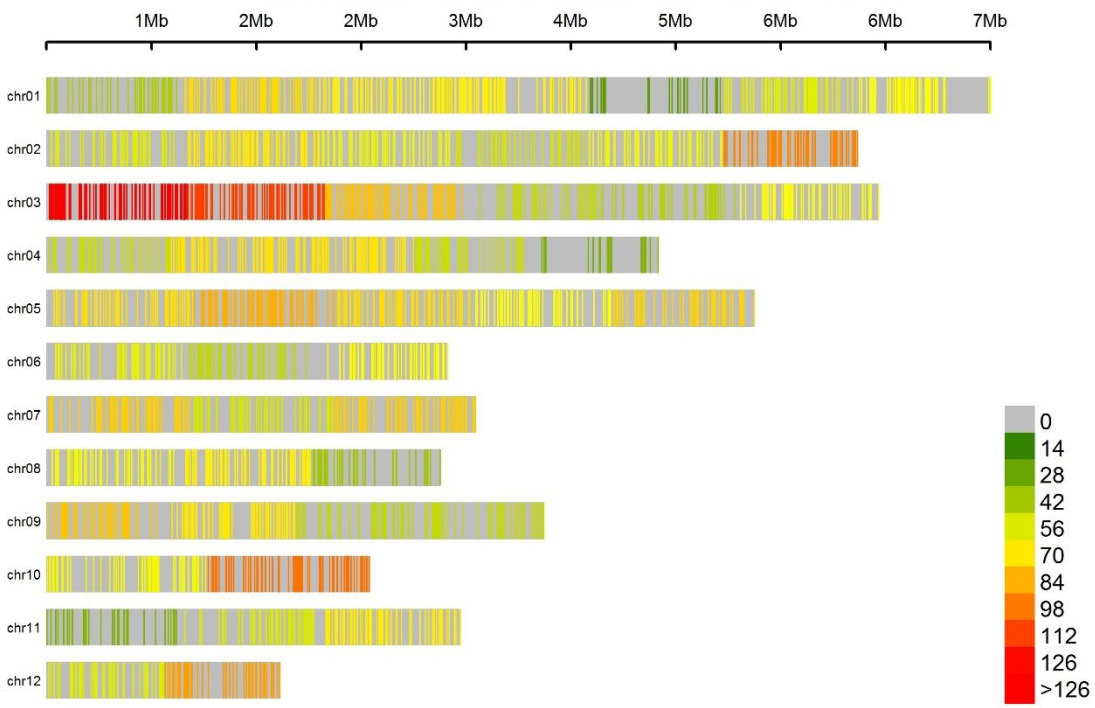
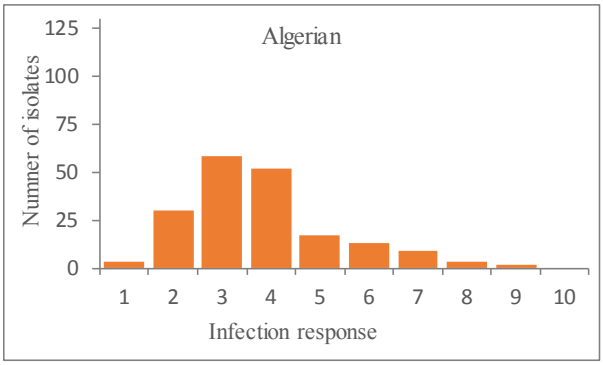
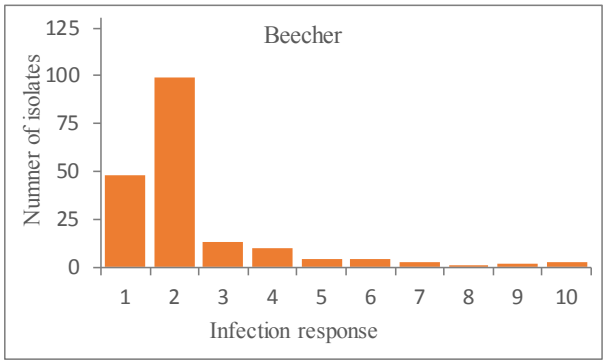


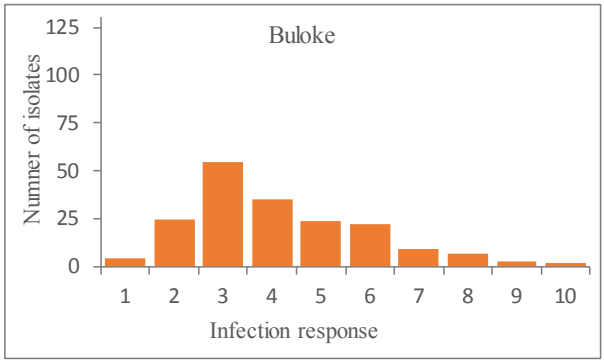
Figure 6. Manhattan plots of the genome-wide analysis conducted to identify markers associated with virulence in *Pyrenophora teres* f. *teres*. The Bonferroni significance threshold (LOD > 4.8) is indicated by the solid red line.

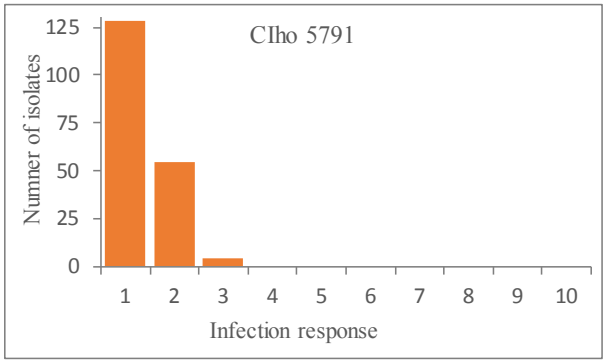


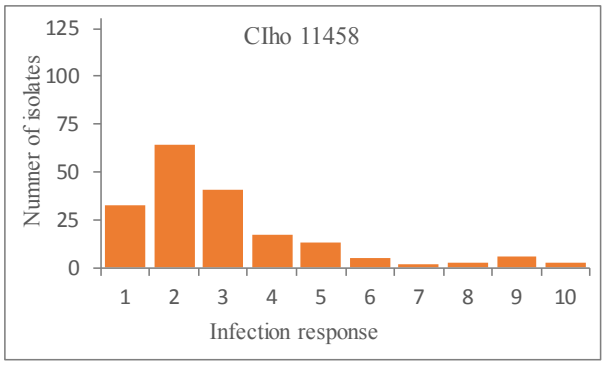
Supplementary Figure 1. Distribution and density of DArTseq™ markers used in genome-wide association mapping across the 12 *Pyrenophora teres* f. *teres* chromosomes

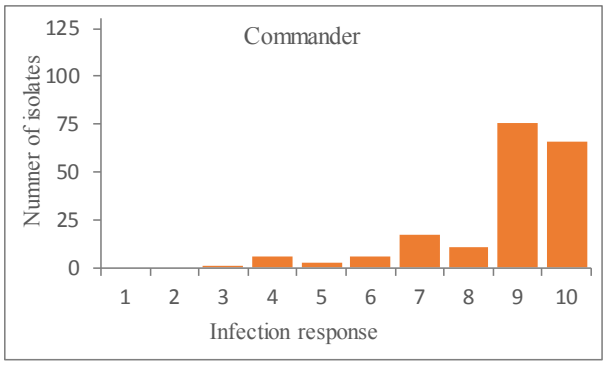

Algerian
Numner of isolates
125
100
75
50
25
0
1 2 3 4 5 6 7 8 9 10
Infection response

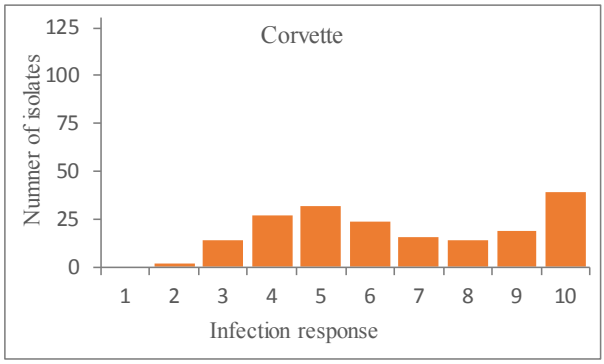

Beecher
Numner of isolates
125
100
75
50
25
0
1 2 3 4 5 6 7 8 9 10
Infection response

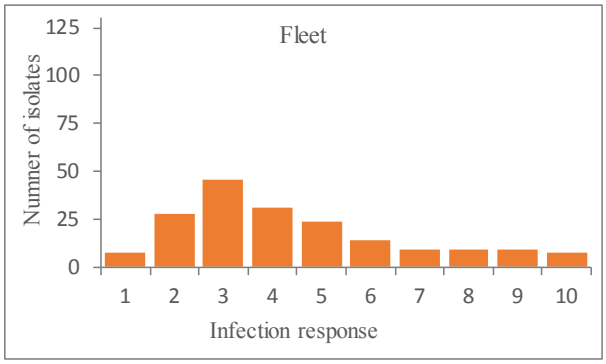

Buloke
Numner of isolates
125
100
75
50
25
0
1 2 3 4 5 6 7 8 9 10
Infection response

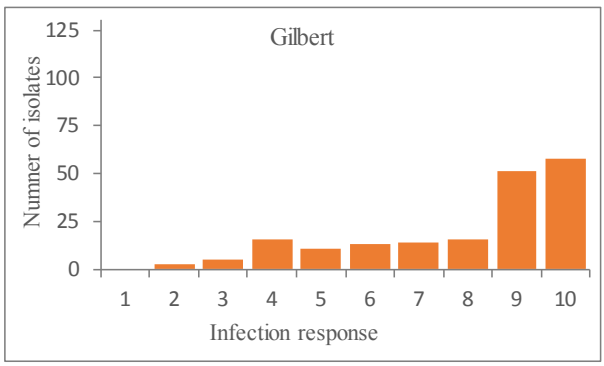

CIho 5791
Numner of isolates
125
100
75
50
25
0
1 2 3 4 5 6 7 8 9 10
Infection response

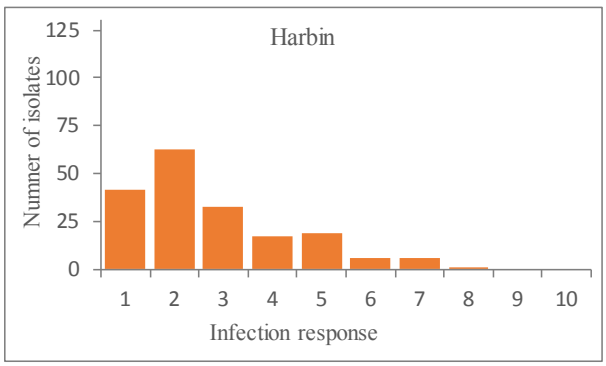

CIho 11458
Numner of isolates
125
100
75
50
25
0
1 2 3 4 5 6 7 8 9 10
Infection response

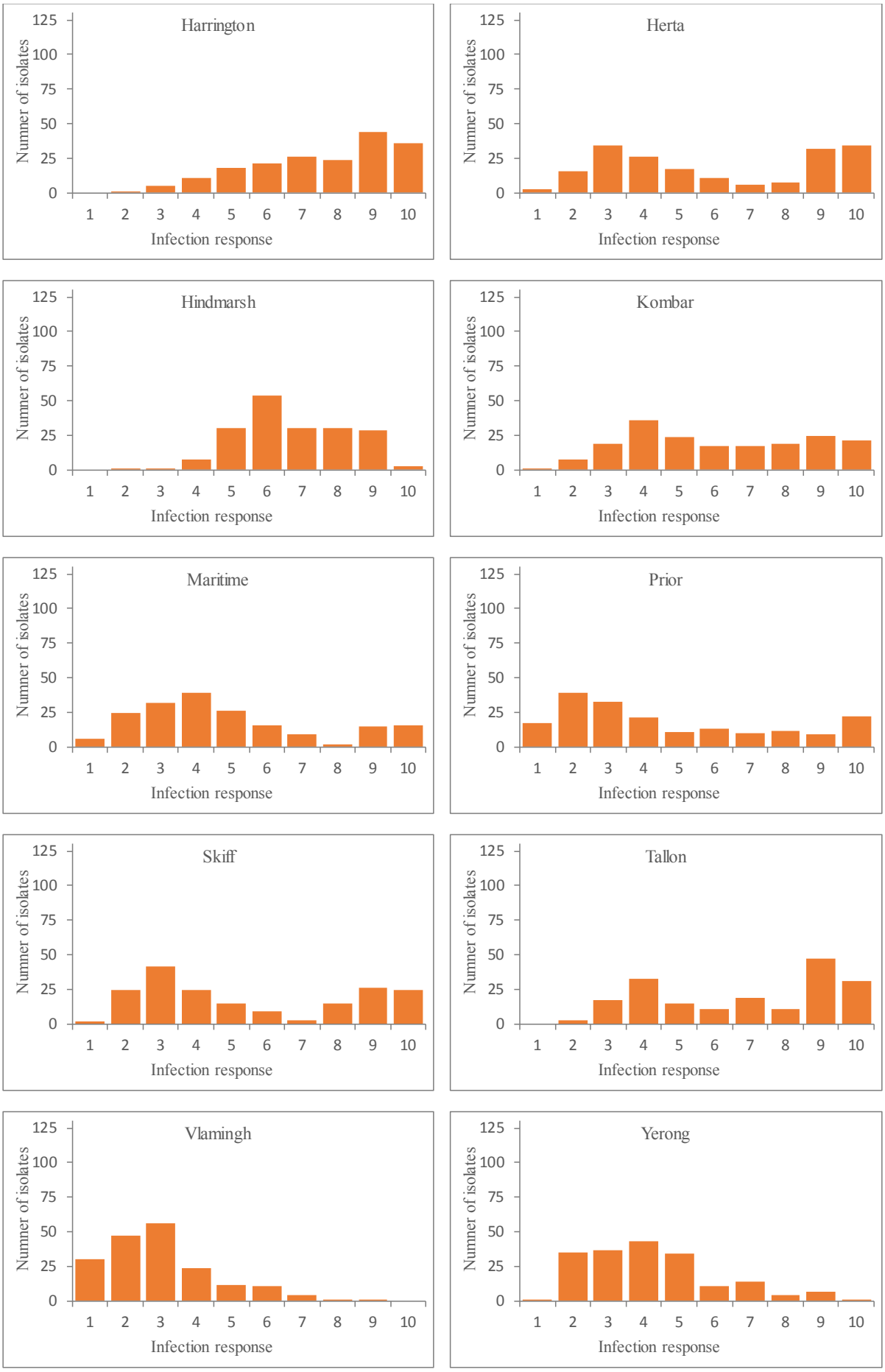

Commander
Numner of isolates
125
100
75
50
25
0
1 2 3 4 5 6 7 8 9 10
Infection response


Corvette
Numner of isolates
125
100
75
50
25
0
1 2 3 4 5 6 7 8 9 10
Infection response


Fleet
Numner of isolates
125
100
75
50
25
0
1 2 3 4 5 6 7 8 9 10
Infection response

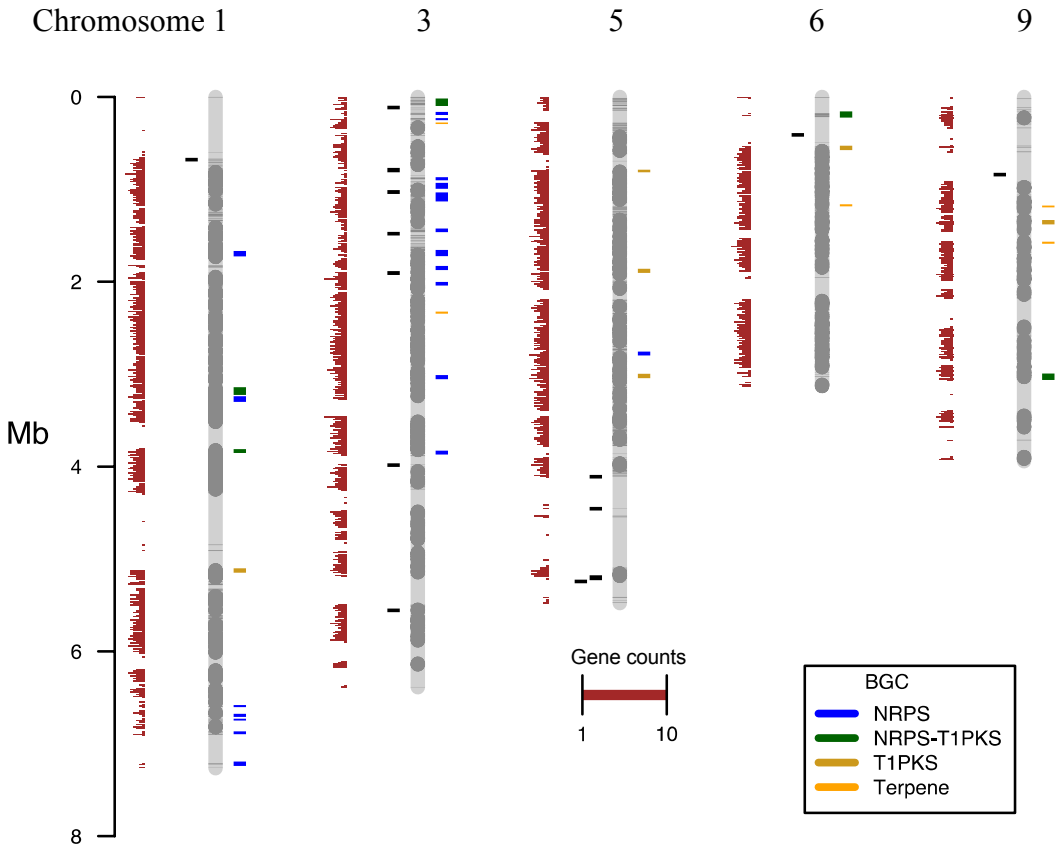

Gilbert
Numner of isolates
125
100
75
50
25
0
1 2 3 4 5 6 7 8 9 10
Infection response


Harbin
Numner of isolates
125
100
75
50
25
0
1 2 3 4 5 6 7 8 9 10
Infection response

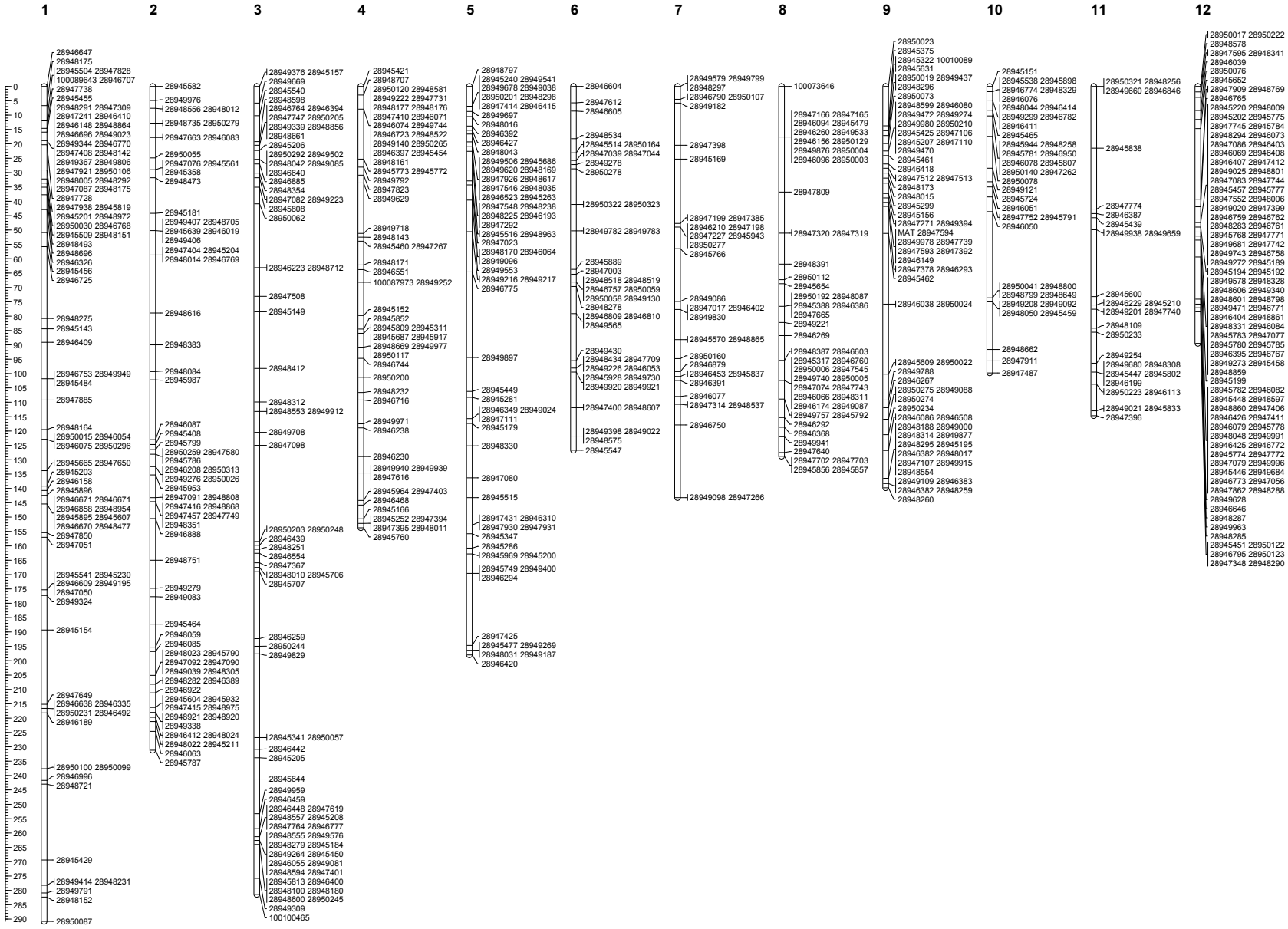


Supplementary Figure 2. Distribution of infection responses of *Pyrenophora teres* f. *teres* isolates

across 20 different barley genotypes



Supplementary Figure 3. *Pyrenophora teres* f. *teres* isolate W1-1 chromosome overview of QTL regions. Chromosomes 1, 3, 5, 6 and 9 are shown with histograms of gene counts in a 10 kb window (red histogram), QTL regions (black) on the left of each chromosome, and biosynthetic gene clusters on the right of each chromosome.


1
2
3
4
5
6
7
8
9
10
11
12
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
105
110
115
120
125
130
135
140
145
150
155
160
165
170
175
180
185
190
195
200
205
210
215
220
225
230
235
240
245
250
255
260
265
270
275
280
285
290
28946647
28948175
28945504 28947828
100089643 28946707
28947738
28945455
28948291 28947309
28947241 28946410
28946148 28948864
28946696 28949023
28949344 28946770
28947408 28948142
28949367 28949806
28947921 28950106
28948005 28948292
28947087 28948175
28947728
28947938 28945819
28945201 28948972
28950030 28946768
28945509 28948151
28948493
28948696
28946326
28945456
28946725
28948275
28945143
28946409
28946753 28949949
28945484
28947885
28948164
28950015 28946054
28946075 28950296
28945665 28947650
28945203
28946158
28945896
28946671 28946671
28946858 28948954
28945895 28945607
28946670 28948477
28947850
28947051
28945541 28945230
28946609 28949195
28947050
28949324
28945154
28947649
28946638 28946335
28950231 28946492
28946189
28950100 28950099
28946996
28948721
28945429
28949414 28948231
28949791
28948152
28950087
28945582
28949976
28948556 28948012
28948735 28950279
28947663 28946083
28950055
28947076 28945561
28945358
28948473
28945181
28949407 28948705
28945639 28946019
28949406
28947404 28945204
28948014 28946769
28948616
28948383
28948084
28945987
28946087
28945408
28945799
28950259 28947580
28945786
28946208 28950313
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28948059
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28948023 28945790
28947092 28947090
28949039 28948305
28948282 28946389
28946922
28945604 28945932
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28948921 28948920
28949338
28946412 28948024
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28949376 28945157
28949669
28945540
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28946716
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28945760
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28949697
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28947292
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28947023
28948170 28946064
28949096
28949553
28949216 28949217
28946775
28949897
28945449
28945281
28946349 28949024
28947111
28945179
28948330
28947080
28945515
28947431 28946310
28947930 28947931
28945347
28945286
28945969 28945200
28945749 28949400
28946294
28947425
28945477 28949269
28948031 28949187
28946420
28946604
28947612
28946605
28948534
28945514 28950164
28947039 28947044
28949278
28950278
28950322 28950323
28949782 28949783
28945889
28947003
28948518 28948519
28946757 28950059
28950058 28949130
28948278
28946809 28946810
28949565
28949430
28948434 28947709
28949226 28946053
28945928 28949730
28949920 28949921
28947400 28948607
28949398 28949022
28948575
28945547
28949579 28949799
28948297
28946790 28950107
28949182
28947398
28945169
28947199 28947385
28946210 28947198
28947227 28945943
28950277
28945766
28949086
28947017 28946402
28949830
28945570 28948865
28950160
28946879
28946453 28945837
28946391
28946077
28947314 28948537
28946750
28949098 28947266
100073646
28947166 28947165
28946094 28945479
28946260 28949533
28946156 28950129
28949876 28950004
28946096 28950003
28947809
28947320 28947319
28948391
28950112
28945654
28950192 28948087
28945388 28946386
28947665
28949221
28946269
28948387 28946603
28945317 28946760
28950006 28947545
28949740 28950005
28947074 28947743
28946066 28948311
28946174 28949087
28949757 28945792
28946292
28946368
28949941
28947640
28947702 28947703
28945856 28945857
28950023
28945375
28945322 10010089
28945631
28950019 28949437
28948296
28950073
28948599 28946080
28949472 28949274
28949980 28950210
28945425 28947106
28945207 28947110
28949470
28945461
28946418
28947512 28947513
28948173
28948015
28945299
28945156
28947271 28949394
MAT 28947594
28949978 28947739
28947593 28947392
28946149
28947378 28946293
28945462
28946038 28950024
28945609 28950022
28949788
28946267
28950275 28949088
28950274
28950234
28946086 28946508
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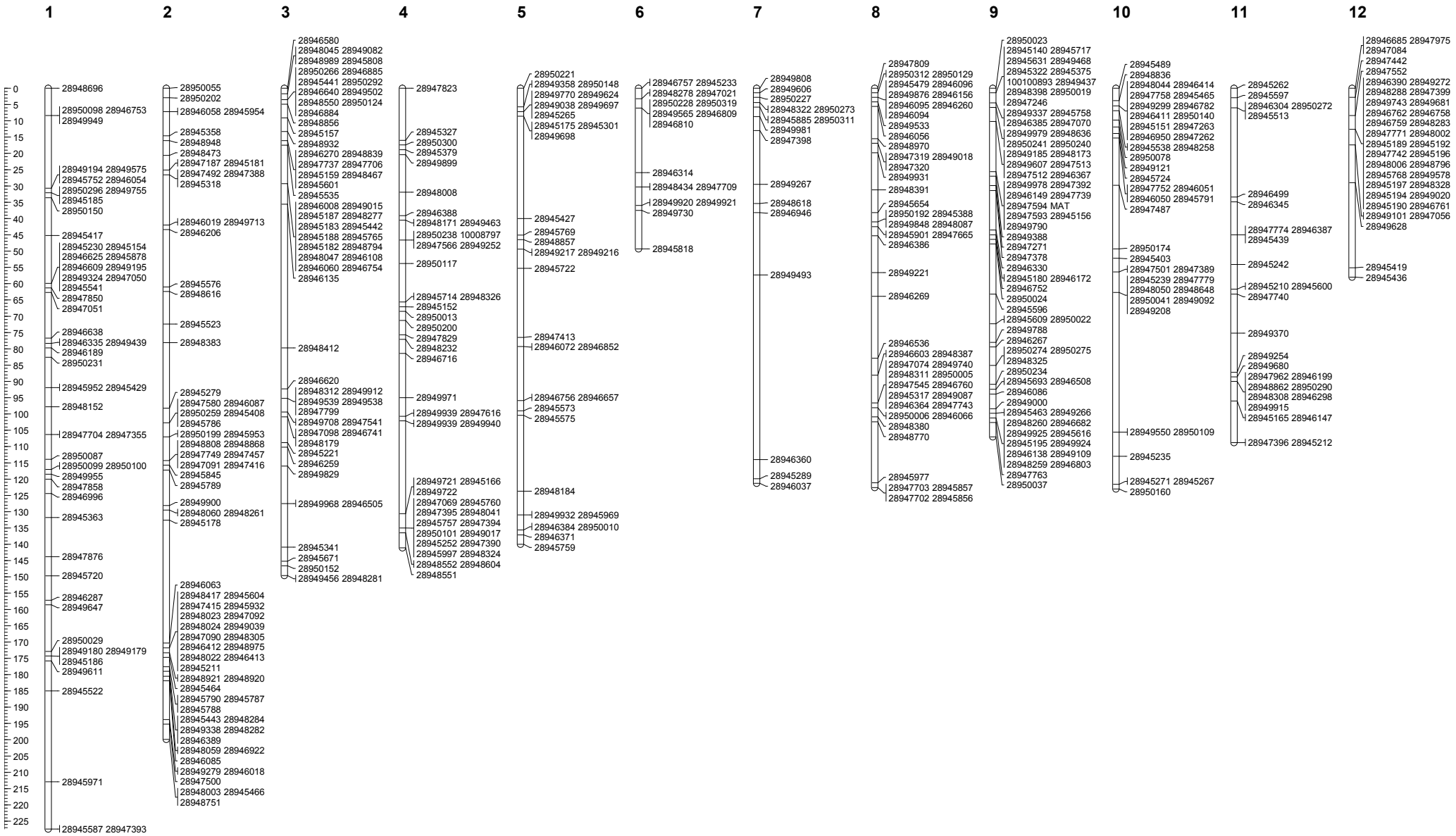
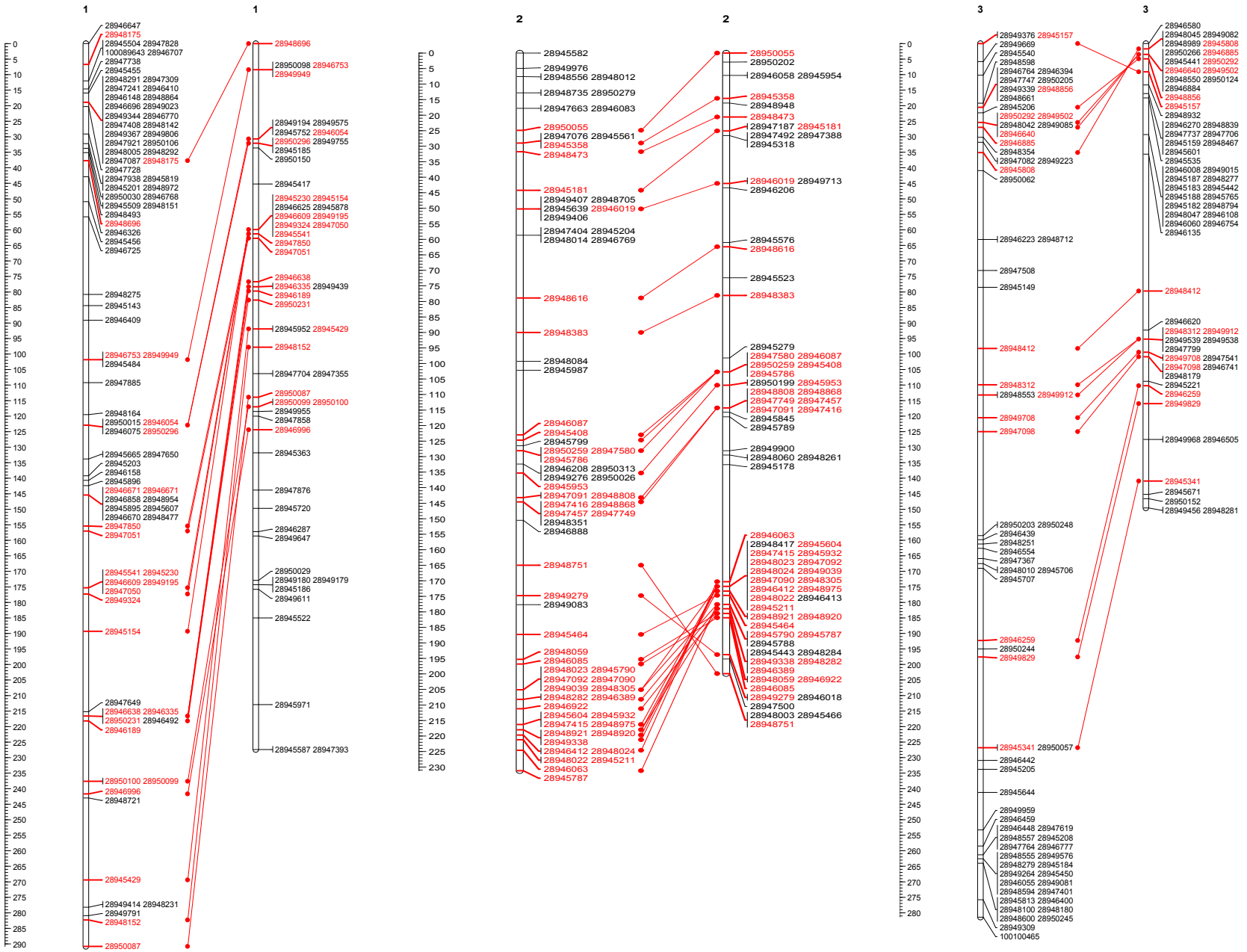
Supplementary Figure 4. Genetic map of population NB029/HRS09122



Supplementary Figure 5. Genetic map of population NB029/N0B85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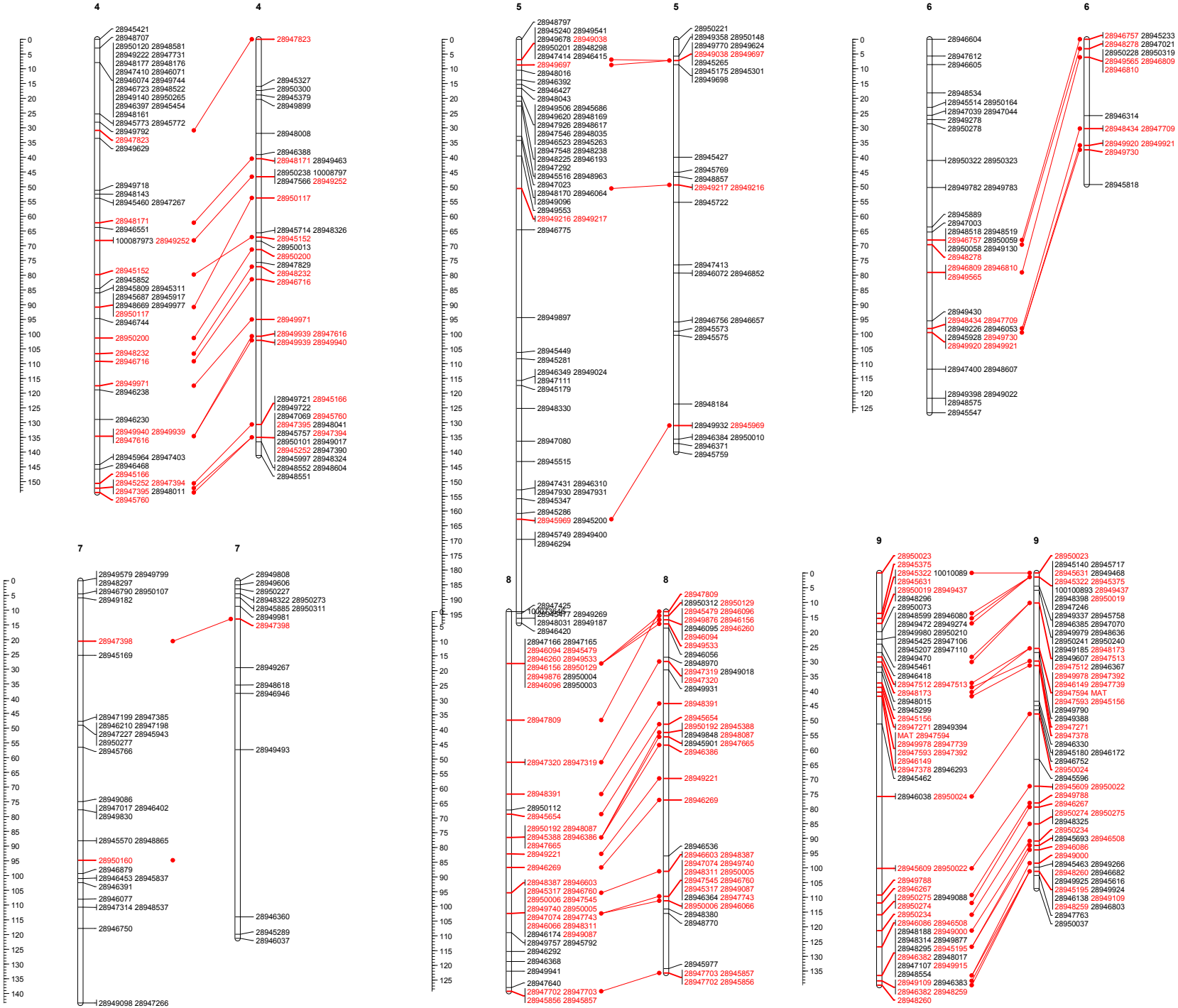
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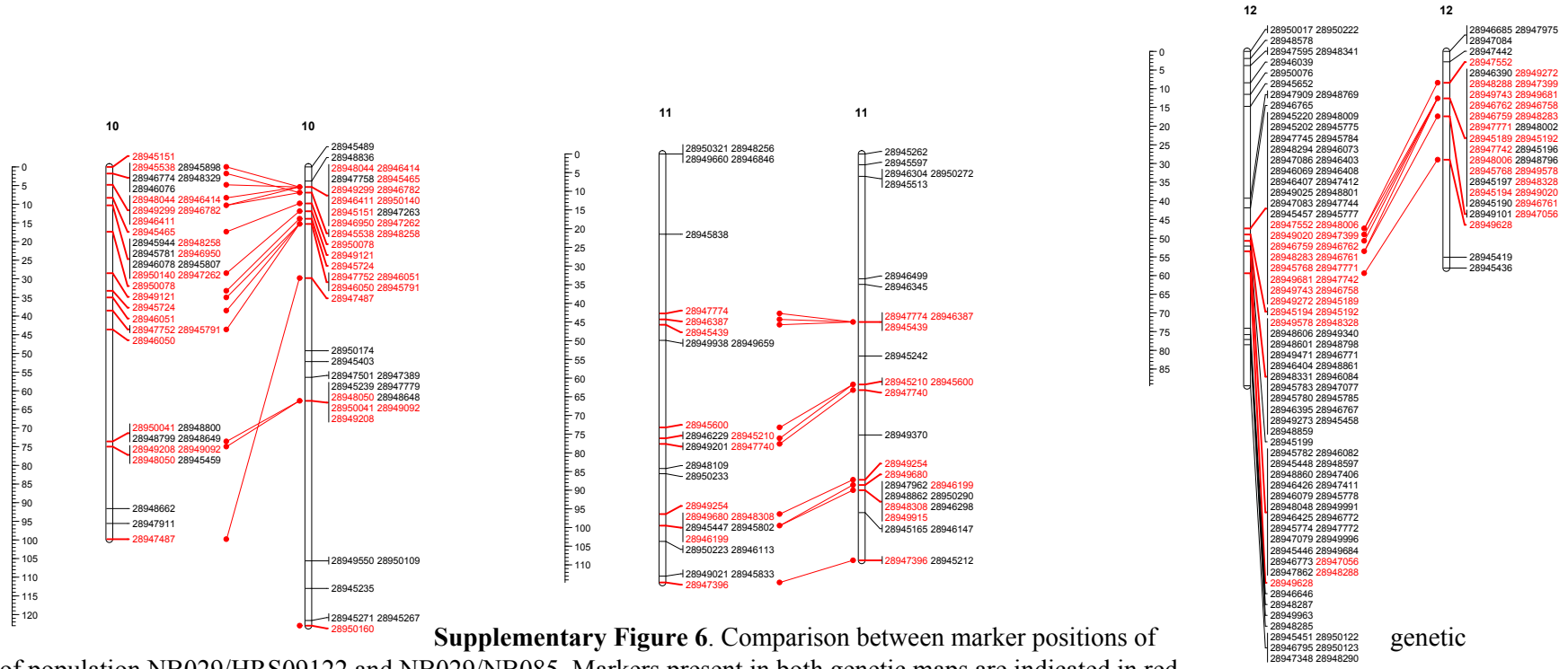
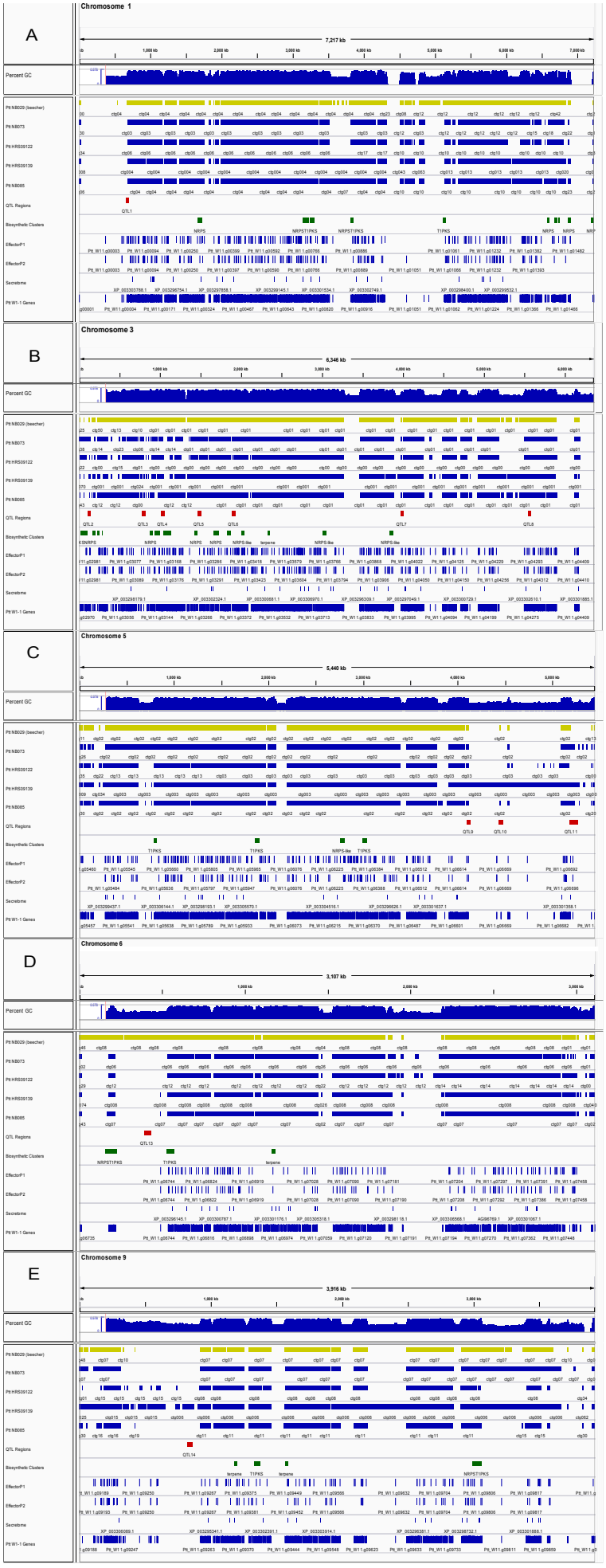


Supplementary Figure 6. Comparison between marker positions of genetic maps of population NB029/HRS09122 and NB029/NB085. Markers present in both genetic maps are indicated in red.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Percent GC
Pt NB029 (beecher)
Pt NB073
Pt HRS09122
Pt HRS09139
Pt NB085
QTL Regions
QTL1
Biosynthetic Clusters
NRPS
NRPS/T1PKS
NRPS/T1PKS
T1PKS
NRPS
NRPS
NRPS
EffectorP1
EffectorP2
Secretome
Pt W1-1 Genes
B
Chromosome 3
6,346 kb
Percent GC
QTL Regions
QTL2
QTL3
QTL4
QTL5
QTL6
QTL7
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Biosynthetic Clusters
NRPS
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C
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Percent GC
QTL Regions
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D
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E
Chromosome 9
3,916 kb
Percent GC
QTL Regions
QTL14
Biosynthetic Clusters
terpene
T1PKS
terpene
NRPS/T1PKS

Supplementary Figure 7. *Pyrenophora teres* f. *teres* isolate W1-1 overview of QTL, genes and isolate genome alignments for chromosomes 1, 3, 5, 6 and 9. Panels A-E represent the chromosomes of *Ptt* W1-1 with association mapping QTL for chromosomes 1, 3, 5, 6 and 9 respectively. The tracks for each panel are in order from top to bottom, W1-1 chromosome length, percent GC bar plot in 10 kb window, *Ptt* isolate genome alignments for NB029 Beecher virulent (yellow), NB073, HRS09122, HRS09139, NB085 association mapping QTL regions (red), predicted biosynthetic gene clusters (green), predicted effector genes for EffectorP version 1 and version 2, and the gene annotations for W1-1.