Association Mapping of Seedling Resistance to Spot Form Net Blotch in a Worldwide Collection of Barley

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ABSTRACT

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Spot form net blotch (SFNB), caused by the necrotrophic fungal pathogen *Pyrenophora teres* f. *maculata*, is an important foliar disease of barley in major production regions around the world. Deployment of adequate host resistance is challenging because the virulence of *P. teres* f. *maculata* is highly variable and characterized minor-effect resistances are typically ineffective against the diverse pathogen populations. A world barley core collection consisting of 2,062 barley accessions of diverse origin and genotype were phenotyped at the seedling stage with four *P. teres* f. *maculata* isolates collected from the United States (FGO), New Zealand (NZKF2), Australia (SG1), and Denmark (DEN 2.6). Of the 2,062 barley accessions phenotyped, 1,480 were genotyped with the Illumina barley iSelect chip and passed the quality controls with 5,954 polymorphic markers used for further association mapping analysis. Genome-wide association mapping was utilized to identify and map

resistance loci from the seedling disease response data and the single nucleotide polymorphism (SNP) marker data. The best among six different regression models was identified for each isolate and association analysis was performed separately for each. A total of 138 significant ($-\log_{10}P$ value > 3.0) marker-trait associations (MTA) were detected. Using a 5 cM cutoff, a total of 10, 8, 13, and 10 quantitative trait loci (QTL) associated with SFNB resistance were identified for the FGO, SG1, NZKF2, and DEN 2.6 isolates, respectively. Loci containing from 1 to 34 MTA were identified on all seven barley chromosomes with one locus at 66 to 69 cM on chromosome 2H common to all four isolates. Six distinct loci were identified by the association mapping (AM) analysis that corresponded to previously characterized SFNB resistance QTL identified by biparental population analysis (*QRpt4*, *QRpt6*, *Rpt4*, *Rpt6*, *Rpt7*, and a QTL on 4H that was not given a provisional gene or QTL nomenclature). The 21 putative novel loci identified may represent a broad spectrum of resistance and/or susceptibility loci. This is the first comprehensive AM study to characterize SFNB resistance loci underlying broad populations of the barley host and *P. teres* f. *maculata* pathogen.

Net blotch of barley (*Hordeum vulgare* L.) caused by the necrotrophic fungal pathogen *Pyrenophora teres* Drechsler (anamorph: *Drechslera teres* [Sacc.] Shoem.) is a destructive pathogen in many barley-growing regions throughout the world (33). This pathogen is separated into the net and spot forms based on predominant disease symptoms with the causal agents being *P. teres* f. *teres* and *P. teres* f. *maculata*, respectively (57). Spot form net blotch (SFNB) has emerged as a major leaf spot disease in several barley-growing regions of the world including regions of Australia, Canada, Europe, South Africa, and recently the United States (29,30,33,41,42,62). Although SFNB had previously been reported in the Upper Midwestern United States, it was not considered a problem (34). However, since it was identified in North Dakota in 2006, it has become a major concern for growers in the Northern Great Plains due to what may be increased virulence as indicated by comparison with isolates collected in other parts of the world (49).

The prevalence and increase of SFNB epidemics may be due to a combination of different factors including minimum or no-till practices, high-density cropping, cultivation of predominantly susceptible varieties, and shifts of virulence in the pathogen populations due to selection by popularly grown varieties (33,42). In years when environmental conditions are conducive to disease and susceptible varieties are grown, SFNB can cause 10 to 40% yield losses in barley (27,30,41,47). Chemical control, cultural

practices, and host resistance are all commonly used to manage SFNB (9,28,43,71), yet deployment of high-quality resistant cultivars would be the most efficient and environmentally friendly means of disease management.

Wide ranges of pathogenic variability in *P. teres* f. *maculata* populations have been reported within and across distinct geographic regions (21,58,62,64). Based on what is known in other well-characterized cereal host–Dothidiomycete necrotrophic pathosystems (16,18,50), it is expected that *P. teres* produces a diversity of necrotrophic effectors (previously known as host specific/selective toxins) that target distinct susceptibility genes in barley resulting in quantitative susceptibility (34,42). Resistant varieties lacking these multiple sensitivity genes are uncommon, thus sources of resistance that are effective against diverse SFNB populations are rare.

Quantitative trait loci (QTL) analysis of SFNB infection types on biparental barley mapping populations identified resistance/susceptibility genes on barley chromosomes 2H (45), 4H (17,20), 5H (39), 6H (20), and 7H (20,68,69). These QTL and the markers linked with resistance may be specific to these genotypes and may represent interactions specific to narrow populations of both the host and pathogen.

Association mapping (AM) presents an alternative approach to biparental mapping that can efficiently capture diverse marker-trait associations (MTA) (15,48,75). This approach has been used effectively in diverse crop plants for many traits including complex yield traits in rice (1), resistance genes/QTL in maize (32), wheat (8,19,63), potato (36) and iron deficiency chlorosis (IDC) in soybean (37,66), flowering time in Arabidopsis (3,11), and quality

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traits in canola (24,76). In barley, different marker types have been identified that are associated with phenotypes including resistance to the diseases spot blotch, Fusarium head blight, powdery mildew, and leaf rust (6,40,54). Loci contributing to other important yet complexly inherited agronomic traits including malting quality (5,22), yield and yield stability (31), flowering time (25), water-stress resistance (26), salt tolerance (51), and winter-hardiness (65) have also been identified utilizing AM.

The objective of this study was to use the AM approach on a large population of barley genotypes from around the world to identify loci that contribute to resistance against diverse SFNB isolates collected from four important barley-producing regions. The identification of single nucleotide polymorphism (SNP) markers associated with SFNB resistance loci and/or lacking susceptibility genes from resistant lines identified from the world barley core collection (BCC; USDA-ARS National Small Grains Collection) will facilitate the deployment of effective and durable SFNB resistance via marker-assisted selection or genome-wide selection strategies.

MATERIALS AND METHODS

Plant materials and phenotyping. A geographically diverse sample of 2,062 BCC accessions comprising cultivars, breeding lines, landraces, and genetic stocks were obtained from the National Small Grain Collection, Aberdeen, Idaho. The lines were grown in the greenhouse at the USDA, Fargo, North Dakota during 2011 and 2012. Three seeds of each barley line were planted in single cones (3.8 cm diameter and 20 cm long) and were placed into cone racks bordered with the susceptible barley cultivar Robust. Each cone contained three seedlings and was evaluated collectively as a single replicate. Barley lines Clho-14219 and PI 67381 were used as resistant checks and the cultivar Robust was used as the susceptible check.

Four geographically diverse isolates of *P. teres* f. *maculata* FGOB10Ptm-1 (FGO), SG1, NZKF2, and DEN 2.6 collected in the United States, Australia, New Zealand, and Denmark, respectively, were used to evaluate the barley lines for SFNB disease reaction. Fungal inoculum preparation, inoculation, and incubation used for the AM analyses described in this manuscript were performed and described in Neupane et al. (49), which is published as an adjoining manuscript. Three independent replicates were performed for each isolate and the mean of the three replicates was considered for further analysis. Disease was evaluated 7 days after inoculation using the 1 to 5 rating scale as described in Neupane et al. (49).

Genotyping. Of the 2,417 barley accessions genotyped with the barley 9k Illumina Infinium iSELECT assay through the triticeae coordinated agriculture project (T-CAP), 2,062 of the barley accessions were evaluated for their reaction to the *P. teres* f. *maculata* isolates (49). The genotyping was performed and described by Muñoz-Amatriáin et al. (46) and the data were obtained from The Triticeae Toolbox (T3) website (<http://malt.pw.usda.gov/t3/sandbox/barley/>). For this AM analysis we utilized the 6,244 quality SNPs that passed the quality control criteria and 1,480 unique iCore spring barley lines that were previously described (46). Throughout the manuscript the analyses relied on the marker positions, which were previously determined and published as the iSelect consensus map (46).

AM. Imputation and marker properties. All missing genotypic data were imputed using a “likelihood” based imputation with default settings in fastPhase 1.3 (55). The minor allele frequency (MAF) for markers was estimated using the FREQ procedure in SAS 9.3 and the markers that had MAF < 5% were removed from further analysis. The structure, kinship, and AM were also performed separately utilizing all the markers, the markers that have MAF > 1% and the markers > 5% MAF which are reported in the manuscript. From the results, there were very minor changes between the analyses in terms of significant markers or the number of QTL identified.

Linkage disequilibrium (LD) and LD decay. LD was estimated as the squared allele frequencies correlation (R^2) for each of the pair-wise comparison of the markers using the Corr procedure in SAS 9.3. Linkage disequilibrium decay graphs were plotted with genetic distance (centimorgans) versus R^2 for all inter-chromosomal marker pairs using nonlinear regression (53). The expected decay of LD was estimated using the formula described by Mamidi et al. (37)

Population structure, principal component analysis (PCA), and relationship matrix. To prevent linkage bias in estimation of population structure (Q), PCA and relationship matrix (K-matrix), a subset of markers that had an LD < 0.5 with every other marker combination were used (67). STRUCTURE 2.3 was used to estimate the number of subpopulations. The admixture model was used with a burn-in of 20,000 and 50,000 iterations for subpopulation numbers (k) ranging from 1 to 15 considering the allele frequencies to be independent. Five runs for each k value were performed and the posterior probability was determined for each run. The optimum number of subpopulations was determined using the Δk approach (12) implemented in STRUCTURE HARVESTER (10).

PCA was performed in SAS 9.3 to estimate PCs that controlled the population structure in the regression model. The number of PCs that explained ~25% of the cumulative variation in the population were selected to be included as cofactors in the association analysis (37,60). An identity by state (IBS) matrix (73) to control the population relatedness was estimated as a Gower similarity ratio implemented in the distance procedure in SAS 9.3.

MTA model testing. Six different linear regression models were tested for MTAs (Naive, PC, Q, kin, PC+kinship, Q+kinship [37]). Three models that did not have kinship were estimated in SAS 9.3 and three models that had the kinship matrix as a random effect were estimated using GEMMA 0.90 (74). Three general-linear models (GLMs) considered only the fixed effects while the remaining three mixed-linear models (MLMs) considered both the fixed and random effects. The underlying linear equation for the sixth model was

$$Y = X\alpha + Q\beta + Kv + \varepsilon$$

In this model, Y was a vector for phenotypic observation, X was a matrix of alleles of the markers, α was the fixed effects related to the SNP markers, Q was the population structure, β was a vector of the fixed effects related to population structure, K was the subpopulation numbers, v was a vector of the random effects related to the relatedness among the individuals, and ε was a vector of the residual effects. For each model, the positive false discovery rate (pFDR) was estimated for all markers using the PROC MULTTEST in SAS 9.3 to correct for multiple MTA (61). For the selection of best model, a rank based mean square difference (MSD) was used (37) based on the suggestion of Yu et al. (72) of random errors. MSD values were calculated between the observed P values and expected P values. Expected P values were estimated by dividing the rank of observed P value with number of makers used in this study. The model with the lowest MSD value was considered the best model. Markers were considered significant if the P value was less than 0.001. The amount of phenotypic variation (R^2) was estimated for each significant marker using a simple regression using the REG procedure in SAS 9.3. Multiple R^2 values for significant markers were calculated using stepwise regression implemented in the Reg procedure in SAS 9.3 (38). The allelic means of the significant markers were estimated in SAS 9.3 using the Means procedure. Further, a QTL was defined as the region that harbors multiple significant markers ($P < 0.001$) within a distance at which the whole genome LD decay stabilizes. The position of the significant markers was obtained from a core consensus map developed by Muñoz-Amatriáin et al. (46).

RESULTS

Phenotypic analysis across barley accession. A total of 2,062 spring BCC accessions were evaluated for their reaction to the four *P. teres* f. *maculata* isolates (FGO, NZKF2, SG1, and DEN 2.6) by Neupane et al. (49). The manuscript describing the phenotypic analyses and results is published as an adjoining manuscript (49). Of the 2,062 accessions reported in Neupane et al. (49), only 1,480 accessions were utilized in our analysis due to marker data not passing quality control and redundant genotypes being eliminated as determined by Muñoz-Amatrián et al. (46).

Marker properties. Of the 2,062 barley accessions evaluated for their reaction to the *P. teres* f. *maculata* isolates (49), 1,947 barley accessions were genotyped with a total of 6,244 SNP markers. Of the 1,947 spring barley accessions genotyped, 467 were removed due to genetic redundancy or inconsistent passport data, leaving 1,480 spring barley accessions in the AM analysis (46). Approximately 0.43% of the missing genotypic data were imputed. Out of 6,244 SNPs, only 5,954 markers that had an MAF > 0.05 were used for further analysis. Based on the LD coefficient (r^2 ; correlation squared between markers), 4,402 markers that had LD > 0.5 with at least one other marker were removed for estimating the confounding factors for population structure and relatedness.

LD. A nonlinear regression model estimates the average genome-wide LD decay across the genome using the interchromosomal comparison of LD. The whole genome-wide LD decay was extended up to 5 cM at $r^2 \geq 0.1$ (Fig. 1).

Population structure, PCA, and relationship matrix. A subset of 1,842 markers that had an LD < 0.5 with any other marker in the subset was used to analyze the population structure, PCA, and relationship matrix. The Bayesian-based clustering approach in STRUCTURE revealed eight subpopulations by the Δk approach (Fig. 2). The number of barley accessions in each subpopulation varied between 94 (subpopulation 4) and 238 (subpopulation 2). Accounting for this population structure in the AM analysis reduces the number of false-positive QTL identified.

Six PCs that explained about 25% of the variation were used as cofactors in the mixed model. The first PC explained 7.92% of the variation followed by PC2 that explained 6.12%.

Fig. 1. Pattern of linkage disequilibrium (LD) decay created by plotting r^2 values against the genetic distance (cM) for the whole genome. The curve shows nonlinear regression of r^2 on a weighted genetic distance.

AM analyses. FGO isolate. For the AM analyses, the four isolates were analyzed separately and the average score of the three replicates for each isolate was used for the phenotypic data. Out of the six models tested for the FGO isolate, the model with only kinship (IBS) was the best with an MSD of 0.001. A total of 29 SNP markers were found to be significantly associated with resistance against isolate FGO at a cutoff of $P < 0.001$. The 29 markers were located on all seven barley chromosomes, with one marker on chromosome 1H, two on 2H, six on 3H, one on 4H, two on 5H, four on 6H, and four on 7H (Table 1). There was no marker position information for the remaining nine markers. The P values of significant markers had a range of 4.37E-09 to 3.43E-04. The MAF for the significant markers ranged from 7.91 to 49.80. The R^2 (phenotypic variation) for all significant markers was up to 4.5% (Table 1). Based on a cutoff of 5 cM, a total of 10 loci associated with resistance were identified. Of the loci associated with resistance, one was identified on chromosome 1H (114.3 cM), two on chromosome 2H (69.55 and 137.44 cM), one on 3H (99.26 to 99.66 cM), one on 4H (99.68 cM), one on 5H (31.86 cM), one on 6H (59.01 to 60.21 cM), and three on 7H (26.35 to 26.92, 109.0, and 133.84 cM) (Fig. 3D). From the stepwise regression, 17 markers were included in the model and cumulatively explained 18.78% of the phenotypic variation. All 10 loci associated with resistance contained markers that were included in the stepwise regression.

SG1 isolate. For the SG1 isolate, the model with structure and kinship (IBS) was the best model with MSD = 0.000668. A total of 17 markers were significant of which two are on 1H, five on 2H, one on 3H, two on 4H, two on 5H, and one on 6H. The remaining four markers did not have any position information (Table 2). The MAF values for all significant markers had a range of 6.08 to 32.97. The P values for the significant markers ranged between 1.60E-05 and 3.76E-05. The R^2 (phenotypic variation) for all significant markers was up to 5.68% (Table 2). There were eight loci identified that contained markers significantly associated with resistance with two being located on chromosome 1H (50 and 98.45 cM), one on 2H (65.71 to 66.11 cM), one on 3H (88.17 cM), two on 4H (32.43 and 47.17 cM), one on 5H (31.86 cM) and one on 6H (101.83 cM) (Fig. 3C). From the stepwise regression, seven markers were included in the model and cumulatively explained 7.74% of the phenotypic variation.

NZKF2 isolate. Out of the six models tested, the best model for the isolate NZKF2 data were structure and kinship (IBS) with an MSD = 0.001829. From the 54 markers identified as significant, two were located on 2H, 12 on 3H, 21 on 4H, one on 5H, three on 6H, and seven on 7H (Table 3). The remaining eight markers did not have

Fig. 2. A graph with Δk and number of subpopulations. The Bayesian-based clustering approach in STRUCTURE revealed eight subpopulations by the Δk approach (12). The peak represents the appropriate number of subpopulation.

marker position information. The P values of the markers had a range of 3.75E-12 to 9.77E-04. The MAF values for all significant markers ranged from 5.14 to 49.19. The R^2 (phenotypic variation) for all significant markers was up to 15.97% (Table 3). A total of 13 loci associated with resistance to NZKF2 were identified, two on 2H (23.76, 69 cM), three on 3H (53.4, 99.26 to 103.86, and 153.39 cM), three on 4H (53.67 to 57.32, 103.48 to 103.58, and 117.13 cM), one on 5H (111.56 cM), one on 6H (59.21 cM), and three on 7H (78.07, 133.84, and 145.68 to 150.36 cM) (Fig. 3B). From the stepwise regression, 23 markers were included in the model and together explained 44.35% of the phenotypic variation. All 113 loci containing markers associated with resistance were included in the stepwise regression.

DEN 2.6 isolate. Out of the six models tested for the DEN 2.6 isolate, the best model identified was structure and kinship (IBS) with an MSD = 0.000577. A total of 38 markers were highly significant with one marker on 1H, one marker on 2H, four markers on 3H, 20 on 4H, one on 5H, three on 6H, and one on 7H (Table 4). The remaining seven markers did not have any marker position information. The P values ranged between 9.65E-15 and 4.99E-04. The MAF values for all significant markers ranged from 5.16 to 46.22. The R^2 (phenotypic variation) for all significant markers was up to 14.78% (Table 4). Analysis of the isolate DEN 2.6 data identified a total of six loci with markers associated with resistance with one locus being on 1H (88.25 cM), one on 2H (69 cM), three on 3H (53.42, 65.16, and 150.19 to 154.47 cM), three on 4H (53.77 to 54.95, 59.22, and 96.6 to 96.99 cM), one on 5H (111.56 cM), one on 6H (59.21 cM), and one on 7H (0 cM) (Fig. 3A). From the stepwise regression, 20 markers were included in the stepwise regression model. The significant markers included in the stepwise regression

together explained 37.1% of the phenotypic variation for this isolate and markers from all 10 loci were included.

DISCUSSION

The BCC represents a worldwide base of diversity allowing for the use of historical recombination for association mapping of variation in responses to isolates of *P. teres* f. *maculata*; the cause of SFNB. The distribution of SFNB infection types across the BCC accessions analyzed indicated that susceptibility or resistance is polygenic in nature consistent with previous studies (33,44,68,69). The phenotyping data coupled with robust genotyping of the BCC accessions challenged with diverse *P. teres* f. *teres* isolates allowed for the capture of multiple MTAs. This AM analysis is the first report of AM of SFNB resistance in barley and includes worldwide diversity of the host challenged with isolates from regions around the globe.

The power of a mixed model to harness historical recombination events using AM is dependent on phenotyping, markers, population structure, and relatedness, thus requiring the testing of multiple models to determine which ones performed best for the specific isolate being analyzed (4,14,37). The mixed model which accounted for both population structure (Q) and kinship (K) was the best model for the Australian isolate SG1, the New Zealand isolate NZKF2, and the Denmark isolate DEN 2.6, whereas the model which only accounted for kinship was the best model for the U.S. isolate FGO. The AM analyses utilizing these models identified a total of 138 significant ($-\log_{10}(P)$ value > 3.0) MTAs. Using a 5 cM cutoff to define a QTL, a total of 10, 8, 13, and 10 loci associated with SFNB resistance/susceptibility were identified for FGO, SG1, NZKF2, and DEN 2.6, respectively. A total of 10 of the 41 loci identified were common to two or more of the isolates, resulting in a total of 27 distinct loci with six consistent to previously identified QTL and the remaining 21 representing putative novel SFNB resistance loci.

The analysis for MTAs was also conducted utilizing the more stringent positive false discovery rate (pFDR) < 0.1 (Tables 1 to 4). These analyses resulted in a reduction from the 138 significant markers using P value down to 105 significant markers using pFDR. The more stringent pFDR analysis reduced the number of significant loci identified to 7, 1, 13, and 7 loci for FGO, SG1, NZKF2, and DEN 2.6, respectively. The reduction of the FGO, SG1, and DEN 2.6 isolates from 10, 8 and 10 to 7, 1, 7 significant loci, respectively, is due to the higher stringency of pFDR analysis. The MTAs defining the locus on Ch 2H at ~69 cM (Fig. 3) was the one locus that was common to all four isolates and remained significant for SG1 using both P value and pFDR. Two other markers at one of the seven remaining loci that was significant using P value and were not significant with pFDR corresponded to the previously identified *Rpt6* QTL (Fig. 3). Since these two independent phenotyping data sets both identified these specific MTAs corresponding to a known resistance locus, it is unlikely that the loss of this locus using pFDR was due to a false association when using the P value. Thus, the P value analysis that identified two significant common markers between isolates SG1 and FGO indicates that these two isolates may carry effectors that target common host susceptibility genes underlying this locus, but these MTAs were eliminated when the pFDR was used. This suggested that when running association analyses on a diverse host population for complex quantitative traits the stringent pFDR may eliminate some important MTAs or loci so here we reported all loci identified using the less stringent P value analyses. However, the pFDR values were left in the tables for comparison.

We detected MTAs that corresponded to six loci on four chromosomes that were previously identified and mapped at low resolution by biparental QTL analyses. A common locus for isolates NZKF2 and DEN 2.6 was identified on chromosome 4H at position 53.67 to 59.22 cM explaining up to 16% of the phenotypic variation. There are 36 significant markers for the NZKF2 and DEN 2.6

TABLE 1. Single nucleotide polymorphism markers significantly associated with resistance/susceptibility to *Pyrenophora teres* f. *maculata* isolate FGO

Marker	Chr ^a	cM ^{a,b}	R^2 (%) ^c	MAF ^d	P value ^e	pFDR ^f
11_21392	1H	114.3	0.0330	13.446	3.09	0.152
12_11504	2H	69.55	0.0185	9.392	3.02	0.170
SCRI_RS_155161	2H	137.44	0.0003	31.62	3.75	0.069
SCRI_RS_221787	3H	99.26	0.0006	26.55	4.24	0.039
SCRI_RS_164704	3H	99.26	0.0004	26.42	3.73	0.069
SCRI_RS_133339	3H	99.56	0.0002	27.64	3.67	0.069
SCRI_RS_211929	3H	99.66	0.0007	27.43	4.25	0.039
SCRI_RS_235791	3H	99.66	0.0006	27.5	4.13	0.042
12_30423	3H	99.66	0.0013	32.43	3.56	0.080
SCRI_RS_131671	4H	99.68	0.0044	45.473	3.12	0.152
SCRI_RS_108416	5H	31.86	0.0033	39.26	4.66	0.038
SCRI_RS_205100	5H	31.86	0.0033	39.39	4.27	0.039
SCRI_RS_213566	6H	59.01	0.0316	26.892	3.11	0.152
SCRI_RS_188243	6H	59.21	0.0279	8.24	8.36	0.000
SCRI_RS_186193	6H	59.21	0.0252	10.27	6.25	0.001
12_30144	6H	60.21	0.0417	32.64	4.22	0.039
12_30530	7H	26.35	0.0192	36.01	3.55	0.080
SCRI_RS_179528	7H	26.92	0.0091	41.689	3.08	0.152
SCRI_RS_112204	7H	109	0.0086	49.8	3.76	0.069
SCRI_RS_202130	7H	133.84	0.0191	35.81	4.44	0.039
SCRI_RS_195914	N/A	N/A	0.0258	11.28	4.04	0.047
SCRI_RS_136604	N/A	N/A	0.0372	8.72	3.77	0.069
SCRI_RS_45644	N/A	N/A	0.0450	7.91	3.67	0.069
SCRI_RS_237419	N/A	N/A	0.0183	33.85	3.51	0.083
SCRI_RS_224297	N/A	N/A	0.0185	33.78	3.46	0.088
SCRI_RS_158011	N/A	N/A	0.0185	33.716	3.37	0.105
SCRI_RS_188305	N/A	N/A	0.0361	8.784	3.34	0.107
SCRI_RS_209824	N/A	N/A	0.0418	32.973	3.28	0.118
SCRI_RS_156620	N/A	N/A	0.0000	41.959	3.16	0.149

^a N/A indicates that the marker was not anchored to the consensus map.

^b Genetic distances in centimorgans (cM).

^c Phenotypic variation explained by individual markers.

^d Minor allele frequency (MAF).

^e $-\log_{10}(P)$ value for each individual marker calculated from the QK mixed model.

^f Positive false discovery rate (pFDR).

isolates at this locus with the markers for NZKF2 having the highest significance of any MTAs in the analysis. Neither of the genes underlying these SNP markers are predicted to encode susceptibility target/biotrophic resistance-like proteins, as characterized in the *Stagonospora nodorum*–wheat pathosystem (13). However, there are other significant SNP markers in the region in resistance-like genes such as leucine-rich receptor-like protein kinases. This region resides in the same region as *QRpts4* as reported by Grewal et al. (20), which explained 21% of the phenotypic variation in a doubled haploid (DH) population of CDC Dolly (susceptible) and TR251 (resistant). The QTL region reported by Friesen et al. (17) on chromosome 4H using the NZKF2 isolate on the DH population of SM89010/Q21861 is proximal to this region, but is close to the NZKF2, DEN 2.6, and FGO common locus at 99.68 to 103.58 cM that explained 8.3% of the phenotypic variation and the NZKF2 specific locus at 117.13 cM explaining 11.7% of the phenotypic variation.

Using the FGO and SG1 isolates, two identical markers were identified (SCRI_RS_108416 and SCRI_RS_205100) on chromosome 5H at position 31.86 that explained 0.1% of the phenotypic variation. This locus is probably the *QRpt6* QTL reported by Manninen et al. (39) that explained up to 84% of the phenotypic variation in a DH population derived from Rolfi (susceptible) and CI 9819 (resistant) using Finnish isolates. The reason for low phenotypic variation in our study might be due to the different populations and different isolates.

The MTAs detected on chromosome 6H at position 58.9 to 60.2 cM were common for DEN 2.6, NZKF2, and FGO and the phenotypic variation explained by this common locus was as high as 4%. This locus is located at a similar genetic interval as the previously described *QRpt6* QTL reported by Grewal et al. (20) in the Canadian breeding line TR251. This locus from line TR251 also exhibited resistance to net form net blotch (NFNB). Qamar et al. (52) also identified an NFNB resistance locus at the *QRpt6* region

Fig. 3. Association mapping of spot form net blotch (SFNB) isolates on the world barley core collection (BCC). Panels **A** to **D** show Manhattan plots generated from the disease reaction to four isolates of *Pyrenophora teres* f. *maculata*: **A**, isolate DEN 2.6 from Denmark; **B**, isolate NZKF2 from New Zealand; **C**, isolate SG1 from Australia; and **D**, isolate FGO from Fargo, ND (United States). All colored pixels represent single nucleotide polymorphism markers from the 9K Illumina genotyping on the seven barley chromosomes; from blue at the top as chromosome 1H and light blue at the bottom as chromosome 7H. All markers above the LOD threshold of 3.0 (P value($-\log_{10}$)) were significantly associated with SFNB resistance/susceptibility. Resistance/susceptibility loci are designated as colored bars. Orange bars are loci common to several isolates, yellow are specific to a single isolate, and gray are loci corresponding to quantitative trait loci (QTL) regions previously identified by biparental mapping. **E**, A map of the seven barley chromosomes is shown as colored vertical bars with chromosome designations given. The 27 resistance loci detected in these analyses are shown as horizontal bars with approximate centimorgan values given to the left and known SFNB resistance genes/QTL nomenclatures provided on the right.

on chromosome 6H, cosegregating with microsatellite marker Bmag0173 and Bmag0807 (52). The highly significant markers SCRI_RS_188243 (gene *MLOC_73854.4*) and SCRI-RS-186193 (gene *MLOC_16471.1*) for the FGO, NZKF2, and DEN 2.6 isolates were identified on chromosome 6H at 59.2 cM. These two common markers, with the gene designations *MLOC_73854.4* and *MLOC_16471.1*, are predicted to encode an unknown protein and an SPLA/Ryanodine receptor (SPRY) domain-containing protein, respectively. These two markers explained up to 2.8 and 2.3% of the phenotypic variation, respectively. From this AM panel data, it is premature to consider the genes containing these SNP markers as candidate host susceptibility or resistance genes; however, there are other markers and genes annotated in the region that code for R-like genes including NBS-LRR and serine/threonine protein kinase genes.

The *Rpt4* gene located on the long arm of chromosome 7H was the first SFNB resistance gene described in the cultivar Galleon. Later, *Rpt4* was mapped in several breeding lines and varieties such as CI9214, Keel, Chebec, and Tilga (68,69); however, this gene was not utilized in Australian breeding programs due to its lack of effectiveness at the adult plant stage (68). We identified one locus at the *Rpt4* region (26.4 to 26.9 cM) that explained up to 3% of the phenotypic variation with the U.S. isolate FGO, but interestingly was not detected using the Australian isolate SG1. This QTL may represent the seedling resistance gene *Rpt4* identified on the long arm of chromosome 7H (position 6.9 to 25.6 cM) flanked by the restriction fragment length polymorphic markers Xpsr117(D) and Xcdo673 (68). A minor effect locus at position 0.7 cM was also detected on chromosome 7H against isolate DEN 2.6 and a locus common to isolates FGO and NZKF2 was identified at position 132.6 to 133.8 cM explaining up to 5.1% of the phenotypic variation. We also identified two other loci at 109 cM and 145.68 to 150.36 cM close to this region that were identified using the FGO and NZKF2 isolates, respectively. Any of these loci could be the same as the *QRpt7* gene reported by Williams et al. (69) on chromosome 7H at position 116 to 134 cM. The identification of all six well-characterized SFNB resistance loci suggests that these AM analyses were robust and that the remaining 21 novel resistance loci may represent putative new sources of SFNB resistance or lack of susceptibility that could also be utilized in resistance breeding efforts.

TABLE 2. Single nucleotide polymorphism markers significantly associated with resistance/susceptibility to *Pyrenophora teres* f. *maculata* isolate SG1

Marker	Chr ^a	cM ^{a,b}	R ² (%) ^c	MAF ^d	P value ^e	pFDR ^f
11_20810	1H	50.00	0.001	38.649	3.17	0.271
SCRI_RS_188909	1H	98.45	0.008	27.365	3.22	0.262
SCRI_RS_151535	2H	65.71	0.000	27.905	3.98	0.147
SCRI_RS_175065	2H	65.81	0.000	24.73	3.76	0.157
SCRI_RS_154617	2H	66.11	0.000	23.58	4.80	0.064
11_10325	2H	66.11	0.000	24.73	3.52	0.181
11_10733	2H	66.11	0.000	24.865	3.36	0.207
SCRI_RS_159340	3H	88.17	0.042	10.068	3.65	0.157
12_30907	4H	32.43	0.005	11.351	3.11	0.287
SCRI_RS_9296	4H	47.17	0.003	20.338	3.09	0.287
SCRI_RS_108416	5H	31.86	0.008	39.257	3.71	0.157
SCRI_RS_205100	5H	31.86	0.008	39.392	3.48	0.181
SCRI_RS_151574	6H	101.83	0.000	30.946	3.04	0.300
SCRI_RS_209824	N/A	N/A	0.057	32.97	4.65	0.064
SCRI_RS_139690	N/A	N/A	0.020	6.08	4.42	0.071
11_20336	N/A	N/A	0.000	49.662	3.82	0.157
SCRI_RS_8401	N/A	N/A	0.001	47.568	3.45	0.181

^a N/A indicates that the marker was not anchored to the consensus map.

^b Genetic distances in centimorgans (cM).

^c Phenotypic variation explained by individual markers.

^d Minor allele frequency (MAF).

^e $-\log_{10}(P \text{ value})$ for each individual marker calculated from the QK mixed model.

^f Positive false discovery rate (pFDR).

The last remaining SFNB QTL previously reported by Molnar et al. (45) was reported as a major resistance gene on chromosome 2H. However, the location was not precise so it is difficult to determine if any of the 2H loci identified in this AM analysis are the same as that reported by Molnar et al. (45). We detected MTAs on chromosome 2H at ~24, 69, and 137 cM (Fig. 3). The locus identified at ~69 cM was the only locus identified with all four isolates tested (Fig. 3), and explained up to 5.6% of the phenotypic variation. Thus, this may be the best candidate for the major resistance reported by Molnar et al. (45), but due to the low

TABLE 3. Single nucleotide polymorphism markers significantly associated with resistance/susceptibility to *Pyrenophora teres* f. *maculata* isolate NZKF2

Marker	Chr ^a	cM ^{a,b}	R ² (%) ^c	MAF ^d	P value ^e	pFDR ^f
SCRI_RS_152744	2H	23.76	0.004	39.46	3.03	0.095
12_20878	2H	69	0.071	5.14	3.12	0.084
SCRI_RS_127994	3H	53.42	0.083	25.2	5.03	0.005
12_30785	3H	53.42	0.084	24.19	4.21	0.019
SCRI_RS_221787	3H	99.26	0.001	26.55	3.41	0.061
SCRI_RS_164704	3H	99.26	0.001	26.42	3.25	0.071
SCRI_RS_225641	3H	99.46	0.002	27.64	3.01	0.098
SCRI_RS_133339	3H	99.56	0.001	27.64	3.84	0.030
SCRI_RS_1355791	3H	99.66	0.001	27.5	4.11	0.020
SCRI_RS_211929	3H	99.66	0.001	27.43	3.95	0.025
12_30423	3H	99.66	0.001	32.43	3.56	0.052
SCRI_RS_167825	3H	103.46	0.048	29.46	4.14	0.020
SCRI_RS_163092	3H	103.86	0.048	28.04	4.05	0.021
SCRI_RS_156315	3H	153.39	0.030	42.7	3.07	0.091
SCRI_RS_157310	4H	53.67	0.011	37.16	3.38	0.061
SCRI_RS_128723	4H	54.66	0.139	30.95	5.93	0.001
SCRI_RS_155554	4H	54.66	0.119	30.41	5.56	0.002
SCRI_RS_208828	4H	54.66	0.154	37.43	5.53	0.002
SCRI_RS_221172	4H	54.66	0.137	35.2	4.99	0.005
11_20135	4H	54.95	0.087	22.3	11.43	0.000
11_10262	4H	54.95	0.128	43.31	9.57	0.000
12_31462	4H	54.95	0.132	28.24	7.24	0.000
11_20450	4H	54.95	0.055	17.91	4.37	0.016
11_20412	4H	54.95	0.077	46.22	4.28	0.017
SCRI_RS_168496	4H	54.95	0.134	27.36	4.14	0.020
SCRI_RS_141730	4H	54.95	0.160	33.58	4.09	0.020
SCRI_RS_228477	4H	54.95	0.132	26.89	3.72	0.039
11_10568	4H	54.95	0.138	41.22	3.69	0.039
12_30839	4H	54.95	0.037	28.11	3.50	0.053
11_20020	4H	54.95	0.106	37.84	3.26	0.071
11_10509	4H	55.64	0.065	40.47	3.41	0.061
SCRI_RS_189180	4H	57.32	0.136	47.09	3.49	0.054
SCRI_RS_148330	4H	103.48	0.090	18.65	3.24	0.071
SCRI_RS_192689	4H	103.58	0.017	40.27	4.34	0.016
11_21035	4H	117.13	0.119	47.23	3.04	0.094
12_11298	5H	111.56	0.003	11.69	5.18	0.004
SCRI_RS_188243	6H	59.21	0.018	8.24	7.49	0.000
SCRI_RS_176650	6H	59.21	0.010	20.41	4.56	0.012
SCRI_RS_186193	6H	59.21	0.014	10.27	3.21	0.073
12_11477	7H	78.07	0.003	10.07	3.23	0.071
12_11536	7H	78.07	0.003	10.07	3.23	0.071
12_31000	7H	78.07	0.003	10.07	3.23	0.071
SCRI_RS_202130	7H	133.84	0.006	35.81	4.47	0.013
11_20847	7H	145.68	0.009	40.95	3.55	0.052
11_10687	7H	146.03	0.012	40.54	3.4	0.061
12_10677	7H	150.36	0.019	10.81	3.07	0.091
SCRI_RS_208732	N/A	N/A	0.128	43.51	9.93	0.000
SCRI_RS_147636	N/A	N/A	0.124	44.12	8.74	0.000
11_21017	N/A	N/A	0.062	16.82	4.01	0.022
12_20803	N/A	N/A	0.002	14.26	3.51	0.053
SCRI_RS_156237	N/A	N/A	0.039	31.62	3.35	0.064
SCRI_RS_133327	N/A	N/A	0.120	24.32	3.26	0.071
SCRI_RS_145381	N/A	N/A	0.002	48.78	3.19	0.074
SCRI_RS_146785	N/A	N/A	0.002	49.19	3.17	0.077

^a N/A indicates that the marker was not anchored to the consensus map.

^b Genetic distances in centimorgans (cM).

^c Phenotypic variation explained by individual markers.

^d Minor allele frequency (MAF).

^e $-\log_{10}(P \text{ value})$ for each individual marker calculated from the QK mixed model.

^f Positive false discovery rate (pFDR).

resolution of the mapping in the Leger × CI 9831 DHL population this is only speculation.

We have possibly detected all the major and minor SFNB resistance QTL previously reported on barley chromosomes 2H, 4H, 5H, 6H, and 7H (17,20,23,39,45,68,69) and new QTL present on all seven barley chromosomes. Similarly, NFNB resistance QTL have been identified throughout the genome (33); however, resistance to both forms of the net blotch pathogen were rarely identified in similar locations (20,39), suggesting that the majority of resistances are distinct for the different forms of *P. teres* and in regards to breeding, the two need to be treated as separate diseases. Growing barley cultivars with genetic resistance to SFNB and NFNB is the most sustainable strategy of disease management (33,44). However, the diversity of virulence/avirulence genes within the pathogen populations and complexity of the interaction with host susceptibility targets and resistance genes requires robust genetic characterization of both the host and pathogen to understand the underlying genetic interactions involved. To date, only 11 different genotypes from the primary barley germplasm pool had been genetically characterized for SFNB resistance, thus deeper screening was required to identify diverse resistances or lack of functional susceptibility targets. This was accomplished in this study utilizing AM to capture diverse loci associated with SFNB resistance/susceptibility.

TABLE 4. Single nucleotide polymorphism markers significantly associated with resistance/susceptibility to *Pyrenophora teres* f. *maculata* isolate DEN 2.6

Marker	Chr ^a	cM ^{a,b}	R ² (%) ^c	MAF ^d	P value ^e	pFDR ^f
11_20792	1H	88.25	0.015	31.689	3.02	0.141
12_20878	2H	69.00	0.086	5.135	3.11	0.127
SCRI_RS_127994	3H	53.42	0.077	25.203	3.07	0.131
11_21305	3H	65.16	0.052	37.7	3.95	0.033
SCRI_RS_229623	3H	150.19	0.029	16.55	3.38	0.088
12_10014	3H	154.47	0.053	22.03	3.47	0.086
SCRI_RS_168580	4H	53.77	0.031	16.22	3.39	0.088
SCRI_RS_184107	4H	53.77	0.031	16.22	3.39	0.088
12_30878	4H	53.87	0.024	14.257	3.21	0.111
SCRI_RS_128723	4H	54.66	0.147	30.95	7.51	0.000
SCRI_RS_155554	4H	54.66	0.124	30.41	5.85	0.001
SCRI_RS_208828	4H	54.66	0.148	37.43	5.49	0.002
SCRI_RS_221172	4H	54.66	0.141	35.2	5.47	0.002
11_20135	4H	54.95	0.109	22.3	14.02	0.000
11_10262	4H	54.95	0.145	43.31	10.33	0.000
11_20412	4H	54.95	0.094	46.22	5.76	0.001
11_20450	4H	54.95	0.069	17.91	5.62	0.001
12_31462	4H	54.95	0.124	28.24	5.34	0.002
11_20472	4H	54.95	0.057	20.34	4.46	0.013
12_30839	4H	54.95	0.039	28.11	3.71	0.054
SCRI_RS_168496	4H	54.95	0.130	27.36	3.42	0.088
SCRI_RS_141730	4H	54.95	0.149	33.581	3.08	0.131
SCRI_RS_147712	4H	59.22	0.097	17.43	3.97	0.033
SCRI_RS_163033	4H	59.22	0.095	17.3	3.35	0.092
11_20762	4H	96.6	0.075	37.432	3.22	0.111
12_10666	4H	96.99	0.075	38.51	3.30	0.099
12_11298	5H	111.56	0.001	11.69	4.19	0.021
SCRI_RS_188243	6H	59.21	0.024	8.24	8.77	0.000
SCRI_RS_176650	6H	59.21	0.014	20.41	5.32	0.002
SCRI_RS_186193	6H	59.21	0.020	10.27	4.27	0.018
11_21419	7H	0.00	0.013	41.284	3.14	0.121
SCRI_RS_208732	N/A	N/A	0.144	43.51	10.17	0.000
SCRI_RS_147636	N/A	N/A	0.139	44.12	9.28	0.000
11_21017	N/A	N/A	0.082	16.82	6.10	0.001
SCRI_RS_161627	N/A	N/A	0.015	9.12	3.62	0.063
SCRI_RS_114164	N/A	N/A	0.082	26.351	3.28	0.100
SCRI_RS_156237	N/A	N/A	0.041	31.622	3.18	0.113
SCRI_RS_168610	N/A	N/A	0.098	18.311	3.01	0.142

^a N/A indicates that the marker was not anchored to the consensus map.

^b Genetic distances in centimorgans (cM).

^c Phenotypic variation explained by individual markers.

^d Minor allele frequency (MAF).

^e $-\log_{10}(P \text{ value})$ for each individual marker calculated from the QK mixed model.

^f Positive false discovery rate (pFDR).

A recent review of host specific toxins (also referred to as necrotrophic effectors) produced by the Pleosporales necrotrophic pathogens in the Dothideomycete class suggested that necrotrophic effectors (NE) predominantly interact with dominant host susceptibility gene products (59). These interactions followed the inverse-gene-for-gene model (16) or NE triggered susceptibility (NETS) model (35,56), resulting in quantitative susceptibility to these pathogens which can also be viewed as quantitative recessive resistance. The reports of proteinaceous and nonproteinaceous toxins being produced by *P. teres* f. *maculata* and *P. teres* f. *teres* (reviewed in Liu et al. 2011 [33]) as well as mapping of distinct *P. teres* f. *teres* virulence QTL targeting regions of dominant susceptibility in barley (35,56), suggests that the NETS model may be at least partially responsible for virulence in *P. teres* f. *maculata*. Thus, the accumulation of distinct NE genes spread throughout the genome of the pathogen that theoretically interact with host susceptibility genes, facilitates the pathogens ability to induce disease. The diversity of NEs within pathogen populations explains the variability in virulence of *P. teres* f. *maculata* isolates collected from around the world and may explain why Tekauz (62) was unable to identify effective resistance sources against isolates collected in western Canada.

P. teres f. *maculata* collections from local and global fungal populations show wide variations for virulence on differential barley lines (reviewed in Liu et al. 2011 [33]). This indicates that the variability in virulence gives the pathogen the potential to shift the population toward the prevalence of NEs that target susceptibility factors within popular cultivars resulting in what appears to be virulence on the limited sources of resistance present (2,7,30,33,42,56,62,70). The frequency of virulent genotypes in pathogen populations can rapidly increase as popular cultivars with incomplete resistance or lack of some susceptibility exert selection pressure. Also, these sexual pathogen populations allows for recombination of virulence genes giving rise to new possibly more virulent isolates containing several effectors that were selected for and brought together by recombination. Because of these complex interactions, breeding strategies focused on developing highly resistant lines must combine multiple loci, with some lacking host susceptibility targets and possibly others harboring active resistance loci. Knowledge of these host–pathogen genetic interactions and the genes/loci determining both lack of susceptibility and resistance is critical for intelligent deployment of SFNB resistant lines. These AM analyses provide very important information on these susceptibility/resistance loci but to effectively utilize new loci containing nonfunctional SFNB susceptibility targets and/or resistance from the BCC, further genetic characterization of the host–pathogen genetic interactions through targeted biparental populations of both the host and pathogen is needed. We are further validating the distinct QTL identified by preparing DH and recombinant inbred line populations by crossing susceptible malting cultivars with highly resistant BCC accessions that we determined are missing the susceptibility targets or containing the major resistances. These populations will expedite the development of elite breeding lines that can be utilized in the development of resistant cultivars that can be grown in the upper Midwestern region of the United States and elsewhere, whether the mechanism is through the removal of susceptibility factors or the incorporation of resistance.

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LITERATURE CITED

1. Agrama, H. A., Eizenga, G. C., and Yan, W. 2007. Association mapping of yield and its components in rice cultivars. *Mol. Breed.* 19:341-356.

2. Arabi, M., Al-Safadi, I. E., and Charbaji, B. T. 2003. Pathogenic variation among isolates of *Pyrenophora teres* the causal agent of barley net blotch. *J. Phytopathol.* 151:376-382.
3. Aranzana, M. J., Kim, S., Zhao, K., Bakker, E., Horton, M., Jakob, K., Lister, C., Molitor, J., Shindo, C., Tang, C., Toomajian, C., Traw, B., Zheng, H., Bergelson, J., Dean, C., Marjoram, P., and Nordborg, M. 2005. Genome-wide association mapping in *Arabidopsis* identifies previously known flowering time and pathogen resistance genes. *PLoS Genet.* 1:e60.
4. Atwell, S., Huang, Y. S., Vilhjálmsson, B., Willems, G., Horton, M., Li, Y., Meng, D., Platt, A., Tarone, A. M., Hu, T. T., Jiang, R., Mulyati, N. W., Zhang, X., Amer, M. A., Baxter, I., Brachi, B., Chory, J., Dean, C., Debieu, M., de Meaux, J., Ecker, J. R., Faure, N., Kniskern, J. M., Jones, J. D. G., Michael, T., Nemri, A., Roux, F., Salt, D. E., Tanf, C., Todesco, M., Traw, M. B., Weigel, D., Marjoram, P., Borevitz, J. O., Bergelson, J., and Nordborg, M. 2010. Genome-wide association study of 107 phenotypes in *Arabidopsis thaliana* inbred lines. *Nature* 465:627-631.
5. Beattie, A. D., Edney, M. J., Scoles, G. J., and Rossnagel, B. G. 2010. Association mapping of malting quality data from western Canadian two-row barley cooperative trials. *Crop Sci.* 50:1649-1663.
6. Berger, G. L., Liu, S., Hall, M. D., Chao, S., Muehlbauer, G. J., Baik, B.-K., Steffenson, B., and Griffey, C. A. 2013. Marker-trait associations in Virginia Tech winter barley identified using genome-wide mapping. *Theor. Appl. Genet.* 126:693-710.
7. Bockelman, H. E., Sharp, E. L., and Bjarko, M. E. 1983. Isolates of *Pyrenophora teres* from Montana and the Mediterranean region that produce spot type lesions on barley. *Plant Dis.* 67:696-697.
8. Breseghello, F., and Sorrells, M. E. 2006. Association mapping of kernel size and milling quality in wheat (*Triticum aestivum* L.) cultivars. *Genetics* 172:1165-1177.
9. Brown, M. P., Steffenson, B. J., and Webster, R. K. 1993. Host range of *Pyrenophora teres* f. *teres* isolates from California. *Plant Dis.* 77:942-947.
10. Earl, D. A., and von Holdt, B. M. 2011. STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* 4:1-3.
11. Ehrenreich, I. M., Hanzawa, Y., Chou, L., Roe, J. L., Kover, P. X., and Purugganan, M. D. 2009. Candidate gene association mapping of *Arabidopsis* flowering time. *Genetics* 183:325-335.
12. Evanno, G., Regnaut, S., and Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Mol. Ecol.* 14:2611-2620.
13. Faris, J. D., Zhang, Z., Lu, H., Lu, S., Reddy, L., Cloutier, S., Fellers, J. P., Meinhardt, S. W., Rasmussen, J. B., Xu, S. S., Oliver, R. P., Simons, K. J., and Friesen, T. L. 2010. A unique wheat disease resistance-like gene governs effector-triggered susceptibility to necrotrophic pathogens. *Proc. Natl. Acad. Sci. USA* 107:13544-13549.
14. Flint, J., and Mackay, T. F. 2009. Genetic architecture of quantitative traits in mice, flies, and humans. *Genome Res.* 19:723-733.
15. Flint-Garcia, S. A., Thornsberry, J. M., and Buckler, E. S. 2003. Structure linkage disequilibrium in plants. *Annu. Rev. Plant Biol.* 54:357-374.
16. Friesen, T. L., and Faris, J. D. 2010. Characterization of the wheat-*Stagonospora nodorum* disease system: What is the molecular basis of this quantitative necrotrophic disease interaction? *Can. J. Plant Pathol.* 32: 20-28.
17. Friesen, T. L., Faris, J. D., Lai, Z., and Steffenson, B. J. 2006. Identification and chromosomal location of major genes for resistance to *Pyrenophora teres* in a doubled-haploid barley population. *Genome* 49: 855-859.
18. Friesen, T. L., Faris, J. D., Solomon, P. S., and Oliver, R. P. 2008. Host specific toxins: effectors of necrotrophic pathogenicity. *Cell. Microbiol.* 10:1421-1428.
19. Ghavami, F., Elias, E. M., Mamidi, S., Ansari, O., Sargolzaei, M., Adhikari, T., Mergoum, M., and Kianian, S. F. 2011. Mixed model association mapping for Fusarium head blight resistance in Tunisian-derived durum wheat populations. *G3: Genes Genomes Genetics* 1:209-218.
20. Grewal, T. S., Rossnagel, B. G., Pozniak, C. J., and Scoles, G. J. 2008. Mapping quantitative trait loci associated with barley net blotch resistance. *Theor. Appl. Genet.* 116:529-539.
21. Gupta, S., and Loughman, R. 2001. Current virulence of *Pyrenophora teres* on barley in Western Australia. *Plant Dis.* 85:960-966.
22. Gutierrez, L., Cuesta-Marcos, A., Castro, A. J., von Zitzewitz, J., Schmitt, M., and Hayes, P. M. 2010. Association mapping of malting quality quantitative trait loci in winter barley: Positive signals from small germplasm arrays. *Plant Genome* 4:256-272.
23. Ho, K. M., Tekauz, A., Choo, T. M., and Martin, R. A. 1996. Genetic studies on net blotch resistance in a barley cross. *Can. J. Plant Sci.* 76: 715-719.
24. Honsdorf, N., Becker, H. C., and Ecker, W. 2010. Association mapping for phenological, morphological, and quality traits in canola quality winter rapeseed (*Brassica napus* L.). *Genetics* 53:899-907.
25. Ivandic, V., Hackett, C. A., Nevo, E., Keith, R., Thomas, W. T. B., and Forster, B. P. 2002. Analysis of simple sequence repeats (SSRs) in wild barley from the Fertile Crescent: Associations with ecology, geography and flowering time. *Plant Mol. Biol.* 48:511-527.
26. Ivandic, V., Thomas, W. T. B., Nevo, E., Zhang, Z., and Forster, B. P. 2003. Associations of simple sequence repeats with quantitative trait variation including biotic and abiotic stress tolerance in *Hordeum spontaneum*. *Plant Breed.* 122:300-304.
27. Jayasena, K. W., van Burgel, A., Tanaka, K., Mejewski, J., and Loughman, R. 2007. Yield reduction in barley in relation to spot-type net blotch. *Australas. Plant Pathol.* 36:429-433.
28. Jordan, V. W., and Allen, E. 1984. Barley net blotch: Influence of straw disposal and cultivation methods on inoculum potential, and on incidence and severity of autumn disease. *Plant Pathol.* 33:547-559.
29. Karki, C. B., and Sharp, E. L. 1986. Pathogenic variation in some isolates of *Pyrenophora teres* f. sp. *maculata* on barley. *Plant Dis.* 70:684-687.
30. Khan, T. N., and Tekauz, A. 1982. Occurrence and pathogenicity of *Drehslera teres* isolates causing spot-type symptoms on barley in Western Australia. *Plant Dis.* 66:423-425.
31. Kraakman, A. T. W., Rients, E. N., Petra, M. M., Van den Berg, M., Stam, P., and Van Eeuwijk, F. A. 2004. Linkage disequilibrium mapping of yield and yield stability in modern spring barley cultivars. *Genetics* 168: 435-446.
32. Kump, K. L., Bradbury, P. J., Wissner, R. J., Buckler, E. S., Belcher, A. R., Oropeza-Rosas, M. A., Zwonitzer, J. C., Kresovich, S., McMullen, M. D., Ware, D., Balint-Kurti, P. J., and Holland, J. B. 2011. Genome-wide association study of quantitative resistance to southern leaf blight in the maize nested association mapping population. *Nat. Genet.* 43:163-168.
33. Liu, Z. H., Ellwood, S. R., Oliver, R. P., and Friesen, T. L. 2011. *Pyrenophora teres*: Profile of an increasingly damaging barley pathogen. *Mol. Plant Pathol.* 12:1-19.
34. Liu, Z. H., and Friesen, T. L. 2010. Identification of *Pyrenophora teres* f. *maculata*, causal agent of spot type net blotch of barley in North Dakota. *Plant Dis.* 94:480.
35. Liu, Z. H., Holmes, D. J., Faris, J. D., Chao, S., Brueggeman, R. S., Edwards, M. C., and Friesen, T. L. 2015. Necrotrophic effector-triggered susceptibility (NETS) underlies the barley-*Pyrenophora teres* f. *teres* interaction specific to chromosome 6H. *Mol. Plant Pathol.* 16: 188-200.
36. Malosetti, M., van der Linden, C. G., Vosman, B., and van Eeuwijk, F. A. 2007. A mixed-model approach to association mapping using pedigree information with an illustration of resistance to *Phytophthora infestans* in potato. *Genetics* 175:879-889.
37. Mamidi, S., Chikara, S., Goos, R. J., Hyten, D. L., Annam, D., Moghaddam, S. M., Lee, R. K., Cregan, P. B., and McClean, P. E. 2011. Genome-wide association analysis identifies candidate genes associated with iron deficiency chlorosis in soybean. *Plant Genome* 4:154-160.
38. Mamidi, S., Lee, R. K., Goos, J. R., and McClean, P. E. 2014. Genome-wide association studies identifies seven major regions responsible for iron deficiency chlorosis in soybean (*Glycine max*). *PLoS ONE* 9: e107469.
39. Manninen, O. M., Jalli, M., Kalender, R., Schulman, A., Afanasenko, O., and Robinson, J. 2006. Mapping of major spot-type and net-type net-blotch resistance genes in the Ethiopian barley line CI9819. *Genome* 49: 1564-1571.
40. Massman, J., Cooper, B., Horsley, R., Neate, S., Dill-Macky, R., Chao, S., Dong, Y., Muehlbauer, G., and Smith, K. 2011. Genome-wide association mapping of Fusarium head blight resistance in contemporary barley breeding germplasm. *Mol. Breed.* 27:439-454.
41. Mathre, D. E. 1997. Compendium of Barley Diseases, 2nd ed. American Phytopathological Society, St. Paul, MN.
42. 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 Pasture Sci.* 60:303-315.
43. McLean, M. S., Howlett, B. J., and Hollaway, G. J. 2010. Spot form of net blotch, caused by *Pyrenophora teres* f. *maculata*, is the most prevalent foliar disease of barley in Victoria, Australia. *Australas. Plant Pathol.* 39: 46-49.
44. McLean, M. S., Howlett, B. J., Turkington, T. K., Platz, G. J., and Hollaway, G. J. 2012. Spot form of net blotch resistance in a diverse set of barley lines in Australia and Canada. *Plant Dis.* 96:569-576.
45. Molnar, S. J., James, L. E., and Kasha, K. J. 2000. Inheritance and RAPD tagging of multiple genes for resistance to net blotch in barley. *Genome* 43:224-231.
46. Muñoz-Amatrián, M., Cuesta-Marcos, A., Endelman, J. B., Comadran, J., Bonman, J. M., Bockelman, H. E., Chao, S., Russell, J., Waugh, R., Hayes, P. M., and Muehlbauer, G. J. 2014. The USDA barley core collection: Genetic diversity, population structure, and potential for genome-wide association studies. *PLoS ONE* 9:e94688.

47. Murray, G. M., and Brennan, J. P. 2010. Estimating disease losses to the Australian barley industry. *Australas. Plant Pathol.* 39:85-96.
48. Myles, S., Peiffer, J., Brown, P. J., Ersoz, E. S., Zhang, Z., Costich, D. E., and Buckler, E. D. 2009. Association mapping: Critical consideration shift from genotyping to experimental design. *Plant Cell* 21:2194-2202.
49. Neupane, A., Tamang, P., Brueggeman, R., and Friesen, T. 2015. Evaluation of a barley core collection for spot form net blotch reaction reveals distinct genotype-specific pathogen virulence and host susceptibility. *Phytopathology* 105:509-517.
50. Oliver, R. P., Friesen, T. L., Faris, J. D., and Solomon, P. S. 2012. *Stagonospora nodorum*: From pathology to genomics and host resistance. *Annu. Rev. Phytopathol.* 50:23-43.
51. Pakniyat, H., Powell, W., Baird, E., Handley, L. L., Robinson, D., Scrimgeour, C. M., Nevo, E., Hackett, C. A., Caligari, P. D. S., and Forster, B. P. 1997. AFLP variation in wild barley (*Hordeum spontaneum* C. Koch) with reference to salt tolerance and associated ecogeography. *Genome* 40:332-341.
52. Qamar, M. A., Liu, Z. H., Faris, J. D., Chao, S., Edwards, M. C., Lai, Z., Frackowiak, J. D., and Friesen, T. L. 2008. A region of barley chromosome 6H harbors multiple major genes associated with net type net blotch resistance. *Theor. Appl. Genet.* 117:1261-1270.
53. Remington, D. L., Thornsberry, J. M., Matsuoka, Y., Wilson, L. M., Whitt, S. R., Doebley, J., Kresovich, S., Goodman, M. M., and Buckler, E. S., IV. 2001. Structure of linkage disequilibrium and phenotypic associations in the maize genome. *PNAS* 98:11479-11484.
54. Roy, J. K., Smith, K. P., Muehlbauer, G. J., Chao, S., Close, T. J., and Steffenson, B. J. 2010. Association mapping of spot blotch resistance in wild barley. *Mol. Breed.* 26:243-256.
55. Scheet, P., and Stephens, M. 2006. A fast and flexible statistical model for large-scale population genotype data: Applications to inferring missing genotypes and haplotypic phase. *Am. J. Hum. Genet.* 78:629-644.
56. Shjerve, R. A., Faris, J. D., Brueggeman, R. S., Yan, C., Zhu, Y., Koladia, V., and Friesen, T. L. 2014. Evaluation of *Pyrenophora teres* f. *teres* mapping population reveals multiple independent interactions with a region of barley chromosome 6H. *Fungal Genet. Biol.* 70:104-112.
57. Smedegård-Petersen, V. 1971. Kgl. Vet. Landbohøjsk. Arsskr., *Pyrenophora teres* f. *maculata* f. nov. and *Pyrenophora teres* f. *teres* on barley in Denmark. Copenhagen, Denmark, Royal Veterinary and Agricultural University, Pages 124-144.
58. Steffenson, B. J., and Webster, R. K. 1992. Pathotype diversity of *Pyrenophora teres* f. *teres* on barley. *Phytopathology* 82:170-177.
59. Stergiopoulos, I., Collemare, J., Mehrabi, R., and De Wit, P. J. G. M. 2013. Phytotoxic secondary metabolites and peptides produced by plant pathogenic Dothideomycete fungi. *FEMS Microbiol. Rev.* 37:67-93.
60. Stich, B., and Melchinger, A. E. 2009. Comparison of mixed-model approaches for association mapping in rapeseed, potato, sugar beet, maize, and Arabidopsis. *BMC Genomics* 10:94.
61. Storey, J. D. 2002. A direct approach to false discovery rates. *J. R. Stat. Soc. Series B Stat. Methodol.* 64:479-498.
62. Tekauz, A. 1990. Characterization and distribution of pathogenic variation in *Pyrenophora teres* f. *teres* and *P. teres* f. *maculata* from western Canada. *Can. J. Plant Pathol.* 12:141-148.
63. Tommasini, L., Schnurbusch, T., Fossati, D., Mascher, F., and Keller, B. 2007. Association mapping of *Stagonospora nodorum* blotch resistance in modern European winter wheat varieties. *Theor. Appl. Genet.* 115:697-708.
64. Tuohy, J. M., Jalli, M., Cooke, B. M., and Sullivan, E. O. 2006. Pathogenic variation in populations of *Drechslera teres* f. *teres* and *D. teres* f. *maculata* and differences in host cultivar responses. *Eur. J. Plant Pathol.* 116:177-185.
65. Von Zitzewitz, J., Cuesta-Marcos, A., Condon, F., Castro, A., Chao, S., Corey, A., Filichkin, T., Fisk, S., Gutierrez, L., Haggard, K., Karsai, I., Muehlbauer, G., Smith, K., Veisz, O., and Hayes, P. 2011. The genetics of winter hardiness in barley: Perspectives from genome-wide association mapping. *Plant Genome* 4:76-91.
66. Wang, J., McClean, P. E., Lee, R., Goos, R. J., and Helms, T. 2008. Association mapping of iron deficiency chlorosis loci in soybean (*Glycine max* L. Merr.) advanced breeding lines. *Theor. Appl. Genet.* 116:777-787.
67. Weber, A. L., Briggs, W. H., Rucker, J., Baltazar, B. M., Sanchez-Gonzalez, J. D., Feng, P., Buckler, E. S., and Doebley, J. F. 2008. The genetic architecture of complex traits in teosinte (*Zea mays* ssp. *paryglumis*): New evidence from association mapping. *Genetics* 180:1221-1232.
68. Williams, K. J., Lichon, A., Gianquitto, P., Kretschmer, J. M., Karakousis, A., Manning, S., Langridge, P., and Wallwork, H. 1999. Identification and mapping of a gene conferring resistance to the spot form of net blotch (*Pyrenophora teres* f. *maculata*) in barley. *Theor. Appl. Genet.* 99:323-327.
69. Williams, K. J., Platz, G. J., Barr, A. R., Cheong, J., Willmsmore, K., Cakir, M., and Wallwork, H. 2003. A comparison of the genetics of seedling and adult plant resistance to the spot form of net blotch (*Pyrenophora teres* f. *maculata*). *Aust. J. Agric. Res.* 54:1387-1394.
70. Wu, H.-L., Dunkle, L. D., and Ciuffetti, L. M. 2003. Genetic variation for virulence and RFLP markers in *Pyrenophora teres*. *Can. J. Plant Pathol.* 25:82-90.
71. Youcef-Benkada, M., Bendahmane, B. S., Sy, A. A., Barrault, G., and Albertini, L. 1994. Effects of inoculation of barley inflorescences with *Drechslera teres* upon the location of seed-borne inoculum and its transmission to seedlings as modified by temperature and soil moisture. *Plant Pathol.* 43:350-355.
72. Yu, J., Pressoir, G., Briggs, W. H., Bi, I. V., Yamasaki, M., Doebley, J. F., McMullen, M. D., Gaut, B. S., Nielsen, D. M., Holland, J. B., Kresovich, S., and Buckler, E. S. 2006. A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. *Nat. Genet.* 38:203-208.
73. Zhao, K., Aranzana, M. J., Kim, S., Lister, C., Shindo, C., Tang, C., Toomajian, C., Zheng, H., Dean, C., Marjoram, P., and Nordberg, M. 2007. An Arabidopsis example of association mapping in structured samples. *PLoS Genet.* 3:e4.
74. Zhou, X., and Stephens, M. 2012. Genome-wide efficient mixed-model analysis for association studies. *Nat. Genet.* 44:821-824.
75. Zhu, C., Gore, M., Buckler, E. S., and Yu, J. 2008. Status and prospects of association mapping in plants. *Plant Genome* 1:5-20.
76. Zou, J., Jiang, C., Cao, Z., Li, R., Long, Y., Chen, S., and Meng, J. 2010. Association mapping of seed oil content in *Brassica napus* and comparison with quantitative trait loci identified from linkage mapping. *Genome* 53:908-916.