Theor Appl Genet (2015) 128:489–499
DOI 10.1007/s00122-014-2447-z

ORIGINAL PAPER

Spot form of net blotch resistance in barley is under complex genetic control

Xuemin Wang · Emma S. Mace · Gregory J. Platz · Colleen H. Hunt · Lee T. Hickey · Jerome D. Franckowiak · David R. Jordan

Received: 6 August 2014 / Accepted: 17 December 2014 / Published online: 10 January 2015

Abstract

Key message **Evaluation of resistance to *Pyrenophora teres* f. *maculata* in barley breeding populations via association mapping revealed a complex genetic architecture comprising a mixture of major and minor effect genes.**

Abstract In the search for stable resistance to spot form of net blotch (*Pyrenophora teres* f. *maculata*, SFNB), association mapping was conducted on four independent barley (*Hordeum vulgare* L.) breeding populations comprising a total of 898 unique elite breeding lines from the Northern Region Barley Breeding Program in Australia for discovery of quantitative trait loci (QTL) influencing resistance at seedling and adult plant growth stages. A total of 29 significant QTL were validated across multiple breeding populations, with 22 conferring resistance at both seedling and adult plant growth stages. The remaining 7 QTL conferred resistance at either seedling (2 QTL) or adult plant (5 QTL) growth stages only. These 29 QTL represented 24 unique genomic regions, of which five were found to co-locate with previously identified QTL for SFNB. The results indicated that SFNB resistance is controlled by a large number of QTL varying in effect size with large effects QTL on chromosome 7H. A large proportion of the QTL acted in the same direction for both seedling and adult responses, suggesting that phenotypic selection for SFNB resistance performed at either growth stage could achieve adequate levels of resistance. However, the accumulation of specific resistance alleles on several chromosomes must be considered in molecular breeding selection strategies.

Communicated by Xiaoquan Qi.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-014-2447-z) contains supplementary material, which is available to authorized users.

X. Wang · C. H. Hunt · D. R. Jordan
Queensland Alliance for Agriculture and Food Innovation, Hermitage Research Facility, The University of Queensland, Warwick, QLD 4370, Australia

E. S. Mace · G. J. Platz (✉) · C. H. Hunt · J. D. Franckowiak
Department of Agriculture, Fisheries and Forestry, Hermitage Research Facility, Warwick, QLD 4370, Australia
e-mail: Greg.Platz@daff.qld.gov.au

L. T. Hickey
Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, St Lucia, QLD 4072, Australia

Present Address:
J. D. Franckowiak
Department of Agronomy and Plant Genetics, University of Minnesota Twin Cities, St Paul, MN 55108, USA

Introduction

Net blotch, caused by *Pyrenophora teres* Drechsler [anamorph *Drechslera teres* (Sacc.) Shoem], is an important foliar disease of barley (*Hordeum vulgare*) worldwide (Liu et al. 2012; McLean et al. 2009). Infection with net blotch results in economic loss through reduced thousand-kernel weight and test weight (Mathre 1997). Based on the type of leaf lesion, net blotch has been divided into two forms: spot form of net blotch (SFNB, also referred to as spot type net blotch), caused by *Pyrenophora teres* f. *maculata* (*Ptm*), and net form of net blotch (NFNB), caused by *Pyrenophora teres* f. *teres* (*Ptt*) (Smedegård-Petersen 1971). Both diseases over-season on infected stubble and have increased globally with the adoption of reduced or conservation tillage practices and increased frequency of barley cropping (McLean et al. 2009).

SFNB is considered a significant foliar disease of barley crops in America, Australia, Canada, Denmark, Finland, Hungary, Morocco, Norway, South Africa, Tunisia and Turkey (McLean 2011). Recent changes in the distribution of SFNB within countries have been observed. For instance in Australia, SFNB was first recorded in Western Australia in 1977 (Khan and Tekauz 1982); however, it is now present throughout all barley growing regions in Australia (Hollaway and McLean 2006; McLean et al. 2010; Platz and Usher 2006). Yield losses caused by SFNB have become increasingly severe in recent years (Liu et al. 2012). Potential average annual losses to SFNB have been estimated at AUD$192 million in Australia (Murray and Brennan 2010).

Even though SFNB can be managed by application of fungicides and cultural practices, the most cost-effective, practical and environmentally sustainable measure to reducing the impact of this disease is through the use of genetically resistant cultivars (McLean et al. 2009; Zhou and Steffenson 2013a). QTL conferring resistance to SFNB have been mapped on all chromosomes in barley (Cakir et al. 2011; Friesen et al. 2006; Grewal et al. 2008, 2012; Manninen et al. 2006; Molnar et al. 2000; Williams et al. 1999, 2003); however, all of these studies have used populations derived from bi-parental crosses. Considering the limitations of bi-parental mapping studies to identify multiple resistance alleles, a more efficient and powerful approach for mapping resistance loci is via association mapping (AM) using elite breeding materials. This removes the necessity for constructing large bi-parental populations and hence provides a vehicle for incorporating marker technologies into applied breeding programmes by bridging the gap between development and implementation. AM facilitates the discovery of QTL and possibly the casual polymorphism within the gene responsible for alternative phenotypes (Zhou and Steffenson 2013b). While AM has been applied to determine the underlying loci controlling resistance to spot blotch (Roy et al. 2010; Zhou and Steffenson 2013b), Septoria speckled leaf blotch (Zhou and Steffenson 2013a), powdery mildew (Hickey et al. 2012), and leaf rust (Hickey et al. 2011; Ziems et al. 2014), no studies report the application of AM to dissect the genetics of SFNB resistance in barley.

This study reports on a large-scale analysis of the genetic architecture of resistance to SFNB in seedling and adult plants using an AM approach in elite Australian barley breeding germplasm, which was generated from parental lines originating from Australia, Canada, China, Czech Republic, Ecuador, England, Germany, Japan, Mexico, New Zealand, Syria, Uruguay and the USA. Using globally diverse but related germplasm should greatly improve the breadth and depth of understanding this important trait. Furthermore, our results are integrated with those of previous SFNB studies, providing information that can be used by barley breeders to design selection strategies appropriate for development of cultivars with resistance to SFNB.

Materials and methods

Association mapping populations

The panel used in this AM study consisted of four two-rowed spring barley breeding populations of the Northern Region Barley Breeding Program (NRBBP) in Australia, totalling 898 unique lines, with 60 lines (i.e. 7 %) in common across the four populations. A list of all lines in the four breeding populations has been presented in supplementary Table S1. Breeding Population 1 (BP1) comprised 399 entries, including 386 Stage 2 (F_3:F_5) elite lines and introductions and 13 current Australian cultivars. Stage 2 trials, containing breeding lines undergoing their second year of yield evaluation, are trials that include selected entries from the Stage 1 (initial or preliminary) trials conducted the previous year. Stage 2 breeding lines were selected from Stage 1 yield trials conducted at Hermitage Research Facility (HRF), Warwick, Queensland in 2008. Breeding Population 2 (BP2) consisted of a total of 190 lines selected from 2010 yield trials of both Stage 1 and Stage 2 lines, elite germplasm, parental lines and eight Australian cultivars. Breeding populations 3 and 4 (BP3 and BP4) consisted of 133 and 248 lines, respectively. To enhance diversity in the material being studied, individuals of the four populations originated from, or were progeny from parental lines from, Australia, Canada, China, Czech Republic, Ecuador, England, Germany, Japan, Mexico, New Zealand, Syria, Uruguay and the USA. As the individuals from the four breeding populations were all derived from a single breeding programme, they have high levels of relatedness and shared parentage (ESM Figure S1).

Phenotyping seedlings for resistance

Seedlings were grown in the glasshouse at HRF. Five to seven seeds per line were sown per pot and fertilized twice weekly after emergence. Screening was conducted at HRF using field-collected conidia of two isolates of *Ptm*: SNB331 (only for seedling assessment in 2009) and SNB320, to represent virulences of *Ptm* common within the region.

Inoculation was performed at the two- to three-leaf stage (12–15 days after sowing). BP1 was inoculated with isolate SNB331 applied at 2 ml pot^{-1} in an aqueous suspension of 10,000 conidia ml^{-1} through a commercial airless spray gun (Krebs Model 25T). After inoculation, plants were immediately placed in a fogging chamber at 100 % relative humidity at 19 °C for 24 h, with the first 14 h in darkness,

then transferred to a growth room maintained at a temperature of 24/14 °C (day/night) under a 12-h photoperiod for disease development. Infection type on the second leaf was recorded 10 days after inoculation.

BP2, BP3 and BP4 were inoculated with isolate SNB320 at 3 ml pot^{-1} using a suspension of 6,667 conidia ml^{-1} through a gravity-fed, compressed air spray gun (Will Wick ® Model H828W). Plants were inoculated and maintained in the glasshouse at 25/14 °C (day/night) until infection type was scored 11 days later.

Infection types on the blade of second leaves were recorded using a 1–9 scale, where 1 and 9 indicate highly resistant and highly susceptible disease responses, respectively (Cakir et al. 2011). Entries were not replicated in 2009 (BP1), but were replicated twice in 2011 (BP2), 2012 (BP3) and 2013 (BP4).

Phenotyping adult plants for resistance

For each entry, 15–20 seeds were sown as hill plots in rows with in-row spacing of 50 cm and 76 cm between rows. Spreader rows (4 in 2009; 5 in 2011, 2012, and 2013) of the susceptible cultivar Dash were sown 2–3 weeks before the plots, parallel to and 76 cm from the data rows, so that each row of plots was 76 cm from the nearest spreader row. Treatments were sown in a randomised complete block design with two replicates for all four breeding populations.

Epidemics were initiated by spreading fresh plant material infected with isolate SNB320 over the susceptible spreader rows. Disease development was promoted with sprinkler irrigation applied in late afternoon when temperatures were favourable for disease and high humidity and low wind were forecasted (Ziems et al. 2014). After the epidemics were sufficiently developed to give good differentiation, line reactions were assessed on leaf blades based on whole plots using a 1–9 response scale during the secondary growth stages between 58 (emergence of inflorescence completed) and 75 (medium milk) (Zadoks et al. 1974).

Genotyping

DNA from mixed leaf blade tissue of 30 seedlings of each barley line was extracted using procedures recommended by Diversity Arrays Technology (Yarralumla, ACT 2600, Australia; http://www.diversityarrays.com) and sent to the company for genotyping. For BP1 and BP2, 368 and 155 lines were genotyped using the Barley PstI (BstNI) v 1.7 array, respectively, which in turn provided a total of 1,601 high-quality polymorphic DArT markers (1,411 for BP1 and 1,159 for BP2). BP3 (133 lines) and BP4 (248 lines) were analysed using the DArTseq genotyping-by-sequence (GBS) platform and resulted in a number of 10,608 high-quality polymorphic SNP markers.

Analysis of phenotypic data

For all experiments, except for seedling phenotypes for BP1, a linear mixed model was fitted to the raw data with genotypes as a fixed effect and replicates as random effects. ASReml-R (Butler et al. 2009) used the residual maximum likelihood (REML; Patterson and Thompson 1971) algorithm to provide best linear unbiased estimates (BLUEs) as the predicted values for the breeding lines. Experiments within BP1 and BP2 were analysed as separate trials due to non-congruence of genotypes within those experiments. The four experiments in BP3 and BP4 (seedling and adult screenings) were combined and analysed using a fixed effect for experiment by genotypes interaction.

Association mapping and analysis of linkage disequilibrium

The SFNB predicted BLUEs were used for AM for the latter three breeding populations (BP2–BP4) and for adult plant phenotypes of BP1. Missing values were assigned to lines displaying a mixed disease response. A Linear Mixed Model (LMM) implemented in GenStat 16th edition (VSN International 2011) was applied to investigate correlations between DArT markers and seedling or adult resistance to *Ptm*. The method employed was as described in Ziems et al. (2014). The population structure was investigated using principal component analysis (PCA) and accounted for using the eigenanalysis relationship model. The Wald statistical test was used for each marker to test the null hypothesis that the marker's effect was zero. A threshold of $p < 0.05$ was used as an initial threshold to identify significant marker–trait associations in BP1 and BP2. AM was then conducted on the BP3 and BP4 validation sets, with an initial threshold of $p < 0.05$ applied as previously, and the results combined across all four independent populations. To avoid the potential for false positives while maintaining power, QTL were reported only for genomic regions identified in at least two out of four populations, for both seedling and adult plant assessments separately.

Additionally, linkage disequilibrium (LD) decay was determined in the four germplasm sets, using the eigenanalysis relationship model in GenStat 16th edition, both genome-wide and at specific QTL.

A linear mixed model was fitted containing simultaneous random effects for all markers (Smith 2011; Verbyla et al. 2007). Further models were fitted where highly significant markers were considered as fixed effects. An optimal model was achieved when the set of markers in the fixed part of the mixed model accounted for all of the marker variation in the random part of the model. The resulting set of uncorrelated fixed markers was considered as the final set of QTL regions.

Table 1 Details of the eight SFNB QTL publications included in this study, including population pedigree, population size, number of seedling and adult resistance QTL identified and the corresponding QTL able to be projected onto the consensus map of this study

Reference	Population pedigree	Population size	No. of seedling resistance QTL		No. of adult resistance QTL		Analysis method
			Identified	Projected	Identified	Projected	
Williams et al. (1999)	Galleon/Haruna Nijo	95	1[a]	1	–	–	Unknown
Molnar et al. (2000)	Léger/CIho 9831	93	2	0	–	–	BSA
Williams et al. (2003)	Galleon/Haruna Nijo	92	1	1	4	4	SPRA, SIM, CIM, TLIA
Williams et al. (2003)	CIho 9214/Stirling	98	1	1	1	1	SPRA, SIM, CIM, TLIA
Williams et al. (2003)	Keel/Gairdner	67	1	1	1	1	SPRA, SIM, CIM, TLIA
Williams et al. (2003)	Chebec/Harrington	112	1	1	–	–	SPRA, SIM, CIM, TLIA
Williams et al. (2003)	Tilga/Tantangara	112	1	1	–	–	SPRA, SIM, CIM, TLIA
Williams et al. (2003)	VB9104/Dash	181	1	1	3	1	SPRA, SIM, CIM, TLIA
Friesen et al. (2006)	SM89010/Q21861	120	1	1	–	–	SIM, CIM
Manninen et al. (2006)	Rolfi/CIho 9819	119	2[b]	0	–	–	SIM, CIM
Grewal et al. (2008)	CDC Dolly/TR251	150	3[c]	3	–	–	SIM, MQM
Cakir et al. (2011)	Baudin/AC Metcalfe	178	2[d]	2	4	4	ANOVA, IM
Grewal et al. (2012)	CDC Bold/TR251	150	4	4	–	–	SIM, MQM

Analysis method used included *BSA* bulked segregant analysis, *SPRA* single-point regression analysis, *ANOVA* analysis of variance, *SIM* simple interval mapping, *CIM* composite interval mapping, *TLIA* 2-locus interaction analysis, *MQM* multiple QTL model, *IM* interval mapping

All these studies used doubled-haploids (DH) for SFNB QTL investigations

[a] *Rpt4* was taken as QTL for display purpose

[b] *Rpt6* was taken as QTL for display purpose

[c] Includes 2 QTL conferring resistance at both seedling and adult plant growth stages

[d] Includes 1 QTL conferring resistance at both seedling and adult plant growth stages

Projection of QTL onto an integrated consensus map

Data on SFNB resistance QTL were collated from eight publications. Details of the QTL are provided in Table 1, including population pedigree, population size, number of QTL identified for seedling and adult resistance, number of QTL projected onto the consensus map used for this study and analysis method implemented. In total, 13 doubled-haploid (DH) populations were investigated across the eight mapping studies.

An integrated consensus map was constructed by Ziems et al. (2014) following the method described in Mace et al. (2009) by projecting additional DArT markers onto the consensus map (Wenzl et al. 2006) using bridge markers. After integrating an additional 519 DArT markers from the ND19119-5/PI 642914 (W. Lawson, unpublished) and ND24260/Flagship (Hickey et al. 2011) genetic linkage maps to the barley DArT consensus map, a total of 3,476 DArT markers were positioned across the 7 chromosomes, spanning 1,417 cM with an average marker density of 0.41 cM per marker (Ziems et al. 2014).

The integrated consensus map was then used as the reference map for QTL projection through MapChart (Voorrips 2002). The positions of QTL reported previously were projected onto the consensus map based on flanking markers in common between the individual studies and the integrated map. For cases where flanking markers were not present in the integrated map, their locations were projected based on common markers (Mace and Jordan 2011). The confidence interval (CI) for projected QTL was calculated by applying the formulae $CI = 530/(NR^2)$ for F_2/DH populations (Darvasi and Soller 1997). Only significant ($p < 0.05$) and highly significant ($p < 0.01$) QTL identified previously were projected onto the integrated consensus map (suggestive QTL were excluded).

QTL locations identified in the current study, in addition to those previously identified in the literature, were displayed using MapChart. Co-location of QTL between previous and the current studies was defined as 2 QTL either having overlapping confidence interval (CI) or the mean of the QTL being less than 10 cM apart. Additionally, QTL CI frequency graphs were generated for the two hot-spot regions on 6H and 7H, as described by Mace and Jordan (2011). The values used in the production of the QTL CI frequency graph were the projected CIs around the LOD peak for each QTL. The frequency was calculated by summing the overlapping CIs in 1 cM intervals.

QTL nomenclature

QTL identified in the current study were named according to Grewal et al. (2008) with minor modifications. "*QRptm*" indicated a QTL for resistance to *P. teres* f. *maculata*, followed by "s" or "a" if effective only at seedling or adult plant stage, respectively, and followed by the barley chromosome on which the QTL was mapped. If there were several QTL detected on a chromosome, a number suffix was added.

Terminology

In this publication, seedling resistance denotes resistance observed on leaf blades at seedling stages of plant growth, while adult resistance is resistance observed on leaf blades at adult plant growth stages.

Results

Disease responses to SFNB

The mean disease response ratings to SFNB assessed at the seedling stage for the four trials ranged from 5.0 (BP4) to 5.9 (BP2) (Fig. 1). In comparison, the mean disease response ratings of adult plants in the four trials ranged from 6.1 (BP4) to 6.8 (BP1). The correlation between seedling and adult plant responses to SFNB within each breeding populations was high (>0.6, $p < 0.01$), with the exception of BP1 where the correlation was lower, 0.46 (ESM Figure S2).

Although many of the breeding lines exhibited disease response scores ≥6, lines with lower scores were identified in all four breeding populations (BP1–BP4) and offered a number of potential parental lines for use in breeding for SFNB resistance. In BP1, the number of lines with disease response scores ≤5 for seedling and adult plants was 149 (43.7 %) and 48 (12.0 %), respectively (ESM Table S2). In BP2, the number of lines exhibiting seedling and adult plant disease response scores ≤5 was 73 (39.3 %) and 37 (19.5 %), respectively. The proportion of lines with disease response scores ≤5 for seedlings increased slightly to 48.1 % (64/133) for BP3 and further increased to 65.3 % (162/248) for BP4, whereas approximately one-third of accessions displayed adult plant disease response scores ≤5 in both BP3 (i.e. 40 lines) and BP4 (i.e. 90 lines). The number of lines that displayed high seedling disease response scores, but low disease response scores at the adult plant growth stage was only 9, 7, 9, and 4 in BP1, BP2, BP3, and BP4, respectively. On the other hand, 98, 21, 33, and 76 lines displayed low seedling disease response scores yet high adult disease response scores in the four populations, respectively. There were 34, 37, 31, and 86 entries exhibiting low disease response scores across both seedling and adult plant growth stages in BP1, BP2, BP3, and BP4, respectively. The 60 genotypes in common across multiple years had similar ranking of disease response scores with limited re-ranking, e.g. correlation between the 32 genotypes in years 2011 and 2012 was 0.89 for both seedling and adult plants.

The heritability of disease response to SFNB at seedling and adult plant growth stages was calculated for all four populations, except for the seedling assay performed for BP1 which was unreplicated. Overall the heritability was very high across both seedling and adult plant assays, ranging from 0.82 to 0.94 across BP2, BP3, and BP4, with the exception of adult plant data collected for BP1 (i.e. 0.48; ESM Table S3).

QTL identification and validation

To avoid the potential for false positives while maintaining power, a detection and validation approach was used, where an initial significance threshold of $p < 0.05$ was used to detect QTL in BP1 and BP2, which were retained only if validated in at least two of the four independent populations. Given the independence of populations and experiments, this approach maintained power to detect associations while providing a strong protection against type 1 errors. Although population structure was accounted for using eigenanalysis, two approaches were used to investigate the potential for structure to modify results. Approach 1 involved conducting a principal component analysis on each of the four breeding populations (ESM Figure S3), with only limited structure observed. Approach 2 involved generating a pedigree diagram of the populations showing the genealogical interrelationships between the lines in the four breeding populations (ESM Figure S1). Analysis of this graph showed the strong relatedness among the different breeding populations, but no major differences in the structure of the material except that resulting from selection during line development. The initial AM analysis, based on BP1 and BP2 only, identified a total of 35 significant genomic regions conferring resistance to SFNB at a threshold of $p < 0.05$ (ESM Table S4). Twenty-nine of these regions were further validated in BP3 and BP4 (Table 2; Fig. 2). The 29 QTL were identified on all seven chromosomes; however, they were not evenly distributed, with 8 QTL located on 7H and only 1 QTL (*QRptm1-1*) located on 1H. Eight QTL (i.e. 28 %) were identified across all four populations, with a further 18 QTL (i.e. 62 %) identified across three of the four populations. The majority of QTL (75 %) conferred resistance at both seedling and adult plant growth stages across populations; only five resistance QTL were unique for adult plants, while 2 QTL were unique at

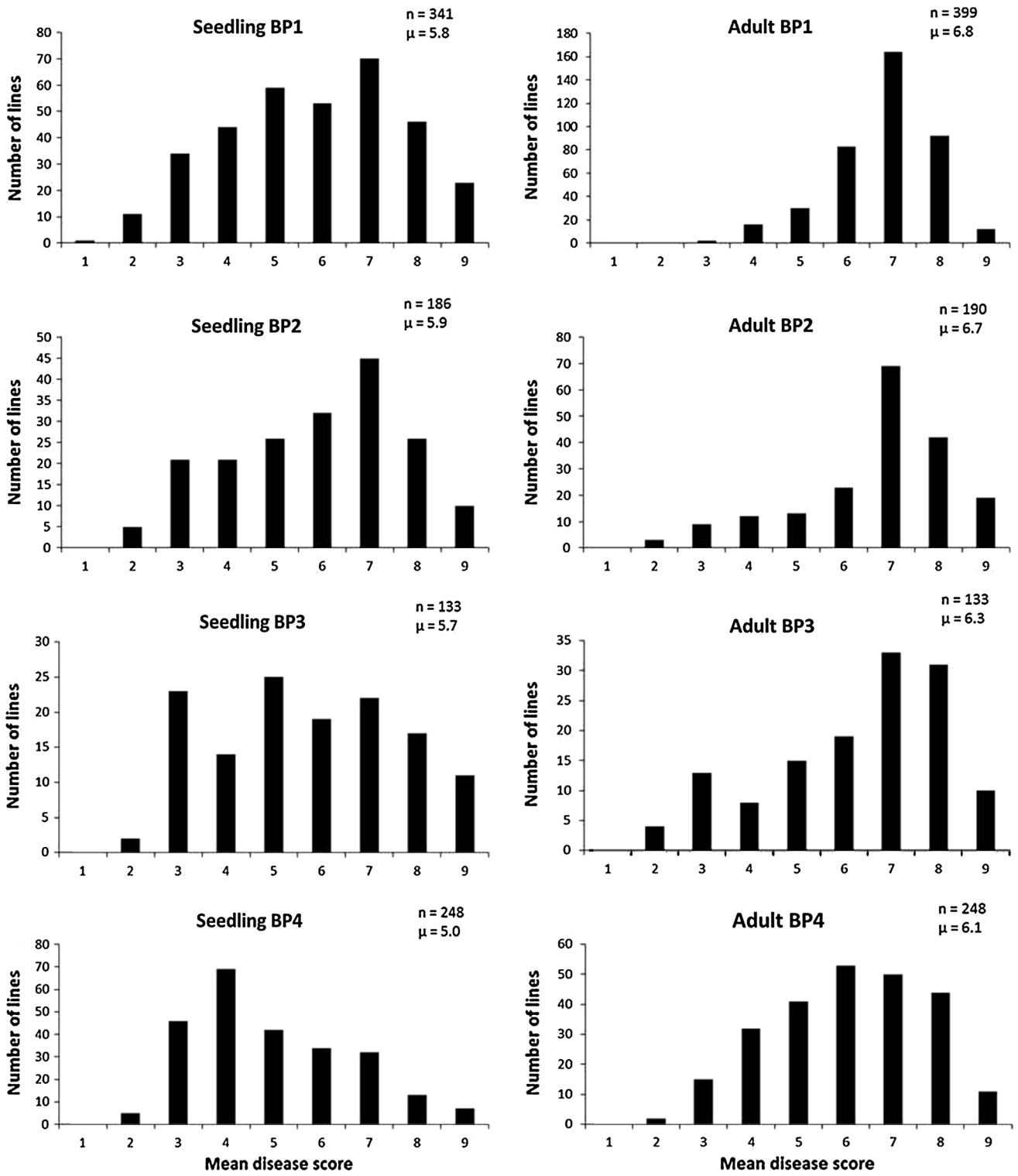

Fig. 1 Frequency distribution of disease response to *P. teres* f. *maculata* assessed at seedling and adult plant growth stages across the four breeding populations (BP1–BP4)

the seedling stage—one QTL on 2H (*QRptms2-4*) and the other on 3H (*QRptms3-2*). When considered in a single population, more than half (53–77 %) of the QTL conferred resistance as seedling and adult plants in BP2–BP4, with a slight difference observed in BP1, where only 2 QTL conferred a similar reaction pattern (ESM Table S5).

Table 2 Summary of SFNB resistance QTL identified and validated at seedling and adult plant growth stages in the four populations (BP1–BP4)

QTL name	Resistance type[a]	Chr.	Bin[b]	Position (cM)[c]	Seedling				Adult plant			
					BP1	BP2	BP3	BP4	BP1	BP2	BP3	BP4
QRptm1-1[d]	S&A	1H	Bin05	58.61	NS	2.2*	**,[e]	*	1.6*	2.6*	**	*
QRptm2-1	S&A	2H	Bin03	23.33	**2.5*,**[f]	NS	NS	*	**NS**	2.8*	**	*
QRptm2-2	S&A	2H	Bin07	70.30	NS	**2.6***	*	**NS**	**NS**	**3.4****	**NS**	*
QRptma2-3	A	2H	Bin13	157.70	NS	NS	NS	NS	1.3*	**NS**	*	NS
QRptms2-4	S	2H	Bin14	169.36	**NS**	NS	*	**	NS	3.0*	**NS**	NS
QRptm3-1	S&A	3H	Bin01	47.07	NS	**NS**	*	*	1.5*	**NS**	NS	**
QRptms3-2	S	3H	Bin01	57.61	**2.6***	**NS**	*	NS	**NS**	NS	**NS**	NS
QRptm3-3	S&A	3H	Bin11	158.20	NS	NS	**	*	NS	2.7*	NS	*
QRptm3-4	S&A	3H	Bin14	198.04	3.4**	2.4*	**NS**	*	NS	3.1*	**	**
QRptm4-1	S&A	4H	Bin04	86.00	**2.7***	NS	*	*	**NS**	NS	**	*
QRptm4-2	S&A	4H	Bin08	128.90	**NS**	**NS**	**	**	NS	**2.5***	**	**
QRptm5-1	S&A	5H	Bin02	33.70	**NS**	**2.5***	**NS**	**	**NS**	**3.3****	*	**NS**
QRptma5-2	A	5H	Bin06	83.99	**NS**	**NS**	NS	*	**1.5***	**NS**	*	**NS**
QRptma5-3	A	5H	Bin08	108.40	**NS**	NS	NS	NS	NS	2.5*	*	*
QRptm5-4	S&A	5H	Bin11	151.64	**NS**	NS	**	*	**1.7***	NS	**	*
QRptma5-5	A	5H	Bin14	190.08	**NS**	NS	*	**NS**	**NS**	2.6*	*	NS
QRptm5-6	S&A	5H	Bin15	202.10	**NS**	NS	**	*	NS	2.6*	**	*
QRptm6-1	S&A	6H	Bin01	12.70	2.8*	NS	*	**	NS	NS	**	*
QRptm6-2	S&A	6H	Bin06	73.80	**2.5***	**NS**	**	**	NS	**2.5***	*	*
QRptm6-3	S&A	6H	Bin08	89.20	NS	NS	**	**	1.5*	**NS**	*	*
QRptma6-4	A	6H	Bin11	143.73	**NS**	**NS**	NS	*	**1.4***	**NS**	**	*
QRptm7-1	S&A	7H	Bin01	27.71	3.1*	NS	**	*	NS	NS	**	*
QRptm7-2	S&A	7H	Bin04	66.63	NS	NS	*	**	2.0**	NS	*	NS
QRptm7-3	S&A	7H	Bin06	115.75	NS	2.5*	*	**	**NS**	3.1*	**	**
QRptm7-4	S&A	7H	Bin08	152.90	**NS**	**3.1***	**	**	**1.8***	**3.8****	**	**
QRptm7-5	S&A	7H	Bin09	159.30	2.6*	2.5*	**	**	**1.5***	3.0*	**	**
QRptm7-6	S&A	7H	Bin10	166.60	2.8*	NS	**	**	2.4**	NS	**	**
QRptm7-7	S&A	7H	Bin10	173.08	**NS**	**3.8****	**	**	**NS**	**4.4****	**	**
QRptm7-8	S&A	7H	Bin11	188.88	**NS**	**3.1****	**	**	**1.7***	**3.9****	**	**

Additive effects are calculated as the difference between the effect of the "1" allele minus that of the "0" allele

NS not significant

* Significant at the level of 0.05

** Highly significant at the level of 0.01

[a] QTL conferring resistance to SFNB at both seedling and adult plant stages are marked as S&A, while QTL conferring seedling or adult resistance only are marked as S or A, respectively

[b] Estimated position based on the barley bin maps (Kleinhofs 2006)

[c] Peak position in cM on the DArT consensus map

[d] To avoid potential for false positives while maintaining power, QTL were reported only for genomic regions identified in a minimum of 2 out of 4 populations, for both seedling and adult plant assessments separately

[e] Allele effects in BP3 and BP4 are not presented since small population sizes may result in inaccurate allele effect size in the two populations

[f] Bold indicates a negative association between SFNB reaction and the positive allele at each locus, while non-bold indicates a positive association

In BP1, the largest allele effect at a specific locus for seedling resistance, based on seedling disease response score differences, contributed by highly significant QTL *QRptm3-4*, was 3.4 units on the 1–9 disease response scale, whereas the lowest significant allele effect was 2.5 units. In comparison, the significant allele effects at specific loci for adult resistance were much lower, ranging from 1.3 to 2.4 units on the 1–9 scale (Table 2). In BP2, the lowest

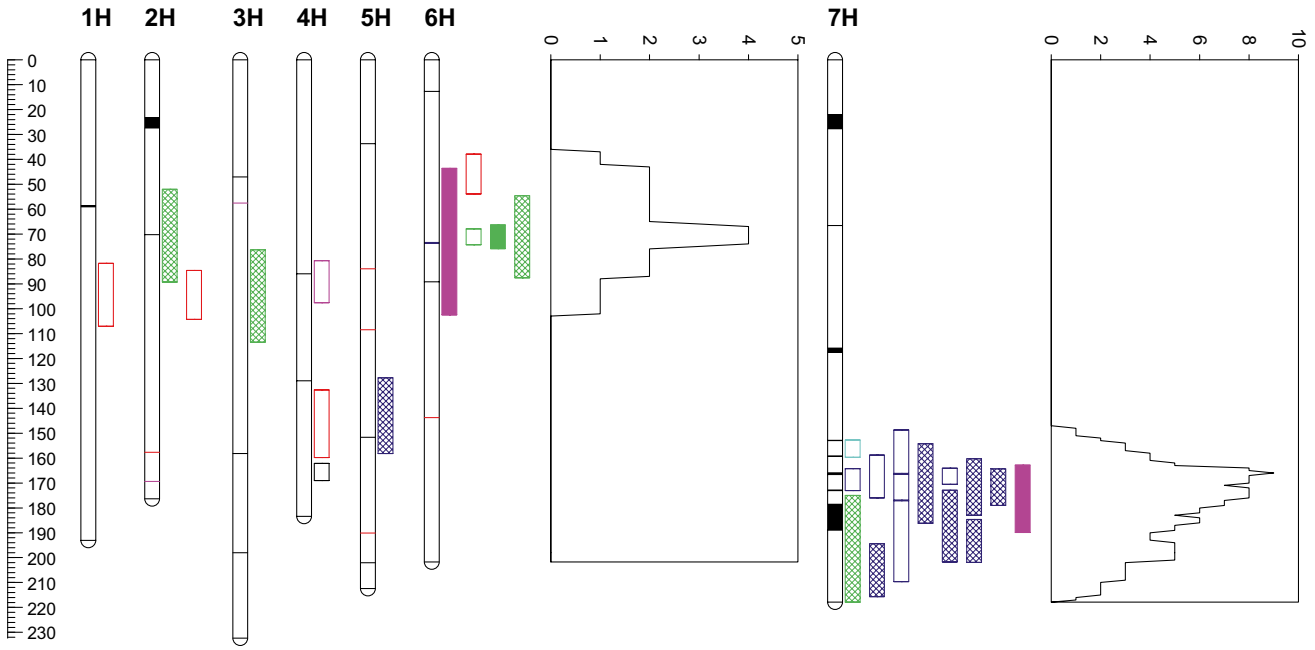

Fig. 2 Projected QTL from the current study onto the barley consensus map and comparison with SFNB resistance QTL identified in previous studies. The *scale bar* to the *left* indicates the length of each chromosome in cM. *Graphs* to the *right* of chromosomes 6H and 7H represent QTL density (number of QTL per cM) reported by previous studies. (1) Segments on chromosome represent the location of significant QTL at the level of $p < 0.05$ identified in this study. *Black* indicates QTL conferring resistance at both seedling and adult plant growth stages; *red* indicates QTL for adult resistance; *pink* indicates QTL contributing seedling resistance. Segments represent intervals between the first and the last markers showing significant associations with the corresponding QTL; thus, the segments of QTL (especially those on 2H and 7H) with large intervals are much thicker. (2) Seedling resistance QTL reported by Grewal et al. (2012); seedling resistance QTL reported by Grewal et al. (2008); seedling resistance QTL reported by Friesen et al. (2006); QTL conferring resistance at both seedling and adult plant growth stages reported by Grewal et al. (2008); seedling resistance QTL reported by Cakir et al. (2011); adult resistance QTL reported by Cakir et al. (2011); QTL conferring resistance at both seedling and adult plant growth stages reported by Cakir et al. (2011); *Rpt4* conferring seedling resistance reported by Williams et al. (1999); seedling resistance QTL reported by Williams et al. (2003); adult resistance QTL reported by Williams et al. (2003)

significant allele effects for seedling and adult resistance were 2.2 and 2.5 units contributed by *QRptm1-1* and *QRptm4-2*, respectively. Interestingly, the highly significant QTL *QRptm7-7* contributed the largest allele effects during both seedling and adult plant assessments, with values of 3.8 and 4.4 units, respectively.

QTL projection onto the integrated consensus map

Eight previous studies identified 34 SFNB resistance QTL/genes; 18 for seedling resistance, 13 for adult resistance and three for resistance at both seedling and adult plant growth stages (Table 1). Of these, 28 QTL were successfully projected onto the integrated consensus map; 14 for seedling resistance, 11 for adult resistance and three for resistance at both seedling and adult plant growth stages. Two studies (Manninen et al. 2006; Molnar et al. 2000) had too few non-sequenced markers (AFLPs and RAPDs) in common with the integrated consensus map, thus were excluded from this analysis. Two resistance loci reported by Williams et al. (2003) were also not included for the same reason. Of these 28 projected QTL, 21 co-located with the 29 QTL identified in the current study (Fig. 2), where co-location of QTL was defined as two QTL between the current and eight previous studies either having overlapping CI or the mean of the QTL being less than 10 cM apart. All 57 SFNB QTL represented 28 unique genomic regions, of which 19 were unique to this study, five co-located between the current and previous studies, and the remaining four were unique to previous studies (Cakir et al. 2011; Grewal et al. 2012; Friesen et al. 2006). In particular, the highly significant genomic region identified on 7HL in the current study co-located with *Rpt4*, the gene previously reported by Williams et al. (1999) as conferring seedling resistance to SFNB.

In the current study, the genomic region on 7HL which exhibited highly significant resistance in three of the four populations consisted of four separate QTL (*QRptm7-4*, *QRptm7-6* to *QRptm7-8*), spanning a 36-cM region. Given the proximity of these regions and the potential for selection during the breeding process to create linkage blocks, there are several explanations for the clustering of the QTL

in this region. To further investigate this region, a mixed model containing random marker effects was fitted and modified to test the independence of marker–trait associations in this region (Smith 2011; Verbyla et al. 2007). In this procedure, significant markers were sequentially transferred to the fixed part of the mixed model. If the linked markers were in LD with the same QTL, then making one marker linked to the same QTL into a fixed effect should reduce the significance of the other marker(s). This was not observed indicating that putative QTL were statistically independent, and all markers were required to explain the data. Further LD analysis was undertaken on the subset of markers at this location for each breeding population. Correlations between the 7HL QTL marker-subset were found to be very low, with LD <0.2 (ESM Figure S4).

Discussion

This study investigated QTL conferring seedling and adult resistance to *Pyrenophora teres* f. *maculata* in four barley breeding populations, identifying and validating a total of 29 QTL across the barley genome. Of the 29 QTL, 22 exhibited resistance at both seedling and adult plant growth stages, while 5 QTL were associated with adult plant resistance and the remaining 2 QTL were detected at the seedling stage only. Although large effects QTL were located on 7H, it would be necessary to accumulate QTL on other chromosomes to achieve higher levels of resistance to SFNB. The study also provided evidence to support the concept that phenotypic selection for SFNB resistance at just one developmental stage could provide effective levels of resistance across growth stages.

Genetic architecture of SFNB resistance

In this study, a total of 29 QTL conferring SFNB resistance were identified across all seven chromosomes. By integrating these results with the 28 projected QTL from eight previous studies, 28 unique genomic regions were identified, indicating that the SFNB resistance is more complex than many previous studies suggest. It appears resistance is controlled by a large number of QTL that vary in effect size, with large effect QTL on 7H. Liu et al. (2011) and McLean et al. (2014) summarised that resistance to SFNB was contributed by major effect genes, along with minor effect QTL, with two named genes (i.e. *Rpt6* and *Rpt4*) with large effects located on 5H and 7H (Manninen et al. 2006; Williams et al. 1999) and one unnamed major gene on 4H (Friesen et al. 2006) having been found to explain 64–84 % of the phenotypic variation individually. On the other hand, multiple minor QTL have been reported for this disease (Cakir et al. 2011; Grewal et al. 2008, 2012; Williams et al. 2003), where the small effect QTL may contribute as little as 5 % of the phenotypic variation. However, since all eight previous studies used bi-parental populations with small numbers of individuals, it is likely that only a subset of the total number of resistance loci was identified. In contrast, AM, with the broad genetic base of the underlying population, provides an opportunity to uncover novel resistance genes and alleles which are not detectable in bi-parental populations.

Analysis of the distribution of previously reported QTL (Cakir et al. 2011; Grewal et al. 2008; Williams et al. 1999, 2003) suggested that 7HL harbours a major QTL for SFNB resistance, as shown by CI frequency distribution on 7H (Fig. 2). However, the subsequent AM in the four breeding populations indicated that the genetic architecture of this region is complex with 4 QTL (*QRptm7-4*, *QRptm7-6* to *QRptm7-8*) within a 36-cM region displaying highly significant resistance in three populations. The result could be due to either the presence of multiple linked QTL, multiple alleles at a single QTL, as an artefact of the local population structure, or indeed some combination of the above. Analysis of LD in the region indicated that the QTL we detected were statistically independent and uncorrelated providing support for the hypothesis that the region contains multiple linked QTL. However, further dissection of the region from multiple source lines is required to confirm this and to help breeders formulate appropriate breeding strategies. For example, if multiple linked QTL are responsible then an approach to accumulate all of the favourable alleles may be warranted; whereas, if multiple alleles are involved, barley breeders will need to select the most effective alleles for a particular target environment.

The QTL allele effect size showed consistency at seedling and adult plant growth stages in both BP1 and BP2, with the QTL on 7HL having the largest allele effects for seedling and adult plant assessments in these two populations. The exception was *QRptm3-4* which contributed the largest allele effect for seedling resistance in BP1. The highly significant QTL *QRptm7-7* exhibited the largest allele effects of 3.8 and 4.4 units on the 1–9 scale rating at seedling and adult plant stages, respectively, in BP2. *QRptm7-4* contributed large allele effects of 3.1 units for seedlings and 3.8 units for adult plants in BP1, while *QRptm7-8* showed large allele effects of 3.1 units for seedlings and 3.9 units for adult plants in BP2. Therefore, the presence of these large effect alleles is highly desirable in breeding populations, because even one of them could enhance the average disease resistance in breeding material from highly susceptible (disease response score of 9) to medium susceptible or medium resistant (with a score of 5–6).

The current study found that SFNB resistance QTL were effective at both seedling and adult plant developmental

stages studied, suggesting that selection for resistance at one developmental stage could achieve appropriate levels of resistance at the other stage. A high proportion of QTL (53–77 %) conferred resistance at both stages in BP2, BP3 and BP4, which is consistent with the correlations between phenotypes observed for the two stages in the three breeding populations. Amongst the 29 QTL identified, 22 QTL conferred both seedling and adult resistance across populations. When classifying resistance types for the QTL regions, there were a few exceptions. For instance, although 2 QTL were considered as seedling resistance QTL, one of them (i.e. *QRptms2-4*) was detected in the adult plant assay in BP2; and 3 out of the 5 QTL conferring adult resistance were also detected at the seedling stage in one population, with *QRptma5-2* and *QRptma6-4* conferring seedling resistance in BP4 and *QRptma5-5* in BP3.

When the same QTL were detected in both seedling and adult plants in the same breeding population, a large proportion of the allele effects were in the same direction. In BP2, all 9 QTL exhibited the same direction of association for the SFNB reactions at the two growth stages. In BP3 and BP4, 11 and 12 QTL, respectively, had the same allele effect direction between seedling and adult plants. Even when two different isolates were used for seedling and adult plant assessments in BP1, 50 % of the QTL still exhibited allele effects in the same direction.

Application of these results for barley breeding

This study identified five highly significant QTL (*QRptm3-4*, *QRptm7-4*, *QRptm7-6* to *7-8*) contributing resistance to SFNB in three populations across seedling and adult plant growth stages; four of them positioned on 7H. This provided evidence to support the concept that alleles for resistance can be accumulated on 7H. However, grouping of breeding lines for analyses based on marker haplotypes in specific regions revealed that selection for one or a few QTL regions alone is not sufficient to provide very high levels of resistance. It is necessary to accumulate some of the small effect QTL to obtain the high levels of resistance, which would be preferred in elite cultivars.

The marker haplotype information generated for individual lines by AM in the barley breeding populations can be used to identify specific lines that are more valuable in subsequent breeding efforts. The haplotypes in genomic regions having large effect QTL can be followed based on identity by descent. Although haplotypes on 7H could provide resistance to SFNB, other QTL would need to be accumulated to achieve high levels of SFNB resistance during cultivar development. Since individuals with various combinations of the QTL can be identified and their accumulative QTL effect on phenotypic scores can be determined, selection of individual lines having better or specific combinations of the QTL for SFNB is possible. Based on marker haplotype profiles near major effect QTL, individual lines can be evaluated for nearby recombinations. Certain donor parents and specific lines would be the preferred sources of major QTL in the ongoing effort to produce high-yielding, locally adapted cultivars with durable resistance to SFNB.

In summary, the genetic architecture of SFNB resistance is contributed by the actions of a complex mixture of major and minor effect genes. The allele effect size of QTL and the majority of same direction of QTL allele effects at seedling and adult plant growth stages, suggested that selection for seedling resistance to SFNB could provide acceptable levels of adult resistance, or vice versa. However, selection strategies need to be considered that facilitate the accumulation of resistance alleles on other chromosomes. In order to confirm whether the genomic region on 7HL contains multiple linked QTL and to understand other haplotypes of this region, further research is required to investigate this region using multiple source lines and to help breeders formulate efficient breeding strategies.

Author contribution statement J.D.F., L.T.H., D.R.J. and E.S.M. managed the project; G.J.P. designed the experiments, and conducted and scored the SFNB screenings; J.D.F. provided the plant materials; X.W., E.S.M. and D.R.J. analysed the data with contributions from J.D.F., C.H.H., L.T.H. and G.J.P; X.W. wrote the manuscript with assistance from all the other authors.

Acknowledgments We would like to give thanks to Ms Janet Barsby (DAFFQ), Mr Ryan Fowler (DAFFQ) and Ms Julie McKavanagh (DAFFQ) for technical assistance in the laboratory and field, and to Mr Michael Hassell (DAFFQ) for generating the pedigree diagram of the four breeding populations. We acknowledge funding support from the Grains Research and Development Corporation (GRDC) of Australia.

Conflict of interest The authors declare they have no conflict of interest.

References

Butler D, Cullis B, Gilmour A, Gogel B (2009) ASReml-R reference manual, Release 3. In: Technical report, Queensland Department of Primary Industries and Fisheries, Brisbane

Cakir M, Gupta S, Li CD, Hayden M, Mather DE, Ablett GA, Platz GJ, Broughton S, Chalmers KJ, Loughman R, Jones MGK, Lance RCM (2011) Genetic mapping and QTL analysis of disease resistance traits in the barley population Baudin x AC metcalfe. Crop Pasture Sci 62:152–161

Darvasi A, Soller M (1997) A simple method to calculate resolving power and confidence interval of QTL map location. Behav Genet 27:125–132

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

Grewal T, Rossnagel B, Pozniak C, Scoles G (2008) Mapping quantitative trait loci associated with barley net blotch resistance. Theor Appl Genet 116:529–539

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

Hickey LT, Lawson W, Platz GJ, Dieters M, Arief VN, German S, Fletcher S, Park RF, Singh D, Pereyra S, Franckowiak J (2011) Mapping *Rph*20: a gene conferring adult plant resistance to *Puccinia hordei* in barley. Theor Appl Genet 123:55–68

Hickey LT, Lawson W, Platz GJ, Fowler RA, Arief V, Dieters M, German S, Fletcher S, Park RF, Singh D, Pereyra S, Franckowiak J (2012) Mapping quantitative trait loci for partial resistance to powdery mildew in an Australian barley population. Crop Sci 52:1021–1032

Hollaway G, McLean M (2006) Spot form of net blotch in Victoria, Australia: distribution, management and resistance screening. In: Turkington TK, Orr D, Xi K (eds) Proceedings of the third international workshop on barley leaf blights. Edmonton, Alberta, pp 42–45

Khan TN, Tekauz A (1982) Occurrence and pathogenicity of *Drechslera teres* isolates causing spot-type symptoms on barley in Western Australia. Plant Dis 66:423–425

Kleinhofs A (2006) Integrating molecular and morphological/physiological marker maps. Barley Genet Newsl 36:66–82

Liu ZH, Ellwood SR, Oliver RP, Friesen TL (2011) *Pyrenophora teres*: profile of an increasingly damaging barley pathogen. Mol Plant Pathol 12:1–19

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

Mace ES, Jordan DR (2011) Integrating sorghum whole genome sequence information with a compendium of sorghum QTL studies reveals uneven distribution of QTL and of gene-rich regions with significant implications for crop improvement. Theor Appl Genet 123:169–191

Mace ES, Rami JF, Bouchet S, Klein PE, Klein RR, Kilian A, Wenzl P, Xia L, Halloran K, Jordan DR (2009) A consensus genetic map of sorghum that integrates multiple component maps and high-throughput diversity array technology (DArT) markers. BMC Plant Biol 9:13

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

Mathre DE (1997) Compendium of barley diseases, 2nd edn. American Phytopathological Society, St. Paul

McLean MS (2011) The epidemiology and control of spot form of net blotch of barley in Victoria, Australia. PhD thesis, The University of Melbourne

McLean MS, Howlett BJ, Hollaway GJ (2009) Epidemiology and control of spot form of net blotch (*Pyrenophora teres* f. *maculata*) of barley: a review. Crop Pasture Sci 60:303–315

McLean MS, Howlett BJ, Hollaway GJ (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 Path 39:46–49

McLean MS, Martin A, Gupta S, Sutherland MW, Hollaway GJ, Platz GJ (2014) Validation of a new spot form of net blotch differential set and evidence for hybridisation between the spot and net forms of net blotch in Australia. Australas Plant Path 43:223–233

Molnar SJ, James LE, Kasha KJ (2000) Inheritance and RAPD tagging of multiple genes for resistance to net blotch in barley. Genome 43:224–231

Murray GM, Brennan JP (2010) Estimating disease losses to the Australian barley industry. Australas Plant Path 39:85–96

Patterson HD, Thompson R (1971) Recovery of interblock information when block sizes are unequal. Biometrika 31:100–109

Platz GJ, Usher TR (2006) Losses to spot form net blotch in north-eastern Australia. In: Turkington TK, Orr D, Xi K (eds) Proceedings of the third International Workshop on Barley Leaf Blights. Edmonton, Alberta, pp 23–27

Roy JK, Smith KP, Muehlbauer GJ, Chao S, Close TJ, Steffenson BJ (2010) Association mapping of spot blotch resistance in wild barley. Mol Breed 26:243–256

Smedegård-Petersen V (1971) *Pyrenophora teres* f. *maculata* f. *nov.* and *Pyrenophora teres* f. *teres* on barley in Denmark. In: Yearbook of the royal veterinary and agricultural university, Copenhagen, pp 124–144

Smith AB (2011) A whole genome approach for QTL detection using a linear mixed model with correlated marker effects. In: Australian applied statistics conference, Palm Cove

Verbyla AP, Cullis BR, Thompson R (2007) The analysis of QTLs by simultaneous use of the full linkage map. Theor Appl Genet 116:95–111

Voorrips RE (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. J Hered 93:77–78

VSN International (2011) GenStat for Windows, 14th edn. VSN International, Hemel Hempstead. http://GenStat.co.uk

Wenzl P, Li H, Carling J, Zhou M, Raman H, Paul E, Hearnden P, Maier C, Xia L, Caig V, Ovesna J, Cakir M, Poulsen D, Wang J, Raman R, Smith KP, Muehlbauer GJ, Chalmers KJ, Kleinhofs S, Huttner E, Kilian A (2006) A high-density consensus map of barley linking DArT markers to SSR, RFLP and STS loci and agricultural traits. BMC Genom 7:206–228

Williams KJ, Lichon A, Gianquitto P, Kretschmer JM, Karakousis A, Manning S, Langridge P, 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

Williams KJ, Platz GJ, Barr AR, Cheong J, Willsmore K, Cakir M, 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

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

Zhou H, Steffenson BJ (2013a) Association mapping of Septoria speckled leaf blotch resistance in US barley breeding germplasm. Phytopathology 103:600–609

Zhou H, Steffenson B (2013b) Genome-wide association mapping reveals genetic architecture of durable spot blotch resistance in US barley breeding germplasm. Mol Breed 32:139–154

Ziems L, Hickey L, Hunt C, Mace E, Platz GJ, Franckowiak J, Jordan D (2014) Association mapping of resistance to *Puccinia hordei* in Australia barley breeding germplasm. Theor Appl Genet 127:1199–1212