Theor Appl Genet (2008) 116:529–539
DOI 10.1007/s00122-007-0688-9

ORIGINAL PAPER

Mapping quantitative trait loci associated with barley net blotch resistance

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Received: 12 March 2007 / Accepted: 28 November 2007 / Published online: 11 December 2007
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Abstract Net blotch of barley, caused by *Pyrenophora teres* Drechs., is an important foliar disease worldwide. Deployment of resistant cultivars is the most economic and eco-friendly control method. This report describes mapping of quantitative trait loci (QTL) associated with net blotch resistance in a doubled-haploid (DH) barley population using diversity arrays technology (DArT®) markers. One hundred and fifty DH lines from the cross CDC Dolly (susceptible)/TR251 (resistant) were screened as seedlings in controlled environments with net-form net blotch (NFNB) isolates WRS858 and WRS1607 and spot-form net blotch (SFNB) isolate WRS857. The population was also screened at the adult-plant stage for NFNB resistance in the field in 2005 and 2006. A high-density genetic linkage map of 90 DH lines was constructed using 457 DArT® and 11 SSR markers. A major NFNB seedling resistance QTL, designated *QRpt6*, was mapped to chromosome 6H for isolates WRS858 and WRS1607. *QRpt6* was associated with adult-plant resistance in the 2005 and 2006 field trials. Additional QTL for NFNB seedling resistance to the more virulent isolate WRS858 were identified on chromosomes 2H, 4H, and 5H. A seedling resistance QTL (*QRpts4*) for the SFNB isolate WRS857 was detected on chromosome 4H as was a significant QTL (*QRpt7*) on chromosome 7H. Three QTL (*QRpt6*, *QRpts4*, *QRpt7*) were associated with resistance to both net blotch forms and lines with one or more of these demonstrated improved resistance. Simple sequence repeat (SSR) markers tightly linked to *QRpt6* and *QRpts4* were identified and validated in an unrelated barley population. The major 6H QTL, *QRpt6*, may provide adequate NFNB field resistance in western Canada and could be routinely selected for using molecular markers in a practical breeding program.

Communicated by M. Kearsey.

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Introduction

Net blotch of barley (*Hordeum vulgare* L.), caused by *Pyrenophora teres* Drechs. [anamorph: *Drechslera teres* (Sacc.) Shoemaker] is an important disease in Canada (Tekauz 1990) and elsewhere (Steffenson 1997). Two types of leaf symptoms occur: the net form (NFNB), caused by *P. teres* f. *teres*, which causes a dark brown reticulate venation pattern that sometimes turns chlorotic; and the spot form (SFNB), caused by *P. teres* f. *maculata*, which results in dark brown circular or elliptical spots accompanied by chlorosis of the surrounding leaf tissue (Khan and Tekauz 1982). Yield losses of 20–30% in susceptible cultivars have been reported in western Canada (van den Berg 1988) and up to 40% in western Australia (Khan 1987). More importantly, infection reduces kernel weight, plumpness and bulk density, negatively affecting malting and feed quality. Deployment of resistant cultivars is the most economic and eco-friendly method for control. However, resistance can be complex, being controlled by a single or several genes depending on the source of resistance, plant development stage and the pathotype used (Ho et al. 1996; Steffenson et al. 1996; Afanasenko et al. 1999; Manninen et al. 2000; Cakir et al. 2003; Williams et al. 2003). Variation in *P. teres* virulence has often been reported (Tekauz 1990; Steffenson and Webster 1992; Peever and Milgroom 1994; Gupta and Loughman 2001). Tekauz (1990) reported

extensive pathogenic variability across western Canada and found most cultivars susceptible to most isolates. The variability observed in *P. teres* and failure to identify lines resistant to all isolates suggest breeding for resistance should emphasize pyramiding resistance genes to develop broad-based durable resistance. There is a need to identify resistance genes and molecular markers linked to these for their rapid introgression into elite lines.

The diversity arrays technology (DArT®) marker technology has shown potential for cost-effective, whole-genome profiling of barley (Wenzl et al. 2004, 2006) and is becoming increasingly popular for linkage map construction. The DArT is based on microarray hybridizations that detect presence versus absence of individual DNA fragments in a representation derived from the total genomic DNA of an organism or a population of organisms (Jaccoud et al. 2001). Wenzl et al. (2004) reported that a barley DArT map was equivalent in quality to an RFLP (restricted fragment length polymorphism) framework map and was rapidly generated. A high density consensus barley DArT map has been published, allowing comparison with new barley DArT maps (Wenzl et al. 2006).

We report mapping of quantitative trait loci (QTL) for net blotch resistance in a doubled-haploid (DH) barley population using DArT® markers, identification of SSR markers linked to major QTL which can be used for marker-assisted selection (MAS), and their validation in an unrelated barley population.

Materials and methods

Plant material

One hundred and fifty anther-culture derived DH lines were developed from the cross CDC Dolly (net blotch susceptible) × TR251 (net blotch resistant). CDC Dolly is a barley cultivar (Canadian Food Inspection Agency registration # 3967, 1 June 1994) from Crop Development Centre (CDC), University of Saskatchewan, Saskatoon, SK, Canada. TR251 (TR229//AC Oxbow/ND7556) is a barley breeding line from Agriculture and Agri-Food Canada (AAFC), Brandon Research Centre, Brandon, MB, Canada.

All 150 DH lines were screened at the seedling stage with NFNB isolates WRS858 and WRS1607 and SFNB isolate WRS857 in the Phytotron at the University of Saskatchewan. TR253 (TR238//TR236/TR234), a highly resistant barley breeding line from the AAFC Brandon Research Centre, was used as a resistant check and Harrington, a highly susceptible barley cultivar from CDC Saskatoon (Harvey and Rossnagel 1984), was used as a susceptible check.

Inoculation

Eight seeds/line were sown in a bunch with three lines per 15-cm pot containing Sunrise® LG3 Mix. Seedlings were raised in a growth chamber at 24/18°C with 16/8 h photoperiod. Three replications of each DH line were planted using an incomplete block design where a random sub-set of DH lines were assigned to each sub-block along-with parents and checks (Harrington and TR253). Due to limitations in screening area, each incomplete block was separated in time.

P. teres isolates (WRS858, WRS1607 and WRS857) were obtained from Dr. A. Tekauz, AAFC Cereal Research Centre, Winnipeg, MB. NFNB isolate WRS858 was collected from Teulon, Manitoba in 1973 and was more virulent than WRS102, a standard long-term check used to screen elite barley lines (Tekauz and Mills 1974). WRS1607, another NFNB isolate was collected from Prince Albert, Saskatchewan in 1985. SFNB isolate WRS857 was collected from Oakbank, Manitoba in 1973 and now is being used as a standard isolate to screen elite barley lines for SFNB (A. Tekauz, personal communication). Isolates were sub-cultured on V8 Juice Agar (V8A) plates and were incubated at 21 ± 1°C using a 12-h photoperiod. After 10–14 days, conidia were harvested by adding 10 mL of sterile distilled water to the Petri plate, scraping the culture with a glass rod and filtering through two layers of cheesecloth. Conidial concentration was adjusted to 10^4 conidia/mL. Plants were inoculated 14 days after planting (three leaf stage) with conidial suspension using a Duray® 2 oz. Pump Sprayer (Goody Products, Inc., Peachtree City, GA). Approximately 5 mL inoculum per pot was applied until the plants were uniformly wet. Inoculated plants were incubated at 21°C in darkness for 24 h at 100% RH. Inoculated plants were then grown at 21/19°C with 16/8 h photoperiod at 75% RH.

Assessment of infection response

Infection response (IR) was scored 8 days after inoculation on a 1–10 scale for *P. teres* f. *teres* (NFNB) (Tekauz 1985). The same scale was used for *P. teres* f. *maculata* (SFNB) but without employing categories 4, 6 and 10. Generally, the most common lesion type was scored. For each isolate, the test was conducted once and repeated only if symptoms were not fully expressed on the susceptible check. Analysis of variance was performed using PROC Mixed of SAS 9.1 (SAS Institute Inc. 2005). Parental lines, checks, and DH lines were considered fixed effects with replications and incomplete blocks nested within replications as random effects. Least significant differences (LSD) were calculated ($P = 0.05$) for each test for mean comparisons. Heritability was calculated for each test using variance estimates

derived from PROC Mixed. The phenotypic variance was $\sigma^2_{\text{DH lines}} + \sigma^2_{\text{residual}}$/replications. For heritability analysis, parental data were removed and all factors were considered random effects.

Field trials

The 150 DH lines were screened at the adult-plant stage in the field in the CDC NFNB disease-screening nursery located at Melfort, SK in 2005 and 2006. TR253 was used as the resistant check and Harrington as the susceptible check. The parents were planted at the beginning and end of every 100-hill set, whereas resistant and susceptible checks were planted after every 50-hill set. Melfort is a hot spot for NFNB (*P. teres* f. *teres*) where the disease is endemic. In both years, there was natural infection of NFNB at the site (no artificial inoculum was used). Spreader rows of the highly susceptible cultivar Harrington were grown all around the experiment to promote pathogen development and spread. In 2005, lines were planted on June 15 as hill plots (10–15 seeds/hill) with experiments arranged in a three-replicate randomized complete-block design. To control weeds, a herbicide tank mix of Frontline*/Puma* Super was applied on 5 July 2005 at commercially recommended rates. NFNB reaction was scored on 8 September 2005 using a 1–9 scale, where 1 is highly resistant and 9 is highly susceptible. In 2006, experiments were planted on 7 June using the same material and design. Achieve* and Prestige* were applied for weed control as a tank mix on 26 June 2006. NFNB reaction was scored on 31 August 2006. Analysis of variance was conducted using PROC Mixed. Parental lines, checks, and DH lines were considered fixed effects and replications as random effects. LSD was calculated ($P = 0.05$) for each test for means comparisons. Heritability estimates were calculated as above.

DArT mapping and QTL analysis

DNA was extracted from leaf samples of the parents and a random set of 90 DH lines using a CTAB DNA extraction method of Procunier et al. (1991) with some modifications. The extraction was scaled down many-fold and tissue was ground directly in the CATB extraction buffer in a 1.5 mL Eppendorf tube without liquid nitrogen. Extracted DNA samples were cleaned with phenol/chloroform. Ten microliters of 100 ng/µl DNA from each sample (90 DH lines with the two parents duplicated at the beginning and middle of the 96-well tray) was sent to Triticarte Pty Ltd (Yaralumla, Australia; http://www.triticarte.com.au). DArT® markers were scored on the 90 DH lines by Triticarte Pty Ltd, probing genomic DNA from individual DH lines against the barley DArT® array. A total of 557 DArT® informative markers were scored and used to construct a high-density genetic linkage map of the CDC Dolly/TR251 population. The map was constructed using JoinMap version 3.0 (van Ooijen and Voorrips 2001) with the Haldane mapping function (Haldane 1919) to convert observed recombination frequencies to genetic distances in centimorgans (cM). For map construction, markers were initially joined into 10 linkage groups at LOD score of 5.0. To improve map robustness, suspect markers consistently displaying recombination values greater than 0.5 in combination with LOD scores greater than 2.5 were removed from further analysis. Markers displaying unusually high frequencies of double cross over events were also removed prior to final map construction. By comparing marker order to the existing barley DArT and SSR/RFLP consensus map (Wenzl et al. 2006), the ten groups were joined into seven linkage groups corresponding to the seven barley chromosomes using LOD score of 3.0 or higher using the "Second Order" mapping in JoinMap 3.0.

For QTL analysis, simple interval mapping (SIM) was performed on least-square means to identify markers most significantly associated with variation in net blotch resistance. To enhance the power of QTL detection, the analyses were repeated using those markers identified by SIM as being significantly associated with net blotch resistance as co-factors for QTL in a multiple-QTL model (MQM) in MapQTL V 5.0. With MQM mapping, additional QTL were identified in some tests. The QTL analysis was repeated by selecting the markers associated with these QTL as co-factors as described by van Ooijen (2004). Permutation tests (1,000 iterations) were performed for each experiment to determine the threshold at which the LOD score became significant ($P < 0.05$) and highly significant ($P < 0.001$) for QTL identification (van Ooijen 2004). Epistatic interactions between QTL were evaluated on least-square means with PROC Mixed of SAS using co-factors identified in the final MQM model. Co-factors and their interactions were considered fixed effects in the model.

QTL nomenclature

"*QRptt*" indicates a QTL for resistance to *P. teres* f. *teres* (NFNB) and "*QRptm*" indicates a QTL for resistance to *P. teres* f. *maculata* (SFNB). "*QRpt*" indicates a QTL for resistance to *P. teres*, i.e., effective against both NFNB and SFNB. "*QRpt*" is followed by "s" or "a" if effective only at seedling or adult-plant stage and followed by the barley chromosome onto which the QTL was mapped.

Identification of SSR markers associated with net blotch resistance

Preliminary QTL analysis indicated a major NFNB seedling and adult-plant resistance QTL on chromosome 6H

and a major SFNB seedling resistance QTL on chromosome 4H. The parent lines were screened with 10 SSR markers previously mapped to chromosome 6H (surrounding the 6H QTL) and 17 SSR markers mapped to chromosome 4H (Ramsay et al. 2000; Wenzl et al. 2006) to identify informative markers polymorphic between the parents. Four and seven polymorphic SSR markers from 6H and 4H, respectively, were screened against the 90 DH lines.

For SSR screening, amplification was performed in a total volume of 25 µL, containing 100 µM each of dNTPs (Gibco BRL), 200 nM of each primer, 50 ng of genomic DNA, 2.0 mM of $MgCl_2$ (Gibco BRL), 1 unit of *Taq* polymerase and 1X of Gibco BRL buffer (50 mM KCL, 20 mM Tris–HCL, pH 8.4). Amplification was performed with a GeneAmp® PCR system 9700 (Applied Biosystems, USA) and consisted of an initial denaturation step at 94°C for 3 min, followed by 37 cycles as follows: a denaturation step at 94°C for 1 min, an annealing step at 55°C for 1 min and an extension step at 72°C for 1 min; ending with an extension period of 72°C for 10 min. Amplified products were initially separated on 2% agarose, 0.5× TBE gel, stained with ethidium bromide and viewed under ultra-violet light. Amplified products were then separated on 6% polyacrylamide gels and viewed with silver staining if polymorphism was not visible on agarose gels. The 11 SSR markers were placed on the DArT® map using JoinMap 3.0. QTL analysis was performed with MapQTL V 5.0 as described earlier.

QTL effect on net blotch resistance

To determine the effect of individual QTL and their combinations on net blotch resistance, the mean IR of all lines with the resistance allele for each QTL or their combinations was calculated and compared with the mean of lines having the susceptible allele for that particular QTL. A *T*-test ($P = 0.05$) was performed to determine statistical significance. For NFNB resistance, IR of lines with *QRpt6* was compared with IR of lines having combinations of *QRpt6* and other QTL or no QTL using a *T*-test.

Validation of SSR markers associated with net blotch resistance

The MEH#486/Harrington population was selected for validation of SSR markers HVM74 and HVM03 linked to the 6H QTL for NFNB resistance and to the 4H QTL for SFNB resistance, respectively. One hundred and forty-eight F_8 lines from the cross were obtained from Dr. W. Legge, AAFC Brandon Research Centre, Brandon, MB. MEH #486 is a 2-row Ethiopian accession, resistant to net blotch (Grewal et al., unpublished data). DNA extraction, PCR and gel conditions were as described earlier. These lines were screened with SSR markers HVM74 and HVM03 and scored on agarose gel. The population was screened with NFNB isolate WRS858 and SFNB isolate WRS857 at the seedling stage in the Phytotron, as previously described. The population was also screened at the adult-plant stage in the field at Melfort in 2006. TR253 and CDC Dolly were used as resistant and susceptible checks, respectively. Remaining experimental conditions were as previously described.

Results

Map construction

Triticarte® scored 557 DArT® markers on the 94 barley DNA samples with a call rate of 95%. After removal of suspect markers and exclusion of third-order markers, a high-density genetic linkage map was constructed using 457 DArT® and 11 SSR markers. Polymorphism information content (PIC) values of the 468 identified polymorphic markers ranged from 0.33–0.50, with a mean of 0.49. Chromosome 7H had the largest number of markers (97), while chromosome 4H had the lowest (27). The final linkage map spanned 926 cM with a mean two-locus interval of 1.98 cM. Chromosome sizes ranged from 104 cM (4H) to 172 cM (5H). There were few large gaps (>20 cM) between markers; one each for chromosomes 1H and 3H and two for 4H.

Disease reaction

TR251 was consistently resistant in all trials and CDC Dolly was susceptible (Table 1) except in the field in 2006 where CDC Dolly had an intermediate reaction. The infection response of resistant and susceptible checks was as expected. NFNB isolate WRS858 was more virulent than isolate WRS1607 at the seedling stage, as evidenced by the significantly higher IR (*T*-test, $P = 0.05$) for CDC Dolly and TR251 (Table 1). Regardless of isolate, or testing environment, large variation was observed in disease response in the CDC Dolly/TR251 population. Little transgressive segregation was observed with the most resistant lines in the DH population, not significantly different from TR251 (Table 1). High heritability for different tests (0.72–0.91) indicated that the majority of the phenotypic variance was due to genetic effects.

QTL associated with NFNB resistance

A major QTL on 6H, designated *QRpt6*, associated with seedling resistance to WRS1607 and explaining 60% of the net blotch variance was identified (Fig. 1). For isolate WRS858, MQM analysis identified *QRpt6* explaining 65% of the variance with additional QTL on 2H-*QRptts2* (8% variance), 4H-*QRpts4* (5% variance) and 5H-*QRptts5* (7%

Table 1 Net blotch infection response (1–10) of parents and checks of the CDC Dolly/TR251 population

Line/test	NFNB				SFNB
	[a]WRS858	[a]WRS 1607	Field 2005	Field 2006	[a]WRS857
TR251	2.8	2.0	1.8	1.4	2.3
CDC Dolly	8.1	6.3	6.5	4.3	7.6
TR253	1.2	1.7	2.2	1.6	2.9
Harrington	8.2	7.9	8.7	7.8	8.9
Population					
Mean	5.4	3.8	4.1	2.7	6.4
Range	2.0−8.3	1.7−7.3	2.0−6.7	1.3−6.0	2.7–8.0
[b]$LSD_{0.05}$ lines	1.4	1.1	1.4	1.4	1.3
$LSD_{0.05}$ lines vs. checks	1.2	0.9	1.4	1.4	1.1
Heritability	0.90	0.91	0.84	0.72	0.82

NFNB Net-form net blotch, *SFNB* spot-form net blotch

[a] *Pyrenophora teres* isolate

[b] Least significant differences ($P = 0.05$)

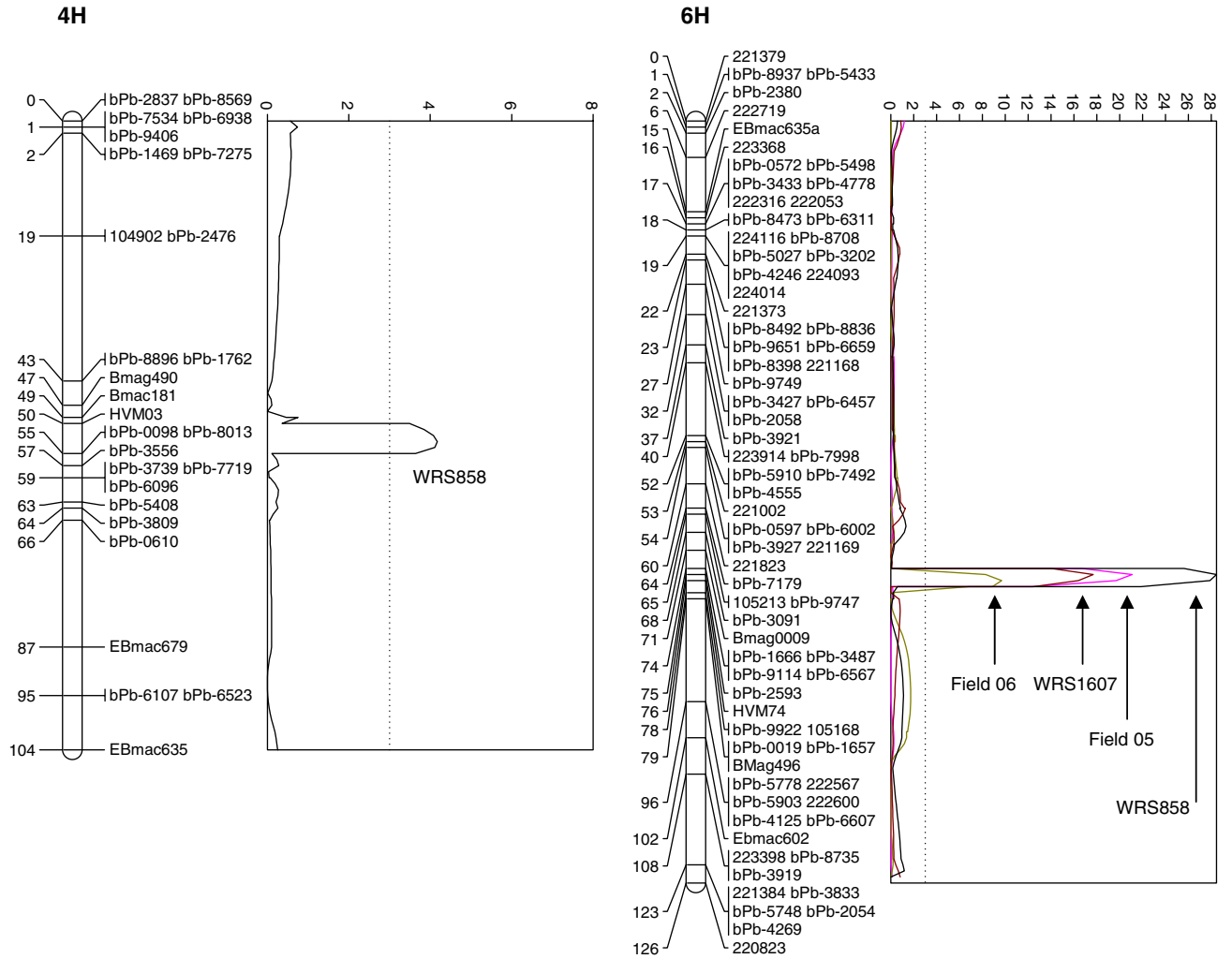

Fig. 1 Multiple-QTL model (MQM) LOD scans of chromosomes where QTL were detected for NFNB resistance in the CDC Dolly/TR251 DH population. *Vertical lines* indicate significance threshold for each experiment, estimated from 1,000 permutations of the data. One QTL on *4H* associated with NFNB (WRS858) resistance. A major QTL on *6H* associated with seedling (WRS858, WRS1607) and adult-plant (Field 2005, field 2006) resistance. (Two additional QTL on 2H and 5H associated with seedling resistance to WRS858 and adult-plant resistance QTL on 3H, 5H and 7H for field 2005 not shown.)

variance) (Table 2). QTL analysis of the adult-plant reaction in the field in 2005 again identified the major QTL, *QRpt6*. Three significant QTL, *QRptta3, QRptta5* and *QRpt7* were detected on 3H, 5H and 7H, respectively (Table 2). A single major adult-plant resistance QTL on 6H, *QRpt6* ($r^2 = 42$ %) was identified from the 2006 field trial. The resistant parent TR251 contributed the resistance allele for all QTL except a seedling resistance QTL for WRS858 on 5H (*QRptts5)*, which was contributed by the susceptible parent, CDC Dolly (Table 2).

Overall, *QRpt6* was identified in all NFNB tests, explaining 42–65% of the phenotypic variation. There was an additive effect on NFNB resistance when *QRpt6* was combined with other seedling and/or adult-plant resistance QTL (Table 3), but in majority of the cases, the resistance provided by *QRpt6* alone was not significantly different from that when it was combined with other QTL. Epistatic effects of QTL were evaluated and showed that interaction between different QTL was not significant except *QRpt6* and *QRpt7* in the field in 2005 ($P = 0.02$) (Table 4). However, reduction in NFNB incidence was greater than that of *QRpt7* or their combinations when *QRpt6* was present. SSR markers HVM74 and Bmag496 were identified as tightly linked to *QRpt6* and SSR marker Bmag9 flanked *QRpt6*, 5 cM from HVM74 (Fig. 1). Lines with HVM74 had similar resistance as lines selected using all three SSR markers (HVM74, Bmag496, Bmag9) (data not shown) suggesting selection of lines with HVM74 should be sufficient for MAS for *QRpt6*.

QTL associated with SFNB resistance

MQM mapping detected a major QTL on chromosome 4H, designated *QRpts4*, explaining 21% of the phenotypic variance for SFNB reaction and a second QTL on 7H (*QRpt7*), explaining 13% of the variance (Fig. 2, Table 2). All SFNB resistance QTL were contributed by the resistant parent, TR251. SSR markers HVM03 and Bmac181 were associated with *QRpts4* and the QTL was flanked by SSR marker Bmag490, 3 cM from HVM03.

Three QTL (*QRpt6*, *QRpts4*, and *QRpt7*) were associated with resistance to both forms of net blotch. Lines combining *QRpt6* with *QRpts4* or *QRpt7* or both, showed significantly better resistance to both forms of net blotch than those having none of these QTL (Table 5). However, lines with TR251 allele at *QRpts4* and *QRpt7* showed significantly better resistance only for SFNB and the field 2006 NFNB test (Table 5).

Validation of SSR markers

MEH#486 exhibited resistance at the seedling and adult-plant stage and Harrington was susceptible (Table 6).

Table 2 QTL identified for net blotch resistance in the CDC Dolly/TR251 population

Test	QTL	Chr	Support interval (cM)	Closest marker[a]	LOD	r^{2b}	Add. Effect[c]
NFNB							
[d]WRS858	*QRpt6*	6H	75−78	HVM74	28.5**	65	1.27 (TR251)
	QRptts2	2H	50−51	bPb-4877	6.8**	8	0.46 (TR251)
	QRpts4	4H	50−54	HVM03	4.2**	5	0.34 (TR251)
	QRptts5	5H	109	bPb-8462	6.2**	7	0.44 (CDC Dolly)
[d]WRS1607	*QRpt6*	6H	75−78	HVM74	17.7**	60	0.86 (TR251)
Field 2005	*QRpt6*	6H	75−78	HVM74	21.1**	60	0.92 (TR251)
	QRptta3	3H	115−119	bPb-9599	3.0*	5	0.28 (TR251)
	QRptta5	5H	89−97	bPb-1462	3.4*	6	0.29 (TR251)
	QRpt7	7H	116−134	222163	3.3*	6	0.29 (TR251)
Field 2006	*QRpt6*	6H	75−78	HVM74	10.7**	42	0.54 (TR251)
SFNB							
[d]WRS857	*QRpts4*	4H	50−54	HVM03	5.7**	21	0.52 (TR251)
	QRpt7	7H	116−134	222163	3.3*	13	0.42 (TR251)
	QRpt6	6H	75−78	HVM74	2.5	6	0.30 (TR251)

* Significant ($P < 0.05$); ** highly significant ($P < 0.001$)

NFNB Net-form net blotch, *SFNB* spot-form net blotch

[a] Marker with maximum LOD (logarithm of the odds)

[b] The amount of total trait variance explained by a QTL at this locus, as %

[c] Additive effect for QTL association and name given in bracket is the parent contributing resistance allele at the QTL

[d] *Pyrenophora teres* isolate

Table 3 Average infection response of lines with NFNB seedling and/or adult-plant resistance QTL from the CDC Dolly/TR251 population

	[a]WRS858	[a]WRS1607	Field 2005	Field 2006	[c]# of lines
Seedling resistance QTL					
[b]*QRpt6*	4.3	3.0	3.4	2.3	51
QRpt6 + *QRptts2*	3.6**	3.0	3.3	2.3	27
QRpt6 + *QRptts2* + *QRpts4*	3.4**	2.9	3.3	2.2	12
QRpt6 + *QRptts2* + *QRpts4* + *QRptts5*	2.8**	2.8	3.7	2.3	7
No SR QTL	7.5**	4.9**	4.3	3.5**	3
Adult-plant resistance QTL					
QRpt6 + *QRptta5*	4.7	3.2	2.9**	2.2	20
QRpt6 + *QRptta5* + *QRptta3*	4.4	3.0	2.7**	2.2	8
QRpt6 + *QRptta5* + *QRptta3* + *QRpt7*	4.5	3.4	2.9	1.9	4
No APR QTL	6.5**	5.1**	6.2**	3.6**	4

** Highly significant ($P < 0.01$) than the value in the first column when read in a respective column

[a] *Pyrenophora teres* isolate

[b] Least square means of lines with *QRpt6* in each test were compared with that of lines with QTL combinations using a *T*-test

[c] Total number of lines with resistance allele at particular QTL or their combinations

Table 4 Least square (LS) means (±standard error) of a significant interaction between *QRpt6* (6H) and *QRpt7* (7H) for the 2005 Field net-form net blotch (NFNB) experiment

Interaction	*QRpt6*	*QRpt7*	LS Mean ± SE
QRpt6 × *QRpt7*	CDC Dolly	CDC Dolly	5.7 ± 0.16
	CDC Dolly	TR251	4.7 ± 0.19
	TR251	CDC Dolly	3.5 ± 0.14
	TR251	TR251	3.3 ± 0.18

The parental allele at each QTL loci is indicated

Allelic variation at HVM74, linked to *QRpt6*, was associated with MEH #486 seedling resistance to NFNB isolate WRS858 and adult-plant resistance in the field (Table 7), indicating *QRpt6* is present in MEH#486. Similarly, HVM03 linked to *QRpts4* was associated with SFNB resistance in this population. Lines from the MEH#486/Harrington population with the MEH#486 allele at the *QRpt6* locus had an IR of 4.0 at the seedling stage compared with 6.8 for lines with the Harrington allele. Similarly, at the adult-plant stage, lines with the MEH#486 allele had an IR of 3.0, whereas lines with the Harrington allele had an IR of 4.5. Lines with *QRpt6* showed significantly better resistance to both forms of net blotch than those without *QRpt6*. Lines with *QRpts4* showed lower IR than those without *QRpts4* but the difference was non-significant. Selection based on both the markers showed better resistance than either or no marker, but the differences were non-significant as compared to lines with *QRpt6* alone except for isolate WRS858 (Table 7), further proving selection based on *QRpt6* alone could give better resistance to both net blotch forms.

Discussion

QTL analysis is useful for locating major and minor genes and to determine their interactions in a segregating population. Most importantly, there is no need for arbitrary classification of lines into discrete resistance or susceptibility classes. A NFNB seedling resistance QTL, *QRpt6*, spanning approximately 3 cM interval on chromosome 6H was identified for isolates WRS858 and WRS1607 (Fig. 1). The same QTL was identified for adult-plant resistance in the 2005 and 2006 field trials. These results confirm earlier reports of a major QTL or gene on chromosome 6H (Steffenson et al. 1996; Manninen et al. 2000; Cakir et al. 2003; Gupta et al. 2004; Friesen et al. 2006). Three additional QTL for seedling resistance to the more virulent NFNB isolate WRS858 were identified on chromosomes 2H, 4H and 5H. Three significant adult-plant resistance QTL, *QRptta3, QRptta5, and QRptta7*, were also identified on chromosome 3H, 5H and 7H, respectively, from the 2005 field trial. This may be due to higher natural infection in 2005 versus the 2006 field trial. QTL for NFNB resistance on 2H (Steffenson et al. 1996; Richter et al. 1998; Cakir et al. 2003; Raman et al. 2003; Ma et al. 2004), 3H (Cakir et al. 2003; Raman et al. 2003; Yun et al. 2005), 4H (Steffenson et al. 1996; Richter et al. 1998; Spaner et al. 1998; Raman et al. 2003; Yun et al. 2005) 5H and 7H (Steffenson et al. 1996; Spaner et al. 1998) have been reported. These results suggest the CDC Dolly/TR251 population is segregating for the majority of resistance QTL, making this population useful for fine mapping or candidate gene validation.

A highly significant seedling resistance QTL-*QRpts4* for the SFNB isolate WRS857 was detected on chromosome 4H and a significant QTL, *QRpt7*, mapped to 7H (Fig. 2).

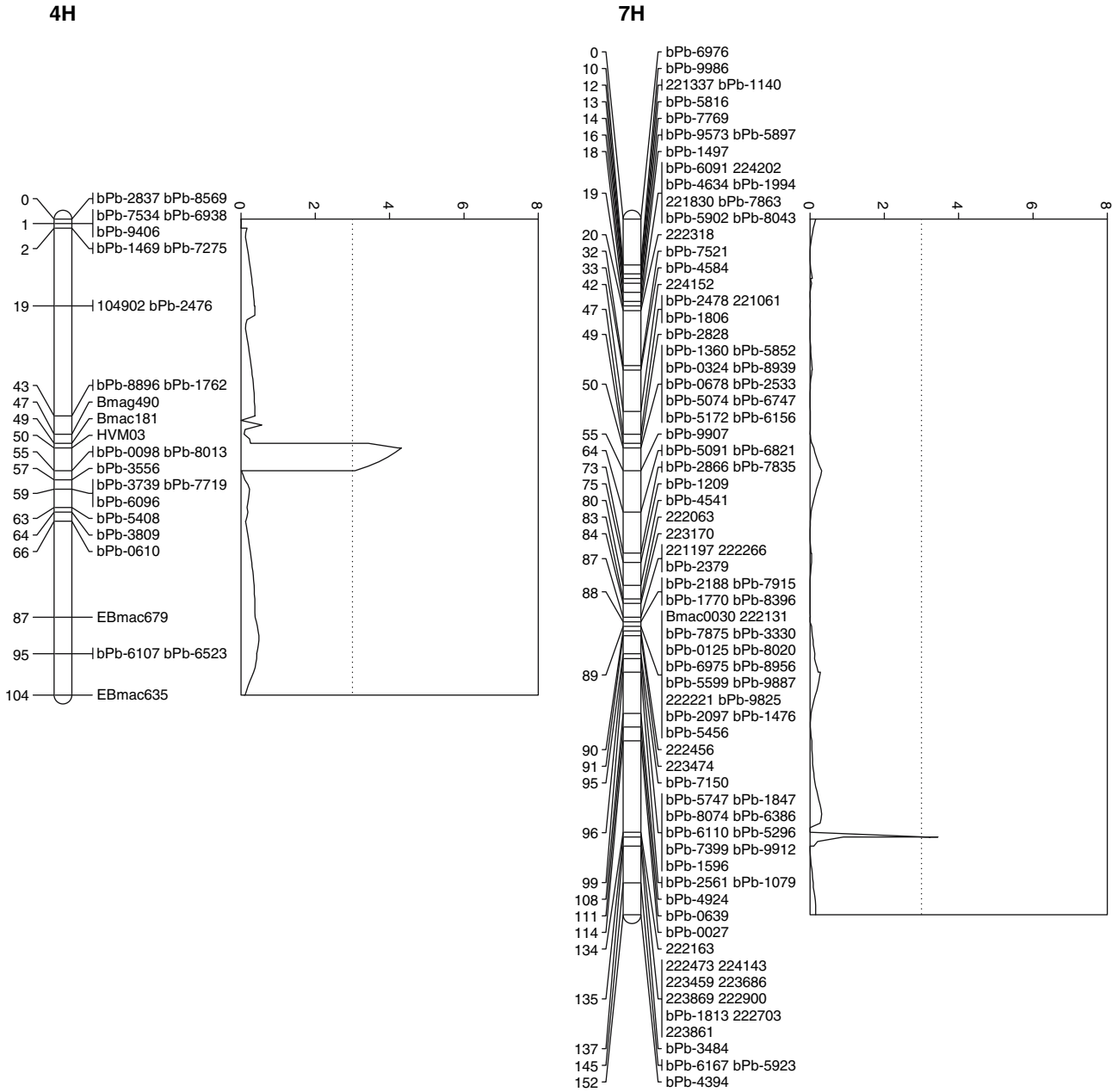

Fig. 2 Multiple-QTL model (MQM) LOD scans of chromosomes where QTL were detected for SFNB resistance in the CDC Dolly/TR251 DH population. *Vertical lines* indicate significance threshold for each experiment, estimated from 1,000 permutations of the data. A major QTL on *4H* and a significant QTL on *7H* associated with SFNB seedling resistance (WRS857)

Williams et al. (1999) mapped a major seedling resistance gene *Rpt4*/QTL, explaining 80% of SFNB reaction variation, on chromosome 7H in cv "Galleon" and the same gene was responsible for SFNB seedling resistance in five other barley lines (Williams et al. 2003). Based on the consensus map (Wenzl et al. 2006), *QRpt7* in the CDC Dolly/TR251 population is at the same location as *Rpt4*, thus the major SFNB seedling resistance QTL, *QRpts4*, identified in this study is novel. Interestingly, Williams et al. (2003) detected adult-plant resistance QTL on chromosome 4H in VB9104 linked to SSR marker HVM03, but that QTL was not effective at the seedling stage. This suggests that *P. teres* f. *maculata* populations in Canada and Australia are different. This was confirmed by screening several Australian barley populations with Canadian net blotch isolates (Grewal et al. unpublished data). Isolate WRS857, used in this study, is a long-term standard Canadian SFNB isolate (A. Tekauz, personal communication) used to screen elite barley lines for SFNB resistance reaction. Recently, Friesen et al. (2006) reported a major SFNB seedling resistance

Table 5 Average infection response of lines with or without net blotch resistance QTL from the CDC Dolly/TR251 population

QTL/test	NFNB					SFNB	
	[a]Allele	[b]WRS858	[b]WRS1607	Field 2005	Field 2006	[b]WRS857	[c]# of lines
QRpt6	R	4.2**	3.0**	3.4**	2.3**	6.3	51
	S	6.8	4.8	5.1	3.3	6.7	39
QRpts4	R	5.2	3.8	4.3	2.7	6.0**	48
	S	5.5	3.8	3.9	2.7	7.0	42
QRpt7	R	5.9	3.9	3.9	2.6	6.0**	28
	S	5.2	3.8	4.3	2.8	6.7	56
QRpt6 + *QRpts4*	R	4.0**	2.9**	3.5**	2.3**	5.6**	25
	S	7.1	4.7	4.8	3.5	7.1	16
QRpt6 + *QRpt7*	R	4.4**	2.8**	3.2**	2.0**	5.3**	14
	S	6.6	4.7	5.5	3.5	6.8	22
QRpts4 + *QRpt7*	R	5.4	3.7	3.8	2.2*	5.1**	15
	S	5.1	3.6	4.0	2.6	7.1	27
QRpt6 + *QRpts4* + *QRpt7*	R	4.4**	2.8**	3.0**	1.9**	4.5**	9
	S	6.8	4.7	5.4	3.5	7.5	7

NFNB Net-form net blotch, *SFNB* spot-form net blotch

* Significant ($P < 0.05$), ** highly significant ($P < 0.01$) using *T*-test between genotypic classes at a particular QTL or their combinations

[a] Resistance (R) or susceptible (S) allele at a particular QTL locus/loci

[b] *Pyrenophora teres* isolate

[c] Total number of lines with R or S allele at particular QTL

Table 6 Net blotch reaction of parents of the MEH#486/Harrington population and checks

Line/Isolate	NFNB		SFNB
	[a]WRS858	Field 2006	[a]WRS857
MEH#486	2.7	1.8	2.8
Harrington	8.4	7.7	7.9
TR253	1.7	1.6	2.2
CDC Dolly	8.8	4.3	7.4
Population			
Mean	5.3	3.7	6.2
Range	2.0−9.3	1.3−7.7	2.3−9.0
[b]$LSD_{0.05}$	1.3	1.5	1.3
Heritability	0.93	0.83	0.90

NFNB Net-form net blotch, *SFNB* spot-form net blotch

[a] *Pyrenophora teres* isolate

[b] Least significant differences ($P = 0.05$)

Table 7 Average infection response of lines from the MEH#486/Harrington population with or without major net blotch resistance QTL (*QRpt6*, *QRpts4*)

QTL/test	[a]Allele	NFNB		SFNB	
		[b]WRS858	Field 2006	[b]WRS857	[c]# of lines
QRpt6	R	4.0**	3.0**	5.7**	79
	S	6.8	4.5	6.8	69
QRpts4	R	5.0	3.6	6.0	62
	S	5.6	3.8	6.4	83
QRpt6 + *QRpts4*	R	3.4**	2.8**	5.4**	25
	S	7.1	4.8	6.9	32

NFNB Net-form net blotch, *SFNB* spot-form net blotch

[a] Resistance (R) or susceptible (S) allele at a particular QTL locus/loci

[b] *Pyrenophora teres* isolate

[c] Total number of lines with R or S allele at particular QTL

** Highly significant ($P < 0.01$) using *T*-test at particular QTL or their combinations

QTL on 4H, explaining 64% of the phenotypic variance. However, the position of that QTL (near the 4H telomere) is not the same as that of the QTL reported here.

DArT markers cannot easily be used for routine MAS, thus there is a need to identify markers linked to highly significant QTL, which could be used for routine MAS. SSR markers are an obvious choice, as they show high polymorphism, are co-dominant, usually chromosome-specific, reliable and simple to assay. Using a DArT/SSR consensus map, we were able to identify SSR markers linked to the major NFNB and SFNB resistance QTL. HVM74 was the closest marker to the NFNB seedling and adult-plant resistance QTL *QRpt6* with both flanked by SSR markers Bmag496 and Bmag9. Similarly, HVM03 was the closest marker to the SFNB seedling resistance QTL, *QRpts4*, and two other markers (Bmac181 and Bmag490) were also linked.

Three QTL (*QRpt6*, *QRpts4*, and *QRpt7*) were effective for resistance to both forms of net blotch. Lines selected based on *QRpt6* or its combinations with two other markers had better resistance to both NFNB and SFNB. For the most part, epistatic interaction between QTL were not significant suggesting primarily additive effects. The major seedling and adult-plant resistance QTL, *QRpt6* may be sufficient to provide adequate field resistance to NFNB and could be selected for on the basis of SSR markers. High heritability for different tests indicated that selection based on molecular markers is feasible. This was validated in an unrelated barley population MEH#486/Harrington where *QRpt6* alone provided better resistance to both forms of net blotch. *QRpt6* in combination with *QRpts4* showed better resistance than *QRpt6* alone, but differences were not significant confirming the epistatic interaction between different QTL was not significant. We propose MAS for *QRpt6* using SSR markers for NFNB resistance, which could be combined with *QRpts4* if SFNB is also important. Molecular markers will also facilitate introgression/pyramiding of different resistance QTL into elite barley lines.

Acknowledgments We are grateful to Rob Stonehouse and Ramon Arango for technical assistance, to Doug Voth for his assistance in field experiments, to Dr. A. Tekauz for supplying net blotch isolates and to Dr. W. Legge for supplying seed of the MEH#486/Harrington population. The work was funded in part by Saskatchewan Agriculture Development Fund Project # 20040386 and the Western Grains Research Foundation Check-off for barley breeding.

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