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Mapping of genomic regions associated with net form of net blotch resistance in barley

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Abstract. Quantitative trait loci (QTLs) associated with resistance to net blotch and their chromosomal locations were determined from analyses of doubled haploid progeny of Alexis/Sloop, Arapiles/Franklin, Sloop/Halcyon, and recombinant inbred lines of Sloop-sib/Alexis. Five QTLs on chromosomes 2H, 3H, and 4H were found to be associated with seedling resistance to the net form of net blotch. In Arapiles/Franklin and Alexis/Sloop populations, 4 significant QTLs explaining 9–17% of the variation in net blotch resistance were detected on 2H and 3H. A major locus, *QRpts4L* accounting for 64% of the variation in infection type, was detected on 4H in the Sloop/Halcyon population. In Sloop/Halcyon, 2 microsatellite markers, EBmac0906 and GMS089, and AFLP marker P13/M50-108, co-segregated and detected maximum variability for net blotch resistance as revealed by bootstrap analysis. EBmac0906 and Bmac0181 were validated in F_2 progeny of an Ant29/Halcyon population and reliably predicted phenotypes of 93% of lines resistant and susceptible to net blotch. These markers may be used within breeding programs to select alleles favourable for net blotch resistance derived from Halcyon.

Additional keywords: seedling resistance, *Pyrenophora teres* f. *teres*, QTL, validation.

Introduction

Net blotch, caused by the fungus *Drechslera teres* (Sacc.) Shoem. f. *teres* Smedeg. (Teleomorph: *Pyrenophora teres* Drechs. f. *teres* Smedeg), is one of the most significant foliar diseases of barley throughout the world (Shipton *et al*. 1973). The disease exists as two forms, the spot form caused by *P. teres* f. *maculata* and the net form caused by *P. teres* f. *teres*, and these can be differentiated on the basis of leaf symptoms (Smedegaard-Petersen 1971). Although the common name often embraces both forms of the disease, in this paper it refers exclusively to the net form and is abbreviated as NFNB. This disease causes a net type lesion, which is characterised by elongate, dark brown blotches often with a net-like pattern. In susceptible seedlings, production of toxins by the pathogen leads to rapid and extensive leaf death. The disease can cause yield losses in excess of 50% and also considerably reduce grain quality (Tekauz 1990; Steffenson *et al*. 1996; Poulsen *et al*. 1999). Identification of sources of resistance to NFNB and an understanding of their genetics are very important in developing resistant varieties (Jalli and Robinson 2000). NFNB resistance is due to either one or several genes and is dependent upon the source of resistance, plant development stage (seedling or adult), and the pathotype used for testing (Mode and Schaller 1958; Wilcoxson *et al*. 1992; Graner *et al*. 1996; Steffenson *et al*. 1996). At adult plant stages, 7–12 quantitative trait loci (QTLs) associated with net blotch resistance have been identified, located on all barley chromosomes except 5H (Steffenson *et al*. 1996; Richter *et al*. 1998).

Most Australian barley cultivars are susceptible or moderately susceptible to one or more pathotypes of the disease (G. J. Platz, unpublished data). However, useful variability for resistance is present in world barley

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germplasm (Tekauz and Buchannon 1977) and has been exploited to introduce useful genes from different resistant sources into barley breeding programs. The cultivars Alexis, Franklin, and Halcyon are being used to introgress desirable genes for disease resistance, malting quality, and growth habit into adapted Australian barley cultivars. Screening of parental lines of populations within the Australian National Barley Molecular Marker Program (NBMMP) with a range of Australian pathotypes of NFNB identified differences in susceptibilities at the seedling stage between Alexis and Sloop, Arapiles and Franklin, and Sloop and Halcyon.

Selection for net blotch resistance in breeding programs has been based on host reaction at the seedling and/or adult plant stage. Molecular markers are recognised as powerful tools for indirect selection and would enhance the efficiency and accuracy of screening for NFNB resistance. Furthermore, quantitative analysis has proven useful for locating genes controlling complex traits and provides a more accurate estimation of gene location than qualitative analysis because of its lower sensitivity to even modest numbers of phenotypic mis-scores (Wright *et al*. 1998). The role and location of QTLs controlling resistance to NFNB in Sloop, Arapiles, Alexis, Franklin, and Halcyon were unknown. Identification of molecular markers associated with major gene loci and QTLs conferring NFNB resistance is imperative for marker assisted selection (MAS) and to facilitate combination of various QTLs to develop higher levels of net blotch resistance. This paper reports the mapping of QTLs for net blotch resistance in 4 populations derived from Alexis/Sloop, Sloop-sib/Alexis, Arapiles/Franklin, and Sloop/Halcyon as part of the NBMMP.

Materials and methods

Mapping populations and genetic maps

Three doubled haploid (DH) mapping populations derived from Alexis/Sloop, Arapiles/Franklin, and Sloop/Halcyon and one population of F_4-derived recombinant inbred lines (RILs) from Sloop-sib/Alexis were generated by the NBMMP. Full linkage maps based upon amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), microsatellite, and single nucleotide polymorphism (SNP) markers were constructed (Barr *et al*. 2003, this issue; Read *et al*. 2003, this issue; D. B. Moody *et al*. unpublished data). These molecular maps were utilised to define the location of QTLs and the linked markers associated with NFNB resistance at the seedling stage.

Pathogen isolates

Screening of parental lines in the NBMMP with a selection of net blotch isolates (Gupta *et al*. 2003, this issue) indicated that isolate NB34 differentiated between Arapiles and Franklin and Alexis and Sloop, whereas NB50 gave superior separation between Halcyon and Sloop. Cultures of NB34 and NB50, preserved in the form of infected dry leaf at –70°C, were retrieved from storage and heat-shocked at 45°C for 3 min. Infected leaf pieces were maintained in a moist dish for 2–3 days at 19°C with 12 h of light (12 L) supplied by 2 blue-black and one cool white fluorescent tube from a distance of 20 cm. Conidia were then picked from the leaf with a sterile needle, cultured on V8 agar, and incubated in the dark at 25°C for 5 days. Plugs were cut from the edges of colonies, placed on peanut oatmeal agar medium (Speakman and Pommer 1986), and incubated for 9 days at 19°C and 12 L as above.

Screening for net blotch resistance

Two replications of 5–7 seeds of each line were sown at 3 sites around the circumference of a 10-cm maxi pot (Garden City Plastics) containing a peat/loam/vermiculite medium (1 : 2 : 1 by vol.) and raised in a glasshouse at 15/25°C and 10–12 h photoperiod. Plants were fertilised weekly after emergence, with single strength Aquasol (Hortico Australia) to avoid any nutrient deficiency. After about 14 days, when seedlings were at the 2-leaf stage, they were inoculated with an aqueous suspension containing 15 000 conidia of the target isolate/mL. Pots were arranged shoulder to shoulder and 200 mL of suspension was applied per m^2 of occupied bench space using an airless spray gun (Krebs Inc.). Inoculated seedlings were incubated in a fogging chamber at 19°C under 14-h dark followed by 10-h light. Seedlings were then returned to the glasshouse and infection response (IR) on the second leaf recorded 8 days later using the scale of Tekauz (1985). IRs used for analysis were means of the 2 replicates.

Statistical analyses

Chi-square analyses were performed to test the goodness-of-fit of the observed over the expected genetic ratios for net blotch infection response.

QTL analysis

The 4 fully constructed linkage maps of the above populations were utilised for QTL analyses. Marker and quantitative phenotypic data from infection response (1–10) were evaluated using MapManager QTX (MMQTX) version 17b (Manly *et al*. 2001). Initially, a single-point regression analysis was performed using the 'QTL Marker Regression' with a threshold of $P = 0.001$. The significant associations between markers and infection response data were tested using likelihood ratio statistics (LRS, Haley and Knott 1992). As a second step, simple interval mapping (SIM) analysis was performed to find evidence of QTLs by MapManager and/or QTL Cartographer (Wang *et al*. 2003) and QGENE (Nelson 1997). Significance thresholds for the test statistics were estimated by 1000 permutations at a significance level of $P = 0.001$ (Doerge and Churchill 1996) by following the algorithm implemented in MMQTX. QTL effects were considered significant either when they exceeded a $\log_{10}$ of the odds ratio (LOD) score of 3.0 or the calculated statistic value by permutation tests (significant at $P = 0.05$, highly significant at $P = 0.001$). The conventional base LOD score was converted to LRS by multiplying it by 4.60 for comparisons as described by Manly *et al*. (2001). QTLs were declared at the genomic position of the test statistic's peak for the main effect and/or their interaction with environment. Bootstrap analysis was performed to estimate a confidence interval and to record the position of the maximum LRS (Visscher *et al*. 1996). In a third approach, composite interval mapping (CIM) was performed to explore the effect of QTL combinations and to separate additional environmental effects that may have reduced the significance of estimated marker trait association (Zeng 1994). To reveal interactions or epistatic effects, 2-locus analysis was performed using $P = 10e^{-6}$ and interactions were tested for significance with an additive model using permutation tests in MMQTX.

Nomenclature of QTLs

A '*QRpt*' indicates a QTL/genomic region for resistance to *P. teres* f. *teres*. This is followed by 's' to indicate a seedling and followed by the barley chromosome arm onto which the QTL/genomic region was mapped.

Validation of microsatellite markers linked with NFNB resistance in Halcyon

An F_2 population comprising 81 plants derived from Ant29/Halcyon was screened for resistance to NFNB as described above. Healthy leaf samples from these were used for validation of 4 microsatellite markers (EBmac0906, Bmac0310, Bmag0353, Bmac0181), which mapped within 10 cM of the locus conferring NFNB resistance in Sloop/Halcyon. These markers have been reported to map closely in barley populations derived from Lina/Canada Park, WB229/Yambla, and Dayton/Harlan Hybrid (Ramsay *et al*. 2000; Raman *et al*. 2002, 2003, this issue). Deoxyribonucleic acid was extracted in 2 mL Eppendorf tubes as described previously (Raman and Read 2000). Microsatellite analyses were performed following PCR conditions as described by Ramsay *et al*. (2000).

Results

Seedling resistance to net form of net blotch

The IRs of all parents were consistent across replicates, although Arapiles showed a 2-unit difference (Table 1). Although most lines gave IRs similar to those reported by Gupta *et al*. (2003), Alexis was higher (6.5 *v.* 4.5) and Arapiles lower (8 *v.* 10) than in the initial parent screening.

After inoculation with the appropriate isolate, all plants developed classical symptoms of NFNB, which allowed discrimination of resistant and susceptible genotypes. The distribution of IRs varied among populations (Fig. 1).

Transgressive segregation for disease response was observed especially in the Alexis/Sloop and Sloop-sib/Alexis populations (Fig. 1). This indicated that the parents carried different resistance genes.

QTL analyses

QTL analysis by SIM detected 5 QTLs/genomic regions on 2H, 3H, and 4H, contributing 7–64% phenotypic variance for net blotch resistance (Table 2).

Alexis/Sloop

In the DH population of Alexis/Sloop, one significant QTL, *QRpts3L* from Alexis flanked with P14/M61-154 and P13/

Table 1. Seedling infection response of mapping parents to 2 pathotypes of net blotch (scale 1–10 where 1 is resistant and 10 is susceptible)

Parent	Isolate	Reaction to *P. teres* f. *teres*	
		Replicate 1	Replicate 2
Alexis	NB34	6	7
Sloop	NB34	9	9
Franklin	NB34	3	3
Arapiles	NB34	7	9
Halcyon	NB50	3	3
Sloop	NB50	9	9

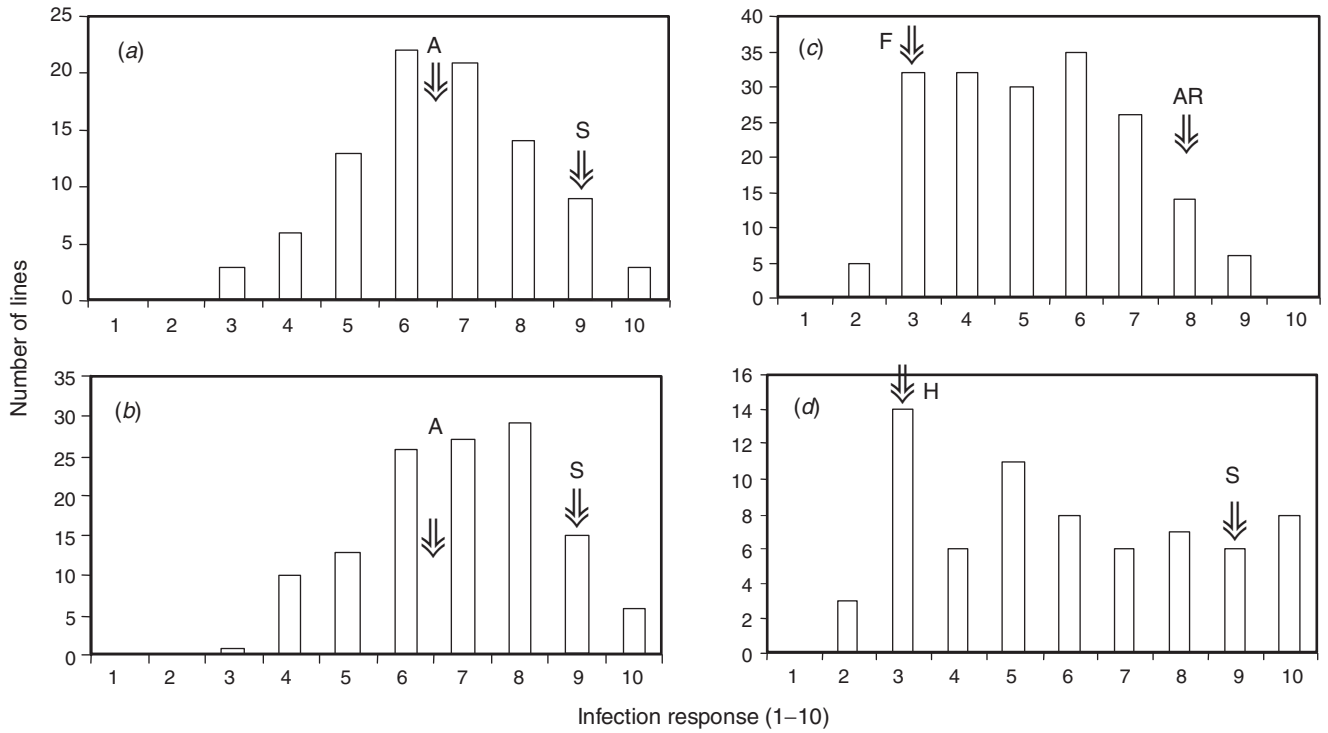

Fig. 1. Frequency distribution of phenotypes for net form of net blotch resistance in 4 segregating barley populations: (*a*) Alexis × Sloop DH population; (*b*) Sloop-sib × Alexis RI lines; (*c*) Arapiles × Franklin DH population; and (*d*) Sloop × Halcyon DH population. Parental means indicated for Alexis (A), Sloop (S), Arapiles (AR), Franklin (F), and Halcyon (H).

Table 2. QTLs identified for net form of net blotch resistance from 4 barley populations using simple interval mapping by Map Manager

QTL	Marker interval	Chromosome	Closest marker[A]	Interval analysis LRS	r^{2B} (%)	Add. effect[C]	λ^2 Statistics[D] $P < 0.05$ ($P < 0.001$)
			Alexis/Sloop-DH				
QRpts2S	P12/M50-241 P13/M47-399	2HS	P13/M47-399	11.7	11	0.56 (Sloop)	12.9 (19.7)
QRpts3L	P14/M61-154 P13/M50-331	3HL	P14/M61-154	18.5	17	0.80 (Alexis)	
			Sloop-sib/Alexis-RIL				
QRpts2S	P14/M48-94 P12/M50-199	2HS	P12/M50-199	12.4	9	0.49 (Sloop)	12.7 (19.2)
QRpts3L	P14/M61-154 ABG4	3HL	P14/M61-154	17.4	13	0.61 (Alexis)	
			Arapiles/Franklin-DH				
QRpts2S	P13/M47-399 P14/M47-299	2HS	P14/M47-299	22.9	13	0.90 (Franklin)	12.6 (20.9)
QRpts2L	P11/M62-160	2HL	P11/M62-160	12.5	7	0.46 (Franklin)	
QRpts3La	P11/M62-261 Bmag0225	3HL	P13/M62-299	28.7	16	0.69 (Arapiles)	
QRpts3Lb	P11/M62-212 P13/M48-312	3HL	P13/M60-185	16.3	9	0.52 (Franklin)	
			Sloop/Halcyon-DH				
QRpts4	P11/M48-105 ABG472	4H	P13/M50-108	94.4	64	2.03 (Halcyon)	13.0 (24.7)
QRpts6L	WG0622-2	6HL	WG0622-2	10.3	11	0.85 (Halcyon)	

[A]Markers detecting maximum LRS (likelihood ratio statistics).
[B]r^2, total trait variance explained by a QTL.
[C]Additive regression coefficient for the association. Name given in parentheses is the parent contributing the favourable allele.
[D]The threshold for highly significant ($P < 0.01$) and significant ($P < 0.05$) associations as determined using permutation tests.

M50-331 (LRS = 18.5, r^2 = 17%), was detected on 3HL (Table 2, Fig. 2*b*). However, another non-significant 'weak' QTL from Sloop, reaching close to the level of significance (LRS = 11.7/LOD > 2.5, r^2 = 11%), was identified on 2HS (Table 2, Fig. 2*a*).

In the RILs of Sloop-sib/Alexis, a similar QTL, *QRpts3L* flanked with P14/M61-154 and ABG4, was detected on 3HL (Fig. 2*d*). The AFLP locus XP14/M61-154 detected maximum variability (LRS = 17.4, r^2 = 13%) as revealed by bootstrap analysis (Table 2). SIM indicated that *QRpts3L* from Alexis increased the phenotypic variation for resistance to NFNB. Another non-significant weak QTL approaching the level of significance (LRS = 12.4, r^2 = 9%) was also detected with MMQTX on 2HS in RILs of Sloop-sib/Alexis (Table 2). This weak QTL—*QRpts2Sa* in Fig. 2*c*—was significant, attaining an LOD score of >3.0, when interval mapping was carried out with QGene. This software also detected a genomic region, designated as *QRpt2sb*, which had an LOD score of 3.0 (Fig. 2*c*).

Arapiles/Franklin

SIM and CIM showed evidence for a total of 4 QTLs affecting NFNB resistance in the DH population of Arapiles/Franklin. One highly significant QTL, designated as *QRpts2S*, from Franklin, was flanked with P13/M47-399 and co-segregating markers P14/M47-299 and P14/M48-332 (LRS = 22.9, r^2 = 13%) on 2HS. A second QTL, *QRpts2L*, nearing the level of significance (LRS = 12.5/LOD = 2.7) and explaining 7% of the phenotypic variance, was detected at XP11/M62-160 on 2HL (Table 2). A third highly significant QTL, *QRpts3La*, flanked with P11/M62-261 and Bmag0225 markers (LRS = 28.7, r^2 = 16%), from Arapiles, and a fourth significant QTL (*QRpts3Lb*) with marker intervals of P11/M62-212 and P13/M48-312 detecting 9% of phenotypic variance, were identified on 3HL (Table 2). At 3 of the 4 QTLs linked with NFNB resistance, alleles from Franklin were in the direction of increasing resistance, and an allele from Arapiles at the *QRpts3La* locus increased resistance to isolate NB34. Similar QTLs were detected using the QTL Cartographer program.

Sloop/Halcyon

A major gene locus, *QRpts4L* from Halcyon, flanked by P11/M48-105 and ABG472 on chromosome 4H, exhibited significant association with NFNB resistance to isolate NB50 (Fig. 3). The P13/M50-108 marker revealed

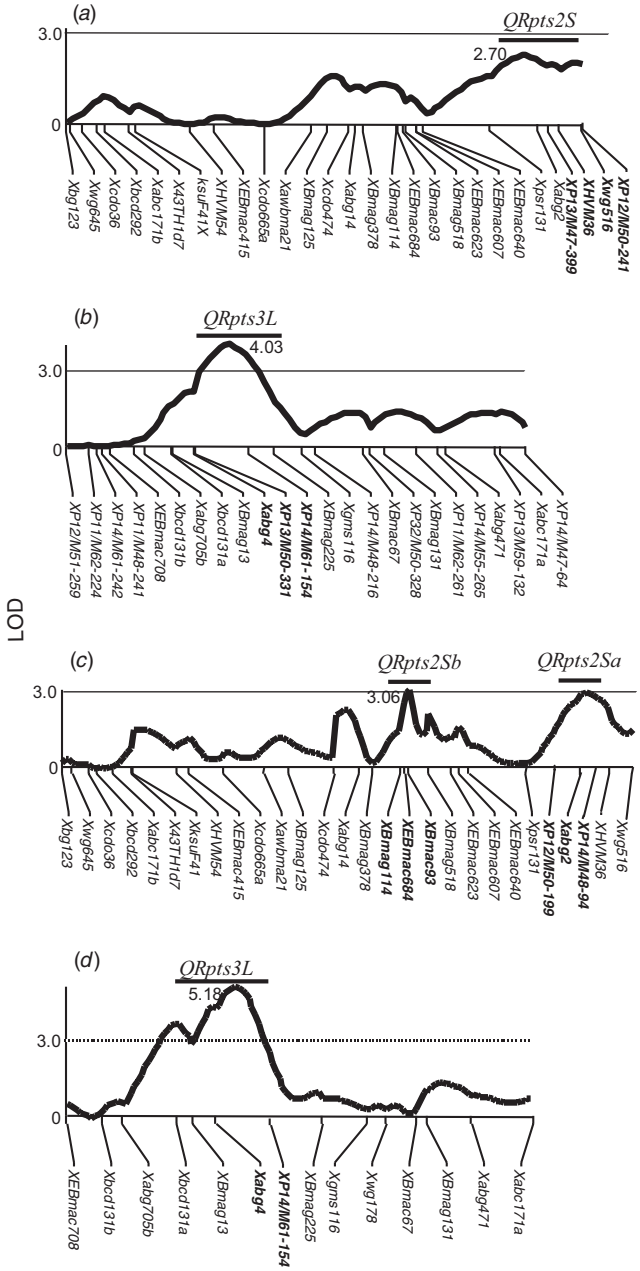

Fig. 2. Map location of QTL associated with net form of net blotch resistance identified using QGENE. Region at or above the threshold LOD score (3.0) shows significant association. (*a*) *QRpts2S* on chromosome 2H in Alexis × Sloop DH population; (*b*) *QRpts3L* on chromosome 3H in Alexis × Sloop DH population; (*c*) *QRpts2Sa* and *QRpts2Sb* on 2HS in RILs of Sloop-sib × Alexis; (*d*) *QRpts3L* on chromosome 3H in RILs of Sloop-sib × Alexis. Note that for clarity, marker density has been reduced. The actual LOD score of *QRpts2Sa* was 5.19.

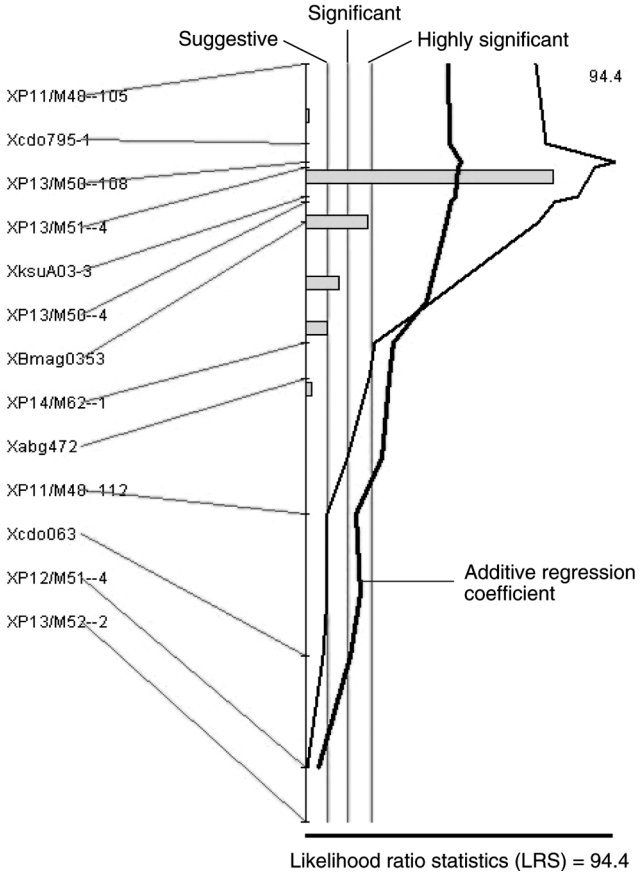

Fig. 3. A major gene associated with net form of net blotch resistance in Sloop × Halcyon DH population on chromosome 4H, detected with SIM using MMQTX. The thresholds for highly significant ($P < 0.001$ = LRS > 24.7), significant ($P < 0.05$ = LRS > 13.0), and suggestive ($P = 0.63$) as determined using permutation tests are indicated. The histogram indicates the output from bootstrap analysis showing maximum LRS within an identified genomic region, *QRpts4L*.

maximum LRS (94.4) as detected by bootstrap analyses and accounted for 64% of the total variation of net blotch resistance (Table 2).

Similar results were found with simple regression analysis using QTL Cartographer. The AFLP marker P13/M50-108 co-segregated with P14/M16-154, P13/M51-4, and microsatellite markers EBmac0906 and GMS089. These mapped in a cluster in the Sloop/Halcyon DH population (Read *et al*. 2003). A weak QTL peak on locus Xwg0622b, syn. 'Xwg0622-2', on 6HL approached close to the level of significance (LRS = 10.3) and explained 11% of the phenotypic variation, but was not statistically significant (Table 2). The Halcyon allele at this locus conferred NFNB resistance. Two-locus analysis showed non-significant interactions.

Validation of 'QRpts4'

Results of the validation experiment comprising 81 plants of an F_2 population derived from Ant29/Halcyon showed that microsatellite markers EBmac0906 and Bmac0181 may be used for marker assisted selection. The primer sequences of these microsatellite markers were:

Bmac0181F: 5′-ATAGATCACCAAGTGAACCAC-3′

Bmac0181R: 5′-GGTTATCACTGAGGCAAATAC-3′

EBmac0906F: 5′-CAAATCAATCAAGAGGCC-3′

EBmac0906R: 5′-TTTGAAGTGAGACATTTCCA-3′

Both the markers EBmac0906 and Bmac0181 co-segregated co-dominantly in F_2 progeny of the Ant29/Halcyon population. Chi-square analysis indicated that these segregation ratios (20 : 43 : 18) fit a single gene model. Resistant and susceptible phenotypes could be correlated with 93% reliability with the respective marker allele associated with resistance/susceptibility to NFNB. In some cases, resistant and intermediate resistant phenotypes to NFNB could not be correlated with the expected marker alleles. F_3 progeny testing is being performed to confirm the genotypes of F_2 families. Other markers, such as Bmac0310 and Bmag0353 that mapped within 5–10 cM of EBmac0906 in a DH population of Canada Park/Lina (Ramsay *et al*. 2000; Raman *et al*. 2003), were also polymorphic in the F_2 population of Ant29/Halcyon.

Discussion

QTL mapping, utilising molecular linkage maps, has proven to be a useful tool for locating major and minor genes, and investigating the parental origins of the favourable QTL alleles controlling genetic variation for NFNB resistance at the seedling stage. In this study, significant putative QTLs for NFNB resistance were detected on chromosomes 2H, 3H, and 4H in 4 barley populations derived from Alexis/Sloop, Sloop-sib/Alexis, Arapiles/Franklin, and Sloop/Halcyon. Several qualitative and quantitative genes conferring net blotch resistance at the seedling stage have been previously reported on 1H, 2H, 3H, 4H, 6H, and 7H. Major genes for net blotch resistance in barley, *Pt.d/Rpt3.d* on chromosome 2H (Bockelman *et al*. 1977; A. Graner and A. Tekauz, unpublished data) and *Pt1* syn. *Pt.a/ Rpt1* and *Rpt2c* on chromosome 3H, have been reported (Schaller 1955; Mode and Schaller 1958; Khan and Boyd 1969*a*; Bockelman *et al*. 1977; Wilcoxson *et al*. 1992; Graner *et al*. 1996). On chromosome 2HS, a QTL for resistance to net blotch of adult plants has been described (Pecchioni *et al*. 1996; Steffenson *et al*. 1996). Other qualitative genes for disease resistance including *Ryd2* for resistance to barley yellow dwarf virus (Collins *et al*. 1996), *Rhy*, *Rh*, and *Rrs* for scald resistance (Graner and Tekauz 1996; Graner *et al*. 2000; Genger *et al*. 2003, this issue), and *rym 4*, *rym5*, and *rym11* for virus resistance to strains of *BaMMV* and *BaYMV* (Graner and Bauer 1993; Bauer *et al*. 1997; Graner *et al*. 1999), also mapped close to *Pt.a/Pt.1* gene for net blotch resistance on the proximal region of 3HL. On chromosome 4H, a large region of the genetic map of Sloop/Halcyon covering ~50 cM exhibited significant association with NFNB resistance (Fig. 4). In this region, several genes such as *Ml-g* and *ml-o* for resistance to *Blumeria graminis* f. sp. *hordei* (Görg *et al*. 1993), QTLs for quantitative resistance to *Puccinia striiformis* f. sp. *hordei* and net blotch in both seedling and adult plant stages, were identified (Hinze *et al*. 1991; Chen *et al*. 1994; Steffenson *et al*. 1996; Manninen *et al*. 2000). Interestingly, in both the populations derived from Alexis and Sloop (DH/RILs) and Arapiles/Franklin, the same genomic regions of linkage map/QTLs were detected on 2H and 3H (Table 2, Fig. 4). Alexis and Franklin are both derivatives of Triumph; Sloop and Arapiles both have Proctor and CI3576 in their pedigree and Franklin also has Proctor in its ancestry. Identification of the same genomic regions/loci suggests that there is a possibility of the same genes or gene clusters associated with NFNB resistance. Hence our results corroborated with previous findings that disease resistance regions are conserved on the genome (Graner *et al*. 2000). Furthermore, investigations using genetic and molecular analyses have shown that resistance genes and their analogues/homologues are also clustered in genomes of different species (Parniske *et al*. 1997; Meyers *et al*. 1998).

All of the QTLs identified in Alexis/Sloop, Sloop-sib/Alexis, Arapiles/Franklin, and Sloop/Halcyon, occurred at 5 genomic regions that correlated with previously identified major genes/QTLs conferring net blotch resistance. In Fig. 4, the estimated locations of the ‘putative’ QTLs identified for NFNB resistance in this experiment are compared with those previously reported.

Mean IRs for Alexis and Arapiles differed between these experiments and the initial parent screening (Alexis 6.5 and 4.5, Arapiles 8 and 10, respectively). In the parent screening, inoculum was applied with an airbrush, but as the changes in IRs of the parents were in opposite directions, it is unlikely that this was the reason for disagreement. Inoculum was applied evenly and at the same rate in all experiments so the variation between experiments was most likely a factor of environment. Khan and Boyd (1969*b*) documented that the resistances of some genotypes were particularly sensitive to environmental influences and it is our experience that this occurs more often with genotypes that are moderately resistant to moderately susceptible, e.g. Alexis.

Isolate NB34 is virulent on the parents Sloop and Arapiles, moderately virulent on Alexis, and avirulent on Franklin. In this study, both Sloop and Arapiles appeared to have a significant QTL on chromosome 2H and 3H, respectively. The alleles at these loci are obviously inadequate to impart resistance to these varieties in the seedling stage. The QTL on 3H in Alexis appears to explain the moderate level of resistance in that variety; however, some 25% of the progeny derived from Alexis and Sloop parents were more resistant than the Alexis parent. It appears that recombination of the allele(s) on 2H from Sloop with the allele(s) on 3H from Alexis may have given these lines

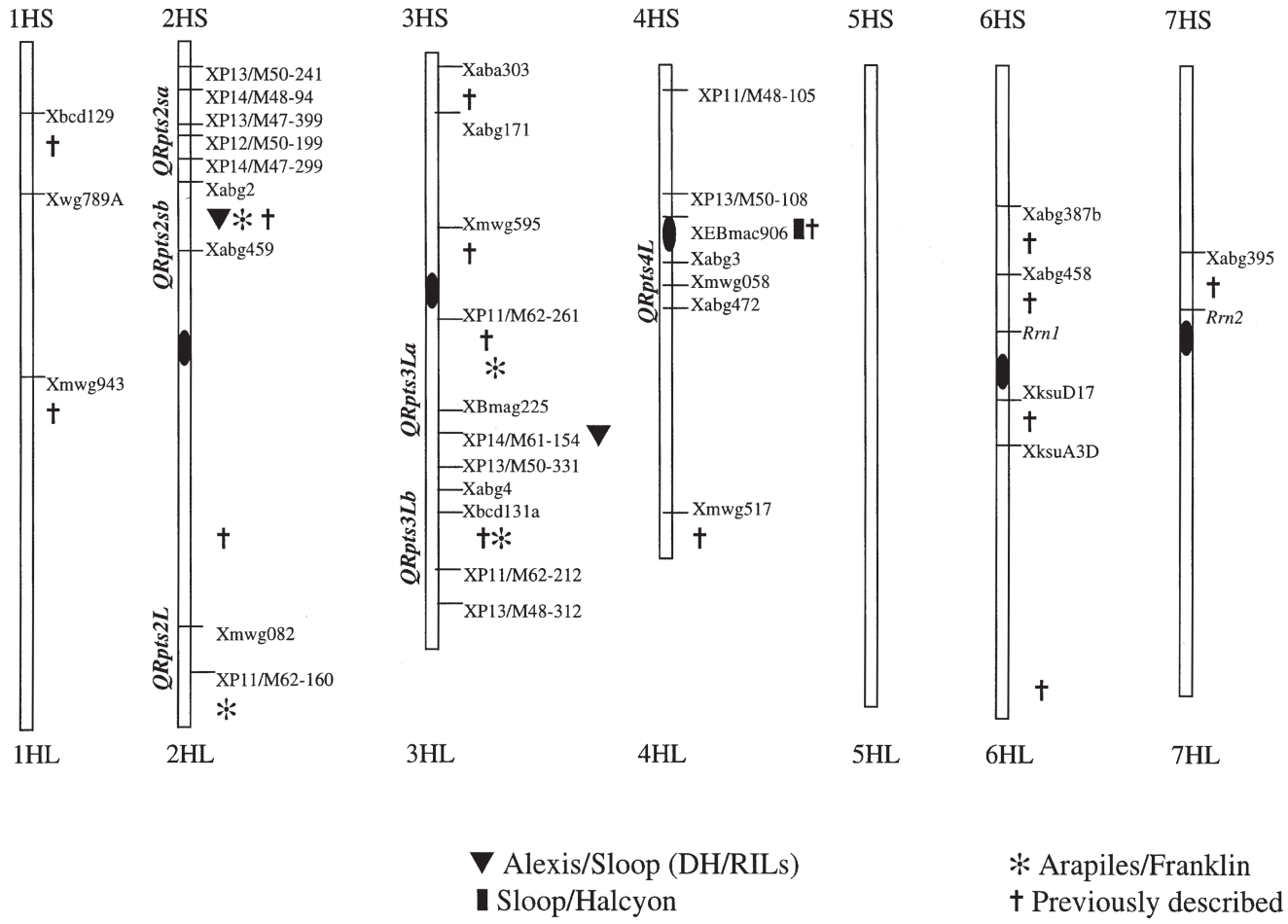

Fig. 4. Barley chromosomes showing mapped chromosome regions associated with net blotch resistance (distances are not marked).

resistance superior to Alexis. Cherif and Harrabi (1993) showed transgressive segregation of progeny of 2 susceptible parents, so it is reasonable to expect that a high proportion of resistant segregants could result from a cross between a moderately susceptible and a susceptible line, each carrying different but complementary genes for resistance.

The resistance in Franklin appears to be conditioned by alleles on chromosomes 2H and 3H. Franklin is strongly resistant to NB34 isolate of net blotch (IR = 3) and compares with the most resistant progeny of the Alexis/Sloop population. It is suggested that the alleles at these loci have a complementary gene action and can be further enhanced when combined with the relatively ineffective allele on 3H from Arapiles.

In this study, individual QTLs explained 7–64% of the phenotypic variation. Some of the QTL regions with an LOD score of ≥2.5 in DH populations from Alexis/Sloop and Sloop/Halcyon were very close to reaching levels of significance (Table 2). Therefore it is possible that the presence of other 'potential QTLs' that may have had a significant association with the trait, could not be detected due to the specific environments encountered, pathotype used, inoculum density/unit area, relatively small population sizes, and high threshold levels (Beavis 1994). In the present investigation, trait–marker associations were made only at the seedling stage and this may not translate to resistance in the adult plant stages. However, for some resistances, seedling resistance to net blotch is closely correlated with adult plant resistance (Tekauz 1986). Steffenson *et al*. (1996) found that the QTL on chromosome 4H, presumably the one identified in the Sloop/Halcyon population, functioned in both seedling and adult plants.

Besides determining chromosomal locations, QTL analysis has enabled us to determine genetic control of NFNB by identifying linked genomic regions, thereby eliminating the need to make arbitrary infection classes from frequency distributions. QTL mapping using linkage maps also provided markers that can be used directly for marker assisted selection, as most of the maps used in this study were constructed using both RFLP and microsatellite markers. For example, in the Sloop/Halcyon population, a major QTL explaining 64% of NFNB resistance is

associated with microsatellite markers EBmac0906 and GMS089, providing markers suitable for MAS without the need for further manipulation such as conversion into polymerase chain reaction format. RFLP and AFLP markers are costly to implement for routine MAS in breeding programs. On the other hand, microsatellite markers are highly polymorphic, co-dominant, less costly to assay, and are better suited to automation, therefore providing a superior alternative.

Our preliminary results on the validation of molecular markers associated with NFNB have indicated that the linked markers may be used to select loci for NFNB resistance from Halcyon. Furthermore, the microsatellite markers mapped in the QTL region exhibited high polymorphic information content values in Australian and European barley germplasm (Raman *et al*. 2003), indicating that these markers can be used in different genetic backgrounds. Recently, consensus maps of barley have been developed (Karakousis *et al*. 2003, this issue) and this will allow further selection of markers mapped closer to the QTLs identified.

From these studies, the information generated on chromosomal location and identification of markers linked to genes conferring resistance to NFNB will allow the Australian barley breeding programs to introgress these genes into adapted germplasm and select lines carrying the favourable alleles using marker assisted selection. Since *P. teres* is a highly variable pathogen (Steffenson and Webster 1992), it is important to combine different QTLs and major genes to develop broad spectrum and high levels of resistance. Genomic regions identified in this study will facilitate in (*1*) monitoring introgression of pathotype-specific major genes/QTLs and epistatic gene interactions, (*2*) pyramiding of different genes conferring resistance to *P. teres*, (*3*) establishing pleiotrophy with other traits, and (*4*) pedigree-based association mapping.

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