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Disease resistance genes in a doubled haploid population of two-rowed barley segregating for malting quality attributes

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Abstract. Malting barley (*Hordeum vulgare* L.) improvement involves selection for many quality traits, but the search for and deployment of resistance genes has continued to be an equally important endeavour. As an aid to phenotypic selection in breeding programs, gene mapping can serve to characterise genes known to exist in elite breeding lines. In the present study, 180 doubled haploid lines derived from the cross of VB9524/ND11231*12 were screened for disease resistance under field and greenhouse conditions. Quantitative trait locus (QTL) mapping and classical genetic linkage approaches were used to identify and map QTLs for resistance to powdery mildew (*Blumeria graminis* f.sp. *hordei*), net form of net blotch (*Pyrenophora teres* f. *teres*) and stem rust (*Puccinia graminis* f.sp. *tritici*). The analyses offered a comparison between QTL mapping and traditional genetic linkage analysis. Both approaches identified a QTL for powdery mildew resistance on chromosome 1H, which mapped to the approximate genomic location of the *Mla6* gene. Similarly, both methods identified a major QTL for resistance to net form of net blotch on chromosome 6H and for stem rust resistance on chromosome 7H. The QTL for stem rust resistance on 7H mapped to the approximate location of the *Rpg1* gene. Classical linkage analysis identified the 3 QTLs with major effects, but was unable to detect 3 other loci with minor effects.

Additional keywords: two-row barley, molecular markers, QTL, linkage analysis, powdery mildew (*Blumeria graminis* f.sp. *hordei*), stem rust (*Puccinia graminis* f.sp. *tritici*), net form of net blotch (*Pyrenophora teres* f. *teres*).

Introduction

Barley (*Hordeum vulgare* L.) used for malt is subjected to more quality constraints than most other crops and is one of only a few field crops sold consistently on the basis of cultivar identity. Consequently, malting barley improvement involves selection for many quality traits, but disease resistance is also of major interest. Diseases caused by fungi and viruses can affect the profitability of barley production by reducing final yield and by lowering grain quality, both of which result in a lower financial return to the grower (Chelkowski *et al*. 2003; Williams 2003). Because malting barley production generally requires low input costs in order to be profitable, reliance on expensive chemical control is not economical and can be potentially harmful to the environment and consumer. The search for and deployment of resistance genes therefore continues to be just as important as is selection for quality attributes in malting barley improvement.

Over 120 major resistance genes against 15 fungal pathogens, 4 viruses, and 2 pests (the green bug aphid *Schizaphis graminum*, and the cereal cyst nematode *Heterodera avenae*) have been identified in barley (see Chelkowski *et al*. 2003). Considerable efforts have been made to identify and map the chromosomal locations of these genes in the barley genome, and to date, 69 have been mapped. A recent review of these efforts (Chelkowski *et al*. 2003; Williams 2003) showed that in most cases advances were made by using molecular markers to locate and measure the effects of the individual underlying genes, *quantitative trait loci* (QTLs) (Tanksley 1993; Lander and Schork 1994).

Gene mapping can also serve to characterise genes already known to exist in elite breeding lines. Knowledge of the effectiveness of such resistance genes and diagnostic DNA markers, along with detailed characteristics of genetic stocks, can improve breeding strategies for malting barley. The doubled haploid (DH) population used for this research is from the cross of VB9524/ND11231*12, specifically constructed to identify regions of the barley genome that influence variations in grain protein concentration (Emebiri *et al*. 2003, 2004). However, it has also been found to possess major genes for malting quality improvement (Emebiri *et al*. 2004). As part of the Australian National Barley Molecular Marker Program, the DH lines were screened for resistance to

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a number of diseases under field and greenhouse conditions. The aim in this paper was to map genes for resistance to powdery mildew (*Blumeria graminis* f.sp. *hordei*), stem rust (*Puccinia graminis* f.sp. *tritici*), and net form of net blotch (*Pyrenophora teres* f. *teres*) segregating in the population and to tag these with molecular markers.

Materials and methods

Mapping population

DH lines were produced from the F_1 generation of a cross between VB9524 and ND11231*12 made in 1997. From over 500 DH lines that were produced, 180 were chosen at random for genetic linkage map construction and field trials. Parents used for the cross originated from breeding programs at the Department of Primary Industries, Victoria, Australia, and the North Dakota State University, Fargo, USA, respectively.

Disease assessment for resistance to powdery mildew (Blumeria graminis *f.sp.* hordei)

Data on powdery mildew resistance were obtained from assessments made in 2003 under natural epidemics. Two replicates of each line were sown as hill plots at 0.5 m by 0.75 m in-row and between-row spacing at Gatton Research Station (27°32′46″S, 152°19′43″E), Qld. Host resistance to a heavy natural infection of powdery mildew was scored on a 0–9 scale where 0 is immune and 9 is very susceptible, at Zadoks growth stage 31–32. The 0–9 scale is explained as follows:

0 Immune, no sign of infection;
1 Very resistant, occasional necrotic lesion from hyersensitive response to infection;
2 Resistant, necrotic lesions from hypersensitive response to infection, no or very little mycelium;
3 Moderately resistant, few colonies, slight to moderate mycelial development but with little or no sporulation, chlorotic or necrotic spots may develop;
4
5 Moderately susceptible, moderate to abundant mycelial development with moderate sporulation, some chlorosis;
6
7 Susceptible, abundant mycelial development with heavy sporulation, little chlorosis;
8
9 Very susceptible, abundant mycelial development, abundant sporulation, little or no tissue response.

Low infection types normally give low disease levels. The values of 4, 6, and 8 were used where ratings could not be clearly allocated to the values described above.

Disease assessment for resistance to stem rust (Puccinia graminis *f.sp.* tritici)

The same plots were used for phenotyping stem rust resistance. Paired rows of hill plots were separated by double rows of stem-rust-susceptible wheat lines, which were artificially inoculated with *Puccinia graminis* f.sp. *tritici*, pathotype ANZ 343-1,2,3,5,6 (G. Platz, pers. comm.). A heavy epidemic resulted and plots were scored twice, after anthesis (15 October) and 8 days later (23 October), using the 0–9 scale as above.

The 0–9 scale for stem rust used here encompasses both reaction type and amount of disease. Again, these are usually strongly correlated where a low reaction type results in low disease levels. In cultivated barleys, there appears to be much less variation in reaction type than with, say, wheat. In the population tested here there was little difference in reaction type, so scores are more an indication of the amount of disease in a line. The epidemic was quite heavy and quite uniform.

Disease assessment for resistance to net form of net blotch (Pyrenophora teres *f.* teres)

After screening the parents with 8 net blotch isolates comprising at least 5 pathotypes, isolate NB77 was found to give the best differentiation. Consequently, parents and progeny were screened as seedlings (GS 12.3) in the greenhouse for resistance to isolate NB77 using the method described by Raman *et al*. (2003).

Molecular marker genotyping

Methods used for DNA extraction and linkage map construction for the population were described by Emebiri *et al*. (2003). The original VB9524 / ND11231*12 linkage map was constructed with 270 markers, which comprised 197 amplified fragment length polymorphism (AFLP) markers, 23 restriction fragment length polymorphism (RFLP) markers, 43 Simple Sequence Repeat (SSR) markers, 6 SSR-derived expressed sequence tag (EST) markers, and 1 random amplified polymorphism DNA (RAPD) marker. For QTL analysis, redundant markers were deleted from the map and locus files, leaving 211 markers (Fig. 1) that covered 2024.4 cM of the genome, at an average interval length of 9.9 cM.

QTL localisation by classical linkage analysis

For this purpose, disease scores were first converted into standard scores using the formula:

$$Z_i = \frac{(Y_i - Y_{.i})}{S_d}$$

where Z_i is the standard score of genotype i, Y_i is the raw score, $Y_{.i}$ is the mean score of all genotypes, and S_d is the standard deviation. The 2 genotypic groups obtained with this standardisation were coded as ‘A’ or ‘B’, depending on the parental phenotypes. Genetic analysis was then performed using LINKEM (Vowden *et al*. 1995). We adopted the nomenclature of Raman *et al*. (2003) for QTL designation and aligned these loci with those for known genes using the barley gene map of Franckowiak (1996).

QTL analysis by interval mapping

Composite interval mapping (CIM) was used for QTL detection and estimation of QTL effects. Cofactors were determined by the SELECT procedure in PLABQTL (Utz and Melchinger 1996), using F-to-Enter and F-to-Delete of 15. A critical logarithm-of-odds (LOD) threshold of 3.6 was chosen for declaring a putative QTL significant, ensuring a comparison-wise error rate of $P < 0.001$ and an experiment-wise error rate of $P < 0.05$. All putative QTLs were examined for the presence of digenic epistatic effects. The proportion of the phenotypic variance explained by all QTLs was determined by calculating an adjusted coefficient of determination (R^2_{adj}) from multiple regression, after fitting a model including all detected QTLs (Utz *et al*. 2000).

Cross validation

The method of cross validation was suggested by Utz *et al*. (2000) to evaluate QTL mapping results because estimates of individual QTL effects and the proportion of genotypic variance explained by the QTLs can be severely inflated, leading to an overly optimistic assessment of the results. Using the PLABQTL software, a 5-fold cross validation was applied in the present study. Four subsets were combined to form the estimation set (ES) for QTL detection and the estimation of genetic effects. The remaining subset formed the test set (TS) in which predictions derived from ES were tested for their validity by correlating predicted and observed data. Subsets were formed randomly 20 times,

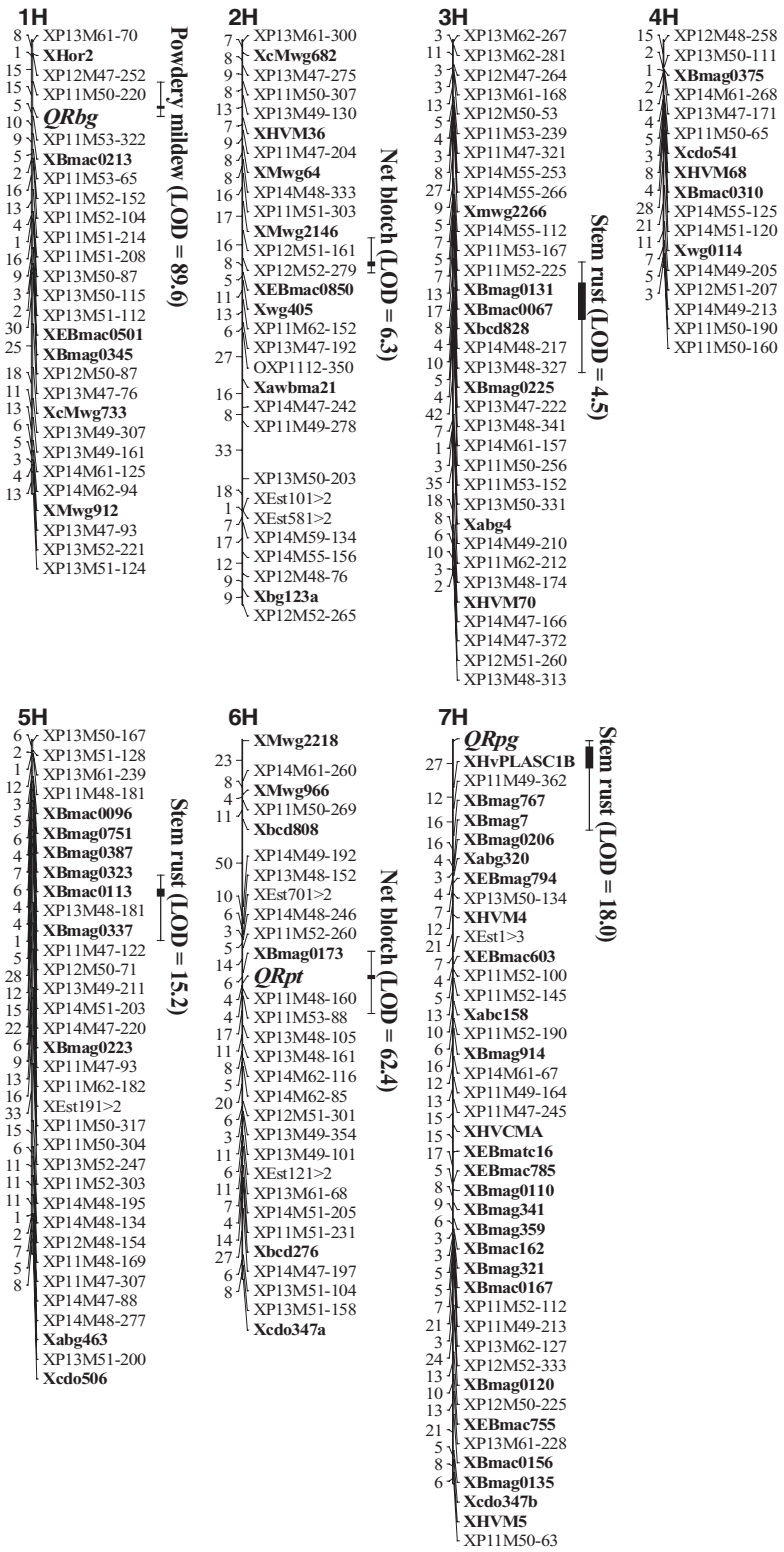

Fig. 1. Genetic linkage map of the doubled haploid population constructed from a cross between VB9524 and ND11231*12. The map includes loci for powdery mildew (*QRbg*, *1H*), net form of net blotch (*QRpt*, *6H*), and stem rust (*QRpg*, *7H*). Distances are in cM (calculated using the Kosambi mapping function). Markers with bold fonts represent framework loci mapped in other barley populations.

yielding 100 iterations, and subsequently, unbiased estimates of QTL position, effect, and explained variation were obtained from the median of the distribution.

Results

Patterns of phenotypic distribution for disease scores are presented in Fig. 2. For powdery mildew, the disease resistance (DR) scores ranged from 0.5 to 9.0 on the scale of 0–9. The frequency distribution showed a distinct bimodal structure centred on DR scores of ≤4 and ≥5, with mean score of 4.2 and standard deviation (s.d.) of 3.0. When standardised by removing the mean and dividing by the s.d., 96 of the DH lines were similar to the resistant parent, VB9524 (i.e. with std. DR scores ≤–0.1), and 86 had positive scores. The resultant positive and negative scores fitted a phenotypic ratio of 1 : 1 (chi-square = 0.56; $P = 0.46$), indicating the segregation of a single major gene for powdery mildew resistance.

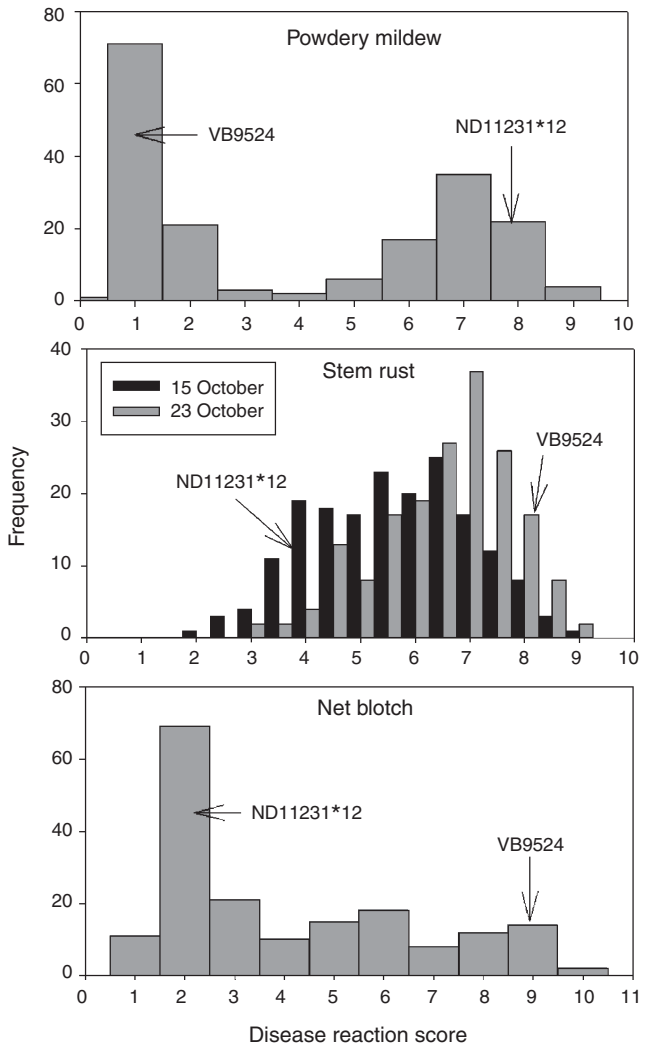

Fig. 2. Frequency distribution of the disease scores obtained for powdery mildew, stem rust, and net form of net blotch in 180 doubled haploids of the cross of VB9524/ND11231*12.

Based on a 2 × 2 contingency table of marker alleles by DR scores, the locus for powdery mildew (*QRbg*) showed significant linkage (LOD score = 37.1; recombination fraction (RF) = 0.06 ± 0.02) to the AFLP marker, XP11M53-322, which mapped to the short arm of chromosome 1H (Fig. 1). The whole genome was then scanned using the composite interval mapping procedure (Jansen and Stam 1994; Zeng 1994) to search for additional QTLs in the population. The results confirmed the location of *QRbg*, but did not identify any additional QTLs at a LOD threshold ≥3.6. *QRbg* was positioned at the interval between the 2 AFLP markers, XP11M50-220 and XP11M53-322 (Fig. 1), with a LOD score of 89.6. The effect of the QTL explained approximately 72% of the phenotypic variation (Table 1), with VB9524 contributing the favourable alleles for resistance.

Stem rust resistance was measured twice after anthesis to account for the influence of differences in maturity amongst the progeny. Although the DR scores at the second stage were noticeably skewed towards the susceptible parent (Fig. 2), there was a highly significant rank correlation ($r = 0.90$; $P < 0.001$) between the 2 scores. Genetic linkage analysis of the individual data gave identical results, so results presented are based on the average disease score from the 2 assessments. After standardisation, 87 of the DH lines had negative DR scores, and 93 had positive scores. The 2 disease classes showed a highly significant fit to the expected ratio of 1 : 1 (chi-square = 0.20; $P = 0.65$), indicating genetic control by a single nuclear gene.

The locus for stem rust resistance (*QRpg*) was linked to segregation at the SSR marker, XHvPLASC1B, by classical linkage analysis (LOD score = 10.4; RF = 0.25 ± 0.03), which mapped to the short arm of chromosome 7H (Fig. 1). The whole genome was then scanned using the composite interval mapping technique, and this identified 2 additional QTLs on chromosomes 3H and 5H (Fig. 1, Table 1). All favoured alleles for stem rust resistance were derived from ND11231*12. The QTL on chromosome 3H was not strongly supported by cross validation, with a LOD score >3.6 occurring only 28 out of the 100 iterations. However, there was a significant interaction ($P = 0.02$) between the 3H locus and that of 7H, such that individuals with ND11231*12 alleles at both loci were more resistant (average DR score = 4.7) than individuals with the 7H allele (DR = 5.7). The effect of individual QTLs accounted for 9–39% of the phenotypic variation, and 54% collectively (Table 1).

The distribution of DR scores for net form of net blotch in the population showed a skewness towards the resistant parent, ND11231*12 (Fig. 2). After the data were standardised, 102 of the DH lines had negative DR scores and 76 had positive scores. The 2 disease classes showed a slight deviation from the expected ratio of 1 : 1 (chi-square = 3.20;

Table 1. Summary of QTL parameters associated with disease resistance to powdery mildew, stem rust, and net form of net blotch in 180 doubled haploids of the cross between VB9524 and ND11231*12

Chr.	Left marker	Position (cM)	LOD score	Additive effect	s.e.	Partial R^2 (%)	Source of resistance
			Powdery mildew resistance				
1H	XP11M53-322	40	52.26	2.64	0.118**	73.70	VB9524
R^2adj. (%): Calibration						73.70	
Validation						71.76	
Bias						1.94	
			Stem rust resistance				
3H	XBmag0225	154	3.80	–0.30	0.076**	9.30	ND11231*12
5H	XP14M51-203	84	12.26	–0.66	0.089**	26.90	ND11231*12
7H	XHvPLASC1B	12	19.00	–0.70	0.077**	38.50	ND11231*12
R^2adj. (%): Calibration						52.29	
Validation						45.71	
Bias						6.58	
			Net blotch resistance				
2H	XP12M52-279	128	7.37	–0.73	0.102**	22.80	ND11231*12
6H	XP11M48-160	138	49.54	–2.33	0.101**	75.20	ND11231*12
Interaction				0.46	0.104**	10.20	
R^2adj. (%): Calibration						76.30	
Validation						70.04	
Bias						6.26	

**$P < 0.001$ for significance of additive effect.

$P = 0.07$). Analysis of the 2×2 contingency table mapped the locus to a position close to the SSR marker, XBmag0173 (LOD score = 32.88; RF = 0.07 ± 0.02) on chromosome 6H (Fig. 1). Using the composite interval mapping approach, an additional QTL was located on chromosome 2H, which had a relatively minor effect ($R^2 = 23\%$) compared with the 75% explained by the locus on 6H. There was also a significant ($P < 0.01$) interaction between the loci on chromosomes 2H and 6H, which accounted for an additional 10% of the phenotypic variation. A final simultaneous fit of the individual QTLs and their interaction accounted for 76% of the phenotypic variation, and results from cross validation indicated that this estimate could have been upwardly biased by 6.3% due to sampling errors (Table 1).

Discussion

In the present study, we have identified and mapped the chromosomal location of 3 major disease resistance genes using classical genetic linkage and QTL mapping approaches. Three other loci were detected using composite interval mapping (Table 1), but these could not be corroborated by classical linkage analysis. Unlike most other studies (e.g. Backes *et al*. 2003), the sources of resistance in the present study are elite breeding lines with high malting quality attributes (Emebiri *et al*. 2004). The genotype VB9524 is an advanced selection from a cross of Arapiles with Franklin. It is significantly lower in grain protein concentration than either parent (L. C. Emebiri and D. B. Moody, unpublished), but was rejected for release due to a blue aleurone characteristic observed during malting. ND11231*12 is a 2-row barley line from the breeding program at North Dakota State University (NDSU). The line is early maturing with good extract, moderate diastatic power but low in α-amylase activity (J. D. Franckowiak, pers. comm.).

The validation of putative QTLs can be achieved by aligning the identified chromosomal locations to genomic regions where cloned genes have been mapped or where QTLs have been identified in other populations. The QTL identified for powdery mildew in the present study (Fig. 1) mapped to the approximate genomic location of the *Mla6* gene (Kleinhofs 2004). In addition, since the VB9524 parent contributed the resistance factor, and VB9524 is derived from a cross between Arapiles and Franklin, we aligned our QTL map to that of the Arapiles/Franklin population (Fig. 3). Five of the markers were common, but the region of interest was not adequately covered in the Arapiles/Franklin map. Nevertheless, the results showed a significant QTL (LOD > 3.6) for powdery mildew within the approximate location identified in this population.

The major QTL identified for stem rust resistance on chromosome 7H corresponds to the map location of the *Rpg1* gene (Kilian *et al*. 1994). The *Rpg1* gene confers resistance to many pathotypes of the stem rust fungus *Puccinia graminis* f.sp. *tritici*. It is considered durable because it has been effective in protecting North American barley cultivars from stem rust for over 60 years (Spaner *et al*. 1998; Brueggeman *et al*. 2002; Horvath *et al*. 2003). Chevron and Peatland

are the original sources of *Rpg1* (Horvath *et al*. 2003), but the nucleotide sequence of the gene is identical to that of Bowman, which is also resistant. Bowman is a close relative of ND11231*12 through one of its parents, ND4994-15 (J. D. Franckowiak, pers. comm.).

Four genes were described by Chelkowski *et al*. (2003) for reaction to *Puccinia graminis* f.sp. *tritici* in barley, but only the map locations of *Rpg1* and *rpg4* are often reported. *Rpg1* was mapped to the short arm of chromosome 7H by Kilian *et al*. (1994) and has recently been cloned (Brueggeman *et al*. 2002), and Borovkova *et al*. (1995) mapped the *rpg4* gene to chromosome 5H. Spaner *et al*. (1998) reported a QTL for stem rust on chromosome 4H. A QTL identified in the present study on chromosome 3H (Fig. 1), with significant effects on stem rust resistance (Table 1), has not been previously reported. This QTL might be linked to a new gene conferring resistance to the pathotype used. Fox and Harder (1995) reported that genes with minor effects may augment the resistance conferred by *Rpg1*, and this could explain the durable nature of the resistance.

QTLs for resistance to net blotch have been identified in other mapping populations by Sato *et al*. (1996), Steffenson *et al*. (1996), Richter *et al*. (1998), Spaner *et al*. (1998), Manninen *et al*. (2000), Cakir *et al*. (2003), Raman *et al*.

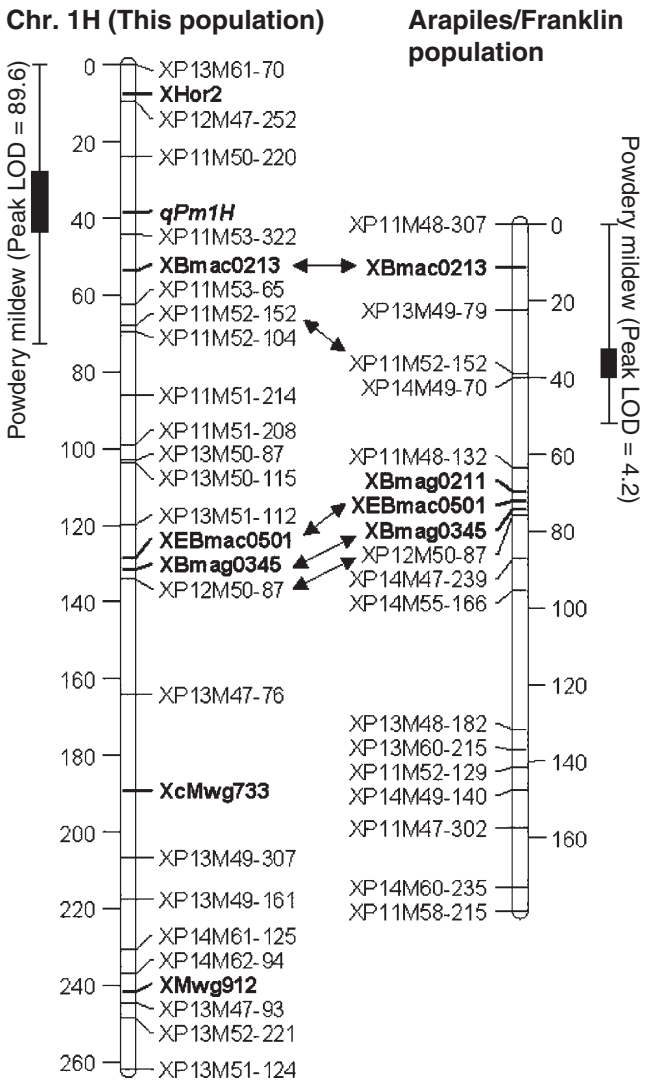

Fig. 3. Comparative mapping of QTLs for powdery mildew on chromosome 1H across different genetic populations. The Arapiles/Franklin map was constructed using data obtained as part of the Australian National Barley Molecular Marker Program. MapChart software (Voorrips 2002) was used to graphically position QTLs with support interval (drop = 1.0 LOD) on the linkage map.

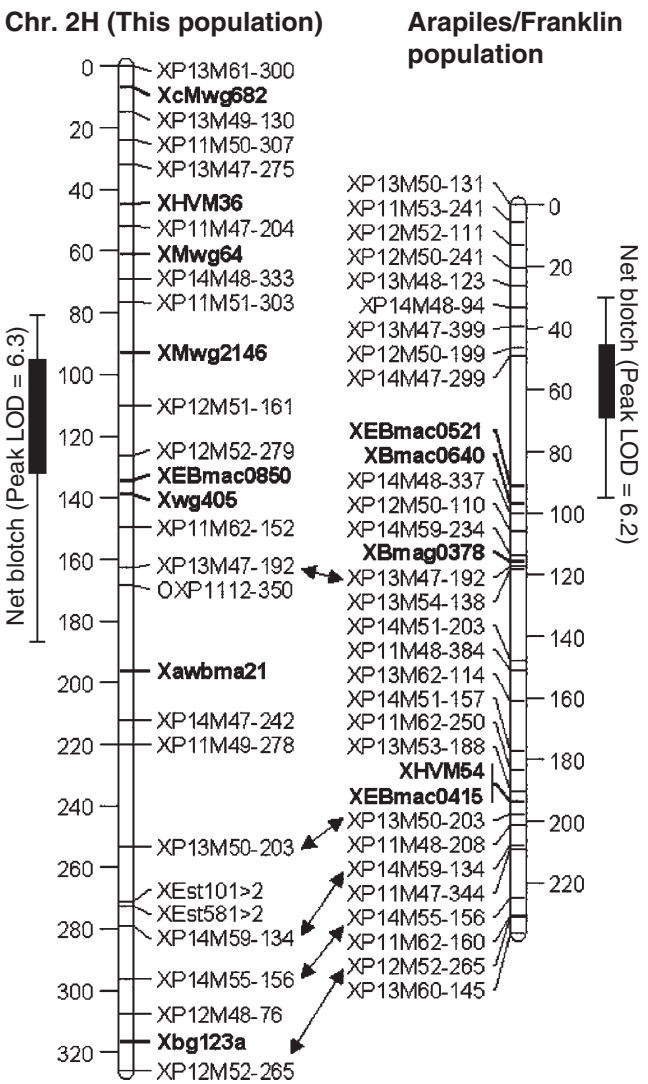

Fig. 4. Comparative mapping of QTLs for net form of net blotch on chromosome 2H across different genetic populations. The Arapiles/Franklin map was constructed using data obtained as part of the Australian National Barley Molecular Marker Program. MapChart software (Voorrips 2002) was used to graphically position QTLs with support interval (drop = 1.0 LOD) on the linkage map.

(2003), and Ma *et al*. (2004). The QTL identified in the present study on chromosome 6H mapped to the approximate genomic location identified by Manninen *et al*. (2000) using retrotransposon markers. Recently, Ma *et al*. (2004) also reported the identification of a major QTL ($R^2 = 64\%$) at the region of chromosome 6H, using RFLP markers. Marker technology in breeding programs is moving towards more user-friendly systems and the SSR marker (XBmag173) identified in the genomic region would be more economical and enable high sample throughput. Joint segregation of net blotch infection scores with XBmag173 showed a LOD score of 35 for linkage and an estimated RF of 0.059 ± 0.018.

The QTL on chromosome 2H with relatively minor effect on net blotch resistance (Table 1) has also been reported. A similar region was identified in the Tallon/Kaputar population (Cakir *et al*. 2003), the Alexis/Sloop DH population, and the recombinant inbred lines of Sloop-sib/Alexis population (Raman *et al*. 2003). Raman *et al*. (2003) also associated the chromosome 2H region with net blotch resistance with pathotype NB34 in the Arapiles/Franklin population, with Franklin as the source of resistance. Alignment of the Arapiles/Franklin map with that of the present population showed that the 2 regions might be identical (Fig. 4). Both Arapiles and Franklin were reported to carry separate QTLs for net blotch resistance on chromosome 3H (Raman *et al*. 2003), but there was no evidence of its presence in the VB9524/ND11231*12 population.

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