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Mapping of adult plant resistance to net form of net blotch in three Australian barley populations

A. Lehmensiek[A], *G. J. Platz*[B], *E. Mace*[B], *D. Poulsen*[B], *and M. W. Sutherland*[A,C]

[A]Centre for Systems Biology, University of Southern Queensland, Toowoomba, Qld 4350, Australia.
[B]Queensland Department of Primary Industries and Fisheries, Hermitage Research Station, Warwick, Qld 4370, Australia.
[C]Corresponding author. Email: marksuth@usq.edu.au

Abstract. Net form of net blotch (NFNB), caused by *Pyrenophora teres* Drechs. f. *teres* Smedeg., is a serious disease problem for the barley industry in Australia and other parts of the world. Three doubled haploid barley populations, Alexis/Sloop, WI2875-1/Alexis, and Arapiles/Franklin, were used to identify genes conferring adult plant resistance to NFNB in field trials. Quantitative trait loci (QTLs) identified were specific for adult plant resistance because seedlings of the parental lines were susceptible to the NFNB isolates used in this study. QTLs were identified on chromosomes 2H, 3H, 4H, and 7H in both the Alexis/Sloop and WI2875-1/Alexis populations and on chromosomes 1H, 2H, and 7H in the Arapiles/Franklin population. Using QTLNetwork, epistatic interactions were identified between loci on chromosomes 3H and 6H in the Alexis/Sloop population, between 2H and 4H in the WI2875-1/Alexis population, and between 5H and 7H in the Arapiles/Franklin population. Comparisons with earlier studies of NFNB resistance indicate the pathotype-dependent nature of many resistance QTLs and the importance of establishing an international system of pathotype nomenclature and differential testing.

Introduction

Net blotch, caused by the fungus *Pyrenophora teres*, is a serious production problem for the barley (*Hordeum vulgare* L.) industry both in Australia and elsewhere (Graner *et al*. 1996; Steffenson *et al*. 1996; Manninen *et al*. 2000; Gupta *et al*. 2003). Two forms of net blotch are recognised: the net form caused by *P. teres* f. *teres* and the spot form caused by *P. teres* Drechs. f. *maculata* Smedeg. Cluster analysis of spot and net form isolates obtained from different Sardinian landraces of barley has separated the isolates into 2 strongly divergent groups corresponding to the net and spot forms (Rau *et al*. 2003). Lesions of net form of net blotch (NFNB) initially appear as minute spots or streaks and then spread to form narrow, dark brown longitudinal streaks. Transverse lines may also form, giving the lesions a net-like appearance (Parry 1990). Lesions may be surrounded by areas of chlorosis and large areas of dead tissue may be present. Lesions of spot form of net blotch are of dark brown colour and elliptical in shape surrounded by a chlorotic margin (Parry 1990). As it can be difficult to distinguish between spot and net form lesions, several polymerase chain reaction (PCR)-based assays have been developed that differentiate spot form and net form isolates (Williams *et al*. 2001; Leisova *et al*. 2005; Keiper *et al*. 2007). NFNB can cause a substantial reduction in grain quality and yield losses approaching 100% are reported, although losses in the order of 10–40% are more typical (Mathre 1997).

P. teres f. *teres* is a highly variable pathogen and at least 13 different pathotypes have been identified in Australia (Platz *et al*. 2000). This variability, combined with the adoption of reduced or zero tillage practices, has increased the incidence of NFNB significantly in recent years. A major objective of the Australian barley breeding program is to increase resistance to this disease in commercially grown barley varieties. Cultivated barley lines that are resistant to NFNB at both the seedling and adult growth stages have been identified (Gupta *et al*. 2003). Mapping of the resistance genes or quantitative trait loci (QTLs) in these lines would facilitate their pyramiding in new barley cultivars.

Several Australian studies have sought to identify QTLs for seedling resistance (SLR) to NFNB (Cakir *et al*. 2003; Raman *et al*. 2003; Emebiri *et al*. 2005). Seedling resistance is initially observed in 2–3-week-old seedlings challenged with fungal inoculum in glasshouse pot trials and is expressed through to maturity in the field. Cakir *et al*. (2003) identified a SLR QTL with a large effect on chromosome 6H and 2 lesser QTLs on chromosomes 2H and 3H in a population derived from the cross Tallon/Kaputar. Based on the NFNB reactions to isolate NB34, Raman *et al*. (2003) reported QTLs for NFNB SLR from 3 mapping populations: Alexis/Sloop, WI2875-1(a Sloop sib)/Alexis, and Arapiles/Franklin. In the first population, a QTL on chromosome 3HL was contributed by Alexis and a possible QTL on 2HS was contributed by Sloop. The same 3HL and 2HS QTLs were identified in the WI2875-1/Alexis population. In the Arapiles/Franklin population, 1 QTL was detected on 2HS, 2 were located on 3HL, and a further QTL was identified on 2HL. All resistance QTLs in this cross were contributed by Franklin with the exception of one of the QTLs on 3HL. In a separate study using the isolate NB77, a QTL for SLR to NFNB was identified in

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a similar region on 2HS from the Franklin-derived line, VB9524 (Emebiri *et al*. 2005).

Since seedling assays fail to detect adult plant resistance (i.e. resistance that is manifested in mature plants but not in the younger, seedling stages), trials to assess adult plant resistance (APR) are sown in the field with ratings taken after heading. The existence of APR to NFNB has been documented in barley field and glasshouse trials both in Australia and overseas (Tekauz 1985; Jonsson *et al*. 1998; Platz 2001) and this type of resistance has been effective against NFNB in southern Australia for several decades. In North America, Steffenson *et al*. (1996) have identified QTLs for resistance expressed in field trials on chromosomes 2H, 3H, 4H, 5H, 6H, and 7H in a Steptoe/Morex population. Only one Australian study to date has reported on QTLs conferring resistance to NFNB in adult plants (Cakir *et al*. 2003). This study resulted in the identification of a QTL ($R^2 = 65\%$) in the region of marker Ebmac874 on chromosome 6H in a Tallon/Kaputar population, suggesting expression of the previously identified SLR QTL at this location.

Here we investigate the genetic control of APR to NFNB in 3 Australian barley populations. Our goal was to determine which QTLs were the major contributors to resistance in the field in order to discover which independent genetic regions might be combined with SLR loci in a marker-assisted selection program seeking to provide stable resistance against the NFNB pathogen.

Materials and methods

Plant material and linkage maps

Two doubled haploid (DH) populations, Alexis/Sloop and Arapiles/Franklin, and one population of recombinant inbred lines established by single seed descent, WI2875-1/Alexis, were screened for resistance in field plots for reaction to NFNB. These populations were developed by the Australian National Barley Molecular Marker program (NBMMP, Barr *et al*. 2003; D. B. Moody *et al*., unpublished data). Sloop (breeding line WI2875-22) and WI2875-1 (designated Sloop-sib) were reselected in the F_6 from the F_2-derived breeders' line WI2875 (Barr *et al*. 2003). Sloop, WI2875-1, and Arapiles demonstrate classical APR to certain NFNB isolates, against which they are susceptible as seedlings yet resistant at adult growth stages. Sloop was used as the male parent in the Alexis/Sloop DH population, whereas selection WI2875-1 was used as the female parent in the recombinant inbred population, WI2875-1/Alexis. The Alexis/Sloop (Al/S) population consists of 111 lines, the WI2875-1/Alexis (W/Al) population consists of 153, and the Arapiles/Franklin (Ar/F) population of 225 lines. Linkage maps for all 3 populations had previously been constructed (Barr *et al*. 2003; Willsmore *et al*. 2006) using restriction fragment length polymorphisms (RFLPs), simple sequence repeats (SSRs), and amplified fragment length polymorphisms (AFLPs).

Pathogen isolates and NFNB APR screening

The 3 populations were screened in the field at the Hermitage Research Station, Queensland. Each population was phenotyped in both 2004 and 2005, while the Ar/F population was also screened in 2003. Treatments (lines) were randomised and grown in 2 replicates. Plots were sown as either short rows (0.5 m with 0.5 m in-row gap) or hill plots at 0.5-m centres, parallel with and 75 cm distant from spreader rows of a NFNB-susceptible cultivar. Spreader rows were inoculated with field-collected conidia of isolates NB329 (2003) and NB329 and NB333 (2004), and diseased straw of NB330 (2005). Three different isolates were used due to issues of insufficient inoculum availability at the time. Earlier tests across a range of resistant host genotypes had indicated that these isolates were of the same pathotype (G. J. Platz, unpublished). Epidemics were promoted using supplementary sprinkler irrigation. Disease reaction was scored after flowering when the level of disease in susceptible lines appeared to be at a maximum. Notes were taken using a 0–9 scale based on amount of disease and lesion size: 0, immune; 3, moderately resistant; 5, moderately susceptible; 7, susceptible; and 9, very susceptible. Disease readings were taken once in 2003 and 2004 and twice, 2 weeks apart, in 2005.

QTL analysis

For the QTL analyses, several AFLP markers that clustered together were removed from the maps. The total number of markers used in the Al/S map was 191, while 200 and 253 markers were used in the W/Al and Ar/F maps, respectively. Two software packages were used for the QTL analyses, namely Windows QTL Cartographer Version 2.5 (Wang *et al*. 2006) and QTLNetwork-2.0β (Yang and Zhu 2005). Data were averaged across replicates for composite interval mapping analysis by QTL Cartographer (QTLCart) and results were produced for each year. With QTLNetwork (QTLNet), data for all replicates and years were entered and analysed simultaneously. Epistatic interactions were also examined. QTL effects were considered to be significant if the log-likelihood (LOD) score was ≥ 3 or $P < 0.0002$. The naming convention for the identified QTL uses the format '*QNFNBAPR.AL/S-2H*', indicating a QTL for resistance to NFNB, followed by 'APR' or 'SLR', indicating whether it is an adult plant or seedling resistance QTL. This is followed by the cross in which the QTL was identified and the chromosome on which the QTL was mapped.

Results

Phenotyping

Prior to the field assessments, all parental lines were inoculated with isolate NB330 in seedling pot trials. All parents scored a highly susceptible seedling disease rating of 10, based on a 1–10 scale (Tekauz 1985) (Table 1).

In the field, the disease severity on adult plants was higher in 2005 (assessments 1 and 2) than in 2003 and 2004 (Fig. 1, Table 1). Mean scores for the Ar/F population were 5.7, 6.4, 4.0, and 3.1, respectively. In all 3 populations only a small number of lines had slightly lower scores than the resistant parent. Distributions of the phenotypic scores are presented in Fig. 1.

QTL analysis

Prior to QTL analysis, several AFLP markers that clustered together were removed from the maps. The resulting total genetic

Table 1. NFNB seedling (SLR) and adult plant reaction (APR) values of the parental lines and mean values of the populations across 3 years

Isolates NB330, NB329, and NB333 have the same seedling pathotype

Isolate	2003		2004	2005 (1)	2005 (2)
	SLR NB330	APR NB329	APR NB329 & NB333	APR NB330	APR NB330
Alexis	10		6.5	7.5	8.0
Sloop/WI2875-1	10		1.5	4.0	4.5
Arapiles	10	2	3.5	4.5	6.3
Franklin	10	5.5	5.0	7.5	8.0
Al/S			2.5	5.5	6.1
W/Al			3.0	6.1	6.7
Ar/F		4.0	3.1	5.7	6.4

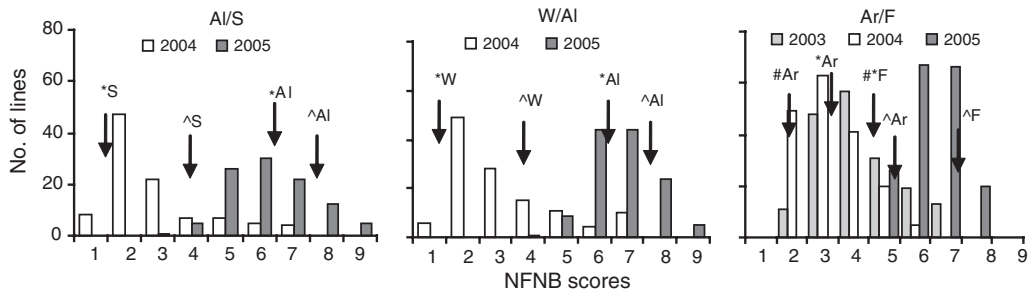

Fig. 1. Frequency distribution of NFNB scores of the Al/S, W/Al, and Ar/F populations across several years (2005 = average of 2 assessments). Parental scores are indicated (Al, Alexis; S, Sloop; W, WI2875-1; Ar, Arapiles; F, Franklin; #, 2003; *, 2004; ^, 2005).

map distances of the maps were 1281, 1521, and 1250 cM for the Al/S, W/Al, and Ar/F maps, respectively, with an average distance of 6.7, 7.6, and 4.9 cM between markers for each of the maps.

For the QTL analyses, the data from individual years were analysed using the composite interval mapping function in QTLCart. The full set of data was also analysed simultaneously by QTLNet to produce an overall percentage phenotypic variance explained across all years. Summaries of the analyses are presented in Table 2. LOD scores and the percentage phenotypic variance explained are listed for the QTLCart analyses. The phenotypic variances explained are listed for significant ($P < 0.0002$) QTL detected by QTLNet analyses.

Alexis/Sloop

In the Al/S population, NFNB resistance QTLs were identified on chromosome arms 3HL, 4HL, 7HS, and 7HL and in the centromeric region of chromosomes 2H and 4H. QTLs with the highest LOD scores were located on chromosomes 2HC and

Table 2. QTL Cartographer results for the populations Al/S, W/Al, and Ar/F given in LOD scores (LOD) and % variance explained (%var) for each year

Significant QTL effects computed by QTL Network (All) for the combined data are given in % variance explained

Chr.	Alexis/Sloop					WI2875-1/Alexis					Arapiles/Franklin						
	2004		2005		All	2004		2005		All	2003		2004		2005		All
	LOD	%var	LOD	%var	%var	LOD	%var	LOD	%var	%var	LOD	%var	LOD	%var	LOD	%var	%var
1HS											3.3	10.9	3.07	12.10	2.88	8.90	3.7
2HS															3.18	16.40	1.3
2HC	5.2	14.5	11.2	19.2	9.6	3.21	10.7	3.61	8.50								
3HL	5.3	17.6	15.6	30.4	16.6	2.82	9.6	3.97	11.0	17.2							
4HC	3.7	10.7	7.5	14.0	10.6												
4HL			4.6	9.8		2.57	7.4	4.83	12.10								
5HS						2.38	8.0	4.05	12.30	6.4							
7HS	2.8	7.3	5.9	8.7	3.7					3.0					3.01	6.90	3.7
7HL			7.0	11.0	5.0					2.7							

3HL (LOD scores in 2005 were 11.2 and 15.6, respectively; Table 2). The highest variances explained by these QTLs were 19 and 30% for the 2HC and 3HL QTLs, respectively. For all QTLs, APR alleles were contributed by Sloop with the exception of the QTL on 7HS, which was from Alexis. Similar results were computed by QTLNet with QTLs located in the same region as those indicated by QTLCart (Table 2, Fig. 2). A difference was observed on chromosome 4H with only one QTL being detected by QTLNet (flanking markers P13/M50-110 and P13/M51-252), whereas 2 QTLs were detected by QTLCart, i.e. *QNFNBAPR.Al/S-4Ha* and *QNFNBAPR.Al/S-4Hb* (Fig. 2). Overall the variance explained calculated by QTLNet was lower than the variance explained calculated by QTLCart.

WI2875-1/Alexis

The QTLs on chromosome regions 2HC, 3HL, and 4HL associated with NFNB APR in the W/Al population were located in the same regions as those in the Al/S population (Fig. 2). A QTL was located on chromosome 5HS, which was not identified in the Al/S population. The highest LOD score of 4.8 was observed for the QTL on 4HL and the variance explained by this QTL was 12% (Table 2). Several differences were observed between the results produced by QTLCart (2004 and 2005) and QTLNet (Table 2). The QTLs on 2HC and 4HL were not identified by QTLNet. The QTLs on 7HS and 7HL in the same regions as the QTLs in Al/S were identified with QTLNet, but were not detected by QTLCart. With QTLNet, the highest variance (17.2%) was explained by the QTL on 3HL. The QTL on 7HS was the only QTL contributed by the parent Alexis; all other QTLs were contributed by W2875-1.

Arapiles/Franklin

Analyses of the data with QTLCart indicated that a QTL on chromosome 1HS explained 9–12% of the phenotypic variance across the different years (Table 2, Fig. 2). QTLs were also identified from the 2005 single-year data on chromosomes 2HS and 7HS, explaining 16.4 and 6.9% of the phenotypic variance, respectively. The 1HS, 2HS, and 7HS QTLs were confirmed by the QTLNet analyses, but the percentage variance explained was less than with QTLCart. All QTLs were contributed by the

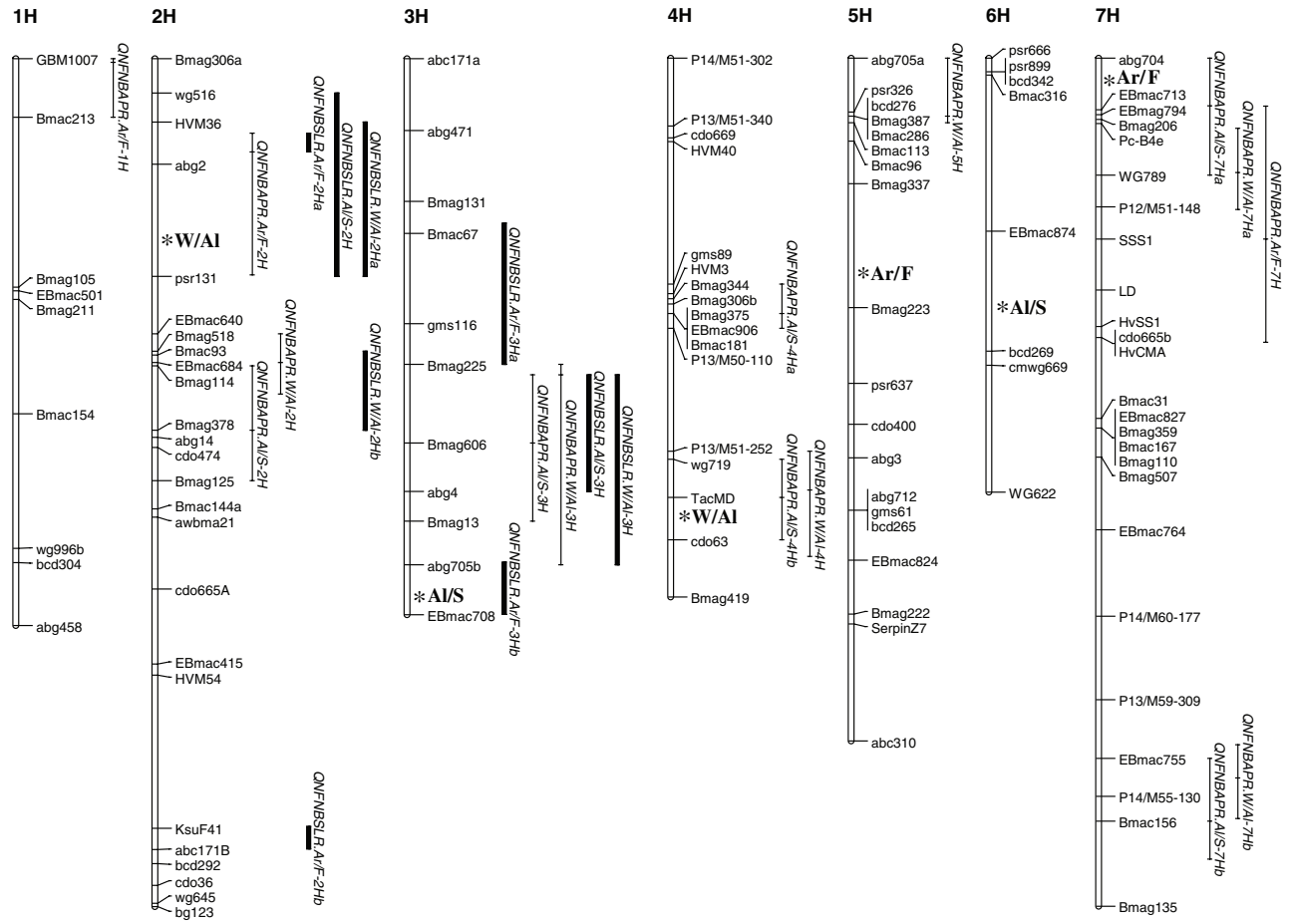

Fig. 2. Approximate chromosomal locations of NFNB QTLs for APR (thin bars) and SLR (thick bars) (Raman *et al*. 2003) for barley populations Al/S, W/Al, and Ar/F. Map construction was based on segregating markers across the 3 populations. Map distances are not given because marker positions are approximations. * Denotes epistatic interactions in the indicated populations.

resistant parent Arapiles. Only the 7HS QTL was located in the same region as a QTL from the Al/S and W/Al populations (Fig. 2).

Epistatic interactions

QTLNet identified several epistatic interactions of which several were significant at $P < 0.0002$. An interaction explaining 5.1% of the phenotypic variance in the Al/S population was observed between regions on chromosome 3H (Sloop) and 6H (Alexis) which, when considered alone, did not have additive effects (Fig. 2). Another epistatic interaction was indicated between loci on 2H and 4H from Alexis in the W/Al population. This interaction contributed 4.2% to the phenotypic variance. In the Ar/F population a significant interaction was observed between a region on chromosome 5H and a region coincident with the 7HS QTL, explaining 11.8% of the phenotypic variance. Both regions were contributed by Arapiles.

Discussion

We have investigated 3 Australian barley populations for genetic regions controlling APR to NFNB. Overall, more QTLs were identified in the 2005 field trials and in almost all cases LOD scores were higher. This is probably linked to the higher levels of disease severity recorded in 2005 in comparison with the other years (Fig. 1). In all 3 populations only a small number of lines had lower scores than the more resistant parent (Fig. 1). Given the error inherent in a visual scoring system, this suggests a lack of transgressive segregation, a conclusion supported by the results of the QTL analysis, which indicates that the relatively susceptible parents in each population donated, at most, one minor APR QTL.

Six QTLs associated with NFNB APR were identified in the Al/S and W/Al populations, with 5 of these occurring in the same genomic regions in both populations (2HC, 3HL, 4HL, 7HS, and 7HL), suggesting that largely the same genes were contributing to the expression of resistance. The Al/S and the W/Al populations are closely related since Sloop and WI2875-1 are different F_6 selections derived from the breeding line WI2875, a Norbert/Schooner cross; however, Al/S was produced as a doubled haploid population while the W/Al population consists of recombinant inbred lines. One difference observed between the Al/S and W/Al populations was the QTL on 4HC contributed by Sloop in the Al/S population, but not detected in the W/Al population. A second difference was a QTL on 5HS contributed by WI2875-1 in the W/Al population, but not detected in the Al/S population. These effects could be due to the presence of different chromosomal regions in the sibling lines Sloop and WI2875-1. To test this theory, we examined the SSR haplotype for WI2875-1 and Sloop in these QTL regions. Different size alleles (data not shown) were observed for markers (Bmag606, Bmag375, and Bmac181) on 4H, suggesting that the 4HC chromosomal region associated with resistance in Sloop was not present in WI2875-1. In contrast the haplotype on 5HS was the same in both lines. The QTL on 5HS was significant in the W/Al population in only one of the 2 years (LOD > 3) and expression may be environmentally dependent.

The Ar/F population bears similar parentage to the Al/S and W/Al populations. Both the NFNB APR susceptible parents Alexis and Franklin are derivatives of Triumph. Arapiles (breeding line/Domen) and Sloop both have Proctor and CI3576 in their pedigrees (Raman *et al*. 2003). Despite these genetic similarities between the populations, of the 3 QTLs detected in the Ar/F population, only *QNFNBAPR.Ar/F-7H* overlapped with QTLs in the Al/S and W/Al populations. This suggested that at least 2 unique QTLs for NFNB APR resistance were expressed in Ar/F and were not significant in the other 2 populations.

Prior to the field assessments of the populations, all parents were assessed for seedling resistance by inoculating them with isolate NB330 when they were 23–24 days old. All parents had a disease score of 10, indicating that they were susceptible at the seedling stage. Raman *et al*. (2003) tested the same parents for seedling resistance using the isolate NB34. With this isolate, Alexis and Sloop had disease ratings of 6.5 and 9, respectively, and Franklin and Arapiles had disease ratings of 3 and 8, respectively. Raman *et al*. (2003) mapped the genomic regions associated with NFNB SLR in the same 3 populations and found that similar regions were involved in SLR to NFNB in all 3 populations. In this case, Alexis and Franklin were the parents that donated the SLR alleles. The locations of the SLR QTLs on chromosomes 2H and 3H are illustrated in Fig. 2 (thick bars). The seedling QTLs on 2HS in the W/Al (*QNFNBSLR.W/Al-2Hb*) and Ar/F (*QNFNBSLR.Ar/F-2H*) populations and on 3HL in the Al/S (*QNFNBSLR.Al/S-3H*) and W/Al (*QNFNBSLR.W/Al-3H*) populations were in a similar location as the APR QTLs identified in these populations in this study. The SLR QTL on 2H was contributed by Franklin, whereas the APR QTL was contributed by Arapiles, and the SLR QTL on 3H was contributed by Alexis, whereas the APR QTL was contributed by Sloop. These differences almost certainly result from the use of isolates from apparently different pathotypes in the 2 studies. For example, isolate NB34 in the SLR study (Raman *et al*. 2003) is avirulent on Franklin (rating = 3) and moderately virulent on Alexis (rating = 6.5), while isolate NB330 used in this study is virulent on both these lines (rating = 10, Table 1). Afanasenko *et al*. (2007) tested isolates obtained from several different geographical regions on 12 resistant barley accessions and also found that seedling resistance is pathotype-specific.

The 2H region associated with APR to NFNB in the Al/S and W/Al populations also appeared to be associated with SLR in the Tallon/Kaputar population (determined using isolate NB97) (Cakir *et al*. 2003). An SLR QTL identified on 4H in the Halcyon/Sloop and Steptoe/Morex populations (Steffenson *et al*. 1996; Raman *et al*. 2003; Read *et al*. 2003) was in the same region as *QNFNBAPR.Al/S-4Ha*. Thus, it seems that even though different sets of genes may be involved in SLR and APR, some of the genomic regions involved are similar. It remains to be discovered whether QTLs in different populations, which co-locate, are allelic or represent different members of a gene cluster. A QTL for SLR that was also associated with resistance in adult plants was identified on chromosome 6H in several studies (Steffenson *et al*. 1996; Richter *et al*. 1998; Spaner *et al*. 1998; Manninen *et al*. 2000; Cakir *et al*. 2003; Emebiri *et al*. 2005; Friesen *et al*. 2006). This QTL was not detected in our study. The APR QTLs on chromosomes 2HS, 3HL, and 7HS identified in our study were in a similar region to the APR

QTLs identified in a North American study (Steffenson *et al*. 1996) using the Steptoe/Morex population. These QTLs were not detected in seedlings and may be loci that operate only as APR QTLs.

Two QTL analysis packages were used in this study: QTLCart to analyse data from individual years and QTLNet to analyse the data across all years. At most loci, similar results were obtained from both programs; however, there were some differences. A QTL on 2HC in the W/Al population, which was significant according to QTLCart, was not identified by QTLNet. Conversely, QTLs on 7H in the W/Al cross were detected only by QTLNet. Such differences in QTL estimations by different software packages were also observed by Ma *et al*. (2006). Overall the percentage of phenotypic variance explained was lower in the QTLNet analysis than in the QTLCart analyses. This difference could have been due to the different methods used by the 2 programs to calculate the phenotypic variance. QTLCart uses the coefficient of determination of a QTL by regression analysis to calculate the phenotypic variance, whereas the QTLNet method uses the variance of the additive effect divided by the phenotypic variance (J. Yang, pers. comm. 2006). The percentage of phenotypic variance explained may also have been different because it was calculated across all years with QTLNet and for individual years with QTLCart.

Significant epistasis was detected in the Al/S population between regions on 3HL and 6HC using the analysis available in QTLNet. These regions may be coincident with QTLs identified in other studies. A SLR QTL on 3HL was previously identified in the Ar/F population by Raman *et al*. (2003). SLR has also previously been identified in the vicinity of the 6HC region by Steffenson *et al*. (1996) in the Steptoe/Morex population and by Friesen *et al*. (2006) in the Q21861/SM89010 population. In the W/Al population, epistasis between regions on 2H and 4H was significant. The 2H region is near a previously reported SLR QTL for NFNB and the region on 4H is near an APR QTL for NFNB (Fig. 2). In the Ar/F population, an interaction was observed between the APR QTL on 7HS and a region on 5H. Several epistatic interactions were identified by QTLNet, but because most of these explained less than 4% of the phenotypic variance, the genomic regions involved would probably not be critical targets in a marker-assisted selection program.

A confounding factor in comparing this work with earlier studies has been pathogen variability and the use of different pathogen isolates by different research groups. Given that a race structure is recognised but poorly characterised in *P. teres* (Platz *et al*. 2000; Gupta *et al*. 2003; Afanasenko *et al*. 2007; Serenius *et al*. 2007), thorough testing of promising resistant materials against a wide range of isolates is essential. It is equally essential that the *P. teres* pathotypes used in genetic and molecular studies are clearly identified, as this can have a significant effect on interpretation and comparison of the data. Following discussions in Edmonton, Canada, at the 3rd International Workshop on Barley Leaf Blights in July 2006, attempts are underway to establish an international differential set of host lines for determination of NFNB pathogenic races, coupled with an international naming convention for each race identified. Such a differential set will aid the identification of which pathogenic races individual isolates belong to and enable researchers to identify race-specific and race non-specific (if they exist) QTLs for both seedling and adult plant resistance.

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