Molecular mapping as a tool for pre-emptive breeding for resistance to the exotic barley pathogen, *Puccinia striiformis* f. sp. *hordei*

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Abstract. Barley stripe rust (BSR), caused by *Puccinia striiformis* f. sp. *hordei*, has been a major disease problem to the barley industry worldwide that has not, to date, been detected in Australia. This paper describes the mapping of stripe rust resistance genes in Tallon/Kaputar (TK) and Arapiles/Franklin (AF) populations. The paper also reports on the usefulness of markers associated with alternative sources of resistance previously identified in the varieties Orca and Shyri in the USA. Stripe rust screening was conducted at the adult plant stage in Toluca, Mexico, for 2 years. Two major quantitative trait loci (QTLs) were found on chromosomes 2H and 5H in both populations. One region on chromosome 5H was highly significantly associated with resistance to stripe rust ($R^2 = 68\%$ and 34% in TK and AF, respectively). The QTL on chromosome 2H accounted for 36% of the variation in TK and 10% of the variation in the AF population. These associations were consistent over both years. Further work will involve screening for additional markers in the target regions to identify polymorphism that can be used to select for multiple resistances in the absence of the pathogen.

Additional keywords: SSR, AFLP, RFLP, genetic mapping, stripe rust.

Introduction

There are several *formae speciales* adapted to the native *Hordeum* species and capable of infecting certain wheat and barley cultivars (Wellings *et al.* 2000). The form of the pathogen specialised on barley (*Puccinia striiformis* f. sp. *hordei*, *Psh*) has been a major threat to barley production in the United States, South America, and central Europe since the 1960s (Dubin and Stubbs 1986; Marshall and Sutton 1995). Under severe conditions, yield losses of up to 70% have been reported in barley (Dubin and Stubbs 1986). Stripe rust is a potential threat to the barley industry in many production areas of the world, including Australia, where it is not currently present. In Australia, past experiences with the introductions of exotic plant pathogens such as wheat stripe rust (caused by *Puccinia striiformis* f. sp. *tritici*; Wellings *et al.* 1987) and chickpea aschochyta blight have highlighted the need to develop contingency plans for managing incursions of exotic pests and pathogens.

A research program funded by the Australian Grains Research Development Corporation (GRDC) has recently been established to test breeding populations for resistance against barley stripe rust (BSR) as a safeguard against this pathogen breaching quarantine barriers and becoming established in Australia. Australian barleys tested with race 24 of *Psh* at Toluca, Mexico, have proved to be generally susceptible (C. Wellings, unpublished data). Thus, the challenge is to develop barley germplasm with resistance to *Psh* in the absence of the disease. Pre-emptive resistance breeding strategies may take either of two approaches: selection for resistance in foreign disease nurseries, or the use of linked disease resistance markers to allow selection in the absence of the pathogen. The former approach will be limited to the selection among fixed lines, and may suffer from a dependency on single gene resistance that may not be durable. The latter is limited by the availability of suitable markers and knowledge of the level of resistance associated

with each marker. Markers have the benefit of allowing the selection of resistance alleles in backcrossing programs aimed at the introgression of exotic germplasm into locally adapted barley populations.

Two dominant genes and 3 recessive genes have been described conferring resistance to *Psh*. A dominant gene identified in European winter barley, *Yr4*, has been mapped to chromosome 1H (Von Wettstein-Knowles 1992). Molecular markers on chromosomes 4H and 5H showed strong associations with resistance quantitative trait loci (QTLs) to stripe rust (Chen *et al.* 1994; Hayes *et al.* 1996). QTLs for adult plant resistance to uncharacterised field isolates of *Psh* were located on chromosomes 1H, 5H, and 7H (Thomas *et al.* 1995). Significant QTLs for resistance to stripe rust have also been reported on chromosomes 1H, 2H, 3H, 4H, 5H, and 6H (Toojinda *et al.* 1998, 2000).

Mapping populations have been developed in Australia from the crosses Tallon/Kaputar and Arapiles/Franklin. Tallon and Franklin both possess a moderate level of resistance to BSR race 24 (D. Moody, unpublished data). The European variety Triumph is a common parent of Tallon (Triumph/Grimmett) and Franklin (Shannon/Triumph) and is the presumed source of BSR resistance in these two varieties. Previous studies on resistance to *Psh* race 24 have been based on resistance derived from Orca and Shyri (Toojinda *et al.* 1998). The objective of this study was to identify markers closely linked to resistance to *Psh* in the 2 Australian barley mapping populations and compare their location to previously reported resistance QTLs derived from Orca and Shyri. This knowledge can then be used to develop an efficient pre-emptive breeding strategy.

Materials and methods

Australian mapping populations

Mapping population Tallon × Kaputar (TK) (Cakir *et al.* 2003, this issue) is described in detail elsewhere in this issue. The Arapiles × Franklin (AF) population was developed by the barley breeding program of the Victorian Institute for Dryland Agriculture (D. Moody, unpublished data). The population was constructed by doubled haploidy using anther culture technique and included 150 lines for the stripe rust phenotyping.

USA germplasm for BSR resistance

QTLs for *Psh* resistance derived from Shyri have been located on chromosomes 1H and 6H (Toojinda *et al.* 2000), whereas QTLs derived from Orca have been located on chromosomes 4H and 5H (Toojinda *et al.* 1998). The polymorphism of markers associated with *Psh* resistance QTLs in breeding lines derived from Orca and Shyri was compared with elite, *Psh*-susceptible Australian parental material. The *Psh*-resistant breeding lines, derived from the cross (Orca/ Harrington//Harrington)/D1-72 (Shyri/Galena), were kindly provided by Prof. Patrick Hayes, Crop and Soil Science, Oregon State University, Corvallis, USA.

Australian parental germplasm

Selected elite parental genotypes from the Victorian barley breeding program (see Table 1) were assessed for BSR resistance at Toluca,

Mexico. Markers within the genomic regions associated with BSR resistance in the USA germplasm were assessed for polymorphism among these parental genotypes.

Phenotyping

The doubled haploid (DH) lines and parents were assessed for adult plant resistance in Toluca, Mexico (2600 m a.s.l.), in 1999 and 2000 for TK and 2000 and 2001 for AF. They were planted in unreplicated double rows, 1 m in length. A field epidemic was initiated by inoculating spreader rows (comprised of 15 extremely susceptible barley genotypes) with a stripe rust isolate presumed to be characteristic of race 24 as described by Dubin and Stubbs (1986). Disease severity was rated at DGS59 (Feekes stage 10.5) as a percentage of leaf area affected on a whole plot basis.

Statistical analysis of phenotypic data

Disease phenotype data from each year were subjected to spatial analysis to maximise the precision with which genetic effects were estimated using the statistical software GENSTAT (GENSTAT 5 Committee 1993).

Broad-sense heritability (h^2) of stripe rust resistance was calculated based on the average disease severity of individual DH lines. Years were used as replications. Analysis was conducted using ANOVA and restricted maximum likelihood (REML) (GENSTAT 5 Committee 1993).

Marker analysis of Australian mapping populations

A genetic map with 263 DNA markers was used to identify marker loci associated *Psh* resistance in the TK population (Cakir *et al.* 2003, this issue). The genetic map of the AF population included 239 markers (D. Moody, unpublished data). QTL analyses were performed using the software packages MapManager (Manly *et al.* 2001) and Qgene (Nelson 1997). A threshold LOD (logarithm of odds ratio) score of 3.0 was chosen for declaring the existence of a QTL. Wherever appropriate, simple regression and interval mapping analyses were used to identify and verify the associations. Analyses were conducted for each year separately and for the 2 years combined.

Assessment of markers associated with BSR resistance in USA germplasm

Genomic DNA was extracted from approx. 2 g of young leaf tissue with 1% sarkosyl, 100 mM TRIS–HCl, 100 mM NaCl, and 2% polyvinyl-polypyrrolidone following the method of Rogowsky *et al.* (1991). Markers CDO057, *Bmy1*, Hor1/2, and BG123, previously associated with resistance to *Psh*, were assessed. Additional markers were selected based on published linkage maps of barley (Kleinhofs *et al.* 1993; Chen *et al.* 1994; Thomas *et al.* 1995; Ramsay *et al.* 2000; Toojinda *et al.* 2000) (see Fig. 2). Detailed restriction fragment length polymorphism (RFLP), simple sequence repeat (SSR), and sequence characterised amplified region (SCAR) marker information was acquired from Graingenes (http://wheat.pw.usda.gov/index.shtml) and the Australian National Barley Molecular Marker program (NBMMP) reports.

RFLP screening of the lines for polymorphism was carried out using 8 restriction enzymes (*Eco*RI, *Eco*RV, *Dra*I, *Hind*III, *Bam*HI, *Xba*I, *Xho*I, and *Sac*I), using standard procedures (Sambrook *et al.* 1989). SSR polymorphism was assessed following the method of Karakousis *et al.* (2000). Products were denatured and run on 8% denaturing polyacrylamide gels (Sequagel, National Diagnostics). *Bmy1* amplification was carried out according to Erkkila *et al.* (1998). *Hor2* amplification was carried out in 25 μL comprising 0.2 mM dNTPs (Sigma), 1 mM MgCl$_2$, 20 pmol each primer, 0.5 U RedTaq (Sigma), and 50 ng template. Amplification was carried out using 47 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 45 s, and

Barley lines	Stripe rust reaction	Barley lines	Stripe rust reaction
Skiff	100	VB9725	80
Tantangara	100	Stirling	80
Schooner	100	Mundah	80
VB9613	100	O'Connor	100
VB9727	100	Brindabella	40
VB9728	100	BCD12	10
VB9729	100	BCD47	20
Arapiles	100	BCD69	5
Chebec	100	BCD84	10
Sloop	100	Tallon	10
Wyalong	100	Kaputar	80
Harrington	80	Gairdner	1
Barque	80	Franklin	1

extension at 72°C for 1 min followed by a final extension at 72°C for 5 min. *Bmy1* and *Hor2* amplification products were separated on 1% agarose gels.

Results

Tallon × Kaputar population

The parental lines differed widely in their resistance to *Psh*. Tallon was resistant with a disease severity score of 10% and Kaputar was susceptible with a disease severity score of 80% (Table 1). These disease severity scores were identical for both years. The DH population showed wide variation for resistance to stripe rust in both years (Fig. 1). In both years, the distribution of disease severity scores over lines showed some tendency of biomodality, suggesting the presence of a major gene. However, 5 lines in 1999 and 3 lines in 2000 showed an intermediate levels of severity (45–55%), indicating the presence of additional genes for resistance to *Psh*. Resistance in the TK population had a high level of broad sense heritability ($h^2 = 0.82$). The genotype × year interaction was not significant.

Markers from 2 different chromosomes were associated with *Psh* resistance in the TK population. In particular, the region on chromosome 5H was highly significantly associated with resistance (highest $R^2 = 68\%$) (Table 2). Markers on chromosome 2H were also associated with resistance to stripe rust (highest $R^2 = 36\%$) (Table 2). The close linkage of markers with disease resistance in both of these regions was consistent over both years.

Simple regression analysis of individual markers revealed 4 markers in an 8.4-cM region on chromosome 2H and 8 markers in a 33.5-cM region on chromosome 5H to be significantly associated with resistance to *Psh* (LOD > 5.0) (Table 2). To determine the additive effect of these 2 QTLs, a stepwise multiple regression analysis was conducted on the average disease severity scores over the 2 years. In the model, the marker with the highest R^2 value was included from each QTL. The percent variation explained by these 2 QTLs was 76%. When the corresponding model was fitted for each year separately, the percent variation explained was 76% for 1999 and 82% for 2000 (results not shown).
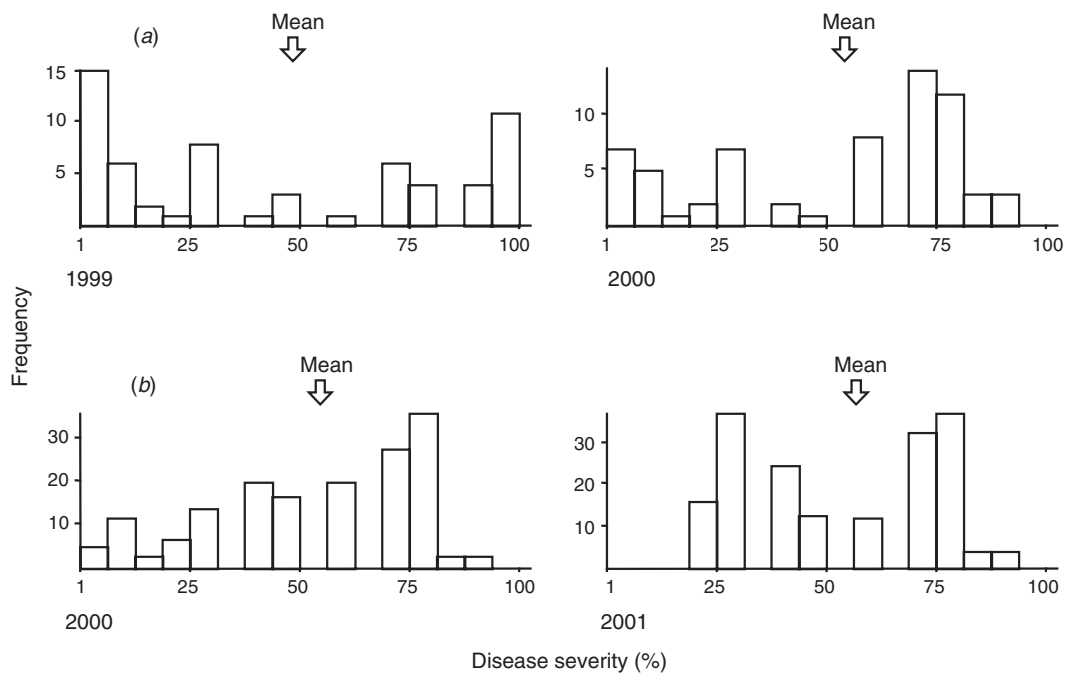

Fig. 1. Distribution of disease severity data for barley stripe rust from the testing of DH lines in Toluca, Mexico (*a*) in 1999 and 2000 for TK population and (*b*) 2000 and 2001 for AF population.

Table 2. The most significant markers associated with stripe rust resistance in Tallon × Kaputar (TK) and Arapiles × Franklin (AF) populations, and statistical values associated with each marker

Marker	Population	Chromosome	R^{2A}	LOD[B]	P^C	AA[D]	aa[E]	Add[F]
Bmag0114	TK	2H	0.36	6.03	0.0000	33.94	74.09	20.07
EBmac0607	TK	2H	0.35	5.67	0.0000	34.91	75.88	20.48
p11m54T105	TK	2H	0.34	5.57	0.0000	34.80	75.00	20.10
p13m62KT134	TK	2H	0.33	5.21	0.0000	33.60	72.50	19.44
p12m54K314	TK	5H	0.68	15.21	0.0000	25.60	80.00	27.20
GSM061	TK	5H	0.58	11.37	0.0000	28.00	77.93	24.96
p11m49K058	TK	5H	0.52	9.76	0.0000	29.03	77.19	24.08
p14m50K081	TK	5H	0.43	7.42	0.0000	31.62	75.22	21.79
p11m47T199	TK	5H	0.45	8.07	0.0000	31.73	76.20	22.23
p12m55T063	TK	5H	0.39	6.58	0.0000	33.79	76.62	21.41
p11m61K233	TK	5H	0.37	5.95	0.0000	32.80	72.73	19.96
p14m54K189	TK	5H	0.36	5.33	0.0000	28.44	67.92	19.74
AT_CAC332	AF	2H	0.10	3.23	0.0001	59.25	44.64	7.30
AT_CAA299	AF	2H	0.10	3.14	0.0002	59.17	44.77	7.19
GMS061	AF	5H	0.34	9.20	0.0000	68.93	42.46	13.23
AT_CAG172	AF	5H	0.32	12.07	0.0000	69.32	43.29	13.01
AT_CAC134	AF	5H	0.32	11.56	0.0000	67.70	42.25	12.72
AG_CTG198	AF	5H	0.31	12.01	0.0000	69.39	43.46	12.96
AG_CTT226	AF	5H	0.30	10.93	0.0000	69.18	44.23	12.47
AA_CCC303	AF	5H	0.29	10.18	0.0000	66.92	42.47	12.22
AA_CCG282	AF	5H	0.28	10.23	0.0000	68.19	43.98	12.10
AA_CCC175	AF	5H	0.26	8.97	0.0000	68.08	44.24	11.92
AA_CCC246	AF	5H	0.26	9.42	0.0000	67.02	43.66	11.67
AT_CAC259	AF	5H	0.26	8.73	0.0000	65.90	43.32	11.28

[A]Phenotypic variation explained by each marker.
[B]Logarithm of odds ratio.
[C]Probability of obtaining LOD ≥ the observed value by chance.
[D,E]Disease severity mean of the DH lines carrying Tallon (A) and Kaputar (a) alleles, respectively.
[F]Additive effect of replacing one allele (a) with the other allele (A).

Arapiles × Franklin population

In field assessments of Australian germplasm at Toluca, the variety Franklin showed a very low level of disease severity (1%). In contrast, the variety Arapiles was highly susceptible, with a disease severity score of 100% (Table 1). Data from the year 2000 growing season revealed lines with intermediate levels of disease severity. Distribution of disease severity data from the year 2001 growing season indicated a bimodal quantitative distribution, suggesting the presence of one major gene and additional genes with smaller effect (Fig. 1). Heritability of the *Psh* resistance was similar to that in the TK population ($h^2 = 0.84$). The genotype × year interaction was not significant.

Two primary QTLs were identified for the *Psh* resistance and were positioned on the same chromosomes as in the TK population. On chromosome 5H there were 10 markers in a 37-cM region that showed significant associations with BSR resistance (LOD > 3.0). Marker GMS061 had the highest R^2 value on this chromosome ($R^2 = 34\%$). This marker had the second highest R^2 value in the TK population ($R^2 = 58\%$). The QTL on chromosome 2H had a smaller effect

($R^2 = 10\%$). There were 2 markers on this QTL that exceeded the LOD score of 3.0 (Table 2).

Assessment of markers associated with BSR resistance in USA germplasm

Chromosome 1H

The *Yr4* resistance locus is located on the short arm of chromosome 1H close to *Hor1* (Thomas *et al.* 1995). Using the RFLP probe Hor1/2 at the *Hor1* locus (Fig. 2), polymorphisms detected with *Eco*RI, *Eco*RV (Fig. 3), and *Dra*I digests were different between resistant and susceptible lines. In addition, amplification using *Hor2* primers resulted in 2 alleles (approx. 1 kb and 800 bp) that differed between resistant and susceptible lines.

Chromosome 4H

Chen *et al.* (1994) identified a QTL with minor effect on the long arm of chromosome 4H (Fig. 2). Marker density on 4H is sparse around the stripe rust QTL; however, polymorphisms were identified between the BSR-resistant USA and BSR-susceptible Australian genotypes using the
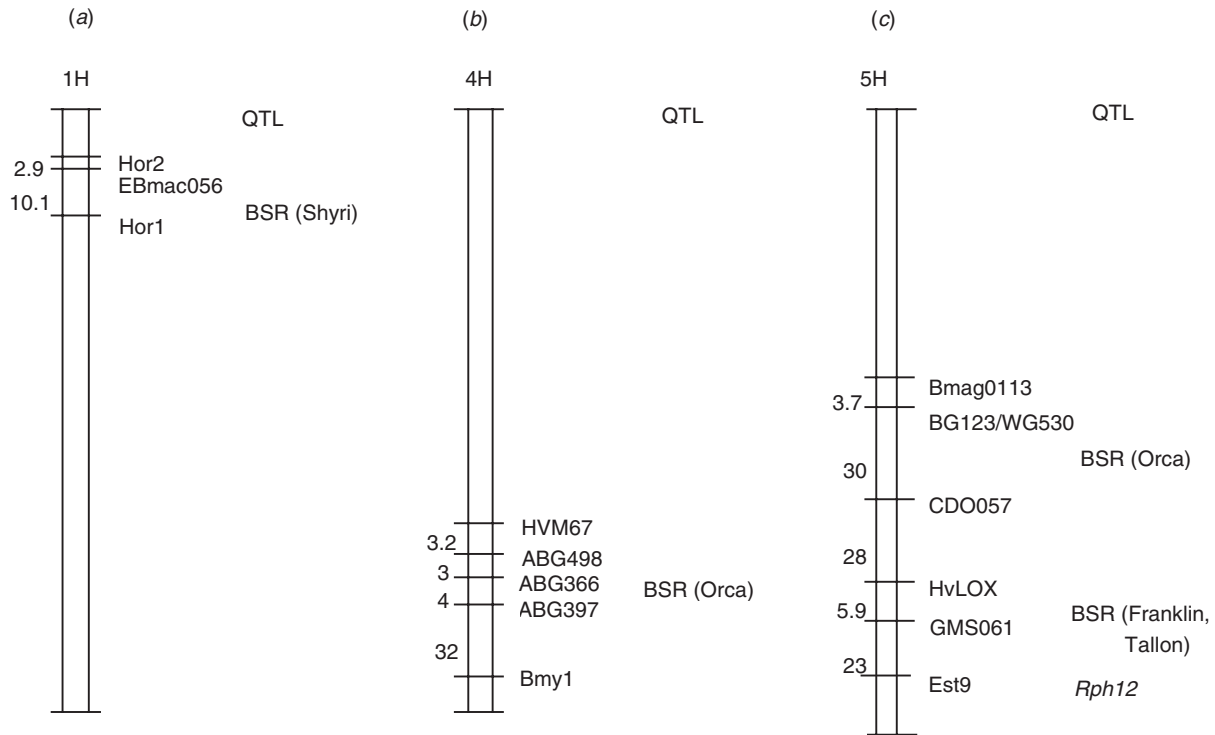
Fig. 2. (*a*, *b*) Locations of barley stripe rust resistance QTLs derived from Shyri (BSR Shyri) and Orca (BSR Orca) with respect to the markers assessed on chromosomes 1H and 4H, respectively. (*c*) Location of the BSR QTL on chromosome 5H of the AF and TK populations (BSR Franklin, Tallon) and its position relative to the QTL for resistance derived from Orca (BSR Orca) and the *Rph12* locus for leaf rust resistance.

Bmy1 SCAR, the ABG366 RFLP, and the HVM67 SSR markers. For *Bmy1*, all *Psh*-resistant lines carried the 500-bp allele, but susceptible lines carried either the 600-bp or 500-bp allele. Markers ABG366, ABG397, and ABG498 would appear to be closely associated with *Psh* resistance on

Fig. 3. Southern analysis of resistant, R, and susceptible, S, lines with *Eco*RV digests probed with Hor1/2. Polymorphic restriction fragments co-segregating with the BSR resistance phenotype are indicated with an arrow. A 1-kb (Promega) molecular weight marker was used. Sample lanes: 1, BCD84; 2, BCD67; 3, VB9613; 4, VB9727; 5, VB0025; 6, VB9729.

chromosome 4H, based on LOD score (Chen *et al*. 1994). However, in this study, no polymorphism was detected using ABG397 and ABG498. The polymorphism detected using the RFLP marker ABG366 identified the absence of a band in the resistant lines that was difficult to use in marker assisted selection. Polymorphism was identified in the SSR marker HVM67. Three alleles of approx. 415, 365, and 345 bp were detected (data not shown). The 415-bp allele was present in all BSR-resistant USA lines and in Franklin, but also occurred in some susceptible lines. The 345-bp allele was rare, occurring only in Gairdner from the samples screened.
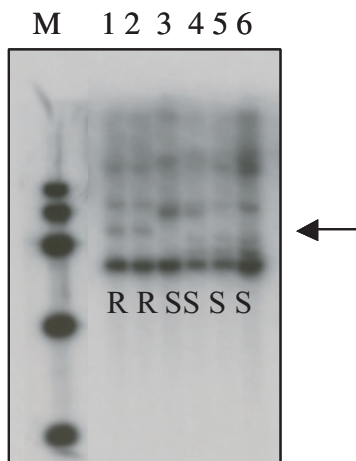
Chromosome 5H

RFLP markers CDO057 and BG123 flank the chromosome 5H locus for *Psh* resistance, with BG123 being proximal to the centromeric region (Fig. 2). These were previously reported to be linked to *Psh* resistance (Chen *et al*. 1994). In this study, CDO057 was highly polymorphic, although not diagnostic, between the USA BSR-resistant and Australian BSR-susceptible genotypes. However, polymorphisms observed with RFLP markers, BG123/*Eco*RV (Fig. 4) and WG530/*Xho*I, appeared to be closely associated with the resistant phenotypes. In addition, 2 alleles were identified using the SSR marker Bmag0113,
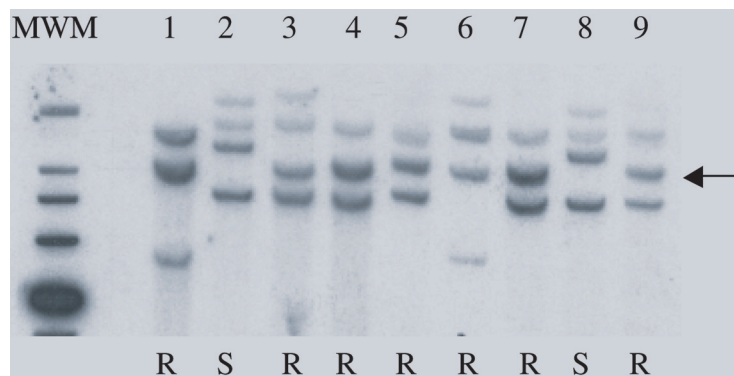
Fig. 4. Southern analysis of resistant, R, and susceptible, S, lines with *Eco*RV digests probed with BG123b. Polymorphic restriction fragments co-segregating with the BSR resistance phenotype are indicated with an arrow. A 1-kb (Promega) molecular weight marker was used. Sample lanes: 1, BCD84; 2, VB9733; 3, Tallon; 4, Franklin; 5, BCD67; 6, BCD47; 7, BCD12; 8, VB0025; 9, VB9729.

although the marker was not polymorphic between several BSR-resistant and BSR-susceptible genotypes.

Discussion

Consistency of disease severity data ($h^2 = 0.82$ for TK and 0.84 for AF) over 2 years of testing confirms that resistance was stable in the disease nursery in Mexico. Similarly, there was a consistent association of the markers in both populations for both QTLs over the 2-year study. A high level of consistency in *Psh* disease severity ratings across 5 environments has also been reported by Toojinda *et al.* (2000).

Quantitative distribution of disease severity data has previously been observed for BSR (Toojinda *et al.* 2000). The presence of DH lines with intermediate resistance in this study may indicate that, in addition to the 2 QTLs for BSR resistance identified, additional genes with smaller effects may be segregating in these populations.

The rust evaluations of mapping populations from this study, as well as those from previous studies (Hayes *et al.* 1996), were done at Toluca, Mexico, where race 24 is presumed to be present. A significant QTL for resistance on chromosome 1H was detected in Shyri derivatives (Toojinda *et al.* 2000), but not in the Triumph-derived (Tallon/Kaputar and Arapiles/Franklin) populations. The *Yr4* gene, which has been mapped to chromosome 1H (Von Wettstein-Knowles 1992), is not effective against race 24, and hence, if *Yr4* were present in either Tallon or Franklin, it would not be expected to be expressed in the screening nurseries at Toluca. Our results indicate that Tallon and Franklin carry a resistance gene, located on chromosome 5H, that provides partial adult plant resistance against race 24 and possibly a second gene, with lesser effect, on chromosome 2H. The occurrence of the QTL on chromosome 5H in Australian varieties, selected in the absence of the BSR pathogen, may not be coincidental. The QTL for BSR resistance maps to the same region as the

leaf rust (*Puccinia hordei*) resistance locus *Rph12* (Park *et al.* 2003, this issue), which has been consciously selected for in the development of both Tallon and Franklin.

The presence of QTLs for resistance to *Psh* on chromosomes 2H and 5H has been reported previously (Chen *et al.* 1994; Hayes *et al.* 1996; Toojinda *et al.* 2000). The QTL mapped on 5H in both TK and AF populations near the marker GMS061 occurs proximal to the QTL identified by Chen *et al.* (1994) derived from Orca. It is not clear whether an additional *Psh* resistance locus has been discovered in Tallon and Franklin. However, the marker genotypes at the GMS061 locus are the same in Franklin and all backcross-derived (BCD) USA lines evaluated in this study. Further work using crosses between Tallon, Franklin, and select BCD lines will determine whether these are allelic or form a cluster of resistance loci. The QTL mapped on chromosome 2H is coincident with a maturity locus, and it has been suggested that the disease scoring may be confounded by plant maturity (D. Moody, unpublished data).

Triumph derivatives, carrying the Triumph-derived chromosome 5H *Psh* resistance, are far more common in Australian germplasm than Orca derivatives carrying the Orca-derived chromosome 5H *Psh* resistance. The introgression of the chromosome 1H Shyri resistance QTL into Australian Triumph derivatives possessing chromosome 5H resistance would potentially enhance the level of adult plant resistance to *Psh* race 24. The transfer of a single QTL would be less disruptive to the malting quality gene pool than a more complex series of introgressions. Further studies would also be useful to determine if chromosome 5H resistance derived from Triumph and Orca is non-allelic and effective against different *Psh* pathotypes and whether the use of both sources of resistance would improve the genetic diversity for BSR resistance, hence providing greater insurance against pathogen evolution.

The stripe rust pathogen *Psh* remains a potential threat to the Australian barley industry, and efforts to develop resistant germplasm as a safeguard against the possible incursion of this pathogen through Australian quarantine barriers are regarded as a priority activity. The use of molecular markers offers the possibility of introducing a diverse range of resistances into breeding germplasm in advance of the possible arrival of the pathogen. Markers for resistance to *Psh* reported on chromosomes 1H, 4H, and 5H from Orca and Shyri were not diagnostic for resistance when assessed on a diverse range of Australian parental material that was characterised for *Psh* response at Toluca, Mexico. However, there was sufficient marker polymorphism to indicate that these markers may be useful for selecting among progeny derived from a known *Psh*-resistant parent in some crosses. This strategy is being investigated with a DH population developed from a cross between BCD84 and VB9733, which will be screened in Toluca in the near future.

Acknowledgments

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