Mapping genes for resistance to *Puccinia hordei* in barley

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Abstract. Six doubled haploid barley populations (Alexis × Sloop, Chebec × Harrington, Arapiles × Franklin, Patty × Tallon, Tallon × Kaputar, and Sloop × Halcyon) and a recombinant inbred population (WI2875-1 × Alexis) were assessed for response to selected pathotypes of the barley leaf rust pathogen, *Puccinia hordei*, at the seedling growth stage. Resistance genes were postulated for the parents of each population based on their reaction to selected pathotypes. In most cases, the resistance genes postulated in the cultivars were validated by QTL mapping analyses of the progeny populations. The resistance genes detected and mapped were *Rph2*, *Rph3*, *Rph4*, *Rph12*, and *Rph19*. The chromosomal locations of these 5 genes were consistent with previous reports, with *Rph2* mapping near to the centromere on the short arm of chromosome 5H, *Rph4* mapping to chromosome 1H, *Rph12* mapping to the long arm of chromosome 5H, and *Rph3* and *Rph19* mapping ~30 cM apart on the long arm of chromosome 7H.

Additional keywords: Hordeum vulgare, leaf rust, disease, QTL mapping.

Introduction

Australian barley crops can be affected by 3 rust diseases. Stem rust (caused by *Puccinia graminis* f. sp. *tritici*, *P. graminis.* f. sp. *secalis*, or the 'scabrum' stem rust, a form regarded to be a somatic hybrid between f. spp. *tritici* and *secalis*) tends to be a problem when inoculum levels are high due to epidemics in crops such as wheat and triticale. A new form of stripe rust, first detected in 1998 and referred to as barley grass stripe rust, can infect some barley genotypes, particularly Skiff and several of its derivatives (Wellings *et al.* 2000). Leaf rust of barley is caused by *Puccinia hordei* and, although sporadic in occurrence, occurs in all barley-growing regions of Australia. Resistance to this disease is known, and to date, 19 loci that confer seedling or major gene resistance to the disease have been characterised. Although virulence has been detected to nearly all of these genes in at least some parts of the world, *Rph3*, *Rph7*, *Rph14*, *Rph15,* and *Rph18*, as well as some combinations of the defeated genes, remain

effective in Australia (Cotterill *et al.* 1995; R. F. Park, unpublished data). *Rph16* and *Rph17* have not been tested in Australia because the lines have not been available.

Mapping populations based on several diverse barley genotypes were produced as part of the Australian National Barley Molecular Marker Program. Multi-pathotype testing of the parental genotypes of these populations with a range of Australian *P. hordei* pathotypes indicated the presence of seedling resistance genes in some parents, several of which were uncharacterised. This paper presents the results of mapping studies conducted to characterise further the resistance genes detected.

Methods

Mapping populations

Seven doubled haploid (DH) mapping populations were studied: Alexis × Sloop (AL/SL), Chebec × Harrington (CH/HAR), Arapiles × Franklin (AR/F), Galleon × Haruna Nijo (G/HN), Patty × Tallon (P/T),

Tallon × Kaputar (T/K), and Sloop × Halcyon (S/HAL). A population of recombinant inbred lines (RIL) from the cross WI2875-1 × Alexis (Barr *et al.* 2003*a*, this issue) was also tested in conjunction with tests of the AL/SL population. WI2875-1 is a sister line of Sloop.

Pathogen isolates

Nine pathotypes of *P. hordei* were used in multi-pathotype tests of all parents, to postulate the resistance genes present in each, or in tests of mapping populations (*viz.* pathotype 200P–, culture S3088=518; 200P+, 900149=570; 210P+, 900041=482; 231P+, 810039=486; 243P+, 760462=537; 253P–, 760462=490; 4610P+, 900380=491; 5610P+, 970073=520; 5653P+, 010189=561). Pathotype nomenclature followed the notation system of Gilmour (1973), with the addition of ‘P–’ or ‘P+’ to indicate avirulence or virulence, respectively, on cv. Prior (*Rph19*).

All isolates originated from Australian pathogenicity surveys of *P. hordei* conducted from 1976–2001, and are maintained in cryogenic storage at the Plant Breeding Institute Cobbitty Rust Collection. A differential set comprising the standard set used in Australasian surveys as described by Park and Karakousis (2002) was included in tests with each isolate.

Greenhouse procedures

Seedlings were raised and inoculated with *P. hordei* as described previously (Park and Karakousis 2002). Following inoculation, plants were incubated in a greenhouse compartment with natural lighting and temperature maintained within the range 20–25°C. The responses of parental lines and derivative DH lines were determined at 11 days post-inoculation, and were recorded as infection types (IT) using a 0–4 scale similar to that used in studies of *Puccinia triticina* (McIntosh *et al.* 1995).

Inheritance studies

Infection types of 3+ or higher were interpreted as indicating susceptibility in the host, and those of 3 or less were interpreted as indicating resistance. For quantitative trait locus (QTL) analyses, resistance and susceptibility were coded as 1 and 0, respectively, unless otherwise stated. Standard chi-square tests were used to test the validity of genetic models with observed data.

Marker genotyping of populations

The mapping populations were genotyped using molecular markers such as RFLPs, SSRs, and AFLPs. Details of molecular genetic map construction and QTL analyses for the populations Alexis × Sloop and WI2875-1 × Alexis (Barr *et al.* 2003*a*), Chebec × Harrington (Barr *et al.* 2003*b*), Galleon × Haruna Nijo (Karakousis *et al.* 2003*a*), Tallon × Kaputar (Cakir *et al.* 2003), and Sloop × Halcyon (Read *et al.* 2003) are published elsewhere in this issue. Molecular analysis of Patty × Tallon was essentially carried out according to Cakir *et al.* (2003); analysis of Arapiles × Franklin was carried out by D. B. Moody *et al.* (unpublished data).

Genetic maps for each population were used to identify QTLs associated with the genes for resistance to different leaf rust pathotypes. QTL analyses were performed using the software packages

Table 1.   Infection types produced in tests of selected barley genotypes infected with Australian isolates of *Puccinia hordei*

Cultivar/line (gene)	Pathotype[A]								Postulated resistance gene(s)
	200P–	210P+	231P+	243P+	253P–	4610P+	5610P+	5653P+	
Alexis	;N	;N	;N	;12–C	;1N	;N	;N	;12–C	*Rph3* or *7*?
Arapiles	;12+C	3+	3	12CN	12–CN	3+	3+	3+	*Rph19* +?
Chebec	;1N	3+	3+	12+N	;1N	3+	3+	3+	*Rph19*, +?
Franklin	;1+N	12–C	;1++N	;12–CN	12–CN	33+	3+	3+	*Rph12*
Halcyon	;1+N	;1+N	;1+N	3+	3+	;1+N	;12–N	3+	*Rph2*
Kaputar	;1N	;1N	;1+N	3+	3+	;1+N	;1+N	3+	*Rph2*
Patty	2+CN	3+	3+	3+	3+	3+	33+C	33+	?
Sloop	;1+N	3+C	33+	XN	;1N	2++3C	3+C	2++3C	*Rph19*, +?
Tallon	;;+N	;1–N	;1++N	;1–N	;1++N	3+	3+	3+	*Rph12*
Gus	3+	3+	3+	3+	3+	3+	3+	3+	
Sudan (*Rph1*)	;12N	;12CN	3+	3+	3+	;12N	;1+CN	3+	
Peruvian (*Rph2*)	;12-CN	;12–CN	;12–CN	X++3	X++	;12–CN	;12N	X++3	
Estate (*Rph3*)	;1–	;1=	;1+C	;1–	;1–	;1=	;1–	;;1–	
Gold (*Rph4*)	;12–	3+	3+	;1+	3+	3+	3+	3+	
Magnif 104 (*Rph5*)	;N	;1–N	;1++N	;–N	;–N	;N	;N	;–N	
Bolivia (*Rph2+6*)	;12–N	;12–CN	;12N	X++3	X+	;1N	;1+N	3+	
Cebada Capa (*Rph7*)	;-N	0;	;N	;-N	;–N	0;	;–N	;N	
Egypt 4 (*Rph8*)	3+	3+	3+	3+	3+	3+	3+	3+	
Abyssinian (*Rph9*)	;1–CN	;12–C	;12–CN	;1+CN	12C	3+	3+	3+	
Clipper BC8 (*Rph10*)	3+	;1++C	;1+C	33+	3	12–C	33+	12++C	
Clipper BC67 (*Rph11*)	3	3	3	33+	3	3–	3	33-	
Triumph (*Rph12*)	;1++N	;1+CN	;1++N	;1+N	;1++N	3+	3+	3+	
PI 531849 (*Rph13*)	;N	;N	;N	;N	;N	;N	;N	3+	
PI 58476 (*Rph14*)	12C	12CN	12+C	;12–C	;12–	12+C	12+C	;12–C	
Prior (*Rph19*)	;1–N	3+	3+	;1-N	;1N	3+	3+	3+	
Cantala (*RphC*)	3+	3+	3+	12+	12–C	3+	3+	3+	

[A]Details are given in the text.

5H

P13/M51-127
abg705a
P14/M51-294
psr326
P14/M51-201
bcd276
P14/M55-370
P13/M48-180
P14/M61-98
P13/M49-211
P13/M54-356
Bmag387
Bmac286
Bmac113
Bmac96
Bmag337
Bmag223
P12/M54-118
P13/M59-224
P14/M51-434
psr637
cdo400
P14/M55-135
P14/M55-146
abg3
bcd265
abg712
gms61
P14/M49-171
P13/M61-198
P13/M62-224
P13/M61-386
P13/M61-388
P13/M50-305
P13/M54-207
P14/M48-195
Bmag222
P13/M59-191
SerpinZ7
P14/M51-211
P13/M55-131
P11/M51-333
MCG002.G11
P11/M62-179
P13/M51-349
abc310
P14/M48-197
P13/M59-186
P13/M59-69
P12/M50-298
P13/M51-199
P11/M51-215
P13/M62-421
P11/M51-193

6.78   0.0
3.0
LOD

7H

abg704
P12/M51-73
P14/M47-278
P13/M51-134
P14/M47-357
P13/M51-264
P14/M47-315
P13/M61-73
P11/M62-141
P14/M61-138
P14/M60-191
P13/M48-85
bcd129
P13/M48-208
P11/M62-353
P12/M51-121
Bmag206
Pc-B4e
P11/M51-341
wg789
P12/M51-148
P13/M51-107
55TH2e7.seq
P14/M47-164
P14/M59-167
P14/M60-133
SSS1
LD
P13/M59-110
P12/M51-200
cdo665b
HVCMA
P14/M48-150
P11/M48-367
P12/M54-56
P14/M59-215
Bmac31
Bmag359
EBmac827
Bmac167
Bmag507
P14/M55-102
P14/M60-177
P14/M60-154
P11/M51-163
P12/M50-223
P13/M59-309
P12/M51-360
P14/M49-187
P13/M51-203
P13/M59-118
P14/M47-355
EBmac755
wg420
P13/M47-300
P14/M55-130
P11/M51-161
P14/M55-344
Bmag135

8.65   0.0
3.0
LOD

Fig. 1. QTLs on chromosomes 5 and 7 associated with resistance to *Puccinia hordei* pathotype 231P+ in a WI2875-1 × Alexis recombinant inbred population, including regression analysis scan and thresholds for significant associations (likelihood of odds).

Table 2.   Pedigrees of barley cultivars used in generating doubled haploid populations

Cultivar	Pedigree
Alexis	Br.1622/Triumph
Arapiles	Noyep/Proctor//CI3576/Union/4/Kenia/3/Research/2/Noyep/Proctor/5/Domen
Chebec	(USA) Orge Martin/2 *Clipper(86)//Schooner (Aust)-(Orge Martin *Clipper#2)/86*Schooner
Dash	(Chad/Joline)/Cask
Franklin	Shannon/Triumph
Halcyon	Warboys/Maris Otter
Harrington	Klages x Gazelle/Betzes//Centennial
Kaputar	5604/1025/3/Emir/Shabet//CM67/4/F3 Bulk Hip
Patty	Volla*Athos
Sloop	WI2468/Norbert//Golden Promise/WI2395/3/Schooner
Tallon	Triumph/Grimmett
VB9524	Franklin/Arapiles

MapManager QTX (Manly *et al*. 2001) and Qgene (Nelson 1997). QTL effects were considered significant when they exceeded a logarithm of odds ratio (LOD) score of 3.0 ($P = 0.001$) and/or the calculated statistic value by permutation tests (significant at $P = 0.05$, highly significant at $P = 0.001$). Significant associations between markers and rust response were also tested using likelihood ratio statistics (LRS). The LRS can be converted to a LOD score by dividing by 4.61. Wherever appropriate, simple regression and interval mapping analysis were used to find associations.

Results

Three of the parental genotypes (Galleon, Harrington, and Haruna Nijo; data not shown) lacked detectable seedling resistance with the rust cultures used, and consequently, the Galleon × Haruna Nijo population was not tested. The ITs of the 9 remaining cultivars to the 8 pathotypes are given in Table 1. On the basis of the pattern of response to the isolates, plus the IT phenotype, leaf rust resistance genes were postulated for each host genotype (Table 1). The data obtained were in some cases unequivocal (e.g. Alexis).

Alexis × Sloop

Multi-pathotype tests of Alexis and Sloop clearly indicated the presence of a highly effective seedling resistance gene in Alexis (possibly *Rph3* or *Rph7*), and *Rph19* in Sloop (Table 1).

The AL/SL DH population and the WI2875-1 × Alexis RIL population were tested with pathotype 231P+, which is virulent for *Rph19*. The observed segregation in the DH population appeared to conform to a 2-gene model (62 resistant:18 susceptible, ($\chi^2_{3:1} = 0.27$, $P > 0.70$). The RIL population was scored as 86 resistant:16 susceptible, which supported a three gene model ($\chi^2_{7:1} = 0.95$, $P > 0.50$). When tested with pathotype 5653P+, both the DH population (32 resistant:45 susceptible, $\chi^2_{1:1} = 2.19$, $P > 0.20$) and the RIL population (45 resistant:51 susceptible, $\chi^2_{1:1} = 0.38$, $P > 0.70$) conformed to single-gene models.

The rust response data for the RIL population tested with pathotype 231P+ were mapped as described by Barr *et al.* (2003*a*), and 3 genomic regions contributing to the resistance
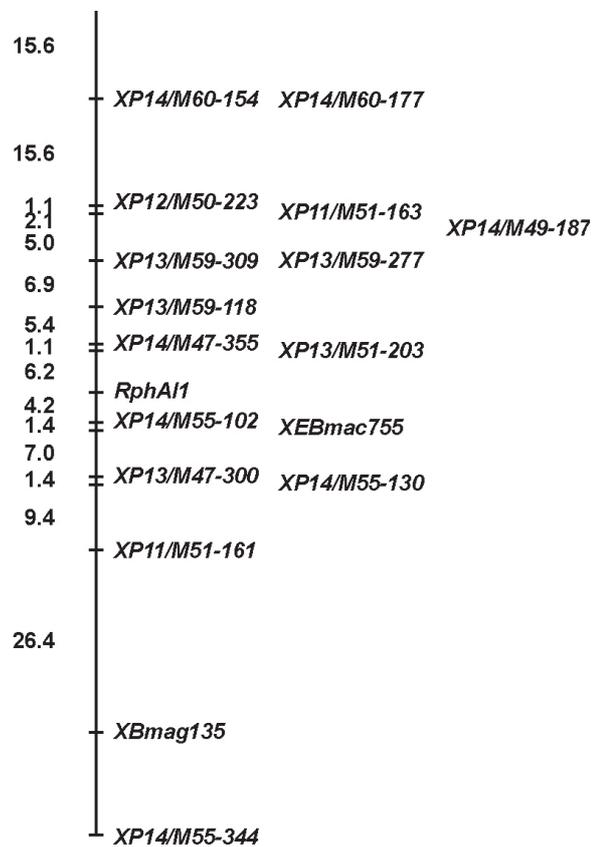
Fig. 2.   Partial map of barley chromosome 7H based on a WI2875-1 × Alexis recombinant inbred population showing the genetic location of a leaf rust resistance gene, *RphAl1*, considered to be *Rph3*, along with map distances (cM).

were identified, 2 of which mapped to chromosome 5H and the third to chromosome 7H (Fig. 1). These results, taken together with those obtained from multi-pathotype tests and the pedigree of Alexis (Table 2), suggest that the 3 loci corresponded to the resistance genes *Rph2*, *Rph3*, and
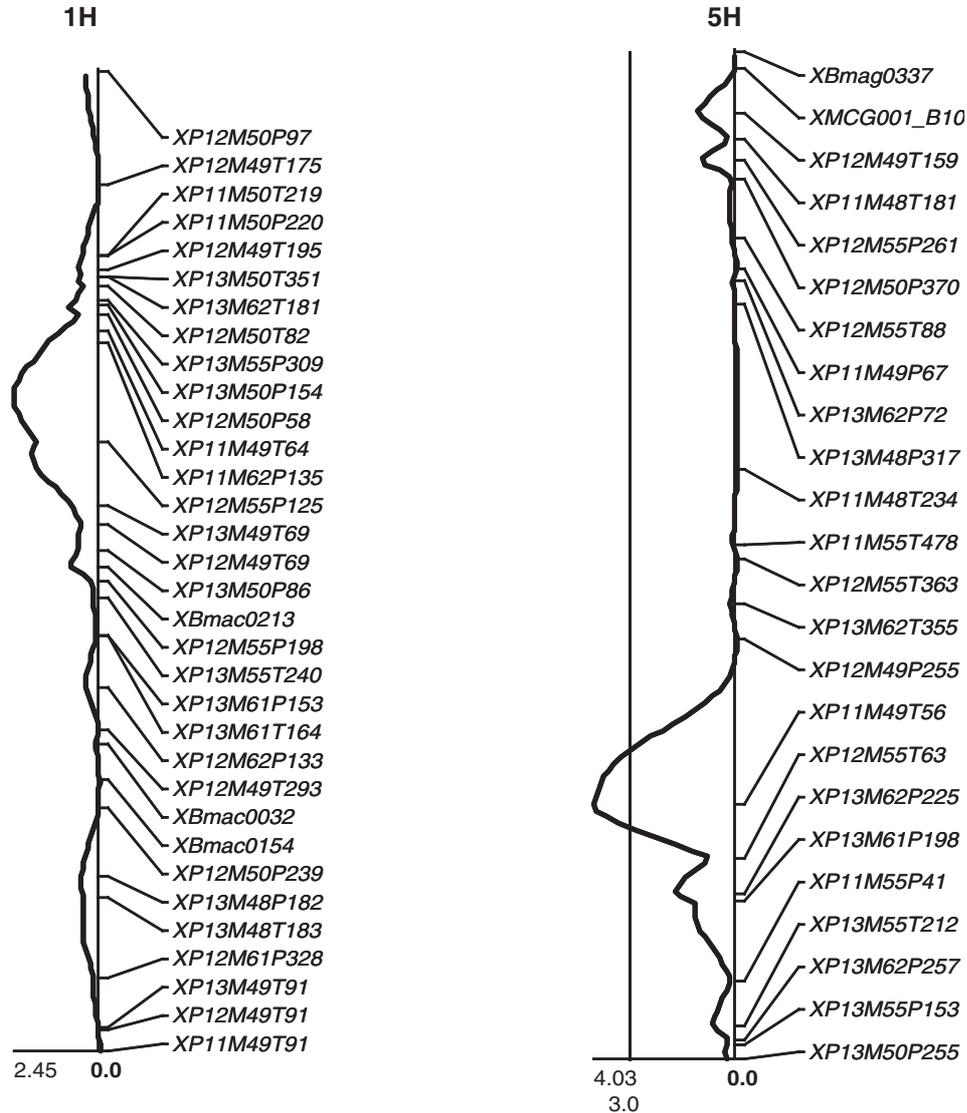
Fig. 3. QTLs on chromosomes 1H and 5H associated with resistance to *Puccinia hordei* pathotype 200P– in a Patty × Tallon doubled haploid population, including regression analysis scan and thresholds for significant associations (likelihood of odds).

Rph12, all of which would have been contributed by Alexis. The single gene identified with pathotype 5653P+ mapped to the long arm of chromosome 7H (LOD score = 16) and was flanked by the AFLP markers *XP13/M51-203* and *XP14/M55-102* at distances of 6.2 cM and 4.2 cM, respectively (Fig. 2). This was likely *Rph3*.

Chebec × Harrington

The DH lines from this cross were either resistant (54) or susceptible (59) when tested with pathotype 243P– (920636=507). This ratio is consistent with segregation at a single locus ($\chi^2_{1:1} = 0.22$, $P > 0.70$). The locus mapped to the long arm of chromosome 7H and was given the designation

Rph19 and an allele designation *Rph19.ah* by Park and Karakousis (2002).

Arapiles × Franklin

The results of multi-pathotype tests supported the presence of resistance gene *Rph12* in Franklin, and *Rph19* in Arapiles (Table 1). The population segregated 55 resistant:7 susceptible when tested with pathotype 253P– which is avirulent for both of these genes. This was a good fit to a 3-gene model ($\chi^2_{7:1} = 0.08$, $P > 0.80$). The pedigree of Franklin includes cv. Shannon (Table 2), which is believed to carry *Rph4*, and so it is possible that this gene may also be present in Franklin. However, the putative third gene

detected in the A/F population with pathotype 253P– cannot be *Rph4* because this pathotype is virulent for *Rph4*. The third gene could be an uncharacterised resistance gene present in many Australian barley cultivars, including Noyep and Research, both of which were used in the development of Arapiles (Table 2), for which pathotype 253P– is avirulent (Cotterill *et al.* 1994).

The mapping conducted with this population (D. B. Moody *et al.* unpublished data) did not identify a significant QTL. However, one region on chromosome 5H was nearly significant (LOD score = 2.7) and is likely to correspond to *Rph12*. The reason(s) for the lack of significant QTLs in this population is not clear.

Patty × Tallon

The cultivar Patty appeared to carry a leaf rust resistance gene of intermediate effect (IT 2+CN to 33+C depending upon pathotype), and Tallon was postulated to carry *Rph12* (Table 1). The intermediate phenotype was difficult to score in the P/T mapping population. Attempts were made to classify lines for this phenotype in 2 further tests by using 2 pathotypes virulent for *Rph12* (pathotypes 4610P+ and 5610P+); however, results were not reproducible and consequently lines showing this phenotype were treated as susceptible. When tested with pathotype 200P–, 74 lines were scored as resistant and 23 as susceptible, a good fit to a 2-gene model ($\chi^2_{3:1}$ = 0.09, $P > 0.80$). Similarly, a test with pathotype 211P+ also conformed with a 2-gene model (66 resistant:27 susceptible, $\chi^2_{3:1}$ = 0.81, $P > 0.50$); however, there were differences in the response of some lines to the 2 pathotypes, with some being susceptible to one and resistant to the other, and *vice versa*. This indicates that at least 1 of the 2 genes detected by the 2 pathotypes differed.

Mapping identified 2 significant loci for the data with pathotype 200P–. The locus on the long arm of chromosome 5H most likely corresponds to gene *Rph12* (Fig. 3; marker interval *XP11M49T56–XP12M55T63*, LRS = 10.8–18.9). The second locus on chromosome 1H could be associated with *Rph4* (marker interval *XP11M62P135–XP12M55P125*, LRS = 10.2–11.1). Analysis of the data from the screening with pathotype 211P+ revealed 2 significant regions on chromosome 5H, one on the short arm (marker interval *XP13M62P72–XP13M48P317*, LRS = 6.2–9.5) and one on the long arm (marker interval *XP13M62P212–XP13M61P198*, LRS = 15.8–17.2) (Fig. 4). Although the locus on the long arm is most likely *Rph12*, that on the short arm had a low LRS value and it is unclear at present if it is of any significance.

Tallon × Kaputar

On the basis of multi-pathotype tests, Tallon was postulated to carry *Rph12* and Kaputar, *Rph2* (Table 1). The T/K doubled haploid population was tested with pathotype 200P+, avirulent for both *Rph2* and *Rph12*. The segregation

5H

XBmag0337
XMCG001_B10
XP12M49T159
XP11M48T181
XP12M55P261
XP12M50P370
XP12M55T88
XP11M49P67
XP13M62P72
XP13M48P317
XP11M48T234
XP11M55T478
XP12M55T363
XP13M62T355
XP12M49P255
XP11M49T56
XP12M55T63
XP13M62P225
XP13M61P198
XP11M55P41
XP13M55T212
XP13M62P257
XP13M55P153
XP13M50P255

4.03   0.0
3.0
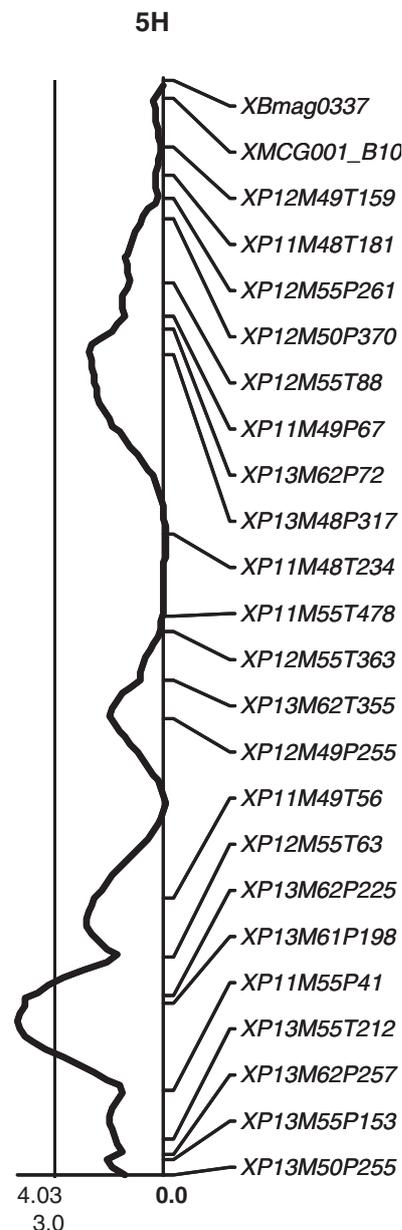
Fig. 4. QTLs on chromosome 5H associated with resistance to *Puccinia hordei* pathotype 211P– in a Patty × Tallon doubled haploid population, including regression analysis scan and thresholds for significant associations (likelihood of odds).

conformed best to a 3-gene model (54 resistant:11 susceptible, $\chi^2_{7:1}$ = 1.16, $P > 0.30$), although there was also evidence for a 2-gene model ($\chi^2_{3:1}$ = 2.26, $P > 0.20$). In view of the results obtained for the Patty × Tallon population, and the pathogenicity of pathotype 200P+, it was expected that the third gene segregating in this population was *Rph4*.

Mapping analysis with the data from pathotype 200P+ revealed a single significant QTL on chromosome 5H, and one locus on chromosome 2H that was just below the significant LOD score of 3.0 (Fig. 5). The locus on the long
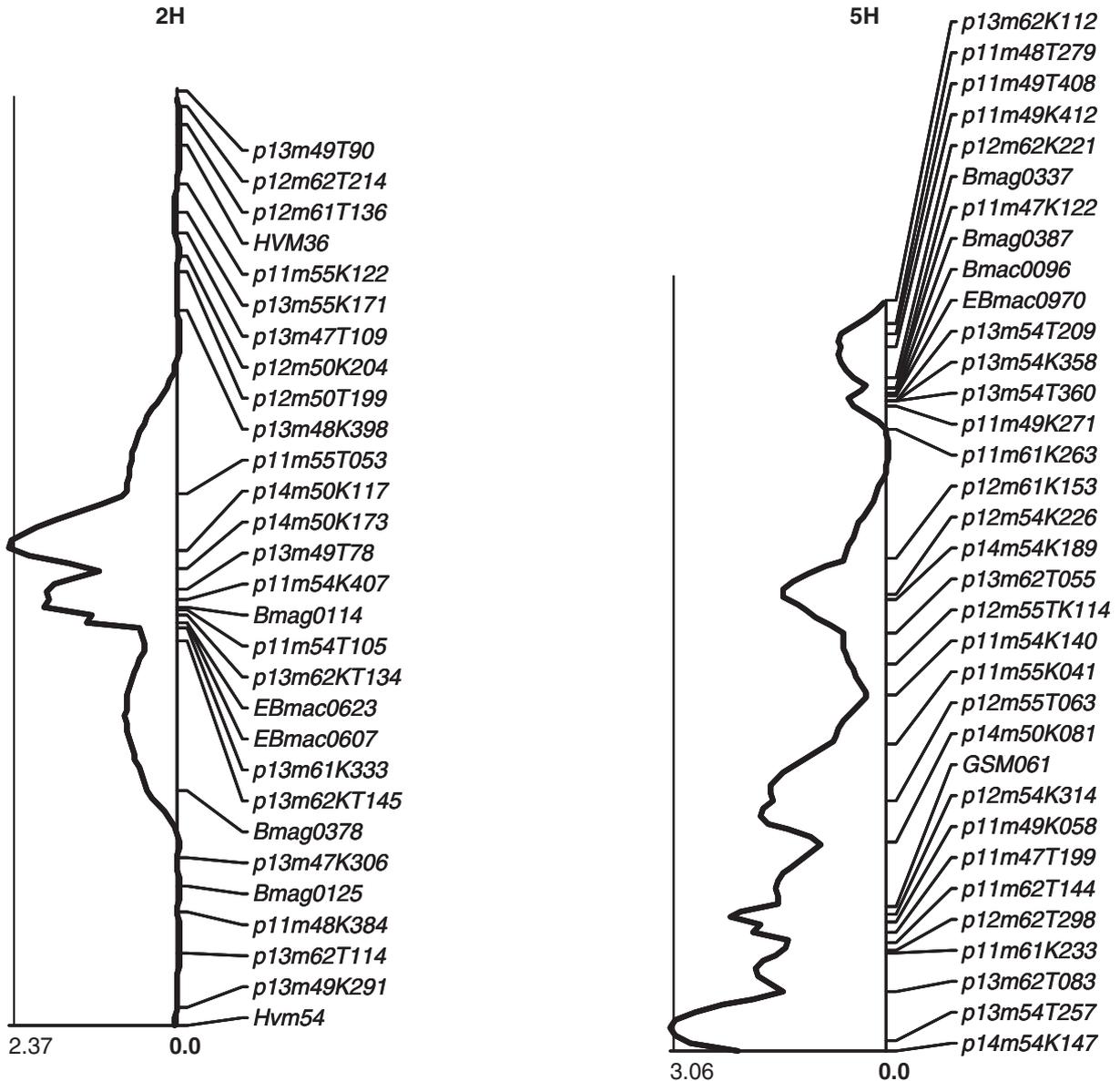
Fig. 5. QTLs on chromosomes 2H and 5H associated with resistance to *Puccinia hordei* pathotype 200P+ in a Tallon × Kaputar doubled haploid population, including regression analysis scan and thresholds for significant associations (likelihood of odds).

arm of chromosome 5H (marker interval *p13m62T083–p13m54T257*, LRS = 10.2–15.2) most likely corresponds to *Rph12*. A region on the short arm of chromosome 5 (marker interval *p12m54K226–p14m54K189*, LRS = 7.3–7.5) could be associated with *Rph2*; however, the LOD score for this was low (Fig. 5). The expectation that the third locus corresponded to *Rph4* was not corroborated by the QTL analysis, which failed to detect any significant association with chromosome 1H on which *Rph4* is located (Tan 1978). Further testing is required to clarify the QTLs identified on chromosome 2H and on 5H. The locus on 2H showed a significance level of just under the threshold (marker interval *p11m55T053–p14m50K117*, LRS = 11.7–12.6), which may have resulted from the smaller population size. The validity of both will be tested further using a larger population from the same cross.

Sloop × Halcyon

Multi-pathotype tests suggested the presence of *Rph19* in Sloop and *Rph2* in Halcyon (Table 1). There was also evidence of a second gene in Sloop, conferring a low infection type of 2++3C to 3+C; however, the expression of this resistance was often not clear and may have been affected by temperature.
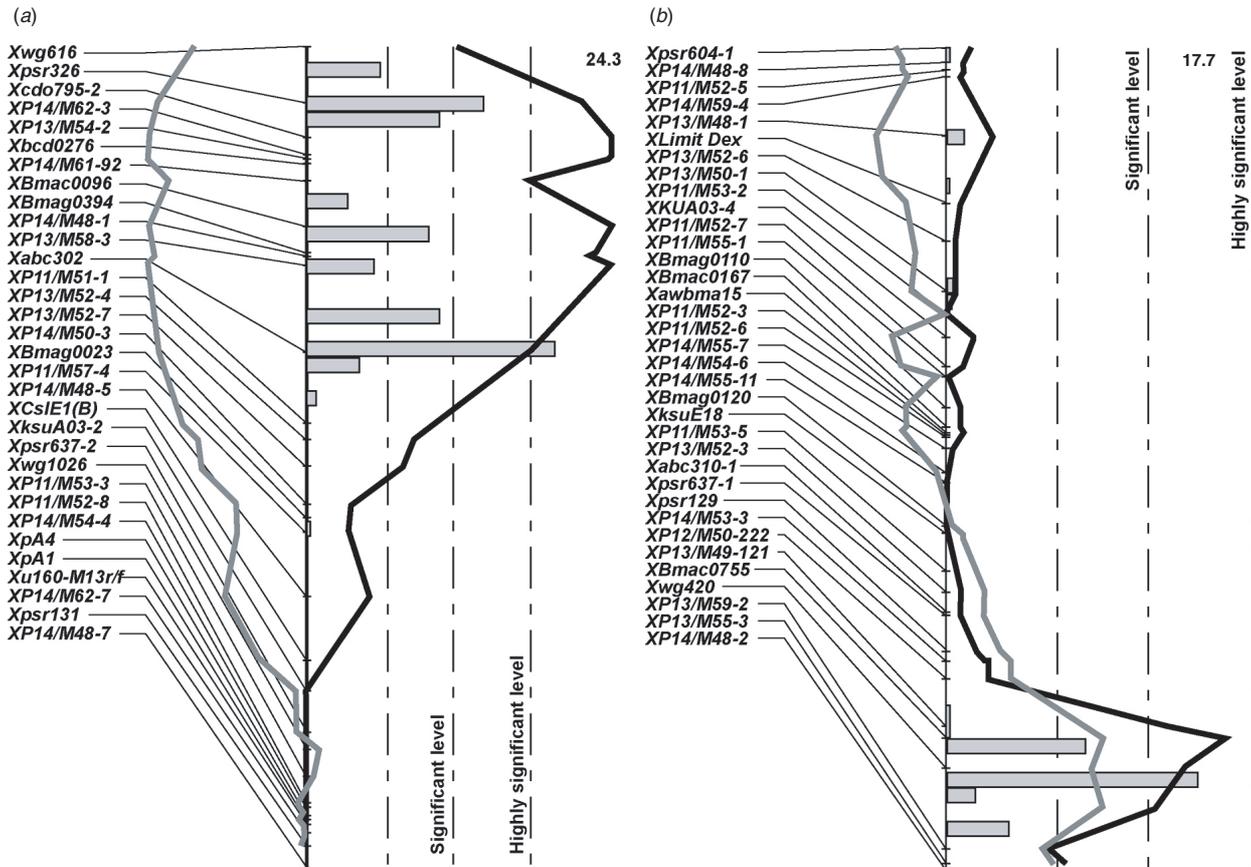
Fig. 6. QTLs on chromosome (*a*) 5H and (*b*) 7H associated with resistance to *Puccinia hordei* pathotype 200P– in a Sloop × Halcyon doubled haploid population, including regression analysis scan and thresholds for significant associations (likelihood ratio statistic).

The S/HAL population was tested initially with pathotype 200P–, and the data obtained fitted a model for the segregation of 3 independent genes (107 resistant : 12 susceptible, $\chi^2_{7:1} = 0.64$, $P > 0.50$). The low infection types associated with these genes varied from ;N, ;1N to 12+C. A satisfactory fit to a 2-gene model was obtained in the second test of the population with pathotype 210P+ (71 resistant : 33 susceptible, $\chi^2_{3:1} = 2.51$, $P > 0.20$). Given the pathogenic characteristics of pathotypes 200P– and 210P+, it was concluded that the genes *Rph2*, *Rph19* and a third uncharacterised gene were detected with pathotype 200P–, and that *Rph2* and the uncharacterised gene were detected with pathotype 210P+.

The S/HAL population was also tested with pathotype 5653P+, virulent on Halcyon but producing IT 2++3C to 3+C on Sloop (Table 1), to score this intermediate phenotype. The data obtained did not fit any genetic model (39 resistant : 76 susceptible), probably indicating that this phenotype was not scored reliably.

Mapping was conducted on rust data using single-point regression analysis as described by Read *et al.* (2003). Simple and composite interval mapping of the rust response

of this population identified 1 or 2 strong QTLs associated with resistance to leaf rust. On the basis of infection response to pathotype 200P–, 2 significant QTLs were identified. The first, contributed by Halcyon, was located near the centromere of chromosome 5H. The significant marker interval was *P14/M50-1–ABC302*, and the markers *P14/M62-3*, *CDO795-2*, *P13/M58-3*, and *Bmac96* had the maximum LRS of 24.3 and explained 23% of the phenotypic variance ($P_{0.05} = 11.9$, $P_{0.001} = 17.8$). This presumably corresponded to the *Rph2* locus (Fig. 6*a*). The second was contributed by Sloop and was located on the long arm of chromosome 7H. The significant marker interval for this QTL was *P13/M49-121–WG420*, and the marker *Bmac755* had the maximum LRS of 16.2 and explained 16% of the phenotypic variance ($P_{0.05} = 11.9$, $P_{0.001} = 17.8$). This was concluded to be *Rph19* (Fig. 6*b*).

The results of multi-pathotype tests of the parents Sloop and Halcyon suggested that only *Rph2* would be detected when the population was tested with pathotype 210P+, and although the chi-square test supported the segregation of 2 genes, only 1 major QTL on chromosome 5H was detected. The significant marker interval was *P11/M53-1–P11/M57*
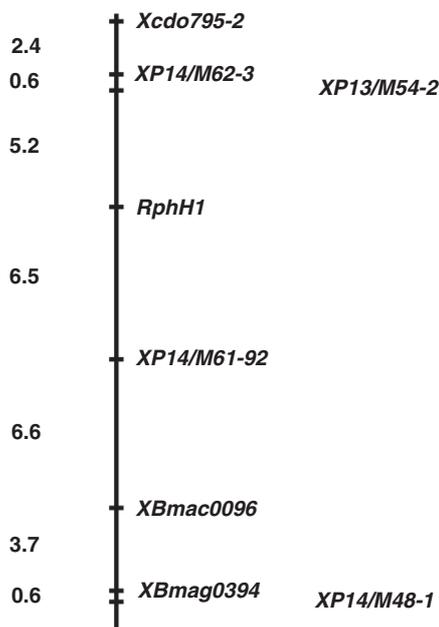
Fig. 7. Partial map of barley chromosome 5H based on a Sloop × Halcyon doubled haploid population showing the genetic location of a leaf rust resistance gene, *RphH1*, considered to be *Rph2*, along with map distances (cM).

BCD267 with a maximum LRS of 156.9, and explained 85% of the phenotypic variance ($P_{0.05} = 13.4$, $P_{0.001} = 20.8$). When mapped as a single trait, this resistance was flanked by the markers *XP13/M54-2* and *XP14/M61-92* at distances of 4.9 cM and 6.5 cM, respectively (Fig. 7).

Composite interval mapping using 'background loci' did not identify any other significant QTL in the S/HAL population. However, a significant interaction (LRS = 38.4) was observed between *XBmac96* on chromosome 5H and *Xbmac755* on 7H, associated with leaf rust resistance to pathotype 200P–. Overall, the LRS for the interaction between these 2 loci was 79.4 at the 10 e^{-6} level of significance, and LRS values for the main effect of both loci were 23.9 and 15.6, respectively. No significant interactions were found with pathotype 210P+.

Discussion

Preliminary multi-pathotype tests of 38 Australian barley cultivars by Cotterill *et al*. (1994) indicated that many had seedling resistance to *P. hordei*, and it was suggested that some cultivars possessed *Rph2*, *Rph4*, *Rph12*, or one or more uncharacterised resistance gene(s). In the present study, which included predominantly Australian barley cultivars, the genes *Rph2* (Halcyon, Kaputar), *Rph3* (Alexis), *Rph12* (Franklin, Tallon), and *Rph19* (Arapiles, Chebec, Sloop) were postulated on the basis of multi-pathotype testing, and results from the molecular mapping studies provided further support for these determinations. The mapping studies also revealed the possible presence of an uncharacterised seedling resistance in Kaputar (chromosome 2H). Although the mapping populations utilised in the present study were not initially generated to study leaf rust resistance, those that were polymorphic for resistance to this disease were extremely useful in determining chromosomal locations for the genes detected.

Multi-pathotype testing using well-characterised pathogen isolates permits resistance gene postulation and provides a sound basis for subsequent genetic analyses (Knott 1989). It is, however, restricted by the virulence combinations available. For example, mapping studies using the Patty × Tallon population implicated the presence of *Rph4* in Tallon. This could not be established by multi-pathotype tests because all available Australian pathotypes of *P. hordei* that are virulent for *Rph12* are also virulent for *Rph4* (R. F. Park, unpublished data). Similarly, the presence of resistance in Alexis that was highly effective to all pathotypes prevented any firm postulations regarding the identity of the resistance of this cultivar or the presence of any additional seedling resistance genes. The genetic analysis of the WI2875-1 × Alexis population, coupled with the QTL analysis, provided good evidence for the presence of *Rph3* in Alexis in combination with *Rph2* and *Rph12*, the latter being masked by *Rph3* in initial multi-pathotype tests.

Rph12 was mapped to the long arm of chromosome 5H by Borovkova *et al*. (1998) using both molecular and morphological markers, and the *Rph2* locus was mapped to the centromeric region of the same chromosome, where it showed close linkage with several markers on the short arm (Borovkova *et al*. 1997). Both locations were confirmed in several populations in the present study. The markers associated with the *Rph2* locus in the WI2875-1 × Alexis RIL population mapped to the centromeric region in a consensus map of this chromosome based on 6 doubled haploid populations (Karakousis *et al*. 2003*b*, this issue). Steffenson and Jin (1996) presented evidence for the presence of at least 3 alleles at the *Rph2* locus. On the basis of specificity to a wide array of Australian pathotypes of *P. hordei*, the alleles present in Halcyon and Kaputar appear to be the same, and show the same specificity as the allele present in the differential cultivar Peruvian (R.F. Park, unpublished data). A test of allelism between Kaputar and Peruvian using 200 F_2-derived F_3 families confirmed allelism or close repulsion linkage (H. Miah and R. F. Park, unpublished data).

Initial work conducted by Cotterill *et al*. (1994) indicated the presence of an uncharacterised resistance gene in the Australian cultivar Prior, which showed the same specificity as a resistance gene in the differential cultivar Reka 1. A test of allelism between Reka 1 and Prior indicated that these 2 genes were either the same or very closely linked (Park and Karakousis 2002). Subsequent multi-pathotype testing indicated that this gene was also present in cv. Chebec.
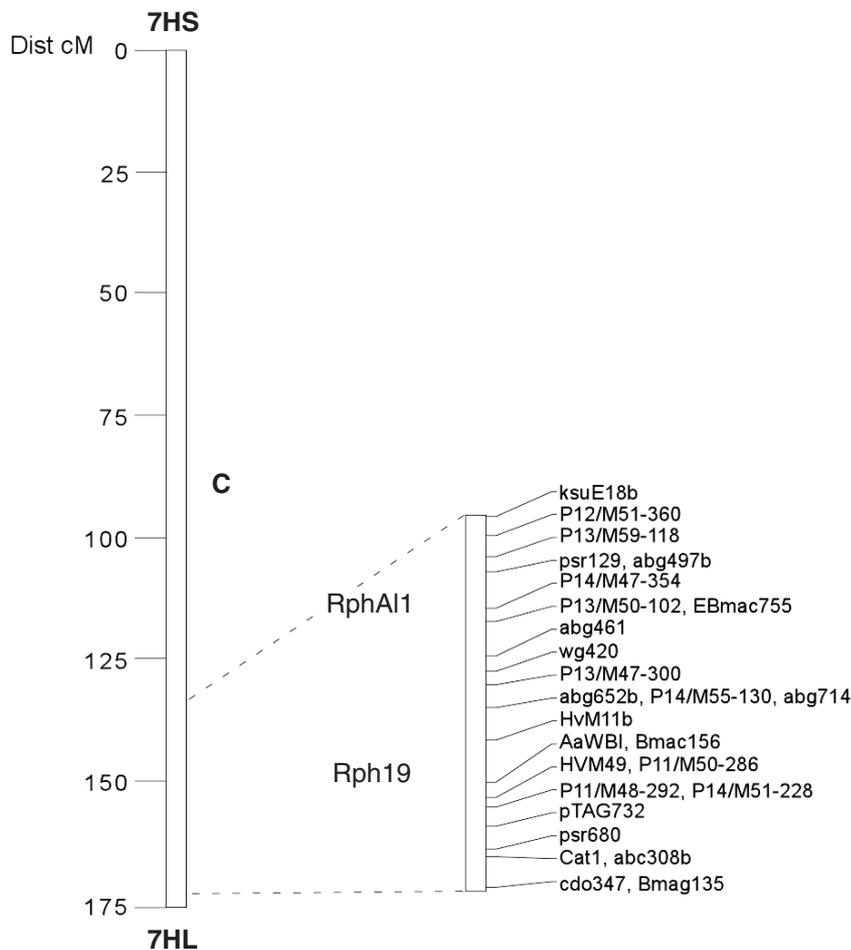
Fig. 8. Partial consensus map for chromosome 7H showing relative positions of *Rph19* and *RphA11* (regarded as *Rph3*), based on the rust responses of a doubled haploid population of Chebec × Harrington and a recombinant inbred population of WI2875-1 × Alexis, and marker data from 6 doubled haploid populations (see Karakousis *et al.* 2003). C, Centromere.

Mapping studies positioned the gene to the long arm of chromosome 7H and it was designated *Rph19* (Park and Karakousis 2002). On the basis of multi-pathotype tests, *Rph19* was also postulated to be present in Arapiles and Sloop. The results presented here for Sloop are consistent with this in showing that the gene present is also located on 7H (Fig. 6b). A linkage study of *Rph3* and *Rph19*, based on seedling tests of an intercross between Prior (*Rph19*) and Estate (*Rph3*), indicated that the 2 genes displayed linkage of 28 ± 4.3 cM (Park and Karakousis 2002). The association of *Rph3* and *Rph19* was also supported by the results obtained here for the WI2875-1 × Alexis RIL population (Fig. 1). A gene considered to be *Rph3* was flanked by 3 markers that also mapped to the region of *Rph19* in the Chebec × Harrington population (AT/CAA355, AG/CAT102, and AG/CAA300; Fig. 2 in Park and Karakousis 2002), and this gene mapped ~30 cM from *Rph19* in the Chebec × Harrington population (Fig. 8). Similarly, the marker *WG240*, located 13.2cM distal to *Rph19* in the

Chebec × Harrington population (Park and Karakousis 2002), mapped close to the QTL associated with *Rph3* in the WI2875-1 × Alexis RIL population (Fig. 2) and was a flanking marker of this locus in the Sloop × Halcyon population (Fig. 6).

Virulence to all of the genes identified in this study has been detected in Australian pathogenicity surveys of *P. hordei*, with the exception of *Rph3* (R. F. Park, unpublished data). *Rph3* has not been deployed in Australia to date. In New Zealand, virulence for *Rph3* was detected following the release of cv. Liberty, believed to possess this gene (Cromey and Villjanen-Rollinson 1995). Similarly, virulence for *Rph12* increased rapidly in Australian populations of *P. hordei* following the releases of several cultivars with this gene (Franklin, released in 1989; Tallon, in 1991; Lindwall, in 1997; Fitzgerald, in 1997; Gairdner, in 1997). The deployment of single seedling resistance genes should therefore be avoided. Resistances that are genetically more complex may be more durable than single-gene

seedling resistances. The effectiveness of seedling resistance genes *Rph3*, *Rph7*, *Rph14*, *Rph15*, and *Rph18* in Australia raises the possibility that 2 or more could be combined to provide greater durability, and molecular markers for these genes would be very useful for this purpose. Furthermore, it is possible that some of the parents used to produce the DH populations may have additional adult plant resistance. D. B. Moody *et al*. (unpublished data) found evidence of a QTL for adult plant resistance in the Tallon × Kaputar population. Further field testing of these populations at adult plant growth stages should be conducted to explore this possibility more fully.

Acknowledgments

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