

A comparison of the genetics of seedling and adult plant resistance to the spot form of net blotch (*Pyrenophora teres f. maculata*)

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Abstract. Spot form of net blotch (SFNB) (*Pyrenophora teres f. maculata*) is an economically damaging foliar disease of barley in many of the world's cereal-growing areas. The gene *Rpt4* that confers seedling resistance to SFNB has been mapped on the long arm of chromosome 7H, but no genes for adult plant resistance (APR) have been identified. A lack of field resistance to SFNB in breeders' lines selected for *Rpt4* led us to investigate the genetics of APR to this disease. Five doubled-haploid populations were phenotyped for seedling and adult plant reaction. Markers linked to *Rpt4* explained a large part of the seedling variation, but little of the APR. In 2 mapped populations, major quantitative trait loci (QTLs) for APR distal to *Rpt4* on chromosome 7H were identified. QTLs contributing to APR on chromosomes 4H or 5H were also identified in each population. Association of the 5H QTL with a gene for cereal cyst nematode resistance and the probable effect of this linkage on the historical development of cultivars with adult plant resistance to SFNB is discussed.

Additional keywords: *Hordeum vulgare*, disease resistance, APR, QTL mapping, genetics.

Introduction

The spot form of net blotch (SFNB), caused by the fungus *Pyrenophora teres* (Drechs.) forma *maculata* (Smedegaard-Petersen), is a foliar disease of barley found on crops grown in Europe, Canada, USA, South Africa, the Mediterranean region, and Australia (Tekauz and Buchannon 1977; Khan and Tekauz 1982; Karki and Sharp 1986; Louw *et al.* 1995). The spot form was differentiated from the net form of net blotch (*P. teres f. teres*) by Smedegaard-Petersen (1971) on the basis of leaf symptom type. SFNB has been responsible for severe disease outbreaks in Canada (Tekauz and Buchannon 1977) and in Australia (Poulsen *et al.* 1999; Wallwork 2000). In Western Australia, Khan and Tekauz (1982) estimated that SFNB caused losses in grain yield of up to 25%.

The use of resistant varieties is the most economic method of control of SFNB, although little is known about the genetics of adult plant resistance (APR) to the disease. The gene *Rpt4* that confers seedling resistance to SFNB has

been mapped on the long arm of chromosome 7H (Williams *et al.* 1999), and markers linked to a SFNB seedling resistance locus have been located on chromosome 2H by linkage with the *Vrs1* locus controlling spike type (Molnar *et al.* 2000).

In Australia, barley improvement programs have been breeding for resistance to SFNB but finding that there was a low recovery of progeny with APR equivalent to the donor parents. Marker-assisted selection (MAS) has been used extensively by those programs to rapidly introgress disease resistance genes into elite lines, and *Rpt4*, in particular, has been targeted using markers in several breeding programs. However, the phenotypic response to MAS for *Rpt4* has been disappointing, with the heritability of APR in *Rpt4*-positive material being relatively low. This has suggested that one or more unmapped genes, not detected in seedling assays of mapping populations, must contribute to APR in the field. This paper describes the mapping and validation of quantitative trait loci (QTLs) for adult plant resistance to

SFNB, the mapping of seedling resistance to the *Rpt4* locus in 4 additional genotypes, and an evaluation of the contribution of *Rpt4* to APR in 5 populations.

Materials and methods

Plant materials and disease screening

Galleon/Haruna Nijo and Chebec/Harrington doubled-haploid (DH) populations have previously been described and mapped (Karakousis *et al.* 2003, this issue). The DH populations Mundah/Keel and VB9104/Dash were produced by Phil Davies (SARDI), Tilga/Tantangara by Barbara George-Jaeggli (QDPI), and CI9214/Stirling by Sue Broughton (Agriculture WA). Segregating F₂ populations were generated from the crosses Dairokkaku/WI3141 and Tilga/Keel. Additionally, a subset of single seed descent F₇ lines derived from the BC₁ population Gairdner/Keel//Gairdner was generated and used for a graphical genotyping study. The pedigrees and SFNB seedling and adult plant reaction of the lines used in this study are presented in Table 1.

Seedling screening

Keel/Gairdner, Dairokkaku/WI3141, Tilga/Keel, Galleon/Haruna Nijo, and Chebec/Harrington populations were phenotyped for seedling resistance in controlled-environment rooms as described in Williams *et al.* (1999), with ratings of 1 and 9 indicating highly resistant and highly susceptible plants, respectively. Tilga/Tantangara, CI9214/Stirling, and VB9104/Dash were screened by inoculating plants at the 2-leaf stage with a suspension of field-collected conidia at 15000 conidia/mL. Conidia were suspended in water and applied using an airbrush at 200 mL/m². Plants were incubated for 24 h at 19°C with an initial period of 14 h dark, and then transferred to a growth chamber (Tilga/Tantangara) or the glasshouse for symptom development. The growth chamber was maintained at 24/14°C and 12-h photoperiod, and glasshouse temperatures ranged from 15 to 25°C. Notes were taken 10–12 days after inoculation using a scale of 1–9 where 9 was very susceptible.

Adult plant resistance

The Galleon/Haruna Nijo population was sown as paired rows in an irrigated disease nursery at Turrefield in South Australia (SA) in 2002. Two incompletely duplicated replicates were assessed after flowering using a 1–9 scale reflecting disease severity. The VB9104/Dash population was sown as a single replicate in the Turrefield nursery in 2001. This population was also affected by scald (*Rhynchosporium secalis*), which made assessment of some plots more difficult than others. The Mundah/Keel population was assessed in naturally infected 4-row, 5-m plots at Tuckey, SA, in 2000. Tilga/Tantangara, VB9104/Dash, and CI9214/Stirling were assessed in a field nursery at Warwick, Qld, where the epidemic was initiated by artificial inoculation of susceptible spreader rows and promoted with sprinkler irrigation.

Genetic mapping and graphical genotyping

Mapping of Galleon/Haruna Nijo and Chebec/Harrington is described elsewhere in this issue (Karakousis *et al.* 2003). For other populations, DNA extraction was achieved using a DNA mini-prep method adapted from Rogowsky *et al.* (1991). Variations to the method were as described below. For DNA extraction, 750 µL of extraction buffer and phenol-chloroform-isoamyl alcohol (25:24:1) were used. The extraction buffer was 0.1 M Tris-HCl (pH 8), 10 mM EDTA, 0.1 M NaCl, and 1% Sarkosyl. Deoxyribonucleic acid was precipitated by the addition of 0.1 vol. of 3 M sodium acetate (pH 4.8) and 1 vol. of propan-2-ol. Genotyping of DH lines and marker nomenclature used in

the VB9104/Dash population were the same as reported elsewhere in this issue (Cakir *et al.* 2003). The map of the VB9104/Dash population contained 192 markers in 9 linkage groups at the time of this analysis. Partial maps were constructed of Tilga/Tantangara, Keel/Gairdner, and CI9214/Stirling, by genotyping 3 simple sequence repeats (SSRs) with known linkage to *Rpt4*.

SSR markers developed and mapped by Ramsay *et al.* (2000) were amplified using a 'touchdown' profile (30 s at 94°C, 30 s annealing, 1 min at 72°C). The annealing temperature for the first cycle was 65°C, reducing by 1°C per cycle for the next 10 cycles. The remaining 24 cycles were with annealing at 56°C, in 10-µL reaction mixes containing 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.0 mM dNTP, 1.875 mM MgCl₂, 15 ng of each primer, 0.5 U of *Taq* DNA polymerase (Life Technologies), and 16 ng template DNA. Polymerase chain reactions (PCRs) were performed in a PTC-225 (MJ Research) thermocycler. SSRs were separated on 1-mm-thick 6% polyacrylamide gels at constant 300 V for 3 h. Gels were stained with ethidium bromide and visualised under UV light before photography.

Analysis of SFNB data by single-point regression analysis, simple interval mapping, composite interval mapping, and 2-locus interaction analysis was conducted using Map Manager QTX (Manly *et al.* 2001). A permutation test is used to establish the significance of the likelihood ratio statistics (LRSs) generated by the interval mapping procedures. The 'suggestive', 'significant', and 'highly significant' threshold values of the permutation test correspond to the 37th, 95th, and 99.9th percentiles, respectively (Manly *et al.* 2001). Bootstrap re-sampling was used to estimate a confidence interval for QTLs. Interval mapping was performed on multiple re-sampled datasets and the positions of maximum LRSs plotted as a histogram overlaying the interval mapping figure (Manly *et al.* 2001).

Graphical genotyping was conducted on lines developed from a backcrossing programme using BC₁ families from the recurrent parent Gairdner and the donor parent Keel. MAS was used to select for the markers linked to cereal cyst nematode (CCN locus *Ha4*) and seedling resistance to spot form net blotch (SFNB locus *Rpt4*) from Keel, and key traits in the Gairdner recurrent parent were also selected by association with linked markers, namely, malt extract on 5H and 2H, BYDV resistance on 3H, and β-amylase isoform on 4H (see Fig. 3). In addition, phenotypic selection was applied for APR to SFNB (from Keel) and 'Gairdner type'. Forty-seven SSRs (Ramsay *et al.* 2000) distributed across the genome were used to genotype elite lines that had survived selection through to F₇.

Results

Seedling resistance

Seedling and APR resistance scores for the parents of the populations used for this study are reported in Table 1.

Five DH populations from crosses between SFNB-resistant and susceptible lines were phenotyped using seedling bioassays (Table 2). Previously published data for the Galleon/Haruna Nijo population (Williams *et al.* 1999) are included for comparison. All populations except Galleon/Haruna Nijo and VB9104/Dash appeared to segregate for a single gene for seedling SFNB resistance ($P > 0.05$). Each of these populations was tested with molecular markers to determine if the resistance matched the known location of *Rpt4* on chromosome 7H.

The resistance in the CI9214/Stirling, Keel/Gairdner, and Tilga/Tantangara populations was observed to align with markers known to be linked to the *Rpt4* locus on the long arm

Table 1. Lines used in this study, their pedigrees, and seedling and field reaction to *P. teres f. maculata*
1, most resistant; 9, most susceptible

Line	Pedigree	Seedling reaction	Field reaction ^A
Chebec	Orge_Martin/Clipper//Clipper/3/Schooner	3.5	6
CI9214	Korean land race	1.5	3
Dairokkaku	Japanese land race	2.0	4
Dash	Chad/Joline//Cask	7.0	9
Gairdner	Onslow/Tas83-587	7.0	8
Galleon	Clipper/Hiproly//3*Proctor/ CI3576	2.7	3
Harrington	Klages/3/Gazelle/Betzes// Centennial	7.0	8
Haruna Nijo	Satsuko Nijo/(K-3/G-65)	7.0	8
Keel	CPI18197/Clipper//WI2645	2.0	2
Stirling	Dampier//(A14)Prior/Ymer/3/Pirolina	6.0	7
Tantangara	AB6/Skiff	9.0	7
Tilga	Forrest/Cantala	1.5	4
VB9104	Europa/IBON#7.148	3.0	4
WI3141	CCN4-10*WI2875-3-1D-17	1.0	7

^AApproximate APR score, based on separate trials.

of chromosome 7H (Table 2). For the Chebec/Harrington DH population, the seedling SFNB reaction data were mapped with respect to 340 markers on its genetic map, with a major QTL also mapping to chromosome 7H at approximately the same location as *Rpt4* (Table 2). Markers linked to *Rpt4* explained 27–74% of the variance for seedling SFNB reaction in these populations (Table 2). The *Rpt4*-linked marker *Bmag120* showed no association with seedling SFNB reaction scored independently by two groups at Turretfield in 2000 and Hermitage Research Station in 2001 in the VB9104/Dash population, although mapping of seedling resistance scores with respect to 192 molecular markers showed that distal markers on the long arm of chromosome 7H explained 9% of the observed variation (Table 2). Weaker QTLs for seedling resistance were also observed on chromosomes 1H and 3H in the VB9104/Dash population (not shown).

In order to examine the allelic relationships between *Rpt4* and the seedling resistance expressed in Tilga and Dairokkaku, 100 F₂ plants from each of the crosses

Dairokkaku/WI3141(*Rpt4*+) and Tilga/Keel(*Rpt4*+) were phenotyped for seedling SFNB reaction. No susceptible individuals were observed from either cross (data not shown).

Adult plant resistance

Five DH populations were phenotyped for SFNB APR in field plots. The relationship between seedling and APR was determined for each cross (Table 3). The slope of the linear regression between seedling and APR scores ranged from 0.39 in Galleon/Haruna Nijo DHs to 0.73 in the Keel/Gairdner single-seed descent lines.

A genetic map of the Galleon/Haruna Nijo cross was used to identify QTLs contributing to APR to SFNB. QTLs were identified on chromosomes 5H and 7H by simple interval mapping (Table 3). Bootstrap analysis showed that whereas the 5H QTLs had a clearly defined confidence interval within 12 cM (not shown), the significant 7H QTLs were distributed along the long arm and many marker loci along the short arm of this chromosome also had a suggested

Table 2. Quantitative trait loci for seedling resistance to spot form of net blotch

Population	Segregation (Res : Sus)	χ^2 ^A	Map location ^B	Support interval	LRS ^C (SIM)	R ² ^D
Galleon/Haruna Nijo	34:58	6.2	7H: 117–141 cM	AWBMA11–PSR129	98**	65
CI9214/Stirling	55:43	1.46†	7H: 112–145 cM	AWBMS22–EBmac755	70.4**	52
Keel/Gairdner	32:35	0.13†	7H: 100–145 cM	Bmag120–EBmac755	36.6**	46
Chebec/Harrington	48:64	2.28†	7H: 115–125 M	PSR637b–P11M48-384	152**	74
Tilga/Tantangara	55:57	0.03†	7H: 100–112 cM	AWBMS22–Bmag120	24.4**	27
VB9104/Dash	8:173		7H: 145–170 cM	P13M47V300–Bmag135	16.1*	9

*Significant ($P = 0.05$); **highly significant ($P = 0.01$) (Manly *et al.* 2001).

^AResults of Chi-square analysis; † segregation ratio does not differ from 1:1 expected for single gene in DH population ($P > 0.05$).

^BApproximate position on consensus map (Karakousis *et al.* 2003, this issue).

^CLikelihood ratio statistic for the association of the trait with this locus, as determined by simple interval mapping.

^DThe amount of the total trait variance that would be explained by a QTL at this locus, as %.

Table 3. Quantitative trait loci for adult plant resistance to spot form of net blotch

Population (and seedling/APR ^A)	Map location ^B	Support interval	LRS (SIM) ^C	R ² ^D	LRS (CIM) ^E	R ²
Galleon/Haruna Nijo (0.39)	7H:					
	153–162 cM	PSR547–ABC321	13.9*	18	12.9†	13
	125–135 cM	ABG652b–AT/CCA 228	15.4*	20	14.7†	15
	167–172 cM	AaWBI–Rlch4B	19.7**	27	15.2*	17
	5H: 30–32 cM	ABC254–Exo2a	15.3*	19	9.6†	10
	4H: 70–73 cM	WG114–BCD1130	0	0	7.0†	8
VB9104/Dash ^F (0.67)	7H: 145–170 cM	P13M47V300–Bmag135	33.7**	17	37.6**	17
			<i>26.8**</i>	<i>14</i>	<i>28.1**</i>	<i>13</i>
	5H: 20–40 cM	P12M47D63–Bmag337	0.2	0	18.8**	9
			<i>1.0</i>	<i>1</i>	<i>0.2</i>	<i>0</i>
	4H: 65–70 cM	HVML03–P12M62V260	14.6*	8	17.2**	8
			<i>18.7**</i>	<i>10</i>	<i>21.7**</i>	<i>10</i>
CI9214/Stirling (0.70)	7H: 112–145 cM	AWBMS22–EBmac755	12.0*	35		
Keel/Gairdner (0.73)	7H: 112–145 cM	AWBMS22–EBmac755	18.5**	32		
Tilga/Tantangara (0.67)	7H: 100–112 cM	Bmag120–AWBMS22	5.2†	7		

^ASlope of the linear regression line through seedling and APR scores for each population.

^BApproximate position on consensus map (Karakousis *et al.* 2003, this issue).

^CLikelihood ratio statistic for the association of the trait with this locus, as determined by simple interval mapping.; † suggestive ($P = 0.63$); *significant ($P = 0.05$); **highly significant ($P = 0.01$) (Manly *et al.* 2001).

^DThe amount of the total trait variance that would be explained by a QTL at this locus, as %.

^ELikelihood ratio statistic for the association of the trait with this locus, as determined by composite interval mapping (Manly *et al.* 2001).

^FResults in plain font are from the Turretfield 2000 trial, whereas those in italics are for the same population scored at Hermitage Research Station in 2001.

($P = 0.63$) contribution to SFNB APR (Fig. 1). Composite interval mapping (CIM) confirmed the significance of the 5H and 7H QTLs, as well as revealing a QTL of minor effect on chromosome 4H. A 2-way interaction analysis at high stringency ($P = 1.0e-6$) revealed a positive interaction (LRS = 35.6) between the most significant QTL on chromosome 7H and AWBMA28, the most distal marker of the short arm of chromosome 2H (not shown). To determine the effect of the APR QTL on chromosomes 5H and 7H identified in the Galleon/Haruna Nijo population, APR scores for DHs with both, either, or neither QTL were graphed. The QTLs provided greatest resistance when combined, suggesting that the 2 QTLs are additive in effect (Fig. 2).

Separate QTL analysis of the Turretfield and Hermitage field data for the VB9104/Dash population revealed a QTL with significant effect on APR on chromosome 7H of VB9104. This QTL was 20 cM distal to *Rpt4*, but was at approximately the same location as the APR QTL from Galleon (Table 3). Another significant QTL for expression of APR at both sites was detected on chromosome 4H of VB9104. CIM confirmed the significance of the 4H and 7H QTLs. Additionally, CIM of the Turretfield data revealed a QTL of minor effect on chromosome 5H, close to the APR QTL identified in Galleon (Table 3).

A further 3 populations were phenotyped for adult plant resistance, but were not fully mapped. Markers genotyped near the *Rpt4* locus in DHs from CI9214/Stirling,

Keel/Gairdner, and Tilga/Tantangara explained less of the phenotypic variation for APR than for seedling resistance in these populations (Table 3).

SSR genotyping of 22 elite F₇ lines from the Gairdner/Keel//Gairdner backcross population shows that, on average, 70% of alleles came from Gairdner, compared with the expectation, if no selection applied, of 75% (Fig. 3). Keel introgression segments are clearly visible around the chromosome 5H and 7H regions for CCN and SFNB resistance, respectively, with other segments on 1H, 2H, and 4H from Keel also common. Large segments of Keel alleles on chromosome 7H were also evident in some lines (Fig. 3).

Discussion

Using a combination of candidate markers and F₂ crosses, we have shown that the 5 barley lines CI9214, Keel, Chebec, Diariokkaku, and Tilga have SFNB seedling resistance at or near *Rpt4*, the gene originally identified in the cultivar Galleon (Williams *et al.* 1999) (Table 2). Each of these genotypes has quite different recent pedigrees, with apparently diverse sources of SFNB resistance. This suggests that to date, only one locus for seedling resistance to SFNB has been definitively mapped. Another locus has been tentatively mapped on chromosome 2H through association of seedling resistance-linked RAPD markers with the *Vrs1* locus for spike type (Molnar *et al.* 2000). All other references to net blotch resistance appear to relate to the net form of net blotch caused by *P. teres f. teres*. In

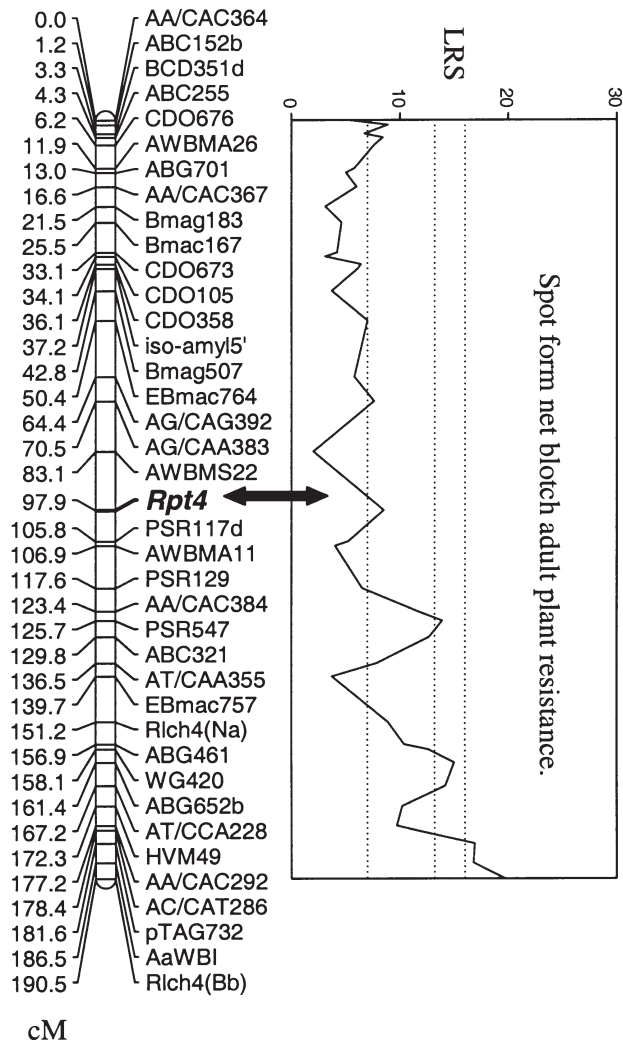


Fig. 1. Interval map of QTLs for adult plant resistance to spot form of net blotch on chromosome 7H from the Galleon/Haruna Nijo cross, compared with position of the seedling resistance gene *Rpt4* on the genetic map (arrow). Vertical dotted lines indicate suggestive (9, $P = 0.63$), significant (12, $P = 0.05$), and highly significant (16, $P = 0.001$) thresholds for the likelihood ratio statistic (LRS; Manly *et al.* 2001) trace.

Australia, only one major pathotype of *P. teres* f. *maculata* has been found, so it cannot be determined if different alleles of *Rpt4* exist.

Following marker-assisted selection of breeding lines for *Rpt4*, it was observed that field levels of resistance to SFNB were lower than expected. It was hypothesised that *Rpt4* alone was not providing adequate APR and that supplementary genes were contributing to the higher levels of APR observed in some genotypes. Five DH populations were phenotyped for adult plant, as well as seedling, resistance to SFNB. Regression analysis showed that seedling resistance and APR were not highly correlated. In addition, QTL analysis showed that although *Rpt4*

contributed to APR, it had much less effect on disease suppression at this stage of plant growth than in seedlings.

When field resistance data were used for QTL analysis in the mapped Galleon/Haruna Nijo population, 3 closely linked QTLs with significant effects were observed on chromosome 7H and were derived from Galleon. None of the QTLs was at the same location as *Rpt4*, with the closest being 20 cM distal (Fig. 1). In addition to the significant QTLs on the long arm of chromosome 7H, the analysis suggested that loci distributed along the short arm of this chromosome were also having an effect on SFNB resistance. Another significant QTL for APR was detected on chromosome 5H in the Galleon/Haruna Nijo cross. This QTL is near the *Ha4* gene for cereal cyst nematode (Barr *et al.* 1998). Many feed barley varieties bred for southern Australia descend from the Egyptian landrace CI3576. It is a carrier of the *Ha4* allele, and the feed varieties Galleon and Barque were bred to carry CCN resistance by selecting for *Ha4*. Keel was derived from a separate introduction from Egypt and has subsequently also been found to carry the *Ha4* allele for CCN resistance (K. J. Williams, unpublished work). All of these lines have excellent APR to SFNB and hence the finding that SFNB and CCN are linked fits well with breeders' experiences. Interestingly, Chebec derives its CCN resistance from the *Ha2* gene on chromosome 2H (Kretschmer *et al.* 1997) and is susceptible as an adult plant, although it has seedling resistance, in common with Schooner, one of its parents.

When seedling and field data were used for QTL analysis in the mapped VB9104/Dash population, a QTL with statistically significant effects on both seedling and APR was observed on chromosome 7H of VB9104. This QTL was 20 cM distal to the location of *Rpt4*, but was at approximately the same location as the APR QTL of Galleon. This raises the possibility that a locus closely linked to the *Rpt4* seedling resistance gene contributes to APR, rather than *Rpt4* itself.

Progeny of the VB9104/Dash cross exhibited a relatively low level of seedling resistance, with the strongest QTL mapping close to the strong APR QTL. It is likely that VB9104 does not contain *Rpt4*, but has several weak QTLs, which, when combined, provide some seedling resistance. A significant QTL for APR was detected on the long arm of chromosome 4H of VB9104, linked to the *mlo*-derived SSR marker HVMLO3. Intriguingly, the 4H QTL mapped in VB9104 and the 5H QTL mapped in Galleon were observed only in Galleon and VB9104, respectively, when composite interval mapping was performed to counteract the effect of the major QTL on chromosome 7H. This indicates that up to 3 loci may be significantly associated with high levels of APR to SFNB. The data are also consistent with the Tilga/Tantangara study conducted by G. J. Platz and D. Poulsen (unpublished data), in which the APR/susceptible segregation ratio indicated the action of 3 independent genes.

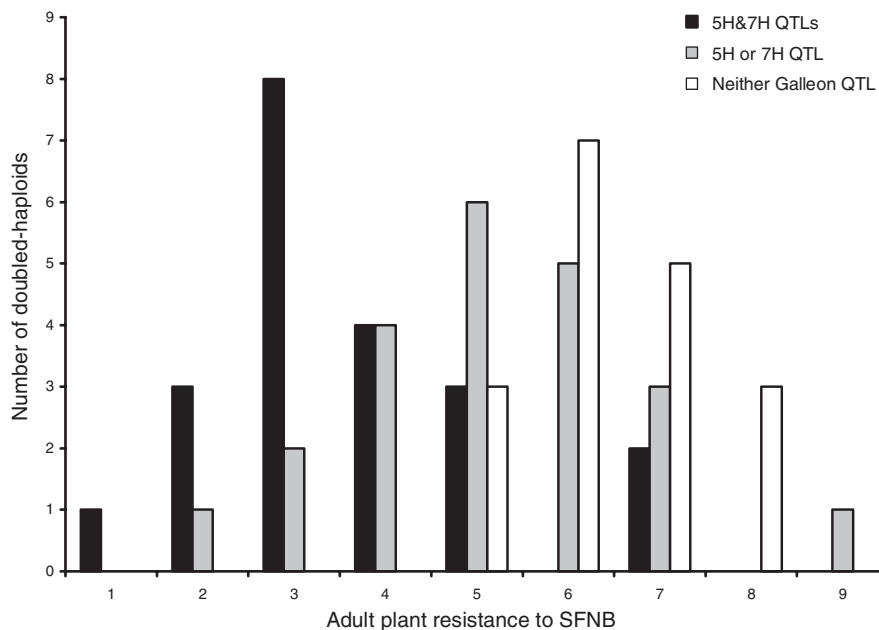


Fig. 2. Histogram of adult plant reaction to spot form of net blotch of Galleon/Haruna Nijo doubled-haploids with neither, one, or both resistance-associated QTL alleles identified in this study.

In an independent study, segregation analysis of the seedling and APR scores from Tilga/Tantangara also indicated the presence of a single gene conferring seedling resistance (G.J. Platz and D. Poulsen, unpublished data). It was observed that, whereas not all plants expressing seedling resistance demonstrated APR, all plants with APR had expressed seedling resistance. As seedling resistance in Tilga maps to the 7H region in the vicinity of *Rpt4*, the data are supportive of the conclusion that a locus in that region influences APR expression.

Plant growth characteristics can have a confounding effect on studies of APR. Kircherer *et al.* (2000) found that a QTL for leaf rust on chromosome 2H co-located with a QTL for heading date, and Spaner *et al.* (1998) found that a QTL for resistance to stem rust, scald, and net blotch on chromosome 4H was also close to genes for time of heading and maturity. The SFNB APR QTLs on 4H and 5H identified in this study map ~20 cM and 2 cM, respectively, from the peaks of 2 major QTLs for basic vegetative period, photoperiod response, and awn appearance on chromosomes 4H and 5H in the Galleon/Huruna Nijo population (Boyd *et al.* 2003, this issue). However, as there appears to have been no direct effects of maturity on disease scores in this population ($R^2 = 0.04$), any association between resistance and these genes is likely to be incidental.

Graphical genotyping of Gairdner/Keel//Gairdner backcross lines was used as an alternative method of determining other regions of the genome with an effect on

SFNB APR. The effect of phenotypic selection for SFNB on the distribution of Keel alleles observed in the graphical genotyping study is speculative, due to the small number of marker alleles determined, but when combined with the results from the mapping and candidate marker studies reported in earlier sections, it seems likely that several regions of 7H, 5H (near *Ha4*), and possibly 1H, 2H, or 4H are involved. These may confer APR to SFNB. Chromosomes 2H, 3H, and 6H carry predominantly Gairdner-type alleles and Gairdner-type alleles are frequent around the regions where MAS was applied for Gairdner quality alleles.

In summary, a lack of field resistance to SFNB in breeders' lines selected for *Rpt4* led us to investigate the genetics of APR to this disease. Five populations were phenotyped for seedling and adult plant reaction. Markers linked to *Rpt4* explained a large part of the seedling variation, but little of the APR. In 2 mapped populations, major QTLs for APR distal to *Rpt4* on chromosome 7H were identified. APR QTLs on 4H or 5H were also identified in each population. A small graphical genotyping study, coupled with breeders' experiences in variety development, supports these findings. Although a search for new seedling resistance loci has revealed that those lines with highly heritable seedling resistance all contain *Rpt4*, we now have identified some possible QTLs for APR that can be tested following marker-assisted selection in breeding for this important disease.

Chromosome	Primer	cM	W13584	W13585	W13586	W13587	W13588	W13599	W13600	W13601	W13602	W13603	BX98A:061D/8	BX98A:061D/12	BX98A:061D/13	BX98A:061D/29	Keel alleles	Gairdner alleles
1H	EBMAC501	37	B	A	A	B	B	B	n/r	B	B	A	B	B	A	A		
	BMAC213	52	A	A/B	A	A	A	A	B	A/B	A	A	A	A	B	A		
	BMAC032	97.3	A	A	A	A	A	A	A	A	A	A	A	A	A	A		
2H	BMAC134	4	B	B	A	B	B	A/B	A	A	B	A	B	A	A	A/B		
	BMAG381	71	A	A	A	A	B	B	A	A	A	A	A	A	A	A		Malt extract
	BMAG378	72	A	A/B	A	n/r	B	A	A	A	n/r	A	A	A	A	A		
	BMAG140	78	A	A/B	A	A/B	B	A	A	A	A	A	A	A	A	A		
	AWBMS56	85	A	A	A	A/B	B	B	A	A	A	A	A	A	A	A		
	BMAG125	89.9	A	A	A	B	A/B	B	A	A	n/r	A	n/r	B	A	A		
	BMAG114	90	A	A	n/r	A	A	A	A	A	B	A	B	B	A	A		
3H	BMAC209	31	A	A/B	A	A	A	A	A/B	A/B	A	A/B	A	A	A	B		
	BMAG006	45	A	A	A	A	A	A	A/B	A	A	A	A	A	A	B		
	BMAG603	45	A	A	A	A	A	A	A	A	A	A	A	A	A	B		
	BMAG112	56	A	A	A	A	A	A	A	A	A	NP	A	A	A	A		Yd2
	HVM060	76	A	A	A	A	A	A	A	A	A	A	A	A	A	A/B		
	BMAG122	90	A	A	A	A	A	A	A/B	A/B	A	A	A	A	A	B		
	EBMAC871	90	A	A/B	A	A	A	A	A/B	A/B	A	A/B	A	A	A	B		
	EBMAC708	115	A	A	A	A	A	A	A	A	A	A	A	A	A	A		
4H	HVM62	115	A	A/B	A	A	A	A	A	A	A	A	A	A	A	A/B		
	GMS089	23	A	A	A	A	A	A	A	A	A	A	B	B	n/r	B		
	BMAC310	29	A	A/B	B	B	B	B	A	A	B	B	A	A	A	B		
	BMAG353	30	B	A/B	B	B	B	B	A	A	B	B	B	B	B	B		
	BMAG384	39	A	A	A	A	A	A	A	A	A	A	B	B	B	B		
	HML0H1A	70	A	A/B	B	A	A	A/B	A	A	A	B	A	A	B	A		
	BMAG419	86	A	B	NP	A	NP	A	A	A	A	NP	A	A	B	A		
5H	BMY1	102	A	A/B	A	A	A	A	A/B	B	A	A	A	A	A/B	B		Bamy1
	BMAC113	29	A	B	B	A	A	A	A	A	A	A	A	A	A	B		
	BMAG387	29.5	A	B	B	A	A	A	A	A	A	A	A	A	A	B		
	BMAC096	33	A	B	B	A	A	A	A	A	A	A	A	A	A	B		
	GMS061	33	A	B	A	A	A	A	B	A	B	A	A	A	A	B		
	BMAG222	58	A	B	n/r	B	A	A	B	B	A	A	A	A	A	B		CCN Ha4
	HVLEU	79	A	B	B	A	A	A	A	A	B	B	B	A	A	A/B		
6H	GMS01	105	A	A	n/r	n/r	A	A	n/r	A/B	n/r	n/r	A	A	n/r	B		Malt extract
	BMAC316	8	A	B	n/r	B	A	B	A	A	n/r	A/B	A	A	B	A		
	BMAG173	73	A	A	A	A	A	A	B	A	A	A	A	B	B	B		
	BMAC040	145	n/r	A	A	A	A	A	A	A	A	A	A	A	A	B		
7H	BMAG206	16	A	B	B	A	B	B	B	A	B	A	B	A	A	A		
	BMAC167	65	B	*	A	B	B	B	B	A	B	A	B	B	A	A		
	EBMAC827	65	B	A	A	B	B	B	n/r	B	B	A	B	B	A	A		
	BMAG321	70	B	A/B	A	B	B	B	B	B	B	A	B	B	A	A		
	BMAG507	74	B	A	A	B	B	A	B	B	B	A	B	B	A	A		
	BMAG217	79	B	A	A	B	B	B	B	B	B	A	B	B	A	A		
	HVSS1	79	B	B	A	B	A/B	A/B	A	A	B	B	B	B	B	A		
	AWBMS37	80	B	A	A	B	B	B	B	B	B	A	B	B	A	A		
	HVCMA	85	B	B	A/B	B	B	B	A	A	B	B	B	B	B	A		
	BMAG 120	100	A	B	B	NP	B	B	n/r	B	A	B	A	A	n/r	NP		SFNB Rpt4
	BMAG135	170	A/B	B	B	n/r	A	n/r	B	B	n/r	B	n/r	n/r	n/r	n/r		
SFNB APR			R	R	R	MR	R	R	R	R	MR	R	MR	MR	MS	MS		
%Gairdner			7	62	78	63	60	65	73	76	64	80	67	65	79	56		

Fig. 3. Graphical genotypes of single-seed descent F₇ lines derived from the BC₁ population Gairdner/Keel//Gairdner selected for CCN locus *Ha4* and SFNB locus *Rpt4* from Keel, and malt extract on 5H and 2H, BYDV resistance on 3H, and β-amylase isoform on 4H from Gairdner, APR to SFNB from Keel, and ‘Gairdner type’. Forty-seven SSRs (Ramsay *et al.* 2000) distributed across the genome were used to genotype elite lines that had survived selection through to F₇. Key: A, Gairdner; B, Keel; A/B, heterozygous allele of Gairdner/Keel; NP, non-parental allele; n/r, no result.

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