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Genetic mapping and QTL analysis of disease resistance traits in the barley population Baudin × AC Metcalfe

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Abstract. A genetic map of barley (*Hordeum vulgare* L.) with 163 amplified fragment length polymorphism and 69 simple sequence repeat (SSR) markers was constructed using a population of 178 doubled haploid lines from a cross between the varieties 'Baudin' and 'AC Metcalfe'. Linkage groups were assigned to barley chromosomes using published map locations of SSR markers as reference points. The total length of the map was 1307.2 cM with an average interval length of 5.6 cM between markers. The genetic map was used to locate quantitative trait loci (QTLs) for several disease resistance traits. The population was tested for spot type net blotch, caused by *Pyrenophora teres* f. *maculata*, and net type net blotch, caused by *Pyrenophora teres* f. *teres*, in greenhouse experiments and in a range of field environments in Western Australia and Queensland. The response of the lines to leaf rust (caused by *Puccinia hordei* L.) at adult plant growth stages was evaluated in Western Australia. Significant marker–trait associations were found for seedling resistance and symptom severity in adult plants to these diseases. A new locus conferring resistance to *P. teres* f. *maculata* at both seedling and adult plant stages was detected on the short arm of chromosome 6H. From the seedling testing against *P. teres* f. *teres*, five highly repeatable QTLs were detected, on chromosomes 2HS, 2HL, 3HS, 4HL, and 6HS. For leaf rust, one highly significant QTL was detected on chromosome 2HL. The markers within these QTL regions present an opportunity for marker-assisted selection for these traits in barley-breeding programs.

Additional keywords: AFLP, barley, leaf rust, net type net blotch, spot type net blotch, SSR.

Introduction

Net type net blotch (NTNB), caused by *Pyrenophora teres* f. *teres*, and spot type net blotch (STNB), caused by *P. teres* f. *maculata*, cause substantial yield losses in barley-growing regions in Australia and worldwide (Khan 1989; Steffenson *et al*. 1991; Mathre 1997; Park *et al*. 2003; Murray and Brennan 2010). Leaf rust caused by *Puccinia hordei* L. occurs in all barley-growing regions of Australia (Park *et al*. 2003). Combined yield losses due to NTNB, STNB, and leaf rust across barley-growing regions of Australia have been estimated to range from 4.5% (southern region) to 13.6% (northern region), with an average annual cost of AU$83 million to Australian farmers, with STNB causing the highest yield losses nationally (Murray and Brennan 2010). One of the aims of barley-breeding programs is therefore to develop new barley varieties with resistance to multiple diseases including NTNB, STNB, and leaf rust.

Molecular markers can be used to genetically map loci that affect both simple and complex traits and for marker-assisted plant breeding (Varshney and Tuberosa 2007). For barley (*Hordeum vulgare* L.), many genetic maps are available (e.g. Karakousis *et al*. 2003; Wenzl *et al*. 2006; Hearnden *et al*. 2007; Stein *et al*. 2007; Close *et al*. 2009), providing

Abbreviations: AFLP, Amplified fragment length polymorphism; DH, doubled haploid; LOD, likelihood-of-odds; MAS, marker-assisted selection; NTNB, net type net blotch; SSR, simple sequence repeat; STNB, spot type net blotch; QTL, quantitative trait locus.

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chromosome locations for large numbers of restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR), diversity array technology (DArT), and single nucleotide polymorphism (SNP) markers. Numerous studies have been conducted in barley to map loci affecting economically important traits (e.g. Thomas *et al*. 1995; Zhu *et al*. 1998; Marquez-Cedillo *et al*. 2000; Cakir *et al*. 2003*a*; Li *et al*. 2003) and to explore opportunities for pyramiding resistance genes from different sources to achieve durable resistance against one or more diseases (Ayliffe *et al*. 2008).

Molecular genetic studies for the characterisation of resistance against NTNB (Cakir *et al*. 2003*b*; Raman *et al*. 2003; Ma *et al*. 2004; Friesen *et al*. 2006; Manninen *et al*. 2006; Lehmensiek *et al*. 2007; Gupta *et al*. 2010; St. Pierre *et al*. 2010) have reported loci on all of the chromosomes of barley, with a locus on chromosome 6H detected in many genetic backgrounds. Resistance genes expressed at both seedling and adult plant stages have been identified by several research groups in Australia. Both seedling and adult plant resistance genes were found on chromosome 6HS, and additional seedling resistance genes on chromosomes 2H and 3H in a population derived from a cross of Tallon/Kaputar (Cakir *et al*. 2003*b*). Raman *et al*. (2003) detected seedling resistance genes on chromosomes 2H and 3H across three barley populations: Alexis/Sloop, WI2875-1/Alexis, and Arapiles/Franklin. Genetic analysis of adult plant NTNB resistance genes in the same populations (Lehmensiek *et al*. 2007) mapped quantitative trait loci (QTLs) on chromosomes 2H, 3H, 4H, and 7H in the Alexis/Sloop and WI2875-1/Alexis populations, and on chromosomes 1H, 2H, and 7H in the Arapiles/Franklin population.

Resistance to STNB is not as well understood as that to NTNB. Loci conferring seedling resistance have been mapped on chromosomes 2H (Molnar *et al*. 2000), 4H (Friesen *et al*. 2006), and 7H (Williams *et al*. 2003). For adult plant resistance, Williams *et al*. (2003) reported loci on chromosomes 4H, 5H, and 7H from multiple backgrounds, with the 7HL QTL distally located to *Rpt4*. In a more recent study, Grewal *et al*. (2008) detected a minor QTL on chromosome 6H for STNB in the Canadian barley line TR251.

At least 19 loci conferring resistance to leaf rust at the seedling and adult plant stages have been reported (Weerasena *et al*. 2004), several of which are tagged by molecular markers (Borovkova *et al*. 1998; Park *et al*. 2003; Golegaonkar *et al*. 2009). QTLs for leaf rust resistance have been mapped by von Korff *et al*. (2005) on chromosomes 2H, 3H, 4H, 5H, and 7H and by Marcel *et al*. (2007) on all seven chromosomes. More recently, Golegaonkar *et al*. (2009) identified an SSR marker that is closely linked with the *Rph14* seedling resistance locus.

The aim of this research was to genetically map seedling and adult plant disease resistance loci for NTNB, STNB, and adult plant leaf rust resistance in a doubled haploid (DH) population derived from a cross between the barley varieties ‘Baudin’ and ‘AC Metcalfe’ and to identify closely linked molecular markers suitable for use in breeding programs.

Materials and methods

Plant material

A cross was made between the Australian breeding line WABAR2080 (later released as Baudin) and the Canadian breeding line TR232 (later released as AC Metcalfe). A population of 178 DH lines was generated from the F_1 generation of this cross, using anther culture as described by Broughton and Priest (1997). Baudin (pedigree: Stirling/Franklin) is a two-rowed malting barley variety developed by the Western Australian Department of Agriculture and Food and released in 2002. It is a semi-dwarf variety with adaptation to the medium and high rainfall barley-growing regions of Australia and has excellent malting quality. AC Metcalfe (pedigree: AC Oxbow/Manley) is an important Canadian malting barley variety developed at the Agriculture and Agri-Food Canada Experimental Station in Brandon, Manitoba, and registered in 1994.

Greenhouse and field trials for disease assessment

The DH population, parental lines, and local check varieties were evaluated in replicated trials in greenhouses and in field environments in Western Australia and Queensland (Table 1).

Table 1. Disease screening experiments conducted, variables assessed, and pathotypes used for phenotyping of the Baudin/AC Metcalfe parental lines and the population in the greenhouse and field from 2002 to 2004

Location	Year	Environment	Variable assessed	Pathotype used for inoculation
		Net type net blotch		
Warwick, Qld	2002	Glasshouse	Infection type on seedlings	NB50
	2003	Glasshouse	Infection type on seedlings	NB50
	2002	Field	Symptom severity on adult plants	NB324
	2003	Field	Symptom severity on adult plants	NB329
		Spot type net blotch		
Perth, WA	2004	Glasshouse	Infection type on seedlings	95NB104
	2004	Glasshouse	Infection type on seedlings	95NB117
Warwick, Qld	2003	Field	Symptom severity on adult plants	NB320
Esperance, WA	2004	Field	Symptom severity on adult plants	WAC11160[A]
		Leaf rust		
Esperance, WA	2004	Field	Symptom severity at anthesis	Uncharacterised

[A]Western Australian collection number.

Net type net blotch seedling test

Responses to NTNB were assessed in glasshouse experiments conducted at Hermitage Research Station in Warwick, Queensland, in 2002 and 2003. The DH lines, Baudin, AC Metcalfe, and local check varieties were grown in pots with three replications. Ten plants of a single line were grown in each pot. Potting mix consisted of loam, peat, and vermiculite in the ratio 2 : 1 : 1 (by volume), to which the basal fertiliser GF306N (Grow Force Australia) was added at 2.5 kg/m^3. After emergence, plants were fertilised weekly with Aquasol (Yates Australia) solution at ~75 mL/pot. Seedlings were grown in a greenhouse where temperatures ranged from 10 to 25°C with a daylength of ~12 h.

Trials were inoculated with *P. teres* pathotype NB50, which is prevalent in the Gatton region of Queensland. To prepare inoculum, single conidial cultures of each strain were increased on peanut oatmeal agar (POA) (Speakman and Pommer 1986) at 19°C under cool white and near-visible ultraviolet (NUV) light on a 12-h cycle. After 9 days in culture, conidia were washed from the agar surface, filtered through a 330-µm strainer, and resuspended in an aqueous solution containing 12 500 conidia/mL. Approximately 1.125 mL of this suspension was applied per pot with a Krebs airless paint sprayer (Oldfields Pty Limited) when plants were at an average growth stage of 13.5 (Zadoks *et al*. 1974). Inoculated plants were immediately placed in a fogging chamber and held at 100% relative humidity for 24 h (14 h dark, 10 h light) at 19°C, then returned to the greenhouse for disease development. Notes on infection types were scored 9 days after inoculation using the 1–10 scale developed by Tekauz (1985).

Spot type net blotch seedling test

Seedling responses to STNB were assessed in greenhouse experiments in Western Australia after inoculation with *P. teres* pathotypes 95NB104 and 95NB117, which are prevalent in Western Australia (Gupta and Loughman 2001).

Single spore isolation and inoculum production

Leaf tissue with net blotch lesions was cut into fragments 5–10 mm in diameter, surface sterilised in 0.5% sodium hypochlorite solution for 2 min, and then double-rinsed in sterile deionised water for 1 min. Fragments were blotted dry and aseptically transferred to 2% water agar plates. Isolation plates were incubated at 15–18°C with 12 h NUV light/12 h dark. For historical pathotypes, lyophilised culture fragments were transferred to 2% water agar plates and incubated as above. After 3–5 days, a single conidium representing each collection was transferred using a needle under the microscope to POA medium plates and incubated for 2 weeks to induce sporulation.

Inoculum and inoculation of host plants

Conidia were harvested from POA plates by adding sterile distilled water and rubbing with a rubber spatula. The spore suspension was filtered through gauze and adjusted to 2×10^4 conidia/mL. Approximately 2×10^3 conidia from this suspension were applied per plant (1 mL/10 plants of each line) using an airbrush sprayer. The plants were placed in a mist chamber and leaf wetness was maintained at 16–18°C for 24 h, after which plants were returned to the greenhouse for symptom development.

Scoring infection types

Infection types on the second leaves were scored 9 days after inoculation for NTNB and 11 days after inoculation for STNB using the scales of Tekauz (1985).

Adult plant disease assessment

Field trials were conducted at Hermitage Research Station, Queensland, in 2002 and 2003, for both NTNB and STNB, and in Esperance, Western Australia, in 2004, for STNB and leaf rust.

The Hermitage Research Station field trials of DH lines, parental lines, and local check varieties were sown in a randomised block design with two replications; plots were sown as short rows (0.5 m with in-row gap of 0.5 m). In 2002, the trial site was spread with barley stubble infested with *P. teres* isolate NB324. In 2003, the same approach was used, but with isolate NB329 (because insufficient inoculum of NB324 was available that year). Earlier tests across a range of resistant host genotypes indicated that these isolates were of the same pathotype (G. J. Platz, unpubl. data). Epidemics were promoted using supplementary sprinkler irrigation.

The Esperance field trial was conducted according to methods described by Gupta and Loughman (2001). All DH lines, parental lines, and local check varieties were sown in a randomised block design with three replications. Each plot comprised a single 1-m row. Barley straw infected with STNB pathotype WAC11160 was applied at 50 g/m^2 at the seedling (4–5-leaf) stage.

The severity of NTNB and STNB symptoms was assessed in both locations ~10 days before anthesis, using a scale of 1–9 based on the amount of disease and lesion size: 1–2, immune; 3–4, moderately resistant; 5–6, moderately susceptible; 7–8, susceptible; 9, very susceptible.

In the Esperance trial, the severity of naturally occurring leaf rust symptoms was assessed at anthesis, using a modified Cobb's scale (Peterson *et al*. 1948) with categories ranging from 1 (absence of symptoms) to 10 (very severe symptoms).

Molecular marker analysis

DNA was extracted from leaf tissue of each DH line and parent, according to Rogowsky *et al*. (1991). AFLP analysis was conducted according to Vos *et al*. (1995), with minor modifications (Cakir *et al*. 2003*a*). Adapters were as described by Vos *et al*. (1995). The list of pre-selective and selective primers used in this study is given in Table 2. *Pst*I selective primers were labelled with FAM, TET, and HEX fluorophores to allow the assay of three primer combinations on the same gel. Three differently dye-labelled reaction products were mixed together (1 µL each) and air-dried overnight at room temperature. The AFLP PCR products were separated by an ABI Prism™ 373 DNA sequencer (PE/Applied Biosystems). Details for gel preparation and running conditions have been described elsewhere (Cakir *et al*. 2003*a*). Data analysis was carried out using GeneScan™ and Genotyper™ software, with electropherogram data converted into a binary format to indicate the presence or absence of amplified fragments. The binary data were then recoded to represent the maternal and paternal genotypes.

Table 2. Pre-selective and selective AFLP primers, their universal codes, and sequences used in the mapping of the Baudin/AC Metcalfe population

Primer	Universal code	Primer sequence
	Pre-selective primer	
*Pst*I-A	P01	5′-GACTGCGTACATGCAG A-3′
*Mse*I-C	M02	5′-GATGAGTCCTGAGTAA C-3′
	Selective primer	
*Pst*I-AA	P11	5′-GACTGCGTACATGCAG AA-3′
*Pst*I-AC	P12	5′-GACTGCGTACATGCAG AC-3′
*Pst*I-AG	P13	5′-GACTGCGTACATGCAG AG-3′
*Pst*I-AT	P14	5′-GACTGCGTACATGCAG AT-3′
*Mse*I-CAA	M47	5′-GATGAGTCCTGAGTAA CAA-3′
*Mse*I-CAC	M48	5′-GATGAGTCCTGAGTAA CAC-3′
*Mse*I-CAG	M49	5′-GATGAGTCCTGAGTAA CAG-3′
*Mse*I-CAT	M50	5′-GATGAGTCCTGAGTAA CAT-3′
*Mse*I-CCT	M54	5′-GATGAGTCCTGAGTAA CCT-3′
*Mse*I-CGA	M55	5′-GATGAGTCCTGAGTAA CGA-3′
*Mse*I-CTG	M61	5′-GATGAGTCCTGAGTAA CTG-3′
*Mse*I-CTT	M62	5′-GATGAGTCCTGAGTAA CTT-3′

Marker nomenclature was the same as that used by Cakir *et al.* (2003*a*).

The SSR analysis was performed using markers selected from published barley maps (Becker *et al.* 1995; Liu *et al.* 1996; Ramsay *et al.* 2000) based on their distribution along the barley chromosomes. The parents Baudin and AC Metcalfe were assessed for polymorphism using >600 SSR markers, and the DH population was genotyped with 70 polymorphic SSRs. Some SSRs were assayed as described by Cakir *et al.* (2003*a*) and others were assayed using Multiplex-Ready Technology, as described by Hayden *et al.* (2008).

Linkage map construction

Linkage analysis for the AFLP and SSR markers was performed using the software package Map Manager QTX (Manly *et al.* 2001). An initial map was constructed using the 'distribute' function and a minimum likelihood-of-odds (LOD) score of 3. The published map location of SSR markers was used to confirm the integrity of the linkage map. Additional markers were placed on the map manually by searching for markers that were most closely linked to, and which minimised the number of, double crossovers. The integrity of the linkage map was further checked by: (*1*) performing a Chi-square test for segregation distortion using QGene (Nelson 1997) to test whether marker alleles segregated in the expected 1 : 1 ratio for a DH population; (*2*) applying the map-checking functions described by Lehmensiek *et al.* (2005) to identify regions that contained an apparent high number of double crossovers; (*3*) using RECORD software (Van Os *et al.* 2005) to refine the marker orders.

Markers with a high number of double crossovers, or significant segregation distortion, were removed from the map and rescored as necessary. This iterative process of re-scoring and checking was continued until a satisfactory version of the map was reached.

Phenotypic data analysis

All of the phenotypic data were subjected to ANOVA and the restricted maximum likelihood procedure in GENSTAT (GENSTAT for Windows 7th edn, 2003© Lawes Agricultural Trust). Best linear unbiased predictors were derived for each line. Correlation coefficients (r) among the traits were calculated using Qgene software (Nelson 1997).

QTL analysis

The QTLs for the disease response traits were characterised by interval mapping using the software packages Map Manager QTX (Manly *et al.* 2001) and QGene (Nelson 1997). A QTL with a LOD test statistic score between 2.0 and 3.0 was considered suggestive, while a QTL exceeding a LOD test statistic score of 3.0 was considered significant. Analysis for each trait was conducted based on the adjusted mean data from each site.

Results

Linkage map construction

Data for 269 AFLP and SSR markers were used in initial map construction. Of these, 37 markers were excluded due to significant deviations from the expected 1 : 1 segregation ratio or a high incidence of apparent double recombinants. The final linkage map (Fig. 1) consisted of 232 loci: 163 AFLP markers and 69 SSR markers. The number of markers per chromosome ranged from 19 (3H) to 51 (7H), with the number of SSR markers ranging from 5 (4H) to 17 (7H) and the number of AFLP markers ranging from 11 (3H and 6H) to 37 (5H). The total map length was 1307.2 cM, with individual chromosomes ranging in length from 81.2 cM (6H) to 241.3 cM (7H). Most marker intervals were <10 cM, but each chromosome had several longer intervals.

Net type net blotch phenotyping

Seedling screening showed that both parents were susceptible to pathotype NB50 (Table 3), but there was variation among the DH lines (Fig. 2*a*). Disease scores in the DH population had mean values of 8.2 and 9.4 in 2002 and 2003, respectively. The NTNB infection types scored on seedlings were highly reproducible across the two years ($r = 0.77$**; Table 4).

Adult plant screening against pathotypes NB324 and NB329 in consecutive years showed that neither parent exhibited severe symptoms (although AC Metcalfe was slightly more resistant; Table 3). However, variation in disease severity was observed among the DH lines (Fig. 2*b*). The DH population had mean disease severity scores of 2.0 and 2.7 in 2002 and 2003, respectively. The symptom severities recorded in the field trials showed highly significant positive correlations between the years ($r = 0.59$**; Table 4).

Spot type net blotch phenotyping

Seedlings of Baudin and AC Metcalfe exhibited moderate resistance to the 95NB104 and 95NB117 pathotypes, although Baudin was more resistant to pathotype 95NB104 (Table 3). In the DH population, infection types ranged from 1.2 to 7.8, with many of the DH lines having more extreme values than either parent (Fig. 2*c*). Seedling responses to the two pathotypes showed a highly significant positive correlation with each other ($r = 0.61$**; Table 4).

Adult plant symptoms were more severe in Queensland than in Western Australia (Fig. 2*d*). The parents showed

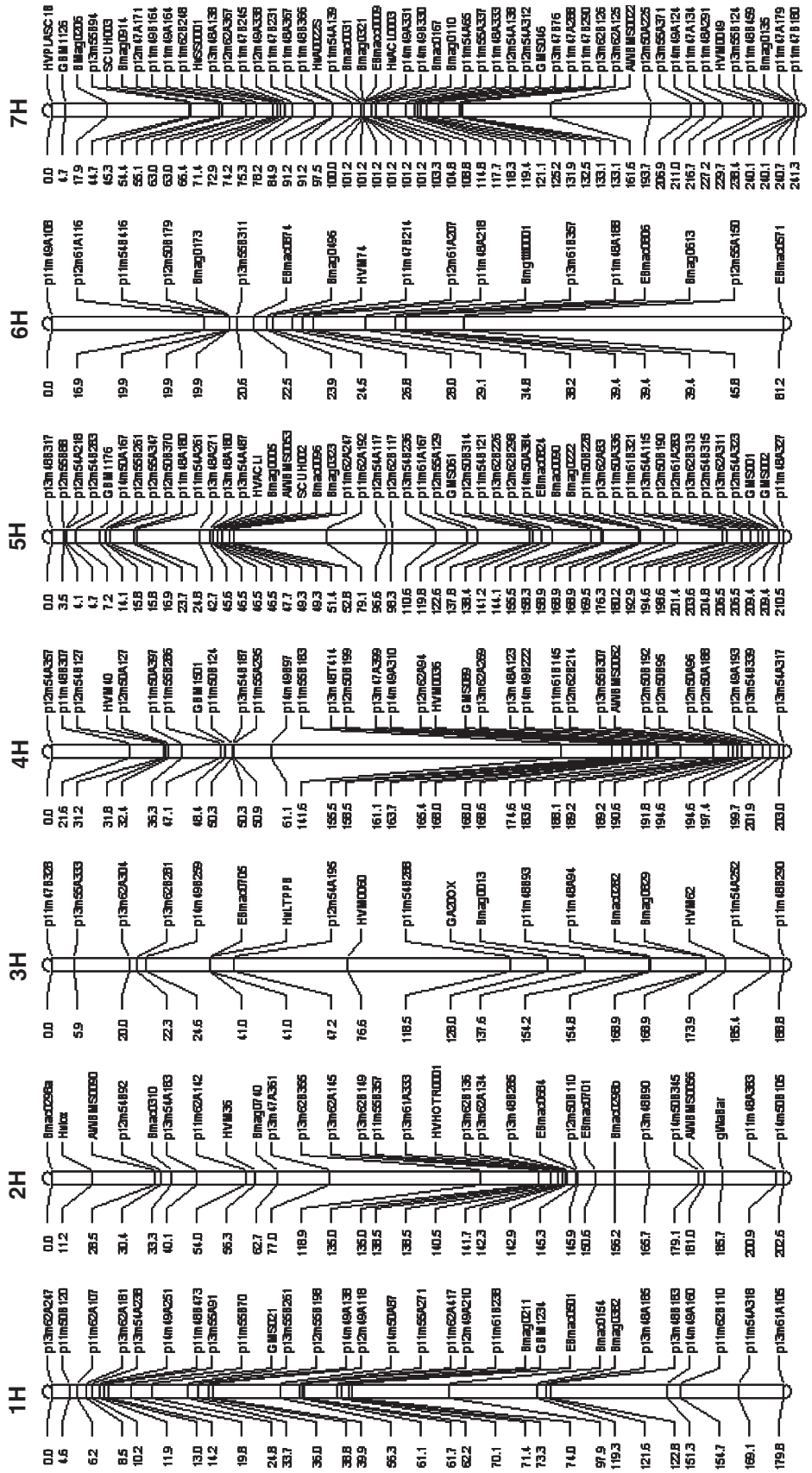

Fig. 1. A linkage map of barley chromosomes based on Baudin/AC Metcalfe mapping population. The genetic map distances in cM are shown on the left. Map distances were calculated with Kosambi function using Mapmanager software. AFLP markers were generated using *Pst*I and *Mse*I primer combinations (Table 1).

Table 3. Disease assessments of barley parental lines Baudin and AC Metcalfe, and minimum, maximum, and mean doubled haploid population values against *P. teres* f. *teres* (net type net blotch), *P. teres* f. *maculata* (spot type net blotch), and *Puccinia hordei* (leaf rust)
Details of the disease scales are described in *Materials and methods*

Year	Screening stage	Pathotype	Baudin	AC Metcalfe	Population mean	Min.	Max.
			Net type net blotch				
2002	Seedling	NB50	9	7	8.2	2.5	10
2003	Seedling	NB50	9	9	9.4	6	10
2002	Adult plant	NB324	2	1	2	1	7.5
2003	AP	NB329	2	1.5	2.7	1	7
			Spot type net blotch				
2004	Seedling	95NB104	5.5	6.5	4.2	2	7
2004	Seedling	95NB117	5.7	5.2	4	2	7
2004	Adult plant	NB320	3	5	6.8	3.5	9
2003	Adult plant	WAC11160[A]	2.9	4.3	5.7	2.7	7.3
			Leaf rust				
2004	Adult plant	Uncharacterised	7.4	4.2	6.1	2	8

[A]Western Australian collection number.

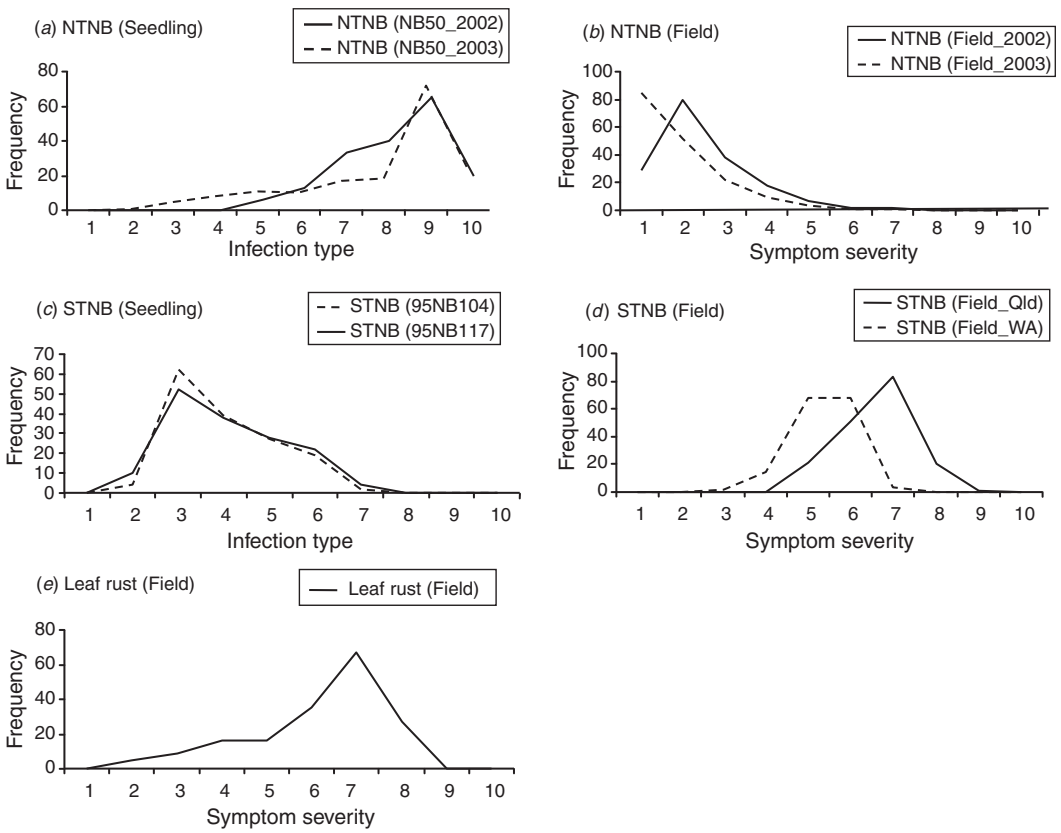

Fig. 2. Frequency polygons showing the phenotypic distributions of disease response traits in the Baudin/AC Metcalfe DH population: (*a*) infection type on inoculated seedlings for net type net blotch (NTNB) of doubled haploid (DH) lines in 2002 (solid line) and 2003 (dashed line); (*b*) symptom severity on field-grown adult plants for NTNB of DH lines in 2002 (solid line) and 2003 (dashed line); (*c*) infection type on seedlings of DH lines inoculated with the pathotypes NB104 (dashed line) and NB117 (solid line) of spot type net blotch (STNB); (*d*) symptom severity on field-grown adult plants for STNB (solid line for Queensland and dashed line for Western Australian environment); and (*e*) symptom severity of leaf rust at anthesis on field-grown DH lines in Esperance, WA.

moderate resistance in both locations (Table 3), and the observations from the two locations were positively correlated ($r = 0.41$**; Table 4).

Leaf rust phenotyping

Adult plant symptom severity in the parental lines showed that AC Metcalfe was more resistant than Baudin, with disease scores

Table 4. Correlation co-efficients (*r*) for the infection responses to net type net blotch (NTNB) and spot type net blotch (STNB) traits in doubled haploid lines of the Baudin/AC Metcalfe population

APR, Adult plant resistance; SLR, seedling resistance. Correlation significantly different from zero: *$P < 0.05$, **$P < 0.01$

Disease	NTNB (APR_2003)	NTNB (APR_2002)	NTNB (SLR_2003)	NTNB (SLR_2002)	STNB (APR_Qld)	STNB (SLR_95NB104)	STNB (SLR_95NB117)
NTNB (APR_2002)	0.59**						
NTNB (SLR_2003)	0.39**	0.35**					
NTNB (SLR_2002)	0.33**	0.34**	0.77**				
STNB (APR_Qld)	0.17*	–0.01	0.03	0.03			
STNB (SLR_95NB104)	0.15	0.15	0.16*	0.20*	0.34**		
STNB (SLR_95NB117)	0.13	0.11	0.081	0.09	0.30**	0.61**	
STNB (APR_WA)	0.05	–0.13	–0.14	–0.07	0.41**	0.39**	0.33**

of 4.2 and 7.4, respectively. The DH population had a mean symptom severity score of 6.1 and a range from 2.0 to 8.0 (Table 3, Fig. 2*e*).

QTL for net type net blotch

The QTLs affecting NTNB infection type at the seedling stage in 2002 and 2003 were highly repeatable. Hence, the mean seedling score for each line was used for QTL analysis. QTLs were detected on chromosomes 2HS, 2HL, 3HS, 4HL, and 6HS. Phenotypic variation explained (R^2) by each QTL ranged from 10 to 19% (Table 5). Resistance alleles were contributed by Baudin for the QTLs on chromosomes 2HS and 6HS, and by AC Metcalfe for chromosomes 2HL, 3HS, and on 4HL (Table 5).

Based on the adult plant data from 2002, a QTL affecting NTNB symptom severity was identified on chromosome 3HS; it explained 10% of the phenotypic variation. An allele inherited from AC Metcalfe contributed to this resistance (Table 5). Two other QTLs were also detected in that year (on chromosomes 5HS and 6HS), each explaining 6% of the phenotypic variance. At those loci, the alleles for resistance were inherited from Baudin. In 2003, a single QTL was detected on chromosome 6HS, explaining 15% of the phenotypic variation and corresponded to the QTL position detected in the previous year.

QTL for spot type net blotch

For seedling STNB resistance, a QTL against both isolates of STNB was detected near marker Bmag0496 on chromosome 6HS. This QTL accounted for 47 and 31% of the phenotypic variation and was highly significant, with LOD scores of 18.5 and 10.71 (Table 5) for 95NB104 and 95NB107, respectively. Baudin was the source of resistance against both isolates.

For adult plant screening conducted in both Western Australia and Queensland, the same QTL on chromosome 6H was detected, with Bmag0496 as the most closely linked SSR marker. The QTL

Table 5. Experimental location and year, screening stage, pathotype, chromosomal location of QTLs, likelihood-of-odds (LOD) scores, % phenotypic variation explained by each marker (R^2), marker with the greatest effect and its map position, and source of resistance for each QTL for disease traits in the barley doubled haploid population Baudin/AC Metcalfe

HRS, Hermitage Research Station, Warwick

Location/year	Screening stage	Pathotype	Chromosome location	LOD score	%R^2	Marker with greatest effect	Map position (cM)[A]	Source of resistance
			Net type net blotch					
HRS, Qld/2002–03[B]	Seedling	NB50	2HS	3.06	10	BMag0740	62.7	Baudin
			2HL	4.27	12	HVMOTR0001	140.5	AC Metcalfe
			3HS	4.11	10	HVM0060	76.6	AC Metcalfe
			4HL	2.56	10	P13M47A399	161.1	AC Metcalfe
			6HS	5.75	19	Bmag0173	19.9	Baudin
HRS, Qld/2002	Adult plant	NB324	3HS	3.39	10	HVM0060	76.6	AC Metcalfe
			5HS	2.14	6	SCU002	49.3	Baudin
			6HS	2.04	6	Bmag0173	19.9	Baudin
HRS, Qld/2003	Adult plant	NB329	6HS	5.36	15	Bmag0173	19.9	Baudin
			Spot type net blotch					
South Perth, WA/2004	Seedling	95NB104	6HS	18.5	47	Bmag0496	23.9	Baudin
South Perth, WA/2004	Seedling	95NB117	6HS	10.71	31	Bmag0496	23.9	Baudin
Esperance, WA/2004	Adult plant	WAC11160	6HS	10.71	31	Bmag0496	23.9	Baudin
HRS, Qld/2003	Adult plant	NB320	2HL	2.4	8	P13M62B149	135.0	Baudin
			3HS	2.13	8	P14M49B259	24.6	Baudin
			6HS	3.52	9	Bmag0496	23.9	Baudin
			7HL	2.11	5	Bmag0135	240.1	Baudin
			Leaf rust					
Esperance, WA/2004	Adult plant	Uncharacterised	2HL	5.13	15	HVHOTR0001	140.5	AC Metcalfe

[A]Genetic distance from the distal end of short arm to the marker position as shown in Fig. 1.

[B]As the results were highly repeatable, the mean of 2002 and 2003 NTNB seedling data were used for QTL analysis.

explained 31 and 9% of the variation in Western Australia and Queensland, respectively. The data from Queensland also revealed three additional QTLs on chromosomes 2HL, 3HS, and 7HL that explained 8, 8, and 5% of the phenotypic variation, respectively. AFLP markers P13M62B149 and P14M49B259 and the SSR marker Bmag0135 had the strongest associations (Table 5). Baudin was the source of resistance for all three QTLs at the adult plant stage.

QTL for leaf rust

For symptom severity of leaf rust on adult plants in the field, a QTL was detected near SSR marker HVHOTR0001 on chromosome 2HL (Table 5). This QTL explained 15% of the phenotypic variation, and the resistance allele was inherited from AC Metcalfe.

Discussion

This study reports on the construction of a genetic linkage map for the Baudin/AC Metcalfe DH population and subsequent QTL analysis for the disease traits NTNB, STNB, and leaf rust.

A genetic linkage map based on 232 AFLP and SSR markers was constructed for the seven barley chromosomes. The total map length was 1307.2 cM. Using AFLP marker technology, we were able map a large number of marker loci using only eight primer combinations. SSR marker technology allowed us to anchor each linkage group to a specific barley chromosome. The relative loci order for the SSR markers were consistent with those previously reported by Ramsay *et al*. (2000) and Varshney *et al*. (2007), indicating the robustness of the genetic map produced.

Several significant QTL regions were identified from the Baudin/AC Metcalfe population for the disease traits studied (Table 5). For NTNB and STNB, QTLs that were common to different locations, years, and pathotypes were detected. Environment-specific loci were also identified (Table 5).

Neither AC Metcalfe nor Baudin exhibited strong resistance against NTNB pathotype NB50, and responses in the population were skewed towards susceptibility (Fig. 2*a*). This could be due to a lack of sufficient seedling resistance in the parental lines. Nevertheless, it was possible to detect QTLs with small effects contributing to seedling resistance on chromosomes 2HS, 2HL, 3HS, 4HL, and 6HS. The resistance alleles came from Baudin at two loci (on 2HS and 6HS) and from AC Metcalfe at the other three loci. Transgressive segregation would therefore have contributed to the phenotypic variation in the DH population. The region on chromosome 6HS also affected resistance against pathotype NB324 in the field. From our results, it was not possible to confirm whether the chromosome 6HS QTL for seedling and adult plant resistances correspond to the same gene. These results were similar to those of Gupta *et al*. (2010), where two QTLs (on 3HS and 6HS) were detected at both seedling and adult plant stages in a population derived from an intercross between cvv. Pompadour and Stirling. QTLs for resistance to NTNB have been detected on chromosome 6HS in at least five other studies (Cakir *et al*. 2003*a*; Ma *et al*. 2004; Friesen *et al*. 2006; Manninen *et al*. 2006; Lehmensiek *et al*. 2007; Gupta *et al*. 2010). Cakir *et al*. (2003*a*) found Bmag0173 to be the closest marker to the 6HS NTNB locus in two barley populations. Friesen *et al*. (2006) also reported Bmag0173 as the closest marker to the resistance locus on 6HS. This 6HS gene has been designated *Rpt5* by Manninen *et al*. (2006). QTLs for resistance to NTNB in seedlings have also been reported on chromosomes 2H and 3H (Manninen *et al*. 2006), at approximately the same locations as those detected in this study. In their study, the markers HVM0060 and MWG803 flanked the 3H QTL. HVM0060 was also the most closely linked marker in this study. Due to a lack of common markers, it was not possible to compare the locations of 2H QTLs detected in each study.

Seedling tests with two STNB pathotypes (95NB104 and 95NB117) resulted in nearly identical phenotypic responses (Fig. 2*c*). A single QTL on chromosome 6HS with effectiveness against both pathotypes was identified, where Baudin contributed the resistance allele. A minor QTL on 6H for STNB was also detected in TR251 (Grewal *et al*. 2008). An allelism test would be required to establish whether the 6H QTLs from Baudin and TR251 were the same resistance gene. Other reports for seedling resistance to STNB have involved QTLs on chromosomes 2H, 5H, and 7H (Williams *et al*. 2003) and 4H (Friesen *et al*. 2006).

The 6HS QTL for STNB was also effective at the adult plant stage at both Esperance and Warwick. Pathotype-specific QTLs were detected on chromosomes 2HL, 3HS, and 7HL with effects at the adult plant stage in Warwick. The 7HL QTL found in this study corresponded in position (near SSR marker Bmag0135) to that reported by Williams *et al*. (2003) (Table 5).

Further research is required to determine whether the 6HS QTL affecting seedling and adult plant infection responses corresponds to the same gene or to closely linked genes. It has been reported that some genes function only in adult plants (Tekauz 1990), whereas others may confer resistance at both seedling and adult stages (Cakir *et al*. 2003*b*).

A QTL for resistance to leaf rust located near marker HVHOTR0001 on chromosome 2H was in a similar location to *Rph15* (Jin *et al*. 1996), *Rph16* (Ivandic *et al*. 1998), and QTLs reported by von Korff *et al*. (2005) and Marcel *et al*. (2007). The leaf rust observations were taken in only one location and year, with no characterisation of the pathotype(s) involved. Additional field trials and/or pathotyping would therefore be required to confirm the 2HL leaf rust QTL and to assess its novelty and utility.

It was evident from the analysis of STNB seedling data that genetic background may have affected the detection of QTLs. Despite both parents showing similar intermediate resistance at the seedling stage, large phenotypic variation was observed in the population. But only one major QTL was detected on the 6HS chromosome, indicating that the interaction between two genetic backgrounds from Baudin and AC Metcalfe may have affected resistance to STNB in the DH population. This was even more evident in the data from the seedling experiments with the pathotype 95NB117. Although Baudin was the less resistant parent, QTL analysis detected only a resistance gene from Baudin. Similar genetic background effects on QTLs have been reported in oat (Holland *et al*. 1997), maize (Blanc *et al*. 2006), and barley (Li and Zhou 2010).

Using SSR markers near QTLs for disease resistance traits, it may be possible to apply marker-assisted selection to improve the resistance of barley against the diseases studied here. Specifically, SSR markers Bmag0173 and HVM0060 may be particularly useful for NTNB; Bmag0496 may be useful for STNB; and

HVHOTR0001 may be useful for leaf rust. These SSR markers are reported to be highly polymorphic in barley germplasm (Ramsay *et al*. 2000). In cases where markers are not polymorphic in the breeding germplasm of interest, it should be possible to select alternative markers via comparison across linkage maps.

As the parental lines are leading malting varieties, the Baudin/AC Metcalfe population has been very useful for identifying important QTLs for several disease and quality traits (J. Panozzo, pers. comm.). The genetic map reported here will assist breeders to select desirable ideotypes from the DH population for a range of disease, agronomic, and quality traits. Markers identified in this study will contribute to marker-assisted barley breeding for improvement of these traits, especially in Australia and Canada.

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