Theor Appl Genet (2005) 111: 313–324
DOI 10.1007/s00122-005-2024-6

ORIGINAL PAPER

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Quantitative trait loci for partial resistance to crown rust, *Puccinia coronata*, in cultivated oat, *Avena sativa* L.

Received: 14 December 2004 / Accepted: 2 April 2005 / Published online: 26 May 2005

Abstract To facilitate the detection of quantitative trait loci (QTLs) for partial resistance to oat crown rust, *Puccinia coronata* f. sp. *avenae* Eriks., a genetic map was generated in a population of 158 F_6-derived oat recombinant inbred lines from a cross of a partial resistance line MN841801-1 by a susceptible cultivar selection 'Noble-2'. The map, developed using 230 marker loci, mostly restriction fragment length polymorphism and amplified fragment length polymorphism markers, spanned 1,509 cM (Haldane) arranged into 30 linkage groups of 2–18 markers each. Four consistently detected major QTLs for partial rust resistance, *Prq1a*, *Prq1b*, *Prq2*, and *Prq7*, and three minor QTLs, *Prq3*, *Prq5*, and *Prq6*, were found in tests involving three field and two greenhouse environments. In addition, two major QTLs for flowering time, *Ftq1* and *Ftq7*, and five weaker QTLs, *Ftq2*, *Ftq3*, *Ftq4*, *Ftq5*, and *Ftq6*, were revealed. Overlapping of the map segments of *Ftq1* and *Prq1* and of *Ftq7* and *Prq7* suggested either linkage between the flowering time QTLs and resistance QTLs or a pleiotropic effect of the *Ftq* QTLs on rust resistance. Relatively low heritability estimates (0.30) obtained for partial resistance to crown rust in the field indicate a potential value for marker-assisted selection.

Communicated by B. Friebe

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Introduction

Crown rust, caused by *Puccinia coronata* f. sp. *avenae* Eriks., is one of the most destructive and widespread diseases in oat (*Avena sativa* L.), decreasing yield and grain quality (Simons 1985; Ohm and Shaner 1992). The most common control of the disease involves the use of cultivars either completely or partially resistant to the pathogen (Simons 1985). The complete-resistance approach is based on monogenic resistance conditioned by major genes that provide race-specific resistance, which can be detected at the seedling stage. Introgression of major genes into new cultivars has been the basic breeding strategy. However, the pathogen can overcome monogenic host resistance within a few years through population virulence shifts. This rapid loss of resistance effectiveness challenged breeders and geneticists to look for more-effective approaches and led to a strong interest in breeding oats with partial, race non-specific, quantitative resistance that may be longer lasting or durable.

A partial-resistance defense mechanism does not prevent infection completely, but does suppress its spread. Genotypes with partial resistance are generally susceptible to the pathogen at the seedling stage. Adult

plants usually demonstrate a range of levels of resistance through some combination of longer latency periods, fewer numbers of pustules per leaf, and fewer spores per pustule (Peturson 1944; Heagle and Moore 1970; Berger and Luke 1979; Luke et al. 1981). The genetic components of partial resistance in different oat genotypes showed various patterns, from a few partially dominant or recessive genes (Luke et al. 1975; Kiehn et al. 1976) to a single partially dominant gene (Harder et al. 1984). However, in most cases, the trait was controlled by multiple genes and was sensitive to environmental differences.

One effective approach to obtain this potentially durable partial resistance is to locate the position of desirable (resistance) genes on a molecular map and then introgress these genomic regions into elite germplasm through marker-assisted selection. A number of quantitative trait loci (QTLs) controlling agronomic traits (Siripoonwiwat et al. 1996; Holland et al. 1997, 2002; Jin et al. 1998; Kianian et al. 1999; Groh et al. 2001b; De Koeyer et al. 2001, 2004) including some QTLs for crown rust resistance (Bush and Wise 1996; Zhu and Kaeppler 2003; Zhu et al. 2003) have been detected in oat during the last decade. Zhu and Kaeppler (2003) reported two QTLs for adult-plant field resistance to crown rust that were consistently identified over 2 years of tests. The source of resistance was attributed to the MAM17-5 parent in which resistance had been introduced from the diploid oat *Avena strigosa* L. Various oat sources may possess different QTLs for resistance to crown rust. In this research, we used a cross of MN841801-1 (resistant) × 'Noble-2' (susceptible). The MN841801 line was identified as a reliable source of partial resistance, based on long-term field tests (Leonard 2002).

In earlier crosses involving MN841801, we had noted a possible association of higher partial resistance and later flowering time among segregates. Because significant correlations between flowering time and resistance have been observed in other analyses of disease resistance (Ma et al. 2000; Zhu and Kaeppler 2003), possible trait correlations and coincidence of QTLs for flowering time and crown rust partial resistance were of interest.

The present study was conducted to (1) detect and characterize QTLs for partial resistance to crown rust in MN841801, (2) detect flowering time QTLs and their possible relationship to the identified rust resistance QTLs, and (3) identify molecular markers linked to the crown rust partial-resistance QTLs to facilitate introgression of this type of resistance into elite oat germplasm.

Materials and methods

Plant materials

A population of 158 F_6-derived F_8 recombinant inbred lines (RILs) was developed by single-seed descent from F_2 plants of a cross between single-plant reselections, MN841801-1 and 'Noble-2', of the parental lines. MN841801 is an oat line that has demonstrated durable resistance to diverse populations of crown rust for more than 30 years (Leonard 2002). 'Noble' (Ohm et al. 1974) is a cultivar with many favorable agronomic traits but highly susceptible to crown rust.

Crown rust evaluation in field tests

Field experiments were conducted in three different environments. The data sets collected in 1997 and 1998 in St. Paul, Minn., USA, and in 1998 in Rosemount, Minn., USA, were designated as SP97PR, SP98PR, and Rm98PR, respectively. The population was grown in two randomized complete blocks, with each RIL entry in each block planted as two adjacent 1.5-meter rows sown with 6 g of seed per row. Every third row and two rows surrounding the blocks were planted with the susceptible cultivar 'Starter' to ensure that each entry had an equal chance to get secondary infection. The parents, MN841801-1 and 'Noble-2', were planted after every ten RIL entries. In St. Paul, the plants were inoculated at a growth stage corresponding to initial panicle emergence of most entries by spraying on three successive days with a urediniospore population bulk. The source of spore bulk and the inoculation method were described by Leonard (2002). Infection intensity was estimated as percentage of pustule area of total leaf area approximately 24–27 days after inoculation. The experimental design in Rosemount was the same as in the St. Paul experiments, except that the test depended on natural infection, and the disease was estimated 10 days after the first pustules appeared on MN841801-1. The score for a replicate was an estimated average severity on five F-1 (leaf below the flag leaf) leaves selected at random in a plot. A computer-simulated scale, based on the modified Cobb scale (Peterson et al. 1948), developed at the USDA Cereal Disease Laboratory, St. Paul, was used as a reference.

Crown rust evaluation in greenhouse tests

A single *P. coronata* rust isolate 93MNB236 demonstrating high virulence to the seedlings of both parents was selected to evaluate disease resistance at the adult stage. Experiments were conducted in 1997 (Gh97PR) and 1998 (Gh98PR). A randomized complete block design with two blocks was used. Each block included one replicate of each F_8 (or F_9) RIL and six replicates of each parent, MN841801-1 and 'Noble-2'. The average value from two plants grown in a pot constituted a replicate for each entry inoculated at the flag-leaf stage. The urediniospores were heat-shocked at 40°C for 10 min, suspended in SOLTROL 170 oil (C10-C15 isoalkanes), and diluted to 5×10^6/ml as estimated from counts under a microscope. Samples of the spores were incubated on

agar overnight, and the percent germination was determined under a microscope to ensure that the germination rate was over 85%. Spores were sprayed on a 10-cm-length marked section of the F-1 leaf. Plants were placed in a dark dew chamber at 100% relative humidity for 18 h, followed by transfer to a greenhouse set at 20°C, with supplemented light to provide 16 h of light per day. Rust severity was recorded about 22 days after inoculation as the number of hand-counted pustules in the marked area followed by conversion to a per-cm^2 basis, using digital images made of the marked leaf area.

Flowering time

Flowering time (heading date) for an entry was estimated in each of three field experiments as the number of days from 1 June to date of 50% panicle emergence. Flowering time was recorded for two replicates of the randomized block design in St. Paul in 1997 (SP97FT), and for only one replicate in 1998 both in St. Paul (SP98FT) and in Rosemount (Rm98FT).

Genetic markers and linkage map construction

Two hundred fifty-nine restriction fragment length polymorphism (RFLP) clones were selected to provide distributed coverage of the 'Kanota × 'Ogle' (KO) oat linkage map (O'Donoughue et al. 1995; Wight et al. 2003). The probes included cDNA clones ACO (oat inflorescence), BCD (barley etiolated leaf tissue), CDO (oat etiolated leaf tissue), UMN (immature oat endosperm tissue), ISU (oat root tissue), and genomic DNA clones OG (oat genomic) and WG (wheat genomic); these have been described previously (O'Donoughue et al. 1995). In addition, nine resistance gene analogues [(RGAs) b1 through b9] isolated from barley (Leister et al. 1999) were provided by Dr. Gary Muehlbauer, University of Minnesota, St. Paul. Two sequence characterized amplified region (SCAR) markers, cdo113s (a marker for a crown rust, *P. coronata*, resistance gene *Pc38*) and *Pg3*-30/33 (a marker for a stem rust, *P. graminis*, resistance gene *Pg3*) were supplied by Dr. Stephen Molnar, Agriculture and Agri-Food Canada, Ottawa, Ont., Canada, and a simple sequence repeat (SSR) marker AM3 was provided by Dr. Graham Scoles, University of Saskatchewan, Saskatoon, Sask., Canada. Detections of polymorphisms for AM3 and Pg3-30/33 were performed using 3% SFR agarose (Amresco, Solon, Ohio, USA) gels, and for cdo113s using 2% regular agarose gels following protocols supplied by the originators of the markers. The SSR AM3 and the SCAR cdo113s have been described previously (Li et al. 2000; Wight et al. 2004).

The DNA was isolated from leaves by the CTAB method (Saghai Maroof et al. 1984; Wise and Schnable 1994). Southern blot hybridization was performed as described by Heun et al. (1991). The restriction enzymes used were *Eco*RI, *Eco*RV, *Bam*HI, *Dra*I, and *Hin*dIII. The amplified fragment length polymorphism (AFLP) analysis was performed as described by Groh et al. (2001a). The AFLP loci were named as described previously (Portyanko et al. 2001) with some modifications. Each AFLP marker name included a Keygene code (http://wheat.pw.usda.gov/ggpages/keygeneAFLPs.html) for the *Pst*I/*Mse*I primer combination followed by a letter referencing the parental source of the fragment (*m* refers to MN841801-1, and *n* refers to 'Noble-2') and the number of a polymorphic band determined for each primer combination starting from the top of the gel. The RFLP loci were designated following Wight et al. (2003, 2004). Multiple polymorphic loci detected by an RFLP probe in MN841801-1 × 'Noble-2' were differentiated by addition of an *x*, *y*, and *z*, based on fragment size, with *x* representing the largest polymorphic fragment in either parent.

A genetic map was generated by MAPMAKER 3.0 (Lander et al. 1987). Linkage groups were established at a minimum LOD score of 3.0 and maximum map distance of 43.7 cM, using the Haldane (1919) mapping function. Loci at LOD <2.0 were assigned to intervals.

Data analysis

Each year–location combination of the three field (SP97PR, SP98PR, and Rm98PR) and two greenhouse (Gh97PR and Gh98PR) environments for rust resistance testing was considered as a single environment for the purposes of statistical analyses. Normality of distribution of the traits analyzed was estimated using Kolmogorov–Smirnov or Shapiro tests (Sokal and Rohlf 1995). Analyses of variance (ANOVAs) were performed for rust severity for each of the single environments as well as across environments. Both RILs and environments were considered as random effects. Variance components were estimated according to Searle (1971). Broad-sense heritability estimates were computed as described by Zhu and Kaeppler (2003). Pearson correlation coefficients (Sokal and Rohlf 1995) were calculated between replicates and between environments for rust severity. Spearman's correlations (Sokal and Rohlf 1995) were used to estimate relationships between environments for flowering time and between flowering time and rust severity. Correlation analyses were based on means for each of the 158 RILs calculated for each of the environments. These means were also used for QTL analyses. The QTL analyses were conducted using PLABQTL 1.1 (Utz and Melchinger 1996) composite interval mapping (CIM). Various models (additive, dominant, with interactions) were tested with the additive model fitting best. For rust severity QTL detection, a LOD threshold of 3.13 was applied that corresponded to theoretical Bonferroni chi squares for genome-wide $\alpha = 0.10$. A more liberal LOD 2.4 was used to detect QTLs for flowering time. Cofactor markers for the final regression were chosen by the software automatically

in the main run followed by minor revisions and several more runs if necessary as recommended (ftp://ftp.uni-hohenheim.de/pub/plabqtl/pq_faq.txt). A support interval with a LOD fall-off 1.0 (default option) was used. Cross-validation tests (command *cross/g 500*) were also performed. A QTL was declared if detected by the CIM procedure in at least two different environments or if detected in one environment by CIM and confirmed by cross-validation tests at the same position in at least one other environment and in both cases, the signs of the additive effects were consistent across environments. The QTLs with overlapping support intervals were assumed to be a single QTL if they belonged to the same trait and to be linked or pleiotropic if they represented different traits. Additive effects were given as positive for QTLs at which MN841801-1 alleles tended to decrease trait values. The proportion of phenotypic variance explained by an individual QTL was estimated by the square of the partial correlation coefficient. The proportion of phenotypic variation explained by all QTLs detected was estimated by multiple regression in a final simultaneous fit. Markers left unlinked on the molecular map were tested for associations with the quantitative traits studied using single-marker regression analysis (Sokal and Rolf 1995). The association was considered valid if single-marker regression was significant at $P < 0.01$ in more than one environment.

Both mapping and rust phenotyping data will be deposited in GrainGenes Database (http://wheat.pw.usda.gov/GG2/index.shtml).

Results

Phenotypic data

A continuous distribution for rust severity both in field and greenhouse experiments indicated that this trait was quantitative with additive inheritance (Table 1; Fig. 1). In all five environments, the distribution deviated somewhat from normal. Because this deviation was not severe, untransformed data were used for the analyses.

Significant differences for partial resistance to crown rust both between the two parents and between parents and RILs were revealed in ANOVA performed for each environment. The ANOVA values across environments (without parents) showed high significance for both genotypic and genotype × environment (G × E) interaction variances (Table 1). The G × E interactions as well as low correlations between replications ($r = 0.19$, $P < 0.05$ for SP97PR; $r = 0.54$, $P < 0.001$ for Rm98PR) and environments (Table 2) probably contributed to the relatively low broad-sense heritability of the trait (Table 1). Because of strong G × E interactions, we examined QTL effects in each environment separately.

Frequency distribution curves of RIL means for flowering time were bimodal in SP97FT and SP98FT (data not shown), so this trait may be mostly under the control of a single Mendelian gene in this population. Because the distribution deviated from normal ($P < 0.01$) and the data for SP98FT and Rm98FT were collected without replication, no ANOVA was performed for flowering time. Spearman correlations between environments ranged from 0.60 to 0.77 ($P < 0.01$ or $P < 0.001$). Spearman correlations between rust severity and flowering time were low and negative for all environments (Table 2).

Table 1 Means and variance components for parents and recombinant inbred lines (*RILs*) for rust severity (percentage) in three field (SP97PR, SP98PR, and Rm98PR) tests or for number of pustules/cm^2 in two greenhouse (Gh97PR and Gh98PR) tests

Environment	Parents		RILs		
	MN841801-1	'Noble-2'	Mean	SD	Range
SP97PR	4.7	26.5	18.4	8.6	2–50
SP98PR	15.0	53.4	30.7	14.6	5–70
Rm98PR	5.1	63.2	26.8	17.6	5–80
Variance components (RILs)					
σ^2_{aG}	59.1*				
$\sigma^2_{G \times E}$	61.3*				
Heritability	0.30				
Gh97PR	4.7	34.9	19.4	11.0	1.4–64.1
Gh98PR	2.8	30.4	19.8	8.9	2.3–41.3
Variance components (RILs)					
σ^2_{G}	43.8*				
$\sigma^2_{G \times E}$	22.9*				
Heritability	0.44				

*Significant at $P < 0.001$

[a]σ^2 Interaction variances, *G* genotype, *E* environment

Linkage map

A total of 230 loci including 114 AFLP, 113 RFLP, 1 SSR, and 2 SCAR markers were used to generate a framework map for QTL analyses. One hundred sixty-seven markers (72%) formed 30 linkage groups (2–14 framework loci each) spanning 1,509 cM (Haldane) of the oat genome, with an average interval length of 11.1 cM. Forty-two markers (18%) were assigned to intervals at LODP < 0.01), showed an excess of the MN841801-1 genotype and nine showed an excess of the 'Noble-2' genotype. Nine of the 12 were distributed among three linkage groups, MN(MN841801 × 'Noble')6, MN17, and MN22, forming distorted regions (Fig. 2). Putative correspondence based on RFLP fragment size, as done in other research (Portyanko et al. 2001), was established among linkage groups of the map and of the KO map including MN3-KO17, MN6-KO6, MN14-KO22, and MN26-KO36.

QTLs for partial rust resistance in field environments

A total of five QTLs for partial resistance for crown rust, *Prq1a, Prq1b, Prq2, Prq3*, and *Prq5*, were detected in

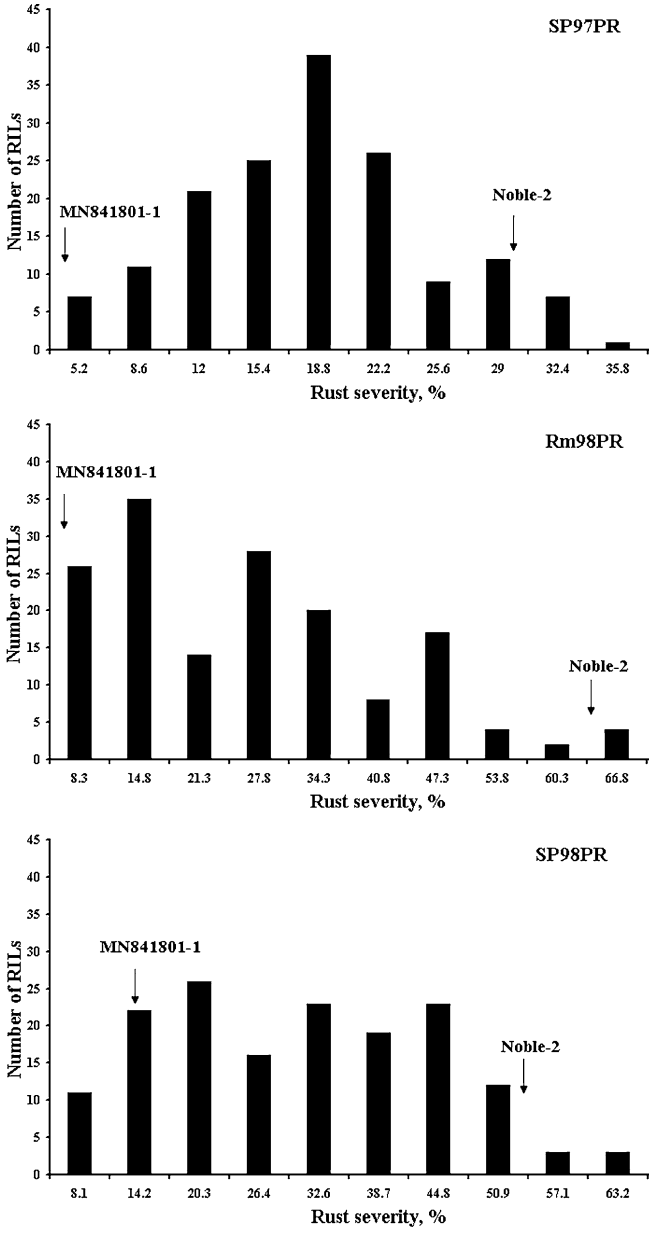

Fig. 1 Frequency distribution of rust severity (percentage) for the 158 $F_{6:8}$ recombinant inbred lines (RILs) from the cross MN841801-1 × 'Noble-2' in the field environments. Positions of parental means are indicated by *arrows*. Environment abbreviations are explained in "Materials and methods"

field environments using the PLABQTL CIM procedure (Table 3; Fig. 2). An additional QTL, *Prq7*, was revealed by single-marker regression analysis (Table 3). An earlier report based on fewer markers and different rust scoring methods identified on linkage group MN3 a single broad peak QTL referred to as *Prq1* (Chen et al. 2000). In the analysis reported here, *Prq1a* and *Prq1b* were detected each in two environments on MN3 (Table 3; Fig. 3). Log-likelihood peaks for these two QTLs were found close to markers cdo1467 and p38m35m6, respectively, and were separated by at least 12 cM (Table 3; Fig. 3). The two support intervals did not overlap indicating independent QTLs (Table 3). In some cases (Rm98PR) selection at first run PLABQTL CIM with both p38m35m6 and cdo1467 as cofactors yielded just one of the QTLs because of mutual absorption of phenotypic effects. The elimination of one of them having a lower "F-to-enter" value allowed detection of both QTLs (Table 3; Fig. 3). The most consistent QTL with a relatively strong effect was *Prq2* on linkage group MN26 (Table 3; Fig. 2). An additional major rust resistance QTL associated ($P < 0.01$) with an unlinked AFLP fragment, p56m48n2, was detected by single-marker analysis in two field environments (Table 3). Two weaker QTLs, *Prq3* and *Prq5*, were found on MN14 and MN6, respectively (Table 3; Fig. 2). The proportion of the total phenotypic variance explained by all CIM-detected QTLs simultaneously was not high in SP97PR and SP98PR (21.8% and 25.9%, respectively), but about double (51.4%) those amounts was detected in Rm98PR (Table 3).

No dominant or epistatic effects were found for rust resistance by tests of the corresponding genetic models offered by PLABQTL.

QTLs for partial rust resistance in greenhouse environments

Five partial-resistance QTLs, *Prq1a*, *Prq1b*, *Prq2*, *Prq6*, and *Prq7*, were detected under greenhouse conditions (Fig. 2; Table 4). All of them but *Prq6* were also found in field tests. As with the field data, the detection of QTLs *Prq1a* and *Prq1b* on MN3 in Gh2PR was affected greatly by choice of cofactors (Table 4). The QTL with the most consistent effect was *Prq2* (Fig. 1; Table 4).

Table 2 Pearson and Spearman correlation coefficients between rust severity in the field (SP97PR, SP98PR, and Rm98PR) and greenhouse (Gh97PR and Gh98PR) experiments and flowering time in the field (SP97FT, SP98FT, and Rm98FT)

	SP97PR	SP98PR	Rm98PR	Gh97PR	SP97FT	SP98FT	Rm98FT
SP97PR					−0.07[a]		
SP98PR	0.36**					−0.14[a]	
Rm98PR	0.44**	0.46**					−0.29[a]**
Gh97PR	0.31**	0.24*	0.40**		−0.29[a] **	−0.36[a] **	−0.22[a]*
Gh98PR	0.29**	0.23*	0.36**	0.53**	−0.24[a] *	−030[a]**	−0.23[a]*

*Significant at $P < 0.01$, **Significant at $P < 0.001$
[a]Spearman coefficients

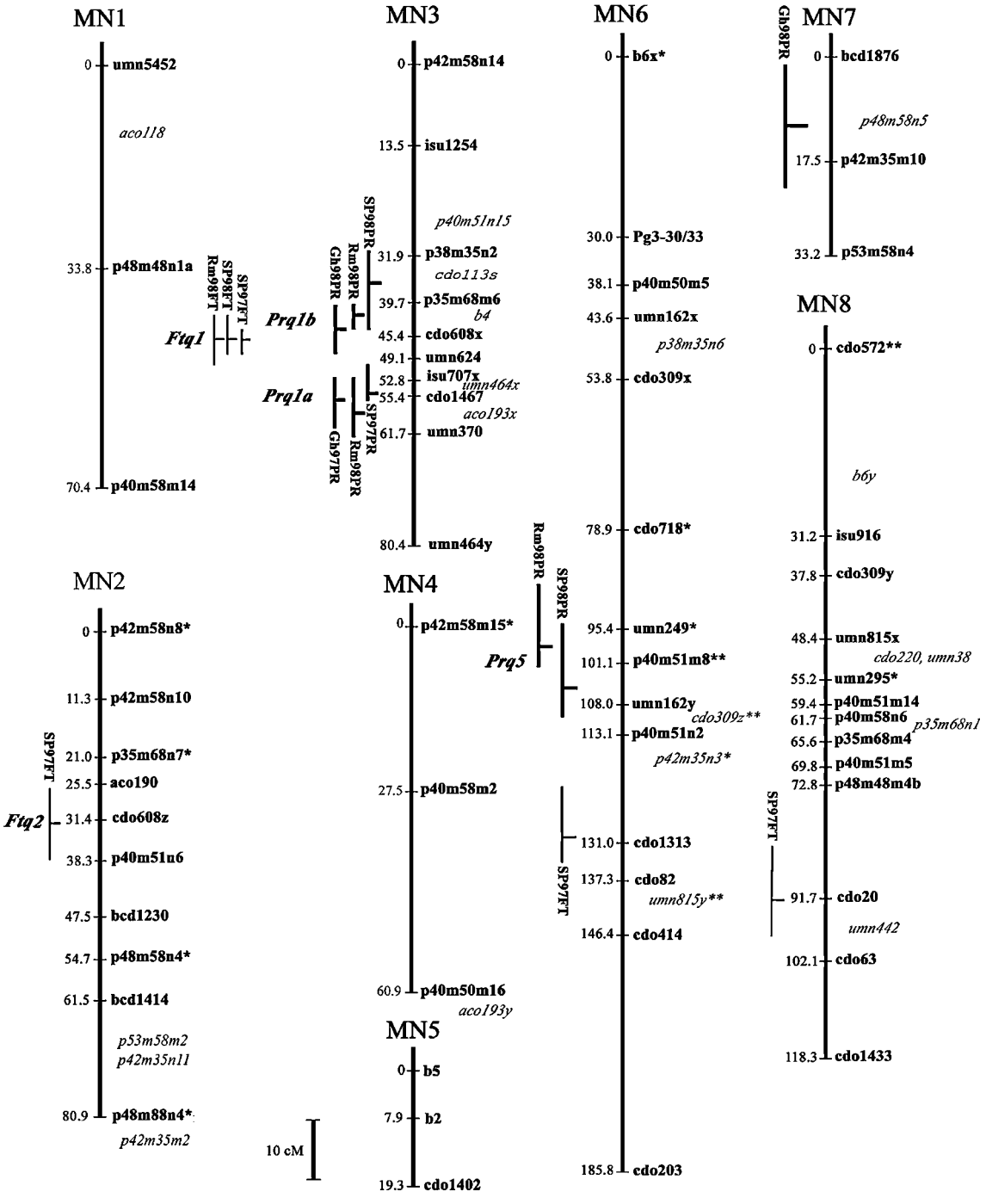

Fig. 2 A molecular linkage map of hexaploid oat based on 158 $F_{6:8}$ RILs from the cross MN841801-1 × 'Noble-2' with mapped quantitative trait loci (QTLs) for partial resistance to crown rust (*Prq*) and flowering time (*Ftq*). Numbers to the *left* of linkage groups are cumulative map distances in cM (Haldane). Marker loci shown in *boldface* were mapped as framework loci at LOD > 2.0. Marker loci shown in *italics* were assigned to intervals only at LODP < 0.05 and $^{**}P < 0.01$. *Bars* to the *left* of linkage groups represent support intervals for the QTLs detected, with *tick marks* showing the LOD peaks. Abbreviations for environments are explained in "Materials and methods"

Prq7 (associated with p56m48n2) was also revealed in all greenhouse data and showed R^2 values higher than in field experiments (Table 4). A new QTL on linkage group MN9, *Prq6*, with relatively weak phenotypic effect undetected in the field tests, was found in each of the greenhouse tests (Table 4). The detection of *Prq6* probably was affected by another QTL with opposite effect discovered on MN9 in Gh98PR. The proportion of phenotypic variance explained by all the CIM-de-

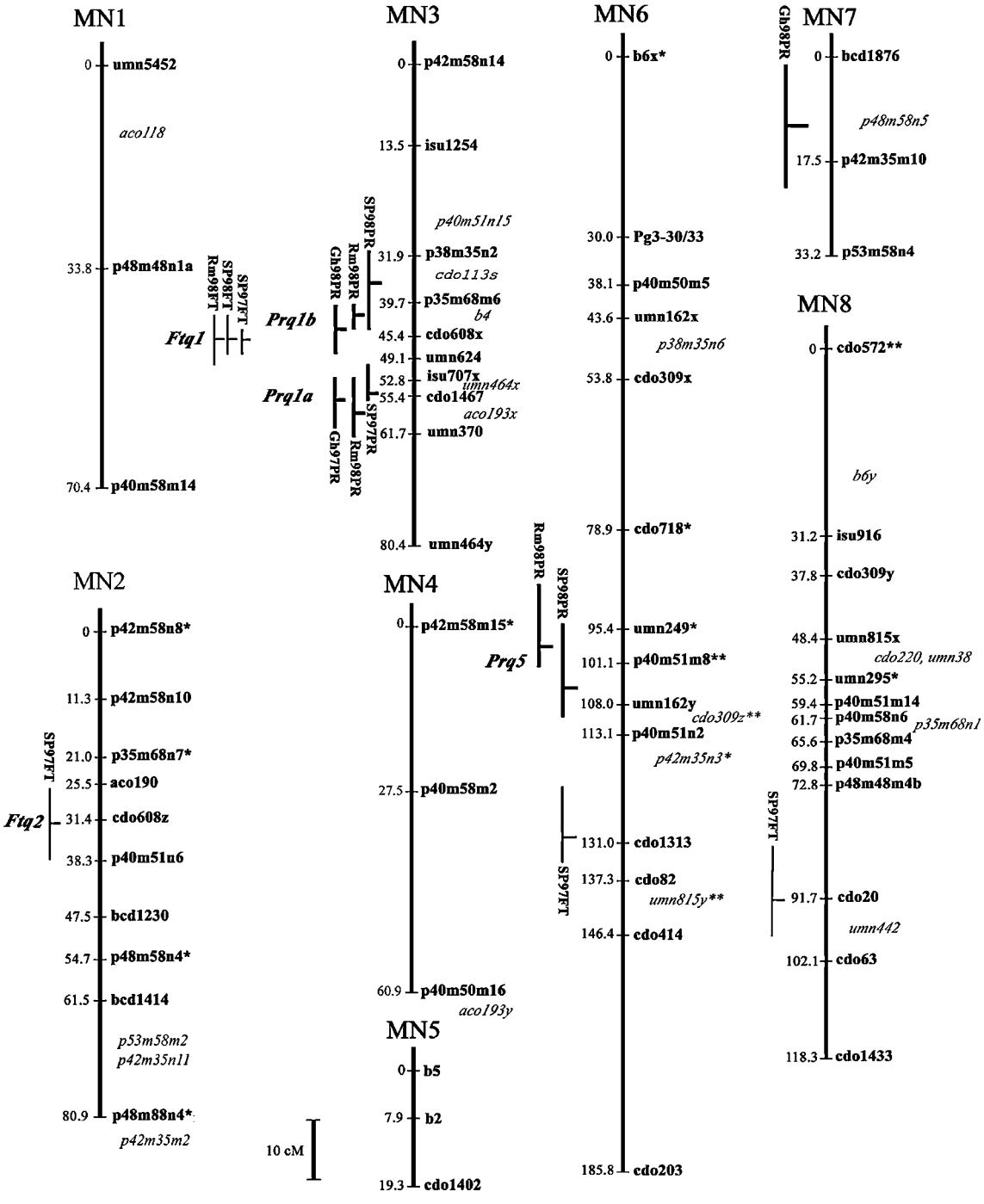

Fig. 2 (Contd.)

tected QTLs revealed in the greenhouse tests ranged from 39.2% in Gh97PR to 44.2% in the Gh98PR data set (Table 4).

QTLs for flowering time in field environments

From previous studies in oat (Siripoonwiwat et al. 1996; Holland et al. 1997, 2002), we knew that there may be many weak QTLs for flowering time (heading date). To avoid excessive type II error (rejection of weak but valid QTLs) for flowering time, we relaxed the significance threshold to LOD 2.4 and accepted any QTL detected in at least one environment by CIM and confirmed in one other environment by CIM or cross-validation tests with the same peak position. A total of seven significant QTLs were detected across all tests (Table 5; Fig. 2). The two most consistent major QTLs across all environments were *Ftq1* and *Ftq7*, with lateness coming from MN841801-1. The *Ftq1* was localized on linkage group MN3 within the marker interval cdo608x–umn62 (Table 5; Fig. 2). *Ftq7* was associated with the unlinked

Table 3 Summary of quantitative trait locus (QTL) analysis for partial crown rust resistance for three separate field environments (SP97PR, SP98PR, and Rm98PR) based on percent pustule area of total leaf area

Linkage group	QTL	SP97PR				SP98PR				Rm98PR			
		Marker[a] (Pos/SI)	LOD	R^2×100	Add[b]	Marker[a] (Pos/SI)	LOD	R^2×100	Add[b]	Marker[a] (Pos/SI)	LOD	R^2×100	Add[b]
MN3	*Prq1a*	isu707x(54/50–56)	4.0	7.0	1.7		–			cdo1467(58/52–62)	3.4	6.2	5.2
	Prq1b		–			p38m35n2(36/30–44)	5.9	12.1	5.0	p35m68m6(42/40–44)	18.5	5.2	4.7
MN6	*Prq5*		–[c]			p40m51m8(104/94–110)	4.0	2.9	2.3	umn249(98/88–102)	3.3	7.5	3.4
MN12			–				–			cdo1509(16/12–20)	3.9	8.0	3.4
MN14	*Prq3*	umn856(36/28–48)	3.2	8.6	1.9		–			umn856(30/24–36)	3.7	6.9	3.1
MN26	*Prq2*	umn498(12/8–12)	4.5	9.6	2.0	umn498(12/8–12)	9.2	14.8	5.2	umn23(6/2–10)	11.9	16.8	5.4
Total $R^2 \times 100$				21.8				25.9				51.4	
Unlink $R^2 \times 100$	*Prq7*	p56m48n2		8.5*				0.09				17.3**	

*Significant at $P < 0.01$, **Significant at $P < 0.001$

[a]Name of the flanking (from top of a linkage group) marker/ support interval (LOD > 1.0)

[b]Additive effect

[c]Cross-validation tests have detected a QTL at 100 cM from top of MN6 within an interval of 96–102 cM in 30.8% of cases

AFLP marker p56m48n2 (Table 5; Fig. 2). The remaining five QTLs detected by a combination of CIM and cross-validation tests were minor ones (Table 5). It was also noted that six of the nine QTLs detected in SP97FT were associated with distorted marker loci (Fig. 2). A similar association between distorted regions of the oat genome and heading date QTLs had been reported earlier by Holland et al. (2002).

Pleiotropy/linkage between QTLs

Comparison of QTLs for flowering time and rust resistance showed that the map regions of *Ftq1* and *Prq1b* overlapped (Tables 3, 4, 5; Fig. 2). This correspondence might be due to either tight linkage or pleiotropy. The log-likelihood peaks for the two QTLs differed slightly in all three environments, suggesting that the linkage hypothesis may be preferable. Another oat genomic region, one associated with unlinked AFLP marker, p56m48n2, showed a similar pleiotropy/linkage relation for flowering time and rust resistance in single-marker regression analysis.

Discussion

Genetic basis of partial resistance to crown rust

Low broad-sense heritability estimates (0.30–0.44) for partial rust resistance limits our ability to detect QTLs important across environments. Better QTL detection power probably can be achieved in a larger population and with a correlation between replicates higher than was obtained in SP97PR and Rm98PR. In the current data set, PLABQTL CIM and single-marker regression analysis involving unlinked markers revealed a total of seven QTLs for rust resistance (rust severity). Six were discovered in three field environments and five in two greenhouse experiments with four of the QTLs being in common. These four most consistently identified QTLs, *Prq1a*, *Prq1b*, *Prq2*, and *Prq7*, can be considered major QTLs. *Prq1a* and *Prq1b* were linked on MN3 and being detected simultaneously, they absorbed the effects of each other and demonstrated reduced R^2 values. However, when either of them was tested alternately, they showed phenotypic contributions equal in value (up to 20–21%, Gh97PR) to that of *Prq2* (SP98PR and all greenhouse data sets). A similar contribution was seen for *Prq7* (up to 22.4%, Gh98PR). It should be noted that the R^2 values were generally higher in the greenhouse. The remaining three minor QTLs (R^2 values up to 8.6%, SP97PR) seemed to be environment sensitive. *Prq3* and *Prq5* were detected only in the field, whereas *Prq6* was revealed only in greenhouse tests. In cross-validation, these minor QTLs also showed less sharp log-likelihood peaks than the major QTLs (data not shown) and expanded support intervals, except for *Prq6*

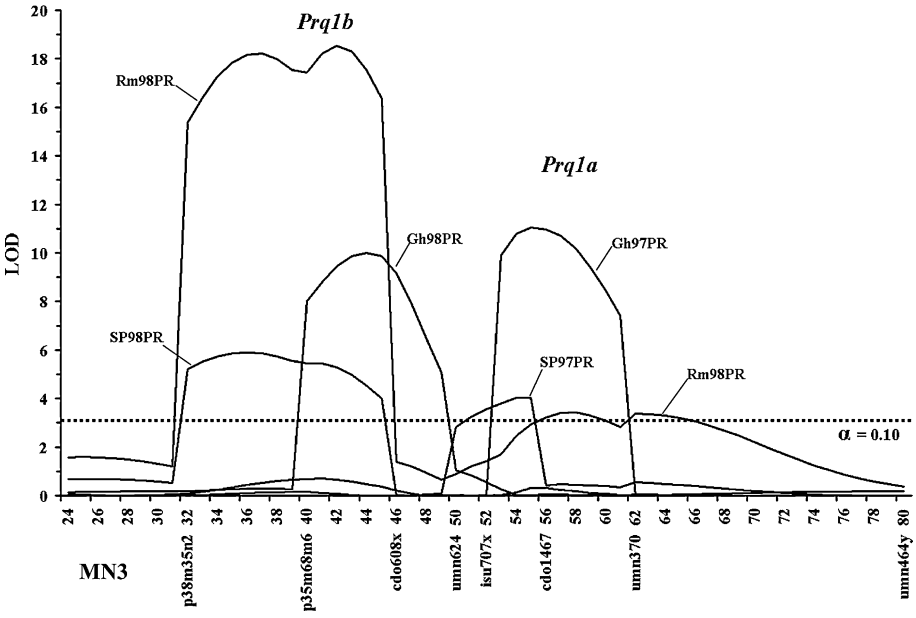

Fig. 3 A fragment of linkage group MN3 with linked QTLs *Prq1a* and *Prq1b*. The *x-axis* shows the distances in centiMorgans (Haldane) from the *top* of MN3 and the marker loci at their appropriate positions. The *curves* superimposed represent the QTL likelihood (*LOD*) scores calculated by the PLABQTL composite interval mapping. The *peaks* representing the highest LOD *scores* indicate the most probable positions of the QTLs. Each of the peaks is labeled using the abbreviated codes of the environments described in "Materials and methods." A *dotted* horizontal line shows the threshold for significance of the QTLs at a genome-wide $\alpha = 0.10$ (LOD = 3.13)

(Fig. 2). Two additional minor QTLs were found each in a single environment (on MN12 in Rm98PR and on MN9 in Gh98PR); however, they were not considered established, because they were not confirmed in at least one more experiment by either CIM or cross-validation. All additive effects were positive suggesting that the 'Noble-2' parent increased the values (rust severity), i.e., all resistance alleles were inherited from MN841801-1. The QTLs discovered by CIM explained from a fifth (SP97PR) to half (Rm98PR) of the total phenotypic variance for rust severity in various experiments. In addition, the unlinked marker p56m48n2 detected a region with an additional major QTL for rust resistance, which was designated as *Prq7*.

The identification of numerous genetic regions influencing partial crown rust resistance in the MN841801-1 source raises the question of the map relationship of these regions to ones containing qualitative and quantitative resistance genes and RGAs localized in other studies. Bush et al. (1994), Bush and Wise (1996, 1998), and Rooney et al. (1994) mapped crown rust resistance *Pc* genes either directly or by marker association to KO linkage groups KO4, KO11, KO12, and KO13 as well as to some unassigned genomic segments. O'Donoughue et al. (1996) identified associated markers for oat stem rust resistance genes *Pg9* and *Pg4*, and by extrapolative mapping also several linked *Pc* genes, and localized them to KO4 and KO3 by comparative mapping. Cheng et al. (2002) placed *Lrk10*-like receptor kinase sequences and other RGAs on KO linkage groups 3_38, 4_12, 5, 6, and 13. Several RGAs were placed on another oat genome map in regions putatively homologous to KO3, 4, 5, 6, 22, and 30 (Portyanko et al. 2001). Zhu and Kaeppler (2003) and Zhu et al. (2003) mapped two QTLs for crown rust resistance introgressed from a diploid oat, *A. strigosa*, in regions with homology to KO15 and KO33. A lack of common markers mapped in various test populations made additional comparisons of resistance gene locations difficult.

Placement in this study of the partial-resistance QTL *Prq1b* to linkage group MN3 close to the RFLP markers cdo608x and cdo1467 and the SCAR marker cdo113s (Fig. 2) indicates a putative correspondence between MN3 and Pendek4838-1, a linkage group identified in a different cross to which Wight et al. (2004) mapped *Pc38* close to the same three markers. We suggest that *Prq1b*, therefore, could be associated with *Pc38* and other resistance genes, including *Pc62* and *Pc63*, clustered with *Pc38* (Harder et al. 1980). A putative correspondence can also be found between some of the resistance genes in the MN6 region containing *Prq5* reported here and RGAs reported previously in KO6. Portyanko et al. (2001) showed that on both arms of linkage group OT8 (putatively homoeologous to KO6) were sequences detected by sorghum RGAs; these were linked in one case with cdo1467 and in the other case with umn162a. Similar duplicated RGA regions on KO6 were found by Cheng et al. (2002). In our research, the RFLP probe umn162 detected duplications on linkage group MN6, which is also putatively homoeologous to KO6. On the upper part of MN6, the umn162x marker was placed not far from Pg-30/33 (Fig. 2), a SCAR marker for the *Pg3*/*Pg9*/*Pcx* cluster of resistance genes (Harder et al. 1995). On the lower portion of MN6, the marker umn162y was tightly linked to *Prq5* (Fig. 2). In contrast to results in our study, O'Donoughue et al. (1996) mapped *Pg9* (and, putatively, the rest of the cluster including *Pg3*) to KO4. However, this discrepancy may be explained by the homeology between KO4 and KO6 and may indicate the presence of similar clusters of resistance genes on both of the chromosomes.

Despite some differences in marker order, probably caused by duplications, our data are in general agreement with the results obtained by Irigoyen et al. (2004),

Table 4 Summary of QTL analyses for crown rust resistance for two separate greenhouse environments (Gh97PR and Gh98PR) based on pustule number per cm^2 of the oat leaf

Linkage group	QTL	Gh97PR				Gh98PR			
		Marker(Pos/SI)	LOD	$R^2\times100$	Add	Marker (Pos/SI)	LOD	$R^2\times100$	Add
MN3	*Prq1a*	cdo1467(56/52–60)	11.0	26.8	4.7		–		
	Prq1b		–			p35m68m6(44/40–48)	10.0	23.7	3.8
MN7			–			bcd1876(12/2–22)	3.2	6.3	2.0
MN9	*Prq6*		–[a]			p40m50m4(80/74–94)	3.6	4.7	2.1
						p42m35n12(122/116–130)	5.7	5.8	−2.3
MN14		p42m58m9a(12/0–24)	3.3	5.7	2.5		–		
MN17			–			p42m35n6(6/2–10)	3.5	6.0	1.7
	Prq2	umn23(4/0–8)	9.0	17.8	3.9	umn23(4/0–8)	9.0	17.8	3.9
Total $R^2\times100$				39.2				44.2	
Unlinked $R^2\times100$	*Prq7*	p56m48n2		14.6*				24.4*	

*Significant at $P<0.001$

[a]Detected at sub-optimal LOD 2.5 ($R^2\times100=3.6$, Add = 1.6) within an interval of 88–102 cM at 94 cM from top of MN9

Table 5 Summary of QTL analyses for flowering time for three field environments (SP97FT, SP98FT, and Rm98FT) with cross-validation

Linkage group	QTL	SP97FT					SP98FT					Rm98FT				
		Marker (Pos/SI)	LOD	$R^2\times100$	Add	Cross-vaidationl (%)	Marker (Pos/SI)	LOD	$R^2\times100$	Add	Cross-validation (%)	Marker (Pos/SI)	LOD	$R^2\times100$	Add	Cross-validation (%)
MN2	*Ftq2*	cdo608z(32/26–38)	4.5	8	0.61**			–			7	–		0		
		p48m58n(60/56–66)	3.0	4.6	−0.45**	12		–			0	–				
MN3	*Ftq1*	cdo608x(46/44–48)	20.7	45.1	−1.344**	94	cdo608x (46/42–48)	17.2	29.9	−2.23**	95	cdo608x (46/42–50)	8.14	25.0	−0.89**	94
							isu1254 (18/0–28)	2.7	3.7	−0.76*	4		–		–	
MN6		p40m51n2(130/122–134)	2.9	5.5	0.37**	34		–			0	–		–		
MN8		Cdo20(92/82–98)	2.5	5.9	−0.38**	10		–			–	–		–		
MN16	*Ftq3*	p4(18/12–18)2m35n1	2.4	4.6	0.34**	16		–			11	–		–		
MN17	*Ftq4*	p38m35m7(88/80–88)	2.5	5.5	0.38**	21		–			4	–		2		
MN22	*Ftq5*	p42m58m11(70/52–80)	3	9.5	0.52**	43		–			1	p42m58m11 (70/56–78)	2.4	3.1	0.29*	26
MN29	*Ftq6*	bcd110(10/6–14)	3.5	7.2	−0.42**	23		–			2	–		9		
Total $R^2\times100$				56.7					48.9					27.1		
Unlinked $R^2\times100$	*Prq7*	p56m48n2		19.3***					30.5***					13.7***		

*Significant at $P<0.05$, **Significant at $P<0.01$, ***Significant at $P<0.001$

who found some clustering of RGAs and genes for resistance to various diseases on linkage group KO17. We found on linkage group MN3 (putatively homologous to KO17) clustering of the locus for cdo113s (a marker for *Pc38*, Wight et al. 2004) with a RGA b4 locus, and the QTL *Prq1b* (Fig. 2). The detection of a QTL for crown rust partial resistance in the field environment Rm98PR (Fig. 2) that mapped in the vicinity of cdo1509 on linkage group MN12 (putatively homoeologous to KO11_41 + 20) also agrees well with an RGA being near cdo1509 on the KO11_41 + 20 linkage group (Irigoyen et al. 2004).

QTLs for flowering time

CIM detected six QTLs for flowering time. Only one of them, *Ftq1*, located on MN3 could be declared as major, being consistent across all environments. Because we found MN3 putatively homologous to KO17, *Ftq1* may correspond to a major QTL for flowering time reported on KO17 (Siripoonwiwat et al. 1996; Holland et al. 1997, De Koeyer et al. 2001) and one reported on a region homologous to KO17 in linkage group OT32 in another study (Holland et al. 2002). A second major QTL, *Ftq7*, was revealed by single-marker analysis through association with unlinked marker p56m48n2. The other five QTLs detected by CIM had very weak effects and only one of them, *Ftq5* on MN22, was detected in two different environments. Four QTLs for heading date were found by CIM in SP97FT only and were confirmed by cross-validation in one or two more experiments. A comparison made between the data obtained in this research and earlier reports (Siripoonwiwat et al. 1996; Holland et al. 1997, 2002; De Koeyer et al. 2001) indicates that most of the regions with identified QTLs for heading date are in good agreement.

A low but significant negative correlation between heading date and rust severity was found in some of the environments in our study. Linkages between *Prq1b* and *Ftq1* or *Prq7* and *Ftq7* or a pleiotropic effect of the *Ftq1* or Ftq7 loci on flowering time and rust resistance are possible explanations of this association. A practical implication is that the genotypes selected as more resistant to crown rust may also be later maturing.

Marker-assisted selection

The present study revealed genomic regions responsible for up to half of the variation associated with durable resistance to crown rust in MN841801-1. A total of seven QTLs for crown rust were identified across diverse environments. The detected QTLs demonstrated additive effects suggesting that marker-assisted selection for field rust resistance may be effective in early generations. The strong G × E interactions found may limit effectiveness of phenotypic selection. Six of the QTLs were detected in two different field environments (St. Paul and Rosemount), with four of them also revealed in the greenhouse. Therefore, these six QTLs most likely have limited environmental sensitivity under field conditions and may be considered good candidates to incorporate into oat breeding programs. The marker-assisted selection approach was designed to help overcome the difficulties of conventional phenotypic selection for traits with low and moderate heritability. The relatively low heritability estimates we obtained for rust severity (0.30–0.44) indicate that marker-assisted selection should be more effective than phenotypic evaluation of the breeding material if informative tightly linked markers can be identified. The results suggest that at least the four most consistent major QTLs for crown rust resistance can be transferred from MN841801 into agronomically important lines using markers.

A considerable proportion of oat genomic regions responsible for field resistance to crown rust remains to be revealed. One of the possible ways to resolve this issue would be to increase the oat genome coverage with markers. Using a mapping population of a larger size may also be helpful. In addition, it would be desirable to perform validation of the detected QTLs in diverse genetic backgrounds and additional test environments.

Acknowledgements This research was supported in part by the Quaker Oats Company, Chicago, Ill. We thank Suzanne Livingston, Roger Caspers, and Richard Halstead for excellent technical assistance.

References

Berger RD, Luke HH (1979) Spatial and temporal spread of oat crown rust. Phytopathology 69:1199–1201

Bush AL, Wise RP (1996) Crown rust resistance loci on linkage groups 4 and 13 in cultivated oat. J Hered 87:427–432

Bush AL, Wise RP (1998) High resolution mapping adjacent to the *Pc 71* crown-rust resistance locus in hexaploid oat. Mol Breed 4:13–21

Bush AL, Wise RP, Rayapati PJ, Lee M (1994) Restriction fragment length polymorphisms linked to genes for resistance to crown rust (*Puccinia coronata*) in near-isogenic lines of hexaploid oat (*Avena sativa*). Genome 37:823–831

Chen G, Portyanko VA, Rines HW, Phillips RL, Leonard KJ, Ochocki GE, Stuthman DD (2000) Identification of QTLs for partial resistance to crown rust of oats. In: 2000 Agronomy abstracts. American Society of Agronomy, Madison, pp 187

Cheng DW, Armstrong KC, Tinker N, Wight CP, He S, Lybaert A, Fedak G, Molnar SJ (2002) Genetic and physical mapping of *Lrk10*-like receptor kinase sequences in hexaploid oat (*Avena sativa* L). Genome 45:100–109

De Koeyer DL, Phillips RL, Stuthman DD (2001) Allelic shifts and quantitative trait loci in a recurrent selection population of oat. Crop Sci 41:1228–1234

De Koeyer DL, Tinker NA, Wight CP, Deyl J, Burrows VD, O'Donoughue LS, Lybaert A, Molnar SJ, Armstrong KC, Fedak G, Wesenberg DM, Rossnagel BG, McElroy AR (2004) A molecular linkage map with associated QTLs from a hulless × covered spring oat population. Theor Appl Genet 108:1285–1298

Groh S, Kianian SF, Phillips RL, Rines HW, Stuthman DD, Wesenburg DM, Fulcher RG (2001b) Analysis of factors influencing milling yield and their association to other traits by

QTL analysis in two hexaploid oat populations. Theor Appl Genet 103:9–18
Groh S, Zacharias A, Kianian SF, Penner GA, Chong J, Rines HW, Phillips RL (2001a) Comparative AFLP mapping in two hexaploid oat populations. Theor Appl Genet 102:876–884
Haldane JBS (1919) The combination of linkage values and the calculation of distances between the loci of linked factors. J Genet 8:299–309
Harder DE, McKenzie RIH, Martens JW (1980) Inheritance of crown rust resistance in three accessions of *Avena sterilis*. Can J Genet Cytol 22:27–33
Harder DE, McKenzie RIH, Martens JW (1984) Inheritance of adult plant resistance to crown rust in an accession of *Avena sterilis*. Phytopathology 74:352–353
Harder DE, Chong J, Brown PD (1995) Stem and crown rust resistance in the Wisconsin oat selection X1588-2. Crop Sci 35:1011–2015
Heagle AS, Moore MB (1970) Some effects of moderate adult resistance to crown rust of oat. Phytopathology 60:461–466
Heun M, Kennedy AE, Anderson JA, Lapitan NLV, Sorrells ME, Tanksley SD (1991) Construction of a restriction fragment length polymorphism map for barley (*Hordeum vulgare*). Genome 34:437–447
Holland JB, Moser HS, O'Donoughue LS, Lee M (1997) QTLs and epistasis associated with vernalization responses in oat. Crop Sci 37:1306–1316
Holland JB, Portyanko VA, Hoffman DA, Lee M (2002) Genomic regions controlling vernalization and photoperiod responses in oat. Theor Appl Genet 105:113–126
Irigoyen ML, Loarce Y, Fominaya A, Ferrer E (2004) Isolation and mapping of resistance gene analogs from the *Avena strigosa* genome. Theor Appl Genet 109:713–724
Jin H, Domier LL, Kolb FL, Brown CM (1998) Identification of quantitative loci for tolerance to barley yellow dwarf virus in oat. Phytopathology 88:410–415
Kianian SF, Egli MA, Phillips RL, Rines HW, Somers DA, Gengenbach BG, Webster FH, Livingston SM, Groh S, O'Donoughue LS, Sorrells ME, Wesenberg DM, Stuthman DD, Fulcher RG (1999) Association of a major groat oil content QTL and an acetyl-CoA carboxylase gene in oat. Theor Appl Genet 98:884–894
Kiehn FS, McKenzie RIH, Harder DE (1976) Inheritance of resistance to *Puccinia coronata avenae* and its associations with seed characteristics on four accessions of *Avena sterilis*. Can J Genet Cytol 18:717–726
Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181
Leister D, Kurth J, Laurie DA, Yano M, Sasaki T, Graner A, Schulze-Lefert P (1999) RFLP and physical mapping of resistance gene homologues in rice (*O. sativa*) and barley (*H. vulgare*). Theor Appl Genet 98:509–520
Leonard KJ (2002) Oat lines with effective adult plant resistance to crown rust. Plant Dis 86:593–598
Li CD, Rossnagel BG, Scoles GJ (2000) The development of oat microsatellite markers and their use in identifying relationships among *Avena* species and oat cultivars. Theor Appl Genet 101:1259–1268
Luke HH, Barnett RD, Pfahler PL (1975) Inheritance of horizontal resistance to crown rust in oat. Phytopathology 65:631–632
Luke HH, Pfahler PL, Barnett RD (1981) Influence of disease severity and environmental conditions on low receptivity of oats to crown rust. Plant Dis 65:125–127
Ma Z, Steffenson BJ, Prom LK, Lapitan NLV (2000) Mapping quantitative trait loci for Fusarium head blight resistance in barley. Phytopathology 90:1079–1088
O'Donoughue LS, Kianian SF, Rayapati PJ, Penner GA, Tanksley ME, Phillips RL, Rines HW, Lee M, Fedak G, Molnar SJ, Hoffman D, Salas CA, Wu B, Autrique E, Van Deynze A (1995) A molecular linkage map of cultivated oat. Genome 38:368–380
O'Donoughue LS, Chong J, Wight CP, Fedak G, Molnar SJ (1996) Localization of stem rust resistance genes and associated molecular markers in cultivated oat. Phytopathology 86:719–727
Ohm HW, Shaner G (1992) Breeding oat for resistance to diseases. In: Marshall HG, Sorrells ME (eds) Oat science and technology, Agronomy monograph No 33. American Society of Agronomy and Crop Science Society of America, Madison, pp 657–698
Ohm HW, Patterson FL, Roberts JJ, Shaner GF (1974) Registration of Noble oats. Crop Sci 14:906
Peterson RF, Campbell AB, Hannah AE (1948) A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. Can J Res Sect C 26:496–500
Peturson B (1944) Adult plant resistance of some oat varieties to physiologic races of crown rust. Can J Res Sect C 22:287–289
Portyanko VA, Hoffman DL, Lee M, Holland JB (2001) A linkage map of hexaploid oat based on grass anchor DNA clones and its relationship to other oat maps. Genome 44:249–265
Rooney WL, Rines HW, Phillips RL (1994) Identification of RFLP markers linked to crown rust resistance genes *Pc91* and *Pc92* in oat. Crop Sci 39:940–944
Saghai Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci USA 81:8014–8018
Searle SR (1971) Linear models. Wiley, NY, p 475
Simons MD (1985) Crown rust. In: Roelfs AP, Bushnell WR (eds) The cereal rusts, vol II. Diseases, distribution, epidemiology, and control. Academic, Orlando, pp 131–172
Siripoonwiwat W, O'Donoughue LS, Wesenberg D, Hoffman DL, Barbosa-Neto JF, Sorrells ME (1996) Chromosomal regions associated with quantitative traits in oat. J Ag Genomics 2 (available at http://www.cabi-publishing.org/jag/papers96/paper396/indexp396. html)
Sokal R, Rohlf FJ (1995) Biometry: the principles and practice of statistics in biological research. Freeman, New York
Utz HF, Melchinger AE (1996) PLABQTL: a program for composite interval mapping of QTL. J Ag Genomics 2: (available at http://www.cabi-publishing.org/jag/index.html).
Wight CP, Tinker NA, Kianian SF, Sorrells ME, O'Donoughue, LS, Hoffman DL, Groh S, Scoles GJ, Li CD, Webster FH, Phillips RL, Rines HW, Livingston SM, Armstrong KC, Fedak G, Molnar SJ (2003) A molecular map in 'Kanota' × 'Ogle' hexaploid oat (*Avena* spp.) enhanced by additional markers and a robust framework. Genome 46:28–47
Wight CP, O'Donoughue LS, Chong J, Tinker NA, Molnar SJ (2004) Discovery, localization, and sequence characterization of molecular markers for the crown rust resistance genes *Pc38, Pc39*and *Pc48* in cultivated oat (*Avena sativa* L). Mol Breed 14:1–13
Wise RP, Schnable PS (1994) Mapping complementary genes in maize: Positioning the *rf1* and *rf2* nuclear-fertility restorer loci of Texas (T)-cytoplasm relative to RFLP and morphological markers. Theor Appl Genet 88:785–795
Zhu S, Kaeppler HF (2003) Identification of quantitative trait loci for resistance to crown rust in oat line MAM17-5. Crop Sci 43:358–366
Zhu S, Leonard KJ, Kaeppler HF (2003) Quantitative trait loci associated with seedling resistance to isolates of *Puccinia coronata* in oat. Phytopathology 93:860–866