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Identification and Validation of Quantitative Trait Loci for Partial Resistance to Crown Rust in Oat

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ABSTRACT

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Management of oat crown rust disease with host resistance is challenging because major gene resistance is generally short lived. Partially resistant oat cultivars could benefit oat growers by providing more durable resistance. The objective of this study was to validate and discover quantitative trait loci (QTL) affecting crown rust resistance in the partially resistant oat line MN841801-1 using conventional and molecular assessments of disease produced in single-race greenhouse inoculations, single-race polycyclic field tests, and under natural infection in disease-conducive environments. Crown rust was assessed on 150 $F_{6:9}$ MN841801-1/'Noble-2' recombinant inbred lines. In total, eight QTL associated with MN841801-1 alleles were detected. Of these, seven matched QTL previously identified while a new QTL (*Prq8*) was detected on linkage group MN13. Four QTL (*Prq1a*, *Prq2*, *Prq7*, and *Prq8*) were consistently detected and predicative genetic assays for these QTL should be developed for future validation in additional genetic backgrounds.

Crown rust, caused by *Puccinia coronata* f. sp. *avenae*, is the most important disease of cultivated oat (*Avena sativa* L.) worldwide, causing significant yield loss and reducing seed quality (24,37,38). Genetic resistance is an effective and economical method of managing crown rust in oat. Many major genes for resistance to crown rust (*Pc* genes) have been identified in cultivated oat and its wild relatives (7,37) but these genes are race specific and have not provided durable resistance even when used in combination (9,11,37). Therefore, alternative strategies are needed to effectively manage crown rust with genetic resistance.

Partial resistance (PR) is defined by a reduction in the amount of disease in spite of a compatible host–pathogen interaction (e.g., a susceptible infection type in cereal rust systems). Additionally, PR is considered to be largely race nonspecific and is typically polygenic (29), thus reducing selection pressure on the pathogen and delaying the evolution of new virulent races (36). In an effort to enhance crown rust resistance, a series of oat lines combining different sources of resistance were developed in the 1960s at the University of Minnesota. Later, 14 lines were selected and retested and several showed good field resistance over several years despite being susceptible as seedlings to >85% of the isolates tested in greenhouse screenings (23). One of the lines, MN841801, remained resistant in field plots in the adult-plant stage, developing <10% as many uredinia as the susceptible check over 7 years of field testing using natural inoculum consisting of a diverse mixture of *P. coronata* races (23).

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The inheritance of PR in MN841801 was studied by Chong (6) using 154 $F_{7:9}$ single-seed-descent lines of the 'AC Assiniboia'/MN841801 population. Chong reported two genes controlling adult-plant crown rust resistance in the population based on rust infection types (ITs) in growth chamber tests (6). Similarly, Portyanko et al. (31) studied the genetics of PR in a line derived from MN841801 (MN841801-1) using a mapping population of 158 $F_{6:8}$ MN841801-1/'Noble-2' (MN) recombinant inbred lines (RILs). They detected four major and three minor quantitative trait loci (QTL) affecting crown rust resistance in the population and suggested that the QTL should be validated in additional test environments.

Measurement of PR can be laborious and time consuming. Traditionally, assessments are based on visual scoring of IT and disease severity or by careful measurement of pustule size, pustules per leaf, latent period, disease progress, and sporulation capacity (3,4,25). Over the past few years, digital image analysis has been used and proved to be a more precise and accurate measure of diseased leaf area (DLA) compared with visual ratings (13,20). More recently, quantification of pathogen DNA content in infected host tissue has been successfully applied to the study of crown rust resistance using a quantitative real-time polymerase chain reaction (q-PCR) assay and single-race field tests (19,20). Single-race field tests allowed a precise evaluation of disease reaction without the confounding effects of multiple races of the pathogen.

The objective of this study was to identify and validate the PR QTL in MN841801-1 using conventional and molecular assessments of disease from single-race greenhouse inoculations, polycyclic single-race field tests, and natural infection in disease-conducive environments.

MATERIALS AND METHODS

Plant materials. The 150 $F_{6:8}$ RILs of the MN population developed by Portyanko et al. (31) were used in this study. To

obtain seed for multilocation trials, the population was advanced to the $F_{6:9}$ generation via bulk increase in Aberdeen, ID. Noble-2, a cultivar with good agronomics (28), is highly susceptible to crown rust.

Fungal isolates and inoculum production. Two *P. coronata* races, LSLG (isolate 93MNB236) and BRCB (isolate CR251), with virulence to both parents at the seedling stage (5,10) were used to evaluate PR. LSLG and BRCB isolates were derived from single-pustule cultures obtained from Dr. Marty Carson at the United States Department of Agriculture–Agricultural Research Service Cereal Disease Laboratory, St. Paul, MN and Dr. James Chong, respectively. The isolates were increased on the susceptible cv. Provena in separate growth chambers to prevent cross-contamination.

Single-race field experiments. The RIL population was sown in irrigated field plots at the University of Idaho Research and Extension Center in Aberdeen, ID in 2007. The Aberdeen 2007 (Ab07) experiments were carried out using a randomized complete block design (RCBD) with two replicates. Each experiment used a different *P. coronata* race. Within each block, 25 to 30 seeds/line were sown in a single hill. The hills were planted 35.5 cm apart on 36-cm centers. A single hill of the susceptible cv. Provena was sown adjacent to each test plot throughout the block as inoculum sources and two plots of each parent were randomly included in each block. In addition, 16 crown rust gene differentials (10) were included at the end of each block and a wheat border was used to reduce edge effects.

To create the environmental conditions necessary for disease development, the blocks were surrounded by a 6-mm polyethylene sheet attached to a wooden frame as described by Jackson et al. (20). Beginning at the fifth-leaf stage, the Provena plots were inoculated at dusk twice per week for three consecutive weeks using a hand-held garden sprayer. The blocks were irrigated 30 min before inoculation, then covered overnight (14 h) with polyethylene sheeting. After the last inoculation, the blocks were covered four additional times at 3-day intervals to promote disease development.

Forty days after the first inoculation, four representative flag leaves per plot were removed and photographed with an Olympus digital camera model SP-510UZ (3,072-by-2,304 pixel resolution) mounted on a portable stand at a fixed distance of 30 cm. A 2-cm^2 section at the midpoint of each leaf was excised and the four samples were pooled for oat and fungal DNA (FDNA) extraction and subsequent DNA estimation. DLA and IT were assessed from the digital images using the ASSESS image analysis software (21). The threshold settings used for DLA estimation from digital images were 103 and 60, as previously described by Jackson et al. (20). These settings take into account pustules and a portion of the adjacent chlorotic area. IT was based on the standard 0-to-4 scale (27) as 0 = no visible symptoms, ; = hypersensitive flecks, 1 = small uredinia surrounded by distinct necrosis or chlorosis, 2 = small-to-medium uredinia surrounded by chlorosis, 3 = medium to large uredinia with or without chlorosis, and 4 = large uredinia without chlorosis. Days to heading (HD) was recorded for each entry as the date on which 50% of the panicles in a hill had emerged from the boot.

In 2008, a single-race field experiment (Ab08) using race LSLG (isolate 93MNB236) was done using the same experimental design as described for the Aberdeen 2007 (Ab07) experiments. Forty days after the first inoculation, the plots were visually scored for IT and for disease severity (DS) using the modified Cobb scale (30) as a reference. HD was also recorded for each entry.

The population was also tested in Canada at the Agriculture and Agri-Food Canada Field Station, Glenlea, Manitoba, in a field experiment in 2008 (MB08). Test plots were seeded in 1-m rows with 0.3-m spacing. The experiment was a RCBD with three replicates. Plots of each parent were randomly planted in each replicate. One week before planting the RIL population, six rows of the susceptible cvs. Makuru and AC Assiniboia, mixed in a 1:3 proportion, were planted 10 m from the boundary of the test plot location in all four directions. A local crown rust epidemic was generated by inoculating the susceptible border plots with urediniospores of *P. coronata* isolate CR251 (race BRCB) when the plants in the border plots were at the tillering stage (Zadoks scale 23) (43). The 10-m separation between the susceptible border plot and test plots was aimed at providing a uniform spread of inoculum into the test plots. The standard 16 crown rust gene differentials were planted at the beginning and end of the experimental block to check for the presence of other races migrating into the plots.

Crown rust severity (DS) of each RIL and parents was assessed by visually scoring the three uppermost leaves of plants in the entire plot. Each of the plots was also scored for IT using the following scale: R = resistant, MR = moderately resistant, MS = moderately susceptible, and S = susceptible. Additionally, digital images (3,264-by-2,448 pixel resolution) of three flag leaves with crown rust symptoms typical of the overall reaction for each plot were individually taken with a Canon digital camera (PowerShot model A720) mounted on a portable stand at a fixed distance of 16 cm. DLA was obtained from the images for individual RILs and parents using the ASSESS program, as previously described.

Single-race greenhouse experiments. Greenhouse experiments were conducted in 2007 (Gh07) using an RCBD with two replicates. In total, 150 RILs were tested for their disease reaction against the same *P. coronata* isolates used in Ab07 and Ab08 field experiments. Two seeds of each RIL were sown in 15-cm pots containing a 1:2:1 (vol/vol/vol) mix of sand, peat moss, and vermiculite. Two replicates of each parent and the susceptible check Provena were included in each block. Once plants were at the five- to six-leaf stage, a 5-cm section at the midpoint of each fifth or sixth leaf was inoculated with a 2.5×10^6 uredinium suspension using the direct application method described by Jackson et al. (20). Leaves were allowed to dry and the plants were then incubated overnight (16 h) in a dew chamber at 15.5 ± 1°C and 100% relative humidity. Ten days after inoculation, leaf sections were excised and digital images were taken from two leaves per line for DLA and FDNA estimation as described for field experiments.

Disease-conducive environment field experiments. An RCBD with two replicates was used to evaluate the RIL population under natural crown rust pressure at the Louisiana State University research station in Baton Rouge in 2007 (LA07) and 2008 (LA08). Additionally, in 2007, the population was evaluated in a grower field in Castroville, TX through cooperation with Texas A&M AgriLife Research (TX07). For each RIL, 4 g of seed was planted in 1-m, two-row plots on 38-cm centers. Plots containing one row of each parental line were planted every 18th row and plots of the susceptible cv. Brooks were planted adjacent to each test plot. The crown rust gene differentials were included in each block to determine the *P. coronata* virulence spectrum. In late March, four representative flag leaves per plot were removed and photographed for DLA estimation as previously described for the Ab07 single-race field experiments. In the LA08 experiment, the plots were assessed for DS and IT twice, beginning at growth stage 50 on the scale of Zadoks et al. (43); first, on leaves flag minus 1, 2, 3, and 4 (lower leaves) for each plot and again, 2 weeks later, on the flag leaves. HD of each entry in the LA08 experiment was recorded as described previously.

DNA extraction and amplification. All single-race field and greenhouse leaf samples were freeze dried and genomic DNA was extracted using cetyltrimethylammonium bromide DNA extraction protocol (39). To estimate the amount of pathogen in each leaf sample a new relative FDNA (RFDNA) estimation technique was used (1). In short, rather than weighing each sample individually, the method of Jackson et al. (20) was modified to allow

the simultaneous amplification of both plant and pathogen DNA (2,40). FDNA and oat DNA were amplified using the TaqMan probe and primers designed for *P. coronata* and oat β-actin, respectively (1). All q-PCR amplifications were conducted in duplicates on an ABI Prism 7000 Sequence Detections System using the Universal Master Mix (Applied Biosystems, Foster City, CA). RFDNA in a sample was estimated using the formula $2^{(hCt - pCt)}$, where hCt is the cycle threshold (Ct) value for the host and pCt is the Ct value for the pathogen in a reaction. Mean RFDNA values were computed for each entry–isolate combination for the field and greenhouse experiments separately.

Data analysis and QTL mapping. For data analysis, traits were defined based on three criteria: (i) experiment (location and year when the test was performed), (ii) inoculum type (single race or natural infection), and (iii) disease measurement. Trait distribution normality was estimated using the Shapiro-Wilk test (35).

Broad-sense heritabilities were calculated per experiment using the genotypic variance divided by the combined genotype + replicate [leaf] + genotype × replicate [leaf] + error variances, as follows: $h^2 = \sigma^2_g/(\sigma^2_g + \sigma^2_r + \sigma^2_{gxr} + \sigma^2_e)$. Broad-sense heritabilities were calculated only for DLA and DS for those experiments for which data were collected for each individual leaf per plant. Mean values across replications were calculated for each RIL and used for QTL analysis and to determine phenotypic correlations in the RIL population. Pearson correlation coefficients between treatments were calculated to determine the relationship between disease measurements evaluated in the different experiments. All statistical analyses were performed using JMP 6.0 statistical software (SAS Institute, Cary, NC).

Genotypic data from a previously published MN linkage map constructed with a total of 230 loci including 114 amplified fragment length polymorphism (AFLP), 113 restriction fragment length polymorphism (RFLP), 1 simple-sequence repeat, and 2 sequence characterized amplified region markers were used for QTL analysis in this study (31). The new map was constructed with MapManager QTXb20 using genotypic data for the MN linkage map (31) available from GrainGenes. Highly heterozygous markers (those containing >30% heterozygotes) were removed and the remaining markers were grouped using the "ri self" linkage evaluation with a search linkage criterion of $P = e^{-6}$. After initial grouping, an attempt was made to join each of the groups together by systematically trying to merge each group to each group in all possible combinations using the distribute function with a linkage criterion of $P = 0.0001$. The best overall marker order was then determined for each of the resulting groups using the ripple function with a linkage criterion of $P = e^{-6}$. The map was then drawn using MapChart 2.2 (41).

Windows QTL Cartographer V2.5 composite interval mapping (CIM) was used to detect QTL in this study (12,44). Experiment-wise significance level (log-likelihood [LOD] = 2.5) was established for QTL detection by running 1,000 permutations for all traits, $\alpha = 0.05$ (12). Forward regression ($P = 0.05$) and a standard CIM model with a 1-centimorgan (cM) walk speed, five control markers, and window size of 5 cM was used for all the analyses. A QTL was declared valid if it was detected with at least two disease measurements in one experiment or if it was detected in the same position in more than one experiment. QTL identified with only one trait were considered putative and in need of further validation to determine usefulness. Multiple intervals mapping was performed on CIM QTL to estimate the total phenotypic variance accounted by the QTL. For comparison purposes, QTL nomenclature in this study follows the convention of Portyanko et al. (31). Because 25 markers were unable to be assigned to MN linkage groups, single-marker analysis (SMA) was used to determine association ($P < 0.01$) between unlinked markers and traits.

RESULTS

Phenotypic distribution. Noble-2 showed higher DLA, RFDNA, IT, and DS than MN841801-1 in all experiments (Table 1). The 150 RILs had a relatively continuous distribution for

TABLE 1. Mean values for infection type (IT), diseased leaf area (DLA) from digital images, relative fungal DNA (RFDNA), and visually estimated disease severity (DS) of the two parents MN841801-1 and 'Noble-2' and recombinant inbred lines (RILs) in single race field and greenhouses tests and in disease-conducive environments under natural disease pressure

Experiment	Race[a]	Trait	Parents		RILs (mean ± SD)[b]	Range	Skewedness	h^{2c}
			MN841801-1	Noble-2				
Single race								
Field tests								
Aberdeen 2007	BRCB	IT	0.3	3.3	1.9 ± 0.75	0.0–4.0	–0.3	…
	…	DLA	1.5	18.8	12.3 ± 9.2	0.5–55.1	1.4	0.38
	…	RFDNA	0.1	16.2	11.2 ± 15.8	0.1–98.9	3.1	…
	LSLG	IT	0	2.5	1.4 ± 0.6	0.0–3.0	–0.2	…
	…	DLA	6.5	18.4	12.5 ± 8.0	1.3–47.3	1.2	0.27
	…	RFDNA	0	34	15.9 ± 28.9	0.0–177.0	6	…
Aberdeen 2008	LSLG	IT	0.8	1.6	1.3 ± 0.4	0.0–2.2	0.6	…
	…	DS	5.4	10.1	7.9 ± 4.5	2.3–26.7	1.7	…
Manitoba 2008	BRCB	IT	0	7	5.2 ± 1.8	0–7	–0.8	…
	…	DS	0.2	36.8	21.7 ± 14.3	1.3–56.7	0.4	0.83
	…	DLA	0	53.1	2.8 ± 2.0	1.7–53.1	1.1	0.13
Greenhouse 2008	BRCB	DLA	0.7	3.9	1.8 ± 1.8	0.02–11.8	2.5	0.1
	…	RFDNA	1.1	8.2	6.8 ± 13.0	0.1–149.0	9	…
	LSLG	DLA	15.7	34.6	25.4 ± 11.4	0.4–51.2	0.2	0.02
	…	RFDNA	30.4	46.1	41.5 ± 32.4	0.8–177.8	1.5	…
Natural infection								
Field tests								
Baton Rouge, LA 2007	Local races	DLA	17	17.2	5.9 ± 4.5	0.3–21.3	1.1	0.23
Castroville, TX 2007	Local races	DLA	2.8	8.8	2.8 ± 2.0	0.3–10.0	1.9	0.14
Baton Rouge, LA 2008	Local races	IT lower leaves	1	7	3.2 ± 1.4	2.0–7.0	1.4	…
	…	DS lower leaves	2	65	16.9 ± 13.1	0–70	1.5	…
	…	IT Flag	0.8	3.6	2.4 ± 0.8	0.0–4.0	–0.3	…
	…	DS Flag	7.0	72.0	34.7 ± 21.6	2.5–86.0	0.5	…

[a] Race of *Puccinia coronata.*
[b] SD = standard deviation.
[c] Broad-sense heritability.

DLA, IT, DS, and RFDNA, as expected for a quantitatively inherited trait. The phenotypic distribution for DLA, RFDNA, and DS was skewed toward resistance, with most RILs falling between the parental lines (Table 1). DLA, DS, and RFDNA traits were transformed for statistical and QTL analysis using the square root, because the transformation improved the normality of the frequency distribution curve. DS and IT were not estimated for the Gh07 experiments because high inoculum concentration used for the swab inoculation prevented an accurate estimation. DLA calculation was not estimated for the LSU08 and Aberdeen 2008 experiments due to high disease level variability between leaves caused by an overall low disease pressure in the experiments. Broad-sense heritability was calculated for Aberdeen 2007, TX07, LA07, and Gh07 DLA and MB08 DS and DLA experiments. Broad heritability estimates were low for all experiments except for MB08 DS. Low heritability estimates were probably due to high variability estimates as a result of uneven distribution of the disease within plots and between replicates (Table 1).

Disease measurements. For RFDNA measurements conducted in 2007 (Ab07 and Gh07), amplification efficiency for the plant–pathogen duplex reaction were ≥86%. In total, 28 independent runs (2,464 reactions) were needed to estimate RFDNA for the experiments. No data calibration was needed to compare results between runs because standard deviations (SDs) were low around the mean efficiency line equation for the FDNA (slope = –3.7, SD = 0.2; intercept = 37.70, SD = 0.70; R^2 = 0.99, SD = 0.01) and host DNA (slope = –3.70, SD = 0.20; intercept = 41.40, SD = 0.70; R^2 = 0.98, SD = 0.01) standards from all 28 plates.

DLA and RFDNA from the field and greenhouse experiments had significant positive correlations (Table 2). Disease measurements from single-race experiments were also positively correlated ($P < 0.05$) with those from field experiments using naturally occurring races (Table 2). Significant correlations ($P < 0.0001$) were also observed between the disease assessments of lower leaves and flag leaves in LA08 (Table 2). Despite using the same *P. coronata* race in the MB08 and the Ab07 experiments, no correlations ($P = 0.05$) were found between MB08 BRCB DS and IT and Ab07 BRCB DLA, IT, or RFDNA. However, positive correlations ($P = 0.05$) were observed between MB08 BRCB DLA and Aberdeen 2007 BRCB DLA, RFDNA, and IT. Positive correlations ($P = 0.05$) were also observed between MB08 BRCB DLA and Ab07 LSLG DLA and RFDNA (Table 2). Correlations between experiments and between disease measurements within an experiment may have been influenced by disease pressure, plant tissue conditions (insect damage or tissue damage), and leaf chlorosis. For example, DLA estimation in the LA07 field test was affected by chlorosis observed on a few of the RILs, including the resistant parental line MN841801-1, resulting in higher DLA estimates than expected based on visual disease assessment (Table 1). This chlorosis may explain, in part, the lower correlation observed between LA07 DLA and disease measurements from other field tests (Table 2). Low disease pressure on flag leaves in LA08 may explain higher correlations between measurements on the lower leaves in LA08 and other tests versus LA08 flag leaves and other tests. Correlations for flowering date between locations (Ab07, Ab08, and LA08) were 0.25 to 0.28 ($P < 0.01$) (Table 3). Low positive and negative correlations were

TABLE 2. Phenotypic correlation coefficients between disease measurements for data collected from field and greenhouse tests in 2007 and 2008[a]

Experiment-isolate-trait	Ab07-BRCB-DLA	Ab07-BRCB-RFDNA	Ab07-BRCB-IT	Gh07-BRCB-DLA	Gh07-BRCB-RFDNA	Ab07-LSLG-DLA	Ab07-LSLG-RFDNA	Ab07-LSLG-IT	Gh07-LSLG-DLA	Gh07-LSLG-RFDNA
Ab07-BRCB-RFDNA	0.4***	…	…	…	…	…	…	…	…	…
Ab07-BRCB-IT	0.38***	0.32***	…	…	…	…	…	…	…	…
Gh07-BRCB-DLA	0.4**	0.18	0.47***	…	…	…	…	…	…	…
Gh07-BRCB-RFDNA	0.28**	0.19*	0.33	0.56***	…	…	…	…	…	…
Ab07-LSLG-DLA	0.42***	0.15	0.08	0.08	0.18	…	…	…	…	…
Ab07-LSLG- RFDNA	0.25*	0.27**	0.11	0.07	0.12	0.38***	…	…	…	…
Ab07-LSLG-IT	0.18	0.2	0.26**	0.17	0.1	–0.04	0.20*	…	…	…
Gh07-LSLG- DLA	0.29**	0.24*	0.26**	0.16	0.18	0.002	0.06	0.28**	…	…
Gh07-LSLG-RFDNA	0.26**	0.2	0.22**	0.15	0.13	0.13	0.18*	0.21*	0.4***	…
Ab08-LSLG-DS	0.08	0.1	0.26**	–0.03	–0.01	0.07	–0.05	0.05	0.12	–0.03
Ab08-LSLG-IT	0.08	0.02	0.13	–0.02	0.04	0.09	0.13	0.06	0.19*	0.04
MB08-BRCB-DS	0.07	0.02	0.08	–0.07	0.12	0.1	0.19*	0.09	–0.02	0.14
MB08-BRCB-IT	–0.02	0.02	0.06	–0.03	0.05	0.04	0.12	0.07	0	0.02
MB08-BRCB-DLA	0.33***	0.2	0.2*	0.05	0.15	0.34***	0.17*	0.09	0.1	0.1
TX07-DLA-flag	0.13	0	0.29**	0.16	0.18	0.3	0.13	0.13	–0.01	0.06
LA07-DLA-flag	0.14	0.2	0.13	0.31***	0.21	0.05	0.05	–0.04	0.09	–0.04
LA08-DS-lower leaves	0.25*	0.12	0.28**	0.22*	0.27**	0.33***	0.18	0.23*	0.27	0.07
LA08-IT-lower leaves	0.19	0.1	0.21*	0.18	0.25*	0.29**	0.17	0.18	0.21	0.02
LA08-DS-flag	0.15	0.1	0.12	0.12	0.21*	0.34***	0.17	0.14	0.05	0.03
LA08-IT-flag	0.02	0.0	0.18	0.13	0.14	0.1	0.11	0.19	–0.02	0

	Ab08-LSLG-DS	Ab08-LSLG-IT	MB08-BRCB-DS	MB08-BRCB-IT	MB08-BRCB-DLA	TX07-DLA-flag	LA07-DLA-flag	LA08-DS-lower leaves	LA08-IT-lower leaves	LA08-DS-flag
Ab08-LSLG-IT	0.43***	…	…	…	…	…	…	…	…	…
MB08-BRCB-DS	0.29**	0.33***	…	…	…	…	…	…	…	…
MB08-BRCB-IT	0.29**	0.41***	0.75***	…	…	…	…	…	…	…
MB08-BRCB-DLA	0.22*	0.17	0.54***	0.43***	…	…	…	…	…	…
TX07-DLA-flag	0.09	0.15	0.16	0.2	0.29**	…	…	…	…	…
LA07-DLA-flag	–0.04	0.02	–0.03	–0.01	0.11	0.21*	…	…	…	…
LA08-DS-lower leaves	0.19	0.19	0.33***	0.3	0.44***	0.5	0.42***	…	…	…
LA08-IT-lower leaves	0.18	0.23*	0.29**	0.29**	0.36***	0.45***	0.4***	0.93***	…	…
LA08-DS-flag	0.18	0.12	0.27**	0.27**	0.41***	0.28**	0.17	0.58***	0.54***	…
LA08-IT-flag	0.17	0.22*	0.38***	0.36***	0.31**	0.19	0.11	0.45***	0.42***	0.52***

[a] DLA= diseased leaf area, RFDNA = relative fungal DNA, IT= infection type, and DS = disease severity (%); Ab = Aberdeen, ID field test; Gh = greenhouse test; MB = Manitoba field station; TX = Texas field station; LA = Louisiana field station, and 07 and 08 = experiment years 2007 and 2008; BRCB= *Puccinia coronata* isolate CR251 (race BRCB) and LSLG = *P. coronata* isolate 93MNB236 (race LSLG); *, **, and *** = significant at the 0.01, 0.001, and 0.0001 levels of probability, respectively.

TABLE 3. Phenotypic correlation coefficients between days to heading (HD) and disease measurements for data collected from field and greenhouse tests in 2007 and 2008[a]

Treatment (experiment-isolate-trait)	Ab07-HD	Ab08-HD	LA08-HD
Ab07-BRCB-DLA	–0.16	…	…
Ab07-BRCB-RFDNA	–0.06	…	…
Ab07-BRCB-IT	–0.07	…	…
Ab07-LSLG-DLA	–0.02	…	…
Ab07-LSLG-RFDNA	–0.11	…	…
Ab07-LSLG-IT	0.12	…	…
Ab08-LSLG-DS	…	–0.04	…
Ab08-LSLG-IT	…	–0.04	…
LA08-lower leaves-DS	…	…	–0.1
LA08-lower leaves-IT	…	…	–0.2*
LA08-Flag-DS	…	…	–0.28**
LA08-Flag-IT	…	…	–0.06

[a] DLA= diseased leaf area, RFDNA = relative fungal DNA, IT= infection type, and DS = disease severity (%); Ab = Aberdeen, ID field test, LA = Louisiana field station test, and 07 and 08 = experiment years 2007 and 2008; BRCB = *Puccinia coronata* isolate CR251 (race BRCB) and LSLG = *P. coronata* isolate 93MNB236 (race LSLG); * and ** = significant at the 0.01 and 0.001 level of probability, respectively.

found between crown rust resistance and heading date whereas, in most cases, the correlations were negative (Table 3).

QTL detected in the field to races BRCB and LSLG. QTL affecting crown rust PR detected in single-race inoculations were associated with MN841801-1 alleles. In the Ab07 field experiment, five QTL (*Prq1a*, *Prq2*, *Prq5*, *Prq6*, and *Prq8*) were detected using CIM of DLA, RFDNA, and IT traits (Table 4; Fig. 1). The two most consistent QTL detected using CIM were *Prq1a* and *Prq2* on linkage groups MN3 and MN26, respectively. BRCB and LSLG DLA and RFDNA detected *Prq1a* with a LOD peak close to markers isu707x, b4, and cdo608x. Support intervals for the QTL detected using RFDNA estimates were reduced compared with QTL detected using DLA by 2 cM for LSLG and by 11 cM for BRCB. In Ab07, *Prq2* was also detected by BRCB and LSLG DLA and RFDNA with a LOD peak close to markers umn498 and umn23. *Prq2* was also detected by IT for BRCB in Aberdeen 2007, explaining 36.1% of the variation. When combined, these two QTL explain 18.8% of the LSLG DLA variation, 20.8% of the LSLG RFDNA variation, 43.2% of the BRCB DLA variation, and 32.1% of the BRCB RFDNA variation in experiment Ab07. A third QTL on linkage group MN13 (*Prq8*) was associated with reduction of LSLG DLA and RFDNA in experiment Ab07 and explained 9.4 and 7.0% of the DLA and

TABLE 4. Quantitative trait loci (QTL) for partial resistance to crown rust identified based on mean disease leaf area (DLA), relative fungal DNA (RFDNA), infection type (IT), and disease severity (DS) measured on 150 recombinant inbred lines of the cross 'MN841801-1/Noble-2' in field inoculations using two *Puccinia coronata* races

Experiment-isolate-trait[a]	QTL marker (peak/interval)[b]	Linkage group	QTL name[c]	LOD	$R^2 \times 100$	Additive[d]
Ab07-LSLG-DLA	cdo608x (70.26/65-79)	MN3	*Prq1a*	4.2	11.7	–0.4
	cdo1502x (15.77/13-20)	MN13	*Prq8*	3.5	9.4	0.0
	umn498 (9.16/ 1-10)	MN26	*Prq2*	2.6	7.1	0.0
	p56m48n2	Unlinked	*Prq7***	…	…	…
Total $R^2 \times 100$	…	…	…	…	25.2	…
Ab07-LSLG-RFDNA	b4 (66.47/ 61-73)	MN3	*Prq1a*	4.2	11.4	–0.1
	cdo1502x (16.77/12.0-20)	MN13	*Prq8*	2.5	7.0	–0.1
	umn23 (7.01/2-10)	MN26	*Prq2*	3.3	9.4	–0.1
	p56m48n2	Unlinked	*Prq7****	…	…	…
Total $R^2 \times 100$	…	…	…	…	27.7	…
Ab07-LSLG-IT	umn249 (85.82/82-94)	MN6	*Prq5*	4.1	10.7	0.0
	p48m88m6 (106.4/94-114)	MN9	*Prq6*	4.0	12.3	0.0
	isu2287 (52.63/46.6-53)	MN12	…	6.6	20.3	0.0
	p56m48n2	Unlinked	*Prq7**	…	…	…
Total $R^2 \times 100$	…	…	…	…	35.1	…
Ab08-LSLG-IT	p42m35n3 (101.8/98-105)	MN6	*Prq5*	5.7	12.3	0.0
Total $R^2 \times 100$	…	…	…	…	12.3	…
Ab07-BRCB-DLA	b4 (67.5/59-76)	MN3	*Prq1a*	3.4	7.2	…
	umn23 (6.01/4-9)	MN26	*Prq2*	13.4	36.0	–0.1
	p56m48n2	Unlinked	*Prq7**	…	…	…
Total $R^2 \times 100$	…	…	…	…	42.0	…
Ab07-BRCB-RFDNA	isu707x (53.7/50-56)	MN3	*Prq1a*	3.1	7.1	–0.1
	umn23 (7.01/4.0-10)	MN26	*Prq2*	8.9	25.0	–0.3
Total $R^2 \times 100$	…	…	…	…	32.6	…
Ab07-BRCB-IT	cdo309x (47.30/35.7-60.0)	MN6	…	3.5	9.4	–0.1
	umn498 (10.16/9-10.0)	MN26	*Prq2*	16.4	36.1	–0.2
Total $R^2 \times 100$	…	…	…	…	40.6	…
MB08-BRCB-DLA	b4 (65.5/63-68)	MN3	*Prq1a*	8.9	20.0	–4.9
	umn5353x (24.2/15-35)	MN13	*Prq8*	4.4	11.5	–4.0
	p56m48n2	Unlinked	*Prq7***	…	…	…
Total $R^2 \times 100$	…	…	…	…	26.6	…
MB08-BRCB-DS	p40m50m11 (48.3/42.0-53.0)	MN10	…	2.6	4.8	–2.8
	cdo1502x (16.8/15.0-20.0)	MN13	*Prq8*	12.9	26.0	–8.1
	cdo1196y (26.2/17.0-33.0)	MN14	*Prq3*	2.6	4.7	–3.1
Total $R^2 \times 100$	…	…	…	…	49.1	…
MB08-BRCB-IT	umn5353y (13.0/11.0-14.0)	MN13	*Prq8*	15.2	32.7	–0.3
	p41m88m5 (1/0.1-7.0)	MN23	…	3.3	7.5	–1.1
Total $R^2 \times 100$	…	…	…	…	25.1	…

[a] Ab = Aberdeen, ID field test; MB = Manitoba field station; TX = Texas field station; LA = Louisiana field station, and 07 and 08 = experiment years 2007 and 2008; LSLG = *P. coronata* isolate 93MNB236 (race LSLG) and BRCB = *Puccinia coronata* isolate CR251 (race BRCB). Total R^2 estimated by multiple interval mapping.

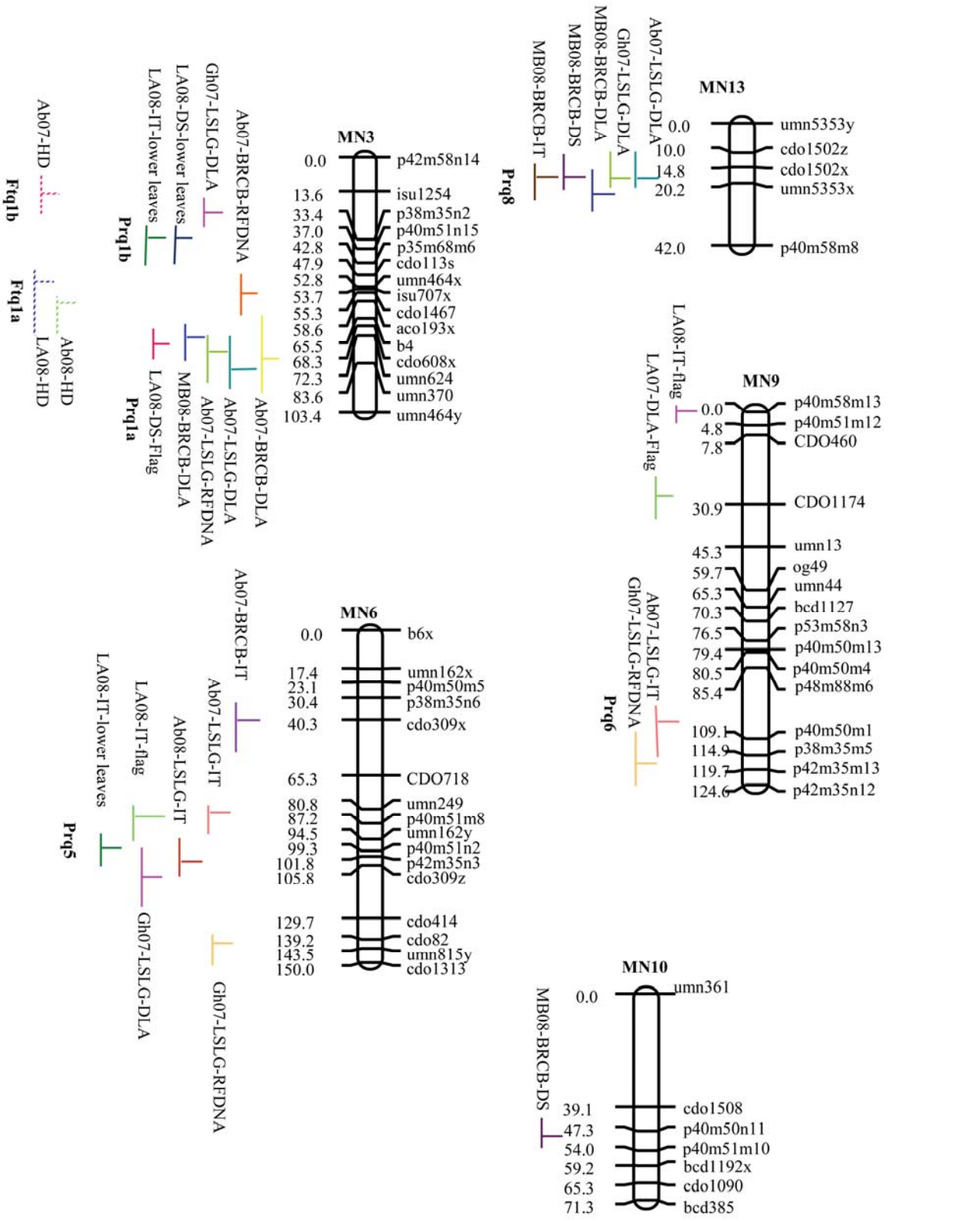
[b] Name of the flanking marker (from top of the linkage group)/support interval (log-likelihood [LOD] > 1.0).

[c] QTL detected by single marker analysis; *, **, ***, and **** = significant at the 0.05, 0.01, 0.001, and 0.0001 levels of probability, respectively.

[d] Additive effect calculated using multiple interval mapping.

RFDNA variation, respectively. Two additional QTL, one on MN6 (*Prq5*) and another on MN9 (*Prq6*), were associated with reduced LSLG IT and explained 10.7 and 12.3% of the IT variation, respectively. Single-marker analysis of unlinked markers revealed significant associations between the AFLP marker p56m48n2 and reduced BRCB DLA and reduced LSLG DLA, RFDNA, and IT (Table 4).

Four QTL (*Prq1a*, *Prq3*, *Prq7*, and *Prq8*) were associated with disease resistance to BRCB in the field experiment in Manitoba (MB08) (Table 4; Fig. 1). Reduction of DLA was associated with



(*Continued on next page*)

Fig. 1. Linkage groups based on 150 $F_{6:9}$ recombinant inbred lines from the cross MN841801-1/'Noble-2' with mapped quantitative trait loci for partial resistance to crown rust (Prq) and flowering time (Ftq). Numbers on the left of linkage groups are cumulative map distances in centimorgans (Haldane). Abbreviations for experiments are explained in Materials and Methods.

QTL *Prq1a*, *Prq7*, and *Prq8*. Together, these three QTL explained >31% of the DLA variation. However, *Prq1a* was not associated with reduced DS or IT in the same experiment. Reduced DS was associated with *Prq8* and a QTL on MN14 (*Prq3*). Together, these two QTL accounted for 45.5% of the DS variation. Reduction of IT was associated with *Prq8* solely. All the QTL identified in the MB08 experiment except *Prq3* were also associated with crown rust resistance in the Ab07 field experiment.

QTL detected in the greenhouse to races LSLG and BRCB. Four QTL (*Prq1b*, *Prq2*, *Prq5*, and *Prq6*) were detected in greenhouse experiments on the basis of DLA and RFDNA (Table 5). *Prq2*, *Prq5*, and *Prq6* were also detected in the Ab07 single-race field experiments (Fig. 1). Reduction of LSLG DLA was associated with *Prq1b* and *Prq5*. *Prq1b* mapped to linkage group MN3 with a LOD peak close to the marker p38m35n2 and explained 7.1% of the variation. Because the *Prq1b* support

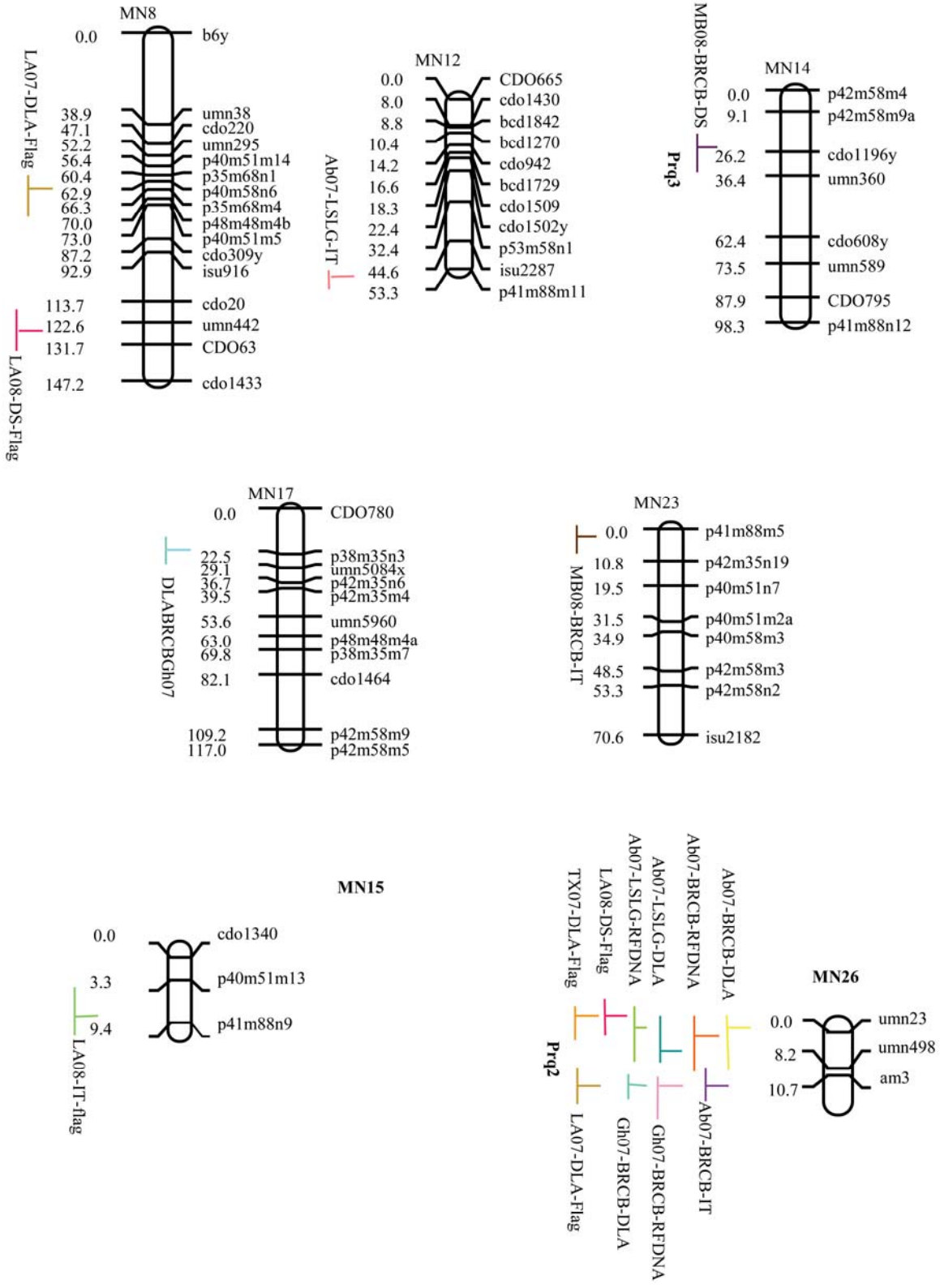


Fig. 1. (*Continued from previous page*)

interval did not overlap with support interval for *Prq1a* (identified in Ab07, LA08, and MB08), they were considered two independent QTL (Fig. 1). *Prq6* on MN9 was associated with reduced LSLG RFDNA and the locus was also associated with a reduction in LSLG IT in the field experiment (Table 4). Reduced BRCB DLA and RFDNA were associated with *Prq2*, which explained 31.7 and 9.7% of the total variation, respectively.

QTL detected in LA07, LA08, and TX07 field experiments. Only *Prq2* was detected under natural disease pressure in LA07 and TX07, accounting for 9.2 and 8.8% of the flag leaf DLA variation in Louisiana and Texas, respectively (Table 6; Fig. 1). In contrast, five QTL were identified in the 2008 Louisiana trial (*Prq1a*, *Pqr1b*, *Prq2*, *Pqr5*, and *Pqr7*). *Prq1a* was associated with a reduction of DS, while *Prq1b* and *Prq2* were detected on the basis of DS of the lower leaves. *Prq5* was associated with a reduction of IT on both flag leaves and lower leaves, while *Prq7* was identified based on DS of lower leaves. All of the QTL identified in disease-conducive environments in Texas and Louisiana were also identified in the single-race inoculations.

Flowering date. Two QTL for HD, *Ftq1b* and *Ftq1a*, on linkage group MN3 were identified in experiments Ab07, Ab08, and LA08 (Table 7). *Ftq1b* was detected in Aberdeen 2007 with a peak close to marker isu1254, whereas *Ftq1a*, with a LOD peak close to markers cdo467 and isu707x, was detected in Ab08 and LA08. In both cases, the QTL increasing HD was associated with MN841801-1 alleles. The locations of the HD QTL overlapped with regions on linkage group MN3 associated with crown rust PR QTL (Fig. 1). A third HD QTL (*Ftq7*) was detected by SMA linked to marker p56m48n2 (Table 7). This marker was also associated with PR (*Prq7*) in Ab07, MB08, and LA08 field experiments (Table 8).

DISCUSSION

PR conferred by MN841801-1. PR is a promising alternative for developing oat cultivars with durable resistance to crown rust disease. Identifying QTL for PR and efficient tools for their selection in segregating populations would greatly benefit breeding efforts. The present study validated seven previously identified QTL across a range of environments using various measures of disease. In addition, a new QTL, *Prq8*, was discovered on linkage group MN13.

Of the eight QTL found in the present study, the two that most consistently detected QTL across experiments by CIM were

TABLE 5. Quantitative trait loci (QTL) for partial resistance to crown rust identified based on mean disease leaf area (DLA) and relative fungal DNA (RFDNA) measured on 150 recombinant inbred lines of the cross 'MN841801-1/Noble-2' in greenhouse inoculations with two *Puccinia coronata* races

Experiment-race-trait[a]	QTL marker (peak/interval)[b]	Linkage group	QTL name	LOD	$R^2 \times 100$	Additive[c]
Gh07-LSLG-DLA	p38m35n2 (33.43/28-37)	MN3	*Prq1b*	2.7	7.1	–2.1
	cdo309z (105.84/99-118)	MN6	*Prq5*	3.4	7.8	–0.9
	p48m58m13 (0.01/0.-2)	MN9	…	5.2	12.8	–0.2
Total $R^2 \times 100$	…	…	…	…	22.7	…
Gh07-LSLG-RFDNA	cdo82 (139.16/134-146)	MN6	…	2.8	6.7	–0.1
	p38m35m5 (117.90/109-122)	MN9	*Prq6*	3.4	8.8	–0.1
Total $R^2 \times 100$	…	…	…	…	10.8	…
Gh07-BRCB-DLA	p38m35n3 (24.49/15.0-29.0)	MN17	…	4.3	10.2	0.0
	umn498 (10.16/9-11)	MN26	*Prq2*	10.4	31.7	0.0
Total $R^2 \times 100$	…	…	…	…	35.8	…
Gh07-BRCB-RFDNA	umn498 (10.16/8-10.0)	MN26	*Prq2*	3.8	9.7	–0.1
Total $R^2 \times 100$	…	…	…	…	9.7	…

[a] Gh07 = greenhouse test in 2007, LSLG= *P. coronata* isolate 93MNB236 (race LSLG), and BRCB= *Puccinia coronata* isolate CR251 (race BRCB). Total R^2 estimated by multiple interval mapping (MIM).
[b] Name of the flanking marker (from top of the linkage group)/support interval (log-likelihood [LOD] > 1.0).
[c] Additive effect calculated using multiple interval mapping.

TABLE 6. Quantitative trait locus (QTL) analysis summary for partial resistance to crown rust based on mean disease leaf area (DLA), infection type (IT), and disease severity (DS) measured on 150 recombinant inbred lines of the cross 'MN841801-1/Noble-2' in disease-conducive environments in Texas (TX) and Louisiana (LA) under natural disease pressure in 2007 (07) and 2008 (08)

Experiment-trait-plant stage[a]	QTL marker (peak/interval)[b]	Linkage group	QTL name[c]	LOD	R^2 (%)	Additive[d]
LA07-DLA-flag	umn498 (9.2 /8-10.0)	MN26	*Prq2*	3.8	9.2	–1.5
Total $R^2 \times 100$	…	…	…	…	9.2	…
TX07-DLA-flag	am3 (0.0/0-5)	MN26	*Prq2*	3.5	8.8	–0.9
Total $R^2 \times 100$	…	…	…	…	8.8	…
LA08-DS-flag	b4 (65.47/61-67)	MN3	*Prq1a*	3.9	9.3	–6.6
	umn442 (124.66/118-132)	MN8	…	3.8	10.0	–1.0
Total $R^2 \times 100$	…	…	…	…	17.5	…
LA08-IT-flag	p40m51m8 (93.19/88-100)	MN6	*Prq5*	4.5	12.3	–0.3
	cdo1174 (30.95/21-38)	MN9	…	2.6	6.6	–0.2
	p40m51m13 (9.28/3-9)	MN15	…	3.2	9.2	–0.2
Total $R^2 \times 100$	…	…	…	…	26.4	…
LA08-DS-lower leaves	p40m51m15 (40/37-48)	MN3	*Prq1b*	6.8	17.3	–2.2
	am3 (5/0-8)	MN26	*Prq2*	2.8	7.7	–4.7
	p56m48n2	Unlinked	*Prq7***	…	…	…
Total $R^2 \times 100$	…	…	…	…	28.5	…
LA08-IT-lower leaves	p35m68m6 (42/37-48)	MN3	*Prq1b*	6.7	16.4	–2.4
	p40m51n2 (99.3/98-102)	MN6	*Prq5*	4.3	9.9	0.0
	p56m48n2	Unlinked	*Prq7***	…	…	…
Total $R^2 \times 100$	…	…	…	…	21.8	…

[a] Total R^2 estimated by multiple interval mapping.
[b] Name of the flanking marker (from top of the linkage group)/support interval (log-likelihood [LOD] > 1.0).
[c] QTL detected by single marker analysis; *, **, ***, and **** = significant at the 0.05, 0.01, 0.001, and 0.0001 levels of probability respectively.
[d] Additive effect calculated using multiple interval mapping.

Prq1a on linkage group MN3 and *Prq2* on MN26. Together, *Prq1a* and *Prq2* explained a total of 30.1% of the phenotypic variation in PR, with averages of 11.1% for Pqr1a and 18.1% for *Prq2*. The new QTL, *Prq8*, was detected in two experiments and was effective against both races tested. Both *Prq8* and *Prq1a* were detected in the MB08 trial and the Ab07 trial, with *Prq1a* accounting for more variation with correspondingly higher LOD. From this result, it appears that *Prq8* has a smaller effect than *Prq1a*. In addition to these three loci, the unlinked AFLP marker p56m48n2 was consistently associated with reducing crown rust in both studies. Thus, PR to crown rust in MN841801-1 may be controlled mainly by four QTL (*Prq1a*, *Prq2*, *Prq7*, and *Prq8*) that were detected in different traits via various measures of disease. The other QTL (*Prq1b*, *Prq3*, *Prq5*, and *Prq6*) appear to be influenced more by the experiment, disease measurement, and the *P. coronata* race used (Tables 4, 5, and 8). For example, *Prq5* and *Prq6* were detected in single-race inoculations with LSLG, which is in agreement with previous results using the same race (31), but were not detected in experiments using race BRCB. This result may suggest race specificity for certain QTL, as has been reported in the barley–*P. hordei* pathosystem (33); however, to rigorously test this hypothesis, near-isogenic lines with various QTL should be developed and tested. *Prq1a*, *Prq2*, *Prq3*, *Prq6*, and *Prq7* were also detected associated with crown rust resistance in a field experiment conducted on 158 $F_{6:8}$ RILs of the MN population in St. Paul, MN in 2007 based on DS (data not shown).

QTL detection and magnitude of their effects may have been influenced by the environment where the experiments were conducted as well as the disease pressure. This may explain the differences and low correlation estimates between the MB08 and Ab07 single-race experiments using race BRCB. Higher disease pressure in MB08 may have hindered the ability of detecting the *Prq1a* and *Prq7* QTL by visual assessment of DS and IT. However, DLA estimation from digital images of the RIL from the same experiment seems to be more sensitive in detecting smaller differences which allowed the detection of a total of three QTL (*Prq1a*, *Prq7*, and *Prq8*).

Disease assessment method also affected the QTL detection. For instance, we detected *Prq6* in the greenhouse based on RFDNA whereas, in the Ab07 field experiments, we only detected the QTL based on IT. Similarly, *Prq5* was detected in the greenhouse experiments based on DLA whereas it was only detected based on IT in the Ab07, Ab08, and LA08 experiments. IT is a semiquantitative measure of disease, where both pustule size and the condition of the host tissue surrounding the pustule are considered. Perhaps, in the field, IT is more sensitive for detection of QTL that have qualitative effects on pustule appearance but

TABLE 7. Quantitative trait locus (QTL) for days to heading (HD) measured on 150 recombinant inbred lines of the cross 'MN841801-1/'Noble-2' in the Aberdeen field experiments in 2007 (Ab07) and 2008 (Ab08) and in Louisiana in 2008 (LA08)

Experiment	Trait	QTL marker (peak/interval)[a]	Linkage group	QTL name[b]	LOD	$R^2 \times 100$	Additive[c]
Ab07	HD	isu1254 (30.6/23-36)	MN3	*Ftq1b*	5.6	19.9	1.0
Ab08	HD	cdo1467 (55.3/55-65)	MN3	*Ftq1a*	5.3	15.5	0.9
Ab08	HD	p56m48n2	Unlinked	*Ftq7***	…	…	…
LA08	HD	isu707x (53.7/51-65)	MN3	*Ftq1a*	7.3	19.6	0.2
LA08	HD	p56m48n2	Unlinked	*Ftq7***	…	…	…

[a] Name of the flanking marker (from top of the linkage group)/support interval (log-likelihood [LOD] > 1.0)
[b] QTL name reflects the name of the QTL for crown rust resistance detected in the same location; *Ftq1a* and Ftqb were detected in the same region as *Prq1a* and *Prq1b*, respectively, *Ftq7* was detected in association with the marker associated with *Prq7*. QTL detected by single marker analysis; ** = significant at the 0.01 level of probability.
[c] Additive effect calculated using multiple interval mapping.

TABLE 8. Single-marker analysis (SMA) summary for eight unlinked markers associated with partial resistance and flowering time for 150 $F_{6:8}$ recombinant inbred lines of the MN841801-1/'Noble-2' cross based on disease leaf area (DLA), relative fungal DNA (RFDNA), infection type (IT), disease severity (DS), and heading date (HD) measured in field and greenhouse single-isolate inoculations using two different *Puccinia coronata* races and in disease-conducive environments under natural disease pressure in 2007 (07) and 2008 (08)

	Marker[b]							
Treatment (experiment-race-trait)[a]	p42m35n5	p56m48n2 (*Prq7*)	umn509y	cdo572	p38m35n1	p40m50n2	p48m88m3	p56m48m1
Ab07-LSLG-DLA	…	****	…	…	…	…	…	…
Ab07-LSLG-RFDNA	…	***	…	…	…		…	…
Ab07-LSLG-IT	…	*	…	…	…	*	…	…
Gh07-LSLG-DLA	…	…	…	**	…	…	…	…
Gh07-LSLG-RFDNA	…	…	**	…	…	…	…	…
Ab07-BRCB-DLA	…	*	…	…	…	…	…	…
Gh07-BRCB-RFDNA	…	…	…	…	*	…	…	…
Ab08-LSLG-DS	…	…	*	*	**	…	…	…
MB08-BRCB-DLA	…	**	…	…	…	…	**	…
MB08-BRCB-DS	*	…	…	…	…	…	…	…
MB08-BRCB-IT	*	…	*	…	…	…	…	*
LA07-local-DLA	…	…	…	…	…	…	…	…
LA08-local-DS-lower leaves	*	**	…	…	…	…	…	…
LA08-local-IT-lower leaves	…	**	…	…	…	*	…	…
LA08-local-IT-flag	*	…	…	…	…	…	…	*
Ab08-HD	*	*	…	…	…	…	…	…
Ab07-HD	…	**	…	…	…	…	…	…
LA08-HD	…	**	…	…	*	…	…	…

[a] Treatments in which one or more markers associated with crown rust resistance or HD were detected by SMA. Ab = Aberdeen field test, Gh = greenhouse test, MB = Manitoba field station, DLA = Louisiana field station, LSLG = *P. coronata* isolate 93MNB236 (race LSLG), and BRCB = *Puccinia coronata* isolate CR251 (race BRCB).
[b] Markers that showed association with crown rust resistance by SMA in more than one experiments or in one experiment but with more than one disease measurement; *, **, ***, and **** = significant at the 0.05, 0.01, 0.001, and 0.0001 probability levels, respectively. Only associations significant at $P < 0.01$ in more than one experiment were considered established.

smaller quantitative effects on disease compared with strictly quantitative measures such as RFDNA, DLA, and DS.

Detection of *Prq5* in single-race inoculations with LSLG and in the LA08 field experiment may be due to the similarity in virulence pattern of LSLG and the virulence pattern observed on the PC differentials in the LA field. The virulence pattern on the PC differential lines of the races in LA08 field showed high virulence on *Pc38*, *39, 40, 51, 56*, and *64* and low disease reaction type on *Pc46*, *50*, *52*, *59* and *68* (data not shown) as occurs for LSLG.

QTL analysis for flowering date. As in previous studies (31,45), QTL for flowering date were associated with crown rust resistance QTL. Portyanko et al. (31) found two major and several minor flowering date QTL. Both of the major QTL were identified in the present study but one (*Ftq1*) was determined to have two separate peaks (*Ftq1a* and *Ftq1b*), roughly corresponding to crown rust resistance QTL *Prq1a* and *Prq1b*. The updated marker order used in our study may account for the ability to discriminate this locus into two peaks. Detection of *Ftq1a* and *Ftq1b* on MN3 is also in agreement with the previous report of a QTL for flowering in the putatively homologous genetic region KO-17 on the 'Kanota'/Ogle157 (KO) population (16) and one reported on OT32 on the Ogle1040/'TAM O-301' (OT) population (17). Our inability to detect other minor QTL reported in previous studies may be due to their small effects.

Comparison of PR QTL in MN841801-1 with QTL or genes from other studies. The genetic region containing *Prq1a* and *Prq1b* (MN3) is homologous with genetic regions on KO-17 in the KO population and OT32 in the OT population (15,32), and the Pendek4838_1 linkage group in the Pendek48/Pendek38 population (42). These linkage groups have previously been associated with crown rust resistance genes, including the *Pc38, 62*, *63, 58* gene complex (15,31,42) and a minor QTL associated with reducing FDNA in the OT population (18). Both of the isolates used in the current study overcome the resistance conferred by *Pc38* and *63* but are avirulent on *Pc58* (data not shown). Nevertheless, because both parents were highly susceptible at seedling stage to both isolates, *Pc58* and *Pc62* cannot be responsible for the PR observed in MN841801-1, because these two genes express their resistance at the seedling stage. The detection of two QTL for PR on the MN3 linkage group in the MN population, in addition to previously described resistance genes on homologous regions in other oat populations (KO and OT), suggests that this region may contain a cluster of diverse crown rust resistance genes. Clusters of resistance genes have been reported in the oat genome, including *Pc38*, *62*, and *63* (14); *Pc39* and *55* (21); *Pc35*, *54*, and *96* (7,26); and *Pc68*, *44*, *46*, *50*, *95*, and *x* (8).

Based on the present study and on previous work (31), *Prq2* on MN26 is perhaps the most consistently detected QTL across experiments. Because there are only three markers on MN26, it is difficult to compare *Prq2* with QTL controlling crown rust resistance identified using other oat maps. However, other resistance loci have also been located in regions homologous to MN26: on linkage group KO36 on the KO map and on linkage group OM15 on the Ogle/MAM17-5 map (45). OM15 is also where the crown rust resistance gene *Pc91* has been localized based on the presence of the linked RFLP marker UMN145 (34,45).

Our results confirm the complex nature of the PR to crown rust of oat due to the interaction of multiple QTL controlling the resistance in the host and possible effects on the efficacy of these QTL from environment and from race specificity. As found in a previous study (18), the use of RFDNA in multiple cases reduced the LOD likelihood interval of the QTL identified compared with the interval identified by the DLA. Using various measures of disease in single-isolate inoculations, we consistently detected QTL, *Prq1a*, *Prq2*, *Prq7*, and *Prq8*, conferring apparently race nonspecific PR in MN841801-1. Future work should focus on discovery of PCR-based genetic markers tightly linked to these QTL for use in developing near-isogenic lines for further study of these important QTL and in practical marker-assisted breeding for crown rust resistance derived from MN841801-1.

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