Theor Appl Genet (2008) 116:517–527
DOI 10.1007/s00122-007-0687-x

ORIGINAL PAPER

Qualitative and quantitative trait loci conditioning resistance to *Puccinia coronata* pathotypes NQMG and LGCG in the oat (*Avena sativa* L.) cultivars Ogle and TAM O-301

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Received: 2 April 2007 / Accepted: 27 November 2007 / Published online: 9 January 2008

Abstract Mapping disease resistance loci relies on the type and precision of phenotypic measurements. For crown rust of oat, disease severity is commonly assessed based on visual ratings of infection types (IT) and/or diseased leaf area (DLA) of infected plants in the greenhouse or field. These data can be affected by several variables including; (i) non-uniform disease development in the field; (ii) atypical symptom development in the greenhouse; (iii) the presence of multiple pathogenic races or pathotypes in the field, and (iv) rating bias. To overcome these limitations, we mapped crown rust resistance to single isolates in the Ogle/TAM O-301 (OT) recombinant inbred line (RIL) population using detailed measurements of IT, uredinia length (UL) and relative fungal DNA (FDNA) estimates determined by q-PCR. Measurements were taken on OT parents and recombinant inbred lines (RIL) inoculated with *Puccinia coronata* pathotypes NQMG and LGCG in separate greenhouse and field tests. Qualitative mapping identified an allele conferred by TAM O-301 on linkage group (LG) OT-11, which produced a bleached fleck phenotype to both NQMG and LGCG. Quantitative mapping identified two major quantitative trait loci (QTL) originating from TAM O-301 on LGs OT-11 and OT-32 which reduced UL and FDNA of both isolates in all experiments. Additionally, minor QTLs that reduced UL and FDNA were detected on LGs OT-15 and OT-8, originating from TAM O-301, and on LG OT-27, originating from Ogle. Detailed assessments of the OT population using two pathotypes in both the greenhouse and field provided comprehensive information to effectively map the genes responsible for crown rust resistance in Ogle and TAM O-301 to NQMG and LGCG.

Communicated by J.-L. Jannink.

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Introduction

Crown rust, caused by *Puccinia coronata*, is the most damaging disease of cultivated oat (*Avena sativa* L.) (Martens et al. 1972; Simons 1985). Many race-specific resistance genes conditioning major effects (Fry 1982) have been characterized from *A. sativa* and *A. sterilis* (Martens and Dyck 1989; Simons et al. 1978). These genes are widely used in breeding programs across North America to control crown rust. Unfortunately, *P. coronata* has rapidly adapted to major gene resistance through population virulence shifts that render most genes either partially or totally useless within 2 years (Chong and Kolmer 1993; Leonard 2003; Leonard et al. 2004, 2005a, b; Leonard and Martinelli 2005).

Several strategies have been proposed to improve durability of resistance to many diseases. These include gene pyramiding (Pederson and Leath 1988), the use of multilines (Mundt 2002), and partial resistance (Parlevliet 1987). Race non-specific or partial resistance is attractive because it does not place extreme selection pressure on the pathogen. To enhance our ability to breed for this resistance, geneticists must identify specific genetic regions associated with resistance and introgress them into germplasm with good agronomic characteristics via marker-assisted selection. Only a limited number of QTLs for partial resistance

to crown rust have been identified (Barbosa et al. 2006; Diaz-Lago et al. 2002; Portyanko et al. 2005; Zhu and Kaeppler 2003; Zhu et al. 2003), probably because of difficulty in developing linkage maps for oat due to its large genome size (Arumuganathan and Earle 1991) and limited availability of genetic markers, most of which are difficult to use (Wight et al. 2003; Portyanko et al. 2001).

The Ogle/TAM O-301 (OT) recombinant inbred line (RIL) mapping population (Portyanko et al. 2001) was used to construct one of the major oat linkage maps, containing 441 genetic markers making up 34 linkage groups (LG). This mapping population has been useful in dissecting crown rust resistance in both Ogle (Brown and Jedlinski 1983) and TAM O-301 (McDaniel 1974). Although Ogle is now generally susceptible in North America, two major genes conferring resistance to specific *P. coronata* races have been mapped in this cultivar using both the Kanota/Ogle (KO) (Bush and Wise 1996) and OT populations (Jackson et al. 2007) to LGs containing homologous markers (Jackson et al. 2007). Additionally, TAM O-301 carries the crown rust resistance gene designated *Pc58* (Simons et al. 1978). Several studies indicated that *Pc58* maintained good levels of resistance over many years (Leonard 2003; Chong and Zegeye 2004). Recently, *Pc58* resistance in TAM O-301 was mapped based on seedling studies to the OT LGs OT-32 and OT-33 as three different genes (Hoffman et al. 2006). Of the three genes, lines containing the allele designated *Pc58a* never gave completely susceptible reactions. Two possible explanations for this were (i) *Pc58a* produced a residual effect even when defeated by pathogen virulence, or (ii) a tightly linked gene or genes conditioned the response.

Field evaluations of the OT RIL population in Texas and Louisiana revealed a distinct "bleached fleck" phenotype in the population (unpublished data), which was not consistently observed in either Ogle or TAM O-301. The phenotype was also absent on the *P. coronata* (Pc) differential line Pc58 in the same environment and thus could be novel. Multiple isolates of *P. coronata* in both field environments, however, made characterization and mapping of the unique phenotype impractical from these field observations.

Precise examination of disease reactions to rust fungi can be difficult in the field because of confounding effects of multiple isolates (Holland and Munkvold 2001). In addition, accurate assessment based on visual ratings is highly variable and biased by the expertise of the rater (Nutter et al. 1993). To overcome these limitations, Jackson et al. (2007) used artificially produced polycyclic field epidemics of a single crown rust isolate virulent on TAM O-301 and avirulent on Ogle coupled with a q-PCR assay (Jackson et al. 2006) to precisely map crown rust resistance in Ogle. Employing the same approach, we sought to characterize and map the "bleached fleck" phenotype conditioned by TAM O-301. We identified *P. coronata* pathotypes that produced this unique phenotype on TAM O-301, but gave susceptible reactions on Ogle, and used both qualitative and quantitative approaches to comprehensively map the genes involved in the OT RIL population.

Materials and methods

Plant material

The RIL population developed to construct the OT linkage map (Portyanko et al. 2001) was increased (F_9) for use in this study. The population was originally developed via single seed descent to the F_6 from which single panicle selections were made to produce F_7 lines. In the present study, $F_{6:9}$ seed were harvested and bulked from F_8 plants which traced back to the original F_6 plants used for mapping.

Fungal isolate and inoculum production

Puccinia coronata isolates designated as pathotypes NQMG (isolate 03MN111) and LGCG (isolate 98MNB245) (Chong et al. 2000) were obtained from the USDA-ARS Cereal Disease Laboratory, St. Paul, MN. Both isolates are avirulent on cultivar TAM O-301 producing bleached flecks with small uredinia (infection types ; to ;1) and virulent on cultivar Ogle producing large uredinia with and without necrosis or chlorosis (infection types 3 to 4). Each isolate was purified from a single pustule and subsequently increased on the susceptible cultivar Provena at different times to prevent contamination.

To prepare fresh inoculum for each test, stored urediniospores of each isolate were suspended in light mineral oil (Soltrol® 170, Isoparaffin), incubated in a 45°C water bath for 10 min, and sprayed onto Provena seedlings. Following inoculation, plants were placed in a dew chamber at 21°C in the dark for 21 h (Politowski and Browning 1975; Simons 1954), then moved to a growth chamber programmed for 13 h light at 25°C and 11 h darkness at 18°C. Approximately 16 days after inoculation, spores were collected using a vacuum collector and resuspended for use in greenhouse and field experiments. A 10^5 urediniospores ml^{-1} inoculum suspension was prepared for the greenhouse experiment by agitating spores in a solution of 0.3% gelatin and 0.3% Tween 20. For field experiments, spores were suspended in light mineral oil, as previously described, and adjusted to 10^5 urediniospores ml^{-1}.

Greenhouse experiment

In 2005, 84 OT RILs were randomly selected from the original OT population of 136 RILs to evaluate crown rust reaction to pathotype NQMG. Eight seeds of each selected line

were sown in 15 cm pots containing a 1:2:1 (V:V:V) mix of sand, peat moss, and vermiculite. One pot per RIL (without replicate) and two pots per parent were planted. After planting, pots were placed in the greenhouse with a 14–15 h photoperiod at 13–21°C. Seedlings were thinned to four seedlings per pot before inoculation. Inoculum was applied directly to a 5 cm^2 section at the mid-point of the fifth leaf with a sterile nylon swab as described by Jackson et al. (2006) and leaves were allowed to dry before plants were incubated in the dew chamber and growth chamber as previously described.

Fourteen days after inoculation, inoculated sections were excised and scanned at 118 samples per cm using a HP Scanjet 4070 Photosmart Scanner. After scanning the sections were freeze dried and DNA was extracted for estimation of fungal DNA (FDNA) content using absolute q-PCR with pathogen-specific primers and protocols developed by Jackson et al. (2006). From each scanned image, crown rust reactions were assessed visually and uredinia length (UL) was measured from six randomly selected uredinia per section ($n = 24$ uredinia).

For the greenhouse experiment nine separate real-time q-PCR runs were done to evaluate the 352 biological samples and the non-biological replicate of each sample. Each individual run consisted of a 96-well plate containing 80 experimental samples and replicates ($n = 2$) of five known FDNA standards, one no-template control and two uninfected oat controls positioned in rows 5 and 9 on the 96-well plate. To accurately combine and analyze data from the nine separate plates, FDNA standards from the same source were used (Jackson et al. 2006) and replicate samples ($n = 8$/RIL and 16/parent) were randomized between plates as previously described by Jackson et al. (2007). Standard deviations (SD) were calculated for the slope, intercept, and R^2 values from regression lines calculated for each plate. Since deviations around the overall means from all ten plates were low (slope = −3.38, SD = 0.2; intercept = 36.30, SD = 0.7; $R^2 = 0.989$, SD = 0.003), no data adjustments were needed between plates prior to further analysis of the overall FDNA data.

Field experiments

A randomized complete block design with two replicates was used to evaluate crown rust reactions of the two pathotypes (NQMG and LGCG) on Ogle, TAM O-301, and the complete 136 OT RIL population. In 2005, an isolated experiment was used to test reactions to NQMG, while two experiments were used to separately test reactions to the two crown rust pathotypes in 2006. All field evaluations were done at the University of Idaho Research and Extension Center in Aberdeen, ID. In both years, 25–30 seed of each line were sown in hill-plots within each block. The plots were planted 35.5 cm apart in rows containing 24 RIL plots and 2 parental plots. Rows were spaced 36 cm apart with a continuous row of Provena planted every third row as a spreader. Additionally, 16 crown rust resistance gene differentials (Chong et al. 2000) were planted in the center of each block and a wheat border was used.

To promote disease development, starting at the fifth leaf stage spreader rows were inoculated using a hand-held garden sprayer following 30 min of irrigation at dusk once per week for five consecutive weeks. After inoculation, each experiment was covered overnight (12 h) with 6 mm polyethylene sheeting attached to a frame. The experiments were subsequently covered four times after the last inoculation at 3-day intervals to promote inoculum spread from spreader rows to plots.

In 2005, two flag leaves best representing the overall disease level of each plot were selected 42 days after the final inoculation (4 leaves/RI line, 8 leaves/parent). Five cm^2 sections were excised from the mid-point of each leaf and scanned as described for the greenhouse experiment. The samples were processed for q-PCR analysis and images were assessed for IT and UL. Fourteen separate real-time PCR runs were used to estimate FDNA from the field samples. The same q-PCR plate setup used for the greenhouse experiment was applied to q-PCR of field samples and comparisons were made between FDNA standards from each plate as previously described. No data adjustment was made prior to further FDNA data analysis since the SDs around the overall slope, intercept, and R^2 means calculated from all 14 plates were low (slope = −3.18, 0.3; intercept = 37.01, 1.4; and $R^2 = 0.987$, 0.008).

In 2006, three flag leaves per hill plot were selected 38 days after the final inoculation. Images of three leaves from each plot were captured with an Olympus C7070 (3072 × 2304 pixels) at a fixed distance of 27.9 cm. IT and UL were recorded from the images.

Qualitative characterization and mapping

The OT RILs were categorized based on the presence or absence of the "bleached fleck" phenotype (Fig. 1) in each experiment. The resulting segregation ratios were compared to expected Mendelian ratios and a Chi-square (χ^2) value was calculated for each experiment. The data were then converted into a "bleached fleck" phenotypic marker for each of the four experiments. The markers were tested for linkage to genetic markers from the previously published OT linkage map (Portyanko et al. 2001) using Mapmaker/EXP 3.0 (Lander et al. 1987) with a maximum likelihood of odds (LOD) score of 4.0 and a maximum distance of 40 cM (Kosambi). The new phenotypic markers were added to the existing linkage group using multipoint

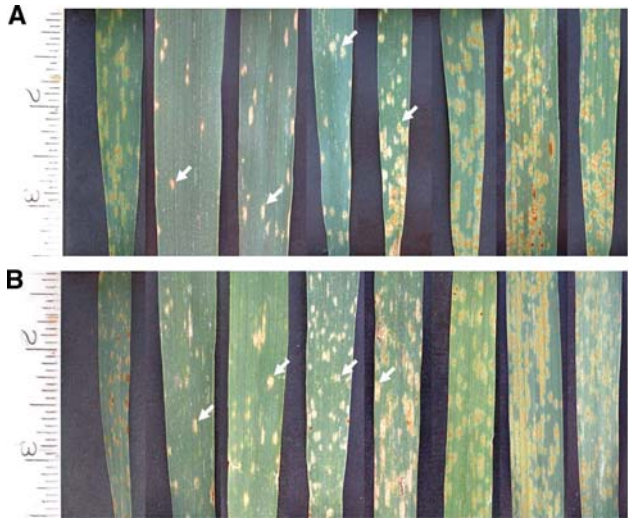

Fig. 1 Phenotypic reactions to *Puccinia coronata* pathotypes NQMG (**a**) and LGCG (**b**) from the field. Bleach fleck presence was scored on plants of the susceptible parent Ogle, the resistant parent TAM O-301, and $F_{6:9}$ recombinant inbred lines OT-86, OT-30, OT-67,OT-84, OT-37, and OT-77 (*left* to *right*). *Arrows* indicate bleached fleck phenotype

linkage analysis with a LOD of 3.0 and a maximum distance of 40 cM (Kosambi).

Quantitative mapping

Means, variances (genotype and environment), and broad sense heritability estimates were calculated for UL in both years and for FDNA estimates in 2005 using the JMP statistical software (SAS Institute, Cary, NC, USA). Broad sense heritability was calculated by dividing the genotypic variance by the combined genotype + environment + genotype × environment variances derived from a mixed model. UL and FDNA variations in the OT RIL population from each experiment were quantitatively mapped using WinQTL Cartographer (Wang et al. 2005). QTL analysis was done using the same OT linkage map as Jackson et al. (2007) with additions of the previously described phenotypic markers. Single marker analysis (SMA), interval mapping (IM), and composite interval mapping (CIM) (Zeng 1994) were performed for each measure of resistance. Forward regression with a threshold value of $P = 0.05$ was used to choose co-factors for CIM. To identify significant QTLs, experiment-wise significance levels were established by running 1,000 permutations for all traits, $\alpha = 0.05$ (Churchill and Doerge 1994). QTL intervals were assigned to the area within one LOD score of the QTL peak. In addition, QTLs were considered major if they were identified in all environments tested in this study. Based on these results, major QTL alleles were inputted into multiple interval mapping (MIM) to estimate the total phenotypic variance accounted for by the QTLs.

Results

Qualitative characterization and mapping of IT

Consistent and uniform crown rust disease pressure from single *P. coronata* isolates of two different pathotypes was achieved in this study by artificial inoculation and manipulation of the environment. Reactions on the 16 differentials planted within each field experiment matched the expected reactions (Chong et al. 2000) for the corresponding pathotype that was applied. In all four experiments, medium to large uredinia with limited chlorosis were recorded on Ogle, while bleached flecks with small uredinia were recorded on TAM O-301 (Fig. 1). Although the bleached fleck phenotype was seen in all environments, it was less clear in the greenhouse experiment, sometimes appearing chlorotic rather than bleached white. With the exception of two segregating RILs, the bleached fleck reaction was consistent among individual lines across experiments. Segregation ratios of RILs with and without bleached flecks did not differ from the 1:1 Mendelian model for a single dominant gene across experiments (Table 1). Classifications of RILs were the same in greenhouse and field experiments inoculated with NQMG in 2005. In the 2006 experiment inoculated with NQMG, only two RILs differed in reaction from the 2005 experiments. Similarly, only five RILs differed in reaction to LGCG and NQMG in 2006. Overall, the pooled data were a good fit to a single gene model and the data were homogenous across environments and years (Table 1).

Two-point linkage analysis using Mapmaker/Exp 3.0 (Lander et al. 1987) revealed linkage of five RFLP probes and one AFLP marker from the OT linkage map (Portyanko et al. 2001) to the four bleached fleck phenotypic markers (*Pc pathotype NQMG 2005 GH, 2005 FD, 2006 FD, and pathotype LGCG 2006, FD*). All six of the genetic markers were previously mapped to the OT linkage group OT-11 (Portyanko et al. 2001). The bleached fleck phenotypic markers were added to OT-11 using the "place" command (LOD 3.0). After the markers were placed the best overall order was determined by breaking the markers on the LG into subsets of six and "comparing" all possible orders (LOD 3.0). The resulting exercise placed all four markers on OT-11 within a region 4.1 to 8.1 cM from RFLP probe BCD1823A (Fig. 2).

Quantitative mapping of UL and FDNA

Compared to Ogle, TAM O-301 had shorter mean uredinia in all four experiments and reduced mean FDNA in the two experiments in which FDNA was measured (Table 2). Means of both measurements from individual RILs were consistent between experiments (Table 2). Broad sense

Table 1 Segregation of $F_{6:9}$ Ogle x TAM O-301 recombinant inbred lines (RIL) to different crown rust pathotypes in greenhouse or field experiments[a]

Year	Exp.	Pathotype	No. of lines[b]		χ^2 (1:1) ratio	
			S	*R*	Value	Probability
2005	Greenhouse	NQMG	38	46	0.76	0.90–0.75
2005	Field	NQMG	64	69	0.19	0.90–0.75
2006	Field	NQMG	66	67	0.01	0.975
2006	Field	LGCG	65	68	0.07	0.90–0.75
		$F_{6:9}$ Total ($df = 4$)	233	250	1.03	
		$F_{6:9}$ Pooled ($df = 1$)			0.60	0.90–0.75
		Heterogeneity ($df = 3$)			0.43	0.95–0.90

[a] The greenhouse experiment contained four subsamples ($N = 84$ lines). The field experiment in 2005 was a complete randomized block design with five replicates ($N = 136$ lines), and the field experiments in 2006 were complete randomized block design with two replicates ($N = 136$ line). Parents were randomized within each greenhouse and field replicate

[b] *S* susceptible; *R* resistant, bleached fleck phenotype

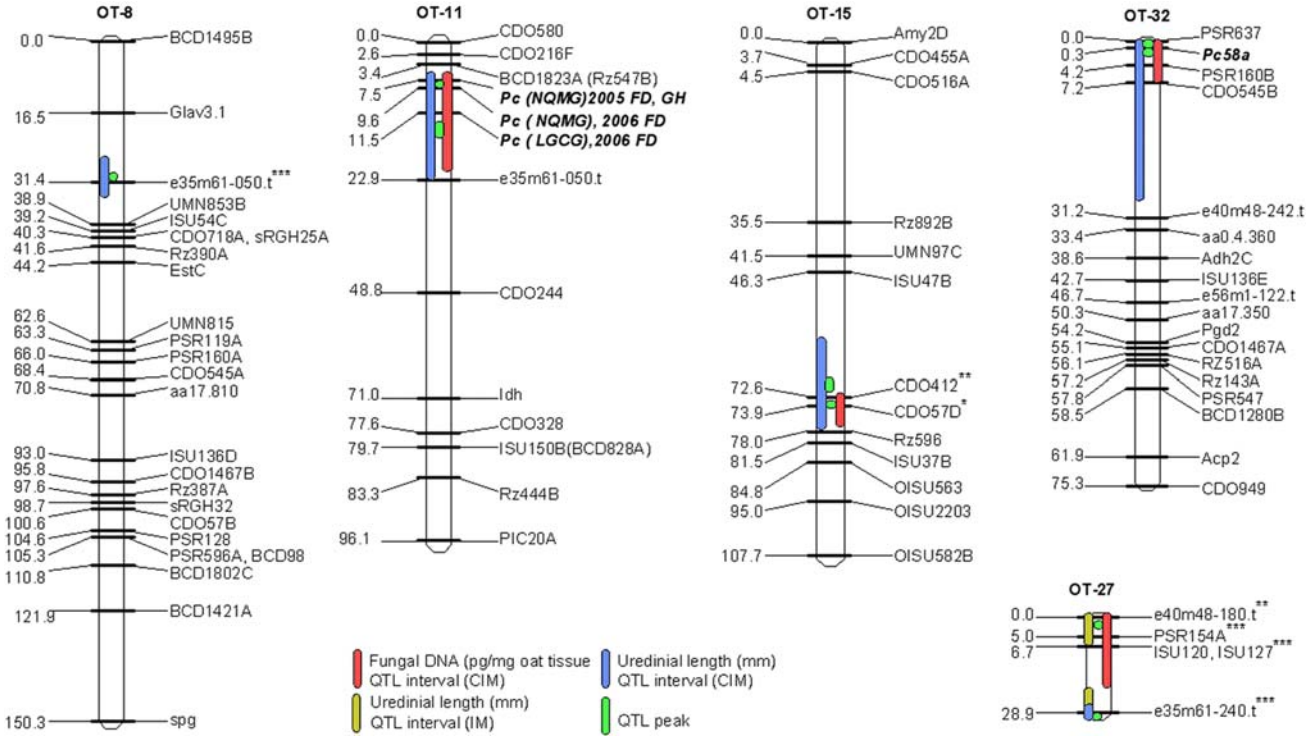

Fig. 2 Linkage map of qualitative resistance genes (*Pc*) conferring resistance to *P. coronata* pathotypes NQMG and LGCG and quantitative trait loci (QTL) detected in the Ogle x TAM O-301 recombinant inbred line population conferring crown rust resistance. The candidate qualitative resistance locus on OT-11 (*Bold*, *italics*) was based on visual ratings of a bleached fleck conferred by TAM O-301 and was mapped using multi-point linkage analysis (Mapmaker EXP/3.0). QTL peaks (*green blocks*) and intervals (*red blocks*) were detected based on fungal DNA estimates (q-PCR) in the greenhouse and field using composite interval mapping (CIM) WinQTL Cartographer. QTL intervals detected using CIM (*blue blocks*) and interval mapping (IM) (*yellow blocks*) were detected based on uredinia length (mm). The linkage map was constructed using; (i) RFLP markers from oat (CDO and UMN), barley (BCD), maize (ISU), and rice (RZ); (ii) AFLP markers (e and aa); (iii) isozyme markers (Idh, Spg, Pgd, and Acp); (iv) a resistance gene analog from maize (pLrk) and sorghum (sRgh) from a previous study (Portyanko et al. 2001). Genetic distances (*left*) were generated using Mapmaker 3.0 (Kosombi)

heritability estimates indicated that 55.6% of the reduced ULs and 67.0% of the reduced FDNA estimates were due to genetic components (Table 2).

Composite interval mapping analysis identified major QTL alleles on OT-11 and OT-32 reducing UL in all four experiments (Table 3). The QTL on OT-11 mapped to a

Table 2 Means and variance components based on uredinia length and fungal DNA measurements from parents and recombinant inbred lines (RILs) inoculated with different *Puccinia coronata* pathotypes over two years in greenhouse (GH) and field (FD) experiments

Trait[a]	Pathotype/Year/Environment	Parents		RILs
		Ogle	TAM O-301	Mean
Uredinia length				
NQMG-05-GH		4.8	0.8	2.0
NQMG-05-FD		3.3	0.9	1.5
NQMG-06-FD		4.5	0.3	2.0
LGCG-06-FD		3.6	0.6	1.5
	Variance components[b] (RILs)			
	σ^2_{G*}	120.7		
	$\sigma^2_{G\times E*}$	12.1		
	Heritability	55.6		
Fungal DNA				
	NQMG-05-GH	907.0	48.1	684.0
	NQMG-05-FD	589.0	47.4	663.6
	Variance components[b] (RILs)			
	σ^2_{G}*	4.0		
	$\sigma^2_{G\times E}$*	1.9		
	Heritability	67.0		

[a] Mean uredinia length (mm) was based on 6 random uredinia measurements taken from leaves of four individual plants per parent and RIL ($N = 20$). Fungal DNA (pg) was estimate from 5 cm^2 samples harvested from four individual plants of each parent and RIL using q-PCR

[b] σ^2 interaction variances, *G* genotype, *E* environment

* $P < 0.001$

19.3 cM region between the RFLP probe BCD1823A and the AFLP marker e35m61-050.t, with a LOD peak (5.5–20.7) 2.1 cM from the bleached fleck phenotypic marker designated *Pc* (*LGCG*), *2006* FD (Fig. 2). This QTL explained an average of 39.3% of the UL variation over all four experiments (Table 3). The QTL on OT-32 mapped to a 27.0 cM region between the RFLP probe PSR637 and the AFLP marker e40m48-242.t, with a LOD peak (6.5–13.7) approximately 0.3 cM from the crown rust resistance gene *Pc58a* (Fig. 2). This QTL explained an average of 21.7% of the UL variation over all experiments (Table 3). Together the major QTLs on OT-11 and OT-32 explained 46.9 and 67.7% of the UL variation to NQMG in 2005 greenhouse and field experiments, and 76.5 and 41.9% of the UL variation in 2006 field experiments using NQMG and LGCG, respectively (Table 3).

Of the major QTLs, a similar region on OT-32 was associated with a reduction in FDNA in both greenhouse and field experiments where FDNA measurements were made in 2005, while the same region on OT-11 was associated with reduced FDNA in only the greenhouse (Table 4). The FDNA QTL on OT-32 mapped to a 7.2 cM region between RFLP probes PSR637 and CDO545B, with a LOD peak (4.2–4.4) 0.2 cM from *Pc58a*. In comparison to the region detected using UL, the region based on FDNA was 19.8 cM shorter (Fig. 2). The QTL explained approximately 12.2% of the FDNA variation (Table 4). The FDNA QTL on OT-11 mapped to a 18.4 cM region between the RFLP probe BCD1823A and the AFLP marker e35m61-050.t, with a LOD peak (5.2) 0.3 cM from *Pc* (*NQMG*) *2006 FD*. In comparison to the region detected using UL, the region based on FDNA was 0.9 cM shorter (Fig. 2). The QTL explained 26.4% of the FDNA variation in the greenhouse. Although the QTL on OT-11 was not associated with reduced FDNA in the field experiment using CIM, a QTL was detected in the same region using IM (data not shown). Overall, the major QTLs on OT-11 and OT-32 explained 25.9% of the FDNA variation in the greenhouse.

Three minor QTLs were detected in this study; one each on OT-15 and OT-8 conferred by TAM O-301 and one on OT-27 conferred by Ogle (Fig. 2). The QTL on OT-15 was detected based on UL in the 2005 field experiment inoculated with pathotype NQMG and 2006 field experiment inoculated with pathotype LGCG (Table 3). In each experiment the QTL was mapped to a 24 cM region between RFLP probes ISU412 and Rz596, with a LOD peak (3.9–4.1) 6.3 cM from RFLP probe CDO412 (Fig. 2). The QTL explained approximately 9.0% of the UL variation in both experiments (Table 3). A similar QTL region on OT-15 was detected using FDNA estimates. The QTL was reduced by 20.6 cM and mapped to a 4.6 cM region between RFLP probes CDO412 and Rz596, with a LOD peak (2.8) directly associated with RFLP probe CDO57D (Fig. 2). The QTL explained 6.9% of the FDNA variation in the field. Although the QTL on OT-15 was not detected using CIM in 2005 greenhouse experiments (UL and FDNA) and in 2006 field experiments inoculated with NQMG (UL), RFLP probes CDO412 ($P = 0.001$) and CDO57D ($P = 0.05$) were significantly associated with a reduction in both traits using SMA (Fig. 2).

The QTL on OT-27 was detected based on UL and FDNA estimates in the greenhouse experiment. Based on

Table 3 Quantitative trait locus (QTL) analysis summary for crown rust resistance based on mean uredinia length (mm) measured on leaves of Ogle x TAM O-301 $F_{6:9}$ recombinant inbred (RI) lines with different *Puccinia coronata* pathotypes over 2 years in greenhouse (GH) and field (FD) experiments

Experiment (Pathotype/ Year/Environment)	Linkage group	QTL marker[a] (peak/interval)	LOD[b]	R^2 (%)[c]	Additive effect
NQMG-05-GH					
	OT-11	BCD1823A(13.6/3.6–22.0)	5.5	32.7	0.85
	OT-27	e35M61–240.t (28.8/22.8–28.9)	3.3	38.8	−1.05
	OT-32	PSR637(0.01/0.0–24.0)	6.5	23.7	0.72
				Total (%) [d] 46.9	
NQMG-05-FD					
	OT-11	BCD1823A(13.6/3.6–22.9)	16.6	44.1	0.83
	OT-15	ISU147B(70.3/61.5–78.0)	3.9	8.0	0.35
	OT-32	PSR637(2.0/0.0–25.0)	9.1	17.4	0.52
				Total (%) 67.7	
NQMG-06-FD					
	OT-11	BCD1823A(15.6/3.6–22.6)	20.7	54.7	1.3
	OT-32	PSR637(2.0/0.0–23.0)	10.0	19.6	0.8
				Total (%) 76.5	
LGCG-06-FD					
	OT-8	e35m61-050.t(31.4/27.0–34.0)	2.6	3.8	0.26
	OT-11	BCD1823A(13.6/3.6–22.3)	10.8	25.5	0.66
	OT-15	ISU147B(66.3/54.0–75.5)	4.1	10.0	0.41
	OT-32	PSR637(2.0/0.0–27.0)	13.7	26.0	0.68
				Total (%) 41.9	

[a] Name of the flanking marker to the left of the QTL peak and interval (cM)

[b] QTLs were detected using WinQTL Cartographer CIM and were based on a LOD threshold of 2.48 (1,000 permutations and a type I error of 5%)

[c] Percent of the phenotypic variation explained by the QTL

[d] Portion of the total variance explained by the significant QTLs OT-11 and OT-32 using MIM

Table 4 Quantitative trait locus (QTL) analysis summary for crown rust resistance based on mean fungal DNA estimates from diseased leaves of Ogle x TAM O-301 $F_{6:9}$ recombinant inbred (RI) lines with different *Puccinia coronata* pathotypes in greenhouse (GH) and field (FD) experiments in 2005

Experiment (Pathotype/ Environment)	Linkage group	QTL marker[a](peak/ interval)	LOD[b]	R^2 (%)[c]	Additive effect
NQMG-GH					
	OT-11	BCD1823A (9.3/3.6–22.0)	5.2	26.4	327.8
	OT-27	e40M48-180.t (2.0/0.0–16.7)	3.9	14.7	−443.9
	OT-32	PSR637 (0.01/0.0–7.2)	4.2	13.3	235.8
				Total (%) [d] 25.9	
NQMG-FD					
	OT-15	CDO57D (73.9/72.6–76.0)	2.8	6.9	235.8
	OT-32	PSR637 (0.01/0.0–7.2)	4.4	11.1	631.9

[a] Name of the flanking marker to the left of the QTL peak and interval (cM)

[b] QTLs were detected using WinQTL Cartographer CIM and were based on a LOD threshold of 2.5 (1,000 permutations and a type I error of 5%)

[c] Percent of the phenotypic variation explained by the QTL

[d] Portion of the total variance explained by the significant QTL on OT-11 and OT-32 using MIM

UL, the QTL mapped to a 6.1 cM region associated with the AFLP marker e35m61-240.t. The QTL had a LOD peak (3.3) 0.1 cM from the AFLP marker (Fig. 2) and explained 38.8% of the UL variation in the greenhouse experiment (Table 3). Based on FDNA estimates, the QTL on OT-27 mapped to a 16.7 cM region on the opposite end of the

small linkage group, with a LOD peak (3.9) 3.9 cM from AFLP marker e40m48-180.t (Fig. 2). The QTL explained 14.7% of the FDNA variation in the greenhouse experiment. Although the QTL was not detected in 2005 and 2006 field experiments, all the markers on OT-27 were significantly associated with reduced UL and FDNA (P = 0.01–0.001) using SMA. Additionally, a QTL at the same locations were detected in the experiments using IM (Fig. 2).

The QTL on OT-8 was only detected in the 2006 field experiment inoculated with LGCG and was mapped to a 7.0 cM region between the sequence tagged site Glav3.1 and the RFLP probe UMN853B,with a LOD peak directly associated with AFLP probe e35m61-050.t (Fig. 2). This QTL explained 3.8% of the UL variation to pathotype LGCG in the field (Table 4). Additionally, the AFLP marker e35m61-050.t was significantly associated (P = 0.001) with reduced UL and FDNA in all experiments using SMA (Fig. 2).

Discussion

The main objective of this study was to characterize and map a unique "bleached fleck" phenotype segregating in the OT RIL population when exposed to naturally occurring isolates in Texas and Louisiana. Genetic characterization from the field data was impossible because the reaction was ambiguous in the resistant parent and the RILs, possibly due to interference from numerous *P. coronata* pathotypes present in each field (Chong and Kolmer 1993; Leonard 2003). To overcome this effect, two isolates of different pathotypes (NQMG and LGCG) were chosen that clearly produced the targeted phenotype on TAM O-301. Both were used in detailed genetic studies in the greenhouse (NQMG) and in the field with a technique enabling single-isolate epidemics to develop. Such experiments in the field have been difficult to conduct (Holland and Munkvold 2001), especially in environments where natural sources of inoculum can migrate into plots. However reactions of the 16 Pc differential oat lines (Chong et al. 2000) within each experiment of the present study indicated the isolates used in each trial remained uncontaminated.

Using our single-isolate experiments the bleached fleck phenotype was consistently expressed on TAM O-301 in four different experiments. The clarity and consistency of the reaction across parents and OT RILs in our experiments allowed mapping of the phenotype to three putative loci 4.1 to 8.1 cM from RFLP probes BCD1823A and RZ547B on LG OT-11. Differences in the map locations were due to variations in the intensity of bleaching associated with infection sites. For instance, two RILs that had bleached flecks surrounding uredinia when inoculated with NQMG in the greenhouse and in the field appeared to have chlorotic halos surrounding uredinia when inoculated with LGCG. No data adjustments were made between experiments because all contradictory reactions, i.e. reactions that differed between pathotypes, were consistent between replications within each experiment. Aside from *Pc58*, additional loci in TAM O-301 reducing crown rust infection by different isolates have been reported on LGs OT-32 and OT-2 (Jackson et al. 2006). However, it appears the gene we identified in this study conditioning the bleached fleck was independent of any known resistance genes in TAM O-301. The bleached fleck was also not seen on any of the 16 Pc differential oat lines (Chong et al. 2000) tested within each experiment, including the *Pc58* differential, and could thus be a new gene with specificity for certain rust isolates including the two used in this study.

In the present study, 12 genetic markers and 3 phenotypic markers were used to construct LG OT-11 (Fig. 2). The addition of the phenotypic markers allowed placement of two genetic markers (AFLP marker e35m61-050.t and RFLP probe ISU150B) not previously placed on the original OT-11 LG (Portyanko et al. 2001). Of the 12 genetic markers, the RFLP probe CDO580 was also mapped onto Kanota/Ogle (KO) LG KO-4 (Fig. 3; Wight et al. 2003). OT-11 and KO-4 may be homologous (Jackson et al. 2006) and previous studies have placed phenotypic and genotypic markers associated with crown and stem rust resistance on KO-4. Crown rust resistance conferred by Ogle was mapped approximately 30 cM from CDO580 (KO4; Bush and Wise 1996; O'Donoughue et al., unpublished), and the stem rust resistance gene *Pg9* (O'Donoughue et al. 1996) and a receptor-like kinase gene, *Lrk10* (Cheng et al. 2002), were mapped to the same region (Fig. 3). If OT-11 and KO-4 are homologous, then the genetic area containing the bleached fleck gene identified in this study could be closely associated with a genetic region rich in plant defense genes. Future work should be done to study the relationship between OT-11 and KO-4, specifically targeting regions identified in this work and others.

In addition to the bleached fleck, our single-isolate experiments and detailed measurements of UL and FDNA revealed variations in crown rust infection between parents and OT RILs that were not apparent in previous field observations based on diseased leaf area (DLA) and infection types (IT). Based on assessments of UL and FDNA we were able to identify two major QTLs conferred by TAM O-301. Of these, one major QTL conferred by TAM O-301 was detected on LG OT-11 which contained all three phenotypic markers for the "bleached fleck". Based on this data, it is likely that the same gene(s) controlling the bleached fleck also reduce UL and FDNA. Whether additional pathogenesis-related genes are tightly linked to the bleached fleck marker and responsible for this reduction

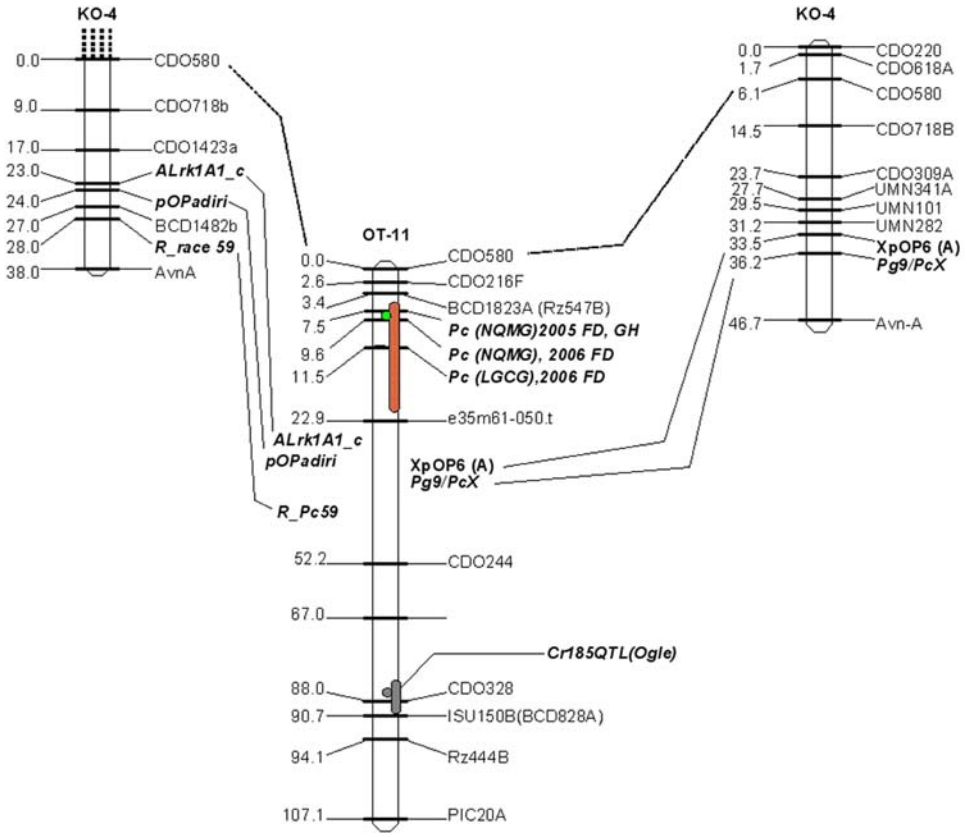

Fig. 3 Localization of a resistance gene analog and *Pg9*/*Pcx* resistance genes onto the Ogle/TAM O-301 (OT) linkage group (LG) OT-11. Mapping of the analogous sequence to a *Lrk10*-like receptor kinase (*ALrk1A1_c*; *left*) and stem rust (*Pg9*) and crown rust (*Pcx*) resistance genes (*right*) onto Kanota/Ogle (KO) LG KO-4 were from two different reports (Cheng et al. 2002; O'Donoughue et al. 1996)

remains to be determined. A more detailed genetic study of this region is needed.

The second major QTL identified in this study reducing UL and FDNA mapped to LG OT-32. QTL peaks based on both assessments were closely associated with the *P. coronata* resistance gene *Pc58a*. In mapping *Pc58*, three genes were identified using six *P. coronata* isolates different from those used in this study, and were designated *Pc58a*, *b*, and *c* (Hoffman et al. 2006). No susceptible reactions were observed on RILs containing *Pc58a*. The authors speculated that a fourth gene tightly linked to *Pc58a* might exist. This suggests that either *Pc58a* and/or other genes tightly linked to *Pc58a* are contributing to a reduction in UL and FDNA resulting from infection by *P. coronata* pathotypes NQMG and LGCG. The genetic region on OT-32 containing *Pc58a* and the QTL interval found in this study includes RFLP probes PSR637, PSR160B, and CDO545B. The availability of sequence information for each of these probes (NCBI) will expedite more detailed molecular genetic studies of this region. Currently, work is underway to develop STS and/or SNP markers for *Pc58a*.

In addition to the two major QTLs our detailed assessments of UL and FDNA allowed the identification of two putative QTLs on OT-8 and OT-15 conferred by TAM O-301 and one putative QTL on OT-27 conferred by Ogle. These QTLs, however, were not detected using CIM in all experiments possibly because of the large effects present from the QTLs on OT-11 and OT-32. These major QTLs explained 76.5% of the total variation, making the detection of minor QTLs difficult. The presence of other QTLs with large effects could also be why no QTLs were detected using CIM on OT-27 in the field experiments. Alternatively the putative QTLs could be artifacts based on the limited numbers of OT RILs used in this study. This possibility is more likely in the case of the QTL on OT-8, since it was detected in only one environment, compared to QTLs identified on OT-15 and OT-27 which were identified in at least two environments.

In this study, heighten resolution was observed between parents when using FDNA estimates versus UL measurements. In the 2005 experiments, mean differences between the parents were an average of three times greater based on FDNA estimates (15-fold) than UL measurements (five-fold). This result is consistent with previous reports where differences in FDNA estimates between resistant and susceptible soybean, rice, and oat genotypes were much greater than from conventional measurements of disease signs and symptoms (Gao et al. 2004; Qi and Yang 2002; Jackson et al. 2006, 2007). Additionally, in this study QTL intervals were consistently more acute based on FDNA estimates. This result was consistent with a previous report where data generated by q-PCR improved mapping

accuracy and precision (Jackson et al 2007). Although q-PCR estimates provided higher resolution between parents and was more precise at measuring QTL intervals than UL measurements in this study, QTLs identified by UL measurements account for more of the phenotypic variation. Overall, both measurements provided a very detailed examination of crown rust disease and allowed a comprehensive interrogation of disease resistance in the OT population to the crown rust isolates used in this study.

The present study revealed the power of using controlled single-isolate experiments coupled with detailed measurements of UL and FDNA to dissect the genetic components controlling resistance to crown rust infection. Successful mapping of the unique "bleached fleck" phenotype conferred by TAM O-301 on OT-11 was due to our ability to screen the population using only isolates producing the desired phenotype. Additionally, the identification of the QTL on OT-32 associated with *Pc58a* and putative QTLs further reducing crown rust infection was due to our detailed measurements of UL and FDNA. Results from this study add to the relatively limited knowledge of mapped crown rust resistance genes and QTLs. Furthermore, this work demonstrates the impact of new precise phenotyping for molecular mapping of disease resistance.

Acknowledgments The authors thank Kathy Satterfield, Irene Shackelford, and Rebecca Caldera for their technical expertise in laboratory and field experiments. We are also grateful to Denise Wedel, Randy Gamble, and the staff of the University of Idaho Research and Extension Center for field plot maintenance. This manuscript is dedicated to the memory of Dr. Patrick Fenn, the doctoral adviser of Eric Jackson.

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