Mapping of the Oat Crown Rust Resistance Gene *Pc39* Relative to Single Nucleotide Polymorphism Markers

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Abstract

Crown rust, caused by *Puccinia coronata* f. sp. *avenae* Eriks. (*Pca*), is among the most important oat diseases resulting in significant yield losses in many growing regions. A gene-for-gene interaction is well established in this pathosystem and has been exploited by oat breeders to control crown rust. *Pc39* is a seedling crown rust resistance gene that has been widely deployed in North American oat breeding. DNA markers are desired to accurately predict the specific *Pc* genes present in breeding germplasm. The objectives of the study were as follows: (i) to map *Pc39* in two recombinant inbred line (RIL) populations (AC Assiniboia/MN841801 and AC Medallion/MN841801) and (ii) to identify single nucleotide polymorphism (SNP) markers for postulation of *Pc39* in oat germplasm. *Pc39* was mapped to a linkage group consisting of 16 SNP markers, which placed the gene on linkage group Mrg11 (chromosome 1C) of the oat consensus map. *Pc39* cosegregated with SNP marker

GMI_ES01_c12570_390 in the AC Assiniboia/MN841801 RIL population and was flanked by the SNP markers avgbs_126086.141 and GMI_ES15_c276_702, with genetic distances of 1.7 and 0.3 cM, respectively. In the AC Medallion/MN841801 RIL population, similar results were obtained but the genetic distances of the flanking markers were 0.4 and 0.4 cM, respectively. Kompetitive Allele-Specific PCR assays were successfully designed for *Pc39*-linked SNP loci. Two SNP loci defined a haplotype that accurately predicted *Pc39* status in a diverse panel of oat germplasm and will be useful for marker-assisted selection in oat breeding.

Keywords: *Avena sativa*, cereals and grains, crown rust, cultivar/resistance, disease management, fungi, field crops, marker-assisted selection, oat, pathogen diversity, *Pc39*, *Puccinia coronata* f. sp. *avenae*, single nucleotide polymorphism

Oat (*Avena sativa* L.) is an important cereal crop grown in many regions of the world. This crop is used for human food and livestock consumption (Gnanesh et al. 2013; Simons 1985) and is increasingly receiving interest because of its nutritional benefits. Crown rust is caused by the fungus *Puccinia coronata* f. sp. *avenae* Eriks. (*Pca*) and is a common disease of oat that can cause severe damage (Simons 1985). In Canada, oat crown rust is most common in the eastern prairie region (Manitoba and eastern Saskatchewan), southern Ontario, and southwestern Quebec (Chong and Brown 1996; McCartney et al. 2011). Estimated yield losses in the eastern prairie region in 2001 to 2005 averaged 5.1% annually, with yield losses up to 11.2 and 8.8% reported in 2001 and 2005, respectively (Chong et al. 2008). In the United States, 20% yield losses have been reported in Minnesota and Louisiana (Carson 2011). Although crown rust outbreaks can be reduced by a combination of several methods, such as elimination of alternate hosts (*Rhynchospora* spp.), early seeding, and in-crop foliar fungicide application, the development of cultivars with host resistance is recognized as the most effective, efficient, economical,

and environmentally friendly method to manage this disease (McCallum et al. 2007; McCartney et al. 2011; Nazareno et al. 2018; Simons 1970). A gene-for-gene interaction underlies the host–pathogen interactions in the oat–*Pca* pathosystem, with evidence of physiological specialization dating back 100 years (Hoerner 1919). The lifespan of a new and widely deployed resistance gene has often been 5 years or less as the result of increased frequency of *Pca* races virulent on the new resistance gene (Carson 2011; Nazareno et al. 2018).

Cultivars with race-specific, seedling resistance genes have been the primary means to control crown rust in North America (Gnanesh et al. 2014; Nazareno et al. 2018). These genes have been deployed either singly or in combinations. There are more than 100 described resistance genes designated with *Pc* gene symbols to date (Cabral et al. 2014; Sanz et al. 2013), but many could be the same or allelic because their chromosomal locations have not been determined. Most *Pc* genes are dominant, but some are partially dominant or recessive (Cabral et al. 2014; Simons et al. 1978). Initially, breeders utilized *Pc* genes from cultivated oat to develop new resistant cultivars (Gnanesh et al. 2013; Martens and Dyck 1989). Many *Pc* genes have been introgressed and deployed from accessions of the wild hexaploid *A. sterilis* collected in Israel and other Mediterranean countries during the 1960s and the early 1970s (Leonard et al. 2004). More than 16 genes from *A. sterilis* have been utilized in oat cultivars in North America (Leonard 2003). In the eastern prairie region of Canada, *Pc38*, *Pc39*, *Pc48*, and *Pc68* have been deployed in oat cultivars and remain common (McCallum et al. 2007). *Pc39* was introgressed into cultivated oat from the *A. sterilis* accession F366 collected in Israel (Fleischmann and McKenzie 1968) and was the first *A. sterilis* *Pc* gene to be used in Canada resulting in the release of cultivar Fidler in 1980 (McKenzie et al. 1981). Subsequently, the *Pc38* and *Pc39* genes were deployed in combination in the cultivars Dumont, Riel, Robert, AC Marie, AC Prekness, and Rebel from 1982 to 1993 (McCallum et al. 2007). *Pc68* was later added to develop cultivars with the *Pc38/39/68* gene pyramid. The

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first of these cultivars was AC Assiniboia (Brown et al. 2001), which was followed by AC Medallion, Pinnacle, and Ronald (Duguid et al. 2001; Mitchell Fetch et al. 2003a, b).

Pc39 is a dominant crown rust resistance gene effective at the seedling growth stage (Fleischmann and McKenzie 1968) that has been mapped relative to restriction fragment length polymorphism (RFLP) markers (Wight et al. 2005). Virulence to the *Pc38/39* gene combination was >87% by 1992 on the Canadian prairies (Chong and Seaman 1994). During 2002 to 2006, 57 to 93% isolates from wild oat in the prairie region were virulent to *Pc38* and *Pc39* (Chong et al. 2008). Yet in other regions of the world, *Pc39* maintains relatively high effectiveness to the native *Pca* population. For instance, *Pca* isolates collected from field trials at five locations in the Czech Republic from 2004 to 2006, 0 of 79 *Pca* isolates were virulent on *Pc39* and the resistance gene was recommended for use in oat breeding (Jiráková and Hanzalová 2008). Similarly, *Pc39* was highly resistant to natural *Pca* populations in field trials in Tunisia, with virulence present at low levels in 1 of 24 environments (Hammami et al. 2010). Paczos-Grzęda and Sowa (2019) assessed virulence using Polish *Pca* isolates collected from 2013 to 2015. A total of 31% (2014), 16% (2015), and 16% (2016) of *Pca* isolates were virulent on *Pc39*. Virulence data in other growing regions were not available. However, *Pc39* is likely useful in many growing regions where the gene has not been previously deployed. In such situations, *Pc39* should be deployed in combination with other effective *Pc* genes to prevent an increase in virulence to *Pc39*.

Pyramiding major resistance genes is seen as a valid strategy to prolong the effectiveness and durability of race-specific seedling resistance genes and has attracted the interest of oat breeders and pathologists. Resistance gene pyramids can be identified in indoor seedling inoculations with carefully selected *Pca* isolates. One isolate is needed for each gene desired in the pyramid, in which this isolate is avirulent on the targeted gene and virulent on the other genes in the pyramid. This strategy is not practical to pyramid three or more *Pc* genes or to pyramid *Pc* genes to which no virulence exists. Marker-assisted selection (MAS) simplifies the pyramiding of resistance genes because any number of resistance genes can be identified, provided that closely linked markers have been developed. DNA markers are available for >18 oat crown rust resistance genes (Gnanesh et al. 2014). The chromosome location of many *Pc* genes is not known, since a chromosome-anchored linkage map was only recently developed in 2013 (Bekele et al. 2018; Chaffin et al. 2016; Oliver et al. 2013). This has hampered the identification of the allelic relationships among the *Pc* genes. The crown rust resistance genes *Pc91* (Gnanesh et al. 2013), *PcKM* (Gnanesh et al. 2015), *Pc45* (Kebede et al. 2018), *Pc53* (Admassu-Yimer et al. 2018), and an adult plant resistance gene derived from MN841801 (Lin et al. 2014) were mapped relative to single nucleotide polymorphism (SNP) markers, which positioned these genes in the oat genome. There is a strong need in oat breeding and genetics to map all *Pc* genes onto the oat genetic map to reveal allelic relationships and identify linkages among *Pc* genes and other economically important genes. This knowledge will facilitate the construction of desirable gene combinations.

The objectives of this study were as follows: (i) to study the inheritance of the major seedling resistance gene *Pc39* in the cultivars AC Assiniboia and AC Medallion, (ii) to genetically map the resistance gene to a specific oat chromosome, (iii) to develop Competitive Allele-Specific PCR (KASP) markers suitable for MAS of *Pc39*, and (4) to evaluate the effectiveness of these KASP markers to predict *Pc39* status in a diverse panel of oat germplasm.

Materials and Methods

Plant material. The segregation of the seedling crown rust resistance gene *Pc39* was investigated in two recombinant inbred line (RIL) populations with varying numbers of progeny. The two populations were derived from the crosses AC Assiniboia/MN841801 (AsbMN) and AC Medallion/MN841801 (MedMN), of which the AsbMN population consisted of 143 F₈-derived RILs and the MedMN population consisted of 121 F₆-derived RILs. AC

Assiniboia (pedigree = *Pc68/7**Robert) is a tan-hulled oat (*A. sativa* L.) cultivar developed at the former Cereal Research Centre (Agriculture and Agri-Food Canada) in Winnipeg, Canada (Brown et al. 2001). AC Medallion (pedigree = *Pc68/7**Dumont) is a white-hulled oat (*A. sativa* L.) cultivar also developed at the former Cereal Research Centre (Duguid et al. 2001). Both AC Assiniboia and AC Medallion possess the crown rust resistance gene combination *Pc38*, *Pc39*, and *Pc68*, which was highly effective against the *Pca* population on the Canadian prairies at the time of registration of these cultivars. The MN841801 experimental oat line was developed by Paul Rothman at the University of Minnesota in the early 1970s. This line has been resistant to diverse populations of crown rust in rust nurseries for >35 years (Leonard 2002). The pedigree of MN841801 is MN841801 Florad/Coker 58-7/3/C17558//Black Mesdag/Aberdeen 101. The differential lines for *Pc38* (pedigree = CAV 2648-4/4*Pendek), *Pc39* (pedigree = CAV 5165/4*Pendek), and *Pc68* (pedigree = Makuru*2//CAV 4904/2*Sun II) were also included as checks for inoculation experiments.

A panel of 74 crown rust-susceptible oat lines and cultivars from the Collaborative Oat Research Enterprise (CORE) project (Esvelt Klos et al. 2017) were used for haplotype analysis with the goal of identifying KASP assays useful for MAS of *Pc39*. The susceptibility of these oat cultivars and breeding lines was verified by testing them with the same *Pca* isolate, 2015118 (race LMBG), that was used to map *Pc39*. Leaf tissue collection and DNA extraction was conducted as outlined below. These oat lines were tested with KASP assays interrogating SNPs linked to *Pc39*.

Evaluation of crown rust reaction. *Pca* isolate 2015118 (race LMBG; virulence/avirulence formula = *Pc38*, 40, 56, 68/39, 45, 46, 48, 50, 51, 52, 54, 58, 59, 62, 64) was used to study the segregation of *Pc39* in two RIL populations (AsbMN and MedMN) and 74 oat lines in the haplotype panel. *Pca* isolate 2015118 was collected in the eastern Prairie region of western Canada in August 2015 from wild oat (*A. fatua*). Seedlings were inoculated at the one-leaf stage by spraying urediniospores (4 mg/450 µl) suspended in light industrial oil (Bayol; Esso Canada, Toronto, ON, Canada) onto the leaves of the plants. Approximately five seedlings from each RIL of the AsbMN and MedMN populations were inoculated in independent experiments. The AsbMN population was inoculated in two experiments (i.e., 10 plants tested per RIL), whereas the MedMN population was inoculated in a single experiment (i.e., five plants tested per RIL). Inoculated plants were incubated in a Percival model I-60D dew chamber (Percival Scientific, Perry, IA, USA) overnight at 15°C and then transferred to a greenhouse with the temperature maintained between 18 and 22°C and 16 h of daylight supplemented with high-pressure sodium lighting (canopy-level light intensity = 450 µmol m⁻² s⁻¹). Crown rust infection type (IT) was scored 12 to 14 days after inoculation on a 0 to 4 scale (Murphy 1935): IT scores of 0 (immune), ; (fleck), 1, and 2 were considered resistant, and ITs of 3 and 4 were considered susceptible.

DNA marker analyses. After disease symptoms were rated, leaf tissue was collected from the third and fourth leaves (i.e., noninfected) into envelopes. The tissue was lyophilized and stored at -80°C until DNA extraction. High-quality genomic DNA was extracted from this lyophilized tissue using the DNeasy Plant DNA extraction kit (Qiagen, Toronto, ON, Canada). DNA was quantified using the fluorescent dye Hoechst 33258. Fluorescence was measured with a Tecan Infinite 135 F500 reader (Tecan Group Ltd., Männedorf, Switzerland).

The AsbMN population, AC Assiniboia, AC Medallion, and MN841801 were genotyped using genotyping-by-sequencing (GBS) following the protocol described by previous researchers (Huang et al. 2014). Genomic DNA was digested using two restriction enzymes, *Pst*I (CTGCAG) and *Msp*I (CCGG), and barcoded adaptors were ligated to each DNA sample in a 96-well plate. The 96 samples of a plate were then pooled into a single library and amplified by PCR. Two pooled libraries were generated to accommodate the number of genotypes tested in this study. A single midoutput lane of NextSeq 500 (Illumina, San Diego, CA, USA) was used to sequence each pooled library. Single-end sequenced

reads of 150 bases were generated. Haplotag software (Tinker et al. 2016) was used to call haplotype-based SNPs at approximately 240,000 loci. The AsbMN population was previously genotyped with the 6K oat (Tinker et al. 2014) and 90K wheat (Wang et al. 2014) Infinium iSelect SNP assays (Lin et al. 2014). All genotyping data were merged to construct a new linkage map for the AsbMN population, which identified SNPs linked to *Pc39*.

KASP markers were designed for *Pc39*-linked SNPs (GBS and 6K oat Infinium) using PrimerPicker Lite software (LGC Genomics LLC, Beverly, MA, USA) and tested on both RIL populations (Supplementary Table S1). The KASP assay included 2.5 µl of DNA (15 ng/µl), 2.5 µl of 2× KASP reaction mix, and 0.07 µl of primer mix. Each primer mix consisted of two forward primers and one

reverse primer diluted to a concentration of 100 pmol/µl. A 5-µl reaction volume was used for PCR with the following conditions: initial denaturation at 94°C for 15 min, 94°C for 20 s, 10 touchdown cycles over 61 to 55°C for 1 min (dropping down by 0.6°C per cycle), and 26 cycles of 20 s at 94°C followed by an extension for 1 min at 55°C. PCR samples were maintained in the dark at 4°C until an Omega FLUOstar scanner (BMG LABTECH GmbH, Offenburg, Germany) determined the fluorescence of each sample. KlusterCaller software (LGC Genomics LLC) analyzed the fluorescence data to identify clusters for scoring SNP alleles.

Statistical and linkage analyses. For each RIL population, the crown rust IT data were tested for fit to Mendelian segregation ratios by χ^2 analysis. Linkage maps were constructed with MapDisto version 1.7.7 software (Lorieux 2012). Genotypes and markers with >10% missing marker data and heterozygous RILs were excluded from linkage analysis. A minimum logarithm of odds score of 3 and a maximum recombination fraction of 0.3 were used as criteria to determine linkage groups. The Kosambi mapping function (Kosambi 1943) was used to convert recombination fractions to centimorgans. MapChart 2.1 software (Voorrips 2002) was used to illustrate linkage maps.

Results

Segregation of crown rust reaction. The *Pc39* differential line and the *Pc39* donor parents AC Assiniboia and AC Medallion had resistant IT scores (IT score = ; or ;1) when inoculated with *Pca* isolate 2015118 (race LMBG) in the seedling inoculation experiments

Table 1. Segregation ratios of the AC Assiniboia/MN841801 (AsbMN) and AC Medallion/MN841801 (MedMN) recombinant inbred line (RIL) populations when inoculated with crown rust isolate 2015118^a (race LMBG^b)

RIL population	HR (n) ^c	Segregating (n)	HS (n) ^d	HR/HS ratio	χ^2	P value
AsbMN	80	3	60	1:1	2.64	0.10
MedMN	61	1	59	1:1	0.03	0.86

^a Crown rust isolate number at Morden Research and Development Centre.

^b Race designation according to the standard North American system of nomenclature of *Pca* (Chong et al. 2000).

^c HR = homozygous resistant.

^d HS = homozygous susceptible.

Fig. 1. Linkage maps showing the common location of the *Pc39* crown rust resistance gene. “Consensus Map” refers to Mrg11 (chromosome 1C) of the chromosome-anchored consensus genetic map (Bekele et al. 2018). Loci in bold are present in more than one linkage map. Lines between linkage maps highlight the colinearity of the maps. The ruler on the left of the linkage maps is scaled in centimorgans.

(Supplementary Fig. S1). The *Pc38* and *Pc68* differential lines and the parent MN841801 had the same IT as the susceptible control cultivar Makuru (IT score = 4). The AC Assiniboia/MN841801 (AsbMN) RIL population segregated 80 homozygous resistant (HR), three segregating, and 60 homozygous susceptible (HS), which is consistent with a single gene controlling resistance (Table 1). The AC Medallion/MN841801 (MedMN) RIL population segregated 61 HR, one segregating, and 59 HS, which is also consistent with a single gene controlling resistance (Table 1). Both of the mapping populations (AsbMN and MedMN) fit a 1:1 resistant/susceptible segregation ratio, indicating a single segregating gene. The reactions of the parents and differential lines indicate that the segregating gene was *Pc39*.

***Pc39* marker development and linkage analysis.** The parental lines and the AsbMN RIL population were genotyped using GBS in this study and were previously genotyped with the 6K oat and 90K wheat Infinium iSelect SNP assays (Lin et al. 2014). Among

these, a total of 522 SNP loci were identified to be linked to *Pc39*. Sixteen SNPs closely linked to *Pc39* were selected for KASP marker development. The sequences of the KASP PCR primers are reported in Supplementary Table S1. Using these data, a linkage group was created for the AsbMN mapping population using the *Pc39* scoring data and 16 newly designed KASP markers (Fig. 1). *Pc39* cosegregated with KASP marker GMI_ES01_c12570_390_kom235 and was bracketed by markers avgbs_126086.1.41_kom234 and GMI_ES15_c276_702_kom237 with genetic distances of 1.7 and 0.3 cM, respectively. The AsbMN *Pc39* linkage map including all SNP loci is presented in Supplementary Table S2. To confirm these results, the 16 KASP markers were tested in the MedMN population. Thirteen of 16 markers were polymorphic between the parental lines and were mapped in this population (Fig. 1). Again, *Pc39* cosegregated with KASP marker GMI_ES01_c12570_390_kom235 and was flanked by the markers avgbs_126086.1.41_kom234 and GMI_ES15_c276_702_kom237 with genetic distances of 0.4 and

Table 2. Evaluation of the predictive accuracy of single nucleotide polymorphism markers linked to the *Pc39* gene in a diverse set of oat lines

Oat line	IT ^a	<i>Pc39</i> carrier	avgbs_95390.1.37_kom242	avgbs2_192806.1.35_kom241	GMI_ES03_c17298_427_kom238	GMI_ES15_c6153_392_kom239	GMI_ES15_c276_702_kom237	GMI_ES01_c12570_390_kom235	avgbs_126086.1.41_kom234	GMI_ES15_c694_635_kom231	GMI_ES11_c2261_56_kom230	GMI_ES01_c719_992_kom229	GMI_ES01_c10157_312_kom228	GMI_ES03_c11209_534_kom227
AC Assiniboia	;	Yes	A ^b	A	A	A	A	A	A	A	A	A	A	A
AC Medallion	;	Yes	A	A	A	A	A	A	A	A	A	A	A	B
Makuru	4	No	B	B	A	B	B	B	A	B	A	A	A	B
MN841801	4	No	B	B	B	B	B	B	B	B	B	B	B	B
00Ab7006	4	No	B	A	B	B	B	B	A	–	A	A	A	B
00Ab7085	4	No	B	A	B	B	B	B	A	A	A	A	A	B
02Ab6655	4	No	B	A	B	B	B	B	A	A	A	A	A	B
02HO-139	4	No	B	A	B	B	B	B	A	A	A	A	A	B
95Ab12770	4	No	B	B	A	B	B	B	A	A	A	A	A	B
95Ab13050	4	No	B	A	B	B	B	B	A	A	A	A	A	B
98Ab7265	4	No	B	A	B	B	B	B	A	A	A	A	A	B
99Ab10937	4	No	A	A	A	B	B	B	A	–	A	A	–	B
99Ab10987	3	No	B	A	B	B	B	B	A	A	A	A	A	B
99Ab11098	4	No	B	A	B	B	B	B	A	A	A	A	A	B
99Ab11227	3	No	B	A	B	B	B	B	A	A	A	A	A	B
99Ab11391	4	No	B	A	B	B	B	B	A	A	A	A	A	B
Andrew	4	No	A	A	A	B	B	B	A	B	B	B	B	B
ARDENTE	4	No	B	A	A	B	B	B	A	B	A	A	A	B
BARRA	4	No	B	A	A	B	B	B	A	B	A	A	A	B
Biri	4	No	B	A	B	B	A	B	A	B	B	A	–	A
Bond	4	No	A	B	B	B	A	A	A	–	B	A	B	A
Brown 1409-164	4	No	B	A	B	B	A	A	A	B	B	A	B	A
Bullion	4	No	B	B	A	B	B	B	A	B	A	A	A	B
CILLA	4	No	B	A	A	B	B	B	A	B	A	A	A	B
CIRCLE	4	No	B	A	A	B	B	B	A	B	A	A	A	B
Columbia	4	No	B	B	B	B	A	A	A	B	B	A	–	A
Derby	4	No	B	B	A	B	B	B	A	B	A	A	A	B
Firth	4	No	A	A	A	A	A	B	A	A	B	A	B	B
FREJA	4	No	B	A	B	B	A	A	A	A	A	A	A	B
Gere	4	No	B	A	A	B	B	B	A	A	A	A	A	B
GN04399	4	No	B	A	A	B	B	B	A	B	A	A	A	B
Grane	4	No	B	A	A	B	B	B	A	B	A	A	A	B
Grenader	4	No	B	B	B	B	A	A	A	B	B	A	B	A
GUNHILD	4	No	B	B	A	B	B	B	A	B	A	A	–	B
HA05AB34-48	3	No	B	A	A	B	B	B	A	A	A	A	A	B
HA05AB35-16	3	No	B	A	B	B	B	B	A	A	A	A	A	B
HA05AB36-33	4	No	B	A	A	B	A	B	A	B	A	A	B	B
HA05AB38-22	3	No	B	A	B	B	B	B	A	A	A	A	A	B
HA05AB41-38	4	No	B	A	A	B	B	B	A	A	A	A	A	B

(Continued on next page)

^a Infection type (IT) based on inoculation with oat crown rust isolate 2015118 (race LMBG). IT scores of 0 (immune), ; (fleck), 1, and 2 were considered resistant, and ITs of 3 and 4 were considered susceptible.

^b A is the AC Assiniboia allele, B is the MN841801 allele, and – is missing data.

0.4 cM, respectively. Comparison of the present linkage maps with the previously published oat consensus genetic map (Bekele et al. 2018) showed that *Pc39* was located on linkage group Mrg11 of the oat consensus map (chromosome 1C) at 3.7 cM (Fig. 1).

Evaluation of *Pc39*-linked SNPs in diverse germplasm. A set of 74 oat lines and cultivars from the CORE project (Esvelt Klos et al. 2017) were tested with 12 of the newly developed KASP markers closely linked to *Pc39* (Table 2). These CORE lines were planted in the greenhouse and inoculated with crown rust isolate 2015118 (race LMBG). AC Assiniboia and AC Medallion were resistant to this isolate because of the presence of *Pc39* as described above, whereas the remaining oat lines were susceptible and did not carry *Pc39*. Genotyping results revealed that the markers GMI_ES15_c6153_392_kom239 (prediction accuracy = 97%), avgbs_95390.1.37_kom242 (prediction accuracy = 85%), and GMI_ES03_c11209_534_kom227 (prediction accuracy = 85%) were the most predictive of *Pc39* since the AC Assiniboia allele was detected in only a few of the susceptible

lines for these markers. Together GMI_ES15_c6153_392_kom239 (approximately 0.8 cM from *Pc39*) and GMI_ES01_c12570_390_kom235 (cosegregating with *Pc39*) correctly differentiated the *Pc39* status of all of these oat lines. These two markers will be useful for MAS.

Discussion

The current study developed new KASP markers tightly linked to the crown rust resistance gene *Pc39* in two RIL populations. *Pc39* was also placed on linkage group Mrg11 (chromosome 1C) of the oat consensus map. Several newly designed KASP markers accurately predicted most of the oat lines that are susceptible to the *Pca* race LMBG. These markers will be useful for MAS of *Pc39* and will facilitate pyramiding *Pc39* with other *Pc* genes in future breeding. The genes *Pc39* and *Pc55* were previously reported to be very closely linked or allelic (Kiehn et al. 1976), so the SNP markers developed in this study could also be used to map *Pc55* and confirm the association

Table 2. (Continued from previous page)

Oat line	IT ^a	<i>Pc39</i> carrier	avgbs_95390.1.37_kom242	avgbs2_192806.1.35_kom241	GMI_ES03_c17298_427_kom238	GMI_ES15_c6153_392_kom239	GMI_ES15_c276_702_kom237	GMI_ES01_c12570_390_kom235	avgbs_126086.1.41_kom234	GMI_ES15_c694_635_kom231	GMI_ES11_c2261_56_kom230	GMI_ES01_c719_992_kom229	GMI_ES01_c10157_312_kom228	GMI_ES03_c11209_534_kom227
HA05AB42-20	4	No	B	A	B	B	B	B	A	A	A	A	A	B
HA05AB53-40	4	No	B	A	B	B	B	B	A	A	A	A	A	B
HA05AB9-52	3	No	B	A	B	B	B	B	A	A	A	A	A	B
HA08-03X31-1	3	No	B	A	B	B	B	B	A	A	A	–	A	B
IL02-5630	4	No	B	B	B	B	A	A	A	B	B	A	B	A
IL2250-14 (PI641977)	4	No	A	B	A	B	B	B	A	B	B	B	B	B
IL3555 (PI641970)	4	No	A	B	B	B	B	–	–	A	A	A	B	B
Kapp	4	No	B	A	A	B	B	B	A	A	A	A	A	B
Kolbu	4	No	B	A	A	B	B	B	A	B	A	A	A	B
LAO-1134-022	4	No	B	B	A	B	B	B	A	B	A	A	A	B
LAO-1136-024	4	No	B	B	A	B	B	B	A	B	A	A	B	B
LAO-1136-056	4	No	B	A	B	B	A	A	A	B	A	–	A	B
Lena	3	No	B	A	B	B	B	B	A	A	A	A	A	B
Lennon	4	No	A	B	A	A	A	B	A	A	B	A	B	B
LIPOPLUS	3	No	B	A	B	B	A	A	A	A	A	A	A	B
Moholt	4	No	B	A	B	B	A	A	A	B	B	A	B	A
ND001397	4	No	A	A	A	B	B	B	A	A	A	A	A	B
Nes	4	No	B	A	A	B	B	B	A	B	A	A	A	B
Nudist	4	No	B	A	A	B	B	B	A	B	A	A	A	B
Odal	4	No	B	A	B	B	A	A	A	A	A	A	A	B
Ogle	4	No	B	B	B	B	A	A	A	B	B	A	B	A
Olam	4	No	B	A	A	B	B	B	A	B	A	A	A	B
PI266887-1	4	No	B	B	B	B	B	A	B	B	B	B	B	B
PI263412-1	4	No	B	B	B	B	A	A	A	B	B	B	B	B
POL	4	No	B	B	B	B	A	A	A	B	B	A	B	A
Porter	4	No	B	A	A	B	B	B	A	B	B	B	B	B
Putnam 61	4	No	A	B	A	B	B	B	–	A	A	A	A	B
Racoon	4	No	B	A	A	B	B	A	A	A	A	–	A	B
Ringsaker	4	No	B	A	B	B	A	A	A	B	B	A	B	A
SW KERSTIN	4	No	B	A	A	B	B	B	A	B	A	A	A	B
SW VAASA	4	No	B	A	A	B	B	B	A	A	A	A	A	B
Tippecanoe	4	No	A	B	A	B	B	B	A	A	A	A	A	B
Tyler	4	No	A	B	A	B	B	B	A	B	B	B	B	B
VAO-58	4	No	B	A	A	B	B	B	–	B	A	A	A	B
Zuton	4	No	B	A	B	B	B	B	A	A	A	A	A	B
Lines (n)			74	74	74	74	74	74	74	74	74	74	74	74
Misclassified (n)			11	50	36	2	18	16	67	33	53	62	48	11
Missing data			0	0	0	0	0	1	3	3	0	3	4	0
Prediction accuracy (%)			85	32	51	97	76	78	6	54	28	13	31	85

of these genes. The present study adds to a growing body of literature that has positioned *Pc* genes on a SNP-based oat consensus map. Information regarding the distribution of *Pc* genes throughout the genome and allelic relationships among genes will deepen as additional genes are mapped.

Pc39 was previously mapped relative to RFLP markers in two populations, Pendek-39/Pendek-48 and OT328/Dumont (Wight et al. 2005). In that study, *Pc39* was tightly linked to the RFLP isu2287 in both populations. Interestingly, this RFLP was placed in five locations on the oat consensus map, one of which was Mrg11 at 8.8 cM (Bekele et al. 2018; Chaffin et al. 2016). Therefore, the results of our study are consistent with Wight et al. (2005). However, the present study conclusively located *Pc39* on consensus map linkage group Mrg11 (chromosome 1C), which was not possible based upon the previous RFLP maps.

A quantitative trait locus (QTL) conditioning crown rust resistance in oat was previously mapped to the Mrg11 linkage group (Esvelt Klos et al. 2017). This QTL was detected using genome-wide association mapping in a panel of spring oats tested in field nurseries in North Dakota, Minnesota, and Manitoba. This QTL mapped on Mrg11 based on the SNP loci Avgbs_222153 (at 21.9 cM) and GML_ES17_1rc7896_489 (at 16.2 cM). When taken together, the results of Esvelt Klos et al. (2017) and the present study suggest that *Pc39* and the QTL detected by association mapping are different. The most predictive marker for *Pc39* (GML_ES15_c6153_392) and the marker cosegregating with *Pc39* (GML_ES01_c12570_390) were both tested by Esvelt Klos et al. (2017) and were not significantly associated with crown rust reaction. This seems highly unlikely if *Pc39* was responsible for the QTL effect detected on Mrg11 by Esvelt Klos et al. (2017). Since the seedling resistance gene *Pc39* and the crown rust resistance QTL on Mrg11 reported in Esvelt Klos et al. (2017) are linked on the same linkage group, this genome region can be used as a combination in the future breeding programs to obtain oat cultivars that possess resistance to multiple *Pca* isolates.

In summary, the race-specific crown rust resistance gene *Pc39* was mapped to oat consensus map linkage group Mrg11 (oat chromosome 1C) based on linkage to SNP markers. This is the first report of the chromosomal location of *Pc39*. The newly developed KASP markers linked to *Pc39* will facilitate the pyramiding of this gene with other crown rust resistance genes. These findings improve our understanding of the genetic basis of crown rust resistance in oat and will facilitate MAS of *Pc39* to develop disease-resistant oat cultivars. The potential allelism or linkage of *Pc39* and *Pc55* should be investigated in future research.

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