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ORIGINAL PAPER

Mapping of the oat crown rust resistance gene *Pc91*

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Abstract Crown rust is an important disease of oat caused by *Puccinia coronata* Corda f. sp. *avenae* Eriks. Crown rust is efficiently and effectively managed through the development of resistant oat varieties. *Pc91* is a seedling crown rust resistance gene that is highly effective against the current *P. coronata* population in North America. The primary objective of this study was to develop DNA markers linked to *Pc91* for purposes of marker-assisted selection in oat breeding programs. The *Pc91* locus was mapped using a population of F7-derived recombinant inbred lines developed from the cross 'CDC Sol-Fi'/'HiFi' made at the Crop Development Centre, University of Saskatchewan. The population was evaluated for reaction to *P. coronata* in field nurseries in 2008 and 2009. *Pc91* mapped to a linkage group consisting of 44 Diversity Array Technology (DArT) markers. DArTs were successfully converted to sequence characterized amplified region (SCAR) markers. Five robust SCARs were developed from three non-redundant DArTs that co-segregated with *Pc91*. SCAR markers were developed for different assay systems, such that SCARs are available for agarose gel electrophoresis, capillary electrophoresis, and Taqman single nucleotide polymorphism detection. The SCAR markers accurately postulated the *Pc91* status of 23 North American oat breeding lines.

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Introduction

Crown rust is the most widespread and damaging disease of oat (*Avena sativa* L.) worldwide (Simons 1985), and is caused by the fungus *Puccinia coronata* Corda f. sp. *avenae* Eriks. Estimated yield loss due to crown rust in the eastern Prairie region of Canada was 5.1% annually in the 5-year period from 2001 to 2005 (J. Chong, unpublished data). Crown rust can be managed with a combination of early seeding, host resistance, in-crop foliar fungicide application, and elimination of alternate hosts (*Rhamnus* spp.). Host resistance is considered the most effective, economical, and environmentally friendly control method. Host–pathogen interactions in the oat-*P. coronata* pathosystem are based upon a gene-for-gene interaction, with evidence of physiological specialization in the pathosystem dating back nearly a century (Hoerner 1919). Consequently, crown rust resistance based upon major genes has broken-down without exception following widespread deployment (McCallum et al. 2007). Despite this problem, interest in major gene resistance remains strong because crown rust control is very effective prior to the buildup of virulent individuals in the *P. coronata* population. Pyramiding major resistance genes has attracted the interest of oat breeders and pathologists because it is expected to prolong the effectiveness of resistance genes.

Pc91 is a major crown rust resistance gene effective at all stages of plant development. Amagalon is the original source of *Pc91* and is a progeny from a cross between tetraploid *Avena magna* (genome AACC) and diploid *Avena longiglumis* (genome AlAl) (Rooney et al. 1994b). 'HiFi' was the first oat variety released carrying *Pc91* and was released for commercial production in North Dakota and Minnesota, USA in 2001 and in western Canada in 2006. In the USA, virulence to *Pc91* occurred in 1 of 77 cultures collected from spring oat and 1 of 61 cultures collected from winter oat grown in 2008 (Carson 2009). In Canada, virulence to *Pc91* was identified in 2002 and 2003 over a survey period from 2002 to 2006 (Chong et al. 2008). More recently, virulence to *Pc91* was identified in 1 of 859 single-pustule isolates collected in Canada from 2007 to 2009 (Chong, unpublished data). *Pc91* remains effective but is expected to eventually breakdown due to increased frequency of virulence to the gene in the *P. coronata* population.

Efficient pyramiding requires appropriate selection pressure on all genes desired in the pyramid. Effective crown rust resistance can be readily selected in field disease nurseries containing multiple races. However, these tests do not differentiate oat lines carrying one effective resistance gene from lines carrying two or more resistance genes. Seedling tests with carefully selected isolates allows for the pyramiding two effective genes, but finding appropriate isolates to pyramid 3 or more genes becomes difficult. Typically, oat breeders would like to pyramid the most effective resistance (i.e. genes to which virulence is very low). Marker-assisted selection (MAS) simplifies this process because the presence of any number of resistance genes can be detected. However, the lack of high quality, high throughput markers in oat has limited the identification of markers linked to desirable traits. The development of the Diversity Array Technology (DArT) marker platform has greatly expanded the number of markers available for oat genetic research and marker numbers are expected to continue to grow with the development of single nucleotide polymorphism (SNP) markers. With these advances, trait selection will increasingly move towards MAS in the future.

Resistance gene postulation utilizes the gene-for-gene specificity between host resistance genes and pathogen avirulence genes to predict which resistance genes are present in a host genotype (Person 1959; Loegering et al. 1971). However, gene postulation can be complicated by epistatic interactions between resistance genes and is not readily amenable to analysis of adult plant resistance genes (Kolmer 1996). Resistance gene postulation can also be conducted with DNA markers linked to resistance genes (McCartney et al. 2004, 2005). This strategy overcomes some problems associated with traditional gene postulation, such as gene interactions and the plant stage of resistance gene expression. A combination of both approaches yields the most accurate postulations (McCartney et al. 2005).

The objectives of this study were to: (1) map *Pc91* relative to DArT markers (2) develop sequence characterized amplified region (SCAR) markers suitable for marker-assisted selection of *Pc91*; and (3) evaluate the utility of the SCAR markers for *Pc91* postulation in North American oat germplasm.

Methods and materials

Plant material

A population of 100 F_7-derived recombinant inbred lines (RILs) was developed from the cross 'CDC Sol-Fi'/'HiFi' made at the Crop Development Centre, University of Saskatchewan. 'HiFi' is a high beta-glucan spring milling oat variety developed by North Dakota State University with the pedigree Amagalon/4/M23/RL3038//'Otana'/3/'Froker'/RL3038//RL3038/'Hudson'/5/MN78142/4/W80-20/3/Hudson/'Lang'//'Dal' (McMullen et al. 2005). 'HiFi' inherited *Pc91* from Amagalon, a derivative of a cross between tetraploid *Avena magna* (genome AACC) and diploid *Avena longiglumis* (genome AlAl) (Rooney et al. 1994b). 'CDC Sol-Fi' is a high beta-glucan spring milling oat variety developed by the Crop Development Centre, University of Saskatchewan with the pedigree N979-5-1/OT366. 'CDC Sol-Fi' is susceptible to crown rust in field tests, but possesses some crown rust resistance in seedling tests.

Evaluation of crown rust reaction

Crown rust reaction was determined in four field tests at Saskatoon, Saskatchewan, Canada and Guelph, Ontario, Canada in 2008 and 2009. The 2008 tests consisted of a single replicate in each environment. The 2009 tests consisted of 11 × 10 alpha lattices with 2 replicates per environment. The 100 'CDC Sol-Fi'/'HiFi' RILs, 'CDC Sol-Fi', 'HiFi', 'AC Morgan', 'CDC Dancer', and 'Leggett' were evaluated in all environments. The 2009 environments also included 'CDC Orrin', 'AC Ronald', a Pc91 differential line, a Pc94 differential line, and a Pc96 differential line. Experimental units were hill plots seeded with approximately 30 seeds.

In Saskatoon, the spreader rows were seeded to 'AC Morgan', a highly susceptible variety which does not carry major resistance genes. The Saskatoon nurseries were inoculated with a mixture of urediniospores of local *P. coronata* isolates. Urediniospores were suspended in

Bayol (Esso Canada) and inoculated with a Micron Microfit HerbiFlex herbicide applicator (Micron Sprayers Ltd, Bromyard, UK). The Guelph nurseries were inoculated with aeciospores produced on common buckthorn (*Rhamnus cathartica* L.). The spreader rows were seeded to a mixture of susceptible varieties.

In Saskatoon, crown rust severity was evaluated with the modified Cobb scale (Peterson et al. 1948) and infection type as follows: R, resistant with necrotic flecks; MR, moderately resistant with small uredinia surrounded by necrosis/chlorosis; MS, moderately susceptible with medium to large uredinia surrounded by chlorosis; and S, susceptible with large uredinia lacking chlorosis or necrosis. In Guelph, crown rust was evaluated on a 0–9 scale: 0, 1, 2 were resistant with a few uredinia on the lower leaves; 3, 4 had additional uredinia, but not over halfway up the plant; 5, 6 had some uredinia on the flag leaf; 7, 8, 9 were susceptible, with varying levels of uredinia on the flag leaf.

DNA extraction and marker analysis

Ninety random RILs of the 'CDC Sol-Fi'/'HiFi' population were selected for Diversity Arrays Technology (DArT) marker analysis (Diversity Arrays Technology Pty. Ltd, Yarralumla, Australia). DNA was extracted using a modified CTAB extraction protocol (Murray and Thompson 1980). For each line, coleoptiles were collected from five to six plants and DNA extracted as a bulk. Six hundred and forty-seven polymorphic DArTs were identified. DArTs with a Q value <80 and more than 10% missing data were excluded, resulting in 386 DArTs for linkage analysis. DArT marker sequences were compared using BLASTN 2.2.23+ (Zhang et al. 2000) to identify redundant DArTs.

SCAR markers were developed for 18 non-redundant DArTs. PCR primers were developed from the published DNA sequences for corresponding DArT clones (Tinker et al. 2009). Primers were designed to be 18–23 bases in length and have an annealing temperature between 52 and 60°C (Table 1). Standard PCR reactions were performed for amplicons to be separated in agarose. Standard PCR reactions were conducted in 15 µl volumes and included 50 ng of template DNA, 0.75 U of Taq DNA polymerase (Genscript, Piscataway, NJ, USA), 1× PCR buffer containing $MgCl_2$ (Genscript, Piscataway, NJ, USA), 200 µM each dNTP, and 6 pmol each forward and reverse primers (Invitrogen, Carlsbad, CA, USA). The reaction mixture was denatured at 94°C for 3 min, followed by 40 cycles of 94°C for 1 min, 52°C for 1 min, 72°C for 1 min, with a final extension step of 72°C for 5 min. PCR products were separated on 1.5% agarose gels and run in TBE buffer.

PCR reactions were modified when capillary electrophoresis was conducted on an ABI Prism® 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The modified PCR reaction was the same as the standard PCR reaction, but contained 1.5 pmol modified forward primer, 2 pmol 6-FAM/HEX/NED-labelled M13 primer (5′ → 3′, CACGACGTTGTAAAACGAC; Applied Biosystems, Foster City, CA, USA). The modified forward primers had a 5′, 19 nucleotide M13 tail (5′ → 3′, CACGACGTTG TAAAACGAC) (Schuelke 2000). PCR amplicons were resolved in an ABI Prism® 3130 Genetic Analyzer with GeneMapper® v4.0 and GeneScan®-500 ROX™ as an internal size standard (Applied Biosystems, Foster City, CA, USA).

A TaqMan SNP assay, oPt-0350-cdc4, was developed for DArT clone oPt-0350. The oPt-0350-cdc1 primer pair produced a single, robust fragment in susceptible genotypes 'CDC Sol-Fi' and OT3031, and resistant genotypes 'HiFi' and OT2048. Fragments from a single PCR amplification of each of the four genotypes (bulks of 5 seedlings each) were ligated into the pCR4-TOPO vector (Invitrogen TOPO TA cloning kit for sequencing) and transformed into TOP10 *Escherichia coli* chemically competent cells. Three clones per genotype were sequenced (Sanger) at the Plant Biotechnology Institute (Saskatoon, SK, Canada). DNA sequences were aligned using the software DNAMAN (Lynnon Biosoft). Six sequences from resistant genotypes were aligned revealing two variable nucleotides, while the six aligned sequences from susceptible genotypes revealed four variable nucleotides. Deduced consensus sequences for the resistant (585 nt) and susceptible genotypes (574 nt) were aligned revealing 33 SNPs plus three small insertion/deletions. A 3 nt indel (+CCG in resistant) at position 346 was selected for TaqMan assay design (Fig. 1). The indel and surrounding DNA sequence was analysed for assay design potential using ABI Primer Express 3.0. The bi-allelic ABI minor groove binder (MGB) assay consists of two locus amplification primers (forward, 5′ → 3′, CACAAGCATA

Table 1 Summary of SCAR markers linked to *Pc91*

Primer pair	Phase	Electrophoresis	DArT	Forward (5′ → 3′)	Reverse (5′ → 3′)
oPt-0350-cdc1	NA	Agarose	oPt-0350	CTACCAAATGTAAAAGGTGTATC	TCCGGACATTCCCGGAGTGTT
oPt-0350-cdc2	Co-dominant	Capillary	oPt-0350	M13-CACCTTCAAGGTAGTGTTTGG	AGGCGCAAACTCAATCTTG
oPt-0350-cdc3	Dominant-coupling	Agarose	oPt-0350	GGACTATCTAGTTTATGGAGGAG	AGGCAAAACGAGCAGTGTAA
oPt-9234-cdc1	Dominant-repulsion	Agarose	oPt-9234	TAGGCGAAGTAACTCAAGTAGC	CTTGTATTGTGCGTTGGAA
oPt-9546-cdc1	Dominant-repulsion	Agarose	oPt-9546	AACGGAAGAAATAAAAAAGGGA	TATAAATGGATGTTGTAGTAGGG

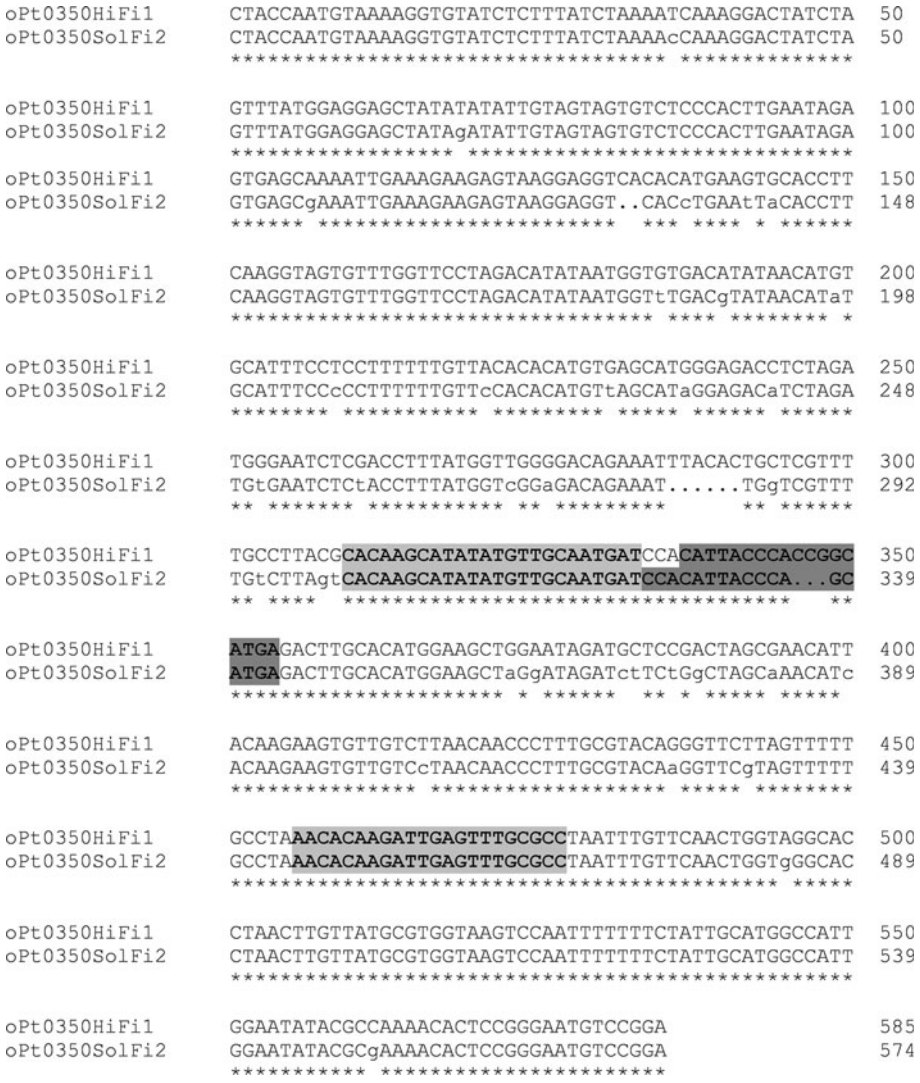

Fig. 1 DNA sequence alignment of the cloned 'HiFi' and 'CDC Sol-Fi' amplicons derived from oPt-0350-cdc1. The 'HiFi' sequence is the consensus sequence from three 'HiFi' reads and three OT2048 reads, and the 'CDC Sol-Fi' sequence is the consensus sequence from three 'CDC Sol-Fi' reads and three OT3031 reads. *Lightly shaded* sequences are the forward and reverse primers for oPt-0350-cdc4. *Darkly shaded* sequences are the allele-specific Taqman probes for oPt-0350-cdc4

TATGTTGCAATGAT; reverse, 5′ → 3′, GGCGCAAAC TCAATCTTGTGTT), plus two allele-specific dual-labelled probes (5′ → 3′, VIC-CATTACCCACCGGCATGA-NFQ [targets 'HiFi' allele]; 5′ → 3′, FAM-CCACATTACCC AGCATGA-NFQ [targets 'CDC Sol-Fi' allele]). TaqMan assays were performed in 20 µl volumes at 1× assay concentrations with 1× Maxima Probe/ROX qPCR Master Mix (Fermentas), and 50 ng genomic DNA template. The reaction mixture was denatured at 94°C for 10 min, followed by 40 cycles of 94°C for 15 s, 60°C for 1 min. Baseline fluorescence was standardized via ROX.

Construction of the linkage map

CarthaGene 1.0 R (de Givry et al. 2005) was used to create a linkage map from the DNA marker data. Loci were placed into linkage groups using a minimum LOD score of 3 and a maximum distance between markers of 30 cM. Marker order was determined using a combination of "build", "algogen", "greedy", "flips", and "polish" commands. Graphical linkage groups were generated with MapChart 2.1 software (Voorrips 2002).

Postulation of *Pc91* amongst oat germplasm

The SCARs oPt-0350-cdc2, oPt-0350-cdc3, oPt-0350-cdc4, oPt-9234-cdc1, and oPt-9546-cdc1 were used to screen a panel of 23 North American oat lines to test the utility of the markers in a broader set of germplasm. Details on these oat lines are presented in Table 2.

Results

Segregation of crown rust reaction

Crown rust developed well in Guelph 2008 (Fig. 2a), Guelph 2009, and Saskatoon 2009 (Fig. 2b), but reached

Table 2 Oat lines (with pedigrees) used to evaluate efficacy of *Pc91* markers

Oat line	Pedigree
'HiFi'	Amagalon/ND820712//ND852107/3/ND900118
Pc91	Amagalon/ND820712
SM7	Amagalon/4*'Ogle'
'Souris'	Amagalon/ND820712//ND852107/3/ND900118
'Stainless'	ND931475/'AC Assiniboia'//'HiFi'
OT2041	ND931475/'AC Assiniboia'//'HiFi'
OT2048	'Ronald'/'HiFi'
OT2052	'HiFi'/'AC Gwen'
OT2054	'HiFi'/'AC Gwen'
99P26-BY1D	'Riel'/ND931475//'HiFi'
'Ogle'	'Brave'//'Tyler'/Egdolon 23
'Leggett'	OT294/Pc94
OT2045	'Ronald'//ND931475/'AC Assiniboia'
OT2055	'Ronald'//ND931475/'AC Assiniboia'
'Ronald'	W89329 (dwarf)/'AC Medallion'
'CDC Sol-Fi'	N979-5-1/OT366
'CDC Minstrel'	OT293/'CDC Dancer'
OT3019	OT2000/SA96482
OT3028	'Goslin'/SA96400
'CDC Dancer'	OT344/W90279
'CDC Weaver'	OT369/OT2007
OT7051	CR245-Dw//'Paul'/'AC Kaufmann'
SA04429	OT389/ND910569

lower severity in Saskatoon 2008. Crown rust reaction was bimodal in all environments, allowing for the classification of RILs into resistant and susceptible classes. The classification of lines was consistent between environments. The 'CDC Sol-Fi'/'HiFi' population segregated 45 homozygous resistant (HR): 2 segregating: 53 homozygous susceptible (HS) RILs based on the field data. This data is consistent with a single gene controlling resistance (expected ratio = 49 HR: 2 segregating: 49 HS, $X^2 = 0.72$, Pr = 0.70).

Linkage analysis

Pc91 mapped within a 58.1 cM linkage group consisting of 44 DArTs in the 'CDC Sol-Fi'/'HiFi' population (Fig. 3). *Pc91* co-segregated with 18 of these DArTs. This linkage group was compared to the 'Kanota'/'Ogle' linkage map containing DArTs (Tinker et al. 2009). The 'CDC Sol-Fi'/'HiFi' *Pc91* linkage group aligned with the 'Kanota'/'Ogle' KOD_1_3_38_X3, KOD_1_3_38_breakpoint, and KOD_1_3_38_X1 linkage groups (Fig. 3). Nineteen of 21 DArTs that were immediately flanking *Pc91* in the 'CDC Sol-Fi'/'HiFi' linkage group were present in the 'Kanota'/'Ogle' KOD_1_3_38_breakpoint linkage group. The remaining two flanking DArTs, oPt-0120 and oPt-12215, were present in the 'Kanota'/'Ogle' KOD_2 linkage group. These two markers are underlined in the 'CDC Sol-Fi'/'HiFi' linkage group in Fig. 3.

All DArTs in the *Pc91* linkage group were compared using BLAST, except oPt-0120 and oPt-5552 for which DNA sequence were not available. Twenty-six of 42 DArTs were classified as redundant and interrogate 11 genetic loci (Supplementary Table 1). Redundant DArT pairs had *E* values of 0, an average maximum identity of 99.4%, and a minimum alignment length of 380 bp. Redundant DArTs co-segregated in the *Pc91* linkage map, except oPt-18233 with oPt-12195, oPt-14381, and oPt-16558. The DArT oPt-18233 mapped 0.6 cM from its corresponding redundant DArTs. Inspection of the scoring data revealed a single putative singleton scoring error for oPt-18233 relative to oPt-12195, oPt-14381, oPt-16558, and other flanking markers. The true map position of oPt-18233 is most likely 25.8 cM on the *Pc91* linkage map, co-segregating with oPt-12195, oPt-14381, oPt-15259, and oPt-16558. Cumulatively, this data suggests that the DArT marker data selected for linkage mapping was accurate.

SCAR development

SCAR markers were designed for 18 non-redundant DArTs linked to *Pc91*. Thirty-eight primer pairs were initially tested and separated on agarose. Seventeen primer pairs produced amplicons in both 'HiFi' and 'CDC Sol-Fi', 15 primer pairs produced amplicons in either 'HiFi' or 'CDC Sol-Fi', and six primer pairs did not produce an amplicon. PCR amplicons were cloned and sequenced from 'HiFi' and 'CDC Sol-Fi' for the oPt-0350-cdc1 primer pair. Alignments for oPt-0350-cdc1 are shown in Fig. 1. Two SCARs were developed from oPt-0350. The SCAR oPt-0350-cdc2 targets 2 indels (3 and 6 bp) between the 'HiFi' and 'CDC Sol-Fi' sequences. This SCAR is co-dominant and was visualized on an ABI Prism® 3130 Genetic Analyzer. The SCAR oPt-0350-cdc3 is a dominant marker that can be visualized on agarose (Fig. 4). The oPt-0350-cdc3 PCR amplicon is in coupling with *Pc91*. For this marker, the 3′ end of the reverse primer targets the 6 bp indel between the 'HiFi' and 'CDC Sol-Fi' sequences. The Taqman marker assay oPt-0350-cdc4 targeted a 3 bp indel, as described in "Materials and methods" section. The SCARs oPt-9234-cdc1 and oPt-9546-cdc1 are two additional high-quality markers designed for agarose electrophoresis, but their amplicons are in repulsion with *Pc91* (Fig. 4).

Postulation of *Pc91* using DNA markers

The utility of five *Pc91* markers was evaluated on a set of 23 North American spring oat lines. The synthetic

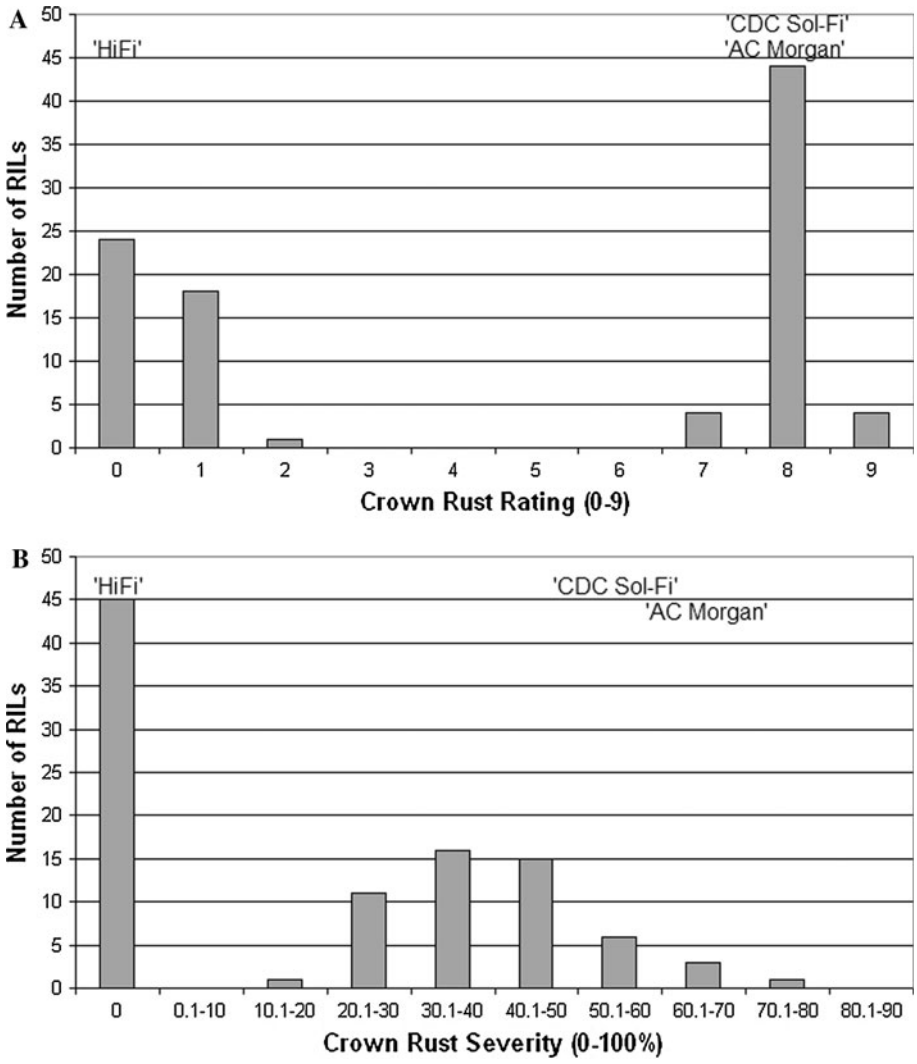

Fig. 2 Crown rust reactions of the 'CDC Sol-Fi'/'HiFi' population in **a** Guelph, 2008, and **b** Saskatoon, 2009. Reaction of check lines is indicated by placement above the corresponding crown rust reaction bin

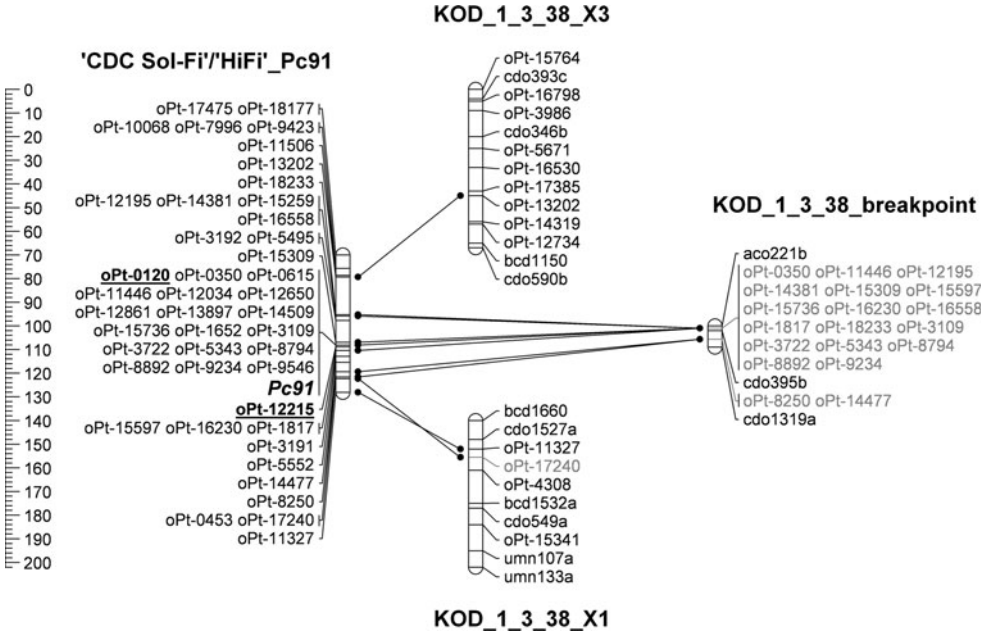

Fig. 3 Alignment of the 'CDC Sol-Fi'/'HiFi' *Pc91* linkage group with the 'Kanota'/'Ogle' linkage groups KOD_1_3_38_X3, KOD_1_3_38_breakpoint, and KOD_1_3_38_X1. *Underlined* oPt DArTs aligned with the 'Kanota'/'Ogle' KOD_2 linkage group. *Black* oPt DArTs on the 'Kanota'/'Ogle' linkage groups were framework markers, while *grey* oPt DArTs were placed within the framework maps (Tinker et al. 2009). An *inset ruler* indicates map distances in cm

Table 3 Evaluation of *Pc91* markers for gene postulation amongst North American oat germplasm

Oat Line	*Pc91* status	oPt-0350-cdc4	oPt-0350-cdc2	oPt-0350-cdc3	oPt-9234-cdc1	oPt-9546-cdc1
'HiFi'	Carrier	b	354[a]	265	Null	Null
Pc91	Carrier	ab	345, 354	265	444	705
SM7	Carrier	ab	345, 354	265	444	705
'Souris'	Carrier	b	354	265	Null	Null
'Stainless'	Carrier	b	354	265	Null	Null
OT2041	Carrier	b	354	265	Null	Null
OT2048	Carrier	b	354	265	Null	Null
OT2052	Carrier	b	354	265	Null	Null
OT2054	Carrier	b	354	265	Null	Null
99P26-BY1D	Carrier	b	354	265	Null	Null
'Ogle'	Not carrier	a	345	Null	444	705
'Leggett'	Not carrier	a	345	Null	444	705
OT2045	Not carrier	a	345	Null	444	705
OT2055	Not carrier	a	345	Null	444	705
'Ronald'	Not carrier	a	345	Null	444	705
'CDC Sol-Fi'	Not carrier	a	345	Null	444	705
'CDC Minstrel'	Not carrier	a	345	Null	444	705
OT3019	Not carrier	a	345	Null	444	705
OT3028	Not carrier	a	345	Null	444	705
'CDC Dancer'	Not carrier	a	345	Null	444	705
'CDC Weaver'	Not carrier	a	345	Null	444	705
OT7051	Not carrier	a	345	Null	444	705
SA04429	Not carrier	a	345	Null	444	705

[a] Length of PCR amplicon(s) in base pairs

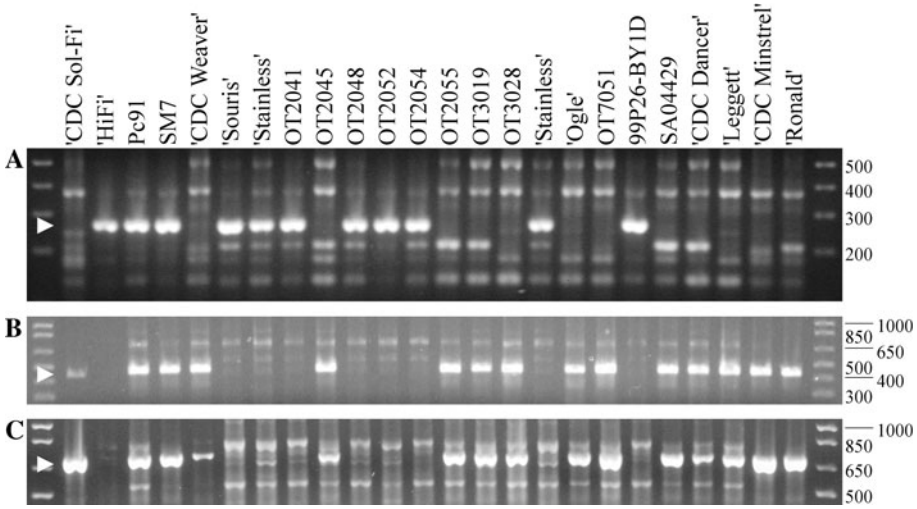

Fig. 4 Agarose gel images of SCARs evaluated on the panel of oat germplasm: **a** oPt-0350-cdc3, **b** oPt-9234-cdc1, and **c** oPt-9546-cdc1. *Arrowheads* identify the relevant DNA marker band. Sizes of DNA *ladder bands* are in bp

hexaploid oat line Amagalon is present in the pedigree of all germplasm carrying *Pc91* (Tables 2, 3; Fig. 4). The five *Pc91* markers accurately differentiated oat lines without *Pc91* and oat lines with *Pc91*, when the gene was inherited from 'HiFi'. The Pc91 differential line and SM7 had both products for the co-dominant markers oPt-0350-cdc2 and oPt-0350-cdc4, and the amplicons for the dominant markers oPt-0350-cdc3, oPt-9234-cdc1, and oPt-9546-cdc1 (Fig. 4). This preliminary result suggested heterogeneity in these lines. To investigate this result further, DNA was extracted from multiple individuals of the Pc91 differential line and SM7 to be re-tested with oPt-0350-cdc4. All plants of both lines produced the same results as initially tested, which eliminated the possibility of heterogeneity in these lines.

Discussion

The present study mapped *Pc91* to a linkage group consisting of 44 DArTs. Eighteen DArTs co-segregated with *Pc91*, 11 of which were non-redundant. Five PCR-based markers were developed from three non-redundant DArTs that co-segregated with *Pc91*. These markers accurately postulated *Pc91* amongst a diverse set of North American spring oat germplasm. This data suggests that the *Pc91* markers will likely be polymorphic in many oat breeding populations segregating for *Pc91*. Different marker types were developed such that *Pc91* markers are available for agarose electrophoresis, capillary electrophoresis, and TaqMan SNP detection. These markers are expected to be useful for marker-assisted selection of *Pc91* and will assist pyramiding *Pc91* with other effective *Pc* genes.

The 'CDC Sol-Fi'/'HiFi' *Pc91* linkage group aligned with the 'Kanota'/'Ogle' KOD_1_3_38_X3, KOD_1_3_38_breakpoint, and KOD_1_3_38_X1 linkage groups. These 'Kanota'/'Ogle' linkage groups are assumed to be involved in the 7C-17 translocation (Δ7C-17) (Tinker et al. 2009). This would place *Pc91* on chromosome 7C or 17, which is not consistent with the previous placement of *Pc91* to chromosome 18 (Rooney et al. 1994b). Rooney et al. (1994b) had located *Pc91* 4.5 cM from a 6.0 kb *Eco*RI fragment identified by the RFLP marker UMN145 in a consensus map derived from the crosses Amagalon/Ogle-1 and Amagalon/Starter-1. The authors did not indicate which lines carry the 6.0 kb *Eco*RI fragment. Rooney et al. (1994a) placed *Eco*RI fragments detected by UMN145 on chromosomes 16 (3.0 kb fragment), 18 (6.0 kb fragment), and 3C (12.0 kb fragment) in monosomics of *A. byzantina* cv. 'Kanota'. Since the UMN145 clone hybridizes to at least three loci in the oat genome and the oat germplasm in question is not closely related, the 6.0 kb *Eco*RI fragment linked to *Pc91* may not correspond to the same locus as the same 6.0 kb fragment that is present in Kanota. This would explain the discrepancies between these studies. The assignment of linkage groups to chromosomes remains a problem for oat that hopefully will be resolved in the future through cytogenetic analyses and the development of integrated SNP, DArT, and RFLP maps.

The number of DArTs linked to *Pc91* was surprising. *Pc91* originates from an alien introgression involving the oat line Amagalon, a derivative of a cross between tetraploid *Avena magna* (genome AACC) and diploid *Avena longiglumis* (genome AlAl) (Rooney et al. 1994b). *Pc91* is believed to have been contributed by the *Avena magna* parent (Rothman 1984). *Avena magna* has been proposed as the progenitor of the A and C genomes of *A. sativa* (Ladizinsky and Zohary 1971), but more recently *Avena insularis* was proposed as a more likely progenitor of the A and C genome (Ladizinsky 1998). Regardless, the *Pc91* introgression may not recombine well with the *A. sativa* genome, which could explain the number of DArTs co-segregating with *Pc91*. The DArTs co-segregating with *Pc91* aligned with the KOD_1_3_38_breakpoint linkage group. This linkage group also consisted of numerous DArTs in the 'Kanota'/'Ogle' population. The KOD_1_3_38_breakpoint linkage group was assumed to be the site of the 7C-17 translocation (Δ7C-17), which inhibits recombination in that region of the 'Kanota'/'Ogle' population (Tinker et al. 2009). Cumulatively, the data suggests that the *Pc91* introgression is inhibiting recombination in the region, which explains the large number of co-segregating DArTs.

The *Pc91* SCARs worked well for postulating *Pc91* when inherited from 'HiFi'. The SCARs did not work as well when *Pc91* was inherited from Amagalon. In the latter case, the *Pc91* SCARs identify products similar to 'HiFi' and 'CDC Sol-Fi' in the Pc91 differential and SM7, which suggested heterogeneity in the seed source of these two lines. This was based on data from five SCAR markers developed from three non-redundant DArTs. Analysis of multiple individuals of these lines with oPt-0350-cdc4 determined that the 'HiFi' product and 'CDC Sol-Fi'-like product were present in each individual. This eliminated the chance of heterogeneity, and suggested the region is duplicated. A tandem duplication is most likely because SM7 is a backcross derivative of Amagalon. If the duplicated regions were not linked, then the 'CDC Sol-Fi'-like product would probably have been eliminated during the backcross development of SM7. The tandem duplicated region could be the result of an unequal crossover event in the development of Amagalon or could be the native state of the region in one of the progenitors of Amagalon. The copy of the region generating the 'CDC Sol-Fi'-like products for oPt-0350-cdc4, oPt-0350-cdc2, oPt-9234-cdc1, and oPt-9546-cdc1, must have been eliminated in one of the two crosses that followed the development of the Pc91 differential line (ND894904) in the subsequent development of 'HiFi'.

Marker development in oat has not kept pace with other cereal crops because of genome complexity, oat research community size, and the limited research investment in the crop. The development of the DArT marker platform is an important first step in closing this gap. The number of DArTs linked to the gene of interest was fortunate in this case, but should not be expected in future oat mapping studies. DArTs were not uniformly distributed over the entire oat genome in the 'CDC Sol-Fi'/'HiFi' population, and substantial portions of the genome were not covered with DArTs (data not shown). In conclusion, this study has demonstrated that DArTs are a valuable tool for mapping in oat.

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