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Discovery, localization, and sequence characterization of molecular markers for the crown rust resistance genes *Pc38*, *Pc39*, and *Pc48* in cultivated oat (*Avena sativa* L.)

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Abstract

Molecular markers for the crown rust resistance genes *Pc38*, *Pc39*, and *Pc48* in cultivated oat (*Avena sativa* L.) were identified using near-isogenic lines and bulked segregant analysis. Six markers for *Pc48*, the closest being 6 cM away, were found in a 'Pendek-*39*' × 'Pendek-*48*' (Pendek3948) population, but none was found in a 'Pendek-*48*' × 'Pendek-*38*' (Pendek4838) population. Three markers for *Pc39* were found in the Pendek3948 population, one of which cosegregated with the gene. This same marker was found to be 6 cM away from the gene in an 'OT328' × 'Dumont' (OT328Du) population. Nine markers for *Pc38* were found in the Pendek4838 population, eight of which are within 2 cM of the gene. One other marker for *Pc38* was found in the OT328Du population; however, comparative mapping suggests that the *Pc38* region in OT328Du is in a different location than that in Pendek4838. A number of markers unlinked to the genes under study formed linkage groups in both the Pendek3948 and Pendek4838 populations. Four of these show homology or homoeology to each other and to the *Pc39* region in Pendek3948. Two RFLP clones closely linked to *Pc38* code for a putative leucine-rich repeat transmembrane protein kinase and a *cre3* resistance gene analogue. This study provides information to support molecular breeding in oat, and contributes to ongoing research into genomic regions associated with fungal pathogen resistance.

Introduction

Crown rust (causal agent *Puccinia coronata* Corda f. sp. *avenae* Eriks.) threatens oat (*Avena sativa* L.) production in many regions of the world. Genetic resistance is the most effective and economical method for controlling the disease. However, by the end of the 1950's, none of the known sources of crown rust resistance present in cultivated oat could provide adequate protection against the disease (Martens and Dyck 1989, Simons 1954). Wild populations of *Avena sterilis* L. in North Africa, the Mediterranean, and the Middle East proved to be a rich source of novel genes (http://www.cdl.umn.edu/res_gene/res_gene.html), many of which have been introgressed into cultivated oat germplasm. A number of these (*Pc38, Pc39, Pc48, Pc51, Pc52, Pc58, Pc59, Pc62*, and *Pc68*) have been, or are, important in breeding for resistance in North America (Chong 2000, Martens and Dyck 1989, McMullen and Patterson 1992).

Table 1. Pedigrees and presence or absence of crown rust resistance genes *Pc38*, *Pc39*, or *Pc48* in the lines and cultivars used in this study.

Line or cultivar	*Pc38*	*Pc39*	*Pc48*	Pedigree	Reference
Pendek	no	no	no	Flamingsgold/Binder	Fleischmann et al. 1971
Pendek-*38*	yes	no	no	*A. sterilis* CAV 2648-4 (CW491-4)/4*Pendek	Chong et al. 2000, Fleishmann and McKenzie 1968
Pendek-*39*	no	yes	no	*A. sterilis* CAV 5165 (F366) /4*Pendek	Chong et al. 2000, Fleishmann and McKenzie 1968
Pendek-*48*	no	no	yes	Pendek*2/ *A. sterilis* CAV 5401 (F158)	Chong et al. 2000, Fleishmann et al. 1971
Dumont	yes	yes	no	Harmon HAM/Double Cross 7[a]	McKenzie et al. 1984
OT328	no	no	no	S79107/Cascade	Howes et al. 1992
Kanota	no	no	no	selection from Fulghum	Salmon and Parker 1921
Ogle	no	no	no	Brave//Tyler/Egdolon 23	Brown and Jedlinski 1983

[a]Double Cross 7 = Kent/Pendek-*38,39*//OT189/Harmon HAM.

Molecular markers have been developed for a number of crown and stem rust genes in cultivated oat, including *Pg3* (Penner et al. 1993a), *Pg9* and *Pg13* (O'Donoughue et al. 1996), *Pc68* (Penner et al. 1993b), *Pc71* (Bush and Wise 1998), *Pc91* and *Pc92* (Rooney et al. 1994), and *Pc94* (Chong et al. 2004). These molecular markers can be used to facilitate pyramiding of the genes, a resistance breeding strategy designed to provide more durable control of crown rust by combining several genes in one cultivar (Pedersen and Leath 1988).

Molecular markers can also be used in counter-selection. The gene *Pc38* would be an excellent choice as a target for removal from new varieties being developed, not simply because the gene has been defeated, but because *Pc38,* or a factor tightly linked to it, is known to suppress the action of the genes *Pc62* (Wilson and McMullen 1997b) and *Pc94* (Chong and Aung 1998). *Pc94* is currently the most effective crown rust resistance gene available in North America (Chong and Zegeye 2004).

A number of crown rust resistance genes are found clustered in the oat genome, including *Pc38*, *Pc62*, and *Pc63* (Harder et al. 1980); *Pc39* and *Pc55* (Kiehn et al. 1976); *Pc35*, *Pc54*, and *Pc96* (Martens et al. 1980, Chong and Brown 1996); and *Pc68, Pc44, Pc46, Pc50, Pc95*, and *PcX* (Chong et al. 1994). Molecular markers developed for any one particular rust resistance gene in a cluster will also be useful for the study of other disease resistance genes found within the same cluster. Comparative mapping of disease resistance loci with reference populations such as those from the hexaploid crosses *A. byzantina* C. Koch cv. 'Kanota' × *A. sativa* L. cv. 'Ogle' (KO) (Wight et al. 2003), Ogle × 'TAM O-301' (Portyanko et al. 2001), and Ogle × 'MAM17-5' (OM) (Zhu and Kaeppler 2003a) increases the number of potential molecular markers available for the resistance genes and furthers our understanding of their organization in the genome.

The main objective of this study was to identify DNA markers linked to the crown rust resistance genes *Pc38, Pc39,* and *Pc48.* The work reported here is part of our continuing effort to locate rust resistance genes and their associated markers within the hexaploid oat genome using comparative mapping. In addition, DNA sequence information from the RFLP clones associated with the rust resistance genes was also obtained, such that user-friendly PCR-based markers might be designed. An analysis of this sequence information is also presented, as many RFLP clones contain cDNA inserts that represent expressed genes.

Materials and methods

Population development

The pedigrees of the *A. sativa* parental lines used in this study and the crown rust resistance genes that they carry are listed in Table 1. Line OT328 has no known crown rust resistance genes. 'Dumont' has crown rust resistance genes *Pc38* and *Pc39.* Pendek-*38*, Pendek-*39*, and Pendek-*48* are 'Pendek' backcross lines carrying the single resistance genes *Pc38, Pc39*, and *Pc48*, respectively. The $F_{2:3}$ populations (F_3 plants from segregating F_2 families) used to find molecular markers linked to *Pc38*, *Pc39*, and *Pc48* were derived from three crosses: Pendek-*39* × Pendek-*48* (Pendek3948), Pendek-*48* × Pendek-*38* (Pendek4838), and OT328 × Dumont (OT328Du). The $F_{2:3}$ families of the OT328Du cross were taken from the same segregating population used to find

molecular markers for the stem rust resistance genes *Pg9* and *Pg13* in three previous studies (Howes et al. 1992; Chong et al. 1994; O'Donoughue et al. 1996). All crosses were made in growth chambers at 18 C with an 18 h/6 h light/dark cycle. A single F_1 plant from each cross was self-pollinated to derive separate F_2 populations. Individual F_2 plants were self-pollinated to produce $F_{2:3}$ families. All panicles of F_2 and $F_{2:3}$ plants were bagged to prevent outcrossing.

Rust resistance evaluation

Races (Chong et al. 2000) LDCB and LLLB of *P. coronata* f. sp. *avenae* were used to test the segregation of the 129 $F_{2:3}$ families of the Pendek4838 cross for *Pc38* and *Pc48*, respectively. Race LDCB is virulent to *Pc48* and avirulent to *Pc38* and *Pc39*. Race LLLB is virulent to *Pc38* and avirulent to *Pc48* and *Pc39*. Races BGBG and LDCB were used to test the segregation of the 135 $F_{2:3}$ Pendek3948 families for *Pc48* and *Pc39*, respectively. Race BGBG is virulent to *Pc39* and avirulent to *Pc48*. Races SJGL and LLLB were used to test the segregation of the 132 $F_{2:3}$ OT328Du families for *Pc38* and *Pc39*, respectively. Race SJGL is virulent to *Pc39* and avirulent to *Pc38*. Seedlings were inoculated by spraying urediniospores of each race suspended in a light industrial oil (Bayol, Esso Canada) (4 mg/450 μl) onto the leaves of the plants. Approximately 24 seedlings from each $F_{2:3}$ family of the Pendek4838 and Pendek3948 crosses were inoculated with each of the two races described above when their primary leaves were fully expanded (one-leaf stage). Approximately 24 seedlings from each OT328Du family were inoculated with LLLB at the one-leaf stage, then with SJGL at the second-leaf stage 7 days later. The inoculated plants were incubated in a Percival Model I-60 dew chamber (Percival Scientific, Iowa, USA) overnight at 15 °C, then grown in a greenhouse maintained at a temperature between 18-22 °C with 16 h of daylight supplemented with fluorescent lighting. Crown rust infection types (ITs) were scored 12 d after inoculation, using a 0 − 4 scale (Murphy 1935): ITs of 0 (immune), ; (flecks), 1, and 2 were considered resistant, and ITs of 3 and 4 susceptible. *Pc38, Pc39,* and *Pc48* are dominant genes and resistant plants in all three crosses typically reacted with a ; or ;1 IT when tested with an avirulent crown rust culture (Chong et al. 2000). All $F_{2:3}$ families were classified as resistant, segregating, or susceptible.

DNA manipulation

DNA purification, restriction enzyme digestion with *Dra*I, *Eco*RI, or *Eco*RV, Southern blotting, and RFLP (Restriction Fragment Length Polymorphism) analyses were done as reported in Wight et al. 2003. RAPD (Random Amplified Polymorphic DNA) analyses were done as reported in Wight et al. 1994. RAPD primers and clones used for RFLP were from sources described in Wight et al. 2003.

The cdo113 SCAR (Sequence Characterized Amplified Region) primers were those described in Larson et al. (1996) for barley. Polymerase chain reaction conditions were as described in Tragoonrung et al. 1992. The 0.7 Kb amplification products were digested with the restriction enzyme *Alu*I at 37 °C overnight. The digestion products were separated by gel electrophoresis through a 6% 7M urea denaturing polyacrylamide gel for 4 h at 60W and visualized using the silver staining protocol from Promega Corp. (www.promega.com).

DNA polymorphism surveys

Putative markers linked to the crown rust resistance genes were identified by screening the parental lines Pendek, Pendek-*38*, Pendek-*39*, Pendek-*48*, OT328, and Dumont for RFLP and RAPD polymorphisms.

DNA samples from Pendek and the Pendek-derived lines were screened with 100 RAPD primers from the University of British Columbia, Vancouver, Canada (UBC set #2). The OT328Du population, previously screened in the search for markers linked to *Pg9* and *Pg13* (O'Donoughue et al. 1996), was screened again using a bulked segregant analysis approach (Michelmore et al. 1991) and 100 RAPD primers from UBC set #5.

Four hundred and ninety two RFLP clones were used to screen all of the parental lines except Pendek-*48*, for which 287 clones were screened. The first clones selected were chosen to give broad coverage of the oat genome, as determined using the KO hexaploid oat reference map of O'Donoughue et al. (1995) and a diploid oat reference map, *A. atlantica* Baum et Fedak × *A. hirtula* Lag. (O'Donoughue et al. 1992). Eighty-two additional clones came from a cDNA library designated 'aco' (Wight et al. 2003). Further clones were selected for more detailed mapping of target regions once a putative location had been identified for each gene.

Table 2. Segregation for oat seedling reactions to races of *Puccinia coronata* f. sp. *avenae* in $F_{2:3}$ families of three crosses.

Population	Race	Resistance gene detected	Number of $F_{2:3}$ families				$\chi^2_{(1:2:1)}$	*P*
			Resistant	Segregating	Susceptible	Total		
Pendek-*48* × Pendek-*38*	LLLB	*Pc48*	31	68	30	129	0.395	0.90–0.80
Pendek-*48* × Pendek-*38*	LDCB	*Pc38*	33	56	40	129	3.000	0.30–0.20
Pendek-*39* × Pendek-*48*	LDCB	*Pc39*	28	82	25	135	6.363	0.05–0.02
Pendek-*39* × Pendek-*48*	BGBG	*Pc48*	23	75	33	135	4.623	0.10–0.05
OT328 × Dumont	SJGL	*Pc38*	26	77	29	132	3.803	0.20–0.10
OT328 × Dumont	LLLB	*Pc39*	43	57	32	132	4.288	0.10–0.05

Polymorphic RFLP bands were named to reflect their relationship with fragments mapped in the KO reference population. If at least one of a pair of polymorphic fragments was the same size as those mapped in the KO population and could be assumed to be allelic, then the locus in the population under study was given the same name as that of the KO locus; e.g., cdo1467a or umn41. If the fragments were different or if a different enzyme had been used to generate them and the relationship was unclear, then the suffixes ‘x’ or ‘y’ were used after the clone name; e.g., isu2287x. The same rule was applied to polymorphic fragments not represented in the KO population but present in both the Pendek3948 and Pendek4838 populations.

Surveys of individual Pendek-48 plants

For the DNA polymorphism surveys, leaves from 12-15 plants were bulked together for large-scale extraction of DNA representing the different parental lines. For additional studies on the Pendek-*48* line, 14 single Pendek-*48* plants were grown and the DNA extracted from each one separately. Southern blots of these DNAs were then prepared as above.

Molecular mapping

Mapping was performed using the exhaustive search method of G-MENDEL Win32 Version 0.8b (Holloway and Knapp 1993) and the compare command of MAPMAKER Version 3.0 for PC (Lander et al. 1987). An LOD of 7.0 was used for grouping. M5 (Multiple Molecular Marker Map Manager) was used for map visualization and comparison (Tinker 1999).

Sequencing of RFLP clones

RFLP clone inserts were sequenced from both the 5’ and 3ends by Canadian Molecular Research Services, Inc., Ottawa, Canada using a Licor IR^2 (Licor, Inc., Lincoln, NE) automated sequencer. The DNA sequences obtained were translated and compared to the GenBank protein sequence database at the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/) using the program BLASTX (Altschul et al. 1997). Sequences of further interest were translated using the Expert Protein Analysis System (ExPASy; http://ca.expasy.org/) ‘translate tool’ and analyzed for common protein domain motifs using the motif scan program from the Swiss Institute of Bioinformatics (Falquet et al. 2002; http://hits.isb-sib.ch/cgi-bin/PFSCAN). Using RPS BLAST, the Conserved Domain Database at NCBI (Marchler-Bauer et al. 2003) was also searched. For DNA sequence alignment and comparison, the program Blast2 (Tatusova and Madden 1999), available from NCBI, was used.

Results

Pathology

Segregation data for *Pc38, Pc39*, and/or *Pc48* resistance in the $F_{2:3}$ families of the Pendek4838, Pendek3948, and OT328Du populations, when tested with the respective crown rust cultures, are shown in Table 2. The chi-square test for goodness-of-fit indicated that the segregation data for *Pc48* resistance in the $F_{2:3}$ populations of Pendek4838 and Pendek3948 to crown rust races LLLB and BGBG, respectively, did not deviate significantly from the 1:2:1 ratio expected for a single gene. The chi-square test for

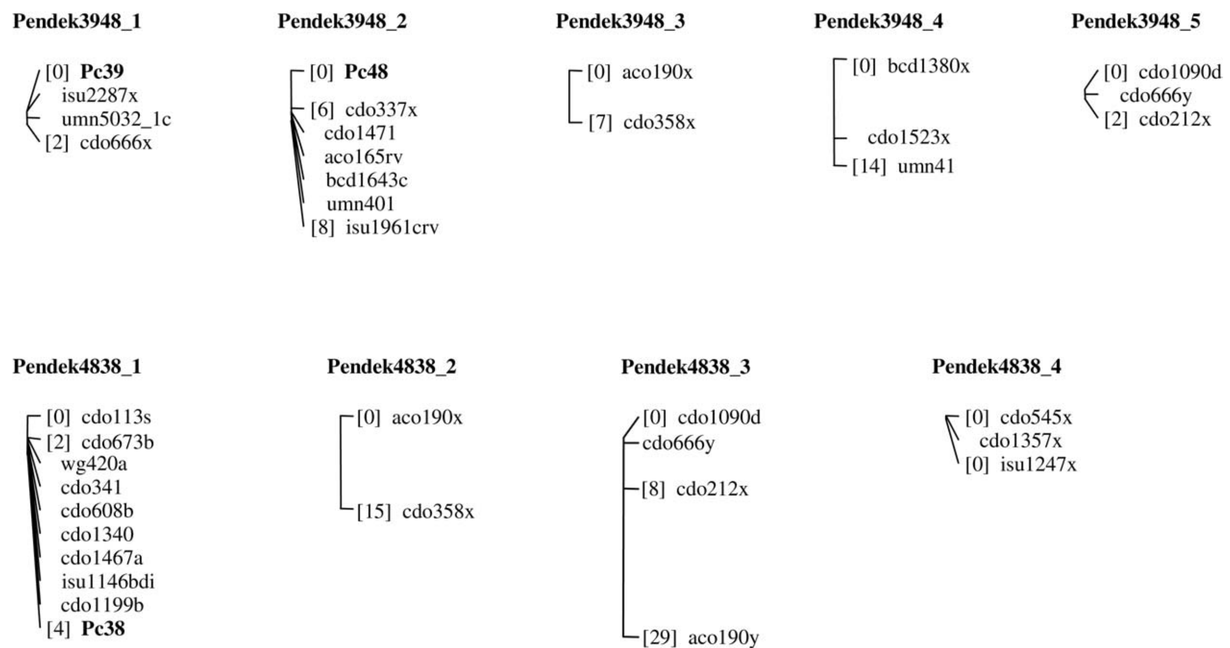

Figure 1. Molecular marker linkage maps for Pendek3948 and Pendek4838. Linkage groups identified in the Pendek3948 population are designated as Pendek3948_x (where × is the group number), and those in the Pendek4838 population as Pendek4838_x. Groups are represented by framework markers with additional markers placed to intervals, in the manner of Wight et al. 2003. Centimorgan distances between framework markers are in square brackets. All markers are named.

goodness-of-fit also indicated that the segregation data for *Pc38* resistance in the $F_{2:3}$ populations of Pendek4838 and OT328Du to crown rust races LDCB and SJGL, respectively, did not deviate significantly from the expected 1:2:1 single gene ratio. While $F_{2:3}$ families of OT328Du segregated to fit a single gene ratio for *Pc39* when tested with LLLB, $F_{2:3}$ families of Pendek3948 segregated to give a poorer fit to the expected 1:2:1 single gene ratio when tested with LDCB, due to an excess of segregating families (Table 2). However, segregation within the $F_{2:3}$ segregating families of Pendek3948 to 1486 resistant and 460 susceptible plants when tested with LDCB gave a good fit to a 3:1 ratio (χ^2 = 1.925, P = 0.20-0.10) for a single dominant gene (*Pc39*). These results, plus the good fit to the expected single gene ratio (χ^2 = 0.395, P = 0.90-0.80) for the segregation of *Pc48* in the $F_{2:3}$ families of Pendek4838, indicated that *Pc38* has no suppressor effect (Chong and Aung 1998; Wilson and McMullen 1997b) on the expression of either *Pc39* or *Pc48*.

Molecular analysis of the Pendek3948, Pendek4838, and OT328Du populations

In the Pendek3948 population, eighteen RFLP clones and three RAPD primers revealed polymorphisms. Using these, the genotypes at 19 RFLP loci and three RAPD loci were determined and the loci mapped (Figure 1). One co-segregating and two closely linked RFLP markers for *Pc39* were found. An additional six RFLP markers were found to link to *Pc48* but at a greater distance: 6 to 8 cM. The remaining eight RFLP markers formed three additional linkage groups. The RAPD markers remained unlinked to either rust resistance locus or the other RFLP or RAPD loci.

In the Pendek4838 population, seventeen RFLP clones, four RAPD primers, and one pair of SCAR primers revealed polymorphisms. Using these, the genotypes at 20 RFLP loci, six RAPD loci, and one SCAR locus were determined and the loci mapped (Figure 1). Eight RFLP markers closely linked to *Pc38* were found. Two of the clones used, cdo1467 and isu1146, have since been determined to be synonymous for the purpose of RFLP mapping (Wight et al. 2003). One SCAR marker, cdo113s, was linked to

Pc38 at a distance of 4 cM. All of the markers linked to *Pc48* in the Pendek3948 population were monomorphic in the Pendek4838 population and no new markers were identified. Nine RFLP markers formed three additional linkage groups, similar to the situation in the Pendek3948 population. Again, the RAPD markers remained unlinked.

The OT328Du population was used previously for the identification of DNA markers linked to the stem rust resistance genes *Pg9* and *Pg13* (O'Donoughue et al. 1996). It also segregates for *Pc38* and *Pc39*, and so had further utility in identifying markers linked to these genes. Additional RAPD primers were monomorphic in this population; however, genotypes were determined at two additional polymorphic RFLP loci. One of these, cdo1385c, linked to the *Pc38* gene. The other marker, isu2287x, linked to the *Pc39* gene. This is the same marker, and the same size marker band, as was found linked to *Pc39* in the Pendek3948 population. However, the other *Pc39* markers identified in the Pendek3948 population were monomorphic in OT328Du.

RFLP clone sequences and gene identification

Sequence information was obtained from all of the mapped RFLP clones. Table 3 contains a list of all of these and their possible identities, based on the information available in GenBank in April, 2004.

Discussion

Pc48 mapping

When compared to the KO hexaploid oat reference map (Wight et al. 2003), the *Pc48* region in the Pendek3948 population (Pendek3948 group 2) is seen to correspond to a region on KO group 22_44+18, based on evidence from five RFLP markers (Figure 2). Also, Pendek3948 group 4 is homologous with KO group 24_26_34, which is homoeologous with KO group 22_44+18 (Wight et al. 2003). This would suggest that the two Pendek3948 groups are homoeologous.

No marker linked to *Pc48* was found in the Pendek4838 population. The presence of faint, extra bands in the bulked DNA of the Pendek-*48* parental line on RFLP blots led us to examine the possibility that Pendek-*48* was a mixture of different genotypes. Southern blots containing DNA from single Pendek-*48* plants and the original bulked sample were prepared. These were probed with a random clone (cdo1435), a clone from the *Pc38* region (cdo608), a clone from one of the smaller linkage groups (cdo545), and a clone from the *Pc48* region (as identified in the Pendek3948 population) (isu1961).

Blots probed with the clone marking the *Pc38* region showed no polymorphism (data not shown). This was also true of blots probed with the clone from one of the smaller linkage groups or the random clone (data not shown). However, blots probed with the clone from the *Pc48* region did show polymorphism, as can be seen in the example presented in Figure 3.

The Southern blot patterns indicate that Pendek-*48* is a mixture of at least two genotypes. Since both populations contained *Pc48* resistance but different alleles at loci mapped to this region, it is probable that the Pendek-*48* genotype used to produce the Pendek4838 population contained a smaller introgressed segment from *A. sterilis* than that used to produce Pendek3948. Such variations in genotype within a line used for breeding can, therefore, have important consequences. It is likely that, with additional surveys, a marker physically closer to the *Pc48* locus could be found using the Pendek4838 population. This variation within the Pendek-*48* line could be exploited by selecting the genotype with the smallest *A. sterilis* introgression as the donor line in a breeding program, thereby reducing linkage drag.

Pc39 locus

When compared to the KO map, the *Pc39* region (as represented by Pendek3948 group 1) appears to be homologous to a region on KO group 37 (Figure 4). This is based on evidence from one RFLP marker, umn5032_1c, which highlights the same sized fragments in the two populations. The three other RFLP markers from KO group 37 were monomorphic in both the Pendek3948 and OT328Du populations.

It is interesting to note that other regions homoeologous to the *Pc39* locus region were found to be segregating in both the Pendek3948 and Pendek4838 populations. One clone identifying a marker linked to *Pc39* on Pendek3948 group 1, cdo666, also produced a marker present in Pendek3948 group 5 (Figure 1, Figure 4). Pendek3948 group 5 is homologous to Pendek4838 group 3 and both of these groups show homology to KO group 16_23, based on one marker with identical fragment sizes in the three populations (cdo1090d). The marker aco190y, also present on

Table 3. RFLP clone identities based on BLASTX comparisons with GenBank database.

RFLP clone	Clone source	GenBank accession #	Associated rust gene	Sequence identity/ GI number (NCBI)	Species matched	Score	E value
aco165	oat	CN180765	*Pc48*	putative RNA-binding protein/ gi:18087662	*Oryza sativa*	228	1e–58
aco190	oat	CN180766	none	no match	NA	NA	NA
bcd1643	barley	CN180783	*Pc48*	senescence-associated putative protein/ gi:18266206	*Narcissus pseudonarcissus*	162	8e–39
cdo212	oat	CN180774, CN180775	none	MDGD synthase type A/ gi:32401379	*Triticum aestivum*	84	3e–16
cdo337	oat	CN180776	*Pc48*	eukaryotic peptide chain release factor subunit 1 (ERF1)/ gi:22331351	*Arabidopsis thaliana*	244	8e–64
cdo341	oat	CN180777	*Pc38*	trigger factor-like protein/ gi:9758119	*Arabidopsis thaliana*	177	1e–43
cdo358	oat	BE439110	none	60s acidic ribosomal protein P0/ gi:6094102	*Zea mays*	67	6e–11
cdo608	oat	CN180778	*Pc38*	putative neutral invertase/ gi:14164543	*Oryza sativa*	330	1e–89
cdo666	oat	CN200320	*Pc39*	cytosolic heat shock protein 90/ gi:32765549	*Hordeum vulgare*	106	1e–34
cdo673	oat	CN180779	*Pc38*	putative leucine-rich repeat transmembrane protein kinase/ gi:15223744	*Arabidopsis thaliana*	228	9e–59
cdo1090	oat	CN180767	none	cytosolic heat shock protein 90/ gi:32765549	*Hordeum vulgare*	272	4e–72
cdo1199	oat	CN180768	*Pc38*	tryptophan synthase beta-subunit/ gi:18481702	*Sorghum bicolor*	501	e–141
cdo1340	oat	CN180769	*Pc38*	expressed protein/ gi:18398662	*Arabidopsis thaliana*	142	4e–33
cdo1385	oat	CN180770	*Pc38*	putative cytochrome P450/ gi:21671941	*Oryza sativa*	63	2e–26
cdo1467	oat	CN180771	*Pc38*	no match	NA	NA	NA
cdo1471	oat	CN180772	*Pc48*	phytochrome A type 3 (AP3)/ gi:130181	*Avena sativa*	74	9e–26
cdo1473	oat	CN180773	*Pc38*	putative cytochrome P450/ gi:21671941	*Oryza sativa*	63	2e–26
isu1146	oat	CN180780	*Pc38*	probable pantothenate kinase/ gi:33112586	*Arabidopsis thaliana*	102	7e–21
isu1961	oat	CN180781	*Pc48*	DnaJ protein homolog ZMDJ1/ gi:7441932	*Zea mays*	343	2e–93
isu2287	oat	CN180782	*Pc39*	P0018C10.26, similar to protein on Arabidopsis thaliana chromosome 3/ gi:21952809	*Oryza sativa*	157	7e–38
umn401	oat	CK780289	*Pc48*	putative cop-coated vesicle membrane protein/ gi:30017578	*Oryza sativa*	256	1e–67
umn5032	oat	CL524684	*Pc39*	no match	NA	NA	NA
wg420	wheat	CN180784	*Pc38*	DW-RGA2 protein (*cre3* resistance gene analogue)/ gi:26986268	*Triticum turgidum* ssp. *durum*	412	e–114

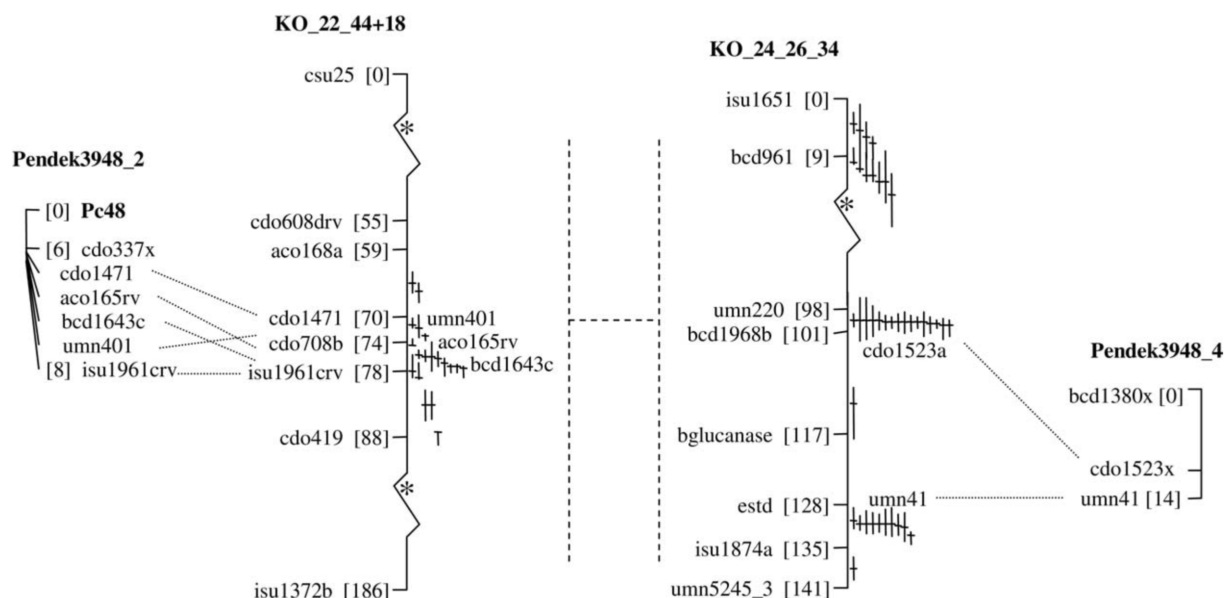

Figure 2. Comparative mapping of the *Pc48* region. Information for the Pendek3948 population is presented as described in Figure 1. Groups representing the KO hexaploid oat reference map are designated KO_x. The placed markers in the KO population are represented by placement bars. Placed markers from KO representing homology between Pendek3948 and KO are also named. The dotted lines connect homologous markers between groups; the dashed lines connect homoeologous regions between groups. A zigzag line indicates that the drawing of a particular group has been shortened for presentation purposes. Asterisks indicate that marker names have also been removed.

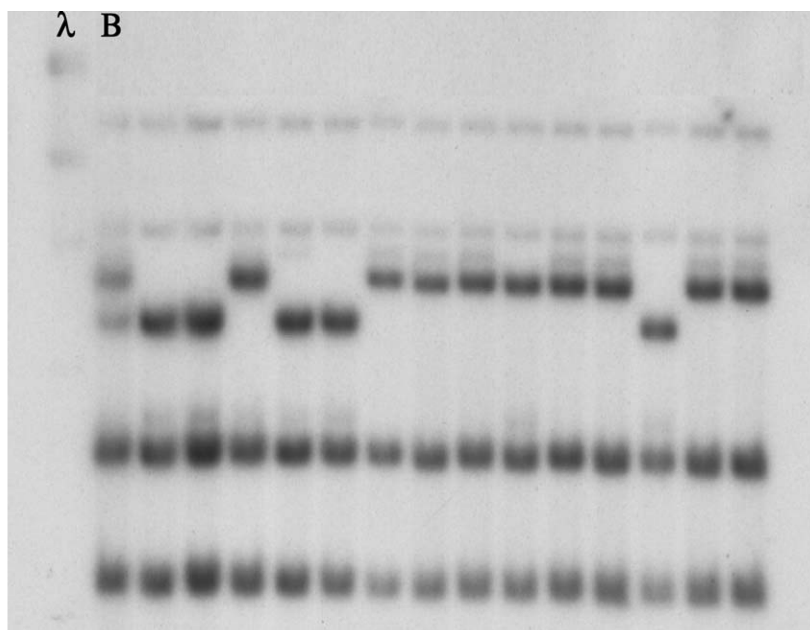

Figure 3. Evidence of heterogeneity in the Pendek-*48* parental line. This Southern blot was probed with isu1961, a marker for the *Pc48* crown rust resistance gene. The first lane contains a Lambda *Hind*III size marker (λ), the second lane contains DNA from a bulk of 12-15 Pendek-*48* plants (B), and the subsequent lanes contain DNA from 14 single Pendek-*48* plants. The restriction enzyme used for DNA digestion was *Dra*I.

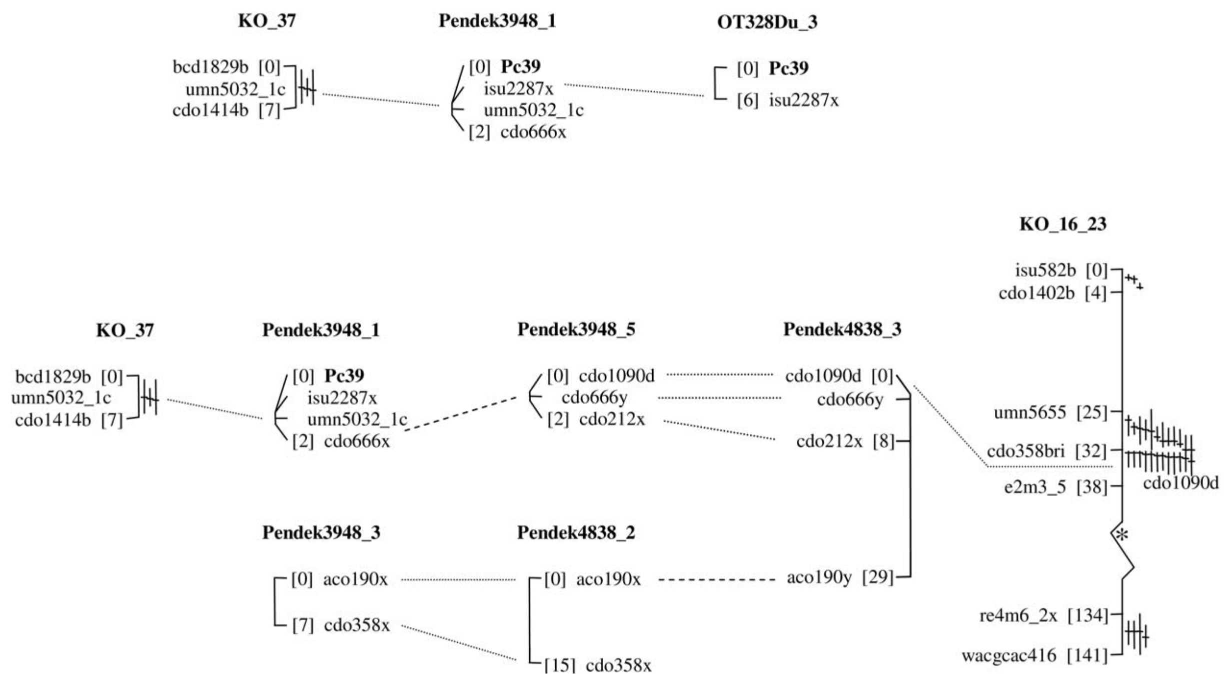

Figure 4. Comparative mapping of the *Pc39* regions and of homologous regions between Pendek3948 and Pendek4838. Linkage groups identified in the OT328Du population are designated OT328Du_x. See Figure 1, Figure 2 for further details.

Pendek4838 group 3, was not polymorphic in the Pendek3948 cross, and it is interesting to note that this marker highlights a region in which the two Pendek-*48* parents differ, as seen on RFLP blots (data not shown). Its homoeologue, aco190x, is present in both Pendek4838 group 2 and Pendek3948 group 3, as is the marker cdo358x. In the KO population, aco190rv, represented by different bands than above, is located on KO group 21+46_31+40, which has one marker in common with KO group 16_23.

Since Pendek4838 groups 2 and 3 contain the same markers as Pendek3948 groups 3 and 5, it is possible that these regions represent *A. sterilis* L. segments brought through from Pendek-*48*, as this parent is common to both populations. It was expected that Pendek-*48* would contain more and longer *A. sterilis* L. segments, as only two backcrosses were made to generate this line instead of the four used to generate the Pendek-*38* and Pendek-*39* lines (Table 1). It is also possible that these extra groups contain genes that have been maintained because they confer some fitness advantage.

Pc38 locus

The *Pc38* region (Pendek4838 group 1) is homologous to a region on KO group 17 (Figure 5). Pendek4838 group 4 may also be part of the same linkage group; however, this cannot be confirmed in the absence of common RFLP bands.

The marker found linked to *Pc38* in the OT328Du cross, cdo1385c, was not found linked to *Pc38* in the Pendek4838 population and is not located on KO group 17, as are the other *Pc38* markers. Instead, cdo1385c is located on KO group 7_10_28, which is homoeologous to group 17 (Wight et al. 2003). It is possible that the band represents a locus homoeologous to cdo1385c that, coincidentally, produces an RFLP band of the same size in the OT328Du population; however, it is also known that the Dumont segment containing the *Pc38* gene is in a translocated position relative to the same segment in other oat cultivars (Leach and McMullen 1989).

The genomes of hexaploid oats are known to contain many rearrangements (Ladizinsky 1970; McMullen et al. 1982; Leach and McMullen 1989) and, because of their polyploid nature, hexaploid oats would seem to tolerate these rearrangements well (Ladizinsky 1970). The different linkage group

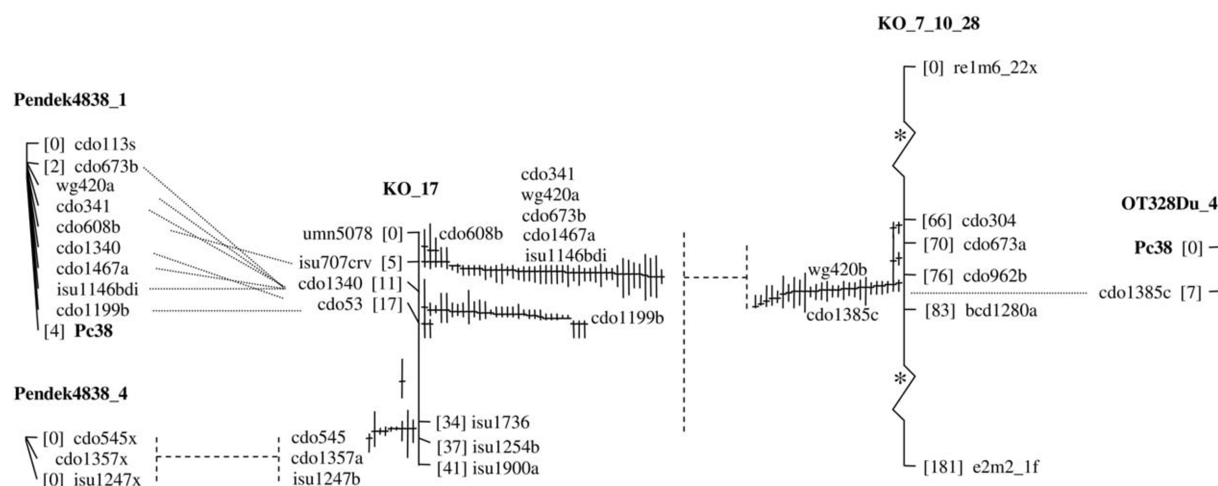

Figure 5. Comparative mapping of the *Pc38* regions. See Figure 1, Figure 2, and Figure 4 for further details.

assignments of the *Pc38* gene in OT328Du and Pendek4838 and the translocation difference between Dumont and other cultivars such as 'Steele' associated with the *Pc38* region (Leach and McMullen 1989) are a case in point. The existence of the translocation is somewhat surprising, since the *Pc38* gene in both Steele and Dumont has come from the same Pendek backcross lines used here (www.ars-grin.gov/, McKenzie et al. 1984). In addition, the cultivar 'Rodney' appears many times in the pedigree of Dumont and in the pedigree of the line OT201, the Steele grandparent containing the *Pc38* gene. The cultivar 'Kent'(USA), to which the Pendek lines were crossed in the development of Dumont, has quite a different pedigree (www.ars-grin.gov/) and, therefore, may be the source of the translocation difference, although this has not been tested. It is also possible that different versions of the Pendek-*38* line exist, as they do for Pendek-*48*.

A quantitative trait locus study of crown rust resistance derived from MAM17-5 in the OM cross highlighted four regions of the genome (Zhu and Kaeppler 2003b). One of these regions links the RFLP marker bcd1280 to crown rust resistance. This marker is found on KO linkage groups 17 (bcd1280b) and 7–10–28 (bcd1280a) in the same regions identified as being homologous or homoeologous to the regions found to contain the *Pc38* gene in Pendek4838 and OT328Du. These are regions of reduced recombination, however, and bcd1280 is found to be more distant from the *Pc38* markers on a diploid oat map, that of *A. strigosa* Schreb. × *A. wiestii* Steud. (Portyanko et al. 2001). In this case, bcd1280 is close to the crown rust resistance region designated PcA (Portyanko et al. 2001). Since the rust resistance gene or genes in MAM17-5 have been derived from *A. strigosa*, it would seem likely that MAM17-5 carries PcA (Zhu and Kaeppler 2003b). However, a simple inversion could bring the PcA and *Pc38* regions together.

RFLP clone sequences and gene identification

The RFLP clones found linked to the *Pc38*, *Pc39*, and *Pc48* genes were sequenced for three reasons. Firstly, RFLP markers provide high quality genotype data, but they are difficult to use for high throughput applications such as marker-assisted selection, and the availability of DNA sequence data is essential for the conversion of RFLPs into PCR-based markers. Secondly, the RFLP clones discussed here (with the exception of umn5032 and wg420) represent cDNAs and, therefore, should contain valuable information concerning expressed genes. Thirdly, the banding patterns of some clones had shown them to be synonymous for the purpose of molecular mapping; however, their similarities at the sequence level had not necessarily been determined (Wight et al. 2003).

The clone cdo1385 marks a band linked to *Pc38* (cdo1385c) in the OT328Du population and also marks a band for *Pg9* (cdo1385f) in the same population (O'Donoughue et al. 1996). This clone was known to be synonymous with cdo1473 (Wight et al. 2003). Both cdo1385 and cdo1473 come from a cDNA library derived from leaf tissue of the oat cul-

tivar 'Brooks' (Heun et al. 1991). Their sequences were found to be identical.

Two clones marking bands linked to *Pc38* in the Pendek4838 population were also discovered to be synonymous: cdo1467 and isu1146. The Brooks library gave rise to cdo1467 but isu1146 comes from a cDNA library derived from root tissue of the oat cultivar 'Lang' (Rayapati et al. 1994). The insert from clone isu1146 has a 98% identity with that of cdo1467 and is approximately 200 bp longer. These clones may represent two different alleles of the same gene or two homoeologues.

With respect to potential gene function, most of the sequences obtained either produced no BLAST hits or code for genes seemingly unrelated to disease resistance (Table 3). It is intriguing, however, that cdo666 and cdo1090 have homology to the heat shock protein *Hsp90*, as it has recently been discovered that this protein is required for RPS2-mediated disease resistance in *Arabidopsis* (Takahashi et al. 2003). It is also interesting that isu1961, found linked to *Pc48*, has homology to another heat shock protein, DnaJ.

Two of the clones marking the *Pc38* gene were found to have homology to well-characterized disease resistance genes. It was reported by Boyko et al. (2002) that cdo673 represents an allene oxydase (sic) synthase (*aos*) gene; however, our translated sequence of the insert from cdo673 was found to have high homology to a putative leucine-rich repeat transmembrane protein kinase gene from *Arabidopsis thaliana*. It also contains the protein kinase domain associated with disease resistance genes of this type (Michelmore and Meyers 1998).

The insert from wg420, a wheat (*Triticum aestivum* L.) genomic clone (Heun et al. 1991), has very high homology to a *cre3* resistance gene analogue derived from durum wheat. It also contains an NB-ARC domain. These domains have been found in both plant disease-resistance genes and in animal genes regulating cell death (van der Biezen and Jones 1998). It has been speculated that plant resistance gene products with this domain may function as part of a cell-death complex (or 'apoptosome') to maintain enzymes required for cell death in an inactive state until cell death is triggered by signals from an invading pathogen (van der Biezen and Jones 1998).

Presence of rust resistance gene regions

While it is improbable that either cdo673 or wg420 relates directly to the source of *Pc38* resistance, they may represent other genes maintained through linkage. These linkages to *Pc38* confirm the importance of the KO group 7_10_28 and group 17 regions of the oat genome with respect to disease resistance and provide us with further evidence of disease resistance gene clustering in oat. It is also intriguing that the *Pc38* markers are associated with the large marker clusters on KO groups 7_10_28 and 17 (Figure 5). Indeed, it is notable that most of the mapped regions described here are associated with regions of the KO reference population map having these marker clusters (Figure 2, Figure 4).

Marker clusters may be indicative of areas of reduced recombination resulting from the presence of centromeres or cytogenetic anomalies such as translocation breakpoints (Wight et al. 2003). A study by McMullen et al. (1982) concluded that cytogenetic irregularities exist within and between lines of both *A. sativa* and *A. sterilis* and that even greater variation exists between the species. McKenzie et al. (1970) provided evidence that the *Pg13* stem rust resistance locus is involved in a translocation and O'Donoughue et al. (1996) noted reduced recombination in the area of this gene in two crosses, including OT328Du. Since the *Pc38* gene is also known to occur in a translocated position, further study of these regions may prove valuable in the search for further disease resistance gene markers and in the development of rust resistance gene deployment strategies by breeders.

Wilson and McMullen (1997a) have already demonstrated the possibilities by taking advantage of the translocation difference between the cultivars Dumont and Steele to produce lines containing both *Pc38* and *Pc63*, which, ordinarily, are allelic or very tightly linked. A better understanding of the structural differences between the genomes of different oat cultivars, and documentation of such in pedigree records, would be particularly useful, not only for the selection and use of markers in marker-assisted breeding, but also for such exploitation of these structural differences.

The markers identified here for *Pc38*, *Pc39*, and *Pc48*, particularly cdo673 and wg420, should prove useful not only for marker-assisted selection, but also for the further study of different regions of the hexaploid oat genome containing disease resistance genes.

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