Recombination Within a 5-centimorgan Region in Diploid *Avena* Reveals Multiple Specificities Conferring Resistance to *Puccinia coronata*

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ABSTRACT

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A set of 100 recombinant inbred lines (RILs) was produced from a cross of diploid ($n = 7$) *Avena strigosa* (CI 3815) with *A. wiestii* (CI 1994), resistant and susceptible, respectively, to *Puccinia coronata*, the causal agent of crown rust. This set of RILs was inoculated with 11 isolates of *P. coronata*. Infection type reactions to eight isolates (PC54, 263, 290, PC62, 202, 325A, PC58, and PC59) fit a 1:1 resistant/susceptible ratio expected for traits controlled by single genes. Reaction to isolate 264B fit a 3:1 resistant/susceptible ratio, suggesting resistance conditioned by two dominant genes. Reaction to isolate 276 fit a 1:3 resistant/susceptible ratio, suggesting control by two dominant genes, whereby one gene inhibits the resistance conferred by the other. All isolates detected resistance specificities that mapped to the *Pca* region in *A. strigosa* for resistance to *P. coronata*. *Pca* is positioned between the oat restriction fragment length polymorphism marker *Xisu2192* and a new randomly amplified polymorphic DNA marker, *XisuC18*, near the end of linkage group A. Five unique specificities within the *Pca* region were differentiated by recombination. The observation of five specificities conferring disease resistance that are linked in coupling is unusual, because such genes generally are linked in repulsion and inherited from multiple donor parents.

Genes in plants that confer resistance (*R*) to fungal pathogens frequently display characteristic gene-for-gene specificity (3,12). This is particularly evident in the resistance of monocotyledonous species to obligate fungal biotrophs, such as *Zea mays* to *Puccinia sorghi* (9,29), *Triticum aestivum* to *Puccinia* species (21,28), and *Hordeum vulgare* to *Erysiphe graminis* (4,11,14,30,37). In many instances, the *R* genes occur in clusters exhibiting various degrees of linkage. Dicotyledons displaying similar patterns of *R* gene organization include flax (*Linum usitatissimum*) for resistance to the flax rust pathogen *Melampsora lini* (10,18,23,31) and lettuce (*Lactuca sativa*) for resistance to the downy mildew pathogen *Bremia lactucae* (13).

P. coronata Corda f. sp. *avenae* Eriks., the causal agent of crown rust, is one of the most important fungal pathogens of cultivated oat (*Avena sativa* L.). The host plant response to this pathogen is influenced by a specific gene-for-gene system (22,33). Individual loci, designated *Pc*, confer resistance to various isolates of the pathogen. However, allelic relationships among *Pc* loci are unclear, and the placement and organization of these loci in the hexaploid oat genome are just beginning to be elucidated (1,25).

This study was undertaken to determine the genetic control of resistance to *P. coronata* within an apparently complex locus in diploid *Avena*. In a previous analysis, a genetic linkage map was constructed with restriction fragment length polymorphism (RFLP) loci in a population of diploid *Avena* segregating for resistance to *P. coronata* (24). A single locus, designated *Pca* for resistance to *P. coronata* in the *A* genome, conferred dominant resistance to nine isolates. This initial study, performed in the F_3 generation, suggests that this locus is complex and that there may be recombination among different resistance specificities within *Pca*. In this report, we demonstrate that the *Pca* region contains at least five closely linked resistance specificities and identify a dominant suppressor of one of these specificities.

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MATERIALS AND METHODS

Plant material. A population of 100 F_2 plants was generated by crossing USDA accessions of *A. strigosa* Schreb. (CI 3815) with *A. wiestii* Stevd. (CI 1994). The parental seed was obtained from D. Wesenberg (USDA-ARS Small Grains Research Facility, Aberdeen, ID). The parents had been characterized previously for resistance to *P. coronata* (34,38). Of the 47 isolates of *P. coronata* in the Iowa State University (ISU) collection, the *A. strigosa* parent was resistant to 41 isolates, and the *A. wiestii* parent was susceptible to 40 of the same isolates. The first 88 recombinant inbred lines (RILs) (of the 100 in the population) were single-seed descent progeny of the 88 F_2 individuals used to construct a genetic linkage map with RFLPs for diploid oat (24). Three F_1 seeds from a single panicle generated the F_2 population used for mapping. RILs were developed by self-pollination of 100 individual

plants from the F_2 through and including the F_6 generation. Self-pollination was ensured by enclosing panicles in glassine bags prior to the emergence of florets from the apical leaf sheath. Chi-square tests of homogeneity were calculated to determine if segregation data from the progeny of each F_1 plant could be combined into a single data set representing the F_6 population of RILs (Table 1).

Inoculum. Propagation of urediniospores and reactions of 33 differentials to each isolate of *P. coronata* were described previously (38). The isolates (Table 1) displayed a distinct differential reaction between the *A. strigosa* (resistant) and *A. wiestii* (susceptible) parents of the RILs. These isolates were selected because they were virulent on the largest number of the differentials, thereby increasing the probability that they would detect unique specificities in the mapping population. The following modifications were used to standardize experimental conditions. Immediately after harvesting, urediniospores were sieved through 0.5 × 0.5-mm mesh, desiccated at 20% relative humidity for 48 h, and stored at –80°C. Spore germination rates were measured on 2% agar-water (wt/vol) plates. Stocks of urediniospores with a minimum of 80% germination were used. Aliquots from the same stock of urediniospores were used for initial and confirmation experiments. A suspension of urediniospores (1 mg/ml) in Isopar M oil was heated at 40°C for 10 min before spraying on seedlings as described previously (1,38).

Detection of infection types. At the F_6 generation after self-pollination, the expected ratio of genotypic classes for two alleles at a single locus is 49 *AA*/2 *Aa*/49 *aa* (7). Assuming monogenic inheritance, approximately half of the RILs were expected to be homozygous resistant, and half were expected to be homozygous susceptible. Therefore, five progeny are sufficient to ascertain the genotype (*AA* or *aa*) at the 95% level of confidence when half of the RIL population are expected to be *aa* (17). For the determination of infection types, 10 seeds of each RIL were placed on moistened filter paper at 4°C for 4 days to allow radicle emergence. By allowing the seeds to imbibe at 4°C, very uniform seedlings were obtained for inoculation. Seven germinated seeds were planted per 6 × 6-cm^2 cell in 36-cell flats (flat size = 28 × 56 cm, Hummert International, St. Louis) to produce at least five seedlings. Markton (*A. sativa*, susceptible to all isolates), CI 3815 (*A. strigosa*), and CI 1994 (*A. wiestii*) were included as controls in each inoculation. Seedlings were inoculated when the first true leaf was twice as long as the coleoptile leaf (usually about 12 days after planting). Flats of inoculated seedlings were maintained for 24 h in a dew chamber at 18 to 21°C in darkness. Flats were then moved to a growth chamber at 18 to 21°C with a 14-h light, 10-h darkness cycle. Fourteen days after inoculation, seedlings were evaluated for their reaction to *P. coronata* by the standard crown rust infection type ratings (ITR) of 0 to 4 as previously described (38). ITRs of 0, 1, or 2 were considered resistant reactions, and ITRs of 3 or 4 were considered susceptible reactions.

Responses of the 100 RILs (at least five seedlings each) to 11 isolates of *P. coronata* were determined twice in separate experiments. Ratios of observed phenotypic classes were compared with expected ratios by chi-square goodness-of-fit tests (Table 1). The RILs in which heterozygotes or putative recombinants were identified were further confirmed by inoculation with the isolates that detected the nonparental phenotype. Confirmation tests were performed with a minimum of 16 seedlings per line to ensure the detection of at least one homozygous recessive, susceptible individual (*aa*) at the 99% level of confidence (17).

DNA isolation, RFLP markers, and DNA gel blot analysis. DNA was isolated from fresh leaves by a modified cetyltrimethylammoniumbromide extraction (27). DNA gel blot analyses were conducted as previously described (39). The oat root cDNAs, ISU2191 and ISU2192, were used to position the RFLP loci, *Xisu2191* and *Xisu2192*, linked to *Pca* (24). Bulked segregant analysis (20) was used to identify additional DNA markers linked to the *Pca* region. Randomly amplified polymorphic DNA (RAPD) marker data were collected with primer sets C and D from Operon Technologies (Alameda, CA). Genomic DNA from 10 RILs resistant to isolate 258 were pooled to form one bulk. The second bulk was composed of DNA from 10 RILs susceptible to isolate 258. Isolate 258 was chosen because the ITR to this isolate gave the best fit to a single locus model in the F_2 (24). Primer pair combinations (780) were screened against these bulks. Polymerase chain reactions (PCR) were conducted in 15-µl reactions in 96-well polycarbonate plates used in a PTC-100 thermal cycler (MJ Research, Watertown MA). PCR conditions were previously described (36), with the addition of 0.05% (vol/vol) NP-40 to minimize nonspecific amplification. Segregation of RAPD loci among 94 RILs was confirmed in replicate experiments. The chi-square goodness-of-fit test was conducted to compare observations with the ratio of 1:1 *A. strigosa* allele/*A. wiestii* allele expected for F_6 RILs.

Data analysis. Data were entered into Map Manager version 2.6 for Macintosh (15) and analyzed for marker order and distance. Map Manager facilitates the analysis of backcross as well as recombinant inbred populations. Resistant reactions were coded as A, and susceptible reactions were coded as B. Chi-square goodness-of-fit values for segregation ratios were calculated, and linkage analysis was conducted on loci for resistance fitting a 1R:1S ratio by the "rearrange" function of Map Manager followed by reiterative analysis of crossovers flanking individual loci. Standard errors for recombination frequencies were calculated by maximum likelihood formulae (32) with the aid of Map Manager.

TABLE 1. Frequencies of phenotypic classes and chi-square goodness-of-fit tests for infection type reaction (ITR) to 11 isolates of *Puccinia coronata* on F_6 recombinant inbred lines of diploid *Avena*

Isolate	ITR[a]		Phenotypic class[b]			χ^2 value					
	CI 3815	CI 1994	Res.	Sus.	Seg.	1R:1S	3R:1S	1R:3S	Probability	Homogeneity χ^2[c]	Probability
PC54	0–1	4	53	44	0	0.83			0.50–0.30	4.14	0.20–0.10
263	0	4	45	44	0	0.01			0.95–0.90	0.81	0.70–0.50
290	0	3–4	50	46	0	0.17			0.70–0.50	1.30	0.70–0.50
PC62	0	4	49	47	0	0.04			0.95–0.90	0.87	0.70–0.50
202	0	4	46	51	0	0.26			0.70–0.50	1.91	0.50–0.30
264B	0	4	67	30	0		1.82		0.20–0.10	2.90	0.30–0.20
276	0	4	21	72	0			0.29	0.70–0.50	1.36	0.70–0.50
258	0	4	58	37	1	4.64			0.05–0.01[d]	3.38	0.20–0.10
325A	0	4	50	42	4	0.69			0.50–0.30	6.50	0.05–0.01
PC58	I	4	53	40	0	1.82			0.20–0.10	1.46	0.50–0.30
PC59	I	3	49	44	0	0.27			0.70–0.50	4.12	0.20–0.10

[a] Seedlings were evaluated for their reaction to *P. coronata* by the standard crown rust ITRs as previously described (36). ITRs of I, 0, 1, or 2 were considered resistant, and ITRs of 3 or 4 were considered susceptible.

[b] Res. = resistant; Sus. = susceptible; and Seg. = segregating.

[c] Test of homogeneity of ITRs were calculated with 2 df.

[d] Significantly different from a 1R:1S ratio at $P = 0.05$. However, when the χ^2 value was calculated for a 3R:1S ratio, the χ^2 (9.85) was highly significantly different at $P = 0.01$.

TABLE 2. Infection-type data from seven *Avena strigosa* (CI3815) × *A. wiestii* (CI 1994) recombinant inbred lines (RILs), the parental lines from which they were derived, and five isolates of *Puccinia coronata* that identified unique loci conferring resistance to crown rust

	Isolate[a]				
RIL number	202	PC62	290	263	PC54
A. wiestii parent	4	4	4	4	4
96	4	4	3	4	*1*
76, 98	4	4	3	*0*	1
73	4	4	*0*	0	0–1
43, 60, 85	4	*0–1*	0	0	0
A. strigosa parent	0	0	0	0	0

[a] Infection type 3 or 4 = susceptible, 0 or 1 = resistant, *0 or 1* = new resistance specificity detected by the isolate. The same infection phenotype was observed for two sets of progeny of each RIL that had been planted and inoculated in independent experiments.

RESULTS

Response to *P. coronata* isolates. The response to each isolate was evaluated within each of three families representing the three F_1 individuals that gave rise to the RIL population. A test for homogeneity was conducted before pooling the data. ITR data for eight isolates (PC54, PC58, PC59, PC62, 202, 263, 290, and 325A) fit a 1R:1S ratio, the ITR data for isolate 264B fit a 3R:1S ratio, and the ITR data for isolate 276 fit a 1R:3S ratio (Table 1). The ITR data for isolate 258 was significantly different than a 1R:1S ratio at $P = 0.05$ ($\chi^2 = 4.64$); however, it was highly significantly different than a 3R:1S ratio at $P = 0.01$ ($\chi^2 = 9.85$).

Utilizing RILs as differential lines. A subset of the RILs was used as differentials to further define isolates from the ISU collection. Utilizing these isolates, the boxing method of McVey and Leonard (19) was used to identify unique resistance specificities

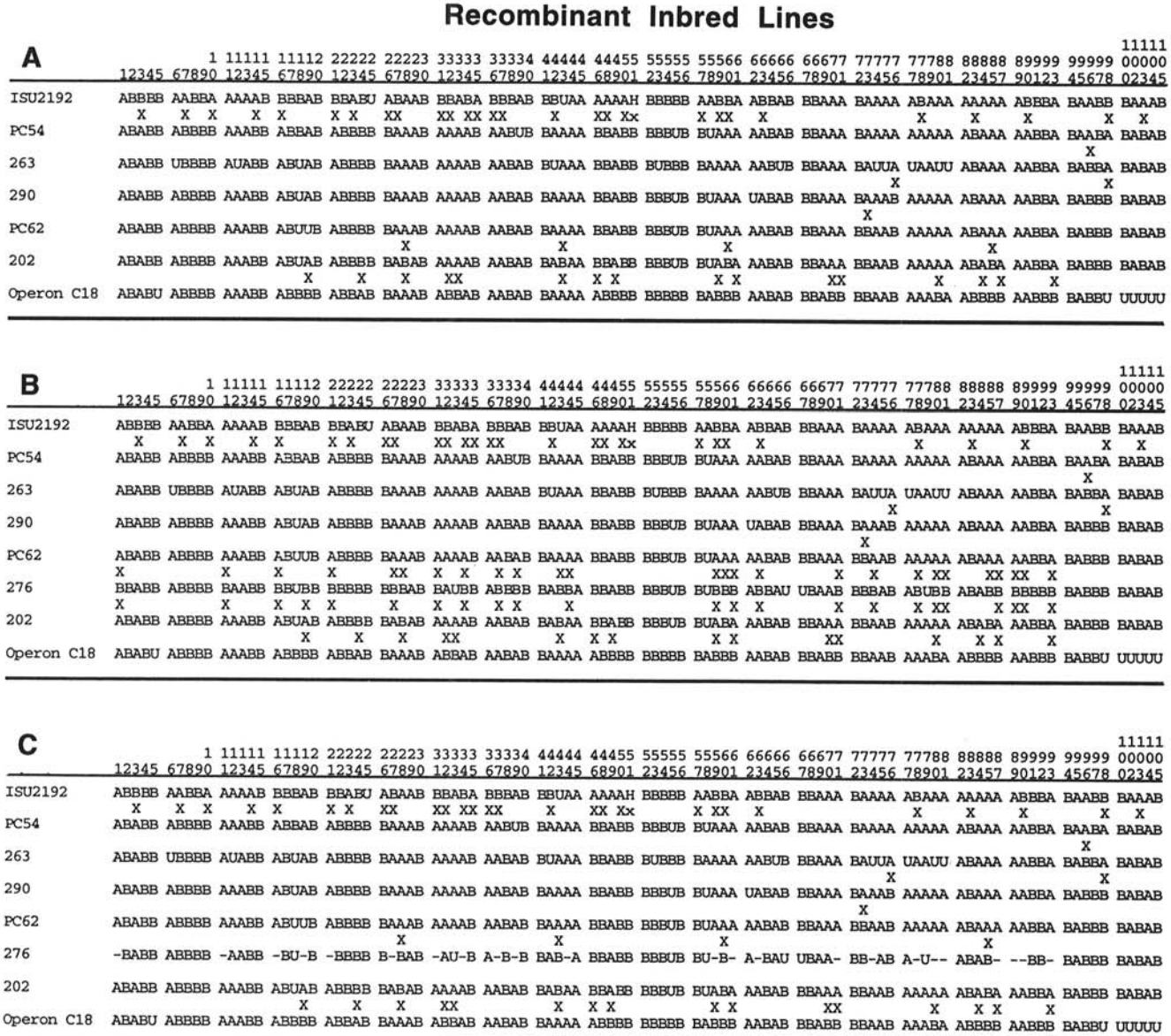

Fig. 1. A-C, Map Manager data set illustrating infection type rating (ITR) of 100 oat recombinant inbred lines (RILs) to six isolates of *Puccinia coronata*. For ITR data and flanking DNA markers, A denotes the phenotype from the *Avena strigosa* (resistant) parent; B denotes the phenotype from the *A. wiestii* (susceptible) parent; H denotes a heterozygote; U denotes a missing data point; and - denotes data removed during further analyses. Numbers across the top denote the individual RILs in the population. Isolates, DNA probes, and primers are listed down the left side. An uppercase X indicates a crossover; a lowercase x indicates a crossover where at least one marker is heterozygous. **A,** Final Map Manager data set containing restriction fragment length polymorphism and infection type data for isolates listed in Table 2. **B,** Reintegration of ITR data from isolate 276 into the final data set (shown in **A**), illustrating the generation of inauthentic double crossovers. **C,** Transformed ITR data from isolate 276, illustrating the most likely position of a locus conferring resistance to this isolate.

in the RILs. This procedure facilitates the identification of the minimum number of unique specificities that are detectable with a defined set of isolates (38). An ITR of 0 or 1 signifies resistant reactions, and an ITR of 3 or 4 signifies a susceptible reaction. Isolates of *P. coronata* headed columns, and RILs were listed as rows. The column with an ITR of 0 farthest down the database became the left column. The other ITRs were ordered in upward steps from left to right based on the number of 0's or 1's per column. Loci conferring unique specificities were identified if an RIL-isolate interaction was different from that of any other locus identified by another RIL-isolate interaction. Table 2 illustrates five unique host-pathogen interaction patterns that detected five specificities when RILs were used as differential lines.

Recombination within the *Pca* region. All of the isolates described above detected loci that mapped to the *Pca* region. Recombination between loci within the *Pca* region was suggested previously based on ITR data of F_3 families of each individual F_2 plant (24). Six of the nine isolates used in the previous study (24), as well as a number of additional isolates, were tested on the F_6 RILs. All of these isolates were avirulent on the *A. strigosa* parent (Table 1). Therefore, in the F_6 generation a line homozygous resistant (R) to one isolate but homozygous susceptible (S) to a second isolate suggested a recombination event between loci conferring different resistance specificities.

To detect recombination within the *Pca* region, ITR data for all isolates that fit a 1R:1S ratio were ordered by the "rearrange" command of Map Manager. A 1R:1S ratio in response to a particular isolate indicated that a single gene or multiple tightly linked genes segregated in the RILs. It was possible to differentiate loci conferring different specificities by analyzing recombinants within individual RILs as described above. Following each rearrangement, the orders were checked reiteratively for more double crossovers than would be predicted by chance, because a locus conferring specificity to a particular isolate might be positioned in the *Pca* region; however, an excess of putative double crossovers would indicate that factors elsewhere in the genome might be influencing the infection type of the plants. This generated "apparent" recombinants that were most likely not authentic. For any order, the ITR data for four isolates (325A, 258, PC58, and PC59) generated an excess of double crossovers. The data for these four isolates confounded the interpretation and, therefore, were removed from the initial recombination analysis.

The ITR data from the remaining five isolates (PC54, 263, 290, PC62, and 202) were ordered in relation to the flanking molecular markers *Xisu2192* and *XisuC18* (Fig. 1A). The ITR of individual lines revealing putative crossovers were rechecked with the isolates that identified the crossover to confirm that these plants had parental ITRs and not an intermediate reaction, because an intermediate reaction might indicate the recognition of more than one specificity and, thus, would not reflect a true recombinant. In all cases involving single crossovers, the recombinant lines displayed a parental ITR. Reaction to five unique isolates was differentiated by recombination, indicating that there were at least five loci conferring specificity to *P. coronata* in the *Pca* region. A summary of the recombination events identified in the individual RILS and the order of the specificities conferred by these loci are presented in Table 3.

Specificity to isolate 276. Response to isolate 276 fit a 1R:3S ratio, suggesting the interaction of two loci. Response to isolate 276 also fit a two-locus model in the $F_{2:3}$ generation (24). According to this model, a dominant allele of an unlinked locus suppresses the expression of a dominant allele at the resistance locus. Table 4 illustrates the expected ratios for genotypes and phenotypes that fit the observed ratios in both experiments with this isolate. *A. strigosa* carries a resistance locus (*R*), and *A. wiestii* presumably carries a suppressor of that resistance (*Su*). *Su* is epistatic to *R*, resulting in a susceptible phenotype when *Su* and *R* are both present. Thus, the genotypes of the original cross were most likely *R/R su/su (A. strigosa)* × *r/r Su/Su (A. wiestii)*. The $F_{2:3}$ progeny fit a 1R:8 segregating:7S ratio, and the F_6 RILs fit a 1R (*R/R su/su*):3S (*R/R Su/Su, r/r Su/Su,* or *r/r su/su)* ratio.

Initial mapping analyses suggested that a locus conferring specificity (*R*) to *P. coronata* was positioned in the *Pca* region in both studies. To determine the most likely position of the specificity conferred by isolate 276, ITR data were reintegrated into the final Map Manager file and arranged reiteratively (Fig. 1B). Twenty-two (25%) of the RILs exhibited a susceptible phenotype, apparently resulting from double crossovers. The number of double crossovers was greater than expected by chance, and, therefore, suggested masking of resistance specificity in *R/R Su/Su* plants (Table 4). The data points involved in these crossovers were removed (RIL 1, 11, 16, 19, 21, 27, 31, 34, 37, 39, 44, 59, 61, 63, 71, 74, 78, 80, 81, 87, 89, 90, and 93), unmasking the effect of the suppressor on the dominant resistance specificity (Fig. 1C). After the removal of the double crossovers, it was evident that the specificity detected by isolate 276 could not be differentiated from the specificity detected by isolate 202. These possibilities will be assessed through additional genetic analysis.

Specificity to isolate 264B. Response to isolate 264B fit a 3R:1S ratio. In the F_6 (self) generation, this ratio was indicative of two independent dominant genes, *R1* and *R2,* interacting so resistance was conferred whenever either dominant gene was present. If homozygosity was achieved at all loci, four genotypes would be expected. RILs with genotypes *R1/R1 r2/r2*, *r1/r1 R2/R2*, or *R1/R1 R2/R2* would be resistant, and RILs with the genotype *r1/r1 r2/r2* would be susceptible. To remove the effect of the resistance specificity located elsewhere in the genome and to posi-

TABLE 3. Summary of recombination events identified from infection type rating (ITR) data of five *Puccinia coronata* isolates on individual oat recombinant inbred lines (RILs)

Isolate	No. of crossovers	cM[a]	Standard error[b]	LOD[c]	Locus designation[d]
PC54					*R84*
	1	0.59	0.59	23.5	
263					*R263*
	2	1.19	0.85	21.8	
290					*R290*
	1	0.53	0.53	26.2	
PC62					*R62*
	4	2.17	1.11	21.7	
202					*R202*

[a] cM = centimorgan. Calculated by Map Manager version 2.6 (15).
[b] Calculated by maximum likelihood formulae of Silver (32) by Map Manager (15).
[c] The LOD score is the $\log_{10}$ of the odds ratio between the presence and absence of linkage between markers.
[d] The locus (*R*) is identified by the isolate that detects it.

TABLE 4. Genotypes and phenotypes expected for a two-locus interaction where a dominant gene (*Su*) is a suppressor of an unlinked dominant resistance gene (*R*) in *Avena*

Parental line[a]	*R/R su/su* (*A. strigosa*)	×	*r/r Su/Su* (*A. wiestii*)
F_1 genotype		*R/r Su/su*	
F_2 genotype	1 *R/R su/su* :	2 *R/R Su/su* :	1 *R/R Su/Su*
		4 *R/r Su/su*	2 *R/r Su/Su*
		2 *R/r su/su*	1 *r/r Su/Su*
			2 *r/r Su/su*
			1 *r/r su/su*
F_3 family phenotype	1 resistant :	8 segregating :	7 susceptible
F_6 RIL genotype	1 *R/R su/su*	:	1 *R/R Su/Su*
			1 *r/r Su/Su*
			1 *r/r su/su*
F_6 RIL phenotype	1 resistant	:	3 susceptible

[a] RIL = recombinant inbred line.

tion the locus determining specificity in the *Pca* region for resistance to 264B, ITR data were reintegrated into the final Map Manager file and rearranged as described above for isolate 276. Again, an apparent excess of double crossovers was revealed, always involving lines that had a resistant infection type. When lines involving these crossovers were removed (RIL 7, 9, 17, 18, 30, 51, 52, 54, 65, 66, 68, 72, 68, 72, and 91), the locus detected by isolate 264B mapped to the *Pca* region and could not be differentiated from the locus detected by isolate PC54 (data not shown).

Specificity to isolates PC59, 325A, PC58, and 258. Four isolates displayed an excess of double crossovers in the Map Manager analysis. This suggested that loci conferring additional specificities were segregating outside the *Pca* region. About half of these double crossovers displayed an intermediate infection type, again suggesting modifiers elsewhere in the genome. Many of these inauthentic crossovers occurred in the same lines as those for isolates 264B (involving a resistant phenotype) and 276 (involving a susceptible phenotype). This may indicate either that the putative modifiers were in the same region of the genome or that factors in the particular RILs allowed full or partial expression of the modification.

To uncover the most likely location of the loci in the *Pca* region detected by these isolates, the ITR data were reintegrated into the Map Manager data file, and lines containing double crossovers were removed from the analysis. When the data were reanalyzed in this way, the locus detected by isolate PC59 could not be differentiated from the locus detected by 263; the locus detected by isolate 325A could not be differentiated from the locus detected by 290; and the locus detected by PC58 could not be differentiated from the locus detected by PC62 (data not shown). An additional unique locus could be detected by specificity to isolate 258, however. This locus could be positioned by unique recombination events between the loci defined by specificity to isolates 263 and 290 (Fig. 1).

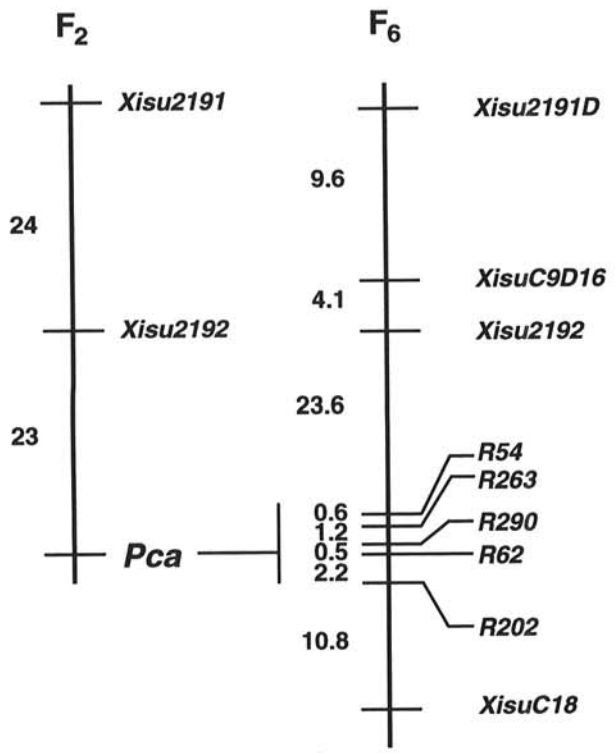

Fig. 2. Genetic maps of the end of linkage group A in diploid *Avena* for F_2 and F_6 generations. Five loci within the *Pca* region were differentiated by recombination in the F_6 generation. The prefix *X* denotes molecular marker loci. The prefix *R* denotes loci for specificity to designated isolates of *Puccinia coronata*. Distances are in centimorgans.

DNA markers linked to *Pca*. All genetic marker loci in Figure 2 fit expected chi-square ratios. Two RFLP loci (*Xisu2191* and *Xisu2192*) were linked in the same orientation with respect to the *Pca* region as in the F_2 generation (24). Because these two markers detected codominant alleles in the F_2 study and six generations of inbreeding were conducted, a ratio of 48.5 (homozygous *strigosa* allele)/3 (heterozygotes)/48.5 (homozygous *wiestii* allele) was expected (17). *Xisu2192* segregated for the two parental classes in a 50:47 ratio. Probe ISU2191 detected a small gene family linked to *Xisu2192* (40). *Xisu2191D*, an RFLP locus detected by ISU2191 and closest to *Xisu2192*, segregated for the two parental classes in a 55:45 ratio. Loci identified by RAPDs were tested against a 1:1 present (*A. strigosa* parent)/absent (*A. weistii* parent) ratio representing the parental classes as expected for a marker scored as a dominant allele. Operon primer C18 was used to amplify a DNA fragment that segregated in a 37:56 present/absent ratio. This fragment was designated *XisuC18* and was positioned 10.8 centimorgans (cM) distal to *Pca*. A pair of Operon primers (C9 and D16) was used to amplify a DNA fragment that segregated in a ratio of 49:38 present/absent. This second fragment was designated *XisuC9D16* and was positioned between *Xisu2191* and *Xisu2192* (Fig. 2).

DISCUSSION

Utilizing defined crown rust isolates and fixed crossovers in homozygous RILs, we provide evidence for a cluster of at least five loci in diploid *Avena* conferring specificity to *P. coronata*. A particular isolate may detect a single gene or multiple tightly linked genes; therefore, we designated the specificities detected by these isolates as loci, with each locus possibly containing more than one gene. For example, a single locus may contain a gene conferring specificity to isolate 202 but also may contain a second gene conferring specificity to isolate 276. If so, the gene conferring specificity to isolate 276 is affected by an independent suppressor, whereas the gene conferring specificity to isolate 202 is not.

All of these loci occur in coupling and originate from the CI 3815 accession of *A. strigosa*. Other studies involving diploid *Avena*, including the same resistant accession (CI 3815), also suggest close linkage between loci conferring specificity to *P. coronata* (16,34). Loci in these early studies were defined only by the accession that carried them and not by map position; therefore, it is difficult to assess if they are the same as those reported here. However, a second locus conferring specificity to isolate 276 was linked to the *Pca* region and was differentiated by recombination in the progeny of a cross between the two *A. strigosa* accessions, CI 3815 and CI 2630 (6). This additional specificity originates from CI 2630 and is 17 cM from the *Pca* region.

The five loci are separated by eight distinct recombination events: six resulting from single crossovers and two from double crossovers. Four events revealed the only crossover between *XisuC18* and *Xisu2191D*, a genetic distance spanning 52.6 cM. The occurrence of double crossovers should be interpreted with caution because these are often indicative of other factors elsewhere in the genome that interfere with the ITR to a particular isolate. For example, RIL 28 was scored as susceptible when inoculated with isolate 202, yet it displayed an intermediate phenotype. Therefore, the putative double crossover generated by this data point could be inauthentic. RIL 43, however, displayed a parental phenotype with isolate 202 and, therefore, most likely contains an authentic double crossover. The fact that this double crossover involves *XisuC18*, a RAPD marker 10.8 cM distal to the *R202* locus, supports this possibility.

The five loci are positioned near the end of linkage group A on the *A. strigosa* × *A. wiestii* map (24) and are arranged in tandem within a 4.5-cM region (Fig. 2). The accumulation of multiple loci conferring resistance on a single chromosome in a self-fertilizing species provides support for the tandem duplication model

for resistance clusters. One explanation is that the *cis* arrangement of *Pc* specificities is derived by tandem duplication followed by mutation of an ancestral *Pc* gene. Sequence duplication, mispairing, and unequal crossing-over have been postulated as mechanisms that produce resistance clusters observed at the ends of chromosomes (35). The multiple locus arrangement could influence breeding strategies as well as the transfer of such regions when they are introduced from wild relatives of cultivated species (4,26). Parents serving as donors of disease resistance should carry *R* loci with the greatest possible number of resistance alleles. When exposed to high pathogen pressures, cultivars with the greatest number of resistance alleles should be more resistant to a larger number of isolates.

The organization of specificity within the *Pca* region is similar to the genetic organization of other resistance clusters. In lettuce, four groups of *Dm* loci confer resistance to *B. lactucae*, and one cluster consists of at least seven *Dm* genes (13). At least 20 alleles or tightly linked genes within 1 cM at the *Mla* locus confer resistance to *E. graminis* (5,9,14,30,37) in barley. Eight *Rp1* alleles within a 0.2-cM cluster confer resistance to *P. sorghi* in maize (9,23,29). Groups of genes conferring resistance to *P. recondita* and *P. graminis* (*Lr2*, *Lr3*, and *Sr9*) have been identified in wheat (19), and linkages such as these are common among genes for resistance to oat crown and stem rusts (2,8,33). Many of the genes in these clusters occur naturally in repulsion, whereas the five loci reported here that confer specificity to *P. coronata* all occur in coupling.

We previously reported that *Pca* was at the end of linkage group A (24). The addition of *XisuC18*, which is 10.8 cM distal to *Pca*, extends the length of this linkage group and provides a flanking molecular marker for use in the analysis of this resistance cluster. Bulks for RAPD analysis were based on F_3 family ITR data for isolate 258. As shown above, analysis of homozygous F_6 RILs revealed that the response to 258 is more complex than was originally thought. This may explain the lack of closer markers when these bulks were used.

Dominant suppressors of resistance to *Puccinia* species have been reported previously for hexaploid *Avena* (33). In this report, we provide further genetic evidence of a dominant suppressor of resistance specificity detected by isolate 276. We show that by careful examination of the data set, it is possible to arrive at a more likely location of resistance to isolate 276. Susceptible individuals (B) exhibiting double crossovers most likely carry a dominant allele of the *Su* locus. In contrast, the resistant individuals (A) would carry a recessive allele of the *Su* locus. None of the other susceptible individuals are diagnostic and can be removed. By transforming these data to reflect the presumed allelic status of the suppressor, it should be possible to place the *Su* locus in reference to other markers. The response of this set of RILs to isolate 276 provides unique opportunities to dissect the genetic basis of epistasis and genetic background effects on the expression of different *Pc* alleles.

To date, we have positioned resistance loci in diploid *Avena* only at or linked to the *Pca* region (6,24). By transforming the data for isolate 264B, it should be possible to position another resistance locus elsewhere in the genome. Linkage group C would be a possible candidate, because other loci for resistance to crown rust have been placed there in hexaploid oat (1).

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