Rds* and *Rih* Mediate Hypersensitive Cell Death Independent of Gene-for-Gene Resistance to the Oat Crown Rust Pathogen *Puccinia coronata* f. sp. *avenae

Gong-Xin Yu,^{1,2} Ed Braun,² and Roger P. Wise^{1,2,3}

¹Interdepartmental Genetics Program, ²Department of Plant Pathology, and ³Corn Insects and Crop Genetics Research, U.S. Department of Agriculture, Agricultural Research Service, Iowa State University, Ames 50011-1020 U.S.A.

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The *Pca* crown rust resistance cluster in the diploid *Avena* genus confers gene-for-gene specificity to numerous isolates of *Puccinia coronata* f. sp. *avenae*. Recombination breakpoint analysis indicates that specificities conferred by the *Pca* cluster are controlled by at least five distinct genes, designated *Pc81*, *Pc82*, *Pc83*, *Pc84*, and *Pc85*. *Avena* plants with the appropriate genotype frequently respond to *P. coronata* by undergoing hypersensitive cell death at the sites of fungal infection. Autofluorescence of host cells in response to *P. coronata* occurs in plants that develop visible necrotic lesions but not in plants that lack this phenotype. Two newly described, non-*Pc* loci were shown to control hypersensitive cell death. *Rds* (resistance-dependent suppressor of cell death) suppresses the hypersensitive response (HR), but not the resistance, mediated by the *Pc82* resistance gene. In contrast, *Rih* (resistance-independent hypersensitive cell death) confers HR in both resistant and susceptible plants. Linkage analysis indicates that *Rds* is unlinked to the *Pca* cluster, whereas *Rih* is tightly linked to it. These results indicate that multiple synchronous pathways affect the development of hypersensitive cell death and that HR is not essential for resistance to crown rust. Further characterization of these genes will clarify the relationship between plant

disease resistance and localized hypersensitive cell death.

Localized cell death, the rapid collapse of pathogen-infected cells and tissues, is a nearly ubiquitous feature of the incompatible interaction between plants and their microbial pathogens. This reaction is termed the hypersensitive response (HR). HR has been proposed to play an important role in disease resistance in that cell death is thought to deprive the pathogen of nutrients, resulting in pathogen containment (Dangl 1995; Heath 1980; Staskawicz et al. 1995). Biochemical and physiological responses are often associated with hypersensitive cell death induced by avirulent pathogens (Dixon and Lamb 1990). One marked response is the biosynthesis and deposition of autofluorescent compounds in and around dead cells (Koga et al. 1980, 1988). These materials are thought to contribute to the limitation of pathogen growth and are nearly always associated with plant cells undergoing pathogen-induced hypersensitive cell death (Hahlbrock and Scheel 1987; Keogh et al. 1980; Koga et al. 1988; Woods et al. 1988).

Early studies hypothesized that localized cell death is involved in plant disease resistance (Dietrich et al. 1994; Jakobek and Lindgren 1993; Jones 1994; Kohm et al. 1993). However, a number of recent examples provide evidence that cell death may be a consequence of, and not the effector, of active disease defense. *Arabidopsis ndr1-1* mutants exhibit an HR even though they are susceptible to avirulent *Pseudomonas syringae* isolates (Century et al. 1995). Conversely, *Rx*-mediated resistance to *Potato virus X* (PVX) does not normally involve localized cell death, but the *Rx* gene is associated with HR in transgenic plants expressing the PVX coat protein elicitor (Bendahmane et al. 1999; Goulden and Baulcombe 1993; Kohm et al. 1993). Moreover, the recent isolation of *dnd* (defense, no death) mutants in *Arabidopsis* demonstrated that gene-for-gene resistance could be separated from the HR in response to avirulent *Pseudomonas syringae* pathogens (Clough et al. 2000; Yu et al. 1998, 2000). Therefore, a general role for localized cell death in the development of plant disease defense remains unresolved.

Puccinia coronata Corda f. sp. *avenae* Eriks., the causal agent of crown rust, is the most important fungal pathogen of cultivated oat (Marshall and Shaner 1992). Several crown rust

Corresponding author: Roger P. Wise; Telephone: +1-515-294-9756; Fax: +1-515-294-9420; E-mail: rpwise@iastate.edu

Current address of Gong-Xin Yu: Mathematics and Computer Science Division, Argonne National Laboratory, 9700 South Cass Avenue, Argonne, IL 60439-4870 U.S.A.

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resistance genes have been identified and positioned in reference to molecular markers (Bush and Wise 1998; Bush et al. 1994; Jellen et al. 1995; O'Donoghue et al. 1996; Penner et al. 1993; Rayapati et al. 1994; Rooney et al. 1994; Wise et al. 1996). These genes, designated *Pc*, typically encode resistance specificity in a gene-for-gene manner (Wise et al. 1996). Yet, little is known about the functional organization of these loci or of their downstream signaling components.

The *Pca* (crown rust) resistance cluster was initially identified in an F_2 population generated by crossing the two diploid oat accessions, *Avena strigosa* (C.I. 3815) and *A. wiestii* (C.I. 1994) (Rayapati et al. 1994). In further studies, it was postulated that distinct *Pca* resistance specificities were controlled by a series of closely linked loci (Wise et al. 1996). In this study, we verified that distinct genes were indeed responsible for the different resistance specificities and determined their order within the *Pca* cluster. Furthermore, we show that separate genes controlled hypersensitive cell death and gene-for-gene resistance to *P. coronata*. Two new genes involved in the *Avena*-*P. coronata* interaction are described. *Rds* (resistance-dependent suppressor of cell death) is an allele from *A. wiestii* that is dominant to the *rds* allele from *A. strigosa* and suppresses hypersensitive cell death (an HR) induced by crown rust isolate 276. *Rih* (resistance-independent hypersensitive cell death) is an allele from *A. strigosa* that is dominant to the *rih* allele from *A. wiestii* and mediates the HR induced by crown rust isolate 290.

RESULTS

Resolution of crown rust resistance genes within the *Pca* cluster.

Recombination breakpoints within the *Pca* cluster were previously postulated from the analysis of infection types (IT)

induced by 11 distinct isolates of *P. coronata* on 100 recombinant inbred (RI) lines (Wise et al. 1996). However, because diverse responses were observed on the *A. strigosa* (C.I. 3815) and *A. wiestii* (C.I. 1994) parents compared with the progeny of the RI population, it was necessary to perform backcross analysis of each putative recombinant individual to authenticate the existence of distinct genes within the *Pca* cluster. To perform this analysis, the seven RI lines that contained putative recombination breakpoints within the cluster were backcrossed once to the susceptible parent C.I. 1994 and selfed to generate large BC_1F_2 populations. Progeny families from the seven BC_1F_2 populations were inoculated with six diagnostic *P. coronata* isolates that contained different combinations of *AvrPc* genes. For each isolate- BC_1F_2 family combination, the IT would be predicted to segregate 3:1 (resistant/susceptible) if the original RI line used in the backcross possessed the resistance specificity to the isolate used for the inoculation. If, however, the RI line did not contain the appropriate resistance specificity, the BC_1F_2 family would be entirely susceptible.

Segregation data from the 42 combinations of the seven BC_1F_2 populations inoculated with the six diagnostic isolates is summarized in Table 1. The linear order of five distinct resistance genes within the *Pca* cluster was determined by the distribution of recombination breakpoints in relation to closely linked flanking markers on linkage group B (Yu and Wise 2000). By comparing all 42 BC_1F_2 combinations, we extended and confirmed our previous RI analyses and the five specificities were arranged as resistance to isolates PC54, 290, 276, 263, PC62, and 202. Hence, the separate genes within the *Pca* cluster that confer specificity to these isolates of *P. coronata* have been designated *Pc81*, *Pc82*, *Pc83*, *Pc84*, and *Pc85* in accordance with the oat crown rust resistance gene nomenclature.

Table 1. Segregation of the infection type reactions in 42 *Puccinia coronata* isolate $\times$ BC_1F_2 population combinations^a

<i>P. coronata</i>			BC_1F_2 populations						
Isolate	<i>R</i> locus ^b	<i>R</i> -gene designation	A8060	A8028	A8073	A8076	A8096	A8085	A8098
PC54	<i>R54</i>	<i>Pc81</i>	17R:6S	20R:7S	23R:8S	17R:8S	12R:5S	13R:6S	14R:8S
290	<i>R290</i>	<i>Pc82-290</i>	15R:6S	18R:4S	25R:6S	16R:8S	11R:4S	0R:29S	0R:22S
276		<i>Pc82-276</i>	17R:8S	19R:9S	34R:13S	17R:10S	11R:4S	0R:32S	0R:27S
263	<i>R263</i>	<i>Pc83</i>	25R:7S	36R:10S	21R:7S	17R:10S	0R:19S	0R:26S	0R:30S
PC62	<i>R62</i>	<i>Pc84</i>	14R:6S	25R:9S	0R:30S	0R:25S	0R:37S	0R:30S	0R:30S
202	<i>R202</i>	<i>Pc85</i>	22R:4S	0R:19S	0R:22S	0R:25S	0R:15S	0R:26S	0R:16S

^a Areas above the dashed line represent isolate $\times$ BC_1F_2 combinations for which the segregation ratio is 3R:1S (R = resistant, S = susceptible), indicating the presence of resistance specificity. Areas below the dashed line represent isolate $\times$ BC_1F_2 combinations in which the population was entirely susceptible. The interface delineated by the dashed line indicates the position of recombination breakpoints between resistance genes in the seven recombinant inbred lines. Infection types (IT) of 0, 1, or 2 were considered resistant. An IT of 3 or 4 were considered susceptible.

^b Previous *R* locus designation (Wise et al. 1996). The *R* locus of isolate 276 was not previously noted.

Table 2. Infection types (IT) of six *Puccinia coronata* isolates on the parental accessions of the cross *Avena strigosa* (C.I. 3815) $\times$ *A. wiestii* (C.I. 1994) and seven recombinant inbred lines that each contain a recombination breakpoint within the *Pca* cluster^a

Isolate	C.I. 3815	C.I. 1994	A8060	A8028	A8073	A8076	A8096	A8085	A8098
P54	1	4	2	2	2	2	2	2	2
290	1 (NLD)	4	1 (NLD)	1 (NLD)	1 (NLD)	2 (NLD)	2 (NLD)	4	3
276	1 (NLD)	4	2	2	2	2	2	3	3
263	1 (NLD)	4	2 (NLD)	2 (NLD)	2 (NLD)	1 (NLD)	4	4	4
PC62	1 (NLD)	4	1 (NLD)	1 (NLD)	3	4	4 (NLD)	3	4
202	1	4	2	4	3	3	3	3	3

^a On a 0-to-4 scale using the method of Murphy (1935). An IT of 0 = nearly immune: no uredinia, necrotic areas, or chlorotic flecks present; 1 = highly resistant: few small (0.1 mm wide by 0.4 mm long) uredinia, and some necrotic areas without uredinia; 2 = moderately resistant: small to medium (0.25 mm wide by 0.4 to 1.2 mm long) uredinia; 3 = moderately susceptible: abundant medium-size uredinia in chlorotic areas, and no necrosis; 4 = completely susceptible: abundant large (≥ 0.25 mm wide $\times$ ≥ 1.2 mm long) uredinia without necrosis or chlorosis. At least three seedlings were planted and inoculated for each line; each inoculation was performed at least three times. NLD indicates macroscopically visible, necrotic lesion development.

Some, but not all, isolates of *P. coronata* elicit necrotic lesion development (NLD) on the diploid *Avena* genus.

P. coronata isolates 290, 263, and PC62 elicited macroscopically visible, NLD on the *A. strigosa* parent line C.I. 3815 and also on all of the resistant RI lines that were recombinant within the *Pca* cluster (Table 2). In contrast, *P. coronata* isolate 276 displayed NLD on C.I. 3815 but not on any resistant RI line that was recombinant within the *Pca* cluster. These observations suggested that NLD may not always be coupled with resistance. The fact that crown rust isolates 202 and PC54 did not elicit NLD either on C.I. 3815 or on any resistant RI line further supported this assumption. In addition, RI line A8096, although susceptible, still exhibited NLD in response to infection by crown rust isolates 290 and PC62.

Macroscopically visible NLD in the *Avena*–*P. coronata* interaction is due to hypersensitive cell death.

Autofluorescence is a hallmark of plant cells undergoing fungus-induced HR. To determine whether macroscopically visible NLD was coincident with HR, and also to assess if microscopic HR was present in the absence of macroscopic NLD, infected leaf samples were subjected to Calcofluor staining 66 h after inoculation and examined by fluorescence microscopy. We performed this analysis on the parents of the diploid *Avena* RI population as well as on several resistant RI progeny that clearly displayed the NLD phenotype (A8013, A8036, A8069, and A8073) or lacked the NLD phenotype (A8016 and A8087). The interaction between fungal hyphae and the plant host cells is illustrated in Figure 1. Upon infection with isolate 276, the macroscopically visible NLD phenotype was coincident with a high incidence of plant cells undergoing HR (exhibiting intense autofluorescence) in the parental line C.I. 3815 and the RI progeny line A8013 (Fig. 1A and C). Necrotic host cells fluoresced bright orange-yellow, whereas *P. coronata* hyphae fluoresced bright blue and unaffected plant cells fluoresced light violet. In contrast, a low incidence of autofluorescent host cells was observed in the susceptible parent line C.I. 1994 (Fig. 1B) and in the resistant plants that did not display (macroscopically visible) NLD (A8016) (Fig. 1D).

To determine the frequency of hypersensitive cell death in response to challenge by *P. coronata*, clusters of necrotic host cells were quantified at sites of fungal infection. Necrotic host cell clusters were observed at most fungal infection sites for the resistant parent C.I. 3815 and RI progeny (A8013, A8036, and A8069) that also exhibited macroscopically visible NLD in response to challenge by isolate 276 (Table 3). For these interactions, the frequency of necrotic cell clusters per infection site ranged from 2.18 to 5.99. In contrast, isolate 276 did not elicit NLD on A8016 or A8087, even though they were very resistant to infection. Accordingly, for these two RI progeny, clusters of necrotic cells were observed rarely at sites of fungal infection at frequencies of 0.18 and 0.17, respectively. Similar results were obtained when plants were challenged with *P. coronata* isolate 290, further supporting the fact that macroscopically visible necrotic lesions are accompanied by host cell autofluorescence (Table 3). Importantly, microscopic autofluorescence was almost never observed in plants that did not show the macroscopic NLD phenotype.

Hypersensitive cell death induced by *P. coronata* isolate 276 is suppressed by a novel gene unlinked to the *Pca* cluster.

We analyzed the segregation of macroscopic HR induced by isolate 276 on the C.I. 3815 × C.I. 1994 RI population. Of the 100 RI lines examined from this cross, 23 were resistant with HR, 28 were resistant without HR, and the remaining 49 lines were susceptible without HR. The observed segregation of HR within the resistant lines was in good fit to a 1:1 ratio (HR/non-HR) ($\chi^2 = 0.49$; $P = 0.48$), expected for the inheritance of a second gene in the RI population controlling HR that is unlinked to the *Pca* locus.

To further characterize this new locus controlling hypersensitive cell death, a resistant non-HR line, RI A8059, was backcrossed to the resistant HR parent C.I. 3815 and selfed to obtain BC₁F₂ lines. The responses of RI A8059, C.I. 3815, the A8059 × C.I. 3815 BC₁F₁ hybrids, and BC₁F₂ population are

Fig. 1. Autofluorescence of diploid *Avena* cells in response to challenge with *Puccinia coronata* isolate 276. Leaf segments were stained with a fluorescent brightener (Cellufluor; Polysciences, Warrington, PA, U.S.A.) and examined by fluorescence microscopy. **A**, The resistant parent C.I. 3815 and **C**, the resistant recombinant inbred (RI) progeny line A8013 illustrate typical autofluorescence of cells undergoing hypersensitive response (HR) upon challenge with isolate 276. Necrotic host cells fluoresced bright orange-yellow, whereas *P. coronata* hyphae fluoresced bright blue, and unaffected plant cells fluoresced light violet. In contrast, no autofluorescent host cells were observed **B**, in the susceptible parent C.I. 1994 or **D**, in resistant plants as evidenced by the non-HR RI progeny line A8016. Scale bars are equal to 30 μ m.

shown in Table 4. The BC₁F₁ hybrid of A8059 × C.I. 3815 was resistant and did not exhibit HR. Segregation of BC₁F₂ progeny without HR to those with HR was in good fit to 3:1 ratio (non-HR/HR). The results of these two genetic analyses indicate that there is a dominant suppressor of HR segregating in response to inoculation with isolate 276 of *P. coronata*. This newly described locus is designated *Rds* (resistance-dependent suppressor of cell death).

We used the 51 resistant, HR-segregating RI lines in the C.I. 3815 × C.I. 1994 RI population to genetically position *Rds* on the diploid *Avena* map. The above genetic analysis indicates that the dominant suppressor *Rds* allele was derived from the *A. wiestii* (C.I. 1994) parent. Therefore, RI lines that exhibited HR were coded homozygous (A) for the recessive *rds* allele from *A. strigosa* (C.I. 3815) and RI lines that did not exhibit HR were coded homozygous (B) for the dominant *Rds* allele from *A. wiestii*, and the data were integrated with 512 previously mapped loci in our Map Manager dataset (Yu and Wise 2000). *Rds* was positioned between *Xisu1410d* and E16M9-8b on linkage group A of the *A. strigosa* × *A. wiestii* RI map, independent of *Pc82* on linkage group B.

A second gene controlling HR in response to isolate 290 is closely linked to the *Pc82* resistance locus.

To further investigate the relationship between HR and gene-for-gene resistance, an expanded population from the A8073 (resistant, HR) × C.I. 1994 (susceptible, non-HR) BC₁F₂ (Table 1) was used to compare the response to inoculation with isolate 290. The typical HR in response to inoculation with isolate 290 is shown in Figure 2. RI line A8073 displayed a high level of resistance and HR, similar to the C.I. 3815 parent. Segregation of progeny that were resistant and exhibiting HR to those that were susceptible and lacking HR was in good fit to a 3:1 ratio ($\chi^2 = 2.15$; $P = 0.14$) (Table 5). The A8073 × C.I. 1994 BC₁F₁ hybrid exhibited both resis-

tance and HR. This indicates that the dominant alleles of genes that confer resistance specificity and hypersensitive cell death originate from A8073. Interestingly, rare resistant non-HR and susceptible HR recombinants were observed among the BC₁F₂ population, suggesting that there is an additional gene that mediates HR that is closely linked to *Pc82-290*.

The resistant non-HR and susceptible HR recombinants were authenticated by molecular marker analysis. Based on

Fig. 2. Segregation of hypersensitive cell death (a hypersensitive response [HR]) on the A8073 × C.I. 1994 BC₁F₂ population in response to the infection by isolate 290. Hypersensitive lesions developed after 7 days on the resistant parent C.I. 3815 and the recombinant inbred line A8073. In contrast, pustules of urediniospores developed on the susceptible parent, C.I. 1994. Although the A8073/C.I. 1994 F₁ hybrid displayed an intermediate response to *Puccinia coronata*, it still developed discernible HR. The susceptible recombinant S233 developed hypersensitive lesions, whereas the resistant, non-HR recombinants did not (data summarized in Table 5).

Table 3. Frequencies of infection sites showing autofluorescence in oat leaf tissues inoculated with *Puccinia coronata* isolates 276 and 290

Plant line	Isolate	Phenotype ^a	Fungal infection sites per cm ²	Necrotic cell clusters per cm ²	Necrotic cell clusters per infection site
C.I. 3815	276	R, HR	106.0	477.0	4.50
C.I. 1994	276	S, non-HR	1,280.4	38.8	0.03
A8013	276	R, HR	653.4	1,425.6	2.18
A8036	276	R, HR	660.0	2,160.0	3.27
A8069	276	R, HR	379.2	2,275.2	5.99
A8016	276	R, non-HR	1,078.4	193.6	0.18
A8087	276	R, non-HR	1,128.0	186.1	0.17
C.I. 3815	290	R, HR	421.2	955.8	2.26
C.I. 1994	290	S, non-HR	692.3	0.0	0.00
A8073	290	R, HR	949.1	1,248.8	1.32

^a R indicates resistance and S indicates susceptible. HR indicates hypersensitive response and indicates presence of macroscopically visible hypersensitive cell death; non-HR indicates absence of macroscopically visible hypersensitive cell death.

Table 4. Hypersensitive cell death in the diploid *Avena* A8059 × C.I. 3815 BC₁F₂ population in response to inoculation of isolate 276^a

Parental line		F ₁ hybrid		χ^2 test		
A8059	C.I. 3815	A8059 × C.I. 3815	BC ₁ F ₂ population ^b	χ^2 value (3:1)	<i>P</i> value	
Resistant, non-HR	Resistant, HR	Resistant, non-HR	Resistant, non-HR 88	0.50	0.48	
			Resistant, HR 25			

^a Four seedlings were evaluated of each parental line. Infection type of 0, 1, or 2 is considered resistant and of 3 or 4 is considered susceptible. HR indicates hypersensitive response and indicates presence of macroscopically visible hypersensitive cell death; non-HR indicates absence of macroscopically visible hypersensitive cell death.

^b Number of plants with each phenotype.

the infection type of A8073 (Table 1) and the linkage map shown in Figure 3, the chromosome on the *Xisu2192* side of *Pc83* in the A8073 RI line must have originated from C.I. 3815 (resistant) and the chromosome on the *Agx9* side of *Pc84* must have originated from C.I. 1994 (susceptible). When A8073 was backcrossed with C.I. 1994 and selfed, recombination would only be detectable on the *Xisu2192* side of *Pc83* because the region between *Pc84* and *Agx9* is homozygous for C.I. 1994-contributed chromosomal segments. The gene that mediates HR originated from A8073; therefore, it must lie on the *Xisu2192* side of *Pc83* (Table 5). Thus, homozygous susceptible HR or homozygous resistant non-HR progeny would have to be recombinant between the diagnostic restriction fragment length polymorphism marker, *Xisu2192* and *Pc83*. Indeed, homozygous susceptible HR recombinants contained *Xisu2192* alleles from A8073 (donated by C.I. 3815) and homozygous resistant non-HR plants contained *Xisu2192* alleles from C.I. 1994, consistent with the scored phenotype of the recombinants.

Linkage distance between the *Pc82* locus (conferring gene-for-gene resistance to isolate 290) and the gene that mediates HR was calculated based on the maximum likelihood method for estimating linkage from the progeny of selfed heterozygotes (Fisher and Balmukand 1928). Based on the observations in Table 5, this gene is separated from *Pc82* by 4.9 ± 2.4 cM. Because hypersensitive cell death was observed on both resistant and susceptible plants, the gene mediating HR in response to isolate 290 was designated *Rih* (resistance-independent hypersensitive cell death).

DISCUSSION

The response triggered by the gene-for-gene interaction is complex and comprises a battery of biochemical, cellular, and physiological responses. Hypersensitive cell death at infection sites is one of the most significant components involved in the plant defense response. In the *Avena* genus, hypersensitive cell death at the site of fungal infection is a typical characteristic of the interaction with *P. coronata*, the causal agent of oat crown rust. In this report, we demonstrate that that *Pca*-mediated, hypersensitive cell death can be regulated by two distinct, but interconnected, signaling pathways.

Two new loci were identified by genetic analysis. The first, *Rds*, has an allele that acts as a dominant negative regulator of hypersensitive cell death induced by *P. coronata* isolate 276 on plants that contain *Pc82* (Fig. 4). Thus, the *Rds* locus may represent a regulatory factor that acts to restrict cell death to discrete clusters of host cells upon pathogen invasion. This would be of obvious benefit to the host, because restricted cell

death would contain the pathogen, yet still leave unaffected cells for normal developmental processes. Moreover, one-half of the *Pc82*-specified, resistant individuals that were identified in the C.I. 3815 $\times$ C.I. 1994 RI population conferred complete resistance against *P. coronata* in the absence of the HR. This indicates that the *Rds*-specified HR is not required for effective gene-for-gene resistance to the crown rust pathogen. The uncoupling of HR from gene-for-gene resistance is analogous to the HR-independent, extreme resistance conferred by potato *Rx* in response to PVX (Bendahmane et al. 1999) or to gene-for-gene resistance in the absence of HR exhibited by the *Arabidopsis dnd1* mutation (Clough et al. 2000; Yu et al. 1998). However, the absence of HR conditioned by the dominant *Rds* allele from *A. wiestii* (C.I. 1994) in *Pc:AvrPc* interactions is most likely not a *DND1*-like gene because the dominant *DND1* allele allows for the expression of HR, not the suppression of it (Clough et al. 2000). In this regard, *Rds* appears to act similarly to wild-type *LSD1* (lesions simulating disease resistance), a dominant allele in *Arabidopsis* that also suppresses the HR (Dangle et al. 1996; Greenberg et al. 1994).

Perhaps even more significantly, *Rih*-mediated HR was triggered in plants challenged with *P. coronata* isolate 290, whether they carried *Pc82*-specified resistance or not. This observation indicates that *Rih*-mediated interactions can be triggered independently of *Pc82*-mediated resistance. Thus, the *Rih* locus functions similarly to *NDRI*, a locus in *Arabidopsis* that triggers an HR in response to *P. syringae* pathogens both with and without the appropriate gene-for-gene-mediated interaction (Century et al. 1995). It is becoming increasingly clear that diverse pathways are utilized to regulate defense responses among plants and their pathogens (Bendahmane et al. 1999; Century et al. 1995; Cole et al. 2001; Hajimorad and Hill 2001; Yu et al. 1998, 2000). Cell death is usually initiated downstream of gene-for-gene recognition (Bent 1996; Hammond-Kosak and Jones 1996). Although there are no previously known resistance signaling genes in the diploid *Avena* genus, genes that inhibit the expression of crown rust resistance have been found in hexaploid oats (Simons 1987). In addition, similar suppressors of leaf rust and stem rust resistance have been identified on D-genome chromosomes of diploid (*Triticum tauschii*) and hexaploid (*T. aestivum* L.) wheats in normally rust-resistant cultivars (Bai and Knott 1992; Kerber and Green 1980). Often, these suppressors are uncovered via interspecific crosses (Hanusová et al. 1995; Kema et al. 1995; Ma et al. 1995). For example, a dominant suppressor in wheat inhibits the phenotypic expression of the *Pm8* powdery mildew resistance gene, which was translocated into wheat on 1RS from rye (Hanusová

Table 5. Hypersensitive cell death in the A8073 $\times$ C.I. 1994 BC₁F₂ population in response to isolate 290^a

Parental line		F ₁ hybrid							χ^2 test	
A8073	C.I. 1994	A8073 $\times$ C.I. 1994		BC ₁ F ₂ population ^b					χ^2 value (9:3:3:1)	P value
Resistant, HR	Susceptible, non-HR	Resistant, HR	Resistant, HR 215	Resistant, non-HR 10	Susceptible, HR 6	Susceptible, non-HR 84			306.30	<0.001

^a Four seedlings were evaluated of each parental line. Infection type of 0, 1, or 2 is considered resistant and of 3 or 4 is considered susceptible. HR indicates hypersensitive response and indicates presence of macroscopically visible, hypersensitive cell death; non-HR indicates the absence of macroscopically visible hypersensitive cell death.

^b Number of plants with each phenotype.

et al. 1995). These gene-specific suppressors most likely represent additional host signaling factors required for the ultimate expression of the resistance phenotype, similar to the *Rds* locus discovered in progeny of the *A. strigosa* × *A. wiestii* interspecific cross described here. However, *Rih* may represent a yet-undiscovered class of genes specifying hypersensitive cell death in cereal crops.

MATERIALS AND METHODS

Mapping populations.

The diploid *Avena* population was generated by crossing *A. strigosa* (C.I. 3815) with *A. wiestii* (C.I. 1994) (Rayapati et al. 1994). *A. strigosa* is resistant to 40 isolates of *P. coronata* in the Iowa State University (ISU) collection, whereas *A. wiestii* is susceptible to the same isolates (Wise and Gobelmann-Werner 1993). One hundred F_{8,9} lines were derived via single-seed descent from an F_{6,8} RI population provided by M. Lee (Iowa State University).

BC₁F₂ populations.

To differentiate genetic factors that control multiple specificities in the *Pca* locus, seven RI lines (A8028, A8060, A8073, A8076, A8085, A8096, and A8098) that contained putative recombination breakpoints within the *Pca* cluster were backcrossed to the susceptible parent C.I. 1994 and selfed to

generate BC₁F₂ populations. To study inheritance of HR induced by crown rust isolate 276, RI A8059 (resistant, non-HR) was backcrossed to the resistant HR parent C.I. 3815. To study the inheritance of HR induced by crown rust isolate 290, a BC₁F₂ population was generated from crossing RI A8073 (resistant, HR) to C.I. 1994 (susceptible, non-HR) and F₁ hybrids were selfed to generate large BC₁F₂ populations.

Rust inoculations.

The PC64, 290, 276, 263, PC62, and 202 *P. coronata* isolates from the ISU collection were used to inoculate the RI, BC₁F₁ hybrids, and BC₁F₂ populations. *P. coronata* inoculum was used at 1.2 × 10⁷ urediniospores per ml and incubation was carried out under our standard conditions (Bush et al. 1994). Disease development was assessed at 7 days after inoculation on a 0-to-4 scale using the method of Murphy (1935). IT of 0, 1, or 2 is considered resistant and of 3 or 4 is considered susceptible. At least 16 plants were used for each BC₁F₁ family test to ensure a 99% probability of observing a susceptible plant if a single dominant gene controls the specificity (Mather 1951).

Microscopy.

In order to observe the response of host tissues to challenge with *P. coronata*, leaf samples were collected 66 h after inoculation and prepared for fluorescence microscopy as described by Rohringer and colleagues (1977). Briefly, the leaf samples were fixed and cleared in boiling lactophenol/ethanol (1:2, vol/vol), rinsed sequentially in 50% ethanol, 0.05 N NaOH, water, and 0.1 M Tris/HCl buffer, pH 8.5. Tissues then were stained for 5 min in 0.1% Cellulofluor (Polysciences, Warrington, PA, U.S.A.), washed in water, and mounted in 25% aqueous glycerol. Samples were observed with a Zeiss inverted epifluorescence microscope (Carl Zeiss, Thornwood, NY, U.S.A.) equipped with a multiband filter set (XF67; Omega

Fig. 3. Map position of *Rds* and *Rih* on linkage groups A and B of the diploid *Avena* genus. Data for *Rds* was compiled into the C.I. 3815 × C.I. 1994 recombinant inbred Map Manager dataset (Yu and Wise 2000) to calculate linkage. Linkage for *Rih* was calculated based on the maximum likelihood method for estimating linkage from the progeny of selfed heterozygotes (Fisher and Balmukand 1928).

Fig. 4. Model for *Rds*- and *Rih*-mediated hypersensitive cell death in response to *Puccinia coronata* f. sp. *avenae*. Upon infection with *P. coronata* urediniospores, expression of *Rds* negatively regulates the level of hypersensitive response (HR) in conjunction with *Pc82*-mediated resistance to *P. coronata* isolate 276. *Rih*-mediated HR occurs in plants challenged with *P. coronata* isolate 290, whether they carried a *Pc82* resistance allele or not. This indicates that *Rih*-mediated interactions can be triggered independently of *Pc82*-mediated resistance.

Optical, Brattleboro, VT, U.S.A.) providing excitation peaks at 390, 486, and 577 nm; dichroic beamsplitters at 400, 485, and 580 nm; and emission maxima at 457, 528, and 633 nm. When prepared as described, necrotic host cells fluoresced bright orange-yellow, whereas *P. coronata* hyphae fluoresced bright blue, and unaffected plant cells fluoresced a faint violet.

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AUTHOR-RECOMMENDED INTERNET RESOURCE

Map Manager dataset: ars-genome.cornell.edu:80/cgi-bin/WebAce/webace?db=graingenes&class=Map_Data&object=Diploid%20Oat%20C%20AFLP&display=text