Interaction of Genes Controlling Avirulence/Virulence of *Magnaporthe grisea* on Rice Cultivar Katy

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ABSTRACT

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Lines of *Magnaporthe grisea* have been developed that are sexually competent and that differ in their avirulence/virulence on one rice cultivar. Two sibling isolates, 70-14 and 70-6, were each virulent on 10 rice cultivars. On rice cultivar Katy, 70-14 was avirulent, and 70-6 was virulent. The 70-14 × 70-6 cross produced 26 avirulent progenies and 28 virulent progenies, which suggested segregation of two alleles at one locus. Intercrosses among the progenies as well as backcrosses and test crosses suggested two avirulence genes and two corresponding suppressors were interacting to control avirulence/virulence on Katy. These avirulence genes and suppressors were designated *P11* and *P12* and *S11* and *S12*, respectively. The proposed genotypes of all the isolates used in this study are given. In addition, *P12*, mating type *MAT1*, and *S12* were loosely linked.

Additional keywords: avirulence/virulence genes, cultivar specificity, linkage, rice blast.

Magnaporthe grisea (Hebert) Barr ((*Pyricularia grisea*) Sacc. (=*Pyricularia oryzae* Cavara)) is considered one of the most important pathogens of rice (*Oryza sativa* L.) (9). The haploid fungus is the casual organism of blast, a destructive disease in rice. The disease is controlled primarily by the development and use of disease-resistant cultivars. The extensive use of blast-resistant cultivars has led to the recovery of new strains of *M. grisea* that render the resistance ineffective (9). Little is known about the mechanism of emergence of the new virulent strains.

Since the discovery of the sexual stage of *M. grisea* (4), there has been considerable interest in genetic analysis of the ability of isolates to be avirulent or virulent on specific rice cultivars (2,3,5,10,11). However, most isolates obtained from rice were female sterile. Crossing and backcrossing an isolate from rice to a highly fertile isolate from weeping love grass (*Eragrostis curvula* (Schrad.) Ness) for several generations did not lead to the development of highly fertile isolates pathogenic on rice (10). Backcrossing did lead to the identification of three unlinked genes that controlled avirulence on three rice cultivars (11). The discovery of a highly fertile hermaphroditic isolate from rice, Guy 11, mating type *MAT1-2* (8), has aided the development of sexually fertile hermaphroditic isolates pathogenic on rice (1–3,5). By sib selection for fertility and pathogenicity to rice over many generations, populations of isolates have been developed that are pathogenic on rice, that are sexually fertile hermaphrodites, and that segregate for avirulence/virulence on a number of rice cultivars (1–3,5).

We have attempted to develop a series of isogenic lines in which the paired lines are both virulent on most rice cultivars but which differ in their avirulence/virulence on one cultivar (1). In this paper, we describe the results of crosses made between isolates

70-14 or 70-15 with 70-6. All three isolates are virulent on 10 cultivars of rice and are avirulent on cultivar IR36. Isolates 70-14 and 70-15 are avirulent and isolates 70-6 and Guy 11 are virulent on rice cultivar Katy. The infection types (IT) on Katy are either small flecks (IT 1–2, considered avirulent) or large lesions, commonly diamond shaped with a gray center (IT 3–4, considered virulent). The differences between infection types on Katy are clear, and isolates are easy to classify by their interaction with Katy.

Earlier research indicated that even though segregation for avirulence/virulence occurred on many rice cultivars in a given cross, there was a preferential recovery of parental gene combinations (3). Segregation for avirulence/virulence on certain cultivars was not observed in some crosses, but test crosses with the progenies showed that segregation had occurred for genes that control expression of avirulence/virulence on those cultivars (2). In a cross of an avirulent isolate with a virulent isolate, the segregation of progenies in a 1 avirulent:1 virulent ratio might suggest that the segregation is the result of two alleles at a single locus. A test of this hypothesis is includes intercrossing the progenies. In this paper, we report the results of crossing isolate 70-14 with isolate 70-6, in which cross the segregation ratio of 1 avirulent:1 virulent progenies was observed. Test crosses did not support this conclusion, however, and it is the identification of these genes and their patterns of interaction with each other that is the subject of this paper.

MATERIALS AND METHODS

Fungal strains. The *M. grisea* isolates used in this study were described previously (1); additional progenies were isolated from several crosses. Guy 11 and 66-10 have been described previously (2,3,8). Isolate names were derived from the cross and isolate numbers (i.e., 70-14 is the fourteenth progeny isolated from cross 70) (2). Isolates to be stored were grown on a complete medium (10) for 1 wk, were dried in a sterile hood, and were stored at −20 C (1,2). Isolates prepared in this manner have been stored for several years and remained viable. To begin a new culture, a small piece of dried mycelia in agar was transferred to a petri plate with complete medium.

Rice cultivars. Seeds of various rice cultivars were kindly supplied by M. A. Marchetti and M. S. Clung (USDA Rice Research Station, Beaumont, TX), S. Linscomb (Louisiana Rice Research Station, Crowley), K. Moldenhauer (Rice Research and Extension Center, Stuttgart, AR), and D. M. Brandon (Rice Experiment Station, Biggs, CA). The rice cultivars used in this study were Bluebelle (CI 9544), IR36 (PI 452175), Katy (PI 527707), L202 (PI 483097), Leah (CI 9979), Lebonnet (CI 9882), Lemont (PI 475833), M103 (PI 527566), M201 (CI 9980), Maybelle (PI 538248), Newbonnet (PI 474580), and S201 (CI 9974).

Crossing *M. grisea*. Crosses were made by placing two isolates about 5 cm apart (mycelia in agar blocks) on oatmeal agar (7) in a petri plate and incubating at 20–22 C with continuous light. Perithecia usually formed and matured in 2–3 wk. Individual perithecia were picked up, crushed between two needles, and dragged across the surface of complete medium agar (1). Individual asci were separated with a bent, sterile glass needle. The ascospores in the asci began to germinate within 6 h. Individual asci with germinated ascospores were transferred to an oatmeal-agar plate. A small colony with conidia usually developed from each ascus in 4–5 days. One conidium was isolated from each colony; thus, one progeny was obtained from each meiotic event (1–3,5). Isolates 70-6, 70-14, 76-3, and Guy 11 were used as testers for determinations of mating type.

Pathogenicity test. Isolates were grown on oatmeal agar for 7–10 days at 22–24 C. The plates were flooded with sterile water containing 0.025% Tween 20. The conidia were suspended in the solution by scraping the plate with a bent needle. The spore suspension was adjusted to approximately 10^5 conidia/ml and was atomized onto rice seedlings at the two- to three-leaf stage. Inoculated plants were covered in a plastic bag to maintain 100% humidity for 36 h and were transferred to a greenhouse held at 25–28 C. About 20–30 plants were inoculated for each isolate-cultivar in each test. The pathogenicity tests were repeated at least twice. The reactions of the plants were scored 6–8 days after inoculation. Four infection phenotypes were recognized: 1 = minute brown to black flecks and spots; 2 = 2- to 3-mm-long brown to black lesions; 3 = usually circular lesions with gray centers, variable in size and shape; and 4 = large, commonly diamond-shaped lesions with gray centers, commonly coalescing into long stripes with gray centers (2,3,5)

RESULTS

Inheritance of avirulence/virulence on 11 of 12 rice cultivars. All the progenies of crosses 70, 76, 77, four subsequent backcrossings to 70-6 and 70-14 (crosses 109 and 131–133), and two sib-crossings between progenies of cross 76 (crosses 102 and 106) were virulent on 10 of the 12 cultivars, showing that all the progenies are lacking genes that give avirulence to the resistance genes in 10 cultivars. All of the progenies from these nine crosses were also avirulent on cultivar IR36.

Analyses of avirulence/virulence on Katy. The only rice cultivar for which there was segregation for avirulence/virulence in crosses 70, 76, 77, and many of the subsequent test crosses and backcrosses was Katy (Fig. 1). All isolates classified as virulent produced large diamond-shaped, water-soaked lesions (ITs 3–4); avirulent isolates produced small brown to black flecks (ITs 1–2) on Katy. The differences in infection types of isolates classified as virulent or avirulent were clear and unequivocal.

An analysis of the segregation of avirulence/virulence on cultivar Katy is given in Figure 1. The 66-10 × Guy 11 cross (cross 70) produced eight progenies that were avirulent and seven progenies that were virulent on Katy. Progeny 70-14, an avirulent isolate, was crossed with its virulent sibling, isolate 70-6, (cross 76), and 54 progenies were obtained: 26 avirulent and 28 virulent on Katy. The 1 avirulent:1 virulent segregation ratio suggested that isolates 70-14 and 70-6 differed by two alleles at one locus. A series of intercrosses and test crosses between progenies of cross 76 were carried out to test the one locus, two alleles hypothesis (Fig. 1). Twenty crosses between avirulent progenies from cross 76 were made with the expectation that only avirulent progenies would be produced because the crosses were homozygous for the avirulence allele. Nineteen crosses produced only avirulent progenies. Cross 151 (76-25 × 76-39) produced 26 avirulent progenies and eight virulent progenies on Katy. The recovery of virulent progenies in a cross of two avirulent isolates shows that the parents had different genes that condition avirulence to Katy. The ratio of 26 avirulent:8 virulent progenies is approximately 3:1, as expected in the segregation of two independent avirulence genes in a haploid pathogen.

Thirty-two crosses were made between an avirulent *MAT1-1* isolate and a virulent *MAT1-2* isolate (Fig. 1). Three crosses were made between a virulent *MAT1-1* isolate and an avirulent *MAT1-2* sibling. All crosses between a virulent and an avirulent isolate showed segregation for avirulence/virulence on Katy. Several crosses in Figure 1 showed segregation that was consistant with 5:3, 1:1, 7:9, and 1 avirulent:3 virulent ratios. Fourteen crosses were made between two virulent isolates, and all the crosses produced only virulent progenies.

The observed segregation ratios are given in Figure 1, and the postulated genotypes and expected ratios are given in Figure 2. The cross number, parental isolates crossed, infection types of parents, segregation among progenies, and chi-square values for fitness to different ratios are given in Table 1. Cross 109 (76-3 × 70-6) gave a ratio of 16 avirulent:39 virulent progenies. Crosses 102 (76-3 × 76-13), 205 (76-3 × 76-15), and 153 (76-25 × 76-15) also produced segregations that approximated 1 avirulent:3 virulent ratios. A 1 avirulent:3 virulent ratio suggests the segregation of an avirulence gene and a suppressor of the avirulence allele (2).

If the results of cross 151 (76-25 × 76-39) suggest that two genes control avirulence (a 3 avirulent:1 virulent ratio), and the results of crosses 102 (76-3 × 76-13), 109 (76-3 × 70-6), 153 (76-

MAT1-2	IT	MAT1-1 66-10	70-14	70-15	76-3	76-9	76-14	76-19	76-25	76-30	76-35	76-37	76-7	76-26
		1	1	1	1	0-1	1+-2	1	1	1	1+-2	1	3-4+	3+-4
76-2	1				6:0									
76-4	1				22:0			12:0		12:0	7:0			
76-6	1												17:19	
76-17	1				15:0	12:0	15:0	21:0	12:0	32:0	5:0			6:3
76-39	1-2+				35:0	12:0	12:0	34:0	26:8	12:0	34:0	16:0		21:9
Guy11	4	8:7	25:23		27:28				15:22					
70-6	3-4+		26:28	29:28	16:39	18:16			32:25					0:12
76-8	3+-4		17:28										0:17	
76-10	4		32:45										0:12	
76-11	3+-4		11:9									18:11	0:35	
76-12	4		13:11						8:13				0:25	
76-13	3+-4		35:19		12:34								0:17	
76-15	3+-4		10:11		12:29				10:19			14:22	0:18	0:9
76-22	4		5:5		18:19								0:20	
76-23	4		13:15						8:14			3:2	0:14	0:13
76-28	3-4+													0:36
76-31	3-4+				15:20								0:19	
76-33	3+-4		34:14											
76-43	3+-4		16:18		16:19				10:10				0:20	
76-44	4											7:10		

Fig. 1. The parents, their infection types (IT), and the observed segregation ratios of avirulence:virulence on rice cultivar Katy among the progenies of 72 crosses. ITs 1–2 = avirulent. ITs 3–4 = virulent. A "+" indicates a predominant infection type.

Isolate # (MAT1-1) / Isolate# (MAT1-2)	Postulated genotypes	66-10 *P11 P12 s11 S12*	70-14 *P11 P12 S11 s12*	70-15 *P11 P12 S11 s12*	76-3 *p11 P12 S11 s12*	76-9 *P11 P12 s11 s12*	76-14 *P12 s12*	76-19 *P12 s12*	76-25 *P11 P12 s11 S12*	76-30 *P12 s12*	76-35 *P12 s12*	76-37 *P11 P12 s11 s12*	76-7 *p11 p12 S11 S12*	76-26 *p11 p12 S11 S12*
76-2	*p11 P12 s12*				1:0									
76-4	*p11 P12 s12*				1:0			1:0		1:0	1:0			
76-6	*P11 P12 s11 s12*												7:9	
76-17	*P11 P12 s11 s12*				1:0	1:0	1:0	1:0	1:0	1:0	1:0			3:1
76-39	*P11 P12 S11 s12*				1:0	1:0	1:0	1:0	3:1	1:0	1:0	1:0		5:3
Guy11	*p11 p12 S11 s12*	7:9	1:1		1:1				7:9					
70-6	*p11 p12 s11 S12*		7:9	7:9	1:3	5:3			1:1					0:1
76-8	*p11 p12 s11 S12*		7:9										0:1	
76-10	*p11 p12 s11 S12*		7:9										0:1	
76-11	*p11 p12 S11 s12*		1:1									5:3	0:1	
76-12	*p11 p12 S11 s12*		1:1						7:9				0:1	
76-13	*P11 p12 S11 S12*		1:3**		1:3								0:1	
76-15	*p11 p12 S11 S12*		1:3*		1:3				1:3			7:9	0:1	0:1
76-22	*p11 P12 s11 S12*		5:3		1:1								0:1	
76-23	*p11 p12 S11 s12*		1:1						7:9			5:3	0:1	0:1
76-28	*p11 p12*													0:1
76-31	*p11 p12 s11 s12*				1:1								0:1	
76-33	*p11 p12 s11 s12*		5:3											
76-43	*p11 p12 S11 s12*		1:1		1:1				7:9				0:1	
76-44	*p11 p12 s11 S12*											5:3		

Fig. 2. The parents, their postulated genotypes, and the expected segregation ratios of avirulence:virulence on rice cultivar Katy among the progenies of 72 crosses. * and ** signify that the observed ratio deviates significantly from the expected, at $P \leq 0.05$ and 0.01, respectively.

25 × 76-15), and 205 (76-3 × 76-15) with segregation ratios of 1 avirulent:3 virulent suggest the segregation of a suppressor, then at least three genes must be segregating in cross 76. The segregation of two avirulence genes and a suppressor of one of the avirulence genes would be expected to give a 5 avirulent:3 virulent segregation ratio. Several crosses, 154 (76-37 × 76-11), 192 (76-26 × 76-39), and 213 (70-14 × 76-33), showed segregations that approximated 5 avirulent:3 virulent ratios. Cross 76 (70-14 × 70-6) gave a ratio (26 avirulent:28 virulent) that was consistent with the expected results of two avirulence genes and two suppressors segregating, i.e., a 7 avirulent:9 virulent ratio. The observed 7 avirulent:9 virulent segregation ratios of crosses 133 (76-8 × 70-14), 131 (76-10 × 70-14), 215 (Guy 11 × 76-25), 140 (76-12 × 76-25), 158 (76-23 × 76-25), and 160 (76-15 × 76-37) support the argument that two avirulence genes and two suppressors were segregating, as in cross 76.

From the results of the intercrosses made among the progenies of cross 76 and of some backcrosses made to each parent (Fig. 1), the postulated genotype for each isolate was deduced based on the segregation observed in all crosses with each isolate. Consideration was given to whether segregation occurred and, if so, to the segregation ratio observed. The postulated genotypes and the expected segregation ratios are given in Figure 2.

Analyses of the isolates with suppressed avirulence gene(s). The crosses involving virulent isolates 76-7 and 76-26, both *MAT1-1*, with their virulent *MAT1-2* siblings produced only virulent progenies (Fig. 1). To explain the data of the crosses between the virulent isolates of *MAT1-2* with their avirulent *MAT1-1* siblings, it was necessary to postulate that several of the virulent *MAT1-2* isolates contained avirulence genes and suppressors of these avirulence genes (Fig. 2). The hypothesis that some virulent isolates contained one or more avirulence gene(s) plus the gene(s) that suppressed expression of the avirulence gene(s) was tested by crossing virulent progenies of four crosses with isolate Guy 11 and/or 70-6.

Cross 109 (76-3 × 70-6; Fig. 2) was postulated to be segregating for *P12* and *S12*. The cross is homozygous for *p11*, and heterozygous for *S11* and *S12*. (A lowercase *p* or *s* indicates a recessive allele). No detection of the segregation of *S11* was expected because both parents contained *p11* (Fig. 2). Segregation of *P12* and *S12* should produce four types of progenies: *P12 s12, P12 S12, p12 s12,* and *p12 S12*. Only progenies with *P12 s12* were expected to be avirulent. One of the three types of virulent progenies was expected to contain an avirulence gene and a suppressor (*P12* and *S12*). Two to six virulent progenies from each of the four crosses were tested for the presence of an avirulence gene and a suppressor (Table 2).

Six virulent *MAT1-1* progenies from cross 109 (76-3 × 70-6) were crossed with Guy 11 (Table 2). In three of the six crosses (251,257, and 253), all progenies were virulent. The remaining three crosses (252, 258, and 259), however, segregated 1 avirulent:3 virulent (Table 2). The recovery of avirulent progenies from these three crosses supports the hypothesis that isolates 109-20, 109-24, and 109-49 contain both *P12* and *S12*. Four virulent *MAT1-1* progenies of cross 205 (76-3 × 76-15) were also crossed with Guy 11 (Table 2). Three of the crosses (261,254, and 262) produced

TABLE 1. Cross number, parent isolates crossed, infection type (IT) of each parent isolate, observed ratios of avirulent:virulent progenies, and chi-square values for the expected ratio for each of 36 crosses in which segregation for avirulence/virulence occurred on rice cultivar Katy

Cross number	Parents		Infection type of parents[a,b]		Observed ratio	Chi-square for expected ratio				
	1	2	1	2	Avir:vir	3:1	5:3	1:1	7:9	1:3[c]
151	76-55	76-39	1	1–2$^+$	26:8	.04	...	...	...	...
70	66-10	Guy 11	1	4	8:7	...	...	...	0.6	...
216	70-14	Guy 11	1	4	25:23	...	...	0.1	...	...
76	70-14	70-6	1	3–4$^+$	26:28	...	...	...	0.4	...
133	70-14	76-8	1	3$^+$–4	17:28	...	...	...	0.7	...
131	70-14	76-10	1	4	32:45	...	...	...	0.2	...
136	70-14	76-11	1	3$^+$–4	11:9	...	...	0.2	...	...
138	70-14	76-12	1	4	13:11	...	...	0.2	...	...
132	70-14	76-13	1	3$^+$–4	35:19	3.7	.02	3.8	8.2	58**
137	70-14	76-15	1	3$^+$–4	10:11	...	...	.04	0.1	6.3*
134	70-14	76-22	1	4	5:5	...	0.7	...	...	...
212	70-14	76-23	1	4	13:15	...	...	0.1	...	...
213	70-14	76-33	1	3–4$^+$	34:14	...	1.4	...	...	...
214	70-14	76-43	1	3$^+$–4	16:18	...	...	0.1	...	...
77	70-15	70-6	1	3–4$^+$	29:28	...	...	...	1.2	...
167	76-3	Guy 11	1	4	27:28	...	...	1.1	...	...
109	76-3	70-6	1	3–4$^+$	16:39	...	...	...	...	0.5
102	76-3	76-13	1	3$^+$–4	12:34	...	...	...	...	.03
205	76-3	76-15	1	3$^+$–4	12:29	...	...	...	...	0.4
206	76-3	76-22	1	4	18:19	...	...	.03	...	...
207	76-3	76-31	1	3–4$^+$	15:20	...	...	0.7	...	...
208	76-3	76-43	1	3$^+$–4	16:19	...	...	0.3	...	...
219	76-9	70-6	0–1	3–4$^+$	18:16	...	1.3	...	...	...
215	76-25	Guy 11	1	4	15:22	...	...	...	0.2	...
163	76-25	70-6	1	3–4$^+$	32:25	...	...	0.9	...	...
140	76-25	76-12	1	4	8:13	...	...	...	0.3	...
153	76-25	76-15	1	3$^+$–4	10:19	...	...	...	...	1.4
158	76-25	76-23	1	4	8:14	...	...	...	0.5	...
159	76-25	76-43	1	3$^+$–4	10:10	...	...	...	0.3	...
154	76-37	76-11	1	3$^+$–4	18:11	...	.002	...	...	...
160	76-37	76-15	1	3$^+$–4	14:22	...	...	...	0.3	...
162	76-37	76-23	1	4	3:2	...	0.5	...	...	...
155	76-37	76-44	1	4	7:10	...	...	...	.05	...
106	76-6	76-7	1	3–4$^+$	17:19	...	...	...	.02	...
217	76-17	76-26	1	3$^+$–4	6:3	0.3	...	...	...	...
192	76-39	76-26	1	3$^+$–4	21:9	...	0.7	...	...	...

[a]ITs 1–2 = avirulent (avir); ITs 3–4 = virulent (vir).
[b]"+" indicates the predominant infection type.
[c]* = $P < 0.05$; ** = $P < 0.01$.

only virulent progenies, and one of the crosses (255) produced a 10 avirulent:14 virulent segregation ratio. Cross 206 (76-3 × 76-22) produced an 18 avirulent:19 virulent segregation ratio (Fig. 1). Two of the virulent progenies from cross 206 were crossed with Guy 11 (Table 2). One of the crosses (256) produced only virulent progenies, and the other (260) produced a 1 avirulent:3 virulent segregation ratio. Five virulent progenies from cross 102 (76-3 × 76-13) were crossed with Guy 11 and with 70-6 (Table 2). Three of the virulent progenies of cross 102 (102-10, 102-30, and 102-34) produced only virulent progenies when crossed with either Guy 11 or 70-6. One of the virulent progenies from cross 102, 102-15, produced 18 avirulent:28 virulent progenies when crossed with Guy 11 and 6 avirulent:18 virulent progenies when crossed with 70-6. The fifth virulent progeny, 102-47, produced a 5 avirulent:6 virulent progeny ratio when crossed with Guy 11 but produced only virulent progenies when crossed with 70-6.

One progeny, 102-15, is of particular interest. The ability to recover avirulent progenies from cross 242 (102-15 × 70-6) and from cross 247 (102-15 × Guy 11) suggests that 102-15 is not only carrying *P12* and *S12* but also *P11* and *S11*. In cross 247, segregation at both *P12* and *S12* loci must have occurred because both 102-15 and Guy 11 contained *S11*. In contrast, segregation at both *P11* and *S11* must have occurred in cross 242 because both 70-6 and 102-15 contained *S12*. These two crosses also allow us to trace the origin of *P11* in cross 102 (76-3 × 76-13) to 76-13 because 76-3 contained only *P12*.

The postulated genotypes of 70-6 and Guy 11 given in Figure 2 and Table 2 were derived from the series of crosses given in Figure 1. The postulated genotype of progenies from crosses 102, 109, 205, and 206 were deduced from the crosses with Guy 11 and 70-6, assuming that the postulated genotypes of Guy 11 and 70-6 are correct.

Linkage analyses of *P12* and *S12* with *MAT1*. The segregation of mating type with avirulence/virulence on Katy is given in seven crosses in Table 3. Crosses 167, 207, 208, 102, 205, and 206 are either homozygous for *p11* or *S11* or both and therefore, should show no segregation for avirulence because of the *P11* locus. Crosses 167, 207, and 208 are heterozygous for *P12* and homozygous for *s12*. The analyses of these three crosses suggest that the *MAT* locus is 16 crossover units from the *P12* locus. Cross 206 is homozygous for *P12* but segregates for *S12/s12*. The data from this cross suggest that *S12* is five crossover units from the *MAT* locus. Crosses 102 and 205 are heterozygous for both *P12/p12* and *S12/s12* in trans. The data suggest that *P12* and *S12* are 40 crossover units apart. The most likely sequence on the chromosome is *P12-MAT1-S12*. This was determined because several crosses that are heterozygous for *P12* and *S12* segregated with a close fit with a 1:3 avirulent/virulent ratio (e.g., crosses 102 and 205; Table 3 and Fig. 2). If *P12* and *S12* were closer to each other, we would expect a deviation from 1 avirulent:3 virulent segregation ratios resulting from a decrease in crossing over between *P12* and *S12*, and more avirulent progenies would have been recovered in crosses 102 and 205. On the other hand, if *P12* and *S12* were farther apart, crossing over would have occurred within a frequency approaching 50%, as we observed in crosses 102 and 205 (Table 2).

DISCUSSION

The segregation ratio of 26 avirulent:28 virulent progenies in cross 76 suggested that 70-14 and 70-6 differed by two alleles at one locus. Intercrosses among their progenies and backcrosses of the progenies to 70-14, 70-6, and Guy 11 showed that segregation was occurring at more than one locus. The postulated segregation at four loci permitted an explanation of all 90 crosses (Figs. 1 and 2; Table 3), both qualitatively (in terms of which crosses showed segregation for avirulence/virulence) and quantitatively (the expected ratios were in agreement with the observed ratios), and was internally consistent, except for crosses

TABLE 2. Segregation of avirulence/virulence on rice cultivar Katy of crosses of Guy 11 and/or 70-6 with virulent, *MAT1-1* progenies from crosses 109, 205, 206, and 102

			MAT1-2 parents						
			Guy 11 (IT 4) *p11 p12 S11 s12*[a]			70-6 (IT 3–4$^+$) *p11 p12 s11 S12*[a]			
			Ratios			Ratios			
Cross number	*MAT1-1* Parent 1	Infection type[b]	Observed Avir:vir	Expected[c] Avir:vir	Chi-square 1:3	Observed Avir:vir	Expected Avir:vir	Chi-square 1:3	Postulated genotypes
251	109-6	3–4$^+$	0:28	0:1	...	...	...	...	*p11 p12* __ __[d]
257	109-17	4	0:15	0:1	...	...	...	...	*p11 p12* __ __
252	109-20	4	8:27	1:3	0.08	...	...	...	*p11* ***P12*** __ ***S12***
253	109-22	3$^+$–4	0:15	0:1	...	...	...	...	*p11 p12* __ __
258	109-24	3$^+$–4	4:11	1:3	0.02	...	...	...	*p11* ***P12*** __ ***S12***
259	109-49	4	5:12	1:3	0.18	...	...	...	*p11* ***P12*** __ ***S12***
261	205-3	3$^+$–4	0:15	0:1	...	...	...	...	*p11 p12 S11* __
254	205-6	4	0:15	0:1	...	...	...	...	*p11 p12 S11* __
255	205-15	4	10:14	1:3	3.66	...	...	...	*p11* ***P12 S11 S12***
262	205-33	4	0:15	0:1	...	...	...	...	*p11 p12 S11* __
260	206-8	3–4$^+$	8:14	1:3	1.51	...	...	...	*p11* ***P12*** __ ***S12***
256	206-12	4	0:15	0:1	...	...	...	...	*p11 p12* __ __
241	102-10	4	...	...	...	0:15	0:1	...	*p11 p12 S11* __
246	102-10	4	0:20	0:1	...	...	...	...	*p11 p12 S11* __
242	102-15	3–4$^+$	...	...	...	6:18	1:3	0	***P11 P12 S11 S12***
247	102-15	3–4$^+$	18:28	1:3	4.89[e]	...	...	...	***P11 P12 S11 S12***
243	102-30	4	...	...	...	0:15	0:1	...	*p11 p12 S11* __
248	102-30	4	0:20	0:1	...	...	...	...	*p11 p12 S11* __
244	102-34	4	...	...	...	0:15	0:1	...	*p11 p12 S11* __
249	102-34	4	0:20	0:1	...	...	...	...	*p11 p12 S11* __
245	102-47	4	...	...	...	0:26	0:1	...	*p11* ***P12 S11 S12***
250	102-47	4	5:6	1:3	2.45	...	...	...	*p11* ***P12 S11 S12***

[a] Postulated genotypes.
[b] Infection types (ITs) 3–4 = virulent (vir). "+" indicates the predominant infection type.
[c] Based on the minimum number of genes required to explain the data.
[d] __ = allele unknown.
[e] $P < .005$.

132 and 137, in which the ratios were significantly different than expected. Cross 132 should theoretically segregate at a 1 avirulent:3 virulent ratio instead of at the observed 35 avirulent:19 virulent (5:3) ratio (Fig. 2; Table 1). One possible explanation for this discrepency could be the preferential recovery of the progenies that were avirulent on Katy. The reasons behind the differences between observed and expected ratios are now under investigation.

The data fit the postulated two avirulence genes, *P11* and *P12* and the two suppressor genes, *S11* and *S12*. *S11* and *S12* suppress the expression of *P11* and *P12*, respectively, but have no phenotype in the presence of *p11* and *p12*, respectively. Suppressors had been postulated previously to explain the origin of avirulent progenies in a cross of two virulent isolates (2,6). The data presented here, gathered from many test crosses, show that the only explanation consistent with all crosses is that there is one suppressor for each of the two avirulence genes. One suppressor of each avirulence allele has been found for each of the avirulence genes identified to date ([2]; A. H. Ellingboe, *unpublished data*).

The suppression we observed appeared to be complete. Isolates with suppressed avirulence genes produced full virulence (ITs 3–4) on Katy, in contrast to the reaction in flax rust, in which intermediate infection types were attributed to incomplete suppression of avirulence genes by suppressors in *Melampsora lini* (6).

Finding a suppressor for each avirulence gene raises a question concerning the interpretations of crosses between virulent and avirulent isolates. Segregation may be occurring at the locus that determines the specificity of the interaction, or it may occur at the locus that controls the expression of the gene that determines the host specificity. Crosses 167, 207, 208, and 206 all showed segregation ratios of 1 avirulent:1 virulent progenies. The segregation in the first three crosses was at the *P12* locus. Cross 206 was homozygous for *P12* but was heterozygous for *S12*. The segregation, therefore, was at the *S12* locus not at the *P12* locus. Several crosses were segregating for avirulence genes but no segregation of avirulence/virulence was observed because they were homozygous for the corresponding suppressors (Fig. 2; crosses 76-7 × 76-13 and 76-7 × 76-22). It is interesting to note that the cross of two avirulent isolates (76-25 × 76-39) that produced virulent progenies was homozgyous for *P11* and *P12* but was segregating for *S11* and *S12*. The segregation of two suppressors in a cross homozgyous for *P11* and *P12* gave the same segregation ratios expected for a cross (76-26 × 76-17) segregating for *P11* and *P12* but homozygous for *s11* and *s12*. The observation of segregation for avirulence/virulence in progenies of a cross is not adequate to conclude the segregation of avirulence genes.

The implications of the distinction between avirulence genes and suppressors of avirulence genes for molecular approaches to the study of avirulence/virulence on specific cultivars is obvious. One reason for the developent of isogenic lines is to assist in the cloning and analyses of genes that control cultivar specificity. The *P12* gene that gave 70-14 its avirulence on Katy would not have been expressed when transformed into 70-6 because 70-6 had a suppressor, *S12*, of *P12*. Isolate 70-14 contained avirulence gene *P11* but avirulence resulting from *P11* was not expressed because *S11* also was present in 70-14. Isolate 70-6 contained *p11* and *s11*. If a clone from 70-14 containing *P11* was transformed into 70-6, it would be expected to be expressed and convert 70-6 to avirulence on cultivar Katy. Although one might assume that the gene that gave 70-14 avirulence on Katy was the same gene that when transformed into 70-6 converted 70-6 into avirulence on Katy, these studies show that the avirulence gene *P12* would not be the gene that transformed 70-6 from virulence to avirulence on Katy. On the other hand, a clone of *S12* from 70-6 should be capable of transforming 70-14 from avirulence to virulence by suppressing *P12*. *S12* would then appear to be a gene for virulence.

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TABLE 3. Linkage of *P12*, *MAT1*, and *S12* in relation to segregation of avirulence/virulence on rice cultivar Katy in crosses 167, 207, 208, 206, 102, and 205

Cross number	Parents	Infection type[a]	Mating type	Observed ratio: Progenies *MAT1-1* Avir:vir	Progenies *MAT1-2* Avir:vir	*MAT1-1*:*MAT1-2*	Avir:vir
167	76-3	1	*MAT1-1*	24:7	0:19	31:19	24:26
	Guy 11	4	*MAT1-2*				
207	76-3	1	*MAT1-1*	10:6	5:12	16:17	15:18
	76-31	4	*MAT1-2*				
208	76-3	1	*MAT1-1*	15:1	0:19	16:19	15:20
	76-43	3^+–4	*MAT1-2*				
				49:14[b]	5:50[b]	63:55[b]	54:64[b]
				Total: *MAT1-P12* = 19/118 = 16% crossing over.			
206	76-3	1	*MAT1-1*	18:2	0:17	20:17	18:19
	76-22	4	*MAT1-2*				
				Total: *MAT1-S12* = 2/37 = 5% crossing over.			
102	76-3	1	*MAT1-1*	13:7	0:23	20:23	13:20
	76-13	3^+–4	*MAT1-2*				
205	76-3	1	*MAT1-1*	10:4	2:25	14:27	12:29
	76-15	4	*MAT1-2*				
				23:11[b]	2:48[b]	34:50[b]	25:59[b]
				Total: *P12-S12* = 40% crossing over.			

[a]Infection types 1–2 = avirulent (avir) and infection types 3–4 = virulent (vir). A "+" indicates a predominant phenotype.
[b]Sum of the crosses.

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