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Mapping of a *Magnaporthe grisea* locus affecting rice (*Oryza sativa*) cultivar specificity

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Abstract *Magnaporthe grisea* causes rice blast, the most important fungal disease of rice. The segregation of genes controlling virulence of *M. grisea* on rice was studied to establish the genetic basis of cultivar specificity in this host-parasite interaction. Full-sib progeny and parent isolates Guy11 and 2539 of *M. grisea* were inoculated onto rice (*Oryza sativa*) cultivar 'CO39' and five near-isogenic lines (NILs) of CO39. Each NIL contained a different single gene affecting resistance to specific isolates of *M. grisea*. No differential interactions between NILs and progeny or parents were observed; parents and progeny pathogenic on CO39 were pathogenic on all five NILs. Segregation ratios of 101 full-sib progeny, 117 progeny from full-sib parents, and 109 backcross progeny, indicated a common single gene affecting pathogenicity on CO39 and the five NILs. A subset of the above 327 isolates (43 full-sib progeny, 37 progeny from full-sib parents, and 32 backcross progeny) were inoculated onto rice cultivar '51583'; all were pathogenic, indicating that cultivar specificity to CO39 was segregating in this population of isolates. The locus controlling cultivar specificity, named *avrCO39*, was mapped to chromosome 1 using a subset of the progeny previously used to construct an RFLP map of *M. grisea*. The closest reported RFLP markers were 11.8 (estimated 260 kb) and 17.2 cM (estimated 380 kb) away and provide starting points on either side of the locus for a "chromosome walk" to clone the locus.

Key words Rice blast · *Magnaporthe grisea* Pathogenicity · Genetic map · Avirulence gene

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Introduction

Rice is a major staple food for approximately two-thirds of the world's population (Vaughn and Sitch 1991). More than 90% of the world's rice is both grown and consumed in developing countries. One of the most devastating diseases of rice is blast caused by *Magnaporthe grisea*. This disease occurs worldwide and yield-losses of 10% are common in blast-infested fields. In the rice-growing areas of the southern United States, growers frequently experience blast-related problems. For example, Texas suffered its worst blast epidemic in 20 years in 1991 (N.G. Whitney, personal communication) while, in Arkansas, a 30% yield loss due to blast occurred in 1987 (C. Rush, personal communication).

Rice blast disease is controlled primarily by the use of resistant cultivars. Complete resistance may be conferred by one or more major genes, whereas partial resistance is controlled by a combination of minor genes (Bonman et al. 1992). *M. grisea* populations are notorious for their ability to rapidly overcome blast-resistant cultivars (Ou 1985). The molecular basis for this ability is only beginning to be understood (Valent and Chumley, In Press).

We have studied the mode of inheritance of cultivar specificity in *M. grisea* on five near-isogenic lines (NILs) carrying different, specific resistance genes and the common recurrent parent CO39 (Mackill and Bonman 1992). The purpose of using the NILs was to examine the possibility of gene-for-gene relationships by studying the interaction between a population of blast isolates and specific resistance genes, each in a common genetic background. Having different resistance genes in a common genetic background minimizes differences in the epistatic interaction between host genotypes, and emphasizes the major single-gene differences between them. We report here data indicating single-gene inheritance for cultivar specificity on CO39. We also report the mapping of this gene to chromosome 1 of the pathogen.

Materials and methods

Rice cultivars

Seed of CO39 and five NILs of CO39, each theoretically differing from the others by only a single gene for blast resistance (Mackill and Bonman 1992), were provided by J. M. Bonman (International Rice Research Institute, Los Banos, Philippines). Each NIL was developed separately by backcrossing a single gene for blast resistance into the recurrent parent, CO39. The five morphologically similar NILs are: 'C101LAC', (*Pi-1*) 'C101A51' (*Pi-2*), 'C104PKT' (*Pi-3*), 'C101PKT' (*Pi-4a*), and 'C105TTP-4' (*Pi-4b*). CO39 is an *indica* rice cultivar that is highly susceptible to most tropical blast isolates.

Seed of 51583 and 'Sha-tiao-tsao' (Leung et al. 1988) was provided by H. Leung (previously at the International Rice Research Institute, Los Banos, Philippines) and increased by A. Marchetti (USDA-ARS, Beaumont, Tex.). Seed of 757 rice accessions was provided by H. E. Bockelman (National Small Grains Collection, USDA-ARS, Aberdeen, Idaho). Seed of cultivars 'M103', 'S201', and 'M201' came from D. M. Brandon (Rice Exp. St., Biggs, Calif.), and 'IR36', 'Leah', 'Dular', and 'Asahi' were provided by C. N. Bollich (USDA-ARS, Beaumont, Tex.).

M. grisea isolates

Isolate 'Guy 11' (Mat 1-2; hermaphrodite), collected from a diseased rice plant in French Guyana,was provided by J.L. Notteghem (Institute de Recherches Agronomiques Tropicales, Montpellier Cedex, France). Isolate '2539' (Mat 1-1; hermaphrodite) was developed in the laboratory of Hei Leung by crossing rice and grass isolates of *M. grisea* and was isolated as a single conidium from cultivar 51583 (Leung et al. 1988). Isolate 2539 has in its pedigree fertile isolates from goosegrass, weeping lovegrass, finger millet, two Japanese rice isolates, two Chinese rice isolates, and two laboratory isolates not pathogenic on rice (Leung et al. 1988). Isolates 2539 and Guy 11 were mated to produce progeny termed "HL" during 1986–87 (Leung et al. 1988). These progeny and isolate 2539 were provided by H. Leung (Washington State Univ., Pullman, Wash.). Progeny termed "MF" were also derived from a cross of isolates 2539 and Guy 11 and were isolated by Mark Farman (University of Wisconsin, Madison) in 1992 to supplement the data from the initial set of "HL" progeny. These two sets of progeny are considered to be full-sib in their relationship. An additional 226 progeny were derived from four backcrosses and five crosses between sibs.

Media and storage of *M. grisea* isolates

Isolates were stored at −20 °C in 6-mm chromatography paper discs (Whatman) as described by Valent et al. (1991). Isolates were grown on oatmeal agar plates for the production of conidia and perithecia. 2YEG agar plates were used to check conidial germination. Oatmeal and 2YEG media were prepared as described (Valent et al. 1991). Water agar (4%) plates were used for the dissection of perithecia and the spreading of asci; 0.1×complete agar plates (0.1% sucrose, 0.06% casamino acids, 0.06% yeast extract, and 1.5% agar) were used to isolate a single germinated conidium from each single-ascus colony.

Mating and progeny production

Matings and progeny production were as described by Ellingboe (1992). The resulting single-conidium isolates, each derived from a single ascus, represent products from single meiotic events. This procedure is thus equivalent to using a single ascospore from each ascus.

Inoculation procedure and disease severity ratings

One-to-four rice seedlings were grown in plastic disposable pots (7.0 ×5.5×5.5 cm) placed in plastic flats (6.5×26×52.5 cm). The growth medium was *Bacto* potting soil (Michigan Peat Co., Houston, Tex.). Seedling growth and inoculation occurred in a growth chamber equipped with fluorescent lights (230 uE/m/sec) set for 16 h photoperiods. Day/night temperatures were 28 °C/21 °C, respectively, and percent relative humidity was 33%. Plants were inoculated at 14 to 18 days after planting (approximately when the third leaf was emerging).

Inoculum was prepared by growing isolates on oatmeal agar plates placed under fluorescent light (20–55 uE/m/sec) at 22 °C for 12 to 16 days. Conidia were detached from conidiophores by gently rubbing the agar surface with a bent glass rod after adding 5 ml of an 0.2% gelatin solution. The spore concentration was adjusted to 10^4/ml and approximately 10^4 conidia/plant were used for inoculations.

Inoculations were made by atomizing conidial suspensions onto rice seedlings in pots placed within each plastic bag. A minimum of 6 ml of conidial suspension was applied within each bag to maintain the high humidity necessary for conidial germination and penetration of the host cuticle. Each bag was sealed immediately following inoculation and placed inside a darkened (0.1–0.002 uE/m/sec) box (87.5×60.0×75.0 cm) within the growth chamber for 24 h. Pots were then removed and allowed to air-dry on racks, after which they were returned to their trays. A gelatin control and isolates Guy 11 and 2539 were used in each experiment as standards.

Seven days following inoculation, ratings for disease severity were taken on the youngest leaf that was expanded at the time of inoculation. Various disease severity rating scales are used by the different groups studying genetic interactions between rice and *M. grisea* (Latterell et al. 1963; Yu et al. 1987; Leung et al. 1988; Ellingboe et al. 1990; Valent et al. 1991; Silue et al. 1992). We based our judgements on the ability of affected tissue to support conidiation under high humidity conditions and thus complete the disease cycle (Valent et al. 1991). Isolates of *M. grisea* that produced lesions that would not sporulate under high humidity were considered non-pathogenic, or avirulent, whereas those that would were considered pathogenic, or virulent. As defined by Valent et al. (1991), virulence is used here to refer to pathogenic ability that is cultivar-specific. To describe the interaction phenotype, a similar numerical system to that of Yu et al. (1987) has been adopted in this paper: Type 0 – no visible symptoms; Type 1 – small dark brown, pin point-sized, nonsporulating lesions; Type 2 – dark brown, nonsporulating lesions 2–3 mm in length; Type 3 – circular, sporulating lesions with tan centers and dark brown margins; Type 4 – large diamond-shaped, sporulating lesions with tan centers and dark brown margins. *M. grisea* isolates giving rise to lesion types 0, 1, and 2 were considered avirulent while those producing types 3 and 4 were considered virulent. In cases of a single leaf having both 1 and 3 lesion-types, the *M. grisea* strain was considered to be virulent because the disease cycle was able to be completed on that leaf. Valent et al. (1991) have made similar observations and conclusions.

The rice genotypes and blast isolates employed, with the numbers of experimental replications and plants used, are given in Table 1. Isolate 2539 was also screened on 765 rice lines in addition to CO39, the five NILs, and 51583. Inoculations of isolate 2539 were repeated (a total of eight plants per line) for 61 of the additional lines.

The repeated inoculation of CO39 with Guy 11 and 2539 showed that specific isolate-cultivar reactions were consistent over time. However, as observed by Bonman et al. (1986), a small number of infection types differed between repeated experiments. These cultivars and isolates were retested to obtain consistent results.

Inoculated leaves were preserved by taping a piece of the leaf onto index cards using transparent tape. Cards were then stored at 22 °C in the dark (Valent et al. 1991).

Mapping

Linkage analysis of cultivar specificity on CO39 was performed as described by Skinner et al. (1993). Sixty-one progeny were used to estimate linkage relationships. The 61 "HL" progeny consisted of 44 gray and 17 buff progeny. Buff isolates have been known to have lost the ability to infect rice and thus do not manifest their genotype for other pathogenicity/virulence factors (Chumley and Valent 1990). The above 17 buff progeny in this study were unable to produce

Table 1 Number of rice plants per cultivar employed to study the pathogenicity/virulence of parent isolates and F1, F2 and backcross progeny of *M. grisea* obtained from a primary cross of isolates 2539 ×Guy11[a]

Rice cultivars	Number of replicates	Total Number of Plants tested per *M. grisea* isolate				
		H[b]	MF[c]	Sib/BC[d]	Guy 11	2539
CO39	4				39	35
CO39	4	14			15	12
CO39	1			14	48	39
CO39 + C105TTP-4	1		10		7	2
Five NILs	1	12			34	27
51583	1	2		4[e]	19	21

[a] Rice seedling pathogenicity assays were conducted as described in Materials and methods
[b] "HL" progeny were developed by Hei Leung (Leung et al. 1988) from a cross of *M. grisea* isolates Guy11×2539
[c] "MF" progeny were developed by Mark Farman from a cross of *M. grisea* isolates Guy11×2539
[d] Sib=F2 progeny of matings between siblings from a cross of *M. grisea* isolates Guy11×2539. BC=backcross progeny of matings between F1 progeny from a cross of *M. grisea* isolates Guy11×2539 and parents Guy11 or 2539
[e] 69 of the 226 Sib/BC progeny were tested

Table 2 Segregation of virulence on rice cultivars CO39 and 51583 in *M. grisea* "HL" progeny obtained from a cross between isolates 2539 x Guy11[a,b]

Cultivar	Disease Reaction[c]		Progeny segregation (A:V)	χ^2 (1:1)
	2539	Guy11		
CO39	1	3	25:18	1.1395
51583	4	4	0:43[d]	

[a] Rice seedling pathogenicity assays were conducted as described in Materials and methods
[b] "HL" progeny were developed by Hei Leung (Leung et al. 1988) from a cross of *M. grisea* isolates Guy11×2539
[c] Interaction phenotypes are: Type 0 – no visible symptoms; Type 1 – small dark brown pinpoint-sized, non-sporulating lesions; Type 2 – dark brown, non-sporulating lesions 2–3 mm in length; Type 3 – circular, sporulating lesions with tan centers and dark brown margins; and Type 4 – large diamond-shaped, sporulating lesions with tan centers and dark brown margins. *M. grisea* isolates causing lesion types 3 and 4 were considered virulent (V) while those causing types 0, 1, and 2 were considered avirulent (A)
[d] Paul Tooley, Al Rossi, and Francis Latterell tested 32 of the "HL" progeny on 51583 and obtained similar results to those reported here (personal communication)

symptoms on either CO39 or 51583. The gray-progeny isolate '6100' was non-pathogenic on CO39, 51583, and all known cultivars for which 2539 is virulent (see Table 3); thus 6100 was eliminated from the analysis. The remaining 43 gray progeny were used to estimate linkage relationships between the *avrCO39* locus and the other markers reported by Skinner et al. (1993).

Since it is assumed that the 17 buff progeny and the 43 gray progeny are random subsets of the 61 progeny data set, differing only in their pigmentation, deleting the 17 from the whole, should not bias the data set either for or against pathogenicity/virulence. Skinner et al. (1993) found that 99 of the 100 markers segregated 1:1. For the same markers, we found that 89 and 83, for the buff and gray subsets, respectively, segregated 1:1 (P=0.05). As a result of sampling errors associated with smaller data sets, the number of non-randomly-segregating markers in these latter estimates may exceed the expected number (five for P=0.05) to respect this null hypothesis (1:1).

Results

Segregation of virulence in "HL" progeny on NILs, CO39, and 51583

Table 2 lists the infection types resulting from inoculation of cultivars CO39 and 51583 with the *M. grisea* isolates 2539 and Guy 11 and 43 "HL" progeny. The lesions produced by each parental isolate on the five NILs and their recurrent parent, CO39, were the same except for Guy 11 on C104PKT, where the disease reaction was more severe (larger lesions; data not shown). The lesion type of a given progeny was the same on each NIL (data not shown). Moreover, if a given progeny was virulent on CO39, it was also virulent on all five NILs. Although each of the NILs and CO39 differ by single resistance genes to rice blast, there was no major differentiating effect of these genes on the family of *M. grisea* isolates tested. Hence, the use of the five NILs essentially gave additional replications for CO39. Figure 1 shows the differential response between isolates Guy11 and 2539 on CO39 and is representative of the differential seen on all five NILs. All isolates described in Table 2 were virulent on cultivar 51583 (see Fig. 3).

Virulence of 2539

The virulence of 2539 on rice was further studied by inoculating 765 additional rice genotypes (757 from the USDA Small Grains Collection plus M103, S201, M201, IR36, Leah, Dular, Asahi, and Sha-tiao-tsai). Table 3 lists the 29 rice genotypes found to be moderately susceptible (lesion types 3 and 4, but with fewer lesions) to *M. grisea* 2539, together with their names, origin, origin of ancestors, and names of known ancestors. The remaining 736 rice accessions were resistant to 2539.

Segregation of virulence in 327 progeny on CO39

Table 4 lists ten crosses (the initial cross 2539×Guy 11, five sib-crosses, and four backcrosses), the lesion types of parents on CO39, and the segregation of virulence on CO39 among progeny of the crosses. Data from the original 43 progeny ("HL" progeny in Table 1) were combined with data from a second set of progeny ("MF") of the same parentage in order to give a larger data set. Combining data sets was justified because chi-square analysis showed that the ratios of the two data sets were not different (chi-square=0.9610).

Table 3 Identification of rice cultivars showing a susceptible disease reaction after inoculation with *M. grisea* isolate 2539[a,b]

Name	CI or PI number	U.S. origin[c]	Origin of one or more ancestors	Known ancestors
Calrose	8988	CA	Japan	Caloro, Calady LadyWright
	9152	TX	Japan Philippines	Caloro,Rexoro Marong Paroc
	9169	LA	Taiwan	Fortuna, Pa Chiam
	9172	LA	Taiwan	Fortuna, Blue Rose, Magnolia Pa Chiam
	9305	CA		
	9311	CA	Japan	Caloro, Blue Rose
	9338	CA	Japan	Caloro
	9339	CA	Japan	Caloro, Blue Rose
	9504	CA		
CS-S4	9835	CA	Japan	Caloro
	9855	LA		
	9938	LA	Taiwan Philippines	Fortuna, Rexoro, Bluebonnet, Pa Chiam, Marong Paroc
Calrose76	9966	CA	Japan	Calrose
M7	9967	CA	Japan	Calrose76, CS-M3
M-302	9976	CA	Japan	Calrose76, M5, CM-M3
	11047	CA		Maxwell
	11049	CA	Japan	Calrose
	11052	CA		M5
Caloro	12027	CA	Japan	Early Wataribune
Calmochi 101	494.104	CA	Japan	Tatsumi Mochi, M7, S6, Calrose 76
A-301	505.817	CA	Philippines	IR22, Della
	506.221	CA		M-101
	506.222	CA	Japan	Calrose76, CS-M3
	506.223	CA		M-101
	506.224	CA		M-101
ST25M	558.501	CA		M-201
M-103	527.566	CA	Japan	SD7, Earlirose M-302, Reimei Calrose 76, CS-M3
M-201	9980	CA	Philippines	IR8, Terso, CS-M3, Kokuhorose
S-201	9974	CA	Japan	Calrose76, CS-M3, S6

[a] Rice seedling pathogenicity assays were conducted and scored as described in Materials and methods. Rice cultivars showing type 3 or 4 interaction phenotypes after inoculation with *M. grisea* isolate 2539 were considered susceptible

[b] Harold Bockelman (USDA Small Grains Collection) provided the information and references shown in this table

[c] CA, California; TX, Texas; LA, Louisiana

Table 4 Segregation of virulence on rice cultivar CO39 in *M. grisea* F2 and backcross progenies derived from a primary cross of isolates 2539 x Guy11[a]

Cross type	Parents	Disease reaction[b]		Progeny segregation	Expected ratio	χ^2
		P1[c]	*P2*[c]	(A:V)		
Initial	2539×Guy11	1	3	53:48[d]	1:1	0.25
Sib	6064×6005	3	3	0:20	0:1	
Sib	6082×6005	1	3	16:20	1:1	0.44
Sib	6095×6061	1	1	31:0	1:0	
Sib	6090×6061	1	1	23:0	1:0	
Sib	6095×6008	1	1	7:0	1:0	
Backcross	6005×Guy11	3	3	0:20	0:1	
Backcross	6008×Guy11	1	3	25:19	1:1	0.82
Backcross	6112×2539	1	1	4:0	1:0	
Backcross	6095×2539	1	1	41:0	1:0	

[a] Rice seedling pathogenicity assays were conducted as described in Materials and methods

[b] Interaction phenotypes are: Type 0 – no visible symptoms; Type 1 – small dark brown pinpoint-sized, non-sporulating lesions; Type 2 – dark brown, non-sporulating lesions 2–3 mm in length; Type 3 – circular, sporulating lesions with tan centers and dark brown margins; and Type 4 – large diamond-shaped, sporulating lesions with tan centers and dark brown margins. *M. grisea* isolates causing lesion types 3 and 4 were considered virulent (V) while those causing types 0, 1, and 2 were considered avirulent (A)

[c] P1and P2 are parent *M. grisea* isolates used in the cross (P1 × P2)

[d] The progeny of the initial cross of *M. grisea* isolates Guy11 and 2539 comprised 48 "HL" progeny and 58 "MF" progeny. "HL" and "MF" progenies were developed by Hei Leung (Leung et al. 1988) and Mark Farman, respectively

As would be expected from a single-gene model for virulence, the 101 progeny resulting from the initial cross segregated 1:1 for virulence (V):avirulence (A). Ratios from sib-matings and backcrosses also did not deviate from the expected. In two A×V crosses, progeny segregated 1:1 in each. In five A×A crosses, only A progeny resulted. In two V×V crosses, only V progeny resulted.

Linkage relationships

Figure 2 shows the linkage relationship between the CO39 avirulence gene (*avrCO39*), 13 RFLP markers, and one isozyme marker. The level of certainty was higher (LOD=4.0, theta=0.20) for the linkage relationships between *avrCO39* and RFLP markers CH5-120H, 1.2H, CH5-131H, and 5-10F than for that between these four markers and the other ten markers (LOD=4.0, theta=0.30) on chromosome 1 (Skinner et al. 1993).

Discussion

A single locus controls virulence/avirulence of *M. grisea* on CO39 and NILs

The segregation of pathogenicity on CO39 and the NILs indicated that a single locus controls pathogenic ability on

CO39. By contrast, all progeny tested were pathogenic on cultivar 51583. We conclude that the gene controlling pathogenicity on CO39 is an avirulence gene as this locus controls a cultivar-specific interaction phenotype. As no differential interaction phenotype was observed on the five isolines studied, avirulence genes corresponding to resistance genes in these isolines were not identified in our mapping population.

Valent et al. (1991) also described a single gene (*Avr1-CO39*) controlling cultivar specificity on CO39. They concluded that this avirulence gene came from isolate '4091-5-8', which is an ancestor of isolate 2539. Isolate 2539 contributed the avirulence gene identified in the present study. As 4091-5-8 is an ancestor of 2539, it is possible that the avirulence genes in 4091-5-8 and 2539 are the same, being identical by descent. Identification of an avirulence gene for CO39 suggests that CO39 has a corresponding resistance gene (Valent et al. 1991).

Some additional studies on the inheritance of cultivar specificity in *M. grisea* have also suggested that this trait is controlled by single avirulence genes (Leung et al. 1988; Ellingboe and Robertson 1990; Silue et al. 1992). However, other studies have indicated a more complex mode of inheritance (Ellingboe, 1992; Silue and Notteghem 1992; Lau et al. 1993). It is interesting to note that, in two cases, the same research group suggested different models depending on the specific parental isolates used. A single-gene model for cultivar specificity has been suggested when evaluating '*Pi-n4*' with progeny derived from isolates Guy11×'ML25' (Silue et al. 1992), whereas a two-gene model was proposed when *Pi-n 4* was evaluated with progeny of isolates Guy 11×'CD128' (Silue and Notteghem 1992). Ellingboe postulated single genes to control avirulence/virulence on 'L202' and 'Bluebelle' when evaluated with progeny of Guy11×'6-28' (Ellingboe et al. 1990), and a two-gene model when L202 and Bluebelle were evaluated with Guy11 x '6-20' derivatives (Ellingboe 1992). The main difference between these studies seems to be the source of avirulence genes. The specific parents for each cross determines how many genes are segregating in the resulting populations. Thus, the inheritance of virulence on rice by *M. grisea* varies with the specific rice and blast genotypes used and can be simple or complex.

Pathogenicity of 2539

Isolates 2539 and Guy 11 were previously reported to be non-pathogenic and pathogenic, respectively, on 51583 (Leung et al. 1988). Our data differ in that isolate 2539 was found to be pathogenic on 51583 (Fig. 3), but the lesion type produced by isolate 2539 on 51583 was unique in that generally fewer lesions were observed. In some replications, the lesion type was similar to those of virulent isolates on the second youngest leaf when the disease reaction on the youngest leaf was the most severe (Fig. 4; Latterell et al. 1963; Valent et al. 1991). This milder lesion type was not manifested on 51583 by isolate Guy 11 or by any

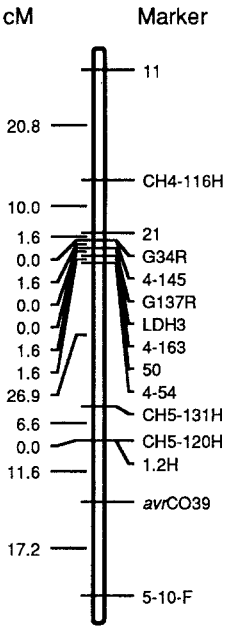
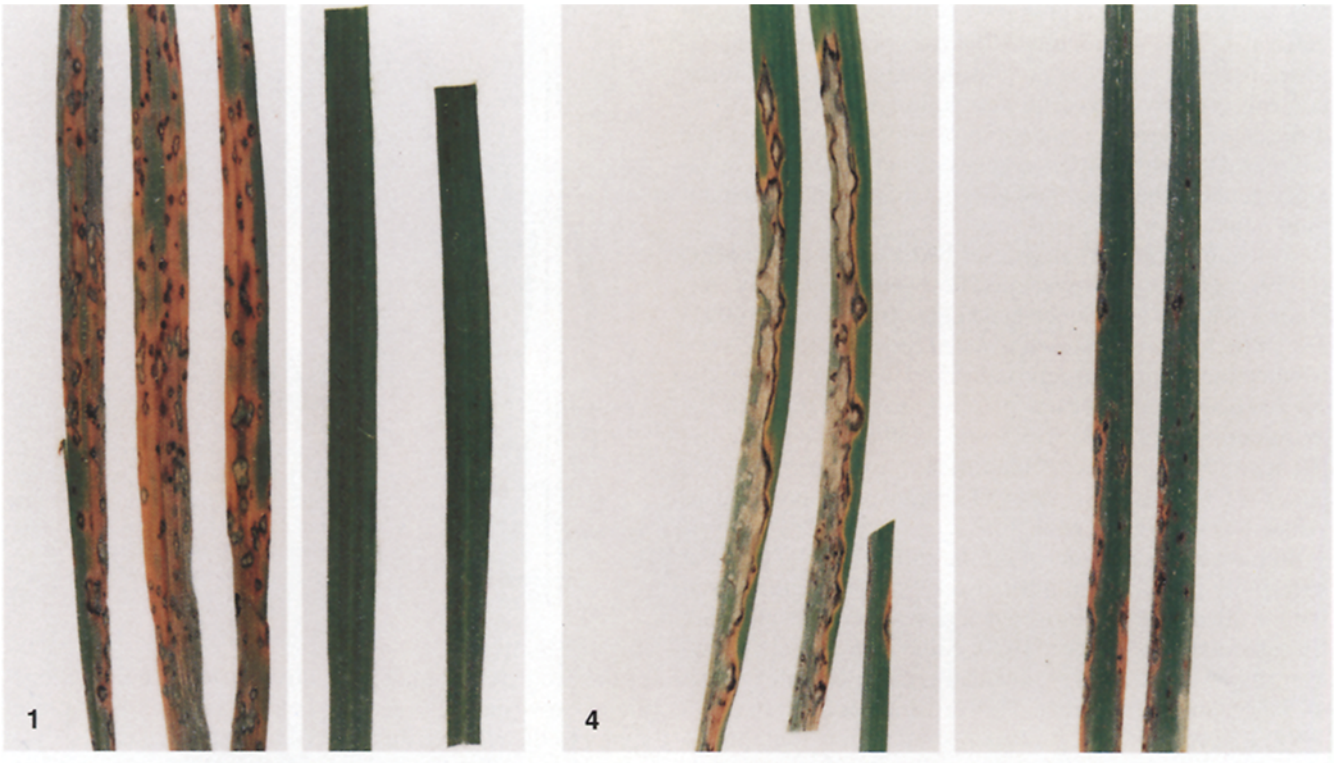


Fig. 2 Genetic mapping of a locus controlling specificity to rice cultivar CO39 to Chromosome 1 of *M. grisea* in the cross Guy11×2539 (Skinner et al. 1993). Map distances in cMs were calculated using the Kosambi mapping function from estimates of recombination fractions using the MAPMAKER program as described in Materials and methods

of the progeny tested. The relative difference in pathogenicity between 2539 and other blast isolates may correspond to the relative differences in resistance to blast isolates observed by Roumen (1992) among the ten rice genotypes he evaluated. The "window" for a virulent reaction of isolate 2539 on 51583 may be small and hence easily missed compared to other rice-blast interactions. Also, the virulence of 2539 may be controlled by a combination of genes, which act epistatically to produce a milder reaction. This "parental" combination of genes may be only rarely manifested in its offspring due to the random segregation of multiple factors.

Isolate 2539 is virulent on at least 30 rice genotypes and avirulent to many more (this study). The pathogenicity of 2539 on some, but not most, rice genotypes, and the differential response of its progeny on CO39 and 51583, implies that it carries genes controlling cultivar specificity which interact with corresponding resistance genes in the host. Isolate 2539 was developed by interbreeding rice and grass pathogenic isolates of *M. grisea* (Leung et al. 1988). For crosses involving *M. grisea* isolates CH104-3 and CH40-1, isolate 2539 may have gained an ability for pathogenicity on rice or lost host-range limiting factors that its grass ancestors carried. CH104-3 and CH40-1 are both Chinese isolates which are pathogenic to rice. CH40-1 was the recurrent parent for a generation of backcrossing to develop isolate 2539 (Leung et al. 1988); isolate CH40-1 is virulent on cultivars IR36, S201, and Dular, while isolate CH104-3 is virulent only on S201 (Kolmer and Ellingboe 1988). We found isolate 2539 to also be virulent on S201 (Table 3), but not on IR36 or Dular (data not shown).

Caloro is present in the pedigrees of over one-half of the 24 California breeding lines found to be susceptible to isolate 2539 (Table 3). That Caloro is a single-plant selection from 'Early Wataribune', a heterogeneous Japanese accession introduced into the U.S. in 1913, points to isolate 2539 possibly inheriting pathogenic ability on these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cultivars from its Japanese rice ancestors, 'Ken 73-01' and 'Ina 168' (Leung et al. 1988). Thus, there are at least four rice-pathogen ancestors of isolate 2539 which could have transmitted rice virulence genes to isolate 2539.

Mapping of a cultivar specificity locus for CO39

The *avrCO39* locus maps to chromosome 1 between CH5-120H and 5-10F which are 11.8 and 17.2 cM, respectively, away from the locus; *avrCO39* was estimated to be separated from the telomere by a recombination fraction of 0.39 (chi-square=5.55, 3 *df*, 90 progeny)(M. Farman and S. A. L., unpublished results).

Sweigard et al. (1993) have mapped three avirulence genes; one for each of the rice cultivars 'Maratelli' (*Avr1-MARA*), 'Tsuyuake' (*Avr1-TSUY*), and 'Yashiro-mochi' (*Avr2-YAMO*). *Avr2-YAMO* has been cloned based on its position in their map (Valent and Chumley, In Press). *Avr1-TSUY* and *Avr2-YAMO* map to opposite ends of chromosome 1 in isolate '4224-7-8', while *Avr1-MARA* maps approximately 19 cM from the end of chromosome 2b. Valent et al. (1992; 1994) have suggested that *Avr2-YAMO* and *Avr1-TSUY* may be unstable due to deletions of the ends of the chromosome on which they reside. *avrCO39* might be expected to be more stable, due to its internal location. Indeed, during the course of this study, we found no evidence for alterations in virulence of any of the isolates reported here.

The degree of relatedness between isolates 4224-7-8 and 2539 is not known. As they are the donors of the avirulence genes discussed above, the *avrCO39* allele may be present on chromosome 1 of 4224-7-8 along with *Avr1-TSUY* and *Avr2-YAMO*.

This study has demonstrated single-gene inheritance of cultivar-specificity between the rice cultivar CO39 and a specific population of blast isolates. The relative map location of this gene has been determined. Yet to be realized, however, are the cloning of this cultivar-specificity locus and the understanding of its function in host resistance. Our current efforts are directed toward this end (Leong et al. In Press).

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Fig. 1 Differential response of leaves of rice cultivar CO39 to inoculation with *M. grisea* isolates Guy11 and 2539. Rice seedlings were inoculated and the disease severity was scored as described in Materials and methods. CO39 was inoculated with *M. grisea* isolates Guy11 (left) and 2539 (right)

Fig. 3 Interaction phenotypes of leaves of rice cultivar 51583 and *M. grisea* isolates 2539 and Guy11, or a gelatin control. Rice seedlings were inoculated and disease severity was scored as described in Materials and methods. Rice cultivar 51583 was inoculated with *M. grisea* isolate 2539 (left), a gelatin control (middle), and Guy11 (right)

Fig. 4 Interaction phenotypes of youngest and second-youngest leaves (at the time of inoculation) of rice cultivar 51583 and progeny '6059' from a cross between *M. grisea* isolates Guy11 and 2539. Rice seedlings were inoculated and disease severity was scored as described in Materials and methods. Shown are lesions on: youngest leaf (left) and second-youngest leaf (right)

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