Evidence of a Gene-for-Gene Relationship in the *Oryza sativa-Magnaporthe grisea* Pathosystem

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ABSTRACT

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A cross was made between two field isolates of *Magnaporthe grisea* pathogenic to rice. Full-sib crosses between F_1 ascospores, followed by backcrosses to the female fertile parent GUY11, produced perithecia fertile enough for tetrad isolation. Genetic analysis of the pathogen revealed that avirulence to the rice cultivar Pi-n°4 is controlled by one gene (*Avr1-Pi-n°4*) unlinked to the gene MAT1-1, which is responsible for mating type. Additionally, resistance of Pi-n°4 was found to be controlled by one gene. Thus, existence of a gene-for-gene relationship was shown for the pathosystem *Oryza sativa-M. grisea*.

Additional keyword: rice blast.

Pyricularia grisea Sacc. (formerly *Pyricularia oryzae* Cavara [18]) is a fungus pathogenic to almost 40 plant species in 30 genera of Poaceae, including the genus *Oryza* (1). This fungus is the causal organism of the blast disease.

Hebert (5) first produced in vitro the perfect state of *P. grisea* and demonstrated that the fungus is heterothallic. Later, he succeeded in making crosses between isolates of *P. grisea* from different hosts, including rice (6). As a result, the perfect state of the fungus is now classified as *Magnaporthe grisea* (Hebert) Barr (2,27,28). Host specificity studies have demonstrated the existence of groups of *M. grisea* isolates with specific pathogenicity to different groups of Poaceae (9).

Since the perfect state of *M. grisea* was first produced in the laboratory, several studies have been conducted on the genetics of the organism. These studies have examined inheritance of isozyme markers (13,15), resistance to fungicides (21–23), host specificity (24,26,29), and pathogenicity to rice (3,12,14). The genetics of avirulence to different rice genotypes have been examined only in a few studies (3,12,14) because of the low fertility of isolates pathogenic to rice (10) and because these isolates often behave only as males in crosses (7,8,12,25). The identification of GUY11, a hermaphroditic isolate pathogenic to rice (14), has made it possible to make crosses with isolates that are pathogenic to rice (3,14).

Among the 470 rice isolates from different geographical origins that are stored at the IRAT/CIRAD plant pathology laboratory in Montpellier, only a few produce fertile perithecia when mated with GUY11. The most fertile cross is GUY11 × ML 25. ML 25 was isolated in 1986 in Mali from the rice cultivar Sintiane diofor. Complementary experiments examined segregation of avirulence to rice and the inheritance of resistance in rice to test Flor's gene-for-gene hypothesis (4) in the *Oryza sativa-M. grisea* pathosystem.

MATERIALS AND METHODS

Isolates of *M. grisea* and crosses. To minimize variations due to extended subculturing on artificial media, dried cultures (parents and progenies) on filter paper were obtained and stored as described by Valent et al (25). Four hermaphroditic isolates, OG 2, IN 1, OG 5 (supplied by H. Kato and pathogenic to finger millet [*Eleusine coracana*] but not to rice), and GUY11 were used to determine the mating type of each isolate. OG 2 and IN 1 are MAT1-1 and OG 5 and GUY11 are MAT1-2 according to the nomenclature of Yoder et al (30). The efficiency of these four isolates has been demonstrated (17). Several characteristics of the parental isolates, GUY11 and ML 25, have been described (14,19). The crosses used for this study are given in Table 1. Crosses were made to improve fertility and to provide parental isolates differing in avirulence to the rice cultivar Pi-n°4.

Mating methods were similar to those described by Valent et al (25). Spore production and matings were done on a paddy flour agar medium (16) (1 L of distilled water, 15 g of agar, and 20 g of paddy flour) with 5×10^5 IU of penicillin added after it was autoclaved at 120 C for 20 min.

Isolation of single ascospores was done on water agar using a glass needle as previously described (25). A trinomial identity was assigned to each ascospore: the first number was the cross

TABLE 1. Segregation of random ascospores from 14 crosses of *Magnaporthe grisea* for avirulence to the rice cultivar Pi-n°4

Crosses	Blast rating[a] P_1	Blast rating[a] P_2	Segregation[b] (v:av)	Expected ratios	χ^2
GUY11 × ML 25	6	1	19:19	1:1	...
1/0/2 × GUY11	1	6	20:21	1:1	0.02[c]
1/0/7 × GUY11	1	6	16:16	1:1	...
1/0/20 × GUY11	1	6	11:20	1:1	2.60[c]
1/0/27 × GUY11	1	6	18:14	1:1	0.50[c]
1/0/7 × 1/0/37	1	5	20:18	1:1	0.05[c]
1/0/13 × 1/0/37	1	5	24:14	1:1	2.60[c]
1/0/4 × 1/0/7	1	1	0:34	0:1	...
1/0/27 × 1/0/31	1	3	2:35	0:1	...
1/0/1 × 1/0/19	5–6	1	16:19	1:1	0.25[c]
1/0/9 × 1/0/19	4	1	19:17	1:1	0.11[c]
32/0/14 × GUY11[d]	1	5–6	18:18	1:1	...
32/0/19 × GUY11[d]	1	6	14:13	1:1	0.03[c]
2/0/3 × GUY11[d]	1	5–6	27:23	1:1	0.32[c]

[a] Ratings of the parental isolates in the order given by the cross description ($P_1 \times P_2$).
[b] Segregation in virulent (v = disease ratings 4–6) and avirulent (av = disease ratings 1–3) progenies.
[c] Not significant.
[d] Crosses where tetrads were isolated.

number, the second the ascus number, and the last the ascospore number. Ascospores were randomly isolated when germination was poor. In these crosses, 30–60 progenies were isolated and the ascus number was assigned the number zero.

Rice cultivars. Three rice cultivars were used in this study. Maratelli is a temperate, japonica-type cultivar that has been grown for a long time in Italy, where it is considered to be very susceptible to local isolates of the rice blast fungus. In artificial inoculation experiments with more than 200 diverse isolates of *P. grisea* that are pathogenic to rice, Maratelli always gave virulent infection types (rating 6, Fig. 1). Because this cultivar has never demonstrated specific resistance to any blast isolate in field tests, Maratelli was used to evaluate the pathogenicity of all *P. grisea* isolates. Pi-n°4 is one of the differential cultivars described by Kiyosawa (11). Indio is another temperate rice cultivar obtained in Italy that is susceptible to *P. grisea*. For the genetic analysis of resistance in Pi-n°4, we used F_2 plants from the cross Pi-n°4 × Indio (seeds supplied by B. Courtois, IRAT/CIRAD, Guadeloupe).

TABLE 2. Segregation for mating type and avirulence to the rice cultivar Pi-n°4 of tetrads isolated from the cross 2/0/3 × GUY11

Isolates	Mating type[a]	Pi-n°4[b]
2/0/3	MAT1-1	−
GUY11	MAT1-2	+
4/1/5	MAT1-1	+
4/1/6	MAT1-1	+
4/1/8	MAT1-1	+
4/1/7	MAT1-1	+
4/1/1	MAT1-2	−
4/1/2	MAT1-2	−
4/1/3	MAT1-2	−
4/1/4	MAT1-2	−
4/2/2	MAT1-1	−
4/2/4	MAT1-1	−
4/2/3	MAT1-1	+
4/2/5	MAT1-1	+
4/2/1	MAT1-2	−
4/2/6	MAT1-2	−
4/2/7	MAT1-2	+
4/2/8	MAT1-2	NT[c]
4/3/1	MAT1-1	+
4/3/2	MAT1-1	+
4/3/3	MAT1-1	+
4/3/4	MAT1-1	+
4/3/5	MAT1-2	−
4/3/6	MAT1-2	−
4/3/7	MAT1-2	−
4/3/8	MAT1-2	−
4/4/1	MAT1-1	+
4/4/2	MAT1-1	+
4/4/4	MAT1-1	−
4/4/5	MAT1-2	+
4/4/6	MAT1-2	+
4/4/7	MAT1-2	−
4/4/3	MAT1-2	−
4/5/6	MAT1-1	−
4/5/2	MAT1-1	−
4/5/7	MAT1-1	−
4/5/3	MAT1-2	+
4/5/1	MAT1-2	+
4/5/4	MAT1-2	+
4/5/5	MAT1-2	+
4/6/3	MAT1-1	+
4/6/4	MAT1-1	+
4/6/5	MAT1-1	−
4/6/6	MAT1-1	−
4/6/2	MAT1-2	+
4/6/7	MAT1-2	+
4/6/1	MAT1-2	−
4/6/8	MAT1-2	−
4/7/3	MAT1-1	+
4/7/6	MAT1-1	+
4/7/2	MAT1-1	−
4/7/7	MAT1-1	−
4/7/4	MAT1-2	+
4/7/5	MAT1-2	+
4/7/1	MAT1-2	−
4/7/8	MAT1-2	−

[a] Mating-type nomenclature after Yoder et al (30).
[b] Infection types scored on cultivar Pi-n°4. + = Virulent progeny (disease ratings 4–6); − = avirulent progeny (disease ratings 1–3).
[c] Not tested.

Plants were grown under greenhouse conditions with temperatures between 20 and 30 C and additional light in winter. Fourteen lines of 12 seeds were sown in flats (45 × 29 × 7 cm) filled with compost (Motex compost no. 7, Inter-humus S.A., Lunel, France).

Inoculation and disease rating. Inoculations were made by injecting 0.2 ml of a spore suspension (5×10^4 spores per milliliter of water) with a syringe through the sheaths of each plant. These plants were grown 3–4 wk in the greenhouse before inoculation (four- to five-leaf stage). Experiments were repeated at least twice. Plants were maintained for 7 days in the greenhouse before symptoms were scored according to a 1–6 scale (Fig. 1) developed by Notteghem (16). Our experience has shown that ratings from 1 to 3 correspond to avirulence, whereas ratings from 4 to 6 correspond to virulence in the pathogen.

Successive inoculations for identification of resistance gene. To determine if the resistance gene we identified in Pi-n°4 was *Pi-ta*[2] (the gene previously described by Kiyosawa [11]), 139 F_2 plants of Pi-n°4 × Indio were inoculated at the 4–5 leaf stage. Half of the F_2 plants were inoculated with ML 25 and the other half with isolate INA 72, which is one of the isolates Kiyosawa used for identification of *Pi-ta*[2] (11). After assessment of disease from the first inoculation, the plants initially inoculated with and resistant to INA 72 were then inoculated with ML 25. Similarly, the plants resistant to ML 25 were inoculated with INA 72. As a control, 10 random plants found resistant to each isolate were inoculated with GUY11, which is virulent on both parents. This was to determine that resistance had not been induced by the first inoculation with an avirulent isolate.

RESULTS

***M. grisea* crosses.** Thirty-eight ascospores were isolated randomly from the initial cross of GUY11 × ML 25 (20). From these F_1 progenies, three types of crosses were made: full-sib crosses, backcrosses between F_1 progenies and GUY11, and crosses between full-sib progenies and GUY11 (Table 1). Fertile backcrosses with ML 25 were not obtained. For each of the 14 crosses, mating types segregated in a 1:1 ratio (data not shown). The crosses 32/0/14 × GUY11, 32/0/19 × GUY11, and 2/0/3 × GUY11 (Table 1) were fertile enough so that tetrads could be isolated.

Two crosses (1/0/4 × 1/0/7 and 1/0/27 × 1/0/31) were made

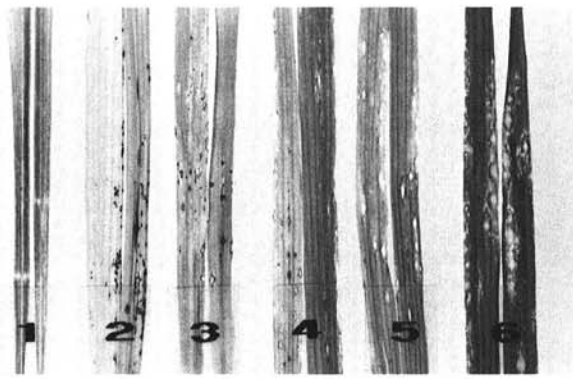

Fig. 1. Disease rating scale for rice blast (after Notteghem [16]). Lesion types 1–3, avirulent reactions; lesion types 4–6, virulent reactions.

between F_1 progenies avirulent on Pi-n°4. The 12 other crosses were made between one virulent isolate and one avirulent on Pi-n°4.

Inheritance of avirulence. The progeny from each cross were inoculated onto the cultivar Maratelli, and all induced a highly susceptible reaction (infection type 6). This pathogenicity phenotype was identical with those induced by the parental isolates GUY11 and ML 25. Thus, the pathogenicity to rice was not lost in the progeny isolates.

All segregations of randomly isolated ascospores and tetrads are given in Tables 1 and 2. In the initial cross, GUY11 × ML 25, progenies segregated as 19 virulent/19 avirulent on the cultivar Pi-n°4. Four backcrosses (1/0/2 × GUY11, 1/0/7 × GUY11, 1/0/20 × GUY11, and 1/0/27 × GUY11) also showed a 1:1 segregation (Table 1). Progenies of four full-sib crosses (1/0/7 × 1/0/37, 1/0/13 × 1/0/37, 1/0/1 × 1/0/19, and 1/0/9 × 1/0/19) also segregated in a 1:1 fashion, indicating that avirulence to Pi-n°4 was controlled by one gene. In 1/0/4 × 1/0/7 (one of two crosses between avirulent progenies; Table 1), only avirulent progenies were found. In the other cross (1/0/27 × 1/0/31), an unexpected result was obtained: 35 ascospores were avirulent and two were virulent. The hypothesis of a single gene segregation was supported by analysis of tetrads obtained in the crosses 32/0/14 × GUY11, 32/0/19 × GUY11, and 2/0/3 × GUY11. Table 2 shows examples of segregations of tetrads isolated in cross 2/0/3 × GUY11. All tetrads segregated 4:4 (virulent/avirulent) and 3:4 or 4:3 if the tetrads were incomplete. One exception was observed with tetrad 35/2 (data not shown), which showed a segregation of 2:5.

Inheritance of resistance in Pi-n°4. When inoculated with ML 25, F_2 seedlings of cross Pi-n°4 × Indio segregated in a 143:36 resistant/susceptible ratio (Table 3), which fits a 3:1 ratio. These data support the hypothesis that one dominant gene controls resistance in Pi-n°4. F_2 seedlings inoculated with two other avirulent progenies (1/0/25 and 1/0/30) also segregated in a 3:1 ratio (Table 3). Successive inoculations with ML 25 and INA 72 (Table 4) on 77 resistant plants from the same F_2 family demonstrated that resistance to one strain is effective against the other.

DISCUSSION

The *M. grisea* isolates used in this study (GUY11 and ML 25) were suitable because they were both isolated from naturally infected rice and were both fully pathogenic to rice. Additionally, they differed in avirulence to various rice cultivars and grew and sporulated abundantly on artificial media. All progenies of the cross between GUY11 and ML 25 exhibited a pathogenic phenotype on rice cultivars that was identical with that of the parents. Fertility in the initial cross was not complete, but full-sib crosses and backcrosses with GUY11 were achieved. Fertility of all of the progenies allowed for identification of mating types. The mating type of the progeny segregated 1:1 in the initial cross as well as in the other 13 crosses.

Through full-sib crosses followed by backcrosses with GUY11, fertility could be enhanced so that tetrads could be isolated in three cases. This result is similar to those obtained by Valent et al (25); however, those researchers improved ascospore germination in *M. grisea* by using full-sib crosses with isolates that did not attack rice.

Segregation of avirulence to the cultivar Pi-n°4, obtained with the cross GUY11 × ML 25, is likely under monogenic control. This result was supported by 13 testcrosses. Although exceptions occurred, the results indicated that one gene, designated *Avr1-Pi-n°4,* conditioned avirulence to Pi-n°4. ML 25 and the avirulent progenies have the allele Avr1-Pi-n°4$^-$; GUY11 and the virulent progenies have the allele Avr1-Pi-n°4$^+$ conferring virulence. Monogenic control of avirulence in *M. grisea* to other rice cultivars has been described (3,14).

The inheritance of resistance of Pi-n°4 to ML 25 and two avirulent progenies (1/0/25 and 1/0/30) indicated that resistance in Pi-n°4 was controlled by one dominant gene (Table 3).

Successive inoculations with ML 25 and INA 72 indicated that the resistance gene is identical or very closely linked to *Pi-ta^2,* the gene Kiyosawa (11) identified in Pi-n°4 using INA 72. Supporting evidence of the identity of this resistance gene could be obtained from the analysis of the progeny of ML 25 × INA 72; however, this cross is impossible because both isolates are of the same mating type.

The monogenic control of avirulence in ML 25 to Pi-n°4 and monogenic dominant resistance in this cultivar is evidence in support of a gene-for-gene relationship (4) in the pathosystem *O. sativa-M. grisea.*

Unexpected segregation ratios, such as the two virulent progenies from the cross 1/0/27 × 1/0/31 and the avirulent one or two ascospores from tetrad 35/2, were obtained. These progenies appeared at a low rate in two crosses and did not disturb the genetic analysis. Leung et al (14) mentioned such a case in their studies with progenies of a cross in which both parental isolates were pathogenic to the cultivar 51583. The genetic basis of occurrence of such mutants is unknown.

TABLE 3. Inoculations of three isolates of *Magnaporthe grisea* to F_2 seedlings from the cross between rice cultivars Pi-n°4 and Indio

Cross	Isolates	F_2 segregation[a] R:S	Expected ratio	χ^2
Pi-n°4 × Indio	ML 25	143:38	3:1	0.17[b]
Pi-n°4 × Indio	1/0/30	109:34	3:1	0.11[b]
Pi-n°4 × Indio	1/0/25	107:33	3:1	0.15[b]

[a]Ratio of F_2 seedlings scoring 1–3 (R, resistant) to those scoring 4–6 (S, susceptible) on a disease rating scale (Fig. 1).
[b]Not significant.

TABLE 4. Test for gene identity

Pi-n°4 × Indio	Isolates	F_2 segregation[a] F:S	Expected ratio	χ^2
One-half F_2	ML 25	49:21	3:1	0.94[b]
One-half F_2	INA 72	46:23	3:1	2.56[b]
F_2 resistant to ML 25	GUY11	0:10	0:1	...
F_2 resistant to ML 25	INA 72	39:0	1:0	...
F_2 resistant to INA 72	GUY11	0:10	0:1	...
F_2 resistant to INA 72	ML 25	38:0	1:0	...

[a]The number of F_2 seedlings scoring 1–3 (R, resistant) and 4–6 (S, susceptible) on a disease rating scale (Fig. 1).
[b]Not significant.

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