Identification of *Magnaporthe grisea* Avirulence Genes to Seven Rice Cultivars

D. Silué, D. Tharreau, and J. L. Notteghem

Laboratoire de phytopathologie CIRAD/CA, BP 5035, 34032 Montpellier Cedex, France.

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ABSTRACT

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To characterize the inheritance of avirulence in *Magnaporthe grisea* to specific rice cultivars, we performed a cross between two field isolates pathogenic to rice. Full-sib crosses followed by backcrosses enhanced the fertility of some matings so that tetrads could be isolated. Genetic analysis of avirulence to the rice cultivars Cica 6, Cica 8, DJ 8-341, IRAT 7, Ku 86, Med Noï, and Tetep was performed with these crosses. Avirulence to rice cultivars DJ 8-341, IRAT 7, Ku 86, and Tetep is controlled by one gene. Two genes are involved in avirulence to rice cultivars Cica 6, Cica 8, and Med Noï. Each of the two segregating genes acts independently and is sufficient to confer avirulence. This is the first report of such a control of avirulence in *M. grisea*. Linkages between avirulence genes and MAT1 and between avirulence genes are suspected. At least six avirulence genes are described.

Additional keywords: gene-for-gene relationship, genetic analysis of avirulence, *Oryza sativa*, rice blast.

Magnaporthe grisea (Hebert) Barr (*Pyricularia grisea* Sacc., formerly *Pyricularia oryzae* Cavara [16]) is an ascomycete pathogenic to rice and to many other monocots (1). Since the discovery of the perfect stage (6), attempts to cross isolates of *M. grisea* originating from rice have rarely been successful. Hebert (6,7), Yaegashi and Nishira (23), and Yaegashi (21) failed to obtain fertile crosses. Kato and Yamaguchi (9) reported a compatible cross, but Valent et al (19) could not reproduce the cross with the same isolates. In addressing this problem, fertility improvement programs were set up to cross infertile isolates from rice to fertile isolates from other grasses. Progeny from such crosses are often nonpathogenic to rice or far less aggressive than the rice pathogen parent from which they came (11,12,19). Moreover, backcrosses and full-sib crosses are not always as fertile as the initial cross (19,20,22). Nevertheless, such fertility improvement programs have allowed genetic analysis of avirulence to rice (10,12,20).

Screening for fertile field isolates pathogenic to rice has allowed us to make three fertile crosses (17). Starting with the cross GUY11 × ML 25, full-sib crosses and backcrosses were successfully made (17), and heredity of avirulence to rice could then be determined (18). For the cultivar Pi-n°4, a gene-for-gene relationship was demonstrated (18). But few things are known about interactions with other rice cultivars. Using the most fertile crosses previously identified (i.e., for which some tetrads were isolated), we studied heredity of avirulence to seven rice cultivars.

MATERIALS AND METHODS

Parental isolates and cultivars. The GUY11 isolate of *M. grisea* from rice has been described previously (12,14,17). Progeny isolates 2/0/3, 32/0/14, and 32/0/19 were obtained from full-sib crosses between first generation progeny of the cross GUY11 × ML 25 (17,18). Tetrads were isolated from the crosses between GUY11 and 2/0/3 (cross number 4), GUY11 and 32/0/14 (cross number 35), and GUY11 and 32/0/19 (cross number 36). Crossing and ascospore isolation methods were identical to those we have already described (14,17).

The origin, morphological type, enzymatic group, and reaction to the four *M. grisea* parental isolates of the eight rice cultivars used in the experiments are given in Table 1. Inheritance of avirulence was only studied with crosses in which parental isolates differed for avirulence to the rice cultivar studied. The parental isolates and their progeny were tested for pathogenicity on the rice cultivar Maratelli. This cultivar is susceptible to all rice pathogen field isolates that we have tested (more than 300).

Spore production and mating type determination. Spore production and matings were done on a rice flour agar medium (13) (1 L of water, 15 g of agar, 20 g of rice flour, and 500,000 IU of penicillin added after autoclaving at 120 C for 20 min). Methods and standard isolates used for mating type identification have already been described (14,17).

Pathogenicity assay. After 10 days of fungal growth on rice flour agar (27 C, 70% relative moisture, and 12 h of light per day), the plates were flooded with distilled water and scraped. The spore suspension obtained was filtered through cheesecloth and adjusted to 25,000 conidia ml^{-1}. Inoculation was performed by syringe injection of the spore suspension between leaf sheaths of 3-wk-old rice plants (four-leaf stage). Injections were done at least two times, and about 15 plants were inoculated each time. Plants were grown under greenhouse conditions (temperature between 20 and 30 C, and additional light in winter) in batches containing compost (Motex compost n°7, Inter-humus S.A., Lunel, France) and watered with nutritive solution containing the main nutritive elements (K, Mg, N, P, S). Inoculated plants were maintained in the greenhouse, and symptoms were recorded 7 days after inoculation by use of a 6-class scale (13,18). By this scale, an isolate is considered virulent when it causes lesions with scores of 4–6, whereas lower disease ratings correspond to avirulent isolates.

RESULTS

Controls. In all the progenies obtained from crosses 4, 35, and 36 (Tables 2–4), the mating type segregated in a 1:1 fashion, as expected.

When inoculated to cultivar Maratelli, parental isolates (GUY11, 2/0/3, 32/0/14, and 32/0/19) and all progenies of crosses 4, 35, and 36 gave type 6 symptoms (Tables 2–4). Therefore, we did not recover any nonpathogenic mutants in these progeny.

Inheritance of avirulence. For cultivar Ku 86, tetrads from all three crosses segregated 4:4, 3:4, 4:3, or 3:5 for virulence/avirulence (Tables 2–4). These segregations are in agreement with a 1:1 segregation, showing that one gene controls avirulence to Ku 86. In the atypical segregation of tetrad 4/8, a 3:5 segregation occurred instead of the expected 4:4.

Avirulence to Tetep was tested with progeny from cross 35, and avirulence to DJ 8-341 and IRAT 7 with progeny from cross 4. On those cultivars segregation was 4:4, 3:4, 4:3, or 3:5. Such 1:1 segregations show that one gene controls avirulence in each case. Again, segregation of the tetrad 4/8 was 3:5 instead of the expected 4:4.

For avirulence to cultivar Cica 6, in cross 4 we obtained segregations 2:6, 2:5, 1:6, 4:4, 0:8, or 3:5; in cross 35, we obtained 2:5, 1:6, 0:8, or 0:7; and for cross 36, we obtained 1:6, 3:4, or 0:7 (Tables 2–5). In tetrads in which only seven ascospores have been isolated, the missing isolate would probably react with cultivars like its sister isolate. Then, segregations can be grouped in three types of tetrads: 0:8 (virulent/avirulent), 2:6, and 4:4. Such tetrads can best be explained by the segregation of two avirulence genes, each gene alone being able to confer avirulence. According to this hypothesis, the avirulent parent of each cross has two different avirulence genes. The tetrads obtained could then be interpreted as parental ditype (4:4, virulent/avirulent), nonparental ditype (0:8), and tetratype (2:6). Although the number of tetrads examined is small, the occurrence of the nonparental ditype in proportions similar to those of the parental ditype suggests that these two genes are independent (Table 5).

For the two cultivars Cica 8 and Med Noï, we obtained different types of tetrads depending on the cross studied. In cross 4 (Table 2), tetrads segregating 4:4, 3:4, 4:3, and 3:5 were obtained for both cultivars. These segregations show that the avirulent parent 2/0/3 differs from GUY11 by only one avirulence gene. In cross 35, tetrads segregating 2:5, 4:4, 3:4, and 0:8 were obtained (Table 3). In cross 36, only tetrads segregating 2:5 and 1:6 were recovered. With the same assumption about missing ascospores for cultivar Cica 6, one can group tetrads from crosses 35 and 36 into three types of tetrads. These tetrads can best be explained by the segregation of two avirulence genes, each gene alone being able to confer avirulence, as already postulated for avirulence to cultivar Cica 6. Avirulence of an isolate can be due to the presence of one of the two avirulence genes in the avirulent allelic form, or by both. Virulence can be caused only by the presence of both avirulence genes in the virulent allelic form. Because of the small number of tetrads obtained, we cannot conclude that those two genes are independent. According to the interpretation of these segregations, avirulent parents differ by their genotypes: isolate 2/0/3 has only one gene conferring avirulence to cultivars Cica 8 and Med Noï, whereas isolates 32/0/14 and 32/0/19 have two avirulence genes each.

Overall, these results show that avirulence of *M. grisea* isolates to a particular rice cultivar could be controlled either by one gene (avirulence of 32/0/14, 32/0/19, and 2/0/3 to cultivar Ku 86; avirulence of 2/0/3 to cultivars Cica 8 and Med Noï; and avirulence of GUY11 to cultivars DJ 8-341 and IRAT 7) or by two independent genes, each gene alone conferring avirulence (avirulence of isolates 32/0/14, 32/0/19, and 2/0/3 to cultivar

TABLE 1. Characteristics of the eight rice cultivars and their reaction to four *Magnaporthe grisea* isolates

Cultivar	Origin	Morphological type[a]	Enzymatic group[b]	*M. grisea* isolate[c]			
				GUY11	2/0/3	32/0/14	32/0/19
Cica 6	Colombia	Indica G5	I	S	R	R	R
Cica 8	Colombia	Indica G5	I	S	R	R	R
Ku 86	Thailand	Special javanica	VI	S	R	R	R
IRAT 7	Senegal	Half dwarf indica	I	R	S	S	S
DJ 8-341	Senegal	Half dwarf indica	I	R	S	S	S
Tetep	Vietnam	Indica G5	I	S	S	R	S
Med Noï	Laos	ND[d]	I	S	R	R	R
Maratelli	Italy	Japonica G2	VI	S	S	S	S

[a] Groups defined by Jacquot and Arnaud (8).
[b] Groups defined by Glaszmann (5).
[c] S = susceptible and R = resistant.
[d] Not determined.

TABLE 2. Segregation for avirulence to rice cultivars Ku 86, Cica 6, Cica 8, Med Noï, DJ 8-341, and IRAT 7 of tetrads isolated from the cross number 4 (2/0/3 × GUY11)

Isolates	Mating type	Ku 86	Cica 6	Cica 8	Med Noï	IRAT 7	DJ 8-341	Maratelli
2/0/3[a]	MAT1-1	2[b]	2	2	2	**6**	**6**	**6**
GUY11	MAT1-2	**6**	**6**	**6**	**6**	2	2	**6**
4/1/5	MAT1-1	3	3	**6**	**5**	2	2	**6**
4/1/8	MAT1-1	3	3	**5**	**5**	2	2	**6**
4/1/6	MAT1-1	**4**	**4**	**5**	**5**	2	2	**6**
4/1/7	MAT1-1	**4**	**4**	**5**	**5**	2	2	**6**
4/1/1	MAT1-2	**4**	2	2	2	**4**	**4**	**6**
4/1/3	MAT1-2	**4**	2	2	2	**4**	**4**	**6**
4/1/2	MAT1-2	2	2	2	2	**4**	**4**	**6**
4/1/4	MAT1-2	1	1	2	2	**4**	**4**	**6**
4/2/2	MAT1-1	**6**	**6**	**5**	**6**	2	2	**6**
4/2/4	MAT1-1	**6**	**6**	**5**	**6**	2	2	**6**
4/2/3	MAT1-1	**5**	3	2	3	2	2	**6**
4/2/5	MAT1-1	**5**	3	3	3	2	2	**6**
4/2/6	MAT1-2	2	2	2	3	**4**	**5**	**6**
4/2/1	MAT1-2	2	2	2	2	**4**	**5**	**6**
4/2/7	MAT1-2	1	1	**5**	**5**	**4**	**5**	**6**
4/2/8	MAT1-2	NT[c]	NT	NT	NT	NT	NT	**6**
4/3/1	MAT1-1	**4**	2	2	3	2	2	**6**
4/3/4	MAT1-1	**5**	2	2	3	2	2	**6**
4/3/2	MAT1-1	1	2	**5**	**5**	2	2	**6**
4/3/3	MAT1-1	1	1	**5**	**5**	2	2	**6**
4/3/5	MAT1-2	2	1	**4**	**5**	**4**	**5**	**6**
4/3/6	MAT1-2	3	3	**4**	**5**	**4**	**5**	**6**
4/3/7	MAT1-2	**5**	2	2	2	**5**	**4**	**6**
4/3/8	MAT1-2	**5**	1	2	1	**5**	**4**	**6**
4/4/1	MAT1-1	**4**	3	2	2	2	2	**6**
4/4/2	MAT1-1	**4**	2	2	3	2	2	**6**
4/4/4	MAT1-1	**5**	**5**	**6**	**5**	**4**	**5**	**6**
4/4/5	MAT1-2	1	1	**4**	**5**	**4**	**5**	**6**
4/4/7	MAT1-2	1	3	**4**	**5**	**4**	**5**	**6**
4/4/6	MAT1-2	1	1	2	2	2	2	**6**
4/4/3	MAT1-2	1	1	2	2	2	2	**6**
4/5/6	MAT1-1	1	1	2	3	2	2	**6**
4/5/7	MAT1-1	1	2	2	3	2	2	**6**
4/5/2	MAT1-1	3	1	**5**	**6**	**4**	**5**	**6**
4/5/3	MAT1-2	**5**	2	3	3	2	3	**6**
4/5/4	MAT1-2	**5**	2	3	3	2	2	**6**
4/5/1	MAT1-2	**5**	**5**	**6**	**6**	**5**	**5**	**6**
4/5/5	MAT1-2	**5**	**6**	**5**	**5**	**5**	**5**	**6**
4/6/3	MAT1-1	3	2	**5**	**5**	2	2	**6**
4/6/4	MAT1-1	2	3	**6**	**5**	2	2	**6**
4/6/5	MAT1-1	**4**	2	2	2	**4**	**5**	**6**
4/6/6	MAT1-1	**4**	1	2	2	**4**	**5**	**6**
4/6/7	MAT1-2	**5**	1	1	2	2	2	**6**
4/6/8	MAT1-2	**5**	2	2	2	2	2	**6**
4/6/1	MAT1-2	2	1	**5**	**5**	**4**	**5**	**6**
4/6/2	MAT1-2	2	2	**5**	**5**	**4**	**6**	**6**
4/7/2	MAT1-1	1	1	1	2	**4**	**5**	**6**
4/7/7	MAT1-1	3	1	2	2	**4**	**4**	**6**
4/7/6	MAT1-1	**5**	**5**	**6**	**5**	**4**	**6**	**6**
4/7/3	MAT1-1	**5**	**5**	**6**	**5**	**4**	**5**	**6**
4/7/4	MAT1-2	**5**	3	3	3	2	2	**6**
4/7/5	MAT1-2	**5**	2	3	3	2	2	**6**
4/7/1	MAT1-2	3	1	**5**	**5**	2	2	**6**
4/7/8	MAT1-2	1	3	**4**	**4**	2	2	**6**
4/8/2	MAT1-1	1	2	2	2	2	2	**6**
4/8/4	MAT1-1	1	1	2	2	2	2	**6**
4/8/5	MAT1-1	1	2	2	2	2	2	**6**
4/8/6	MAT1-1	**4**	**5**	**5**	**5**	**5**	**5**	**6**
4/8/7	MAT1-2	**4**	**5**	**5**	**5**	2	2	**6**
4/8/8	MAT1-2	**4**	**5**	**5**	**5**	2	2	**6**
4/8/1	MAT1-2	1	2	2	2	**4**	**5**	**6**
4/8/3	MAT1-2	1	1	2	2	**4**	**5**	**6**
4/13/3	MAT1-1	1	1	1	3	**5**	**5**	**6**
4/13/4	MAT1-1	1	1	2	3	**5**	**5**	**6**
4/13/5	MAT1-1	**4**	**4**	**5**	**5**	2	2	**6**
4/13/7	MAT1-1	**4**	**4**	**5**	**5**	2	2	**6**
4/13/1	MAT1-2	**5**	2	3	3	2	2	**6**
4/13/2	MAT1-2	**4**	2	2	2	2	2	**6**
4/13/6	MAT1-2	2	2	**6**	**6**	**5**	**5**	**6**

[a] Isolate designation for progeny from crosses. The first number represents the cross number; the second, the tetrad number (0 when random); and the third, the ascospore number.

[b] Disease ratings on a 6-class scale (18). An isolate is considered virulent when it causes lesions scored 4–6 (in bold), whereas lower disease ratings correspond to avirulent isolates.

[c] Not tested.

Cica 6 and avirulence of 32/0/14 and 32/0/19 to cultivars Cica 8 and Med Noï).

Attempted linkage relationships between avirulence genes. We tested linkages between the avirulence genes or between avirulence genes and the mating type gene (MAT1).

All the progeny from crosses 4, 35, and 36 gave the same responses to cultivars Cica 8 and Med Noï (i.e., all progeny avirulent to Cica 8 are also avirulent to Med Noï, and all progeny virulent to Cica 8 are virulent to Med Noï). Similarly, on cultivars DJ 8-341 and IRAT 7 all progeny from cross 4 gave the same responses. These results can be explained by the fact that the same avirulence gene can control avirulence to different cultivars, but strong linkage between avirulence genes cannot be definitely ruled out because of the small number of tetrads studied. Linkages between one avirulence gene to Cica 6 and one avirulence gene to Cica 8 and between MAT1 and one avirulence gene to Ku 86 seem to exist, but confirmation tests are needed to strengthen these hypotheses.

According to the results obtained for the three crosses, and if we assume that genes completely linked can be considered as only one gene, at least six avirulence genes can be described: one for avirulence to Ku 86, one for avirulence to IRAT 7 and DJ 8-341, one for avirulence to Tetep, two for avirulence to Cica 6, and one for avirulence to Cica 8 and Med Noï (the second gene controlling avirulence to Cica 8 and Med Noï is probably also controlling avirulence to Cica 6).

DISCUSSION

Among the 18 tetrads studied, tetrad 4/8 segregated 3:5 (virulent/avirulent) for virulence to the seven cultivars examined. One ascospore seems to have lost its virulence to all resistant cultivars tested, but not its pathogenicity (lesions scored 6 on susceptible cultivar Maratelli). A pleiotropic mutation can account for this abnormal phenotype. In other studies (12,18), such cases were also recorded. Studying these mutants may provide useful

TABLE 3. Segregation for avirulence to rice cultivars Ku 86, Cica 6, Cica 8, Med Noï, Tetep, and Maratelli of tetrads isolated from the cross number 35 (32/0/14 × GUY11)

Isolates	Mating type	Ku 86	Cica 6	Cica 8	Med Noï	Tetep	Maratelli
GUY11	MAT1-2	**6**[a]	**6**	**6**	**6**	**6**	**6**
32/0/14[b]	MAT1-1	1	1	2	1	2	**6**
35/1/1	MAT1-1	**5**	1	1	1	1	**6**
35/1/6	MAT1-1	3	1	3	2	**4**	**6**
35/1/7	MAT1-1	3	1	3	2	**6**	**6**
35/1/2	MAT1-2	1	1	1	1	3	**6**
35/1/5	MAT1-2	3	1	3	3	2	**6**
35/1/3	MAT1-2	**5**	2	3	3	**6**	**6**
35/1/4	MAT1-2	**5**	2	3	3	**6**	**6**
35/2/1	MAT1-2	**5**	1	2	2	**6**	**6**
35/2/2	MAT1-2	**5**	2	2	2	**6**	**6**
35/2/3	MAT1-2	1	2	**5**	**6**	**6**	**6**
35/2/4	MAT1-2	1	2	**5**	**6**	**6**	**6**
35/2/5	MAT1-1	2	1	1	1	2	**6**
35/2/6	MAT1-1	**5**	1	2	1	3	**6**
35/2/7	MAT1-1	**5**	1	2	1	2	**6**
35/3/3	MAT1-1	**4**	1	1	1	3	**6**
35/3/4	MAT1-1	**4**	1	2	1	2	**6**
35/3/5	MAT1-1	**5**	1	2	1	2	**6**
35/3/6	MAT1-1	**5**	1	2	1	2	**6**
35/3/7	MAT1-2	3	3	**5**	**6**	**6**	**6**
35/3/8	MAT1-2	3	2	**5**	**6**	**6**	**6**
35/3/1	MAT1-2	2	2	**5**	**6**	**6**	**6**
35/3/2	MAT1-2	2	3	**5**	**6**	**6**	**6**
35/4/1	MAT1-1	3	1	2	1	3	**6**
35/4/2	MAT1-1	3	1	1	1	2	**6**
35/4/7	MAT1-1	**5**	**5**	**5**	**6**	**6**	**6**
35/4/5	MAT1-1	**5**	**6**	**5**	**6**	**6**	**6**
35/4/6	MAT1-2	2	1	1	1	2	**6**
35/4/4	MAT1-2	1	1	1	1	3	**6**
35/4/3	MAT1-2	**5**	2	3	3	**6**	**6**
35/5/1	MAT1-1	**5**	2	3	3	**6**	**6**
35/5/4	MAT1-1	**5**	2	3	3	**6**	**6**
35/5/7	MAT1-1	3	2	1	1	1	**6**
35/5/5	MAT1-2	2	2	1	2	2	**6**
35/5/3	MAT1-2	1	1	1	1	2	**6**
35/5/6	MAT1-2	**5**	**5**	**5**	**5**	**6**	**6**
35/5/2	MAT1-2	**5**	**5**	**5**	**5**	**6**	**6**
35/6/2	MAT1-1	**5**	1	1	2	NT[c]	**6**
35/6/3	MAT1-1	**5**	1	1	2	NT	**6**
35/6/4	MAT1-1	2	1	1	2	NT	**6**
35/6/6	MAT1-1	2	1	1	2	NT	**6**
35/6/1	MAT1-2	**6**	**6**	**6**	**6**	NT	**6**
35/6/5	MAT1-2	2	2	**4**	**5**	NT	**6**
35/6/7	MAT1-2	2	2	**5**	**6**	NT	**6**

[a] Disease ratings on a 6-class scale (18). An isolate is considered virulent when it causes lesions scored 4–6 (in bold), whereas lower disease ratings correspond to avirulent isolates.

[b] Isolate designation for progeny from crosses. The first number represents the cross number; the second, the tetrad number (0 when random); and the third, the ascospore number.

[c] Not tested.

information on the process of recognition between the host and the pathogen. It would be of interest to know how genetic changes in an isolate can induce avirulence to various cultivars, which probably carry various resistance genes.

Since Flor (4) postulated the gene-for-gene hypothesis for interactions between avirulent pathogens and resistant cultivars, it has been applied to many pathosystems (2). In many cases, this hypothesis is not demonstrated because studies of the heredity of avirulence have yet to be undertaken. But in another study (18), we have shown that Flor's hypothesis could be applied to the *Oryza sativa-M. grisea* pathosystem.

Monogenic control has been demonstrated for avirulence to cultivars DJ 8-341, IRAT 7, Ku 86, and Tetep. Various authors have already shown that the avirulence of *M. grisea* to different rice cultivars is controlled by single genes (3,12,18,20).

We have also shown that avirulence to rice cultivars Cica 6, Cica 8, and Med Noï were controlled by two avirulence genes acting independently. If the gene-for-gene hypothesis is valid for these cultivars, each avirulence gene might correspond to a specific resistance gene in rice cultivars and, therefore, these cultivars should have two different resistance genes. As far as we know, this is the first report of such genetic control of avirulence in *M. grisea*.

Each cross studied could separately describe avirulence genes. In this case, at least 16 avirulence genes could be described. But probably fewer are involved in avirulence to the seven rice cultivars studied. This assumption is supported by two arguments. First, if the gene-for-gene hypothesis is valid, the number of specific resistance genes per cultivar would be particularly high (six for example for cultivar Cica 6). Until now, genetic studies of specific resistance to *M. grisea* have shown that rice cultivars carry few resistance genes (to date no more than three have been reported; see ref. 15 for review). Second, parental isolates 2/0/3, 32/0/14, and 32/0/19 are derived from a unique cross (GUY11 × ML 25). More precisely, 32/0/14 and 32/0/19 are progenies from the same cross. Therefore, it can be assumed that, because of their genetic relatedness, these isolates share the same avirulence genes. So, we propose that at least six avirulence genes are involved in the genetic control of avirulence to the seven rice cultivars studied.

Resistance genes of all these cultivars have not yet been determined for the avirulent isolates we have studied. But we may hypothesize that, if Flor's gene-for-gene hypothesis is valid for all these cultivars, resistance should be controlled by one gene in DJ 8-341, IRAT 7, Ku 86, and Tetep, and by two genes in Cica 6, Cica 8, and Med Noï.

Linkages between avirulence genes have already been reported by other authors (12,20). In this study, complete linkage between avirulence genes was observed. The simplest explanation is that one fungal gene can control avirulence to various cultivars that share the same resistance gene (in a gene-for-gene hypothesis). This hypothesis can be tested by studying the heredity of resistance in these cultivars.

Our study shows that it is of importance to know genotypes of fungal isolates when studying inheritance of resistance in rice cultivars. A good example is given by isolates 2/0/3 and 32/0/14. Both are avirulent to Cica 8 and may be used to study resistance of this cultivar by inoculating an F_2 progeny obtained from a cross between Cica 8 (resistant cultivar) and a susceptible cultivar (susceptible to 2/0/3 or 32/0/14). If Cica 8 carries multiple resistance genes, only one will be detected with isolate 2/0/3, because this isolate has only one avirulence gene. Whereas isolate 32/0/14 will allow identification of two resistance genes. Therefore, characterization of resistance genes strongly depends on the choice of isolates, and genetic analysis of avirulence in isolates is an important step for such studies.

TABLE 4. Segregation for avirulence to rice cultivars Ku 86, Cica 6, Cica 8, Med Noï, and Maratelli of tetrads isolated from the cross number 36 (32/0/19 × GUY11)

Isolates	Mating type	Ku 86	Cica 6	Cica 8	Med Noï	Maratelli
GUY11	MAT1-2	**6**[a]	**6**	**6**	**6**	**6**
32/0/19[b]	MAT1-1	3	2	1	1	**6**
36/1/3	MAT1-1	3	1	1	1	**6**
36/1/6	MAT1-1	1	1	3	2	**6**
36/1/4	MAT1-1	**5**	1	2	1	**6**
36/1/5	MAT1-1	**5**	1	3	1	**6**
36/1/1	MAT1-2	**5**	**5**	**5**	**5**	**6**
36/1/7	MAT1-2	**6**	**6**	**6**	**6**	**6**
36/1/2	MAT1-2	2	**5**	1	3	**6**
36/2/1	MAT1-1	**6**	1	2	2	**6**
36/2/2	MAT1-1	**6**	1	2	2	**6**
36/2/5	MAT1-1	1	2	2	3	**6**
36/2/7	MAT1-1	2	2	2	3	**6**
36/2/3	MAT1-2	3	1	1	2	**6**
36/2/4	MAT1-2	1	1	1	2	**6**
36/2/6	MAT1-2	**6**	**6**	**6**	**5**	**6**
36/3/1	MAT1-2	2	1	**5**	**5**	**6**
36/3/2	MAT1-2	2	2	**5**	**5**	**6**
36/3/6	MAT1-2	2	1	1	1	**6**
36/3/3	MAT1-1	**5**	1	2	1	**6**
36/3/4	MAT1-1	**5**	1	2	1	**6**
36/3/5	MAT1-1	3	2	2	2	**6**
36/3/7	MAT1-1	1	1	1	1	**6**

[a] Disease ratings on a 6-class scale (18). An isolate is considered virulent when it causes lesions scored 4–6 (in bold), whereas lower disease ratings correspond to avirulent isolates.

[b] Isolate designation for progeny from crosses. The first number represents the cross number; the second, the tetrad number (0 when random); and the third, the ascospore number.

TABLE 5. Classification of tetrads from crosses 4, 35, and 36 by parental ditype, nonparental ditype, and tetratype

	Cross 4		Cross 35		Cross 36	
Rice cultivars	GUY11 × 2/0/3		GUY11 × 32/0/14		GUY11 × 32/0/19	
Cica 6	PD[a]	1	PD	0	PD	0
	NPD	2	NPD	3	NPD	2
	T	6	T	3	T	1
Cica 8	PD	9	PD	1	PD	0
	(one gene)		NPD	1	NPD	1
			T	4	T	3
Med Noï	PD	9	PD	1	PD	0
	(one gene)		NPD	1	NPD	0
			T	4	T	3

[a] PD = parental ditype; NPD = nonparental ditype; T = tetratype.

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