Genetics

Inheritance of Avirulence/Virulence in a Cross of Two Isolates of *Magnaporthe grisea* Pathogenic to Rice

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ABSTRACT

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A cross was made between two isolates, Guy 11 and 6-28, of *Magnaporthe grisea* that are pathogenic to rice. Progenies were tested for avirulence/virulence on eight rice cultivars. Both parents and all progeny were virulent on rice cultivar S201. Isolates Guy 11 and 6-28 differed in virulence on seven cultivars. Segregation among the progenies for avirulence/virulence on the seven cultivars indicated that at least seven genes were segregating.

Additional keywords: genetics of host-parasite interactions, *Oryza sativa, Pyricularia oryzae.*

Magnaporthe grisea (Hebert) Barr (*Pyricularia oryzae* Cavara) is economically one of the most important pathogens of rice (*Oryza sativa* L.). The disease, rice blast, is primarily controlled 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 *Magnaporthe*, and, consequently, the previously resistant cultivars have become susceptible (8). The genetic basis of the variability in pathogenicity to rice in *M. grisea* is poorly understood, primarily because of the difficulties in making crosses between isolates pathogenic on rice.

The discovery of the sexual stage of *M. grisea* (4) has led to research efforts to bring the capacity to go through the sexual cycle into isolates of *Magnaporthe* that are pathogenic on rice. Isolates pathogenic on rice have been crossed with isolates pathogenic on other grass species, and the segregation for pathogenicity on rice and other grass species reported (5,7,9,10). We have made similar crosses and have used sib selection for fertility and pathogenicity on rice in several generations (5). Several strains were developed that were pathogenic on rice and also able to intermate (1,5,7). The ratios of pathogenic:nonpathogenic progeny were commonly variable and not consistent with any particular inheritance pattern (1). Therefore, a series of crosses was made between

isolates that are pathogenic on rice obtained in our breeding program with isolates obtained from other sources. One isolate, Guy 11, isolated from rice in Guyana by Dr. J. L. Notteghem, has proved particularly useful. Crosses between Guy 11 and many of our isolates that are pathogenic on rice have usually given some progeny that are not pathogenic on rice. However, when Guy 11 was crossed with progeny from one cross, cross 6, all progeny were pathogenic on the same rice cultivars to which both parents were pathogenic (1). Segregation occurred on several cultivars where the parents differed. The segregation of avirulence/virulence on several cultivars of rice among the progeny of culture 6-28 crossed with Guy 11 is presented in this paper.

MATERIALS AND METHODS

Isolate Guy 11 was obtained from Drs. H. Leung and Sally Leong, with Dr. J. L. Notteghem's permission. The remainder of the cultures have been generated in our research program (1,5).

Crosses were made by placing inoculum of each parent about 3 cm apart on oatmeal agar plates (6). Perithecia usually formed in 2–3 wk. Individual perithecia were crushed between two needles and dragged across the surface of Sc complete agar media (2). Broken perithecia left a trail of asci, which were then separated with a glass needle. Twenty four hours later individual asci from which germ tubes were emerging were transferred to complete agar media. Two to four days later, the conidia were scraped from the colonies with a needle and spread on agar media. Single germinated conidia were isolated 4–6 hr later. Though each conidium is typically three celled, the nuclei have their origin in a single nucleus in the conidiophore (3). Therefore, this procedure gave a single product from each meiotic event. Cultures were stored both on agar slants at 4 C and as dried cultures at −20 C.

Tests of pathogenicity and virulence. Conidia for inoculations were produced following growth of the fungus on autoclaved corn leaves in petri dishes for 5–15 days. Conidia were washed off the leaves by gentle rubbing of the leaves with a glass rod after flooding the plate. Approximately 10^5 conidia per milliliter were atomized onto leaves of plants. Inoculated plants were placed in a dew chamber for 24 hr at 24 C in the dark and then placed in a growth chamber at 24 C with 16 hr of light per day. The reactions of the cultivars were recorded 7 days after inoculation. At least three plants of each cultivar were inoculated with each isolate at each inoculation time. Inoculations were made at least three times. The reaction classes were, 1 = minute black spots, 2 = 2–3-mm length black lesions, 3 = usually circular lesions with gray centers, lesion size quite variable, and 4 = large, commonly diamond shaped lesions with grey centers, commonly coalescing into long stripes with gray centers. Seed of the rice cultivars was kindly provided by M. A. Marchetti, USDA Rice Research, Beaumont, TX 77706.

RESULTS

The infection types on eight rice cultivars following inoculation with the two parent isolates, Guy 11 and 6-28, are given in Table 1. Guy 11 was virulent (infection types 3 and 4) on all cultivars. Isolate 6-28 was virulent on S201 but avirulent (infection types 1 to 2) on the other eight rice cultivars. All 57 progenies from the cross were virulent on S201.

The number of progenies that gave particular infection types on each cultivar is given in Table 1. Thirty progenies gave only black lesions (infection types 1 or 2) and 25 progenies gave either circular or angular lesions with gray centers (infection types 3 or 4) on cultivar L202. The reaction with two isolates was not determined (see Table 2). These two classes of progenies are clearly distinguishable. There was no overlap in phenotypes. Twenty-three progenies gave only low infection type on cultivar Leah and 23 progenies gave only high infection types on Leah. Four progenies gave lesions of infection types 1–3 on Leah. These progenies gave primarily lesions of infection type 1, but an occasional plant had a small round lesion with a grey center. The two progenies with infection types 1–4 and 2–3 also had predominant low infection types. The six progenies with maximum infection type ranges of 1–3, 1–4, and 2–3 are considered avirulent. The six are also clearly different from the three progenies listed as giving infection types 2-4, where the predominant infection type was 3-4, and classified as virulent.

When the data are presented as the maximum range of infection types, it appears that there is a continuous variation in virulence on cultivars Leah, Brazos, Aichi-Asahi, Bluebelle and Newbonnet, and Lemont. However, on each of these cultivars, except Lemont, there is a clear qualitative difference between progenies rated as virulent or avirulent on each cultivar. The segregation ratios presented in Table 1 are based on these differences. The segregation on Lemont is not as clear. Four progenies were rated as infection type 2-3. They contained both infection types in approximately equal frequencies, and this intermediate phenotype was consistent among plants in each replication of the inocu-

TABLE 1. The reaction of eight rice cultivars to inoculation with isolates Guy 11 and 6-28 of *Magnaporthe grisea* and the numbers of progeny with a particular range[a] of infection types on each cultivar

Rice cultivar	Parents: Guy 11	Parents: Guy 28	Infection type: 0-1	1-2	2	1-3	1-4	2-3	2-4	3	3-4	4	Ratio avirulent: virulent	χ^2 for 1:1 ratio[b]
S-201	3-4	3-4	0	0	0	0	0	0	0	0	17	40	0:57	
L202	3-4	1	22	7	1	0	0	0	0	2	5	18	30:25	0.45
Leah	3-4	1-2	19	4	0	4−[c]	1−	1−	3+[d]	0	6	17	29:26	0.16
Brazos	3-4	1-2	11	10	3	2−	1+	1− 3+	6+	3	8	7	27:28	0.01
Aichi-Asahi	3-4	1	13	2	0	3− 1+	1− 3+	0	5+	1	10	16	19:36	5.25*
Bluebelle	3-4	1-2	16	11	1	1−	2−	4+	1− 4+	0	4	11	32:23	1.47
Newbonnet	3-4	1	31	6	0	1+	0	1+	2+	1	5	9	37:19	6.56*
Lemont	3-4	1-2	12	6	2	3−	1+	5	1− 2+	2	8	14	24:27 (:5 intermediate)	

[a]The maximum range of infection types in a minimum of three replicates.
[b]* significant at $P = 0.05$.
[c]The range of infection types with these four progeny was 1-3. The minus indicates that the predominant infection type was the low reading, 1, but one or more replicates had one or few lesions of infection type 3.
[d]The range of infection types was 2-4. The plus indicates that the predominant infection type was for virulence, but one or more replicates had few lesions of low infection type.

lations. Segregation among the progenies could not be classified into only two categories for interaction with cultivar Lemont.

Table 2 gives a summary of the kinds of progeny obtained, and their frequencies. In all pairwise comparisons of the progeny for avirulence/virulence on the seven rice cultivars, there is at least one progeny that is virulent on one cultivar and not on the other, and vice versa. This suggests that the control of avirulence/virulence on each rice cultivar is controlled by a different gene. If seven genes (and possibly eight to explain the third phenotype on Lemont) were segregating, many types of progeny would be expected, and 31 types were recovered (Table 2).

DISCUSSION

Previous research in our laboratory concentrated on the development of isolates of *Magnaporthe* that are sexually compatible and pathogenic to rice (1,5). These isolates were derived from crosses between isolates of *Magnaporthe* pathogenic on rice with isolates of *Magnaporthe* from other grass species. Through sib selection for fertility and pathogenicity on rice it was possible to develop isolates that were sexually compatible and pathogenic on rice (1,5). However, crosses between these isolates, both of which were pathogenic on rice, yielded some progeny that were not pathogenic on rice (1). The genetic basis of progeny nonpathogenic on rice when both parents were pathogenic on rice is unknown. Intercrosses among isolates pathogenic on rice from the pedigrees of sib selections have invariably led to recovery of some progeny that are nonpathogenic (1,5). However, when the progeny of cross 6 that were pathogenic on rice were crossed with Guy 11 (also pathogenic on rice), all progeny were pathogenic on the cultivars to which both parents were pathogenic (1). Cultures 6-28 and Guy 11 were both pathogenic on six cultivars and all progeny were pathogenic and virulent on those cultivars (1). It is with this base for comparison that the segregation of avirulence/virulence on other cultivars was studied.

The recovery of 21 progeny with parental patterns of avirulence/virulence on the eight cultivars indistinguishable from the parents was surprising (Table 2). One possible explanation is that an undetected conidium was associated with some isolated asci and gave rise to the single conidial isolate. This seems highly unlikely because asci are usually separated from each other by moving them across the surface of the agar media with a glass needle, and a conidium adjacent to an ascus is easily visible under the microscope. A second possibility is that spores with parental combinations of genes are preferentially recovered with the procedures used here to recover one progeny from each meiotic event. There may also be preferential segregation in meiosis.

The ratios of the alleles for avirulence and virulence are approximately 1:1. If there is selection for the recovery of particular genotypes, it is probably not at the level of the individual alleles segregating, but particular combinations of alleles. The data suggest that a single locus controls avirulence/virulence on each of seven cultivars (and possibly a second locus for interactions with Lemont). This conclusion is based not so much on the segregation ratios (which vary considerably) as on the observation that there are only two easily discernible infection types among the progeny for reaction on each of six of the seven cultivars. In all pairwise comparisons for avirulence on one cultivar and virulence on the other, and vice versa, both recombinant types were recovered (Table 2).

TABLE 2. Avirulence/virulence of progeny of cross 14, Guy 11 × 6-28, of *Magnaporthe grisea*

	Rice cultivars								
	S201	L202	*Leah*	Brazos	Aichi-*Asahi*	Bluebelle	Newbonnet	Lemont	
Guy 11	+[a]	+	+	+	+	+	+	+	
6-28	+	−	−	−	−	−	−	−	No. of progeny
	+	+	+	+	+	+	+	+	12
	+	−	−	−	−	−	−	−	9
	+	−	−	−	+	−	−	−	2
	+	−	−	−	+	−	−	−	1
	+	−	−	−	+	−	−	+	2
	+	−	−	−	+	−	−	±	1
	+	−	−	+	−	−	−	−	1
	+	−	−	−	−	−	−	+	1
	+	−	−	−	−	−	−	±	1
	+	+	−	−	−	−	−	+	1
	+	+	+	+	+	+	−	+	1
	+	+	+	+	+	+	−	−	1
	+	−	−	+	−	−	−	±	1
	+	−	−	+	−	−	−	+	1
	+	−	−	−	−	+	−	+	1
	+	−	+	−	−	−	+	+	1
	+	+	−	+	+	+	−	−	1
	+	−	−	+	−	−	−	+	1
	+	+	+	+	+	+	−	−	4
	+	+	+	−	+	+	+	±	2
	+	−	+	+	−	−	−	+	1
	+	−	−	−	+	−	−	+	2
	+	+	+	+	+	−	+	−	1
	+	−	−	+	+	−	−	−	1
	+	+	+	+	+	−	+	+	1
	+	+	+	−	+	+	+	+	1
	+	−	−	ND	+	−	−	+	1
	+	−	ND	−	−	−	−	−	1
	+	ND	+	+	+	−	−	−	1
	+	−	−	−	ND	−	+	−	1
	+	ND	ND	ND	+	ND	ND	ND	1

[a]Infection types grouped as given in Table 1. + = virulent, − avirulent.
[b]ND = not determined.

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