Genetic Analysis of Host Species Specificity of *Magnaporthe oryzae* Isolates from Rice and Wheat

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ABSTRACT

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A *Triticum* isolate (pathogenic on wheat) of *Magnaporthe oryzae* was crossed with an *Oryza* isolate (pathogenic on rice) to elucidate mechanisms of their parasitic specificity on wheat. When the pathogenicity of their F₁ cultures (hybrids between a *Triticum* isolate and an *Oryza* isolate) was tested on wheat, avirulent and virulent cultures segregated in a 7:1

ratio. This result suggests that three loci are involved in avirulence of the *Oryza* isolate on wheat. One of the three loci conditioned papilla formation, whereas the others conditioned the hypersensitive reaction. Allelism tests revealed that the locus conditioning papilla formation is *Pwt2* while one of the two loci conditioning the hypersensitive reaction is *Pwt1*. The other locus conditioning the hypersensitive reaction was different from any other known loci and, therefore, was designated as *Pwt5*.

Additional keyword: *Pyricularia*.

Magnaporthe oryzae (1) (anamorph, *Pyricularia oryzae*) is a causal agent of blast disease on gramineous plants. This species is isolated mainly from staple crops such as rice (*Oryza sativa*), foxtail millet (*Setaria italica*), common millet (*Panicum miliaceum*), finger millet (*Eleusine coracana*), oat (*Avena sativa*), and wheat (*Triticum aestivum*). Isolates from each crop are almost exclusively pathogenic on their original host genus (6,8). Kato et al. (6) called this specific parasitism plant species specificity in the blast system following the definition by Heath (3).

M. oryzae isolates with different host ranges are interfertile (6) and, therefore, are amenable to genetic analysis of the plant species specificity. Yaegashi (14) crossed *Eleusine* isolates with weeping lovegrass isolates, and suggested that their specific parasitism on finger millet or weeping lovegrass was conditioned by a single gene. Valent and her coworkers identified major genes controlling avirulence of an *Eleusine* isolate and an *Oryza* isolate on weeping lovegrass, and designated them as *PWL1* and *PWL2*, respectively (13). The cloned *PWL1* and *PWL2* genes resembled classical avirulence genes in that they were highly specific and dominant (4,9). However, weeping lovegrass is a weed that exhibits complicated specificity with *M. oryzae* isolates. For example, it is resistant to some isolates from *Oryza*, *Setaria*, and *Eleusine* and susceptible to other isolates from these three plant genera (6). Genetic mechanisms of plant species specificity on staple crops have not been elucidated.

Our laboratory has performed "pivot turn crossing" for genetic analyses of the plant species specificity (7,10). We chose a *Triticum* isolate (Br48) as the pivot because it is highly fertile with other isolates of *M. oryzae* (unpublished data). Murakami et al. (7) crossed the *Triticum* isolate with a *Setaria* isolate (GFS11-7-2) and produced 80 F₁ cultures. Segregation analyses of the F₁ cultures showed that avirulence of the *Setaria* isolate on wheat is governed by two loci, *Pwt1* and *Pwt2*. *Pwt1*, associated with the lesion color, primarily conditioned the hypersensitive reaction at the cytological level, whereas *Pwt2*, associated with the lesion

number, primarily conditioned papilla formation (7). Subsequently, Takabayashi et al. (10) crossed the *Triticum* isolate with an *Avena* isolate (Br58) and produced 88 F₁ cultures. Segregation analyses of the F₁ cultures showed that avirulence of the *Avena* isolate on wheat is governed by two other loci, *Pwt3* and *Pwt4*. The avirulence and virulence alleles at those four loci were designated as *PWT1* and *pwt1*, *PWT2* and *pwt2*, *PWT3* and *pwt3*, and *PWT4* and *pwt4*, respectively (10).

The most familiar and economically important isolates of *M. oryzae* are those from *Oryza* spp. In the present study, we crossed the *Triticum* isolates with an *Oryza* isolate from Indonesia and performed genetic analyses to elucidate mechanisms of avirulence of *Oryza* isolates on wheat.

MATERIALS AND METHODS

Fungal strains and genetic crosses. Parental field isolates were *M. oryzae* Br48 (*MAT1-1*) isolated from wheat in Brazil and PO12-7301-2 (*MAT1-2*) (5) isolated from rice in Indonesia. Br48 was virulent on wheat and avirulent on rice, whereas PO12-7301-2 was virulent on rice and avirulent on wheat. They were crossed on an oatmeal agar medium (20 g of oatmeal, 10 g of agar, and 2.5 g of sucrose in 500 ml of water) in a petri dish as described previously (7). The resulting F₁ ascospores were isolated from asci as described previously (7) and established as hybrids between a *Triticum* isolate and an *Oryza* isolate (TO cultures). For allelism tests, some of the TO cultures were crossed with 3K1, 3M1, 61M2, or 86Q1, hybrid cultures with known genotypes. Cultures 3K1 (*pwt1*; *PWT2*) and 3M1 (*PWT1*; *pwt2*) were F₁ cultures derived from the cross between Br48 and GFS11-7-2 (an isolate from foxtail millet collected in Japan) (7), and tentatively were designated as hybrids between a *Triticum* isolate and a *Setaria* isolate (TS cultures). Cultures 61M2 (*pwt3*; *PWT4*) and 86Q1 (*PWT3*; *PWT4*) were F₁ cultures derived from the cross between Br48 and Br58 (an isolate from oat collected in Brazil) (10), and tentatively were designated as hybrids between a *Triticum* isolate and an *Avena* isolate (TA cultures).

Plant material and infection assay. *T. aestivum* cv. Norin 4 was used as a test plant. Twelve seeds were sown in vermiculite in a seedling case of 5.5 by 15 by 10 cm, and grown at 22 to 23°C in

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a controlled-environment room with a 12-h photoperiod of fluorescent lighting for 7 to 9 days. Sporulation of *M. oryzae* cultures to be tested was induced as described previously (7). The conidia produced were suspended in water and adjusted to a concentration of 3×10^5 spores/ml with 0.01% Tween 20. The conidial suspension (10 ml/seedling case) was sprayed on the adaxial surface of wheat leaves with an air compressor. The inoculated seedlings were kept in a humid, dark box for 20 to 24 h at 22 to 23°C. They then were transferred to the controlled-environment room mentioned above and incubated further at 22 to 23°C. Five days after inoculation, symptoms were evaluated based on the color and number of lesions. The pathogenicity test was repeated at least three times.

Microscopic observation. The inoculated leaves were fixed in alcoholic lactophenol 48 h after inoculation and observed with a fluorescence microscope as described previously (7). A total of 200 appressoria on two leaves were counted for each culture. This experiment was repeated three times. The incidence of cellular responses was subjected to arcsine transformation and compared by a *t* test.

RESULTS

Production of F_1 cultures between Br48 and PO12-7301-2.

From the cross of the *Triticum* isolate Br48 with the *Oryza* isolate PO12-7301-2, a total of 111 F_1 cultures was obtained. These cultures were derived from 13 mature asci with 8 ascospores and 1 ascus with 7 ascospores. Ascus numbers from 41 through 54 were assigned to these 14 asci. A mature ascus of *M. oryzae* should contain four pairs of sister progenies. All the pairs could be determined easily by the appearance of their mycelial colonies on artificial media. The four pairs were designated by letters K, L, M, and N. By combining the ascus numbers and the letters, the F_1 cultures were designated as 41K1, 41K2, 41L1, 41L2, 41M1, 41M2, 41N1, 41N2, and so on. Here, cultures with the same letter (e.g., 41K1 and 41K2) are sister progenies of a pair.

Genetic analysis of pathogenicity of the F_1 cultures on wheat. Murakami et al. (7) found four lesion types on wheat leaves incubated at 25°C after inoculation: B–, discrete brown pinpoint lesions; B+, confluent brown lesions; G–, discrete green lesions; and G+, confluent green lesions. When the inoculated leaves were incubated at 20°C, the B–, B+, and G– types turned to a completely avirulent phenotype, whereas the G+ type remained a virulent phenotype (10). These results suggested that the B+ and G– types are attenuated phenotypes of avirulence caused by the increased temperature (10). Consequently, avirulence and virulence were discriminated more clearly at 20 than at 25°C. However, the B+ and G– phenotypes give us useful information because they are associated with different cellular reactions (hypersensitive reaction and papilla formation, respectively) and different genes (*PWT1* and *PWT2*, respectively) (7). In the inoculation test at 25°C, the two types of genes can be discriminated by phenotypes. On the other hand, the four types of reactions are sometimes unstable at 25°C because this temperature is the upper limit for expressing the resistant responses. When the inoculated leaves were incubated at more than 25°C, the B+ and G– types became indistinguishable from the G+ type. In the present study, therefore, we chose an intermediate temperature, 22 to 23°C, for the incubation of inoculated leaves.

Wheat cv. Norin 4 was inoculated with the parental field isolates, Br48 and PO12-7301-2, and incubated at 22 to 23°C. Br48 produced many water-soaked, green-to-pale green lesions by 3 days after inoculation. These lesions enlarged and coalesced with time, resulting in the shriveling of the whole leaf by 5 days after inoculation (Fig. 1). This phenotype corresponded to the typical G+ lesion. In contrast, PO12-7301-2 produced a few brown spots without visible centers (Fig. 1). This phenotype corresponded to the typical B– lesion. When Norin 4 was inoculated with the 111 TO

cultures derived from the cross between Br48 and PO12-7301-2, various degrees and types of infection were observed. The TO cultures included 17 cultures that induced no visible reactions on the wheat leaves. Preliminary cytological observation revealed that they had lost the basic capacity of penetration. These 17 cultures were excluded from further analysis. Phenotypes produced by the remaining 94 cultures were classified into the four types (i.e., B–, B+, G–, and G+) (Fig. 1). The B+ type reactions observed in the present study were determined easily as an avirulent phenotype due to the lower temperature during the incubation than that adopted by Murakami et al. (7) (Fig. 1). Similarly, the G– type observed in the present study was discriminated easily from the G+ type and determined as an avirulent phenotype (Fig. 1).

Fig. 1. Four lesion types on wheat cv. Norin 4 infected with *Magnaporthe oryzae* isolates PO12-7301-2, Br48, and their F_1 (45L1, 46N1, 47L1, and 45M1), 5 days after inoculation. B–, discrete brown lesions; B+, confluent brown lesions; G–, discrete green lesions; G+, confluent green lesions.

The avirulent (B–, B+, and G–) and virulent (G+) cultures segregated in a 7:1 ratio (Table 1), suggesting that three loci are involved in avirulence of PO12-7301-2 on Norin 4. The segregation of the brown types (B– and B+) and the green types (G– and G+) fit a 3:1 ratio (Table 1), suggesting that the lesion color is associated with two of the three loci. The avirulence and virulence alleles at these two loci were tentatively designated as *A1* and *a1*, and *A2* and *a2*, respectively. The segregation of the discrete types (B– and G–) and the confluent types (B+ and G+) fit a 1:1 ratio (Table 1), suggesting that the lesion number is associated with the remaining one locus. The avirulence and virulence alleles at this locus tentatively were designated as *B1* and *b1*. Finally, the segregation of the four lesion types fit a

3:3:1:1 ratio (Table 1), indicating that these three loci segregate independently.

Cytological responses associated with the three loci. To elucidate cellular responses conditioned by *A1*, *A2*, and *B1*, inoculated leaves were fixed 48 h after inoculation and observed under a fluorescence microscope. Cytological responses were categorized into four types, A, B, C, and D, as reported previously (7), where A = no reaction of leaf cells, B = the formation of a papilla with yellow fluorescence, C = the hypersensitive reaction of an epidermal cell accompanied by strong yellow fluorescence, and D = the development of infection hyphae with no resistance reactions.

The avirulent parent PO12-7301-2 induced papilla formation in a high percentage (74%) of appressorial penetration sites (Table 2).

TABLE 1. Segregation of lesion types on wheat cv. Norin 4 in F₁ cultures derives from the cross between *Magnaporthe oryzae* isolates, Br48 and PO12-7301-2

Number of F ₁ cultures ^a					χ^2 for ^b			
B–	B+	G–	G+	Total	1:1	3:1	7:1	3:3:1:1
36	38	10	10	94	...	...	...	0.75
74		20		94	31.0**	0.70	6.62*	...
46		48		94	0.04	34.06**	...	...
84		10		94	...	10.34**	0.30	...

^a B–, discrete brown lesions; B+, confluent brown lesions; G–, discrete green lesions; G+, confluent green lesions.

^b Significant at the 5% (*) and 1% (**) levels.

TABLE 2. Cellular responses of wheat primary leaves (cv. Norin 4) to *Magnaporthe oryzae* isolates Br48 and PO12-7301-2 and their F₁ cultures

Culture	Lesion type ^a	Percentage of germlings inducing ^b				HR ratio C/(C+D) ^c
		No reaction (A)	Papilla formation (B) ^c	HR (C) ^d	Hyphal growth (D)	
Parents						
PO12-7301-2	B–	20.2	74.0	5.8	0.0	100.0
Br48	G+	15.2	10.9	30.6	43.3	41.4
F ₁ cultures						
43K1	B–	7.3	58.4	26.2	8.1	76.5
43L1	B–	18.5	76.3	5.2	0.0	100.0
45L1	B–	5.1	94.5	0.4	0.0	100.0
46K1	B–	7.2	85.3	7.5	0.0	100.0
47K1	B–	26.7	71.3	2.0	0.0	100.0
48L1	B–	23.7	70.0	6.3	0.0	100.0
48M1	B–	9.8	83.3	6.9	0.0	100.0
49M2	B–	3.8	92.9	3.3	0.0	100.0
51L1	B–	24.2	72.1	3.7	0.0	100.0
51N1	B–	3.0	87.4	8.8	0.8	92.0
54M1	B–	1.9	54.9	39.3	3.9	91.1
43N2	B+	15.0	19.2	60.3	5.5	91.7
45N1	B+	6.7	12.4	80.2	0.7	99.1
46N1	B+	2.9	10.1	86.0	1.0	98.8
47M2	B+	1.7	14.4	83.9	0.0	100.0
47N2	B+	14.6	7.6	55.6	22.2	71.4
48K1	B+	9.8	18.8	66.9	4.5	93.7
49L2	B+	1.4	14.5	83.1	1.0	98.8
49N2	B+	1.9	12.5	85.6	0.0	100.0
51K1	B+	3.1	21.9	70.5	4.5	94.1
51M1	B+	2.9	20.2	75.7	1.2	98.4
54K1	B+	2.0	8.8	87.2	2.0	97.7
54L1	B+	6.1	14.9	68.8	10.2	87.1
45K1	G–	16.2	79.2	1.8	2.8	38.5
46M2	G–	2.9	94.7	0.8	1.6	33.3
47L1	G–	15.9	74.4	2.4	7.3	25.0
49K2	G–	4.8	90.9	0.7	3.6	16.7
54N1	G–	9.4	83.7	1.0	5.9	14.3
43M1	G+	5.2	11.9	11.9	71.0	14.4
45M1	G+	33.8	19.9	31.6	14.7	68.3
46L1	G+	49.5	13.1	25.3	12.1	67.6
48N2	G+	46.3	11.8	3.2	38.7	7.7

^a B–, discrete brown lesions; B+, confluent brown lesions; G–, discrete green lesions; G+, confluent green lesions.

^b Average of three replications.

^c Significantly high values are enclosed (Table 3).

^d HR = hypersensitive reaction.

Although 5.8% of germings were not stopped at the papilla stage, they subsequently induced the hypersensitive reaction (HR) and failed to colonize wheat leaves. The HR ratio ($C/[C + D]$) was 100%. However, in wheat leaves inoculated with the virulent parent Br48, the incidence of papilla formation and the HR ratio were much lower (10.9 and 41.4%, respectively). Consequently, almost half (43.3%) of the germings successfully colonized epidermal cells.

TABLE 3. Statistical analysis of relationships between lesion types and cellular responses

Lesion type ^a	No. tested ^b	Papilla formation (%) ^c		HR ratio ^c	
		Average	<i>t</i> value ^d	Average	<i>t</i> value ^d
B– and B+	23	44.4	0.44	95.2	9.11**
G– and G+	9	53.3	...	31.8	...
B– and G–	16	79.3	13.03**	74.2	0.09
B+ and G+	16	14.5	...	80.6	...

^a B–, discrete brown lesions; B+, confluent brown lesions; G–, discrete green lesions; G+, confluent green lesions.

^b Number of F₁ cultures tested.

^c Refer to Table 2.

^d Results of *t* test for group of two; ** indicates significant at the 1% level.

TABLE 4. Presumed genotypes of F₁ cultures derived from the cross between *Magnaporthe oryzae* isolates Br48 and PO12-7301-2

Ascus no., F ₁ culture	Lesion type ^a	Genotype ^b		
		<i>A1</i> ^c	<i>A2</i>	<i>B1</i>
43				
K1, K2	B–	ND	ND	+
L1, L2	B–	ND	ND	+
M1, M2	G+	–	–	–
N1, N2	B+	ND	ND	–
45				
K1, K2	G–	–	–	+
L1, L2	B–	+	+	+
M1, M2	G+	–	–	–
N1, N2	B+	+	+	–

^a B–, discrete brown lesions; B+, confluent brown lesions; G–, discrete green lesions; G+, confluent green lesions.

^b ND, not determined; +, avirulence allele; –, virulence allele.

^c Locus.

In the F₁ cultures (TO cultures), the incidence of these resistance reactions was closely related with the lesion types (Table 2). A *t* test showed that the brown and green types were significantly different in the HR ratio, whereas the discrete and confluent types were significantly different in the incidence of papilla formation (Table 3). Consequently, the B– type was characterized by high incidences of papilla formation and high HR ratios (Table 2). The B+ type was characterized by low incidences of papilla formation and high HR ratios, whereas the G– type was characterized by high incidences of papilla formation and low HR ratios. Only when both incidences were low did the infection result in the virulence phenotype, G+ (Table 2). Based on these results, *A1* and *A2* determine lesion color by conditioning the HR, similar to *PWT1*, and *B1* determines lesion number by conditioning papilla formation, similar to *PWT2*.

Allelism tests. In many sets of the TO cultures, it was not possible to infer precise genotypes from their phenotypes. An example of such sets is shown in Table 4. Set 43 is composed of three brown types (K, L, and N) and one green type (M). *A1* and *A2* should be present in two of the three brown types; in other words, one of K, L, and N should carry both *A1* and *A2* while the other two should carry either *A1* or *A2*. It cannot be determined which is the carrier of both genes. From the viewpoint of lesion number, however, this set is composed of two discrete types (K and L) and two confluent types (M and N); therefore, *B1* should be present in K and L. To determine whether the newly identified three avirulence genes include *PWT2*, 43L1 was crossed with 3K1, a TS culture with genotype *pwt1*;*PWT2*. Culture 43L1 should carry two genes (*A1* or *A2*, *B1*) or three genes (*A1*, *A2*, and *B1*), whereas 3K1 carries a single gene, *PWT2*. Let's assume that *A1*, *A2*, and *B1* are different from *PWT2*. If 43L1 carries the three genes, a total of four genes (*A1*, *A2*, *B1*, and *PWT2*) should be involved in the progeny population and, therefore, avirulent and virulent cultures should segregate in a 15:1 ratio. If 43L1 carries the two genes, a total of three genes (*A1* or *A2*, *B1*, and *PWT2*) should be involved in the progeny population and, therefore, avirulent and virulent cultures should segregate in a 7:1 ratio. In fact, all 80 progeny cultures derived from this cross were avirulent on Norin 4 (Table 5). The ratio of avirulent and virulent cultures (80:0) was significantly different from 7:1 or 15:1. These results suggest that 43L1 carries *PWT2*. In other words, one of *A1*, *A2*, or *B1* is *PWT2*. As described above, *A1* and *A2* condition the HR while *B1* conditions papilla formation. Therefore, *PWT2* in

TABLE 5. Segregation of virulence on wheat cv. Norin 4 in progenies derived from crosses between *Magnaporthe oryzae* F₁ cultures

Parent A ^a			Parent B ^b			Number of progeny from A × B						Ratio ^d	χ^2	<i>P</i>
Name	Genotype	Lesion ^c	Name	Genotype	Lesion ^c	B–	B+	G–	G+	Total	(B–,B+,G–):G+			
43L1	?, ?; <i>B1</i>	B–	3K1	<i>pwt1</i> ; <i>PWT2</i>	G–	44	0	36	0	80	80:0	7:1	11.42	<0.01
...	...	...	...	...	...	...	...	...	...	...	...	15:1	5.33	0.01–0.05
45N1	<i>A1</i> ; <i>A2</i> ; <i>b1</i>	B+	3M1	<i>PWT1</i> ; <i>pwt2</i>	B+	0	60	0	0	60	60:0	7:1	8.57	<0.01
45N1	<i>A1</i> ; <i>A2</i> ; <i>b1</i>	B+	61M2	<i>pwt3</i> ; <i>PWT4</i>	B–	35	34	0	11	80	69:11	7:1	0.11	0.50–0.90
45N1	<i>A1</i> ; <i>A2</i> ; <i>b1</i>	B+	86Q1	<i>PWT3</i> ; <i>PWT4</i>	B–	46	21	0	7	74	67:7	15:1	1.30	0.10–0.50

^a F₁ derived from the cross, Br48 (*Triticum* isolate) × PO12-7301-2 (*Oryza* isolate).

^b 3K1 and 3M1, F₁ derived from the cross, Br48 × GFS11-7-2 (*Setaria* isolate); 61M2 and 86Q1, F₁ derived from the cross, Br48 × Br58 (*Avena* isolate).

^c Lesion type: B–, discrete brown lesions; B+, confluent brown lesions; G–, discrete green lesions; G+, confluent green lesions.

^d Expected ratio.

TABLE 6. Genotypes of *Magnaporthe oryzae* field isolates with host species-specific parasitism

Isolates	Original host	Allele ^a				
		<i>Pwt1</i> ^b	<i>Pwt2</i>	<i>Pwt3</i>	<i>Pwt4</i>	<i>Pwt5</i>
PO12-7301-2	<i>Oryza sativa</i>	+	+	–	–	+
GFS11-7-2	<i>Setaria italica</i>	+	+	–	–	–
Br58	<i>Avena sativa</i>	–	–	+	+	–
Br48	<i>Triticum aestivum</i>	–	–	–	–	–

^a Avirulence (+) and virulence (–) alleles.

^b Locus.

43L1 should be *B1*. The 80 progeny cultures showed the discrete types without exception, but contained the brown type (B–) and the green type (G–) in a 1:1 ratio (Table 5). This result suggests that 43L1 carries only one gene for the brown phenotype.

In some sets, genotypes of all four cultures could be determined from phenotypes. Set 45 contained all four phenotypes: B–, B+, G–, and G+ (Table 4). In this case, the brown types (B– and B+) should carry both *A1* and *A2*, whereas the green types (G– and G+) should not carry either. Consequently, 45N was determined as *A1*; *A2*; *b1* (Table 4). When 45N1 was crossed with 3M1, a TS culture with *PWT1*; *pwt2*, all progeny cultures showed the B+ type (Table 5), suggesting that 45N1 carries *PWT1*. Here, *A1* tentatively was designated as *PWT1*. On the other hand, crosses of 45N1 with TA cultures 61M2 (*pwt3*; *PWT4*) and 86Q1 (*PWT3*; *PWT4*) produced virulent (G+) progeny in ratios of 7:1 and 15:1, respectively (Table 5), suggesting that *A2* is different from *PWT3* and *PWT4*. Therefore, *A2* was designated as *PWT5*.

DISCUSSION

We have crossed a *Triticum* isolate (Br48) with a *Setaria* isolate (GFS11-7-2) (7), an *Avena* isolate (Br58) (10), and an *Oryza* isolate (PO12-7301-2) (this study). Genetic analyses of the progenies made it possible to identify five loci (*Pwt1*, *Pwt2*, *Pwt3*, *Pwt4*, and *Pwt5*) involved in the specific pathogenicity of those field isolates on wheat. Results of these studies are summarized in Table 6. The *Oryza* and *Setaria* isolates share two avirulence genes, *PWT1* and *PWT2*, while the *Avena* isolate carries other avirulence genes. This is reasonable because *Oryza* isolates are genetically closer to *Setaria* isolates than to *Avena* isolates. For example, the MAGGY retrotransposon (2) is present in multiple copies in *Oryza* and *Setaria* isolates (11) but absent in *Avena* isolates (*unpublished data*). In the rDNA-ITS2 sequence, *Oryza* isolates are identical to *Setaria* isolates but different from *Avena* isolates (8). The *Triticum* isolate carries none of the five avirulence genes and, therefore, is virulent on wheat (Table 6).

When pathogenicity of the TO cultures were tested on rice cultivars, all 94 cultures were avirulent (data not shown). The difference of the mode of segregation on wheat and rice may be related to the history of their interaction with *M. oryzae*. Rice has been attacked continuously by *M. oryzae* and had to acquire many resistance genes during the long history of interaction. On the other hand, wheat has been cultivated under a dry condition which is unsuitable for infection with *M. oryzae*, and probably did not have to acquire many resistance genes. It was not until the 1980s that *M. oryzae* caused severe damage to wheat production (12). Wheat may be a “living fossil” from the viewpoint of resistance to *M. oryzae*.

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