Analysis of Host Species Specificity of *Magnaporthe grisea* Toward Wheat Using a Genetic Cross Between Isolates from Wheat and Foxtail Millet

J. Murakami, Y. Tosa, T. Kataoka, R. Tomita, J. Kawasaki, I. Chuma, Y. Sesumi, M. Kusaba, H. Nakayashiki, and S. Mayama

First author: Research Fellow of the Japan Society for the Promotion of Science; and second through tenth authors: Faculty of Agriculture, Kobe University, Nada, Kobe 657-8501, Japan.

Current address of M. Kusaba: Faculty of Agriculture, Saga University, Honjo 1, Saga 840-8502, Japan.

Accepted for publication 30 May 2000.

ABSTRACT

Murakami, J., Tosa, Y., Kataoka, T., Tomita, R., Kawasaki, J., Chuma, I., Sesumi, Y., Kusaba, M., Nakayashiki, H., and Mayama, S. 2000. Analysis of host species specificity of *Magnaporthe grisea* toward wheat using a genetic cross between isolates from wheat and foxtail millet. *Phytopathology* 90:1060-1067.

A genetic cross was performed between a *Setaria* isolate (pathogenic on foxtail millet) and a *Triticum* isolate (pathogenic on wheat) of *Magnaporthe grisea* to elucidate genetic mechanisms of its specific parasitism toward wheat. A total of 80 F₁ progenies were obtained from

10 mature asci containing 8 ascospores. Lesions on wheat leaves produced by the F₁ progenies were classified into four types, which segregated in a 1:1:1:1 ratio. This result suggested that the pathogenicity of the F₁ population on wheat was controlled by two genes located at different loci. This idea was supported by backcross analyses. We designated these loci as *Pwt1* and *Pwt2*. Cytological analyses revealed that *Pwt1* and *Pwt2* were mainly associated with the hypersensitive reaction and papilla formation, respectively.

Additional keywords: aggressiveness, *Pyricularia*.

Magnaporthe grisea (Hebert) Barr, is the causal agent of blast disease on many gramineous plants. This fungal species may be divided into groups defined by their restricted ranges of host species (10,11,34), e.g., *Oryza* type pathogenic on rice (*Oryza sativa* L.), *Setaria* pathotype pathogenic on foxtail millet (*Setaria italica* Beauv.), *Panicum* pathotype pathogenic on common millet (*Panicum miliaceum* L.), *Eleusine* pathotype pathogenic on finger millet (*Eleusine coracana* (L.) Gaertn.), and *Digitaria* pathotype pathogenic on crabgrass (*Digitaria sanguinalis* (L.) Scop.). Further, the *Oryza* type is subdivided into races, depending on the rice cultivars they can infect. Host cultivar specificity has also been reported in the *Setaria* pathotype (9). Because of this hierarchic nature of parasitism and high amenability to Mendelian genetic and molecular biological analyses, *M. grisea* has been proposed as a model organism for elucidating molecular and biochemical mechanisms and evolutionary processes of the host-parasite specificity including species and cultivar specificity (30).

The cultivar specificity of the *Oryza* type has been intensively analyzed by many workers. Kiyosawa (13,14) identified 13 rice genes conditioning the resistance to races of the *Oryza* type (rice pathogen). Silue et al. (23) showed gene-for-gene relationships between resistance genes of rice and avirulence genes of the rice pathogen. Recently, Bryan et al. (3) demonstrated an interaction between resistance gene *Pi-ta* and its corresponding avirulence gene *Avr-pita* at the molecular level.

Genetic analyses of the species specificity of *M. grisea* were started by Yaegashi (33). He crossed *Eleusine* isolates with weeping lovegrass isolates and suggested that the pathogenicity of

their progenies on finger millet or weeping lovegrass was conditioned by a single gene. Valent and coworkers identified two major genes, *PWL1* and *PWL2*, which were assumed to be involved in the species specificity of the *Eleusine* pathotype and the *Oryza* type, respectively, toward weeping lovegrass (31). Subsequently, they successfully cloned these genes and showed that they had characteristics similar to avirulence genes that are known to condition cultivar specificity (8,24). Cytological analyses of species specificity were performed by Heath and coworkers (4,5). Heath (4) stressed the importance of concomitant analyses of genetic segregation and cytological events to characterize genes that are involved in infection of various host species with *M. grisea*.

M. grisea populations carry a potential to cause destructive epidemics on plant species which have not been known as its primary hosts. In 1985 severe blast disease occurred on wheat (*Triticum aestivum* (L.) Thell.) fields in Northern Parana state in Brazil (7). Since this first report, the wheat blast disease has spread to other regions, and it is now present in all major wheat-growing regions in this country. Studies on the host range, sexual fertility (29), and fingerprints with repetitive DNA elements (27,28,30,31) have shown that the *Triticum* isolates are distinguishable from the other host-specific pathotypes. Our pathogenicity tests under a strictly regulated condition revealed that the *Triticum* isolates constitute a pathotype of their own. It is intriguing to examine why the other pathotypes could not cause severe disease on wheat and how the *Triticum* pathotype has become pathogenic on wheat.

To address these questions we crossed a *Triticum* isolate with a *Setaria* isolate and produced 80 F₁ progenies composed of 10 complete sets of 8 ascospores (19). We chose a *Setaria* isolate as a parental nonpathogen on wheat since it was highly fertile with *Triticum* isolates. Genetic and cytological analyses of these progenies on wheat are reported here.

Corresponding author: S. Mayama; E-mail address: mayama@kobe-u.ac.jp

Publication no. P-2000-0724-03R

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MATERIALS AND METHODS

Fungal strains and genetic crosses. Two field isolates of *M. grisea* were used as parental isolates for crossing. One was Br48 (*MAT1-1*) isolated from wheat in Brazil and the other was GFSI1-7-2 (*MAT1-2*) isolated from foxtail millet in Japan. Br48 was virulent on wheat and avirulent on foxtail millet, whereas GFSI1-7-2 was virulent on foxtail millet and avirulent on wheat.

Mycelial plugs of isolates to be crossed were placed 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 and incubated at 22°C under continuous fluorescent illumination. After 3 weeks of incubation, perithecia were formed at the intersection of two mycelial colonies. Mature perithecia were picked up from the media with a scalpel under a stereomicroscope and placed in a drop of distilled water on a sterile microscope slide. The perithecia were crushed with a scalpel to release asci, which were transferred to a water agar medium (10 g of agar in 500 ml of water, 2%) with a pipette. Each ascospore was separated from the asci with a fine sterilized glass needle under a micromanipulator. After 24 to 36 h of incubation germinated ascospores were transferred to potato dextrose agar slants. For long term storage, these progenies were transferred to and grown on sterilized barley seeds in vials at 25°C for 1 month and kept in a case with silica gel at 5°C.

DNA manipulations. Genomic DNA of *M. grisea* was prepared as described by Nakayashiki et al. (20). DNA samples were digested with *Bam*HI (Boehringer GmbH, Mannheim, Germany), fractionated in 0.7 to 0.9% agarose gel, transferred to a nylon membrane (MagnaGraph, MSI, Westborough, MA), and hybridized overnight with pEBA18-09, a plasmid containing a homolog of MGR583 (28). The membrane was washed at a high stringency, and then subjected to chemiluminescent detection using Photo-Star Detection Kit (New England Biolabs Inc., Beverly, MA).

Plant material and infection assay. In pathogenicity tests, *Triticum aestivum* (L.) Thell. cv. Norin 4 was used as a test plant. Twelve wheat seeds were sown in vermiculite in a seedling case of 5.5 × 15 × 10 cm, and grown at 25°C in a growth chamber with a 12-h photoperiod for 9 days.

M. grisea isolates or their progenies were grown on oatmeal agar petri dishes for 6 days. Aerial mycelia on the 6-day old cultures were washed off by rubbing mycelial surfaces with cotton balls. The colonies were exposed to near-ultraviolet light (360 nm, 40W) at 25°C for 4 days to induce sporulation. The conidia produced were suspended in water and adjusted to a concentration of 2×10^5 spores per ml. Twenty milliliters of the spore suspension with 0.01% Tween 20 were sprayed on the adaxial surface of primary leaves in a plastic case with an air compressor. The cases were sealed to maintain high humidity and placed in darkness for 20 to 24 h. The inoculated plants were returned to a growth chamber and incubated further at 25°C. Five days after inoculation, symptoms were evaluated based on the color of lesions and the affected area on the leaves. The affected area was rated by 6 progressive grades from 0 to 5: 0 = no visible evidence of infection; 1 = expansion of lesions up to 5%; 2 = 20%; 3 = 50%; 4 = 80%; and 5 = more than 80% of the whole leaf. The pathogenicity test was repeated at least five times for each isolate or progeny.

Microscopic observation. Forty-eight hours after inoculation, the inoculated primary leaves were collected and boiled in alcoholic lactophenol (lactic acid/phenol/glycerol/distilled water/ethanol = 1:1:1:1:8, vol/vol/vol/vol/vol) for 2 min for fixation. Cytological response of the specimens was observed under bright and fluorescent fields of a fluorescence microscope (Olympus, Tokyo, Japan) with an exciter filter B. Observation was focused on appressoria and germings in the process of penetrating epidermal cells. When more than one appressoria were on one epidermal cell, those were excluded from counting. A total of 200 to 500 appressoria on six leaves were counted for each isolate or

progeny. The incidence of cellular responses was subjected to arcsine transformation and compared by a *t* test.

RESULTS

Identification of pairs of sister progenies in the F₁ population. The *Triticum* isolate Br48 was crossed with the *Setaria* isolate GFSI1-7-2 on oatmeal agar media, and a total of 80 F₁ progenies were obtained from 10 mature asci with 8 ascospores. Genomic DNA was extracted from their mycelia, digested with *Bam*HI, and hybridized with pEBA18-09 containing a fragment of the LINE-like element, MGR583. This element was present in multiple copies in GFSI1-7-2 but in a few copies in Br48 (Fig. 1). In the F₁ population most of the bands in the parental isolates segregated in a 1:1 ratio, and their combination produced specific fingerprints for individual progenies. These fingerprints made it possible to identify pairs of sister progenies that were produced by the mitosis of tetrads (Fig. 1). Based on such fingerprint analyses, the F₁ progenies were designated as 1-K1, 1-K2, 1-L1, 1-L2, 1-M1, 1-M2, 1-N1, 1-N2, and so on; here the numeral before the hyphen indicates the ascus (set) number, and progenies with the same letter (K, L, M, and N) are sister progenies of a pair.

Fig. 1. DNA fingerprint profiles of a set of eight F₁ progenies derived from the cross between *Magnaporthe grisea* isolates Br48 and GFSI1-7-2. Genomic DNA was digested with *Bam*HI, electrophoresed, blotted, and hybridized with MGR583.

Genetic analysis of pathogenicity on wheat. Pathogenicity of the parental isolates and the F₁ progenies was examined on wheat cv. Norin 4. The *Setaria* isolate GFSII-7-2 produced a few brown pinpoint lesions without visible centers, and was regarded as avirulent (Fig. 2). In contrast, the *Triticum* isolate Br48 produced many water-soaked patches by 3 days after inoculation. They enlarged and coalesced with time and, finally, the whole leaf shriveled with its color remaining green for 5 days after inoculation (Fig. 2). The F₁ progenies derived from the cross between these parental isolates produced various degrees and types of in-

Fig. 2. Four lesion types on cv. Norin 4 produced by inoculation with *Magnaporthe grisea* isolates GFSII-7-2, Br48, and their progenies (3-K1, 3-L1, 3-M1, and 3-N1). B- indicates a few or discrete brown lesions; B+ indicates many or confluent brown lesions that developed extensively; G- indicates a few or discrete green lesions; G+ indicates many or confluent green lesions that developed extensively.

fection. There were 11 progenies that induced no visible reactions on wheat leaves. Preliminary cytological observation revealed that they were deficient mutants that lost the basic capacity of penetration. Such deficiency appeared in four progenies (two pairs) in each of sets five and eight (Table 1), suggesting that the mutation occurred before the formation of chromatids in these sets. In set seven the deficiency appeared in two progenies (a single pair), suggesting that the mutation occurred between the formation of chromatids and the formation of tetrads. In set four the deficiency appeared in only one sister progeny of a pair, suggesting that the mutation occurred after the mitosis of tetrads. These 11 mutants were excluded from further analysis.

The other 69 F₁ progenies produced visible lesions and could be classified alternatively into one of two types, brown type or green type, based on the color of the lesions. Segregation of the brown type (including the brown pinpoint lesion type as in the parental *Setaria* isolate) and the green type (including the green coalesced lesion type as in the parental *Triticum* isolate) was 35:34, and fitted a 1:1 ratio (data not shown). This result suggested that lesion color was controlled by one major gene.

Furthermore, the degree of the pathogenicity of the populations was analyzed on the basis of affected area of the leaves. In the brown-type progeny, distribution of the index of affected area was almost discontinuous, and the F₁ progenies were clearly grouped into two types, the discrete type (index <1) and the confluent type (index >2) (Fig. 3A). In the green-type progeny the distribution was not discontinuous as a whole (Fig. 3B). When the progenies were compared within each set, however, the two types were distinguishable on the basis of the relative value of the index. In the sets of 1, 3, 4, 7, 9, and 10, the two pairs of sister progenies were split into the discrete type and the confluent type (Fig. 3B). In set six both of the two pairs (M and N) showed more than three of the index and were categorized into the confluent type. However, this appeared to be reasonable because both of the other two pairs (K and L), which produced brown-type lesions, were categorized into the discrete type (Fig. 3A). Similarly, the distribution of the index in set two could be explained by taking account of the whole ascus (Fig. 3A and B). As a whole, segregation of the discrete type and the confluent type fitted a 1:1 ratio, suggesting that there is a major gene involved in the affected area or the number of lesions.

Consequently, infection types of the F₁ progenies fell into one of four categories, which could be distinguished by the color and the affected area of lesions (Fig. 2). The first was type B-, which showed a few or discrete brown pinpoint lesions as observed with the parental *Setaria* isolate. The second was type B+, which showed many or confluent brown lesions that developed extensively in some cases. The third was type G-, which showed a few or discrete green lesions. These lesions usually developed to green islands by 5 to 7 days after inoculation. The fourth was type G+, which showed many or confluent green lesions that developed extensively as observed with the parental *Triticum* isolate.

TABLE 1. Pathogenicity to wheat cv. Norin 4 of F₁ progenies derived from the cross between *Magnaporthe grisea* isolates GFSII-7-2 and Br48

Set no.	Lesion type ^a								Ratio of B-:B+:G-:G+	Tetrad type ^b
	K1	K2	L1	L2	M1	M2	N1	N2		
1	G+	G+	G-	G-	B+	B+	B-	B-	1:1:1:1	T
2	G-	G-	B+	B+	B+	B+	G-	G-	0:2:2:0	NPD
3	G-	G-	B-	B-	B+	B+	G+	G+	1:1:1:1	T
4	...	B+	G-	G-	G+	G+	B-	B-	1:1:1:1	T
5	...	...	B+	B+	B-	B-	...	...	nd	nd
6	B-	B-	B-	B-	G+	G+	G+	G+	2:0:0:2	PD
7	B+	B+	G+	G+	...	...	G-	G-	nd	nd
8	G+	G+	B+	B+	...	...	...	...	nd	nd
9	B+	B+	G+	G+	B-	B-	G-	G-	1:1:1:1	T
10	B+	B+	B-	B-	G-	G-	G+	G+	1:1:1:1	T

^a B- indicates a few or discrete brown lesions; B+ indicates many or confluent brown lesions that developed extensively; G- indicates a few or discrete green lesions; G+ indicates many or confluent green lesions that developed extensively; ... indicates no reaction; and K1 = progeny.
^b T = tetratype; NPD = nonparental ditype; PD = parental ditype; and nd = not determined.

The infection types of the 80 F₁ progenies on cv. Norin 4 are summarized in Table 1. The sets of 1, 3, 4, 9, and 10 contained all of the four types, B–, B+, G–, and G+, and were regarded as tetratypes. Set six contained only the B– and G+ types, and appeared to be a parental ditype. By contrast, set two contained only the B+ and G– types, and appeared to be a nonparental ditype. In the sets of 5, 7, and 8, the type of segregation was not determined exactly because some of their members were the deficient mutants. However, they could be inferred to be tetratypes since they included progenies inducing the B+ or G– types. From these results we hypothesized that the pathogenicity of the F₁ population on wheat (cv. Norin 4) was controlled by two unlinked major genes: one associated with lesion color and the other associated with affected area or lesion number.

This hypothesis was supported by the segregation analysis of the next generation (Table 2). Three of the F₁ progenies were backcrossed with the parental *Triticum* isolate, Br48, and their progenies were isolated randomly. When 3-L2, an F₁ progeny inducing type B– reaction, was backcrossed with Br48, all of the four types appeared in their progenies. On the other hand, a back-

cross of 3-M2, an F₁ progeny inducing the type B+ reaction, with Br48 yielded no progenies inducing the types B– or G–. The segregation of the types B+ and G+ fitted a 1:1 ratio, indicating that a single gene is involved in the B+ type reaction. When an F₁ progeny inducing the type G– reaction was backcrossed with Br48, all progenies produced the green type lesion, but the segregation of the types G– and G+ were not clear due to segregation of intermediate types (data not shown).

Here, we designate the two genetic loci associated with the lesion color and the affected area (or the lesion number) as *Pwt1* and *Pwt2* (after pathogenicity on wheat), respectively. Because dominance of their alleles cannot be determined by classical genetic analyses in this haploid fungus, we tentatively designated alleles from the *Setaria* isolate as *Pwt1s* and *Pwt2s*, and those from the *Triticum* isolate as *Pwt1t* and *Pwt2t*. Then, genotypes of B–, B+, G–, and G+ type progenies are described as *Pwt1s/Pwt2s*, *Pwt1s/Pwt2t*, *Pwt1t/Pwt2s*, and *Pwt1t/Pwt2t*, respectively.

Cytological responses associated with *Pwt1* and *Pwt2*. Primary leaves of the wheat cv. Norin 4 were inoculated with the parental isolates and the F₁ progenies, and observed under a fluorescent microscope 48 h after inoculation. Cytological responses could be categorized into four types, A, B, C, and D (Fig. 4). Type A was characterized by no reaction of the leaf cells. In this case the fungal appressorium seemed to have no capacity to attempt penetration. Type B exhibited the formation of a papilla with yellow fluorescence, which stopped the appressorial penetration of cell walls. Type C showed the hypersensitive reaction of the epidermal cell. Such cells were usually accompanied by strong yellow fluorescence of cell walls or cytoplasm. Type D exhibited no resistance reactions to the appressorial penetration. The appressorium successfully invaded the epidermal cell, followed by the development of infection hyphae.

The avirulent parent GFS11-7-2 induced papilla formation in a high percentage appressorial penetration sites (Table 3). Although about 30% of germings were not stopped at the papilla stage, they subsequently induced the hypersensitive reaction and failed to colonize wheat leaves. The “HR ratio” (percentage of germings inducing the hypersensitive reaction (C) against those invading an epidermal cell (C + D)) was almost 100%. The virulent parent, Br48, also induced papilla formation and the hypersensitive reaction, but their frequencies were low (22.5 and 13.2%, respectively). Consequently, more than half of the germings of Br48 successfully colonized epidermal cells.

In the F₁ progenies the incidence of those resistance reactions was correlated with the segregation of *Pwt1* and *Pwt2*. An example is shown in Table 3. 4-K1 was one of the deficient mutants, so most of its germings were categorized into A (no reaction). Among the other seven progenies of this set, the segregation of *Pwt2* was correlated with the incidence of the papilla formation; *Pwt2s* carriers (4-L1, 4-L2, 4-N1, and 4-N2) induced this reaction in more than 70% of penetration sites, whereas *Pwt2t* carriers (4-K2, 4-M1, and 4-M2) induced the reaction in only 30% of penetration sites. On the other hand, the segregation of *Pwt1* was correlated with the HR ratio; this ratio was almost 100% in *Pwt1s* carriers (4-K2, 4-N1, and 4-N2) but was less than 80% in *Pwt1t* carriers (4-L1, 4-L2, 4-M1, and 4-M2).

TABLE 2. Segregation of lesion types on wheat cv. Norin 4 in BC₁F₁ progenies derived from backcrosses of F₁ progenies with Br48

Cross ^a	Number of progeny				Total
	B–	B+	G–	G+	
3-L2 (B–) × Br48 (G+)	3	7	4	5	19
3-M2 (B+) × Br48 (G+)	0	13	0	15	28

^a Lesion type in parentheses. B– indicates a few or discrete brown lesions; B+ indicates many or confluent brown lesions that developed extensively; G– indicates a few or discrete green lesions; G+ indicates many or confluent green lesions that developed extensively.

Fig. 3. Distribution of the affected area in A, brown types and B, green types resulting from infection of wheat cv. Norin 4 with F₁ progenies derived from the cross, GFS11-7-2 × Br48. The affected area represents the mean value of the index (described in Materials and Methods) obtained from 10 to 20 leaves in two infection assays. Based on the distribution, progenies were grouped into discrete type (●) and confluent type (○). Indices of the parent isolates GFS11-7-2 and Br48 were 0.1 and 5.0, respectively. Tetrad types are indicated in parentheses as T = tetratype; N = nonparental ditype; P = parental ditype; and – = not determined. Deficient mutants that lost the basic capacity of penetration were excluded from the figure.

As a whole, *Pwt1s* carriers showed a higher HR ratio than did *Pwt1t* carriers (Fig. 5A), and the segregation of *Pwt1* was very clear at the cellular level. Similarly, there seemed to be a difference in the frequency of the papilla formation between *Pwt2s* carriers and *Pwt2t* carriers (Fig. 5B). This difference was not so clear as in HR ratio between *Pwt1s* and *Pwt1t* carriers, but was statistically significant in the *t* test. Furthermore, a synergistic

effect was observed on the incidence of these cellular responses when F₁ progenies carried both *Pwt1s* and *Pwt2s* (Fig. 5C). These double-carriers induced the hypersensitive reaction and the papilla formation in very high frequencies compared with progenies carrying only *Pwt1s* or *Pwt2s*. Cytological data of the 69 progenies are summarized in Table 4. Statistical analyses suggested that *Pwt1* and *Pwt2* were mainly involved in the hypersensitive re-

Fig. 4. Cytological responses observed under the bright field (left) or the fluorescence field (right) in epidermal cells of wheat (cv. Norin 4) inoculated with F₁ hybrids **A**, 4-K1, **B**, 4-N2, **C**, 4-K2, and **D**, 4-M2, 48 h after inoculation. **A**, No reaction; **B**, Papilla formation; **C**, Hypersensitive reaction; **D**, Hyphal growth. App = appressoria; Pap = papilla; HR = hypersensitive reaction; IH = infection hyphae. Bars represent 20 μ m.

action and papilla formation, respectively, although they were also associated with the other reaction.

It should be noted that the incidence of the resistance reactions with virulent F₁ progenies was generally higher than their incidence with the parental virulent isolate, Br48. Although the frequencies of papilla formation and HR ratio with Br48 (*Pwt1t/Pwt2t*) were 22.5 and 19.3%, respectively (Table 3), those with the F₁ progenies carrying the same genotype were 38.8% and 47.5% on average, respectively (Table 4).

DISCUSSION

The present results showed that the specific parasitism of the *Setaria* isolate and the *Triticum* isolate on wheat is mainly controlled by two loci, *Pwt1* and *Pwt2*. Valent and coworkers have already cloned two host specificity genes, *PWL1* and *PWL2*, that are supposed to determine the species-specific parasitism of *M. grisea* on weeping lovegrass. In our preliminary test, GFS11-7-2 and Br48 showed intermediate and strong virulence on weeping lovegrass, respectively, suggesting that *Pwt1* and *Pwt2* are different from *PWL* genes that prevent the blast fungus from infecting weeping lovegrass. In addition no PCR products were amplified from those parental isolates with primers designed based on conserved DNA sequences of *PWL* genes (date not shown). It is likely that the genes at the *Pwt1* and *Pwt2* loci are novel, with little or no homology to the *PWL* gene family.

The segregation of these two genes in the F₁ progenies resulted in the production of four genotypes. Two of them were parental types and the others were nonparental types that induced symptoms distinct from either of those induced by the parental isolates. The nonparental progenies were useful for analysis of the relationship between genotypes and phenotypes since they were supposed to carry one of the two target genes. When an F₁ progeny inducing the B+ type was backcrossed with the parental *Triticum* isolate, Br48, the B+ and G+ types segregated in a 1:1 ratio as expected. When an F₁ progeny inducing the G- type was backcrossed with Br48, however, no clear segregation of the G- and G+ types occurred. This may be because the BC₁F₁ generations were composed of random ascospores isolated from several asci. Actually, the distinction between the G- and G+ types was not so clear even in the F₁ generation when it was observed as a random population (Fig. 3B). However, the distinction became more clear when the F₁ progenies were compared within each set.

Cytological studies on the process of blast infection have been mainly focused on rice plant responses (1,2,15,16,17,21,22,25). In the present study, we examined responses of wheat leaves to the blast fungus and found resistance reactions similar to those detected in wheat or barley leaves inoculated with the powdery mildew fungus (12,18,26), i.e., the formation of a fluorescent papilla

and the hypersensitive reaction of an epidermal cell. Interestingly, the responses of wheat leaves at the microscopic level were correlated with those at the macroscopic level in the four genotypes of the F₁ progenies. When the F₁ progenies carried *Pwt1s*, the hypersensitive reaction was induced at most of the penetrated epidermal cells and then the lesions turned brown. When they carried its allele, *Pwt1t*, the hypersensitive reaction was sup-

Fig. 5. Distribution of incidences of cellular responses resulting from infection of wheat cv. Norin 4 with F₁ progenies derived from the cross, GFS11-7-2 × Br48. Closed circles represent carriers of A, *Pwt1s*, B, *Pwt2s*, and C, both *Pwt1s* and *Pwt2s*. Deficient mutants that lost the basic capacity of penetration were excluded from the figure.

TABLE 3. Cellular responses of wheat leaves (cv. Norin 4) to *Magnaporthe grisea* isolates and their F₁ progenies^a

Culture	Percentage of germings inducing				
	No reaction (A)	Papilla formation (B)	HR (C)	Hyphal growth (D)	HR ratio (C/C+D)
Parent					
GFS11-7-2	5.1	64.3	31.1	0.03	99.9
Br48	5.9	22.5	13.2	55.4	19.3
Example of progenies ^b					
4-K1 (...)	92.3	6.0	1.7	0.0	100.0**
4-K2 (B+)	2.7	29.7	67.3	0.0	100.0**
4-L1 (G-)	3.6	77.8*	14.6	4.0	78.5
4-L2 (G-)	6.4	70.3*	12.1	11.1	52.3
4-M1 (G+)	3.7	33.7	29.3	33.3	46.8
4-M2 (G+)	4.0	28.0	40.7	31.3	56.5
4-N1 (B-)	5.3	79.3*	15.3	0.0	100.0**
4-N2 (B-)	16.0	74.5*	9.5	0.0	100.0**

^a *Pwt2s* carriers are indicated by a single asterisk. *Pwt1s* carriers are indicated by a double asterisk.

^b Lesion type is in parentheses. B- indicates a few or discrete brown lesions; B+ indicates many or confluent brown lesions that developed extensively; G- indicates a few or discrete green lesions; G+ indicates many or confluent green lesions that developed extensively; and ... indicates no reaction.

pressed and the lesions remained green. Similarly, when the F_1 progenies carried *Pwt2s*, papilla formation was induced at many infection sites and the lesions were discrete. When they carried its allele, *Pwt2t*, papilla formation was suppressed and the lesions formed extensively on the whole leaf and became confluent. The combination of these cytological responses could account for the appearance of the four lesion types (Fig. 2). These results suggest that *Pwt1* and *Pwt2* are primarily associated with the hypersensitive reaction of epidermal cells and the formation of a fluorescent papilla, respectively.

Heath et al. (5) suggested that the presence of the allele of *PWL1* was correlated with the browning of plant cells around developing colony margins in weeping lovegrass leaves. In the present study the cosegregation of fungal genes with cytological responses was detected at earlier stages of the infection process. Cytological responses determining host-species specificity may be different between plant species and between host-specific pathotypes of *M. grisea*.

At first, *Pwt1* was defined as a gene controlling lesion color, and did not seem to be involved in pathogenicity because the B+ phenotype produced by the *Pwt1s/Pwt2t* genotype appeared to be a virulent reaction while the B- phenotype produced by the *Pwt1s/Pwt2s* genotype was undoubtedly an avirulent reaction. When the infection assay was performed at a lower temperature ($\approx 20^\circ\text{C}$), however, the lesion number and size were decreased especially in the B+ type, and this type became indistinguishable from the B- type. We assume that the primary role of *Pwt1* is the determination of pathogenicity and that the lesion color is merely a superficial result of the determination. At a lower temperature the hypersensitive reaction controlled by the *Pwt1* locus can stop the fungal penetration efficiently and produce brown pinpoint or small lesions without the help by *Pwt2s*. At a higher temperature the hypersensitive reaction mediated by the *Pwt1* locus cannot stop it efficiently under the absence of *Pwt2s*, so it is induced continuously and extensively, resulting in the browning and shriveling of the whole leaf, that is, the B+ type reaction.

At present we have no data suggesting the dominance/recessiveness of the alleles at these two loci. If the alleles from

the *Setaria* isolate are dominant, they should be avirulence genes that induce the hypersensitive reaction of epidermal cells or papilla formation. If the alleles from the *Triticum* isolate are dominant, they should be pathogenicity genes that suppress those resistance reactions. Although further research is needed to determine which is the case, it seems highly probable that *Pwt1*, at least, is an avirulence locus because this locus was closely associated with the hypersensitive reaction, the typical cellular response induced in gene-for-gene interactions.

Valent et al. (32) conducted a series of backcrosses of a weeping lovegrass pathogen with a rice pathogen, and noted that they could not observe the segregation of avirulence genes on rice cultivars until the fourth and fifth generations of the backcrossing program. They suggested that this was due to the segregation of minor genes that determine the extent of lesion development. In the present study the segregation of major genes was very clear in the first generation. We suggest that the host specificity of *M. grisea* on wheat is governed by a relatively simple genetic mechanism. This may be related to the short history of interactions between wheat and the blast fungus.

Nevertheless, the F_1 progenies between the *Triticum* isolate and the *Setaria* isolate, as a whole, seemed to be less virulent on wheat than the parental isolates at the visible level. Cytological data showed that the incidence of papilla formation and the HR ratio with virulent F_1 progenies were relatively higher compared with those with the virulent parent, Br48 (Tables 3 and 4). This may be attributable to reduction of their viability or aggressiveness caused by the reassortment of minor genes involved in virulence. We suggest that hybrids are generally less aggressive than field isolates, and tend to disappear as shown in the powdery mildew of cereals (6).

ACKNOWLEDGMENTS

We thank H. Kato, a former professor at Kobe University, for valuable suggestions and comments. This work was supported by grants from the Ministry of Education, Japan (10460022 and 10556011) and from Research Fellowships of the Japan Society for the Promotion of Science for Young Scientists.

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TABLE 4. Relationship between genotypes of *Magnaporthe grisea* F_1 progenies and cellular responses in wheat leaves

Lesion type ^a	Allele ^b		Papilla formation ^c		HR ratio ^d	
	<i>Pwt1</i>	<i>Pwt2</i>	Average ^e	<i>t</i> value ^f	Average	<i>t</i> value ^f
B- and B+	s	...	60.3 (35)	2.58*	95.4	9.53**
G- and G+	t	...	48.1 (34)		60.0	
B-	s	s	77.5 (16)	3.70**	98.7	7.11**
G-	t	s	58.5 (16)		73.9	
B+	s	t	45.7 (19)	1.42	92.7	8.33**
G+	t	t	38.8 (18)		47.5	
B- and G-	...	s	68.0 (32)	6.31**	86.3	3.05**
B+ and G+	...	t	42.4 (37)		70.7	
B-	s	s	77.5 (16)	7.02**	98.7	3.32**
B+	s	t	45.7 (19)		92.7	
G-	t	s	58.5 (16)	3.69**	73.9	3.91**
G+	t	t	38.8 (18)		47.5	

^a B- indicate a few or discrete brown lesions; B+ indicate many or confluent brown lesions that developed extensively; G- indicate a few or discrete green lesions; G+ indicate many or confluent green lesions that developed extensively.

^b The letters s and t represent alleles derived from the *Setaria* isolate GFSI1-7-2 and the *Triticum* isolate Br48, respectively.

^c Percentage of germplugs inducing papilla formation.

^d Germplugs inducing HR/germplugs that penetrated cell walls $\times 100$.

^e Number of progenies are indicated in parentheses.

^f Results of *t* test for groups of two. * and ** indicate significant at the 5 and 1% levels, respectively.

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