A Gene-for-Gene Relationship Underlying the Species-Specific Parasitism of *Avena/Triticum* Isolates of *Magnaporthe grisea* on Wheat Cultivars

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Accepted for publication 13 June 2002.

ABSTRACT

Takabayashi, N., Tosa, Y., Oh, H. S., and Mayama, S. 2002. A gene-for-gene relationship underlying the species-specific parasitism of *Avena/Triticum* isolates of *Magnaporthe grisea* on wheat cultivars. *Phytopathology* 92:1182-1188.

To elucidate genetic mechanisms of the species-specific parasitism of *Magnaporthe grisea*, a *Triticum* isolate (pathogenic on wheat) was crossed with an *Avena* isolate (pathogenic on oat), and resulting F₁ progeny were subjected to segregation analyses on wheat cvs. Norin 4 and Chinese Spring. We found two fungal loci, *Pwt3* and *Pwt4*, which are

involved in the specific parasitism on wheat. *Pwt3* operated on both cultivars while *Pwt4* operated only on 'Norin 4'. Using the cultivar specificity of *Pwt4*, its corresponding resistance gene was successfully identified in 'Norin 4' and designated as *Rmg1* (*Rwt4*). The presence of the corresponding resistance gene indicated that *Pwt4* is an avirulence locus. *Pwt3* was assumed to be an avirulence locus because of its temperature sensitivity. We suggest that gene-for-gene interactions underlie the species-specific parasitism of *M. grisea*.

Additional keywords: blast fungus, *Pyricularia*.

The host-parasite specificity between plants and microorganisms has been divided into two types, i.e., plant species specificity determining host species range and cultivar specificity determining cultivar range within a given host species (5). The latter was genetically characterized as being controlled by gene-for-gene interactions, while the former was considered as being genetically complex. However, data have been reported that may be interpreted as suggesting that gene-for-gene interactions underlies the plant species specificity (6). In fungal pathosystems, most of such data came from analyses of highly specialized obligate parasites. An example is *Blumeria graminis*, the powdery mildew fungus of cereals, which is composed of several formae speciales specific to plant genera. Tosa (13) and Tosa and Sakai (14) demonstrated that the forma specialis-genus specificity is under the control of gene-for-gene interactions.

Magnaporthe grisea (Hebert) Barr, is the causal agent of blast disease of many gramineous plants. This fungal species includes several forms or subgroups, each of which has a restricted range of host species (9). Representative subgroups are *Oryza* type pathogenic on rice (*Oryza sativa*), *Setaria* pathotype pathogenic on foxtail millet (*Setaria italica*), *Panicum* pathotype pathogenic on common millet (*Panicum miliaceum*), *Eleusine* pathotype pathogenic on finger millet (*Eleusine coracana*), and *Digitaria* pathotype pathogenic on crabgrass (*Digitaria sanguinalis*). Kato et al. (9) called this relationship as species specificity in the blast fungus-gramineous plant system, following the traditional usage. Yaegashi (21) started a genetic analysis of the species specificity by crossing *Eleusine* isolates with weeping lovegrass isolates. Valent and coworkers identified two major genes, *PWL1* and *PWL2*, which were assumed to be involved in the species specificity of an *Eleusine* isolate and an *Oryza* isolate, respectively, toward weeping lovegrass (19). Subsequently, they cloned these genes and

showed that they have characteristics similar to avirulence genes (8,11).

The *M. grisea* population maintains a potential to evolve new host-specific forms. An example is the wheat pathogen, which appeared in 1985 in Brazil (7). Studies on the host range, sexual fertility (18), and fingerprints with repetitive DNA elements (17, 19) have shown that isolates from wheat (*Triticum aestivum*) are distinct from the other host-specific subgroups. Our subsequent pathogenicity test at low temperature (20°C) revealed that the *Triticum* isolates constitute a pathotype of their own (*unpublished data*). Murakami et al. (10) crossed a *Triticum* isolate with a *Setaria* isolate (isolate from foxtail millet) and produced 80 F₁ progeny. Segregation analyses of the progeny revealed that their specificity on wheat is conditioned by two loci, *Pwt1* and *Pwt2* (10).

The *Triticum* isolates employed for those experiments were a portion of a *M. grisea* collection gathered from various plant species in Brazil from 1990 to 1992. This collection contained an isolate from oat (*Avena sativa*). At first we assumed that the *Avena* isolate might be a wheat pathogen that colonized oat through opportunistic infection. However, pathogenicity tests at low temperature (20°C) in a controlled-environment room revealed that it was virulent on oat but avirulent on wheat. On the other hand, *Triticum* isolates were virulent on wheat but avirulent on oat in the same condition. These relationships seemed to constitute a good model system to examine genetic mechanisms of the species specificity in the *M. grisea*-gramineous plant system.

In the present study we crossed a *Triticum* isolate with the *Avena* isolate, and produced an F₁ population composed of 88 random progeny. Segregation analyses of the fungal F₁ progeny and plant F₂ progeny suggested that gene-for-gene interactions underlie the species specificity.

MATERIALS AND METHODS

Fungal strains and genetic crosses. Parental field isolates used for crossing were *M. grisea* Br48 isolated from wheat (*Triti-*

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Publication no. P-2002-0826-01R
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cum isolate) and Br58 isolated from oat (*Avena* isolate). Both isolates were collected in Brazil in 1990 and have been maintained at Kobe University. At 20°C, Br48 was virulent on wheat and avirulent on oat while Br58 was virulent on oat and avirulent on wheat. Br48 and Br58 were *MAT1-1* and *MAT1-2*, respectively, and interfertile.

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. Ascospores were isolated from the perithecia as described previously (10). The progeny were transferred to sterilized barley seeds in vials, grown at 25°C for 1 to 2 weeks, and stored in a case with silica gel at 4°C.

Plant material and infection assay. Test plants were *Triticum aestivum* (L.) Thell. cvs. Norin 4 and Chinese Spring. Seeds were sown in a seedling case (5.5 × 15 × 10 cm) in vermiculite and grown at 20 or 25°C in a controlled-environment room with a 12-h photoperiod for 9 days.

M. grisea cultures to be tested were grown on oatmeal agar media. Aerial mycelia on 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 3 days to induce sporulation. The conidia produced were suspended in water and adjusted to 2 to 3 × 10⁵ spores per ml. Twenty milliliters of the spore suspension with 0.01% Tween 20 were sprayed on the adaxial surface of primary leaves of test plants with an air compressor. The inoculated seedlings were placed in dark, humid boxes at 20 or 25°C for 20 to 24 h and returned to the controlled-environment room regulated at 20 or 25°C. Five (at 20°C) or four (at 25°C) days after inoculation, symptoms were evaluated based on the size and color of lesions. The lesion size was rated by six progressive grades from 0 to 5: 0 = no reaction; 1 = pinpoint spots; 2 = small lesions; 3 = intermediate lesions; 4 = large lesions; and 5 = coalesced lesions. The lesion color was recorded with B (brown), Y (yellow), or G (green). The pathogenicity test was repeated at least three times for each culture.

Microscopic observation. Crosses between *M. grisea* isolates frequently yield mutants that have lost basic capability of penetration and cannot attempt penetration to any hosts (1,2). To check whether the test isolates retain the basic capability of penetration, the inoculated leaves were collected 48 h after inoculation 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. The behavior of germings and responses of leaf cells were observed under bright and fluorescent fields of a fluorescence microscope (Olympus, Tokyo, Japan) with an exciter filter B (10).

RESULTS

Pathogenicity of the parental field isolates. Pathogenicity of the parental isolates, Br48 and Br58, was checked on wheat 'Norin 4' and 'Chinese Spring'. At 20°C these field isolates showed contrasting parasitism on wheat. Br48 produced large, water-soaked, green-to-pale green lesions on both cultivars whereas, Br58 produced a few, brown pinpoint spots (Fig. 1). Their infection types were 4-5G and 0-1B, respectively. As temperature increased to 25°C, the specificity became less clear. Br48 maintained its compatibility with the wheat cultivars at 25°C and caused more severe damage (Fig. 1). The green-to-pale green lesions coalesced, resulting in the shriveling of the whole leaf. The leaf color sometimes turned yellow, but basically remained green to pale green. There were no prominent differences of symptoms between the two cultivars; the infection types were 5G or 5Y on both cultivars. On the other hand, symptoms produced by Br58 were contrasting between these cultivars at 25°C. On 'Norin 4', the symptom was brown pinpoint spots as those at 20°C,

indicating that the incompatibility is still expressed at 25°C. By contrast, lesions on 'Chinese Spring' were large, brown, and coalesced, resulting in shriveling of the leaf. The infection types on 'Norin 4' and 'Chinese Spring' were 1B and 5B, respectively. These results suggested that genetic mechanisms of the species specificity are not uniform among wheat cultivars.

Segregation analysis of pathogenicity in the F₁ population.

Br48 was crossed with Br58 on oatmeal agar media, and 88 random ascospores were isolated. This F₁ population did not contain mutants that lost basic capability of penetration. 'Norin 4' and 'Chinese Spring' were inoculated with the F₁ cultures, incubated at 20°C, and observed 5 days after inoculation. At this temperature, small (type 1 to 2) and large (type 4 to 5) lesions were always accompanied by brown and green colors, respectively. On

Fig. 1. Symptoms on wheat cvs. Norin 4 and Chinese Spring produced by inoculation with *Magnaporthe grisea* Br48 (*Triticum* isolate), Br58 (*Avena* isolate), and representative F₁ progenies. 3M1 and 3K1 are F₁ cultures derived from a cross between Br48 and GFSII-7-2 (*Setaria* isolate) while 66W1 and 70Q3 are from a cross between Br48 and Br58. Their genotypes are shown in parentheses. Inoculated leaves were incubated at 20 and 25°C for 5 and 4 days, respectively.

a leaf with the type 3 infection, however, brown and green lesions sometimes occurred concomitantly. Consequently, we redefined the infection types for the segregation analysis at 20°C as follows: 0 = no reaction; 1 = brown pinpoint spots; 2 = small brown lesions; 3– = brown lesions with an intermediate size accompanied by a few, scattered green lesions; 3+ = green lesions with an intermediate size accompanied by a few, scattered brown lesions; 4 = large green lesions, and 5 = coalesced green lesions.

Assuming that types 0 to 3– and types 3+ to 5 are avirulent and virulent, respectively, their segregation on ‘Chinese Spring’ fits a 1:1 ratio (Table 1), suggesting that the specific parasitism on ‘Chinese Spring’ is controlled by a single locus. On the other hand, the segregation on ‘Norin 4’ fits a 3:1 ratio (Table 1), suggesting that the specificity on ‘Norin 4’ is controlled by two loci. All progeny avirulent on ‘Chinese Spring’ were avirulent on ‘Norin 4’, while progeny virulent on ‘Chinese Spring’ were avirulent or virulent on ‘Norin 4’ in a 1:1 ratio (Table 2). This segregation pattern suggests that one of the two loci detected by inoculation of ‘Norin 4’ is identical to the locus detected on ‘Chinese Spring’. Here, we designate the locus operating on both cultivars as *Pwt3* and that operating on ‘Norin 4’ alone as *Pwt4*. Although dominance/recessiveness of alleles at these loci cannot be determined unequivocally in this haploid fungus, the three avirulent/one recessive segregation suggests that avirulence alleles are dominant. Therefore, we designate avirulence alleles from the *Avena* isolate as *PWT3* and *PWT4* and virulence alleles from the *Triticum* isolate as *pwt3* and *pwt4*.

Allelism tests with the *Pwt* loci. Murakami et al. (10) identified two loci, *Pwt1* and *Pwt2*, which control avirulence/virulence on ‘Norin 4’ in an *F₁* population derived from a cross between the *Triticum* isolate Br48 and *Setaria* isolate GFSI1-7-2. They designated avirulence alleles from the *Setaria* isolate as *Pwt1s* and *Pwt2s*, and virulence alleles from the *Triticum* isolate as *Pwt1t* and *Pwt2t*. Here, we rename these alleles as *PWT1*, *PWT2*, *pwt1*, and *pwt2*, respectively, following the present system of nomenclature.

We examined whether *Pwt3* and *Pwt4* were independent of *Pwt1* by crossing *F₁* progenies. The first cross was performed between 67Q4 and 4K2. 67Q4 is an *F₁* progeny derived from the cross, Br48 × Br58, that was determined as *pwt3*;*PWT4* based on its virulence on ‘Chinese Spring’ and avirulence on ‘Norin 4’. 4K2 is an *F₁* progeny derived from the cross, Br48 × GFSI1-7-2, that was determined as *PWT1*;*pwt2* (10). Among 31 progeny derived from the cross, six were mutants that lost basic capability of penetration and were excluded from further analysis. When ‘Norin 4’ was inoculated with the 25 normal progeny, infection types

were discontinuously distributed with two peaks at 1 and 4 (Table 3). The segregation of avirulent and virulent cultures fits a 3:1 ratio, indicating that *Pwt4* is independent of *Pwt1*.

The second cross was performed between 66W1 and 4K2. 66W1 is another *F₁* progeny derived from the cross, Br48 × Br58, which was chosen as a carrier of *PWT3*. Unfortunately, we could not determine its genotype definitely before crossing. This was due to the fact that *PWT3* carriers showed avirulence on both cultivars irrespective of the alleles at the *Pwt4* locus, and therefore, that genotypes *PWT3*;*PWT4* and *PWT3*;*pwt4* were phenotypically indistinguishable. However, an analysis with this cross, enabled us to determine the genotype of 66W1 as described below. All 31 progeny obtained from this cross retained basic capability of penetration. When ‘Norin 4’ was inoculated with the 31 progeny, avirulent and virulent cultures segregated in a 3:1 ratio (Table 3). This result indicates that *Pwt3* is independent of *Pwt1*. These two loci are enough to produce the bifactorial segregation in the 66W1 × 4K2 cross; if 66W1 would carry *PWT4*, there should occur a trifactorial segregation. Consequently, only one case that can explain the actual results is that the genotype of 66W1 is *PWT3*;*pwt4* and that *Pwt3* is independent of *Pwt1*. When ‘Chinese Spring’ was inoculated with the 31 progeny from 66W1 × 4K2, avirulent and virulent cultures also segregated in a 3:1 ratio (Table 3). All cultures avirulent on ‘Chinese Spring’ were avirulent on ‘Norin 4’ while all cultures virulent on ‘Chinese Spring’ were virulent on ‘Norin 4’. These results indicate that the *Pwt1* locus operates not only on ‘Norin 4’, but also on ‘Chinese Spring’, supporting the previous conclusion that *Pwt1* is distinct from *Pwt4*, which operates on ‘Norin 4’ but not on ‘Chinese Spring’.

Phenotypes conferred by the loci and temperature sensitivity of their alleles. We chose four *F₁* cultures, 3M1 (*PWT1*;*pwt2*), 3K1 (*pwt1*;*PWT2*), 66W1 (*PWT3*;*pwt4*), and 70Q3 (*pwt3*;*PWT4*), for a comparative analysis of phenotypes conferred by those loci. 3M1 and 3K1 are *F₁* progeny derived from Br48 × GFSI1-7-2 while 66W1 and 70Q3 are from Br48 × Br58. At 20°C, 3M1, 3K1, and 66W1, which carry *PWT1*, *PWT2*, and *PWT3*, respectively, produced a few, brown pinpoint spots on both ‘Norin 4’ and ‘Chinese Spring’ as their avirulent parents (GFSI1-7-2 and Br58) did (Fig. 1). On the other hand, 70Q3 carrying *PWT4* induced different reactions on the two cultivars; it produced brown pinpoint spots on ‘Norin 4’ but extended green, water-soaked lesions on ‘Chinese Spring’ (Fig. 1).

As temperature increased, the phenotypes with 3M1, 3K1, and 66W1 changed dramatically. At 25°C, ‘Norin 4’ and ‘Chinese Spring’ inoculated with 3M1 (carrying *PWT1*) turned brown and shriveled 4 days after inoculation (Fig. 1). This phenotype corre-

TABLE 1. Segregation of virulence on wheat cvs. Norin 4 and Chinese Spring in *F₁* progeny derived from the cross between *Magnaporthe grisea* isolates Br48 and Br58

Wheat	Reaction of parental isolates		Number of progeny					Avirulent (0 to 3–)	Virulent (3+ to 5)	χ^2 or	
	Br58	Br48	0-2 ^a	3–	3+	4-5	Total			1:1	3:1
Norin 4	0-1 ^a	4-5	49	15	3	21	88	64	24	18.18 ^b	0.061
Chinese Spring	0-1	4-5	30	13	5	40	88	43	45	0.045	32.06 ^b

^a Infection type evaluated after 5 days incubation at 20°C. 0 = no reaction; 1 = brown pinpoint spots; 2 = small brown lesions; 3– = brown lesions with an intermediate size accompanied by a few, scattered green lesions; 3+ = green lesions with an intermediate size accompanied by a few, scattered brown lesions; 4 = large green lesions; and 5 = coalesced green lesions.

^b Significant at the 1% level.

TABLE 2. The combined segregation on wheat cvs. Chinese Spring and Norin 4 of *F₁* progeny derived from the cross between *Magnaporthe grisea* isolates Br48 and Br58

Wheat		Number of progeny					χ^2 for	
p	q	ApAq ^a	ApVq	VpAq	VpVq	Total	3:1:3:1	2:(0):1:1
Chinese Spring	Norin 4	43	0	21	24	88	33.78 ^b	0.25

^a A = avirulent (infection types 0 to 3–); V = virulent (infection types 3+ to 5). For example, ApVq represents progeny that are avirulent on p and virulent on q.

^b Significant at the 1% level.

sponded to the B+ type reported by Murakami et al. (10). 3K1 carrying *PWT2* showed another phenotype: large but a few, discrete lesions (Fig. 1). On 'Norin 4' such lesions remained green and developed to green islands by 4 to 5 days after inoculation. Lesions on 'Chinese Spring' were green at first and sometimes turned yellow by 4 to 5 days after inoculation, but faint green islands were still visible. This phenotype corresponded to the G-type reported by Murakami et al. (10). When temperature increased to 27 to 28°C, the phenotype produced by 3K1 became indistinguishable from that produced by the parental *Triticum* isolates, Br48 (data not shown). When 'Norin 4' and 'Chinese Spring' were inoculated with 66W1 carrying *PWT3*, the phenotype produced was very similar to the B+ type with 3M1 and different from the G- type with 3K1 (Fig. 1). This phenotypic characteristic suggests that *PWT3* is distinct from *PWT2*. 70Q3 carrying *PWT4* produced completely compatible lesions on 'Chinese Spring' as expected (Fig. 1). Interestingly, its interaction with 'Norin 4' was temperature insensitive, and completely incompatible as observed at 20°C. These characteristics indicate that *PWT4* is distinct from the other avirulence genes.

Taken together, we conclude that *PWT1*, *PWT2*, *PWT3*, and *PWT4* are all different genes. Further, we suggest that they are located at different loci. Although allelism tests have not been performed between *PWT3/PWT4* and *PWT2*, it seems unlikely that avirulence genes with such distinct characteristics are located at the same locus. Then the genotypes of the parental field isolates, GFSI1-7-2, Br58, and Br48 are assumed to be *PWT1*:*PWT2*; *pwt3*:*pwt4*, *pwt1*:*pwt2*:*PWT3*:*PWT4*, and *pwt1*:*pwt2*:*pwt3*:*pwt4*, respectively. A cross between Br48 and GFSI1-7-2 should produce recombinants like 3M1 (*PWT1*:*pwt2*:*pwt3*:*pwt4*) and 3K1 (*pwt1*:*PWT2*:*pwt3*:*pwt4*) while a cross between Br48 and Br58 should produce recombinants like 66W1 (*pwt1*:*pwt2*:*PWT3*:*pwt4*) and 70Q3 (*pwt1*:*pwt2*:*pwt3*:*PWT4*). Each of these four F₁ progeny is different from Br48 at only one locus among the four loci involved. A comparison of genotypes and phenotypes between Br48 and 3M1 (Fig. 1) suggests that *PWT1* confers the high avirulence at 20°C and the B+ reaction at 25°C. Similarly, a comparison between Br48 and 3K1 suggests that *PWT2* confers the high avirulence at 20°C and the G- reaction at 25°C. *PWT3* seems to have a function similar to *PWT1*. *PWT4* appears to confer the cultivar specific, high avirulence at both temperatures.

A hypothesis. Here, we assume that *PWT1*, *PWT2*, *PWT3*, and *PWT4* are functional alleles under the control of gene-for-gene interactions. For *PWT1*, *PWT2*, and *PWT3* the assumption is based on their responses to temperature. Fig. 1 shows that they are temperature sensitive while their virulence alleles are temperature insensitive. It is unlikely that a nonfunctional allele confers a temperature-sensitive phenotype. The temperature sensitivity may not be due to the avirulence alleles themselves but to their corresponding resistance genes. This may be the case, but makes no contradiction to our assumption because this idea presupposes that this system is governed by gene-for-gene interactions between avirulence genes and resistance genes.

We tentatively designate resistance genes corresponding to *PWT1*, *PWT2*, *PWT3*, and *PWT4* as *R1*, *R2*, *R3*, and *R4*, respectively. Using these symbols, a hypothesis was constructed that

explains the obtained results on the basis of gene-for-gene interactions (Table 4). On 'Norin 4' both *PWT3* and *PWT4* are involved in the specificity because this cultivar carries both *R3* and *R4*. On 'Chinese Spring', *PWT3* is involved but *PWT4* is not because this cultivar carries *R3* but does not carry *R4*.

Evidence for a gene-for-gene interaction. The two rows at the bottom of Table 4 look very similar to the typical "quadratic check" which has been used to explain a gene-for-gene relationship. We picked up this quadrat as a model to test the hypothesis.

First, 61M2, an F₁ progeny from Br58 × Br48 with the *pwt3*:*PWT4* genotype, was backcrossed with the parental *Triticum* isolate, Br48 (*pwt3*:*pwt4*). 61M2 is avirulent on 'Norin 4' and virulent on 'Chinese Spring' while Br48 is virulent on both. Among 78 random progeny isolated, eight did not produce conidia, and one was a mutant that lost basic penetration capability. These progeny were excluded from further analysis. When 'Norin 4' was inoculated with the 69 normal progeny, avirulent (infection types 0 to 2) and virulent (infection types 4 to 5) cultures segregated in a clear 1:1 ratio (Fig. 2). This result confirms that the phenotypic difference on 'Norin 4' between the genotypes *pwt3*:*PWT4* and *pwt3*:*pwt4* (Table 4) is controlled by a single locus, *Pwt4*. Those progeny were all virulent on 'Chinese Spring' (data not shown).

Second, 'Norin 4' was crossed with 'Chinese Spring' and their F₂ progeny were inoculated with 61M2, 65X1, and 70Q3 which were randomly chosen from cultures with the genotype *pwt3*:*PWT4*. 'Norin 4' is resistant to these cultures while 'Chinese Spring' is susceptible. With all three cultures, the distribution of infection types was discontinuous in the F₂ population. Resistant and susceptible seedlings occurred in a 3:1 ratio (Table 5), suggesting that the resistance of 'Norin 4' to the genotype *pwt3*:*PWT4* is controlled by a single gene. This gene should be *R4*, the hypothesized resistance gene corresponding to *PWT4*, and is therefore, tentatively renamed as *Rwt4*. When inoculated with 62L1, 63K1, and 68Q2 with the genotype *pwt3*:*pwt4*, the F₂ seedlings were all susceptible (Table 5), suggesting that there is no difference between 'Norin 4' and 'Chinese Spring' in genetic mechanisms conditioning the susceptibility to these cultures. When inoculated with 79Q1, 82Q1, and 88Q1 with the genotype *PWT3*:*PWT4* or

TABLE 4. A hypothesis explaining the interaction between *Magnaporthe grisea* F₁ progeny and wheat cultivars

Genotype of F ₁ progeny ^b	Reaction ^a with	
	Chinese Spring (<i>R3</i> : <i>r4</i>) ^c	Norin 4 (<i>R3</i> : <i>R4</i>)
<i>PWT3</i> : <i>PWT4</i>	A (<i>PWT3</i> - <i>R3</i>) ^d	A (<i>PWT3</i> - <i>R3</i> , <i>PWT4</i> - <i>R4</i>)
<i>PWT3</i> : <i>pwt4</i>	A (<i>PWT3</i> - <i>R3</i>)	A (<i>PWT3</i> - <i>R3</i>)
<i>pwt3</i> : <i>PWT4</i>	V (-)	A (<i>PWT4</i> - <i>R4</i>)
<i>pwt3</i> : <i>pwt4</i>	V (-)	V (-)

^a A = avirulent; V = virulent.

^b Derived from the cross between *M. grisea* isolates Br48 (*pwt3*:*pwt4*) and Br58 (*PWT3*:*PWT4*).

^c Plant genotype.

^d Avirulence gene – resistance gene pair involved in the reaction.

TABLE 3. Segregation of virulence on wheat cvs. Norin 4 and Chinese Spring in progenies derived from crosses between *Magnaporthe grisea* F₁ cultures

Cross ^a	Test cultivar	Number of progeny						Total	Avirulent (0-2)	Virulent (4-5)	χ ² for 3:1	P
		0 ^b	1	2	3	4	5					
67Q4 × 4K2	Norin 4	0	15	3	0	7	0	25	18	7	0.12	0.5-0.9
66W1 × 4K2	Norin 4	2	17	4	0	8	0	31	23	8	0.01	>0.9
66W1 × 4K2	Chinese Spring	1	19	3	0	8	0	31	23	8	0.01	>0.9

^a 67Q4 and 66W1 are F₁ progeny from Br48 × Br58 while 4K2 is an F₁ progeny from Br48 × GFSI1-7-2.

^b Infection type evaluated after 5 days incubation at 20°C. 0 = no reaction; 1 = brown pinpoint spots; 2 = small brown lesions; 3 = brown to green lesions with an intermediate size; 4 = large green lesions; and 5 = coalesced green lesions.

PWT3;pwt4, the F₂ seedlings were all resistant (Table 5), suggesting that identical gene(s) are involved in the resistance of 'Norin 4' and 'Chinese Spring' to these cultures. If the hypothesis is correct, the identical gene should be *R3* corresponding to *PWT3*.

These results lead us to a conclusion that interactions in the quadrat are controlled by a gene-for-gene relationship between *PWT4* and its corresponding resistance gene *R4* (*Rwt4*).

Fig. 2. Segregation of infection types on wheat cv. Norin 4 in progeny derived from a backcross of 61M2 (*pwt3;PWT4*) with Br48 (*pwt3;pwt4*). Infection type 0 = no reaction; 1 = brown pin-point flecks; 2 = small brown lesions; 3 = brown to green lesions with an intermediate size; 4 = large green lesions; and 5 = coalesced green lesions. The ratio of avirulent (infection types 0-2) and virulent (infection types 4-5) progeny was 36:33. χ^2 (1:1) = 0.13, $P > 0.5$.

DISCUSSION

Murakami et al. (10) reported that symptoms of wheat leaves inoculated with F₁ progeny from Br48 × GFS11-7-2 were classified into four categories, B-, B+, G-, and G+, based on lesion color and size. Their pathogenicity test was performed at 25°C. The present study showed that when the pathogenicity was tested at a lower temperature (20°C), the two characters occurred concomitantly; small lesions were accompanied by brown color while large lesions were accompanied by green color. At this temperature, avirulent and virulent phenotypes were clearly discriminated. The B+ and G- types proved to be attenuated phenotypes of avirulence caused by the increased temperature. We suggest that pathogenicity tests with wheat seedlings be performed at a lower temperature (approximately 20°C). This seems reasonable because wheat is a winter crop.

We identified two fungal genes, *PWT3* and *PWT4*, which are involved in the avirulence of the *Avena* isolate (Br58) on wheat cultivars. Interestingly, these genes had contrasting phenotypes; *PWT3* was cultivar nonspecific (at least on 'Norin 4' and 'Chinese Spring') and temperature sensitive, while *PWT4* was cultivar specific and temperature insensitive. The phenotypic differences observed when 'Norin 4' and 'Chinese Spring' were inoculated with Br58 are fully explained by these characteristics of *PWT3* and *PWT4* (Fig. 1). Br58 is highly avirulent on 'Norin 4' even at 25°C due to the temperature-insensitive gene, *PWT4*. However, *PWT4* does not confer avirulence to 'Chinese Spring' due to its cultivar-specific nature. This enabled us to uncover the B+ phenotype conferred by *PWT3* at 25°C.

We presume that *PWT3* and *PWT4* are genes conditioning species specificity. It may sound curious for *PWT4* because its expression is cultivar specific. However, Valent and coworkers in another series of crosses in *M. grisea* have already reported similar results. Valent et al. (20) identified three avirulence genes, *Avr1-CO39*, *Avr1-M201*, and *Avr1-YAMO*. They were derived from a weeping lovegrass pathogen but were expressed in a cultivar-specific manner on rice cvs. CO39, M201, and Yashiro-mochi, respectively. Cvs. CO39 and M201 are known to be highly sus-

TABLE 5. Responses of F₂ seedlings derived from a cross between wheat cvs. Norin 4 and Chinese Spring to F₁ progeny derived from the cross between *Magnaporthe grisea* isolates, Br48 and Br58

F ₁ progeny	Parental reaction		Number of F ₂ seedlings										<i>x</i> ² for 3:1	<i>P</i>
	Norin 4	Chinese Spring	0 ^a	1	2	3	4	5	Resistant ^b	Susceptible ^c	Total			
62L1	5	5	0	0	0	0	6	43	0	49	49	...	...	
63K1	4	4	0	0	0	0	1	20	0	21	21	...	...	
68Q2	5	5	0	0	0	0	10	15	0	25	25	...	...	
79Q1	1	1	0	21	0	0	0	0	21	0	21	...	...	
82Q1	0	1	0	20	0	0	0	0	20	0	20	...	...	
88Q1	1	1	0	58	4	0	0	0	62	0	62	...	...	
61M2	1	5	0	180	17	0	42	19	197	61	258	0.253	0.5-0.9	
65X1	1	5	0	194	9	0	43	20	203	63	266	0.246	0.5-0.9	
70Q3	1	4	0	183	11	0	40	12	194	52	246	1.957	0.1-0.5	

^a Infection type evaluated after 5 days incubation at 20°C. 0 = no reaction; 1 = brown pinpoint spots; 2 = small brown lesions; 3 = brown to green lesions with an intermediate size; 4 = large green lesions; and 5 = coalesced green lesions.

^b Infection types 0-2.

^c Infection types 4-5.

TABLE 6. Genetic mechanisms of interactions between *Magnaporthe grisea* isolates (GFS11-7-2, Br48, and Br58) and wheat cultivars

<i>M. grisea</i> isolates	Genotype ^b	Reaction ^a with	
		Chinese Spring (<i>R1;R2;R3;rwt4</i>) ^c	Norin 4 (<i>R1;R2;R3;Rwt4</i>)
<i>Setaria</i> isolate GFS11-7-2	(<i>PWT1;PWT2;pwt3;pwt4</i>)	A(<i>PWT1-R1, PWT2-R2</i>) ^d	A(<i>PWT1-R1, PWT2-R2</i>)
<i>Triticum</i> isolate Br48	(<i>pwt1;pwt2;pwt3;pwt4</i>)	V (-)	V (-)
<i>Avena</i> isolate Br58	(<i>pwt1;pwt2;PWT3;PWT4</i>)	A (<i>PWT3-R3</i>)	A (<i>PWT3-R3, PWT4-Rwt4</i>)

^a A = virulent; V = virulent.

^b *PWT1, PWT2, PWT3*, and *PWT4* are avirulence genes corresponding to *R1, R2, R3*, and *Rwt4*, respectively.

^c Plant genotype.

^d Avirulence gene - resistance gene pair involved in the reaction.

ceptible to rice isolates and have not been shown to contain blast resistance genes. This fact suggests that *Avr1-CO39* and *Avr1-M201* are absent or very rare in the population of rice blast fungus and are not involved in the cultivar specificity between rice isolates and rice cultivars. Therefore, they must be genes conditioning the species-specific avirulence of the weeping lovegrass pathogen on its inappropriate host, rice, with a cultivar-specific manner of expression. Similar results have also been obtained from genetic analyses of the forma specialis-genus specificity in the powdery mildew fungus (*Blumeria graminis*)–gramineous plant system. Tosa and his coworkers crossed *B. graminis* f. sp. *tritici* (the wheat mildew fungus) with *B. graminis* f. sp. *agropyri* (the wheatgrass mildew fungus) and identified four genes, *Ppm10*, *Ppm11*, *Ppm14*, and *Ppm15*, in their F₁ population (12,13,14). They were derived from the wheatgrass mildew fungus and involved in its species-specific avirulence on wheat. However, their expression was cultivar specific, and two of the four genes were involved in the avirulence on each wheat cultivar in a different combination.

In the present study, we also identified a resistance gene in wheat ‘Norin 4’. We designated it as *Rwt4* because it corresponds to *PWT4*. However, this symbol does not comply with the rules for wheat genes. *Rwt4* will be the first of a series of wheat genes for resistance to *M. grisea*. Under the rules of nomenclature, therefore, we designate it as *Rmg1* (resistance to *M. grisea*) with *Rwt4* listed as a synonym. What is the primary target of this gene? ‘Norin 4’ is susceptible to the *Triticum* isolate Br48 in spite of carrying *Rwt4*. Therefore, *Rwt4* appears to be a gene for resistance to isolates of *M. grisea* that are not wheat pathogens. One may argue that it may be a gene for resistance to other *Triticum* isolates that happen to operate on the *Avena* isolate. However, this seems unlikely because *Triticum* isolates first appeared in Brazil in the 1980s (7). The *Rwt4* carrier Norin 4 is a Japanese cultivar and should have had no chance to interact with the *Triticum* isolates.

‘Norin 4’ carries *RWT4* but ‘Chinese Spring’ does not, which indicates that the resistance mechanism against *M. grisea* isolates infecting other plant species is not uniform among cultivars of a host species. Similar results have been reported in the cereal mildew system. Tosa and his coworkers examined genetic mechanisms of the resistance of wheat cultivars to the wheatgrass mildew fungus, and identified four genes involved in the resistance (14,15,16). Wheat cultivars carried two or three of the four genes in various combinations (14).

PWT4 is obviously a functional allele under the control of a gene-for-gene interaction. *PWT3* also seems to be a functional allele since (i) its expression is temperature sensitive and (ii) avirulence is “dominant” in crosses involving *PWT3*, e.g., *PWT3* × *PWT4* (Table 1) and *PWT3* × *PWT1* (Table 4). There is another fact that favors this idea. The two crosses, Br48 × Br58 and Br48 × GFSI1-7-2, share Br48 as a virulent parent. Therefore, the virulence allele at the *Pwt3* locus detected in the Br48 × Br58 cross should be also inherited in the F₁ population from Br48 × GFSI1-7-2. In fact, this F₁ population showed no segregation at the *Pwt3* locus. If *Pwt3* is a virulence locus (i.e., *pwt3* is functional), only one possible explanation of this fact is that the *Setaria* isolate (GFSI1-7-2) also carries the functional allele conferring the virulence on wheat while the *Avena* isolate (Br58) does not. This possibility cannot be ruled out logically, but seems to be unlikely since the *Triticum* isolate is phylogenetically closer to the *Avena* isolate than to the *Setaria* isolate (Oh et al., unpublished data). *PWT1* and *PWT2* also seem to be functional alleles because of their temperature sensitivity (Fig. 1) and the “dominance” of avirulence in crosses involving them, e.g., *PWT1* × *PWT2* (8 and unpublished data), *PWT1* × *PWT3* (Table 4), and *PWT1* × *PWT4* (Table 4).

Based on these discussions, a hypothetical model was constructed that explains the parasitic specificity of the *Setaria*,

Triticum, and *Avena* isolates on wheat cultivars (Table 6). Genotypes of ‘Chinese Spring’ and ‘Norin 4’ are presumed to be *R1;R2;R3;rwt4* and *R1;R2;R3;Rwt4*, respectively. The *Avena* isolate is avirulent on ‘Norin 4’ because *PWT3* and *PWT4* are recognized by *R3* and *Rwt4*, respectively. ‘Chinese Spring’ lacks *Rwt4* but is still resistant to the *Avena* isolate because *R3* matches *PWT3* in the *Avena* isolate. The *Setaria* isolate is avirulent on these cultivars because *PWT1* and *PWT2* are recognized by *R1* and *R2*, respectively. The *Triticum* isolate is virulent on them because there occurs no corresponding gene pairs.

Although all the data obtained so far favor the idea that *Pwt1*, *Pwt2*, *Pwt3*, and *Pwt4* are avirulence loci, they are not conclusive except for *Pwt4*. For example, the temperature sensitivity of the avirulence shown in Figure 1 could be explained by the collapse of basic resistance of wheat plant at 25°C. This collapse may be caused by malfunction of the resistance mechanisms or by increased aggressiveness of the fungus at the high temperature. These possibilities cannot be ruled out completely, although the high resistance of ‘Norin 4’ to Br58 and 70Q3 favors the idea that the basic or fundamental resistance mechanisms are still functional at 25°C. If the temperature sensitivity is due to the collapse of basic resistance, *Pwt1*, *Pwt2*, and *Pwt3* may or may not be avirulence loci.

Further studies are needed at the molecular level to test the model. Reports on *AVR1-CO39* may be a good example of molecular analyses of the species specificity. This gene was successfully cloned by chromosome walking and proved to be able to confer avirulence on CO39 to the rice pathogen (4). Interestingly, all rice isolates tested, suffered from genetic rearrangements at this locus leading to the loss of function (3), suggesting that those rearrangements had played an important role in the evolution of the rice pathogen and that *AVR1-CO39* is now an avirulence gene involved in the species specificity on rice.

It should be noted that our data suggest involvement of minor genes in our crosses. The segregation of avirulent and virulent cultures was clearer in the backcross generation (61M2 × Br48) than in the first generation (Br48 × Br58) as reported by Valent et al. (20) in a series of backcrosses with a rice isolate. When compared within progeny with the same genotype at the major gene loci, there seemed a tendency that progeny with high aggressiveness (producing large lesions) on wheat were also highly aggressive on oat, and vice versa (unpublished data). Apparently, minor genes involved in such aggressiveness do not have species-specific effects. However, we do not deny that there are also minor genes involved in the species specificity. These genes may function through producing virulence factors. The effect of such minor genes may be small in some pathotypes like the wheat pathogen, which arose recently, but may be large in others like the rice pathogen, which has a long history of interactions with its host.

ACKNOWLEDGMENTS

We are grateful to H. Kato, a former professor at Kobe University, for valuable suggestions and comments; and B. McIntosh, The University of Sydney, for suggestions for wheat gene designation. This work was supported by grants from Ministry of Education, Culture, Sports, Science and Technology, the Japanese Government (grants 10460022 and 10556011).

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