Identification of a Hidden Resistance Gene in Tetraploid Wheat Using Laboratory Strains of *Pyricularia oryzae* Produced by Backcrossing

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ABSTRACT

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In the process (BC₃F₁ generation) of backcrossing an *Avena* isolate of *Pyricularia oryzae* with a *Triticum* isolate, color mutants with white mycelia were obtained. These white mutants lacked virulence on all (31/31) hexaploid and most (28/32) tetraploid wheat lines tested. In a BC₄F₁ population, white and black cultures segregated in a 1:1 ratio, suggesting that the mutant phenotype is controlled by a single gene. Furthermore, the mycelial color was perfectly linked with avirulence in the BC₄F₁ population; white cultures were all avirulent on common wheat (*Triticum aestivum*) ‘Norin 4’ (N4) whereas black cultures were all viru-

lent. White cultures in the BC₃F₁ and BC₄F₁ generations were also avirulent on tetraploid wheat (*T. dicoccoides*) accession ‘KU109’ (Tat4), which was susceptible to all cultures derived from the parental wild isolates through the BC₂F₁ generation. A cross between Tat4 and a susceptible tetraploid (*T. paleocolchicum*) accession ‘KU196’ (Tat14) produced resistant and susceptible F₂ seedlings in a 3:1 ratio against the white cultures. In the F₃ generation homozygous resistant/segregating/homozygous susceptible lines segregated in a 1:2:1 ratio. These results suggest that the resistance of Tat4 to the white cultures is controlled by a single major gene. This gene, tentatively designated as *RmgTd(t)*, is considered to be a hidden resistance gene because it was not detected with the Br58, F₁, BC₁F₁, or BC₂F₁ cultures. Cytological analysis revealed that the moderate resistance controlled by *RmgTd(t)* was associated with a hypersensitive reaction of mesophyll cells.

Blast disease is caused by the filamentous fungus *Pyricularia oryzae* Cavara (*Magnaporthe oryzae* B. C. Couch) (3) and occurs on rice, wheat, and several genera of grasses (14,20). Blast is a new field disease of wheat first reported in Parana, Brazil, in 1985, and later causing considerable yield losses (26). The use of resistant cultivars remains the most effective, economical, and environmentally sound management strategy, at least for rice blast; however, resistant cultivars are lacking for wheat blast, considering that the pathogen has become a major threat to stable wheat production (10,26,27).

P. oryzae consists of several host-specific subgroups or pathotypes (14) such as *Oryza* isolates pathogenic on rice (*Oryza sativa*), *Setaria* isolates pathogenic on foxtail millet (*Setaria italica*), *Triticum* isolates pathogenic on common wheat (*Triticum aestivum*), *Avena* isolates pathogenic on oat (*Avena sativa*) (19), and *Lolium* isolates pathogenic on perennial ryegrass (*Lolium perenne*) (25). This specificity, which determines host species range, has been categorized into plant species specificity, whereas the specificity that determines cultivar range within a given host range has been termed cultivar specificity (7). *P. oryzae* is known to adapt easily to cultivar resistance. During the past 50 years, resistance genes introduced into rice cultivars were quickly overcome by new races of *P. oryzae* (15). On the other hand, plant

species specificity appears to be stable. In the mechanism of plant species specificity, we may find a clue to the establishment of a breeding system for durable resistance.

Silue et al. (21) demonstrated that race-cultivar specificity follows the gene-for-gene relationship (5). The genetic mechanism of host species specificity, however, remains to be elucidated. Crossing of fungal strains belonging to different host-specific subgroups allows identification of avirulence genes involved in host species specificity. Takabayashi et al. (23) crossed a *Triticum* isolate (Br48) with an *Avena* isolate (Br58) and demonstrated that the specificity on wheat was conditioned by two loci, *Pwt3* and *Pwt4*. They designated the avirulence/virulence alleles at these loci as *PWT3/pwt3* and *PWT4/pwt4*, respectively, suggesting that these genes are under the control of gene-for-gene interactions. A corresponding resistance gene to *PWT4* designated as *Rwt4* (*Rmg1*) was also identified. *Rwt4* is the first known wheat gene for resistance to *P. oryzae*. This gene confers the resistance of wheat to inappropriate isolates from *Avena* (23) but is ineffective against *Triticum* isolates. Their interaction was found to be insensitive to high temperature whereas the interaction of *PWT3* with its corresponding putative resistance gene (*Rwt3*) was temperature sensitive and ineffective at high temperatures (23). These conditions provide a good model system to study the genetic mechanism of host species specificity of *P. oryzae*.

PWT3 is assumed to be a fundamental gene that ubiquitously controls avirulence of *Avena* isolates on wheat. To establish the functionality of *PWT3*, attempts were made to produce a near-isogenic strain of *Triticum* isolate Br48 that carries *PWT3* by continuous backcrosses. In the process of backcrossing, a new

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type of fungal gene associated with mycelial color and avirulence was found. This gene was obviously different from *PWT3* but had a characteristic that simulated an avirulence gene because it corresponded to a “hidden” resistance gene, designated here as *RmgTd(t)*. Genetic behavior of these unique “complementary” genes are reported here.

MATERIALS AND METHODS

Plant and fungal materials. Plant materials used for the preliminary screening were 31 hexaploid and 32 tetraploid wheat lines (Supplemental Table 1). Of these 63 lines, *T. aestivum* ‘Norin 4’ (N4), *T. dicoccoides* accession ‘KU109’ (laboratory code Tat4), and *T. paleocolchicum* accession ‘KU156’ (laboratory code Tat14) were chosen as representatives for segregation analyses. Tat14 was used as a susceptible control. *P. oryzae* strains used in the present study were *Avena* isolate Br58, *Triticum* isolate Br48, and progenies derived from their crosses. Br58 and Br48 were collected in Brazil in 1990. These cultures have been maintained on barley seed media (6) at 4°C in the Laboratory of Plant Pathology, Kobe University. They were transferred to a potato dextrose agar slant just before use and grown at room temperature. Slant cultures, 1 week to 3 months old, were used for experiments.

Genetic crosses of fungal strains. A cross between Br48 (pathogenic on wheat) and Br58 (pathogenic on oat) was performed previously (23). To remove effects of genes other than *PWT3*, an F₁ culture (73Q2) with genotype *PWT3;pwt4* was chosen, and backcrossed with Br48 four times (Fig. 1). Progenies produced by backcrosses were checked by DNA fingerprinting. Genomic DNA extracted as described previously (17), were digested with *EcoRI* (Takara Bio, Shiga, Japan), electrophoresed on 0.7% agarose gel in 0.5× Tris-acetate-EDTA buffer, and transferred onto a nylon membrane Hybond N+filter (GE Healthcare, Buckinghamshire, UK). The membrane was hybridized with pPO-H (4) containing a fragment of Pot2 (13). Labeling and detection were performed using the AlkPhos Direct Labeling and Detection System (GE healthcare).

Infection assay. Wheat seed were pregerminated on moistened filter papers for 2 days, sown in vermiculite (Asahi-Kogyo, Okayama, Japan), supplied with liquid fertilizer in a seedling case (5.5 by 15 by 10 cm), and grown at 22°C in a controlled environment room with a 12-h photoperiod of fluorescent lighting for 7 days. Mycelial plugs of fresh slant cultures (3 weeks to 3 months old) were transferred to oatmeal agar plates (oatmeal, 20 g; sucrose, 10 g; and agar, 10 to 15 g in 500 ml of distilled water) and incubated at 22°C for 10 days. Conidia produced as described previously (16) were suspended in distilled water with 0.01% Tween 20 and adjusted to 1 to 2 × 10⁵ spores/ml. The conidial suspension was sprayed on the adaxial surface of 7-day-old primary leaves. The inoculated plants were incubated in a dark, humid box at 22°C for 24 h. Then, they were transferred to a growth chamber controlled at 22°C with a 12-h photoperiod. Disease intensity was evaluated 4 to 5 days after inoculation. Disease ratings consisted of six progressive levels from 0 to 5: 0 = no visible evidence of infection; 1 = pinpoint spots; 2 = small lesions (<1.5 mm); 3 = lesions of intermediate size (<3 mm); 4 = large, typical lesions; and 5 = complete blighting of leaf blades. These lesions were classified into two categories on the basis of their color; that is, brown (B) and green (G). Infection types were represented by the combination of the size (intensity) score and the color code. Infection types 0, 1B, 2B, and 3B were considered to be resistant and infection types 3G, 4G, and 5G to be susceptible. Sometimes lesions of size 2 and 3 were accompanied by both brown and green tissues. These types of lesions, designated as 2BG and 3BG, respectively, were considered resistant according to Hyon et al. (9). Each infection assay was repeated at least three times.

Molecular mapping. Simple-sequence repeat (SSR) markers for the *P. oryzae* genome (29) were screened to find *PWT3*-flanking markers. Polymerase chain reaction (PCR) for the SSR markers was performed in a 15-μl reaction mixture containing 20 to 50 ng of genomic DNA, 1× PCR buffer (10 mM Tris-HCl [pH 8.4], 50 mM KCl, and 1.5 mM MgCl₂), 200 μM each dNTP, 0.5 μM each of the forward and reverse primers, and 1 U of Taq polymerase. All SSR fragments were amplified with a routine PCR program: 5 min at 94°C; 30 cycles of 30 s at 94°C, 30 s at 55°C, and 1 min at 72°C; and 5 min at 72°C for final extension. Amplified products were electrophoresed in 4.0% polyacrylamide gels (Nacalai Tesque, Kyoto, Japan) and the banding patterns were visualized using SILVER SEQUENCE DNA Staining Reagents (DNA Silver Staining System; Promega Corp., Madison, WI). Molecular weight size markers V and VIII (Boehringer, Mannheim, Germany) were used as standard size markers.

SSR markers for the wheat genome (22,24) were screened to identify the chromosome carrying *RmgTd(t)*. Linkage analysis was performed using 86 F₃ lines derived from the cross between Tat4 (resistant) and Tat14 (susceptible), as described by Inoue et al. (11).

Cytological analysis. Inoculated primary leaves were harvested 60 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 specimens were mounted on a slide glass in 50% glycerol and observed under bright and dark fields of an Olympus BX51 microscope (Olympus, Tokyo) fitted with an exciter filter B. Sites with more than one appressoria on one epidermal cell were excluded from counting. This experiment was repeated twice. In total, 300 appressoria on three leaves were counted for each isolate or progeny in each replication. Cell death was visualized with trypan blue staining, as described previously (9).

RESULTS

Appearance and inheritance of a color mutation. 73Q2 (*PWT3;pwt4*), an F₁ culture derived from Br58 (*PWT3*;PWT4) × Br48 (*pwt3;pwt4*), was backcrossed with Br48 (Fig. 1). Geno-

Fig. 1. Pedigree of representative cultures derived from backcrosses of *Avena* isolate Br58 with *Triticum* isolate Br48.

types of the BC₁F₁ cultures at the *Pwt3* locus were determined by spraying them on N4, with which *PWT3* had been first identified (23). Yt3N1, a BC₁F₁ culture avirulent on N4 (*PWT3* carrier), was chosen, and again backcrossed with Br48 (Fig. 1). The resulting BC₂F₁ cultures were sprayed on N4, and an avirulent culture (h12-1-6) was further backcrossed with Br48. In the resulting BC₃F₁ generation, we found several white cultures. In some sets (tetrads), white and black (normal) cultures segregated in a 1:1 ratio. An example is shown in Figure 2. Of eight BC₃F₁ cultures derived from a single ascus (h22-4), four were white and four were black. The white cultures were considered to be color mutants because both parental isolates (h12-1-6 and Br48) produced black colonies. To reveal the inheritance of this mutation, white BC₃F₁ culture h22-4-7 was further backcrossed with Br48. In the resulting BC₄F₁ population derived from 11 asci, white and black cultures segregated in a 1:1 ratio (41:40) (Supple-

mental Table 2), suggesting that this mutation involved a single gene. Fingerprinting with Pot2 (13) showed that all the white BC₃F₁ cultures shared an identical profile with the black BC₃F₁ siblings and their parents (h12-1-6 and Br48) (Supplemental Figure 1).

Association of the color mutation with avirulence. To check the association of the color mutation with pathogenicity, the eight BC₃F₁ cultures derived from ascus h22-4 were sprayed on N4. The four white cultures were avirulent on N4 whereas the four black cultures were virulent (Fig. 2). Furthermore, in the segregating BC₄F₁ population derived from h22-4-7 × Br48, black colony color was perfectly concordant with virulence on N4 (Table 1).

Tetraploid accession Tat4, which is susceptible to all cultures from the parental *Avena* isolate (Br58) through the BC₂F₁ generation, showed a moderately resistant reaction to the white

Fig. 2. Colony color and pathogenicity of BC₃F₁ cultures derived from a single ascus (h22-4-1 through h22-4-8) and their parents (h12-1-6 and Br48), 5 days after inoculation of N4, Tat4, and Tat14.

TABLE 1. Responses of wheat lines to *Avena* isolate Br58, *Triticum* isolate Br48, and progenies derived from backcrosses with Br48

Plant species	Line	Infection type with						Number of BC ₄ F ₁ cultures from h22-4-7 × Br48 ^a			
		Br58	73Q2 (F ₁)	Yt3N1 (BC ₁ F ₁)	h12-1-6 (BC ₂ F ₁)	h22-4-7 (BC ₃ F ₁)	Br48	White A	White V	Black A	Black V
<i>Triticum aestivum</i> (6×)	N4 (Norin 4)	0–1B	3BG	2B	2B	1B	4G	41	0	0	40
<i>T. dicoccoides</i> (4×)	Tat4 (KU109)	5G	5G	5G	5G	3B	5G	41	0	0	40
<i>T. paleocolchicum</i> (4×)	Tat14 (KU156)	5G	5G	5G	5G	5G	5G	0	41	0	40

^a A = avirulent (infection type 0 to 3BG) and V = virulent (infection type 3G to 5G).

BC₃F₁ cultures (Fig. 2; Table 1). This moderately avirulent reaction of the BC₃F₁ cultures on Tat4 was inherited by the BC₄F₁ cultures. The segregation of avirulent and virulent cultures in the BC₄F₁ generation fitted a 1:1 ratio, and was again perfectly concordant with the segregation of colony color (Table 1).

Identification of a hidden resistance gene. To examine whether the white cultures had lost virulence on all tested wheat lines, 31 hexaploid and 32 tetraploid accessions were inoculated with a white BC₄F₁ culture (h31-2-7). The hexaploid lines tested were all resistant to h31-2-7 (data not shown). On the other hand, the tetraploids contained four susceptible lines. *T. paleocolchicum* Tat14 is shown in Figure 2 and Table 1 as an example of these susceptible accessions. Tat14 was susceptible not only to all black cultures from the parents (Br58 and Br48) through the BC₄F₁ generation but also to all white cultures in the BC₃F₁ and BC₄F₁ generations.

These exceptional lines made it possible to identify a resistance gene in tetraploid wheat. Against white culture h22-4-7, Tat4 was resistant (3B) whereas Tat14 was susceptible (5G) (Table 1). When F₂ seedlings derived from Tat4 × Tat14 were inoculated with h22-4-7, resistant and susceptible seedlings segregated in a 3:1 ratio (Table 2). To confirm the single-gene control, a white culture (A1-3) was chosen from the BC₄F₁ cultures and employed for further segregation analysis. Against A1-3, Tat4 was resistant (2B) whereas Tat14 was susceptible (5G). When inoculated with A1-3, the F₂ population from Tat4 × Tat14 showed a clear 3:1 segregation of resistant and susceptible seedlings (Table 2). In F₃ lines derived from Tat4 × Tat14, homozygous resistant, segregating, and homozygous susceptible lines segregated in 20:42:24, fitting a 1:2:1 ratio ($\chi^2 = 0.42$, $P_{2df} > 0.05$). Molecular mapping with these F₃ lines revealed that this gene was located on chromosome 7B (Supplemental Figure 2). On the other hand, this gene was not detected with Br58, 73Q2 (F₁), Yt3N1 (BC₁F₁), or h12-1-6 (BC₂F₁); Tat4 and all F₂ seedlings from Tat4 × Tat14 were susceptible to these cultures (Tables 1 and 2). These observations led us to successfully detect a hidden resistance gene in wheat which was tentatively designated as *RmgTd(t)*.

This gene is not *Rwt3* corresponding to *PWT3*, because Tat4 is susceptible to Br58, 73Q2 (F₁), Yt3N1 (BC₁F₁), and h12-1-6 (BC₂F₁), which are the *PWT3* carriers in the pedigree. Moreover, h22-4-7 (BC₃F₁) and h31-2-7 (BC₄F₁) carried Br48 alleles at both of the MGM130 and MGM134 loci (Fig. 3B) which flanked *PWT3* on chromosome 6 (Fig. 3A). If h22-4-7 carried *PWT3* (the Br58 allele) at the *Pwt3* locus, crossing-over must have occurred in both flanking regions (i.e., between MGM130 and *PWT3* and between *PWT3* and MGM134), which is highly unlikely.

Cytological response associated with *RmgTd(t)*. To obtain a clue to the nature of this resistance gene, cytological analysis was performed. Tat4 and Tat14 were fixed 60 h after inoculation with Br58, h22-4-7, and Br48 and examined cytologically. On both accessions, appressorial penetration by both parental isolates (Br48 and Br58) was blocked at the papilla stage with frequency of 1 to 5% (Table 3). Most of the germings that overcame the papilla stage produced infection hyphae and effectively colonized the epidermal cells, leading to susceptible reactions.

The white culture, h22-4-7, colonized the susceptible accession Tat14 in a similar way. The most prominent cellular response in Tat4 leaves inoculated with h22-4-7 was a hypersensitive reaction of mesophyll cells (Table 3). Those mesophyll cells were accompanied by yellow fluorescence and stained with trypan blue (Fig. 4). This incompatible reaction resulted in failure to cause disease.

Fig. 3. Analysis of backcross progenies with molecular markers. **A**, Genetic map of the *PWT3* region constructed using a BC₁F₁ population derived from 73Q2 × Br48. Markers prefixed with MGM are from Zheng et al. (29). Others were designed using the database of the *Magnaporthe oryzae* 70-15 genomic sequence. **B**, Polyacrylamide gel electrophoresis analysis of backcross progenies. Two *PWT3*-flanking simple-sequence repeat loci (MGM130 and MGM134) were amplified from genomic DNA of Br48 (*Triticum* isolate), Br58 (*Avena* isolate), 73Q2 (F₁), Yt3N1 (BC₁F₁), h12-1-6 (BC₂F₁), h22-4-7 (BC₃F₁), and h31-2-7 (BC₄F₁), and run on a 6% polyacrylamide gel.

TABLE 2. Frequency distribution of infection types with *Avena* isolate Br58 and its backcross progenies in F₂ seedlings derived from a cross between tetraploid wheat accessions *Triticum dicoccoides* accession KU109 (Tat4) and *T. paleocolchicum* accession KU156 (Tat14)

Culture	Number of F ₂ seedlings from Tat4 × Tat14 ^a									χ^2 for 3:1	<i>P</i>	
	0/1B	2B/2BG	3B	3BG	3G	4G	5G	Total	Res			Sus
Br58	0	0	0	0	0	3	37	40	0	40	...	...
73Q2 (F ₁)	0	0	0	0	3	2	33	38	0	38	...	...
Yt3N1 (BC ₁ F ₁)	0	0	0	0	1	5	31	37	0	37	...	...
h12-1-6 (BC ₂ F ₁)	0	0	0	0	0	5	32	37	0	37	...	...
h22-4-7 (BC ₃ F ₁)	19	5	2	0	3	2	3	34	26	8	0.04	0.84
A1-3 (BC ₄ F ₁)	39	33	9	0	5	2	15	103	81	22	0.73	0.39

^a Infection type at 5 days after inoculation: 0 to 3BG = resistant (Res) and 3G to 5G = susceptible (Sus).

DISCUSSION

In the process of producing *PWT3*-segregating populations, we found color mutants with white mycelia. The white mutants lacked virulence on all hexaploid and most tetraploid wheat lines. The avirulent white and virulent black progeny in the BC₄F₁ population segregated in a 1:1 ratio, suggesting single-gene control. Color mutations accompanied by pathogenicity defects

have been frequently observed in the blast fungus. Chumley and Valent (2) described three classes of pigment mutants (albino, rosy, and buff) that appeared spontaneously or were recovered following mutagenesis. These mutants infected plants that had been wounded by abrading the leaf epidermis but failed to infect intact host plants (2) due to defects in melanin biosynthesis in the appressoria (8). Our mutants (white cultures) are apparently different from the pigment mutants that completely lost the basic

TABLE 3. Cytological responses of wheat accessions against *Avena* isolate Br58, *Triticum* isolate Br48, and BC₃F₁ culture h22-4-7 derived from their crosses^a

Culture	Accession ^b	Infection type	Appressoria (%) accompanied by				
			No reaction	Cell wall defense ^c	HR of epidermal cell	Hyphal growth with HR of mesophyll cells	Hyphal growth with no HR
Br58	Tat4	5G	13.3	1.6	0.5	8.0	77.4
	Tat14	5G	17.1	4.6	7.5	3.4	67.7
h22-4-7	Tat4	3B	0.0	1.6	0.0	96.7	1.6
	Tat14	5G	10.9	2.2	2.1	0.0	85.1
Br48	Tat4	5G	5.5	3.1	33.6	0.8	57.5
	Tat14	5G	11.4	3.1	20.0	1.0	65.1

^a Average of two replications. More than 300 appressoria on three leaves were counted in each replication. HR = hypersensitive reaction.

^b Tat4 = *Triticum dicoccoides* accession KU109 and Tat14 = *T. paleocolchicum* accession KU156.

^c Appressoria that attempted penetration but were stopped at the cell wall stage by papilla formation.

Fig. 4. Cytological responses in Tat14 and Tat4 observed under **A**, a blight field and **B**, a dark field or **C**, after trypan blue staining 60 h after inoculation with h22-4-7. **A** and **B** are the same sites. App = appressoria, IH = infection hyphae, and arrowhead indicates dead cell strongly stained with trypan blue. Bars represent 100 μ m.

capacity of penetration. They produced dark-brown appressoria and extended infection hyphae (Fig. 4), indicating that their ability to penetrate host cell walls remains intact. In addition, they are completely virulent on some tetraploid accessions including Tat14 (Fig. 2).

The mutants we have described led us to identify a hidden resistance gene, *RmgTd(t)*, located on chromosome 7B in tetraploid accession Tat4. The intermediate resistance of Tat4 conditioned by *RgmTd(t)* was due to the production of infection hyphae by appressoria, which eventually induced hypersensitive responses of mesophyll cells. *RmgTd(t)* is not *Rwt3* corresponding to *PWT3* because Tat4 is susceptible to *PWT3* carriers such as Br58 and the present F₁, BC₁F₁, and BC₂F₁ cultures. Zhan et al. (28) found two resistance genes, *Rmg2* and *Rmg3*, in 'Thatcher' wheat that are effective against *Triticum* isolates. These genes are located on chromosomes 7A and 6B, respectively. Two other wheat genes, *Rmg4* and *Rmg5*, controlling resistance to the inappropriate species *P. grisea* (*Digitaria* isolates), were located on chromosomes 4A and 6D, respectively (18). Apparently, *RmgTd(t)* is different from these blast resistance genes previously identified in wheat.

The above results led us to infer the original function or targets of *RmgTd(t)*. One possibility is that *RmgTd(t)* is involved in the basic machinery of disease resistance and that the four tetraploid accessions susceptible to the white mutants are defective in this gene. This seems unlikely because the four accessions are resistant to other blast isolates. For example, Tat14 is resistant to *P. grisea* Dig41 isolated from crabgrass. Another susceptible accession, *T. dicoccum* St16, is resistant to *P. oryzae* PO12-7301-2 isolated from rice (data not shown). The second possibility is that *RmgTd(t)* is a pattern recognition receptor recognizing a microbial-associated molecular pattern (MAMP) (1,12). This seems also unlikely because, if this is the case, the white mutants must carry a MAMP which was absent in the wild isolates and newly produced by a mutation during the backcrossing. This idea contradicts the definition of MAMPs, which must be ubiquitous in microbes. The third possibility is that the effect of *RmgTd(t)* was not enough to arrest infection of young seedlings with aggressive wild isolates and became apparent at a later stage, with development of adult resistance, but that the pigment mutation reduced the aggressiveness of the isolates and made its effect detectable at the primary leaf stage. The fourth possibility is that *RmgTd(t)* is a potential resistance gene with no targets or a resistance gene against other microbes but happened to recognize the product of the mutated fungal gene.

It should be noted that incompatibility occurs only when *RmgTd(t)* matches the fungal gene associated with the color mutation. From a genetic viewpoint, therefore, the gene responsible for the pigment and virulence defects looks like an avirulence gene corresponding to *RmgTd(t)* involved in a gene-for-gene interaction. Further studies are needed to clone the gene responsible for the unique characteristics of the mutant.

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