The Rye Mildew Fungus Carries Avirulence Genes Corresponding to Wheat Genes for Resistance to Races of the Wheat Mildew Fungus

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ABSTRACT

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The common wheat cultivar Chancellor and its near-isogenic lines carrying resistance genes to *Blumeria graminis* f. sp. *tritici,* the wheat powdery mildew fungus, were inoculated with 60 F_1 cultures derived from a cross between *B. graminis* f. sp. *secalis*, the rye powdery mildew fungus, and *B. graminis* f. sp. *tritici*. Segregation patterns of avirulent and virulent cultures showed that the F_1 population carries avirulence genes corresponding to *Pm1, Pm2, Pm3a, Pm3b, Pm3c,* and *Pm1a,* resistance genes to races of the wheat mildew fungus. This result indicates that the rye mildew fungus, an inappropriate forma specialis for wheat, carries these avirulence genes.

Additional keywords: gene-for-gene interaction.

Between higher plants and their pathogens we can recognize various levels of specificity, which is considered to result from coevolutionary processes. With the accumulation of experimental data from genetic, cytological, and biochemical studies, some basic concepts of host-parasite specificity have been developed. Heath (4) distinguished two types of host-parasite specificity: one (plant species specificity) determines host species range, and the other (cultivar specificity) determines cultivar range within a given host species. The former is thought to be genetically complex, while the latter is controlled by gene-for-gene interactions (3). Cultivar specificity was considered to be superimposed on the "basic compatibility" resulting from plant species specificity. This model (the basic compatibility model of specificity) has been widely accepted as a basic concept. However, several recent reports have suggested that inappropriate biotypes (parasitizing other plant species) of some pathogens carry avirulence genes that condition cultivar specificity (5). Such data appear to contradict Heath's basic concept.

Blumeria graminis (= *Erysiphe graminis*), the causal agent of powdery mildews of gramineous plants, comprises eight formae speciales, e.g., f. sp. *tritici* (the wheat mildew fungus) parasitic on *Triticum,* f. sp. *secalis* (the rye mildew fungus) parasitic on *Secale,* and f. sp. *agropyri* (the wheatgrass mildew fungus) parasitic on *Agropyron*. These formae speciales are interfertile but show various levels of reproductive isolation (6). In an F_1 population derived from a cross, *B. g.* f. sp. *agropyri* × *B. g.* f. sp. *tritici,* a negative correlation is recognized between virulence range and aggressiveness (6). Based on these facts, Hiura (6) inferred that the wild type of *B. graminis* was originally a Mendelian population that hybridized without any barrier, but that host specialization developed gradually, resulting in the establishment of the formae speciales. Subsequently, Tosa (12) demonstrated that the forma specialis–genus specificity follows the gene-for-gene interactions. This let him conclude that the forma specialis–genus specificity belongs to cultivar specificity rather than plant species specificity. Taking these ideas into consideration, Tosa (13) constructed a model for the evolution of formae speciales and races. The points of the model are: i) each forma specialis establishes basic compatibilities with all genera including "hosts" of other formae speciales, and ii) evolution of formae speciales and races is the process of losing potential avirulence genes that are associated with aggressiveness. If his model is valid, it is not unaccountable that inappropriate formae speciales carry avirulence genes that control cultivar specificity.

Tosa and Tada (17) reported that *B. g.* f. sp. *agropyri,* an inappropriate forma specialis for wheat, carries avirulence genes corresponding to wheat genes for resistance to *B. g.* f. sp. *tritici* (i.e., determining cultivar specificity), and suggested that these resistance genes operate against *B. g.* f. sp. *agropyri*. The objective of this study was to examine whether another inappropriate forma specialis, *B. g.* f. sp. *secalis,* carried avirulence genes corresponding to *Pm1* through *Pm4a,* wheat genes for resistance to races of *B. g.* f. sp. *tritici*.

MATERIALS AND METHODS

Fungal materials. The parental cultures were field isolates, *B. graminis* (DC.) Golovin ex Speer f. sp. *secalis,* Sk-1, and *B. graminis* (DC.) Golovin ex Speer f. sp. *tritici,* Tk-1. Their genotypes were previously reported (14,16). Sk-1 was avirulent on most (358 of 362) wheat cultivars collected from all over the world (15). Sixty F_1 cultures derived from a cross between Sk-1 and Tk-1 (14) were used for inoculation. The F_1 cultures were maintained on an accession of einkorn wheat, *Triticum urartu* Thum. (Urr).

Plant materials. The wheat lines tested were *Triticum aestivum* (L.) Thell. 'Chancellor' (Cc0) and six near-isogenic lines produced by Briggle (1) (Table 1). These lines carry one each of known genes, *Pm1, Pm2, Pm3a, Pm3b, Pm3c,* and *Pm4a,* for resistance to the wheat mildew fungus. All these lines, except Cc2, were highly susceptible to wheat isolate Tk-1 (infection type 4) and highly resistant to rye isolate Sk-1 (infection type 0) (Table 1).

Determination of infection types. Seeds of the wheat lines were sown in soil mix in 1.8 × 35 cm glass test tubes with paper plugs. On the same day conidia of cultures to be tested were transferred to Urr seedlings growing in test tubes. Eight days after sowing, primary leaves were inoculated with conidia from the 8-day-old colonies on Urr seedlings using writing brushes. The seedlings were grown in a controlled-environment room under fluorescent lighting (2,000 to 40,000 lx). The temperature in the room was 22 ± 1°C during the light cycle (13 h) and 20 ± 1°C during the dark cycle (11 h). Eight days after inoculation, infection type was rated using 13 progressive grades from 0 to 4: 0 = no mycelial growth or sporulation; 0^+ = mycelial growth with no visible sporulation; 1^- = conidiophore formation without visible conidial powder; 1, 1^+ = scant sporulation; 2^-, 2, 2^+ = reduced sporulation; 3^-, 3, 3^+ = slightly reduced sporulation; 4^-, 4 = heavy sporulation. Six seedlings were scored for each F_1 culture. There was not much variation of infection types among six seedlings inoculated with the same culture. If they did not show the same infection type, the most frequent infection type was scored. Based on frequency distribution of infection types, the cultures were rated as avirulent or virulent. In this report the terms avirulent and virulent are considered to be only relative. The segregation of avirulent to virulent cultures was analyzed using the chi-square (χ^2) test.

RESULTS

When Cc0 was inoculated with the 60 F_1 cultures, a discontinuous distribution of infection types was produced (Table 2). The border between avirulent and virulent cultures was considered to be located between 1^- and 1. The segregation of avirulent (infection types 0 to 1^-) to virulent (infection types 1 to 4) cultures fit a 1:1 ratio. These results indicate that a major gene is involved in the avirulence of Sk-1 on Cc0.

On Cc1, Cc3a, Cc3b, and Cc4a the segregation of avirulent and virulent cultures in the F_1 population fit a 3:1 ratio (Table 2), indicating that two genes are involved in the avirulence on each of them. All cultures avirulent on Cc0 were avirulent on Cc1, Cc3a, Cc3b, and Cc4a (Table 3) while the other 27 cultures virulent on Cc0 segregated 1:1 for avirulence and virulence on the four near-isogenic lines. These results indicate that an additional gene is involved in the avirulence on each of the four lines compared with avirulence on Cc0. The segregations on Cc1, Cc3a, and Cc4a were independent of one another (Table 4). The segregations on Cc3a and Cc3b were not independent but a few recombinants occurred (Table 4).

On Cc3c the segregation of avirulent and virulent cultures in the F_1 population did not fit a 3:1 ratio (Table 2). However, 5 cultures were virulent on Cc0 and avirulent on Cc3c (Table 3), indicating that an additional avirulence gene is also involved. On Cc2 all 60 cultures produced infection type 0, indicating that the two parental isolates have at least one avirulence gene in common.

TABLE 1. Infection types of wheat lines near-isogenic for wheat mildew resistance genes to *Blumeria graminis* f. sp. *tritici* isolate Tk-1 and *B. g.* f. sp. *secalis* isolate Sk-1

Line[a]	Resistance gene	Tk-1[b]	Sk-1[b]
Chancellor (Cc0)	*Pm10* + *Pm15*[c]	4	0
Norka/8*Cc (Cc1)	*Pm10* + *Pm15* + *Pm1*	4	0
Ulka/8*Cc (Cc2)	*Pm10* + *Pm15* + *Pm2*	0	0
Asosan/8*Cc (Cc3a)	*Pm10* + *Pm15* + *Pm3a*	4	0
Chul/8*Cc (Cc3b)	*Pm10* + *Pm15* + *Pm3b*	4	0
Sonora/8*Cc (Cc3c)	*Pm10* + *Pm15* + *Pm3c*	4	0
Khapli/8*Cc (Cc4a)	*Pm10* + *Pm15* + *Pm4a*	4	0

[a] Cc1–Cc4a are near-isogenic lines of Cc0 (1).
[b] Infection type produced: 0, no mycelial growth or sporulation; 4, heavy sporulation.
[c] *Pm10* and *Pm15* are for resistance to inappropriate formae speciales of *B. graminis* (14,16,17).

DISCUSSION

Cc0 carries two genes, *Pm10* and *Pm15,* for resistance to the wheatgrass mildew fungus (17). *Pm15* also operates against the rye mildew fungus, Sk-1, while *Pm10* does not (14). Therefore, according to the gene-for-gene relationship, the avirulence gene detected on Cc0 in the present study is considered to be *Ppm15* corresponding to *Pm15*. *Ppm15* should be also involved in the avirulence on Cc1, Cc2, Cc3a, Cc3b, Cc3c, and Cc4a, since they are near-isogenic lines of Cc0, and carry *Pm15*. This inference was confirmed by the result that all cultures avirulent on Cc0 (*Ppm15* carriers) were avirulent on Cc1 through Cc4a.

The combined segregation analysis (Table 3) indicated that an additional gene is involved in the avirulence on Cc1 compared with avirulence on Cc0. Since Cc0 and Cc1 have the same genotype except for *Pm1,* any difference in their phenotypes of reaction to powdery mildew can be attributed to *Pm1*. Therefore, we consider that the additional avirulence gene corresponds to *Pm1*. This avirulence gene is apparently derived from Sk-1 since Tk-1 is virulent on Cc1 (Table 1) and avirulence is epistatic to virulence (11). This means that the rye mildew fungus, Sk-1, carries the avirulence gene corresponding to *Pm1*. Similarly, the additional genes involved in the avirulence on Cc3a, Cc3b, Cc3c, and Cc4a are considered to correspond to *Pm3a, Pm3b, Pm3c,* and *Pm4a,* respectively, and to be derived from Sk-1.

Since Cc2 is resistant and Cc0 is susceptible to Tk-1, the resistance of Cc2 is attributable to *Pm2* (Table 1). This means that Tk-1 carries the avirulence gene (here tentatively designated as *Ppm2*) corresponding to *Pm2*. If Sk-1 does not carry *Ppm2,* a cross between Sk-1 (*Ppm15* carrier) and Tk-1 (*Ppm15* noncarrier) would yield progeny that do not carry any of *Ppm2* or *Ppm15* and are, therefore, virulent on Cc2. In fact, no such cultures occurred in the 60 F_1 cultures from Sk-1 × Tk-1 (Table 2). This indicates that Sk-1 carries the avirulence gene corresponding to *Pm2*.

From these results we conclude that rye mildew isolate Sk-1 carries avirulence genes corresponding to *Pm1, Pm2, Pm3a,*

TABLE 2. Distribution of infection types on wheat lines near isogenic for wheat mildew resistance genes in an F_1 population of mono-conidial cultures derived from the cross between *Blumeria graminis* f. sp. *tritici* isolate Tk-1 and *B. g.* f. sp. *secalis* isolate Sk-1

	No. of F_1 cultures producing infection types[a]										χ^2 for	
Line	0	0^+	1^-	1	1^+	2^-	2	2^+ to 4	Avirulent:virulent	Total	1:1	3:1
Cc0	30	3	0	0	6	7	8	6	33:27	60	0.60	12.8*[b]
Cc1	47	1	1	0	3	3	2	3	49:11	60	24.07*	1.42
Cc2	60	0	0	0	0	0	0	0	60:0	60	. . .	. . .
Cc3a	38	4	1	0	9	4	3	1	43:17	60	11.27*	0.36
Cc3b	41	2	1	0	4	8	3	1	44:16	60	13.07*	0.09
Cc3c	37	1	0	4	3	5	6	4	38:22	60	4.27*	4.36*
Cc4a	45	4	2	0	3	2	2	2	51:9	60	29.40*	3.20

[a] 0 to 1^-, avirulent; 1 to 4, virulent.
[b] *, significant at the 5% level.

Pm3b, Pm3c, and *Pm4a,* wheat genes for resistance to races of the wheat mildew fungus. Tosa and Tada (17) indicated that isolate Ak-1 of wheatgrass mildew fungus carries avirulence genes corresponding to *Pm1, Pm2, Pm3a,* and *Pm3b*. From these data we suggest that inappropriate formae speciales generally carry avirulence genes corresponding to race-specific resistance genes. Tosa (13) presented a model suggesting that the evolution of formae speciales would be a process of losing avirulence genes so as not to be recognized by their corresponding resistance genes. According to this model, *Pm1* through *Pm4a,* the genes involved in the race-cultivar specificity in wheat-wheat mildew interactions, should have occurred in the wheat population after formae speciales were established. Thus, the rye mildew fungus and the wheatgrass mildew fungus did not have to lose potential avirulence genes corresponding to those resistance genes in wheat. This is probably why the inappropriate formae speciales carry such avirulence genes.

The fact that the rye mildew fungus, Sk-1, carries avirulence genes corresponding to *Pm1* through *Pm4a* implies that these resistance genes operate against Sk-1. Similarly, *Pm1* through *Pm3b* operate against the wheatgrass mildew fungus, Ak-1 (17). However, they are not main genes that condition the resistance of wheat to inappropriate formae speciales. Such resistance is conditioned by *Pm10, Pm11, Pm14,* or *Pm15* (16,18,19) which do not operate against the wheat mildew fungus. In summary, there are two categories of resistance genes in wheat. One is effective against both the wheat mildew fungus and inappropriate formae speciales. The other is effective only against inappropriate formae speciales and is not involved in "cultivar specificity." Both are under the control of gene-for-gene interactions but different in the distribution of their corresponding avirulence genes (15).

TABLE 3. Segregation on wheat lines near isogenic for wheat mildew resistance genes in an F_1 population derived from the cross between *Blumeria graminis* f. sp. *tritici* isolate Tk-1 and *B. g.* f. sp. *secalis* isolate Sk-1

Lines		No. of F_1 cultures with combination of virulence and/or avirulence					χ^2 for 1:1
p	q	A_pA_q[a]	A_pV_q	V_pA_q	V_pV_q	Total	(V_pA_q:V_pV_q)
Cc0	Cc1	33	0	16	11	60	0.93
Cc0	Cc2	33	0	27	0	60	. . .
Cc0	Cc3a	33	0	10	17	60	1.81
Cc0	Cc3b	33	0	11	16	60	0.93
Cc0	Cc3c	33	0	5	22	60	10.70*[b]
Cc0	Cc4a	33	0	18	9	60	3.00

[a] A, avirulent (infection type 0 to 1⁻); V, virulent (infection type 1 to 4). For example, A_pV_q represents cultures that are avirulent on p and virulent on q.
[b] *, significant at the 5% level.

TABLE 4. Segregation on wheat lines near isogenic for wheat mildew resistance genes among Cc0-virulent F_1 cultures derived from the cross between *Blumeria graminis* f. sp. *tritici* isolate Tk-1 and *B. g.* f. sp. *secalis* isolate Sk-1

Lines		No. of F_1 cultures with combination of virulence and/or avirulence					χ^2 (independence)
p	q	A_pA_q[a]	A_pV_q	V_pA_q	V_pV_q	Total	
Cc3a	Cc3b	9 (4.1)[b]	1 (5.9)	2 (6.9)	15 (10.1)	27	15.78*[c]
Cc1	Cc3a	6 (5.9)	10 (10.1)	4 (4.1)	7 (6.9)	27	0.01
Cc1	Cc4a	11 (10.7)	5 (5.3)	7 (7.3)	4 (3.7)	27	0.06
Cc3a	Cc4a	7 (6.7)	3 (3.3)	11 (11.3)	6 (5.7)	27	0.06

[a] A, avirulent (infection type 0 to 1⁻); V, virulent (infection type 1 to 4). For example, A_pV_q represents cultures that are avirulent on p and virulent on q.
[b] Expected number of F_1 cultures when avirulence on two lines is assumed to be inherited independently.
[c] *, significant at the 5% level.

There are at least two systems that are controlled by similar genetic mechanisms. One is the cereal rust system (7). Sanghi and Luig (9,10) suggested that resistance of wheat to the rye stem rust, *Puccinia graminis* f. sp. *secalis,* is conditioned by two types of resistance genes: one that provides resistance to the rye stem rust but not to the wheat stem rust, *P. graminis* f. sp. *tritici,* and another that provides resistance to the wheat stem rust, which may or may not operate against individual strains of the rye stem rust.

The other system is the *Magnaporthe grisea*–gramineous plant system (6). This fungus comprises several types that are different in host ranges (e.g., pathogenic on rice, finger millet, or crabgrass) (8,21). Yaegashi and Asaga (22) reported circumstantial evidence that the finger millet pathogen (nonpathogenic on rice) carries an avirulence gene corresponding to *Pi-a,* a rice gene for resistance to races of the rice pathogen. This is comparable to the present result that the rye mildew fungus carries avirulence genes corresponding to *Pm1* through *Pm4a*, wheat genes for resistance to races of the wheat mildew fungus. Furthermore, Valent et al. (20) reported that the weeping lovegrass pathogen (nonpathogenic on rice) carries three avirulence genes, *Avr1-CO39, Avr1-M201,* and *Avr1-YAMO,* determining avirulence on rice cultivars CO39, M201, and Yashiro-mochi, respectively. At least two of them, *Avr1-CO39* and *Avr1-M201,* do not seem to occur in the rice pathogen population since CO39 and M201 have not been shown to carry resistance genes to the rice pathogen. The two genes are apparently cultivar-specific, but are not involved in "cultivar specificity." They are comparable to *Pmp10, Pmp11,* and *Ppm14* (avirulence genes corresponding to *Pm10, Pm11,* and *Pm14,* respectively) in the cereal mildew system, since *Ppm10, Ppm11,* and *Ppm14* are specific to wheat cultivars Norin 4, Chinese Spring, and Norin 10 respectively, but are not involved in "cultivar specificity" of the wheat mildew fungus–wheat interactions.

In bacterial systems, some reports suggest, inappropriate pathovars of a given host carry genes conferring cultivar-specific avirulence toward the host (2,5). It is not clear whether these data can be interpreted as mentioned above.

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