GENETICS OF HOST-PARASITE INTERACTION IN BARLEY MILDEW

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Flor (1955) has elucidated the gene-for-gene relationship between pathogenicity in the flax rust fungus *Melampsora lini* (Pers.) Lév. and rust reaction in the host, *Linum usitatissimum* L. According to his hypothesis, for each resistance gene in flax there is a specific gene for virulence in those rusts to which it is susceptible. Moseman (1959, '60, '63), and Moseman & Schaller (1960, '62) have found that for the 8 genes conditioning reaction in the barley varieties, Algerian, Atlas, Black Russian, Goldfoil, Hanna, Kwan, Nepal, and Recard, there are 8 complementary genes for pathogenicity in barley mildew fungus. Hiura, Heta & Tsushima (1961) studied inheritance of pathogenicity of *Erysiphe graminis* f. sp. *hordei* on the barley varieties with known genes for resistance to the avirulent parent cultures of the fungus. They have shown that Flor's gene-for-gene hypothesis is true in barley mildew.

The present paper deals with some of data of the Japanese article (Hiura, Heta & Tsushima 1961) and unpublished additional data for elucidation host-parasite interaction in barley mildew.

MATERIALS AND METHODS

11 cultures of *Erysiphe graminis* f. sp. *hordei* (H2, H4, H5, H6, H7, H9, H10, H11, H12, H13 and H14) were used in this study. The first 8 cultures were single conidium subcultures of the races II, IV, V, VI, VII, IX, X and XI, respectively, used in the previous studies (Hiura 1960). Cultures H12, H13 and H14 were derived from crosses of race I × race IV, race IX × race XI, and race XI × H12, respectively.

14 barley varieties (A. 222, Goldfoil, Hanna, Heil's Hanna, H. E. S. 4, Hoso-mugi C, Kairyo-bozu, Monte Cristo, Nigrate, Russian 12, Russian 66, Russian 74, Six-rowed Chevalier and Turkey 290) were used to differentiate the pathogenicity of haploid progeny cultures obtained from crosses between cultures of the fungus. These 14 varieties, except Turkey 290, had known genes for mildew reactions (Hiura 1960). Turkey 290 had one gene conditioning mildew reaction (unpublished data).

Each of the crosses between cultures of *Erysiphe graminis* f. sp. *hordei* was made on 2 plants of the susceptible barley variety, Kuromugi 148, grown in a 12 cm clay pot for 4–5 weeks in a isolation cage. The cage was 21 × 21 cm and 42 cm in height, enclosed with glass on two sides and white cotton cloth on the

other two sides and top. One of the 2 plants was inoculated with one of cultures to be crossed and the other one was inoculated with the mate. Mildew developed well on those plants 7 days after inoculation. Hybridization was accomplished by transferring conidia with a hair pencil from pustules of one culture onto pustules of the other culture. Cleistothecia formed on the plants 10–14 days after crossing. Leaves with cleistothecia were harvested 20 days after crossing and stored in a desiccator with calcium chloride.

Progeny cultures were obtained from the cleistothecia by the following methods. Mycelial mats with cleistothecia were removed from the leaves and kept on moist filter paper. The moist filter paper was suspended at the top of a glass cylinder, 20 × 7 cm, that enclosed seedlings of Kuro-mugi 148 growing in a 12 cm clay pot. The discharged ascospores fell on and infected the seedlings. Conidia from each pustule developed on the seedlings were transferred with a hair pencil onto a seedling of the susceptible host growing in a 22 × 2 cm test tube plugged with cotton. The cultures in the test tubes were purified by means of single spore isolations, thus obtaining pure haploid progeny cultures of the cross.

The pathogenicity of the progeny cultures on the differential hosts were determined by inoculation with conidia of each test tube culture on the seedlings of the varieties each growing in a 22 × 2 cm test tube. Inoculation was made by using a hair pencil and readings were made 7–8 days following the inoculation. Pathogenicity was divided into 3 classes : A=avirulent, producing an infection type of 0; I=intermediate, producing an infection type of 1 to 3; and V=virulent, producing an infection type of 3–4 to 4. 2 test tubes were used for each test and each test was repeated twice. All experiments in this study were conducted in the greenhouse where the temperature was maintained at approximately 20°C.

RESULTS

1. *Inheritance of Pathogenicity of Mildew Cultures on Barley Varieties Each Having a Single Gene Conditioning Resistance to Mildew*

Progeny Cultures from Cross of Cultures H9 × H14. 9 barley varieties (A. 222, Goldfoil, Hanna, Heil's Hanna, Monte Cristo, Nigrate, Russian 12, Russian 74 and Turkey 290) with single mildew conditioning genes, were chosen as differential varieties for the pathogenicity of the progeny cultures from cross of H9 × H14 because they showed reciprocal reactions to the parent cultures. The pathogenicity of cultures H9 and H14 on the 9 varieties was given in Table 1. The number of genes conditioning the pathogenicity of cultures H9 and H14 on each variety was determined by the pathogenicity of 289 haploid progeny cultures from the cross of cultures H9 × H14 on those varieties (Table 2).

Although the infection types produced on the varieties, Goldfoil, Monte

TABLE 1
Pathogenicity of cultures H9 and H14 of *Erysiphe graminis hordei* on 9 barley varieties

Variety	Pathogenicity of culture	
	H9	H14
A.222	V*	I
Goldfoil	A	I
Hanna	I	V
Heil's Hanna	V	A
Monte Cristo	A	I
Nigrate	I	A
Russian 12	V	I
Russian 74	V	A
Turkey 290	V	A

* V = virulent, producing an infection type of 3-4 to 4;
I = intermediate, producing an infection type 1 to 3;
A = avirulent, producing an infection type 0

TABLE 2
Classification of 289 haploid progeny cultures from cross of cultures H9 × H14 according to their pathogenicity on 9 barley varieties

Differential variety	Observed number of progeny cultures			Value of P for 1 : 1 ratio
	Virulent*	Intermediate	Avirulent	
A.222	135	154		.2-.3
Goldfoil		149	140	.5-.7
Hanna	147	142		.7-.8
Heil's Hanna	147		142	.7-.8
Monte Cristo		133	156	.1-.2
Nigrate		149	140	.5-.7
Russian 12	143	146		.8-.9
Russian 74	142		147	.7-.8
Turkey 290	143		146	.8-.9

* See footnotes, Table 1.

Cristo and Nigrate were not always the same as those produced from inoculation with the parent cultures, it was not difficult to distinguish avirulent from intermediate. On Goldfoil, for example, cultures H9 and H14 produced reaction type 0 and type 1-2, respectively, and the progeny cultures of H9 × H14 produced types 0, 1, 2 and 3. Avirulent (type 0), however, could be sharply distinguished from intermediate (types 1, 2 and 3). The infection types of the progeny cultures on the varieties Hanna, Heil's Hanna, Russian 12, Russian 74, and Turkey 290 were the same as those produced from inoculations with the parent cultures. On A. 222, difficulties were encountered in dividing some

cultures into intermediate and virulent. It was supposed that cultures H9 and H14 differed by one major gene conditioning their pathogenicity and modifying gene or genes affecting the degree of the pathogenicity on A. 222.

In this way, on each variety, except A. 222, the 289 progeny cultures could be separated exactly by their reaction types into only 2 classes : avirulent and either intermediate or virulent, or intermediate and virulent. The observed number of cultures divided into 2 classes on each of 9 varieties fits the 1:1 ratio expected if the parent cultures differed by one gene for pathogenicity on that variety.

The relationships of the genes conditioning pathogenicity on the 9 varieties were determined by comparing the pathogenicity of the 289 haploid progeny cultures on those varieties (Table 3). On each variety, the pathogenicity of

TABLE 3

Relationships of genes conditioning pathogenicity of 289 progeny cultures from cross of cultures H9×H14 on 9 barley varieties

Differential variety		Observed number of progeny cultures				Value of P for 1:1:1:1 ratio
X	Y	V_xV_y*	V_xA_y	A_xV_y	A_xA_y	
A. 222	Goldfoil	72	63	77	77	.5 -.7
A. 222	Hanna	70	65	77	77	.7 -.8
A. 222	Heil's Hanna	65	70	82	72	.5 -.7
A. 222	Monte Cristo	61	74	72	82	.3 -.5
A. 222	Nigrate	66	69	83	71	.5 -.7
A. 222	Russian 12	65	70	78	76	.5 -.7
A. 222	Russian 74	64	71	78	76	.5 -.7
A. 222	Turkey 290	63	72	80	74	.5 -.7
Goldfoil	Hanna	83	66	64	76	.3 -.5
Goldfoil	Heil's Hanna	77	72	70	70	.5 -.7
Goldfoil	Monte Cristo	74	75	59	81	.3 -.5
Goldfoil	Nigrate	73	76	76	64	.7 -.8
Goldfoil	Russian 12	70	79	73	67	.7 -.8
Goldfoil	Russian 74	79	70	63	77	.5 -.7
Goldfoil	Turkey 290	66	83	77	63	.3 -.5
Hanna	Heil's Hanna	67	80	80	62	.3 -.5
Hanna	Monte Cristo	68	79	65	77	.5 -.7
Hanna	Nigrate	70	77	79	63	.5 -.7
Hanna	Russian 12	5	142	138	4	Very small
Hanna	Russian 74	74	73	68	74	.95-.98
Hanna	Turkey 290	77	70	66	76	.7 -.8
Heil's Hanna	Monte Cristo	64	83	69	73	.3 -.5
Heil's Hanna	Nigrate	80	67	69	73	.7 -.8
Heil's Hanna	Russian 12	83	64	60	82	.1 -.2
Heil's Hanna	Russian 74	70	77	72	70	.9 -.95
Heil's Hanna	Turkey 290	75	72	68	74	.9 -.95
Monte Cristo	Nigrate	67	66	82	74	.5 -.7
Monte Cristo	Russian 12	67	66	76	80	.5 -.7
Monte Cristo	Russian 74	58	75	84	72	.1 -.2
Monte Cristo	Turkey 290	70	63	73	83	.3 -.5
Nigrate	Russian 12	80	69	63	77	.3 -.5
Nigrate	Russian 74	63	86	79	61	.1 -.2
Nigrate	Turkey 290	82	67	61	79	.2 -.3
Russian 12	Russian 74	70	73	72	74	.98-.99
Russian 12	Turkey 290	67	76	76	70	.8 -.9
Russian 74	Turkey 290	64	78	79	68	.5 -.7

* V_x and A_x indicate the pathogenicity of progeny cultures on var. X;
V_y and A_y indicate the pathogenicity of progeny cultures on var. Y.

TABLE 4
Linkage relation between genes conditioning pathogenicity of 289 progeny cultures from cross of cultures H9×H14 on barley varieties Hanna and Russian 12

Differential variety X	Differential variety Y	Item	Number of progeny cultures V_xV_y*	V_xA_y	A_xV_y	A_xA_y	Total	Value of P for ratio indicated
Hanna	Russian 12	Observed	5	142	138	4	289	
		Based on 1:1:1:1	72.25	72.25	72.25	72.25	289	Very small
		Based on p=0.0311	4.49	140.01	140.01	4.49	289	.98-.99

* See footnotes, Table 3.

the progeny cultures could be separated into only 2 classes. Therefore, to simplify the Table, the 2 classes have been shown as avirulent and virulent. The observed number of the progeny cultures indicated that 7 genes conditioning pathogenicity on A. 222, Goldfoil, Heil's Hanna, Monte Cristo, Nigrate, Russian 74 and Turkey 290 were inherited independently of each other gene. Only 9 of the 289 haploid progeny cultures differed from the parent cultures in pathogenicity on Hanna and Russian 12. This indicated that genes for pathogenicity on those varieties were linked. The recombination value calculated was 3.11 ± 1.02 per cent and the fit of the observed number to the expected on this basis was very good (Table 4). The 2 genes conditioned the pathogenicity on Hanna and Russian 12 were inherited independently of the other 7 genes.

Progeny Cultures of Crosses between Cultures other than Cultures H9 and H14. In the progeny from cross of H9×H14, one gene was found to condition pathogenicity on each of the barley varieties possessing one gene for resistance to the avirulent parent culture. Is the same true in any other crosses between cultures of the fungus? As shown in Table 5, cultures H2, H4, H5, H7, H11 and H13 were virulent on 2 varieties designated as X and Y, and cultures H4, H6, H11 and H12 were avirulent on the 2 varieties. Each of 8 barley varieties, Kairyo-bozu, H. E. S. 4, Hoso-mugi C, Goldfoil, Monte Cristo, Russian 12, Nigrate and Russian 74 possessed one gene for resistance to the avirulent cultures used. Crosses were made between cultures of virulent and avirulent on 2 varieties (X and Y). In all crosses, except cultures H5×H6, progeny cultures that differed from the parent cultures in pathogenicity on 2 varieties were segregated. This suggested that each parent culture being virulent on 2 varieties had at least 2 genes to condition the pathogenicity on the 2 varieties. In the cross of cultures H5×H6, all 29 progeny cultures showed the same pathogenicity as parent cultures on var. Hoso-mugi C and Russian 12. This indicated that the genes conditioning pathogenicity on those 2 varieties might be allelic or closely linked.

The results shown in Tables 2, 3 and 5 indicated that each gene conditioning pathogenicity on a variety was different from every other gene conditioning pathogenicity on every other variety.

TABLE 5
Segregation of genes conditioning pathogenicity of progeny from crosses between virulent and avirulent cultures of mildew on 2 barley varieties designated as X and Y

Parent culture and cross	Differential variety		Observed number of progeny cultures			
	X	Y	V_xV_y*	V_xA_y	A_xV_y	A_xA_y
H2 $(V_xV_y)\times$H12 (A_xA_y)	Kairyo-bozu	H. E. S. 4	9	4	9	6
H4 $(V_xV_y)\times$H11 (A_xA_y)	H. E. S. 4	Monte Cristo	4	7	10	7
H5 $(V_xV_y)\times$H6 (A_xA_y)	Hoso-mugi C	Russian 12	15	0	0	14
H7 $(V_xV_y)\times$H6 (A_xA_y)	H. E. S. 4	Goldfoil	7	7	6	5
H7 $(V_xV_y)\times$H6 (A_xA_y)	H. E. S. 4	Monte Cristo	6	8	4	7
H7 $(V_xV_y)\times$H6 (A_xA_y)	Goldfoil	Monte Cristo	6	7	4	8
H11 $(V_xV_y)\times$H4 (A_xA_y)	Goldfoil	Nigrate	2	12	6	8
H13 $(V_xV_y)\times$H6 (A_xA_y)	H. E. S. 4	Russian 74	95	99	89	117

* See footnotes, Table 3.

2. *Inheritance of Pathogenicity of Cultures H9 and H14 on the Barley Varieties Each with 2 Genes Conditioning Resistance to the Avirulent Parent Culture*

Russian 66 had 2 genes (Ml_h in Hanna, and Ml_{h4} in H. E. S. 4), and Six-rowed Chevalier had 2 genes (Ml_{r12} in Russian 12, and Ml_{h4} in H. E. S. 4) for resistance to mildew (Hiura 1960). Culture H9 was virulent on H. E. S. 4 and Russian 12 but avirulent on Hanna, and culture H14 was virulent on Hanna but avirulent on H. E. S. 4 and Russian 12. The segregation of 47 haploid progeny cultures obtained from cross of cultures H9 × H14 for pathogenicity on Russian 66 and Six-rowed Chevalier was shown in Table 6. The observed

TABLE 6
Classification of 47 haploid progeny cultures from cross of cultures H9 × H14 according to their pathogenicity on var. Russian 66 and Six-rowed Chevalier each with 2 genes conditioning resistance to the avirulent parent culture

Variety	Observed No. of progeny			Expected ratio	Value of P for ratio indicated
	Virulent	Avirulent	Total		
Russian 66	9	38	47	1 : 3	.3–.5
Six-rowed Chevalier	10	37	47	1 : 3	.5–.7

numbers of virulent and avirulent cultures satisfactorily fit those expected if virulence were conditioned by two genes on those varieties, indicating that progeny cultures segregated for pathogenicity on barley varieties in accordance with the number of genes in the barley varieties that conditioned resistance to the avirulent parent culture. This suggests that for each gene conditioning resistance in the host there is a corresponding gene conditioning pathogenicity in the parasite.

3. *Genes Conditioning Pathogenicity of the Mildew Races Which were Virulent on One and the Same Barley Variety*

A single gene for pathogenicity in a mildew culture could not condition its pathogenicity on a variety or varieties possessing 2 genes for resistance to mildew. Is there any case that different genes in mildew cultures condition their pathogenicity on one and the same variety with one gene for mildew reaction.

Of 5 pairs of cultures, H2 and H4, H3 and H4, H5 and H9, H10 and H11, and H13 and H6, each was virulent on the same barley variety with one gene for mildew reaction. Each pair was crossed. If the pathogenicity of parent cultures on the same variety are conditioned by different genes, avirulent progeny on the variety should segregate. As shown in Table 7, all progeny cultures showed the same pathogenicity as parent cultures. This indicated that 2 cultures being virulent on one and the same variety possessed the same gene for virulence on that variety.

TABLE 7

Pathogenicity of haploid progeny from crosses between mildew cultures each virulent on one and the same variety

Parent culture and cross	Variety on which progeny cultures were tested	No. of progeny	
		Virulent	Avirulent
H2 (virulent on H. E. S. 4) × H4 (virulent on H. E. S. 4)	H. E. S. 4	100	0
H3 (virulent on M. Cristo) × H4 (virulent on M. Cristo)	Monte Cristo	100	0
H5 (virulent on Russian 12) × H9 (virulent on Russian 12)	Russian 12	100	0
H10 (virulent on Nigrate) × H11(virulent on Nigrate)	Nigrate	100	0
H13 (virulent on Hanna) × H6 (virulent on Hanna)	Hanna	400	0

DISCUSSION

Flor's gene-for-gene hypothesis, that is : for each gene conditioning reaction in the host apparently there is a specific gene for pathogenicity in the parasite, is true in barley mildew as far as major genes for pathogenicity of mildew cultures and for resistance of barley varieties are concerned.

Moseman (1963) has shown that 3 genes conditioning pathogenicity on Goldfoil, Hanna and Atlas or Russian 12 in cultures of *Erysiphe graminis* f. sp. *hordei* isolated in Japan and in North America may be the same. Genes found in Goldfoil for resistance to Japanese mildew cultures and to those of North America may be the same because both the genes were linked with hooded in var. Nepal with recombination values of 17.8% and 18.8%, respectively (Hiura 1960, Briggs & Stanford 1943). If the gene-for-gene hypothesis is true, the gene in culture H14 conditioning its pathogenicity on Goldfoil must be the same as that of North American culture 59. 11. Culture H14, however, was

intermediate in pathogenicity on Goldfoil showing reaction type 1-2, and culture 59.11 was virulent on the variety showing reaction type 4. This disagreement might be elucidated as follow : supposing that virulence gene always shows reaction type 4, and reaction type of avirulence gene is determined in accordance with related resistance gene in host. And supposing that Goldfoil has 2 genes Ml_g and Ml_{g2} for resistance to mildew, and Ml_g shows reaction type 0 and Ml_{g2} shows reaction type 1-2, then postulated genotypes of the 4 cultures, H9, H14, 59.11 and 59.20, and their reaction types on Goldfoil are as given in Table 8. In cultures H9 and H14, and cultures 59.11 and 59.20,

TABLE 8

Postulated genotypes for pathogenicity of mildew cultures H9 and H14 isolated in Japan and cultures 59.11 and 59.20 isolated in North America*, and their reaction types on barley variety Goldfoil

on Goldfoil	Japanese cultures		American cultures	
	H9	H14	59. 11	59. 20
Genotype for Pathogenicity	A_gA_{g2}**	V_gA_{g2}	V_gV_{g2}	A_gV_{g2}
Reaction type	0	1-2	4	0

* Moseman, J. G. (1963)

** *V* indicates the gene is virulent to the complementary gene for resistance in the host, and *A* indicates it is avirulent.

each pair differs by one gene conditioning virulence to Ml_g, and although cultures H14 and 59.11 showed different reaction types on Goldfoil, both the cultures may have the same gene V_g complementary to the Ml_g gene in Goldfoil.

In the progeny cultures from the cross of cultures H9 × H14, 9 genes were found to condition their pathogenicity on 9 barley varieties, each with one gene for resistance to the avirulent parent culture. 2 of 9 genes conditioning pathogenicity on Hanna and Russian 12 were linked with recombination value of 3.11 ± 1.02 per cent. Therefore, 8 of 9 genes are able to combine freely, and $2^8 = 256$ biotypes should be expected on 8 barley varieties. 164 of 289 progeny cultures of H9 × H14 were found to be different in pathogenicity on 8 differential varieties. In nature, however, some of physiologic races were prevalent, and in general, not so many races or biotypes could be found. This fact is, from a practical viewpoint, the most interesting problem to be extensively studied.

SUMMARY

For genetic approach to elucidation of host-parasite interaction in barley mildew, genetics of pathogenicity of 11 cultures of *Erysiphe graminis* f. sp. *hordei* was studied on 14 barley varieties with known genes conditioning their reaction

to the mildew cultures.

On barley varieties possessing one gene for resistance to the avirulent parent culture, pathogenicity was conditioned by one gene, and on barley varieties possessing 2 genes for resistance to the avirulent parent culture, pathogenicity was conditioned by 2 genes. Each gene conditioning pathogenicity on a variety was different from every other gene conditioning pathogenicity on every other variety when each barley variety possessed different one gene for mildew reaction. The mildew cultures being virulent on one and the same barley variety, with one gene for mildew reaction, possessed the same gene for virulence on that variety. These results amply proved that Flor's gene-for-gene hypothesis was true in barley mildew.

Linkage relationships were determined for 9 genes conditioning pathogenicity of the fungus on 9 barley varieties. 7 of 9 genes were inherited independently of each other gene. 2 genes conditioning the pathogenicity on Hanna and Russian 12 were linked with recombination value of 3.11 ± 1.02 per cent. The 2 genes were inherited independently of the other 7 genes.

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