INHERITANCE OF RESISTANCE TO MILDEW, ERYSIPHE GRAMINIS HORDEI, IN THE BARLEY VARIETY, BLACK RUSSIAN

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SINCE BIFFEN (1905) first established that resistance to disease-inciting organisms in plants followed Mendelian laws, the entire field of host-pathogen relationships has been subjected to extensive investigations. Numerous among them have been those pertaining to the genetic mechanism of disease resistance. While notable contributions have been made in this field, few attempts have been made to explore the total genetic potential (in terms of resistance) of a host species to a specific pathogen. However, the results of FLOR's work with flax rust, *Melampsora lini* (Pers.) Liv. (FLOR 1947, 1954), and that of BRIGGS and associates (BRIGGS and STANFORD 1938; STANFORD and BRIGGS 1940; FAVRET 1949) with powdery mildew, *Erysiphe graminis hordei* (DC.) Marchal, of barley suggest that the genetic variation in terms of resistance within certain species to specific pathogens is essentially unlimited. At least 22 genes conditioning resistance to flax rust and 9 genes responsible for resistance to a single race of powdery mildew of barley have been identified. In both cases, only a small sample of the total germ plasm has been examined.

The genetics of resistance to race 3 of powdery mildew in 12 varieties of barley has been determined (BRIGGS and STANFORD 1938; STANFORD and BRIGGS 1940; FAVRET 1949). Nine genes, 7 dominant or incompletely dominant and 2 recessive, have been identified. The resistance of 8 varieties was conditioned by a single gene pair, by duplicate gene pairs in 3 varieties and by 3 gene pairs in one variety. The 12 varieties, together with the genes responsible for their resistance are listed below:

Variety	Genes conferring resistance	Variety	Genes conferring resistance
Hanna	Ml_hMl_h	West China	ml_wml_w
Goldfoil	Ml_gMl_g	Psaknon	Ml_rMl_p
Algerian	Ml_aMl_a	Chinerme	Ml_pMl_p; Ml_yMl_y
S.P.I. 45492	Ml_aMl_a	Nigrate	Ml_pMl_p; Ml_yMl_y
Kwan	Ml_kMl_k	Arlington Awnless	Ml_pMl_p; Ml_yMl_y
Monte Cristo	Ml_mMl_m	Duplex	Ml_pMl_p; Ml_hMl_h; ml_dml_d

Linkage between the Ml_a and Ml_k and the Ml_p and ml_d loci has been established (BRIGGS and STANFORD 1938; BRIGGS 1945).

FAVRET (1949) identified two additional genes, Ml_c and ml_n, which impart resistance to races A-1 and A-2, but which are susceptible to race 3. While other single gene segregations have been reported in crosses between resistant and susceptible varieties, their relationships to the genes discussed above is not known. However, the gene identified in the variety Nepal (TIDD 1940) which gives protection against

races 6 and 7 must be different since Nepal is susceptible to race 3. Thus, at least 12 genes are known which confer resistance to the various physiologic races of powdery mildew.

In this paper another gene for mildew resistance which forms an allelic series with one previously identified is discussed. Additional information on the linkage relationships of the various genes is presented.

MATERIALS AND METHODS

The variety Black Russian, C.I. 2202, was found to be resistant to mildew by DR. G. A. WIEBE (private correspondence) in the field at Madison, Wisconsin. While resistant to race 3 in California, its level of resistance is not as high as that possessed by a number of other varieties. Black Russian is a head selection from C.I. 705, an introduction from the Caucasian region of Russia. Except as parental material, it has no commercial value in the United States.

Black Russian was crossed with the susceptible variety Atlas and with the resistant varieties Hanna, Goldfoil, Psaknon, Algerian, Chinerme, Kwan and Selection 175, which are testers for seven of the genes discussed previously. Segregation was studied in the F_2 generation and, in pertinent crosses, confirmed in the F_3 generation.

The hybrid populations, together with their respective parents, were grown in greenhouse benches. Susceptible Atlas checks were included every twentieth row. The plants were inoculated at the three-leaf stage with race 3 of mildew by dusting the spores over the plants from infected plants grown for this purpose. The plants were classified according to the system suggested by MAINS and DIETZ (1930) as follows:

Type 0—Highly resistant, no mycelium evident. Chlorotic or necrotic spots may be developed by some varieties.

Type 1—Very resistant, slight to moderate mycelial development, but with little or no sporulation. Chlorotic or necrotic spots may develop in some varieties.

Type 2—Moderately resistant, moderate mycelial development, accompanied by limited sporulation. Chlorotic or necrotic areas may be formed.

Type 3—Moderately susceptible. Moderate to abundant mycelial development, accompanied by moderate sporulation.

Type 4—Very susceptible. Abundant mycelial development, accompanied by abundant sporulation.

A high level of infection was obtained throughout these tests; the susceptible checks were consistently classified as type 4. The resistant parent, Black Russian generally gave a type 2 reaction, although under certain conditions, which will be discussed later, it was classified as types 0 or 1. In previous investigations at this station no attempt was made to differentiate between types 3 and 4. Phenotypically the plants were either resistant (types 0, 1 and 2) or very heavily mildewed (type 4). Plants were encountered in crosses involving Black Russian which were intermediate between types 2 and 4 and were thus classified as type 3. This was the first indication that the genetic factors responsible for resistance in Black Russian differed from those previously identified.

TABLE 1

The classification of the parents and F_2 plants or F_3 rows, as indicated, of the crosses named and goodness of fit to expected ratios based on independent segregation

Generation	Parent or hybrid	Observed numbers			Ratio	Value of P for ratios indicated
		Resistant	Heterozygous rows or type 3 plants	Susceptible		
	Atlas*			550		
	Black Russian*	510				
F_2	Atlas × Bl. Russian†	155	268	130	1:2:1	>0.20
F_2	Atlas × Bl. Russian‡	129	69	59	1:2:1	<0.01
F_2	Atlas × Bl. Russian§	621		189	3:1	>0.20
	Atlas (rows)			6		
	Bl. Russian (rows)	6				
F_3	Atlas × Bl. Russian (rows)	32	64	28	1:2:1	>0.80
	Hanna (Ml_h)	72				
F_2	Bl. Russian × Hanna	205		17	15:1	>0.30
	Goldfoil (Ml_g)	60				
F_2	Bl. Russian × Goldfoil	197		13	15:1	>0.90
	Chinerme (Ml_p; Ml_y)	55				
F_2	Bl. Russian × Chinerme	369		3	63:1	>0.30
	Psaknon (Ml_p)	150				
F_2	Bl. Russian × Psaknon	1268		57	15:1	<0.01
	Selection 175 (ml_d)	85				
F_2	Bl. Russian × Sel. 175	695		64	13:3	<0.01
	Kwan (Ml_k)	75				
F_2	Bl. Russian × Kwan	617		2	15:1	<0.01
	Kwan (rows)	4				
F_3	Bl. Russian × Kwan (rows)	58	6	0	7:8:1	<0.01
	Algerian (Ml_a)	240				
F_2	Bl. Russian × Algerian	4300		0	15:1	<0.01
	Algerian (rows)	15				
F_3	Bl. Russian × Algerian (rows)	300	0	0	1:2:1	<0.01

* Atlas and Black Russian grown at 20 row intervals throughout all tests.

† Series I—Heterozygous plants positively identified (type 3).

‡ Series II—Heterozygous plants not positively identified (see text).

§ Resistant and type 3 plants combined (Series I and II).

EXPERIMENTAL RESULTS

The segregation obtained in the F_2 and F_3 generations of the various crosses is given in table 1. In the F_2 population of Black Russian × Atlas there were 621 resistant to 189 susceptible plants, which is in agreement with the 3:1 ratio expected for monofactorial segregation (P = 0.20 − 0.10). In the F_3 generation there were 32 resistant, 64 segregating and 28 susceptible families, which is in good agreement with the expected 1:2:1 ratio (P = 0.95 − 0.80) for a single gene difference.

It was soon apparent that the reaction of the resistant parent as well as the heterozygote varied with the environmental conditions under which it was tested. Under

certain conditions considerable mycelial development and sporulation, accompanied by necrotic spotting, was evident on the Black Russina parent (type 2). Under these conditions the heterozygotes showed considerable sporulation (type 3) and could be distinguished phenotypically from the homozygous genotypes, permitting complete classification. The F_2 population tested under these conditions segregated in the ratio of 1 type 2: 2 type 3: 1 type 4 (table 1). In other tests the resistant parent was completely free from mycelial growth (type 0) or showed only occasional flecking (type 1). In these tests the heterozygotes varied from types 1 to 2, and could not be separated phenotypically from the homozygous resistant combination (table 1). That this variation in reaction resulted from an interaction with environmental factors rather than from a variation in infection levels was shown by the consistently high infection on the susceptible progenies and checks. Thus gene expression and the degree of dominance were markedly affected by the environmental conditions.

Although no attempt was made to determine the environmental factors responsible for this variation in gene expression, the level of resistance was always the highest in tests made during February and March and lowest in December and January. While temperatures averaged slightly higher during the latter part of the winter, the greatest differences appeared to be in light intensity and in day length. Consequently, these two factors might be the main cause of the variation.

Segregation for susceptible types occurred in all test crosses, except with Algerian which possesses the Ml_a gene. Agreement between the observed and expected distributions for independent segregation was good for the crosses involving Hanna, Goldfoil and Chinerme, with P values ranging from 0.2 to 0.98 (table 1). Association was suggested in crosses with Kwan (Ml_k), Psaknon (Ml_p), and Selection 175 (ml_d) with P values for independent segregation smaller than 0.01 (table 1).

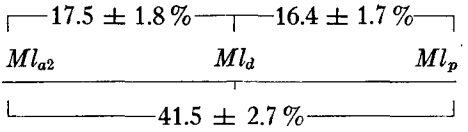
Since no segregation occurred in crosses with Algerian, the gene for resistance in Black Russian is either the same as the Ml_a gene carried by Algerian, closely linked, or an allele of it. Phenotypically, however, the genes could be readily distinguished. The Ml_a gene in Algerian produced a type 0 reaction and was completely dominant in expression. The gene conditioning resistance in Black Russian permitted a type 2 infection when homozygous and under certain environmental conditions, a type 3 reaction when heterozygous. Segregates were readily classified into the respective parental types. If linkage is assumed, the two genes would enter the cross in the repulsion phase. A susceptible type, the double recessive, would result from the union of 2 crossover gametes, the single heterozygotes from the union of a crossover gamete with a parental gamete. Neither type was identified in the F_2 populations or by F_3 progeny tests, suggesting that if recombination occurred, the percentage was extremely small. The combined estimate of linkage by the method of maximum likelihood (ALLARD 1955) from the F_2 and F_3 data, gave a recombination value of 0.00 $\pm$ 1.27 percent, indicating that the true recombination value falls somewhere within the range of 0.00 to 2.54 percent. However, since a test of this kind is not too sensitive, further information on the recombination value can be obtained by examination of the populations on which this estimate was based. In the F_2 population of 4300 individuals (table 1) at least one susceptible plant would have been expected (P = 0.95) if the recombination value was 5.29 percent or

greater. One thousand nine hundred and eighty plants of the above F_2 population were tested under conditions, as discussed previously, which permitted the positive identification of the single heterozygote of the Black Russian gene. In a population of this size at least one such plant would have been expected (P = .95) with a recombination value of 0.30 percent or greater. In neither of the populations were plants of these types observed.

Progenies from 300 random F_2 plants were tested for their reaction to mildew. Recombination products giving rise to the single heterozygotes could be readily identified by segregation ratios of 3 resistant:1 susceptible plant. Positive family classification (P = .999) was assured by populations of 25 or more plants. In a population of 300 F_3 families, at least one such segregating family would have been expected (P = .95) if the recombination value was greater than 1.0 percent. None were found. The F_3 families fell into 3 groups, those homozygous for the parental reactions and those segregating for the parental reactions, in the following ratio: 78 Algerian-like (type 0): 153 segregating (3 type 0:1 type 2): 69 like the Black Russian parent (type 2). The agreement to the expected 1:2:1 ratio attests to the accuracy with which the parental phenotypes could be distinguished (P = 0.95 − 0.50).

The cumulative evidence presented above indicates that the recombination value, if crossing over occurred, was less than 0.30 percent. Such a low value justifies considering the genes conditioning mildew resistance in Algerian and Black Russian as alleles since their expression can be distinguished phenotypically. In conformity with past procedures for assigning symbols to genes conferring resistance to powdery mildew, the allele in Black Russian has been designated Ml_{a2}.

Association between the gene conditioning resistance in Black Russian (Ml_{a2}) with those of Psaknon (Ml_p), Selection 175 (ml_d) and Kwan (Ml_k) was suggested in the test crosses (table 1). The recombination values between Ml_{a2} and Ml_p, and Ml_{a2} and ml_d, calculated from F_2 data (table 1) were 41.5 ± 2.7 and 17.5 ± 1.8 percent, respectively. The Ml_p and ml_d genes were reported by BRIGGS (1945) to be located in linkage group II with 16.4 ± 1.7 percent recombination. The suggested arrangement is as follows:

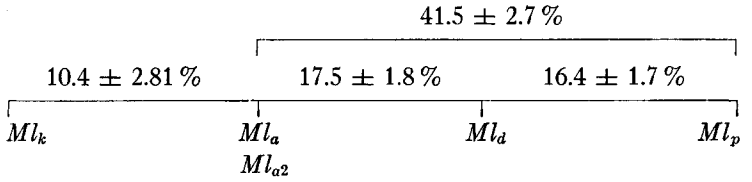


Although STANFORD and BRIGGS (1940) did not report association of the Ml_a locus with either Ml_p and ml_d, the present results are not in conflict with their data. Since the recombination value of 41.5 ± 2.7 percent between Ml_{a2} and Ml_p does not seriously modify the ratio expected with independence, large F_2 populations are necessary to detect it. Only 398 F_2 plants were tested previously in contrast to 1328 plants in the present study.

In the original test cross between Ml_a and ml_d, Duplex was used as the tester variety (STANFORD and BRIGGS 1940). In addition to ml_d, Duplex possesses the genes Ml_p and Ml_h for resistance. Consequently, with four genes segregating an extremely large population would be needed to detect the small departure from the

ratio expected with independence. Substitution of the recombination value of 17.5 ± 1.8 percent between Ml_a and ml_d in the original data gave a satisfactory fit to their observed segregation.

In the test cross with Kwan (Ml_k) 64 F_3 progenies were tested in addition to the F_2 population. Progenies from the singly dominant heterozygotes and the coupling phase heterozygote (with close linkage) would segregate in a ratio of 3 resistant plants:1 susceptible. Six segregating rows were observed. Only progenies from the double recessive F_2 plants would be homozygous susceptible. None were found. The combined estimate of linkage by the method of maximum likelihood from the F_2 and F_3 data gave a recombination value of 10.4 ± 2.81 percent between the Ml_{a2} and Ml_k loci. This estimate is in close agreement with the value of 9.81 ± 4.78 percent reported by BRIGGS and STANFORD (1938) for the Ml_a and Ml_k genes. Cumulative evidence places the Ml_k locus beyond the Ml_a locus in respect to Ml_p and ml_d since no association was detected between them in previous test crosses. With any other arrangement, the proximity of the genes with each other should have been readily established. Thus the following arrangement of the five genes in linkage group II is indicated:



DISCUSSION

Resistance to powdery mildew within the *Hordeum vulgare* species is world-wide in occurrence (MAINS and MARTINI 1932). The differential reaction of many varieties to physiologic races of the pathogen (NEWTON and CHERWICK 1947) suggests that the genetic basis of resistance is extremely diverse. Likewise, the genetics of pathogenicity must be equally diverse. Varieties immune to certain physiologic races are completely susceptible to others.

In the 13 varieties studied thus far, 10 genes have been identified which condition resistance to race 3 of powdery mildew. Incomplete results (SCHALLER unpublished) involving 5 other resistant varieties indicate at least 3 additional genes are present. These varieties represent a fairly random sample of the world germ plasm, including types from China, India, North Africa, Russia and Germany. The number of varieties examined has not been large enough to determine the frequency or the distributional pattern of the genes from any one source.

The nature of resistance imparted by the various genes has not been determined. Phenotypically, however, the majority of them can be readily identified, either by the level of resistance imparted or by their degree of dominance. The Ml_a gene appears to exclude the pathogen (immune reaction) and is completely dominant. The Ml_{a2} genotype permits limited mycelial growth and sporulation and is incompletely dominant. Resistance of the Ml_k gene appears to be one of hypersensitivity to the pathogen, whereby the plant cells adjacent to the infection points are killed, resulting in starvation of the pathogen. Variation in the nature of resistance is also

suggested by the differential gene-race interaction. Although this work is just beginning, it is apparent that the 10 genes exhibit considerable selectivity in regards to the different physiologic races.

It is clearly evident that the factors for resistance are not located at random in the 7 linkage groups. Five of the 10 identified thus far are in linkage group II, two forming an allelic series. Accumulated evidence (SCHALLER unpublished) suggested that one of the others is also in this linkage group, possibly an extension of the allelic series at the Ml_a locus. Only one of the remaining four genes (Ml_h) has been definitely placed in another group, group IV.

The non-random distribution of the genes conditioning resistance to race 3 of powdery mildew is parallel to that found by FLOR (1947, 1954) with flax rust and by BRIGGS and associates (BAKER 1949; BRIGGS 1940; STANFORD 1941) with bunt of wheat. Twenty-two genes imparting resistance to rust in flax have been placed in 3 series, either allelic or closely linked. Four of the seven genes for resistance to race T-1 of *Tilletia caries* (DC.) Tul. in wheat are located in one linkage group. The significance of this distribution pattern of resistance genes within a species is not readily apparent. If each of the genes conditioned resistance to different races occurring within an area, it can be readily seen that a chromosome carrying all of the genes would have a high selective advantage. However, in the majority of the varieties, resistance is conditioned by a single gene pair. If the genes in question have a pleiotropic action and their supplementary effects have a selective value, either independent or complementary, their association in a linkage group would then have a selective advantage. Further investigations with other physiologic races of the same pathogens and with other diseases and hosts are necessary to establish whether the pattern, both as to numbers and arrangements of genes for resistance, suggested by these studies is the rule or the exception.

SUMMARY

A single gene conditioned the resistance of the barley variety, Black Russian, to race 3 of powdery mildew. Segregation occurred in all test crosses, except with the variety Algerian which possess the Ml_a gene for resistance. Sufficiently large F_2 and F_3 populations of the Black Russian $\times$ Algerian cross were grown to detect recombination values greater than 0.3 percent. The gene in Black Russian was considered to be allelic to the Ml_a gene in Algerian, since no crossing over occurred and the two alleles were readily distinguished phenotypically. The gene in Black Russian was designated Ml_{a2}.

Ten genes have now been identified which confer resistance to race 3 of powdery mildew. Evidence was presented to show that at least 5 of the 10 genes are in linkage group II. Their suggested linear arrangement in the linkage group was given.

LITERATURE CITED

ALLARD, R. W., 1955 Tables and formulae to facilitate the calculation of recombination values in heredity. Hilgardia (in press).

BAKER, E. P., 1949 Inheritance of resistance to bunt (*Tilletia caries* (DC.) Tul.) in hybrids involving Turkey wheat selections 10095 and 10097. Ph.D. thesis, University of California, Berkeley, California.

BIFFEN, R. H., 1905 Mendel's laws of inheritance and wheat breeding. J. Agr. Sci. **1:** 4–48.

BRIGGS, F. N., 1945 Linkage relation of factors for resistance to mildew in barley. Genetics **30:** 115–118.

BRIGGS, F. N., 1940 Linkage between the Martin and Turkey factors for resistance to bunt, *Tilletia tritici*, in wheat. J. Am. Soc. Agron. **32:** 539–541.

BRIGGS, F. N., and E. H. STANFORD, 1938 Linkage of factors for resistance to mildew in barley. J. Genet. **37:** 109–117.

FLOR, H. H., 1947 Inheritance of reaction to rust in flax. J. Agr. Res. **74:** 241–262.

1954 Genes for resistance to rust in Victory flax. Agron. J. **43:** 527–531.

FAVRET, E. A., 1949 Herencia de la restencia a *Erysiphe graminis hordei* en cebada II. Estudios con la ranza 3 de California. Revista Inv. Agr. **3:** 31–42.

MAINS, E. B., and S. M. DIETZ, 1930 Physiologic forms of barley mildew, *Erysiphe graminis hordei* Marchal. Phytopathology **20:** 229–239.

MAINS, E. B., and MARY L. MARTINI, 1932 Susceptibility of barley to leaf rust (*Puccinia anomala*) and to powdery mildew (*Erysiphe graminis hordei*) U. S. Dep. Agr. Tech. Bull. 295. 33 pp.

NEWTON, M., and W. J. CHERWICK, 1947 *Erysiphe graminis* in Canada. Canad. J. Research C. **25:** 73–93.

STANFORD, E. H., and F. N. BRIGGS, 1940 Two additional factors for resistance to mildew in barley. J. Agr. Res. **61:** 231–236.

STANFORD, E. H., 1941 A new factor for resistance to bunt, *Tilletia tritici*, linked with the Martin and Turkey factors. J. Am. Soc. Agron. **33:** 559–568.

TIDD, J. S., 1940 Inheritance of resistance to *Erysiphe graminis hordei* in a cross between Featherstone and Nepal barley. Phytopathology **30:** 24–25 (Abstract).