Inheritance of Virulence of Melampsora lini Race 218

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ABSTRACT

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A culture of race 218, *Melampsora lini* was selfed and the progeny were tested for virulence on flax lines each of which contained a pair of genes conditioning rust resistance. Race 218 was crossed with race 22, but only a limited number of F_1 cultures were derived. Race 218 and all of the S_1 cultures resulting from selfing race 218 were virulent on near-isogenic flax lines containing L^5, L^6, L^7, L^9, L^{11}, M^1, M^2, M^4, and P^1 indicating that the genes for virulence were homozygous. Race 218 also was virulent on the line with L^{10}. Segregation for virulence on the line with L^{10} may have been due to a temperature-sensitive factor. Race 218 and all the S_1 progenies tested were avirulent on lines containg L^2, N, P^4, and Kugine indicating that race 218 is homozygous avirulent on lines with these resistance genes. Segregation ratios fit theoretical monogenic recessive ratios on isogenic lines possessing host genes K, L^1, L^3, L^4, L^8, M, M^3, M^5, M^6, N^1, P, P^2, and P^3. Virulence on the line with N^2 probably was conditioned by a single recessive gene since there were only a few more cultures in the virulent category than would fit expected ratios. Two independently inherited recessive genes apparently conditioned virulence on the line with L. Chi-square tests indicated that virulence on several lines with alleles at the L locus and the P locus was linked. Usually virulence on most other lines with alleles at different loci was inherited independently; the few exceptions included virulence on lines with L^8 and M which was apparently closely linked. These linkage patterns indicate that a deletion of a small segment carrying A_L 8 and A_M could cause virulence on flax lines carrying the corresponding resistance genes.

Additional key words: flax rust, fungal genetics, linkage, first generation self (S_1), virulence genes.

The gene-for-gene relationship was probably one of the most significant discoveries in genetics and plant pathology (3). This relationship states that for each gene that conditions reaction in the host there is a corresponding gene in the parasite that conditions pathogenicity (6). Pustule type is conditioned by specific pairs of genes; one in the host and the other in the parasite.

The gene-for-gene relationship implies that the pathogenicity genotype of a rust culture can be established by a study of its selfed cultures on differential lines with single genes conditioning rust resistance (6). This implication made possible the study of heterozygosity of races from the natural *Melampsora lini* (Ehrenb.) Lev. population to determine usefulness of host genes and studies of allelism in the pathogen, thus providing better knowledge of which genes for resistance would be most effective.

Since each gene in either member of a host-parasite system can be identified by its counterpart in the other member of the system, the gene-for-gene relationship has been used to identify major genes conditioning vertical resistance and to develop resistant cultivars (7).

The development of cultivars containing genes for vertical resistance has been one of the most successful methods of controlling plant diseases (7). However, the chief cause of impermanence of disease resistance has been the appearance and rapid distribution of strains of pathogens capable of attacking previously resistant cultivars (1). This happened in flax with *M. lini* on cultivar Bison during the 1930's, cultivar Koto in 1944-45, cultivar Dakota in 1949-51, cultivars with the L gene during 1962-63, and cultivars with the N^1 gene in 1973 (4,9).

The objective of this investigation was to study the inheritance of virulence and linkage patterns of race 218 of *M. lini*. This information is valuable in a breeding program to determine which genes in flax would be most effective for long lasting resistance.

MATERIALS AND METHODS

A urediospore culture of race 218 of *M. lini* was purified by two successive single-pustule isolations. Although original records are unclear it is believed that race 218 is an F_1 of the cross of race 22 by race 6. The culture of race 218 was heterozygous on many of the isogenic lines used and therefore, an ideal culture for a linkage study. The culture of race 218 was used to inoculate Bison flax plants that were approximately 15 cm tall. Teliospores formed when the inoculated plants began to mature and were conditioned to germinate by several alternate freeze-thaw, wet-dry cycles (4). After several cycles, the telia-laden straw was suspended over seedlings of Bison flax. Selfing race 218 was conducted by separately transferring "honeydew" and pycniospores from one pycnial infection of race 218 to another. The cross of race 218 by race 22 was conducted by separately transferring "honeydew" and pycniospores from a pycnial infection of race 218 to a pycnial infection of race 22 or the reciprocal of this.

Aeciospores resulting from selfs or crosses were used to inoculate Bison plants. The 60 resulting urediospore cultures from the self of race 218 and the 10 cultures from the cross of race 22 by race 218 were used to inoculate 29 isogenic flax lines, each with a different gene for resistance (Table 1). Seed of the isogenic lines was obtained from Flor (4). The reaction type expressed on each line was classified on the standard scale of 0 to 4, 12–14 days after inoculation (2). Reaction types 0 to 2 were classified as resistant and 3 and 4 were classified as susceptible for purposes of the genetic analysis. Chi-square tests were used to determine the probabilities of the segregating cultures fitting hypothetical ratios. Chi-square tests for independence were used to determine if pathogen genes were independently inherited. The recombination values were estimated by the product method (8).

RESULTS AND DISCUSSION

Race 218 was virulent on isogenic flax lines containing L^5, L^6, L^7, L^9, L^{10}, L^{11}, M^1, M^2, M^4, and P^1 genes for resistance. All of the first generation (S_1) cultures resulting from selfing race 218 were virulent on isogenic lines containg L^5, L^6, L^7, L^9, L^{11}, M^1, M^2, M^4, and P^1 (Table 1). All 10 of the F_1 cultures from crosses of race 22 × race 218 also were virulent on lines with these genes as would be expected if these genes in *M. lini* race 218 were homozygous for virulence. Race 22 was virulent on all the near-isogenic lines listed in Table 1 except those containing host genes N, L^2, M^6, P^4, or Kugine. Segregation for virulence among the S_1 progeny of race

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218 was detected on the isogenic line containing L^{10}, but race 218 is virulent on the line containing L^{10}. Segregation did not fit expected ratios for a dominant gene or genes. The failure to fit expected Mendelian ratios could be explained by preliminary temperature studies using race 1 where the isogenic line containing L^{10} was susceptible at 21 ± 3 C but resistant at 32 ± 3 C. Variation in greenhouse temperatures during the course of this study may have caused the observed segregation. This temperature effect was not observed for any other isogenic line.

The parental culture of race 218 and all the S_1 progenies tested were avirulent on isogenic lines containing L^2, N, P^4, and Kugine. The failure to observe virulent segregants in S_1 cultures established that race 218 is homozygous avirulent on lines with these genes for resistance. All 10 F_1 cultures from the cross of race 218 × race 22 also were avirulent on isogenic lines with the L^2, N, P^4, and Kugine genes for resistance.

The parental culture also was avirulent on isogenic lines possessing host genes K, L, L^1, L^3, L^4, L^8, M, M^3, M^5, M^6, N^1, N^2, P, P^2, and P^3. Segregation ratios of the selfed cultures fit theoretical monogenic recessive ratios on lines possessing host genes K, L^1, L^3, L^4, L^8, M, M^3, M^6, N^1, P, P^2, and P^3 (Table 1). The F_1 cultures from the cross of race 218 by race 22 segregated on all the above host genes. Although there were only 10 F_1 cultures, all fit a 1:1 ratio ($P > 0.05$) except on the line containing N^1. This exception was probably due to the small number of F_1 cultures.

Segregation ratios on host genes L and N^2 did not fit theoretical monogenic recessive ratios. The failure to fit was due to a higher than normal number in the virulent category on the line containing N^2, but only seven virulent cultures were recorded on the line containing the L gene. Since there were only a few more cultures in the virulent category than would fit the expected ratio on the line with N^2, the failure to fit may be due to chance alone and virulence is probably due to a single recessive gene. The 10 F_1 cultures fit a 1:1 ratio which verifies this monogenic ratio.

The ratio of 50 avirulent to seven virulent cultures on the line with the L gene fit a genetic model that assumes two independently inherited recessive genes for virulence. Previous selfing studies (9) also indicated a digenic ratio on the line with the L gene. Flor (4) explained his observed ratio of 60 avirulent to seven virulent on the line containing the L gene by the fact that Ottawa 770B possessed a gene in the N series conditioning resistance to some Australian races. One of the parental cultures may have been heterozygous for virulence on 770B.

Results obtained when isogenic lines were inoculated with cultures from the selfed race 218 indicated that virulence on most host alleles at different loci were independently inherited, with a few exceptions listed in Table 2. Genes controlling virulence on lines with L^8 and M are apparently closely linked ($P < 0.005$) and appear to be coupled. The S_1 cultures were either all virulent on lines containing L^8 and M or all avirulent, with a few exceptions.

The linkage between $^aL^8$ and ^aM would indicate that these genes for virulence are on the same chromosome in the pathogen while genes for resistance are on different chromosomes in the host. Knowledge of this linkage is important when breeding rust-resistant cultivars since the deletion of a small chromosome segment with $^AL^8$ and ^AM could select for virulence in the pathogen on host genes L^8 and M. The present breeding program which is incorporating M^3 and P^3 into one cultivar should not be affected, since close linkage was not detected between $^aM^3$ and $^aP^3$ in this study. Linkage also was indicated between several other genes including $^aL^1$ and $^aN^2$ ($P < 0.005$), $^aL^8$, and $^aN^2$ ($P < 0.005$), ^aM and ^aP ($P < 0.010$), ^aM and $^aP^3$ ($P < 0.050$), and between ^aM and $^aP^3$ ($P < 0.050$) (Table 2).

Chi-square tests indicated that pathogen genes controlling

TABLE 1. Segregation for pathogenicity among S_1 cultures of *Melampsora lini* derived from selfing race 218 and F_1 cultures of race 22 × race 218

Near Isogenic Line	Response to 218	Distribution of pathogenicity among S_1 cultures						Expected ratio[a]	Goodness of fit	Distribution of pathogenicity among F_1 cultures		Expected ratio[a]
		Avirulent				Virulent				Avirulent	Virulent	
		0 to 0;	0;1 to 0;2	1	2	3	4			0 to 2	3 to 4	
K	0;	23	12	4	4	1	16	3:1	$P > 0.50$	7	3	1:1
L	0	52	1	0	0	2	5	15:1	$P > 0.05$	5	5	1:1
L^1	0;	9	6	11	13	7	14	3:1	$P > 0.05$	7	3	1:1
L^2	0	60	0	0	0	0	0	HA		10	0	HA
L^3	0;	36	1	6	8	6	3	3:1	$P > 0.05$	7	3	1:1
L^4	0	35	2	0	2	6	15	3:1	$P > 0.05$	5	5	1:1
L^5	4	0	0	0	0	0	60	HV		0	10	HV
L^6	4	0	0	0	0	10	50	HV		0	10	HV
L^7	4	0	0	0	0	5	55	HV		0	10	HV
L^8	0	34	6	0	1	4	16	3:1	$P > 0.10$	5	5	1:1
L^9	4	0	0	0	0	0	60	HV		0	10	HV
L^{10}	4	16	7	4	5	13	15	3:1	$P < 0.005$	5	5	1:1
L^{11}	4	0	0	0	0	14	46	HV		0	10	HV
M	0;	38	1	1	0	4	16	3:1	$P > 0.10$	5	5	1:1
M^1	4	0	0	0	0	5	55	HV		0	10	HV
M^2	4	0	0	0	0	4	56	HV		0	10	HV
M^3	0	42	2	0	1	2	13	3:1	$P > 0.99$	7	3	1:1
M^4	4	0	0	0	0	7	53	HV		0	10	HV
M^5	0	20	11	0	8	2	19	3:1	$P > 0.05$	5	5	1:1
M^6	0;	34	3	2	10	8	2	3:1	$P > 0.10$	7	3	1:1
N	0;	59	1	0	0	0	0	HA		10	0	HA
N^1	0;	30	5	2	6	3	14	3:1	$P > 0.05$	2	8	1:1
N^2	12	19	7	5	6	7	16	3:1	$P < 0.05$	3	7	1:1
P	0;	40	4	0	0	5	11	3:1	$P > 0.75$	7	3	1:1
P^1	4	0	0	0	0	4	56	HV		0	10	HV
P^2	0;1	38	4	4	1	4	9	3:1	$P > 0.50$	8	2	1:1
P^3	0;1	41	4	2	0	2	11	3:1	$P > 0.50$	8	2	1:1
P^4	0;	60	0	0	0	0	0	HA		10	0	HA
Kugine	0	42	10	3	5	0	0	HA		10	0	HA

[a]Abbreviations: HV = homozygous virulent, HA = homozygous avirulent.

TABLE 2. Chi-square test for independence between S_1 cultures of race 218 *Melampsora lini* segregating for virulence on host cultivars containing single genes for resistance with linkage indicated

Genes for virulence	*P* value	Recombination estimate (%)	S.E.[a] (%)
$^aL^1$ vs $^aN^2$	< 0.005	27.3	6.9
$^aL^3$ vs $^aL^4$	< 0.005	15.5	5.1
$^aL^4$ vs $^aL^8$	< 0.050	29.9	11.6
$^aL^8$ vs ^aM	< 0.005	6.2	3.2
$^aL^8$ vs $^aN^2$	< 0.005	21.3	12.2
^aM vs ^aP	< 0.010	28.0	7.0
^aM vs $^aP^2$	< 0.050	30.0	7.3
^aM vs $^aP^3$	< 0.050	30.0	7.3
$^aM^6$ vs $^aN^2$	< 0.005	15.7	5.2
$^aN^1$ vs $^aN^2$	< 0.025	29.8	7.3
^aP vs $^aP^2$	< 0.005	7.1	3.4
^aP vs $^aP^3$	< 0.005	7.0	3.4
$^aP^2$ vs $^aP^3$	< 0.005	1.8	1.7

[a]Standard error.

virulence in race 218 on flax lines containing L^3 and L^4 and those containing L^4 and L^8 were not inherited independently. In contrast, the tests indicated that genes controlling virulence on lines containing L^3 and L^8 were inherited independently ($P > 0.100$). Flor (5) previously reported that pathogenicity to lines with L^3, L^4, L^8, and L^{10} was linked.

Flor (4) also reported that pathogenicity on Polk (N^1) was loosely linked with that on Cass (M^3). In the current study, genes conditioning virulence on lines with N^1 and M^3 appear to be inherited independently ($P > 0.50$). My results confirm Flor's (5) findings that pathogenicity on lines dependent on *P* genes for rust resistance is inherited as a unit. In the selfing study with race 218, pathogenicity on lines with *P*, P^2, and P^3 apparently was closely linked since all the S_1 cultures from the self were either virulent on all three host lines or avirulent on all three with a few exceptions (Table 2). These genes for virulence appear to be coupled.

Flor (5) also reported that pathogenicity in the rust fungus on flax lines with *M*, M^3, M^4, *N*, and N^1 was independently inherited. Results of the current study confirm the inheritance on lines with *M*, M^3, and N^1 and also indicate that virulence on lines with M^5 and M^6 is independently inherited.

Flor (5) also reported that pathogenicity in the rust fungus to lines possessing the N^1 and N^2 genes is closely linked. My results confirm linkage between $^aN^1$ and $^aN^2$ but observations do not indicate a close linkage. Linkage was not present between ^aK and genes at other loci.

Although pathogen genes controlling virulence usually were inherited independently, chi-square tests indicated linkage between some pathogen genes. Even though the corresponding genes in the host have been designated as alleles, their pathogen counterparts were independently inherited, which indicates that they are nonallelic or linked with percent recombination indicative of separate genes.

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