

## New Genes for Rust Resistance in Flax

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### ABSTRACT

Two (and possibly three) genes in flax, *Linum usitatissimum*, were identified which condition resistance to all races of the rust pathogen, *Melampsora lini* (Ehrenb.) Lev., including the virulent South American races. Progenies from individual plant selections from accessions C.I. 1888, C.I. 1911, and C.I. 2008 were resistant to races of rust that collectively attack the 26 known genes for resistance. Segregation ratios obtained in the  $F_2$  and testcrosses indicated that resistance of each

selection was monogenic and dominant.  $F_2$  segregations were observed from crosses between three new accessions and five monogenic tester lines, each having dominant alleles for one of the five known rust-conditioning loci. The resistance of C.I. 1888 and C.I. 1911 was found to be conditioned by a gene at the P locus and that of C.I. 2008 by a gene at the M locus. These genes were designated  $P^4$  and  $M^6$ .

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*Additional key words:* disease resistance, breeding, multiple alleles.

Rust incited by *Melampsora lini* (Ehrenb.) Lev. is potentially the most serious disease of flax, *Linum usitatissimum* L. Fortunately, in the principal flax-growing area of the United States, large-scale losses have been avoided through the use of rust-resistant cultivars. Although new races of rust with added virulence periodically evolve, losses have been minimized by rapidly replacing susceptible cultivars with resistant ones.

Twenty-six genes that condition resistance to races of rust have been identified (1, 3). These genes occur as closely linked (or multiple) alleles at five loci, which have been designated by the symbols *K*, *L*, *M*, *N*, and *P*. As the result of the evolution of new races, only six of the 26 known genes condition resistance to all known North American races of rust (2). None of the known genes condition resistance to certain races of rust that occur in Australia, New Zealand, South America, and the United Arab Republic (2, 5). The introduction of broadly virulent races of rust from these countries could be disastrous to the North American flax industry.

The continual evolution of new races of rust, plus concern about the possible introduction of foreign

races, prompted Flor & Comstock (3) to undertake a program to locate sources of resistance to all known races of rust. With the aid of Richard Berquist, Graduate Assistant, they found that most accessions in the World Collection of flax were heterogeneous for reaction to race 1, a race with restricted virulence; but were susceptible to race 22, a race that attacks all but two of the 26 known genes. Of the 367 accessions tested, 58 were heterogeneous or uniformly resistant to race 22. Forty-seven of these 58 accessions were susceptible to races which attack one or both of the genes ( $L^2$ ,  $N^1$ ) known to confer resistance to race 22, thus those accessions did not possess new genes for resistance. The remaining accessions were believed to represent valuable new sources of resistance.

The following study was undertaken to substantiate the findings of Flor & Comstock (3), to determine the number of genes involved in accessions containing undescribed gene(s) for resistance, and to determine the loci of any undescribed genes with respect to the five known rust-conditioning loci.

**MATERIALS AND METHODS.**—New rust-conditioning genes in flax cultivars can be

detected, when monogenic, by the selective pathogenicity of races of *M. lini*. Although there are many races of *M. lini* with known pathogenicity, none will identify all possible combinations of the 26 known resistance genes. Consequently, the presence of a new gene or genes cannot always be established by selective pathogenicity of rust races when two or more host genes are involved in a resistant-type reaction. In such situations, a genetic analysis of an  $F_2$  or a testcross population must be used to establish the existence of previously undescribed genes.

Six accessions from the World Collection of flax (C.I. 1888, C.I. 1911, C.I. 2008, C.I. 2089, C.I. 2226, and C.I. 2259), that were suspected of possessing undescribed genes for rust resistance, were used. Progeny of plant selections from the six accessions were inoculated at consecutive 4-day intervals with races 1, 312, 73, and 22. A resistant reaction to race 1, which has limited virulence, establishes the presence of one or more genes for resistance. Races 22, 73, and 312, collectively, attack all known genes for resistance when monogenic, but will not attack all combinations of known genes for resistance. Plant lines that resisted races 22, 73, and 312 were suspected of possessing undescribed gene(s).

'Bison', a cultivar susceptible to most races of rust, was used as the tester parent in crosses to develop  $F_2$  and testcross populations. Classification of the  $F_2$  and testcross for reaction to races 1 and 22 indicated the number of rust-conditioning genes present in each plant line being analyzed.

Tests were conducted to learn the allelic relationships of the undescribed genes to the known rust-conditioning genes. Plants from the new accessions were crossed with five monogenic host-differential cultivars, each containing a dominant allele at one of the five known rust-conditioning loci.  $F_2$  populations from these crosses were tested for response to race 300, which is avirulent on the five

host-differential cultivars and on the accessions suspected of possessing undescribed genes. Segregation ratios approximating 15 resistant: 1 susceptible suggested independence. We assumed that the undescribed gene was allelic to the host-differential gene involved in  $F_2$ 's that failed to segregate; i.e.,  $F_2$  entirely resistant to race 300.

**RESULTS AND DISCUSSION.**—Progenies from individual plants of three accessions (C.I. 2089, C.I. 2226, and C.I. 2259) suspected from preliminary studies of possessing new genes for resistance were susceptible to races 22 or 73, or both (Table 1). We assumed, then, that these three accessions did not possess new genes for resistance. The data suggested that C.I. 2226 possessed the *N* gene, which is ineffective against race 73; resistance in C.I. 2259 apparently was conditioned by the same genetic system as that of 'Kugine'; and C.I. 2089 contained one of several genes known to confer resistance to races 1 and 312, but which are ineffective against races 22 and 73.

Progenies of individual plants of the three other accessions (C.I. 1888, C.I. 1911, and C.I. 2008) were uniformly resistant to races 1, 22, 73, and 312, which collectively are virulent on monogenic lines possessing all known genes for resistance. These accessions were considered as potential sources of new genes.

The  $F_2$  and testcross populations resulting from crosses between Bison and plant lines of C.I. 1888, C.I. 1911, and C.I. 2008 segregated in 3:1 and 1:1 ratios, respectively, when tested against races 1 and 22 (Table 2).  $F_2$  and testcross segregates resistant to one race were also resistant to the other which confirmed that the resistance of each accession was conditioned by a single dominant gene.

If the resistances of C.I. 1888, C.I. 1911, and C.I. 2008 were conditioned by different alleles at the same locus, their separate identities could not be ascertained with the presently known races of rust. If

TABLE 1. Reaction of individual selections of six flax accessions suspected of containing undescribed genes for resistance when tested to races of rust that collectively attack all known genes for resistance

Accession	Origin and identification	Reaction <sup>a</sup>			
		Race 1	Race 22	Race 73	Race 312
C.I. 1888-8	Hungary (Martoni I.6)	R	R	R	R
C.I. 1888-14	Hungary (Martoni I.6)	R	R	R	R
C.I. 1888-20	Hungary (Martoni I.6)	R	R	R	R
C.I. 1911-4	India (Punjab 53)	R	R	R	R
C.I. 1911-5-2	India (Punjab 53)	R	R	R	R
C.I. 2008-4	Pakistan (P.I. 250568)	R	R <sup>b</sup>	R	R
C.I. 2008-9	Pakistan (P.I. 250568)	R	R <sup>b</sup>	R	R
C.I. 2089-2-2	India (N.P. 15)	R	MS	S	R
C.I. 2089-4-2	India (N.P. 15)	R	MS	S	R
C.I. 2226-1-4	India [N.P. (RR 494)]	R	R	S	R
C.I. 2226-13-2	India [N.P. (RR 494)]	R	R	S	R
C.I. 2259-2	Russia (Swetotsch)	R	MR	MR	R

<sup>a</sup> R = resistant, MR = moderately resistant, MS = moderately susceptible, S = susceptible.

<sup>b</sup> A few virulent-type pustules were observed.

TABLE 2. Segregation for rust reaction in  $F_2$  and testcross populations of crosses between Bison and individual plant selections of C.I. 1888, C.I. 1911, and C.I. 2008

Cross	Generation	Segregation when tested to races 1 and 22 <sup>a</sup>			Goodness of fit to 3:1 or 1:1 ratio <i>P</i> exceeds
		Resistant to both	Susceptible to both	Resistant to one susceptible to other	
C.I. 1888-8 × Bison	Testcross	16	17	0	0.80
	$F_2$	236	58	0	0.04
C.I. 1888-20 × Bison	Testcross	11	12	0	0.80
	$F_2$	136	39	0	0.30
C.I. 1911-4 × Bison	Testcross	9	7	0	0.50
	$F_2$	623	213	0	0.70
C.I. 2008-4 × Bison	Testcross	21	19	0	0.70
	$F_2$	107	36	0	0.90
C.I. 2008-9 × Bison	Testcross	20	16	0	0.50
	$F_2$	97	34	0	0.80

<sup>a</sup> Race 1 is narrowly virulent attacking only resistance due to the  $L^1$ ,  $L^2$ ,  $M^1$ , and  $M^2$  genes. Race 22 is widely virulent attacking resistance due to all known genes except  $N$  and  $L^2$ .

their resistances were conditioned by genes at separate loci, their rust-conditioning genes could be identified by appropriate genetic tests.

All  $F_2$  segregates from C.I. 1888-8 ×  $P^3$ , C.I. 1911-4 ×  $P^3$ , and C.I. 2008-4 ×  $M^3$  were resistant to race 300, a race avirulent on  $P^3$  and  $M^3$ . When tested against race 22, a race virulent on  $P^3$  and  $M^3$ , the  $F_2$  populations segregated in a ratio of three resistant to one susceptible.  $F_2$  populations of crosses between the three accessions and lines monogenic for a gene at the other four rust-conditioning loci ( $K$ ,  $L$ ,  $N$ , and  $M$  or  $P$ ) segregated for reaction to race 300 (Table 3). The ratios generally fit a dihybrid ratio of 15 resistant to 1 susceptible. In some cases, however, the  $F_2$  segregation ratios deviated significantly from the expected 15:1. Even though the deviations resulted from deficiencies of the susceptible class, we concluded that modifying genes were not involved. Rather, we suggest that some of the susceptible plants escaped infection and detection or that there was abnormal disjunction that resulted in a deficiency of one type of gamete. Plonka (4) noted this phenomenon regularly in his studies of inheritance of flower color in flax. Possibly a similar system was operative in our study.

The lack of segregation in the  $F_2$  progenies of C.I. 1888-8 ×  $P^3$ , C.I. 1911-4 ×  $P^3$ , and C.I. 2008-4 ×  $M^3$  establishes that the resistance of these accessions is conditioned by previously undescribed genes at the  $P$  and  $M$  loci. The rust-conditioning gene in C.I. 1911-4 cannot be distinguished from the  $P^4$  gene. The geographic origin of C.I. 1888 and C.I. 1911, however, suggests that the two resistances may be genetically distinct. The rust-conditioning gene in C.I. 2008-4 is designated  $M^6$ .

The  $P^4$  and  $M^6$  genes not only conditioned resistance to all North American races but also conditioned resistance to the South American races (A.M. Rosbaco, Ingeniera Agronoma, Centro

Regional Entrerriano Parans, Entre Rios, Argentina, *personal communication*). All other cultivars with known genes for resistance were susceptible in trials in South America. The discovery of single dominant

TABLE 3. Segregation of  $F_2$  populations of crosses of flax accessions suspected of carrying new genes for rust resistance and flax rust host-differential cultivars having genes for rust reaction at each of the five known loci

Cross	Test race <sup>x</sup>	Number of plants resistant and susceptible		<i>P</i> value
		Observed R:S	Expected R:S	
C.I. 1888-8 × $K$	300	109:9	108:10	>.70
C.I. 1888-8 × $L^6$	300	221:13	219:15	>.50
C.I. 1888-8 × $M^3$	300	1159:21	1100:30	<.01
C.I. 1888-8 × $N^1$	300	2823:20	2815:28 <sup>y</sup>	>.10
C.I. 1888-8 × $P^3$	300	1740:0	1740:0	
	22	972:321	970:323	>.90
C.I. 1911-4 × $K$	300	546:55	563:38	<.01
C.I. 1911-4 × $L^6$	300	228:14	227:15	>.70
C.I. 1911-4 × $M^3$	300	1427:116	1446:97	>.05
C.I. 1911-4 × $N^1$	300	2621:8	2603:26 <sup>y</sup>	<.01
C.I. 1911-4 × $P^3$	300	1513:0	1513:0	
	22	951:323	955:319	>.70
C.I. 2008-4 × $K$	300	187:13	188:12	>.70
C.I. 2008-4 × $L^6$	300	209:11	206:14	>.30
C.I. 2008-4 × $M^3$	300	1510:0	1510:0	
	22	353:123	357:119	>.50
C.I. 2008-4 × $N^1$	300	447:12	430:29	<.01
C.I. 2008-4 × $P^3$	300	932:40	911:61	<.01

<sup>x</sup> The presumed new genes and the five listed genes condition resistance to race 300. Only the undescribed genes condition resistance to race 22.

<sup>y</sup> The ratio was calculated from the known linkage between the  $N$  and the  $P$  locus.

genes that confer resistance to both South American and North American races affords the flax breeder the opportunity to develop flax cultivars containing single genes that condition a wider level of race protection than those presently available.

Although the three accessions were resistant in trials in South America, we do not expect that all of them will remain resistant for a long period of time. Routine screening of C.I. 2008-4 with race 22, a race of South American origin, resulted in the discovery of a few variant type-4 pustules. A laboratory culture derived from these pustules was identical in pathogenicity to race 22 but highly virulent on C.I. 2008-4. This culture was not virulent on either C.I. 1888-8 or C.I. 1911-4. The variant pustules on C.I. 2008-4 are believed to have resulted from single gene mutations within race 22. Regardless of the origin of this variant culture, its detection establishes the existence in the population of *M. lini* of a gene (or genes) capable of overcoming the resistance conferred

by the  $M^6$  gene. The detection of this variant elucidates the pitfalls of postulating the longterm control of flax rust through the use of vertical (monogenic) resistance.

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