Genes for Resistance to Flax Rust in the Flax Cultivars Towner and Victory A and the Genetics of Pathogenicity in Flax Rust to the *L8* Gene for Resistance

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ABSTRACT

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The flax (*Linum usitatissimum*) cultivars Towner and Victory A possess the *L8* and *M4* genes for resistance to flax rust (*Melampsora lini*), respectively. A previous hypothesis was that Towner also has *M4*. Putative *L8* and *M4* lines were derived from Towner by breeding, and these were tested with 18 different strains of flax rust, together with 29 differential cultivars. The putative *M4* line reacted as expected, but the putative *L8* line gave the same reactions as Bison, which carries *L9*. This suggested that the laboratory stock of Towner had *L9* and *M4* rather than *L8* and *M4*. This was confirmed by testing two other cultivars, Bisbee and B^{13} × Towner, which were expected to possess *L8*, with the same 18 strains of flax rust. These gave the same reactions, with one exception, and different reactions to Towner or the putative *L8* line. The exception was caused by *P1*, which was present in Bisbee in addition to *L8* but absent from B^{13} × Towner. These results suggest that the cultivar used as Towner by some previous workers was not Towner, but an unknown cultivar carrying *L9* and *M4*. Consequently, the true stock of Towner may be assumed to possess only *L8*. Progeny from three families of flax rust, previously tested only on the unknown cultivar used as Towner, were tested on B^{13} × Towner, which carries *L8*. These tests revealed that pathogenicity to *L8* was controlled by an avirulence gene pair, *A-L8/a-L8*, and an inhibitor gene pair, *I-L8/i-L8*, here *I-L8* alters avirulence to virulence, and that *I-L8* is linked to previously described inhibitor genes affecting the *A-L1*, *A-L7*, *A-L10*, and *A-M1* avirulence genes.

In flax, *Linum usitatissimum* L., there are at least 30 genes for resistance to flax rust, *Melampsora lini* (Ehrenb.) Lév., which occur at five loci designated K, L, M, N, and P as multiple alleles or pseudo-alleles designated *K* to *K1*, *L* to *L12*, *M* to *M6*, *N* to *N2*, and *P* to *P4*, respectively (2,5,6,10,11). In flax rust, pathogenicity is determined by corresponding avirulence gene pairs designated *A-K/a-K*, *A-K1/a-K1*, *A-L/a-L*, *A-L1/a-L1*, etc., which occur at separate loci, many of which are unlinked (2). However, pathogenicity to *L1*, *L7*, *L10*, and *M1* is also determined by inhibitor gene pairs designated *I-L1/i-L1*, *I-L7/i-L7*, *I-L10/i-L10*, and *I-M1/i-M1*, respectively, in which *I* alters avirulence to virulence, but *i* does not (8).

According to Flor (1), the flax cultivars Towner (CI 1561) and Victory A (CI 1170) possess the *L8* and *M4* genes for resistance to flax rust, respectively. Lawrence et al (8) argue that Towner also has *M4*, but this hypothesis was incompatible with Flor's data (4) unless, as they argue further, Victory A also has *M1* (8). The latter hypothesis is necessary only to accommodate the former, so if the former is invalidated then there is no longer a need for the latter.

To test the hypothesis that Towner carries *L8* and *M4*, a breeding program was initiated to isolate *L8* and *M4* lines from the stock of Towner used by Lawrence in this laboratory. The results of this breeding program have been used first, to determine the resistance genes present in Towner and Victory A, and second, to select an appropriate line of flax monogenic for *L8*. This line was then used to test the pathogenicity to *L8* of progeny in three families of flax rust produced by Lawrence (7,8). The results of these tests reveal that pathogenicity to *L8* is determined not only by an avirulence gene pair, *A-L8/a-L8*, but also by a new inhibitor gene pair, *I-L8/i-L8*.

MATERIALS AND METHODS

Stocks of flax were provided by G. M. E. Mayo. Crosses in flax were performed early in the morning by emasculating flower buds of the female parent just before anther maturity and dusting the styles with pollen from mature anthers of detached flowers from the male parent. Small tags indicating male parentage and the date of crossing were attached to the peduncles. Up to 10 seeds were obtained per capsule. Seeds from different capsules were harvested, stored, and tested separately so that any seed arising from self-fertilization or natural outcrossing could be detected.

Stocks of flax rust were provided by G. J. Lawrence and G. M. E. Mayo. Rust propagation, pathogenicity testing, and scoring of reactions were performed as described by Lawrence et al (8), except that plants selected for susceptibility were scored and infected leaves detached just before sporulation of the rust (7–10 days after inoculation). No rust strains were available that were avirulent to *L8* but not to *M4*, although strains of reverse type were available, so breeding programs involving indirect manipulation of *L8* were devised, using strains virulent to *L8*.

To isolate an *L8* line, the allelic *L2* gene was introduced to

manipulate the undetectable *L8* gene. The flax cultivar Stewart (CI 1072), which carries *L2* (1), was crossed with Towner, and the F_1 (*L2*/*L8 m*/*M4*) was tested for the presence of *M4* to confirm the cross and allowed to self-pollinate (Table 1). Plants presumed to be homozygous for *L8* (*L8*/*L8 m*/*m*) were recovered from the F_2 progeny by selecting for the absence of *L2* and *M4* (Table 1).

To isolate an *M4* line, the F_1 (*L2*/*L8 m*/*M4*) above was crossed to the flax cultivar Dakota (CI 1071), which carries the allelic or pseudo-allelic *M* gene (1). Plants presumed to be heterozygous for *L2* and *M4* (*L2*/*l M4*/*M*) were recovered from the progeny by selecting for the presence of *L2* and *M4*, and these were allowed to self-pollinate (Table 2). Plants presumed to be homozygous for *M4* (*l*/*l M4*/*M4*) were recovered from the progeny of this self-pollination, by selecting for the presence of *M4* and the absence of *L2* and *M* (Table 2).

The putative *L8* and *M4* lines, together with sets of 29 differential cultivars (8), including Towner and Victory A, were tested for their reactions to 18 different and mostly unrelated strains of rust of diverse geographic or hybrid origin.

RESULTS

Towner, Victory A, and the putative *M4* line reacted alike to the 18 strains of flax rust (Table 3), confirming the presence of *M4*. The putative *L8* line, however, reacted the same as Bison (CI 389), which carries the allelic *L9* gene (1). This suggested that either the putative *L8* line possessed *L9* rather than *L8* or, if it had *L8*, that the 18 strains of rust coincidentally showed the same pathogenicity to *L8* and *L9*. The latter hypothesis is obviously the less likely.

However, to test these hypotheses, the reactions of two other cultivars that were expected to possess *L8* were tested with the same 18 strains of rust. One of these cultivars, Bisbee (CI 1336), was a parent of Towner, which was derived from a Bisbee × Bison cross by selection of a line homozygous for *L8* from the F_2 progeny (1). The other, B^{13} × Towner, was derived from Towner by backcrossing 13 times with Bison and selecting for retention of *L8* followed by selfing and selecting for homozygosity of *L8* (1).

TABLE 1. Breeding program for the isolation of an *L8* line from the differential flax cultivar Towner, assuming Towner to be homozygous for *L8* and *M4* as hypothesized by Lawrence et al (8)

Stewart ♀	×	Towner ♂
$\frac{L2\ m}{L2\ m}$		$\frac{L8\ M4}{L8\ M4}$
	F_1	
$\frac{L2\ m}{L8\ M4}$		resistant to rust CH5.105[a]
	self	
$\frac{L2\ -}{-\ -}$ or $\frac{-\ M4}{-\ -}$		$\frac{L8\ m}{L8\ m}$
reaction[b] to rust CH5[c]	−	+
number	57	3

[a] Rust CH5.105, which was avirulent to *M4* but not *L2* or *L8*, was produced by Lawrence (7,8) by selfing rust CH5.
[b] − = No growth of the rust, + = growth.
[c] Rust CH5, which was avirulent to *L2* and *M4* but not *L8*, was produced by Lawrence (7,8) by crossing rusts C and H.

Differences were observed between the reactions of both Towner and the putative *L8* line and both Bisbee and B^{13} × Towner (Table 4), suggesting that Towner and the putative *L8* line do not in fact possess *L8*. The reactions of Bisbee and B^{13} × Towner were alike except for one rust strain, rust CH5.54 (group B3 of Table 4), which was avirulent on Bisbee but virulent on B^{13} × Towner. This suggested that both cultivars possess *L8*, but that Bisbee has an additional resistance gene. Comparing the reactions of Bisbee and the other differential cultivars for the 11 rust strains virulent on B^{13} × Towner (groups A2, B3, B4, and C of Table 4), only those of Akmolinsk (CI 515) were the same as those of Bisbee (Table 4). Akmolinsk carries *P1* (2), so Bisbee presumably carries both *L8* and *P1*.

These results confirm the absence of *L8* from the stock of Towner used in this laboratory by Lawrence et al (8) and suggest that the true stock of Towner was lost and that an unknown cultivar possessing *L9* and *M4* was substituted for it. Use of this laboratory stock as a differential cultivar has been discontinued in favor of B^{13} × Towner, which appears to be monogenic for *L8*.

One immediate use of B^{13} × Towner was to retest the pathogenicity of three families of flax rust produced by Lawrence (7,8) by selfing and intercrossing rusts CH5 and I. These families had previously only been tested on the unknown cultivar used as

TABLE 2. Breeding program for the isolation of an *M4* line of flax from the F_1 of the cross Stewart × Towner, assuming Towner to be homozygous for *L8* and *M4* as hypothesized by Lawrence et al (8)

Stewart × Towner F_1 ♀				Dakota ♂
$\frac{L2\ m}{L8\ m}$		×		$\frac{l\ M}{l\ M}$
reaction[a] to	$\frac{L2\ m}{l\ M}$	$\boxed{\frac{L2\ M4}{l\ M}}$	$\frac{L8\ m\text{ or }M4}{l\ M}$	
rust CH5.64[b]	−	−	+	
rust CH5.105	+	−		
number	2	3	4	
		self		
reaction[a] to	$\frac{-\ M}{-\ M}$	$\frac{L2\ M4}{-\ -}$	$\frac{l\ M4}{l\ M}$	$\boxed{\frac{l\ M4}{l\ M4}}$
rust CH5.105	+	−	−	−
rust CH5.64		−	+	+
rust CH5.87			−	+
number	13	35	3	3

[a] − = No growth of the rust, + = growth.
[b] Rusts CH5.64, CH5.87, and CH5.105, which were avirulent to *L2*, *M*, and *M4*, respectively, were produced by Lawrence (7,8) by selfing rust CH5.

TABLE 3. Patterns of reaction to 18 different strains of rust of the differential flax cultivars Towner, Victory A, and Bison compared to the putative *L8* and *M4* lines

Rust strains		Reaction[a] of				
Group	Strains (no.)	Towner	Victory A	Putative *M4* line	Bison	Putative *L8* line
A	5	−	−	−	−	−
B	11	−	−	−	+	+
C	2	+	+	+	+	+

[a] − = No growth of the rust, + = growth.

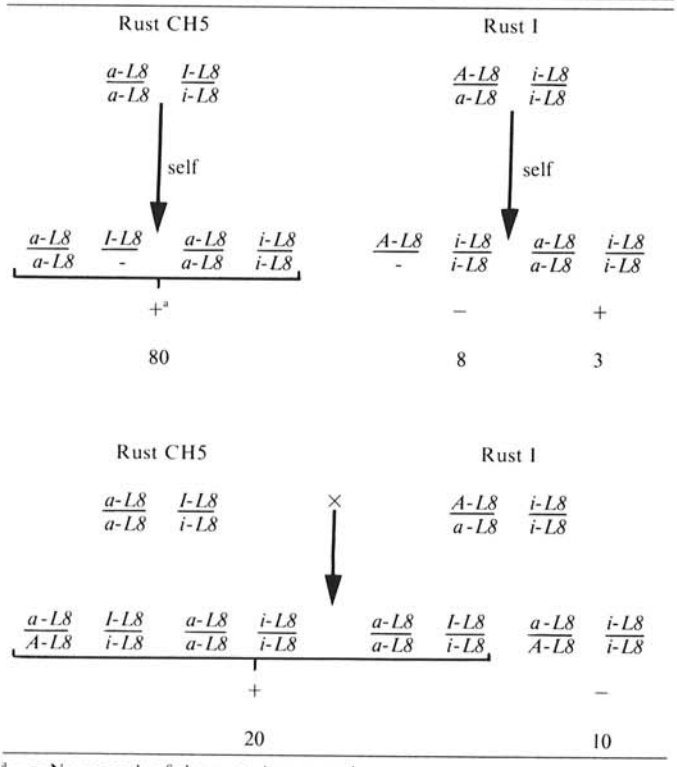
Towner. All 80 progeny from the family obtained by selfing rust CH5 were tested on $B^{13} \times$ Towner and all were virulent, suggesting that rust CH5 is homozygous for the *a-L8* virulence gene. Eleven of the 27 progeny from the family obtained by selfing rust I were tested on $B^{13} \times$ Towner and these segregated 8 avirulent : 3 virulent, which, assuming a binomial distribution based on a 3 : 1 ratio, gives an exact probability of 0.26 of fitting a 3 : 1 ratio, suggesting that rust I was heterozygous for the *A-L8* avirulence gene. Thirty of the 32 progeny from the CH5 × I family (obtained by crossing rusts CH5 and I) were tested on $B^{13} \times$ Towner and these segregated 10 avirulent : 20 virulent, fitting a 1 : 3 ratio ($\chi^2_1 = 1.11$, $p = 0.2$–0.3), suggesting that rust CH5 was not only homozygous for *a-L8* but also heterozygous for an inhibitor gene, *I-L8*, which interacts with *A-L8* to alter avirulence to virulence, and that rust I was not only heterozygous for *A-L8* but also homozygous for a noninhibiting gene, *i-L8* (Table 5).

TABLE 4. Patterns of reaction to 18 different strains of rust of the flax cultivars $B^{13} \times$ Towner, Bisbee, and Akmolinsk compared with the putative *L8* and *M4* lines

Rust strains		Reaction[a] of				
Group	Strains (no.)	$B^{13} \times$ Towner	Bisbee	Akmolinsk	Towner, Victory A, and the putative *M4* line	Bison and the putative *L8* line
B1	3	−	−	−	−	+
A1	2	−	−	+	−	−
B2	2	−	−	+	−	+
B3	1	+	−	−	−	+
A2	3	+	+	+	−	−
B4	5	+	+	+	−	+
C	2	+	+	+	+	+

[a] − = No growth of the rust, + = growth.

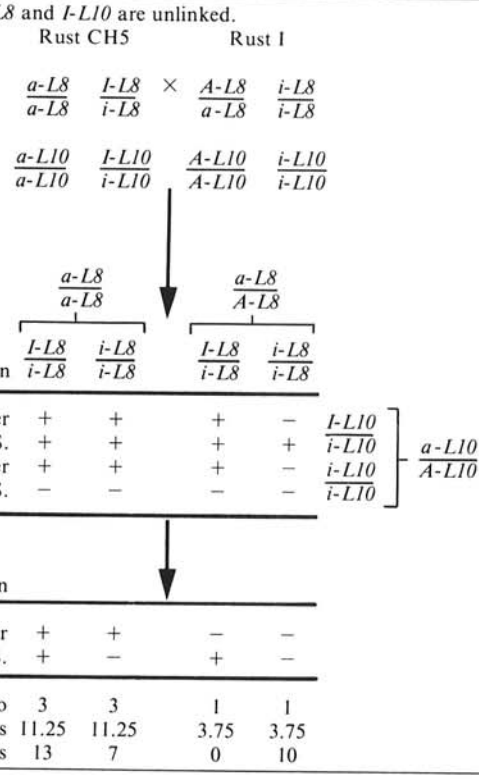
TABLE 5. Pathogenicity of three families of flax rust, obtained by Lawrence (7,8) by selfing and intercrossing rusts CH5 and I, on the flax cultivar $B^{13} \times$ Towner (*L8*)



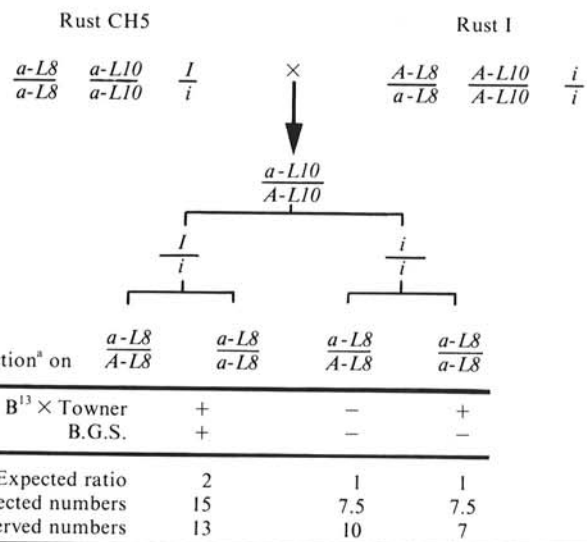
[a] − = No growth of the rust, + = growth.

TABLE 6. Tests of two alternative hypotheses for the joint segregation of genes controlling pathogenicity on the flax cultivars $B^{13} \times$ Towner (*L8*) and B.G.S. (*L10*) in the family of flax rust obtained by Lawrence (7,8) by crossing rusts CH5 and I. Data for the segregation of this family on B.G.S. are from Lawrence (7)

Hypothesis 1[a]. *I-L8* and *I-L10* are unlinked.



Hypothesis 2[c]. *I-L8* and *I-L10* are tightly linked in coupling (i.e., assumed to behave as a single gene *I*).



[a] Hypothesis 1 is not amenable to testing by a χ^2 test for goodness of fit as two of the four classes of progeny have expected numbers less than 5. Such a test would normally proceed to partition a χ^2_3 by subtracting the two χ^2_1 testing the individual segregations on $B^{13} \times$ Towner and B. G. S., which have already been calculated (see text) and shown to be nonsignificant, to indirectly obtain the third χ^2_1 testing for association between the segregations (i.e., for linkage). However, it is possible to test for association between segregations directly, in this case by Fisher's exact test for independence, which gives $p = 0.0011$. This indicates an association between the segregations that is inconsistent with the hypothesis of no linkage between *I-L8* and *I-L10*.

[b] − = No growth of the rust, + = growth.

[c] Hypothesis 2 is amenable to testing by a χ^2 test for goodness of fit. This gives a $\chi^2_2 = 1.13$ with $p = 0.5 - 0.7$, which is consistent with the hypothesis of tight linkage between *I-L8* and *I-L10*.

Rust CH5 has previously been shown to possess inhibitor genes affecting the *A-L1*, *A-L7*, *A-L10*, and *A-M1* avirulence genes (8). Because rust H, one of the parents of rust CH5 (8), is avirulent on $B^{13} \times$ Towner (rust H is in group B1 of Table 4), then *I-L8* must be absent from rust H, so it must have come from rust C, the other parent of rust CH5 (8), which is virulent on $B^{13} \times$ Towner (rust C is in group C of Table 4). The other inhibitor genes also come from rust C (8) and may be related to *I-L8*.

The relationship between *I-L8* and one of these inhibitor genes, *I-L10*, was examined for the CH5 × I family produced by Lawrence (7,8). Because rust CH5 is homozygous for *a-L10* but heterozygous for *I-L10* (8) and rust I is homozygous for *A-L10* and *i-L10* (8), all progeny of the intercross must be heterozygous for *A-L10* (thus providing an avirulent background), but segregating for *I-L10*. Thus, segregation in this family on the differential cultivar B. G. S. (Bolley Golden Selection, CI 1183), which carries *L10* (1), would have been due to segregation of *I-L10* alone. In this way, a segregation of 13 *I-L10*/- : 17 *i-L10*/*i-L10* was determined from Lawrence's data (7) for the 30 progeny tested above, fitting a 1 : 1 ratio ($\chi^2_1 = 0.53$, $p = 0.3$–0.5). Determination of the segregation of *I-L10* allowed consideration of two hypotheses: first, that *I-L8* is not linked to *I-L10*; and second, that *I-L8* is tightly linked to *I-L10*. The data are compatible with the hypothesis of tight linkage and incompatible with that of no linkage (Table 6), so clearly *I-L8* is linked in coupling to *I-L10* in rust CH5. Because each of the 10 progeny avirulent to *L8* was avirulent to *L10* (Table 6), then each of the 10 *i-L8* gametes from CH5 was nonrecombinant for *i-L10*. This gives 0% recombination between *I-L8* and *I-L10* with an upper limit of 30.85% at $p = 0.05$.

DISCUSSION

The finding that the stock of Towner, used in this laboratory by Lawrence et al (8), was not Towner, but a stock possessing *L9* and *M4*, negates their hypothesis that Towner carries *L8* and *M4*. Thus, it may be assumed that the stock of Towner used by Flor in his studies (2–4) only had *L8*. The breakdown of this hypothesis, in turn, eliminates the need for their hypothesis that Victory A carries *M1* and *M4*, since possession of *M1* was invoked only to make Flor's data (4) compatible with the presence of both *L8* and *M4* in Towner. Thus, it may be assumed that the stock of Victory A used by Flor in his studies (2–4) and by Lawrence et al in theirs (8) only had *M4*.

An important consequence of the breakdown of these hypotheses is the reestablishment of Flor's data (4), showing identical segregation on Williston Brown (CI 803), which carries *M1* (1), and Victory A, as the most substantial evidence for the close linkage of *A-M1* and *A-M4* in the rust. This evidence was seriously weakened by the possibility that Victory A shared *M1* with Williston Brown. Another consequence is the removal of evidence for the natural occurrence of two allelic or pseudo-allelic resistance genes, namely *M1* and *M4*, in coupling arrangement, although Mayo and Shepherd (9) have shown experimentally that such an arrangement is possible.

Perhaps one of the most important consequences has arisen from subsequent tests using a stock of flax which does in fact possess *L8*, namely $B^{13} \times$ Towner. This led to the discovery of a new inhibitor gene, *I-L8*, in rust CH5, which alters avirulence to virulence on $B^{13} \times$ Towner. Further analysis revealed that *I-L8* is linked to the *I-L1*, *I-L7*, *I-L10*, and *I-M1* genes of rust CH5, which is consistent with a previous suggestion (8) that the inhibitor genes of rust CH5 may be clustered into a tightly linked group.

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