Genetic Properties of Inhibitor Genes in Flax Rust that Alter Avirulence to Virulence on Flax

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ABSTRACT

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The properties of inhibitor genes in two strains of flax rust (*Melampsora lini*) that alter avirulence to virulence on flax (*Linum usitatissimum*) were investigated by testcross analysis. In rust CH5, a single gene or a tightly linked cluster of genes, designated *I-1*, was shown to inhibit interactions involving the *A-L1, A-L7, A-L8, A-L10*, and *A-M1* avirulence genes. In rust I, a second inhibitor gene, designated *I-2*, inhibited interactions involving the *A-M1* gene and was shown to be allelic or very tightly linked to *I-1*. It is suggested that *I-1* may have two components, one of which is L group specific and the other M group specific, while *I-2* has only one of these.

In flax, *Linum usitatissimum* L., resistance to flax rust, *Melampsora lini* (Ehrenb.) Lév., is determined by at least 30 genes in five series of allelic or closely linked genes designated *K* to *K1, L* to *L12, M* to *M6, N* to *N2*, and *P* to *P4*, respectively (4,6,8,13,14). In flax rust, avirulence is determined by corresponding genes, designated *A-K, A-K1, A-L, A-L1,* etc., some of which are linked (4). Avirulence is usually dominant to virulence, but Flor (2) reports an exceptional F_2 segregation in flax rust in which virulence was apparently dominant to avirulence on the flax cultivar Williston Brown, which carries *M1* (3). The appearance of avirulent F_2 progeny from a virulent F_1 was unexpected because both parents of the F_1 were virulent and did not segregate for avirulence when selfed. To account for these findings, Shepherd (12) postulated that an inhibitor gene, *I-M1*, interacting with an avirulence gene, *A-M1*, resulted in virulence on Williston Brown, and that inhibition of avirulence was dominant to noninhibition. Flor's results could then be explained if one of the parents was *A-M1/A-M1 I-M1/I-M1* and the other was *a-M1/a-M1 i-M1/i-M1* giving an F_1 that was *A-M1/a-M1 I-M1/i-M1* and an F_2 segregation fitting a modified dihybrid ratio of 3 avirulent : 13 virulent. This hypothesis received support from a later study by Flor (5) in which avirulence on Williston Brown (*M1*) was apparently dominant to virulence, with the F_2 segregation observed fitting a monohybrid ratio of 3 avirulent : 1 virulent. This confirmed the existence of an *A-M1/a-M1* gene pair as predicted by Shepherd's hypothesis.

The recent findings of Lawrence et al (11) have expanded and further supported this hypothesis. From segregation in three families of flax rust obtained by selfing and intercrossing rusts CH5 and I, they confirmed the existence of an *I-M1* gene in both rusts and postulated the existence of three new inhibitor genes, *I-L1, I-L7*, and *I-L10*, in rust CH5. When the present study was almost complete, Jones (7) postulated the existence of a fifth inhibitor gene, *I-L8*, in rust CH5.

Lawrence et al (11) found significant associations between segregations involving the inhibitor genes of rust CH5. Because the avirulence genes involved were known to be unlinked, they inferred that the inhibitor genes involved were linked. Similarly, Jones (7) found a significant association between segregations involving the *I-L8* and *I-L10* genes of rust CH5. A hypothesis that Lawrence et al (11) and Jones (7) found attractive was that of tight linkage of the inhibitor genes, but the data, although compatible with this hypothesis, did not exclude relatively loose linkage.

The inhibitor genes may be tightly linked or they may even be a single gene of multiple specificity. However, Lawrence et al (11) exclude *I-M1* from such a multispecific inhibitor gene on the basis of the separate existence of *I-M1* in rust I. This conclusion may not be valid, as their data do not establish the relationship between the *I-M1* genes in rusts CH5 and I. They may be two different unlinked genes or at the other extreme they may be allelic, with *I-M1* in rust I a monospecific allele of a multispecific inhibitor gene in rust CH5.

The aim of this study of the inhibitor genes was twofold: first, to determine if the inhibitor genes in rust CH5 are really one gene or several linked genes; and secondly, to determine if the *I-M1* gene in rust CH5 is allelic to the *I-M1* gene in rust I.

MATERIALS AND METHODS

Stocks of flax (Table 1) were provided by G. M. E. Mayo and stocks of flax rust by G. J. Lawrence. Crosses in flax rust, rust propagation, and scoring of reactions were performed as described by Lawrence et al (11).

Inhibitor testcross 1. To determine if the inhibitor genes in rust CH5 were really one gene or several linked genes, rust CH5, which was heterozygous for *I-L1, I-L7, I-L8, I-L10*, and *I-M1* (7,11), was testcrossed to a rust strain homozygous for the corresponding noninhibiting genes, while ensuring that a background of avirulence was maintained on which inhibitor phenotypes could be detected (Table 2). Because rust CH5 was heterozygous for *A-L1, A-L7,* and *A-M1* and homozygous for *a-L8* and *a-L10* (7,11), the other parent in the cross, besides being homozygous for the noninhibiting genes, had to be homozygous for *A-L1, A-L7, A-L8, A-L10*, and *A-M1* to ensure that all testcross progeny were either heterozygous or homozygous for these avirulence genes.

Rust H (11) satisfied the phenotypic requirements for this parent, being avirulent (i.e., *A/- ii*) on cultivars with *L1, L7, L8, L10*, or *M1* (Table 1). Through selfing and progeny testing, rust H.9.1, homozygous for all five avirulence genes, was selected as the other parent in the cross.

Pycniospores from many donor pycnia of rust H.9.1 (the nonsegregating parent) were mixed before crossing to single pycnia of rust CH5 (the segregating parent), to overcome the inefficiency caused by the mating-type incompatibility system of flax rust (10). One hundred and seventy-seven progeny were produced and tested on flax cultivars carrying *L1, L7, L10,* or *M1* (Table 1) and later a sample of 14 progeny were tested on B^{13} × Towner (*L8*).

Inhibitor gene testcross 2. To determine if the *I-M1* gene in rust CH5 was allelic to the *I-M1* gene in rust I, a rust heterozygous for the *I-L1, I-L7, I-L10,* and *I-M1* genes from rust CH5 and the *I-M1*

gene from rust I was crossed to rust H.9.1 (Table 3). Such a rust was selected from progeny in the CH5 × I family produced by Lawrence et al (11) by crossing rusts CH5 and I. Selection of this rust was based on two assumptions. The first was that the inhibitor genes of rust CH5, in particular *I-L10* and *I-M1*, are inherited as a unit. This assumption was borne out by the results of inhibitor gene testcross 1 reported below. The second was that the *A-M1* and *A-M4* avirulence genes of rusts CH5 and I, which are both *A-M1 A-M4/a-M1 a-M4* (11), are also inherited as a unit. This assumption was supported by published evidence for the tight linkage of *A-M1* and *A-M4* (5,7,11).

Lawrence et al (11) have shown that segregation on B. G. S. (*L10*) for the CH5 × I family was due entirely to the segregation of *I-L10*, so that progeny virulent on B. G. S. (*L10*) must possess *I-L10* and, based on the first assumption above, must also have the *I-M1* gene from rust CH5. Consistent with this assumption these progeny were virulent on Williston Brown (*M1*). The presence of *I-M1* was confirmed for those that were also avirulent on Victory A (*M4*), since, based on the second assumption above, they should possess *A-M1* as well as *A-M4*, but being virulent on Williston Brown (*M1*) they must have *I-M1*.

Five CH5 × I progeny carrying the *I-M1* gene from rust CH5 were selected in this manner. To detect the *I-M1* gene of rust I, these five progeny were testcrossed to rust H.9.1. Rust (CH × I)16 (9) produced testcross progeny avirulent on B^6 × B. G. S. (*L10*); indicating the absence of *I-L10* and, according to the first assumption above, the *I-M1* gene from rust CH5; avirulent on Victory A (*M4*), indicating the presence of *A-M4* and, according to the second assumption above, *A-M1*; but virulent on Williston Brown (*M1*), indicating the presence of the *I-M1* gene from rust I. Rust (CH5 × I)16 was therefore used as the other parent in the cross.

As above, crosses were made between single recipient pycnia of rust (CH5 × I)16 (the segregating parent) and mixtures of pycniospores from many donor pycnia of rust H.9.1 (the nonsegregating parent). Sixty-five progeny were tested on cultivars carrying *L1, L7, L10,* or *M1* (Table 1).

TABLE 1. Flax cultivars used or mentioned in this study and the resistance genes they carry. Compiled from Flor (3) and Jones (7)

Flax cultivar	CI number[a]	Resistance gene
B^{14} × Burke[b]	B^{14} × 1180	*L1*
Barnes	1190	*L7*
B^{13} × Towner[c]	B^{13} × 1561	*L8*
B^6 × B.G.S.[d]	B^6 × 1183	*L10*
Williston Brown	803	*M1*
Victory A	1170	*M4*

[a] Accession number of the Division of Cereal Crops and Diseases, U.S. Department of Agriculture.
[b] A line derived by backcrossing Burke with Bison (CI 389) 14 times.
[c] A line derived by backcrossing Towner with Bison (CI 389) 13 times.
[d] A line derived by backcrossing Bolley Golden Selection to Bison (CI 389) 6 times.

RESULTS

Inhibitor gene testcross 1. A segregation of 79 progeny virulent to *L1, L7, L10,* and *M1* (i.e., inhibited) : 98 avirulent (i.e., noninhibited) was obtained (Table 2) fitting a 1:1 ratio ($\chi^2_1 = 2.04$, $p = 0.1-0.2$). No recombinants were obtained, giving 0% recombination, with an upper limit of 2.06% at $p = 0.05$ calculated using Stevens' tables (1), between *I-L1, I-L7, I-L10,* and *I-M1*. A sample of 14 testcross progeny, chosen so that seven were inhibited by *I-L1, I-L7, I-L10,* and *I-M1* and seven were not, was later tested on B^{13} × Towner (*L8*). This sample was found to segregate 7 virulent (i.e., inhibited by *I-L8*) : 7 avirulent (i.e., noninhibited), in complete accordance with the pattern of inhibition for the other inhibitor genes. This gives 0% recombination between *I-L8* and the other inhibitor genes with an upper limit of 23.16% at $p = 0.05$. Combining these data with those of Jones (7) gives 0% recombination between *I-L8* and *I-L10* with an upper limit of 14.25% at $p = 0.05$. These limited data indicate that *I-L8* is also a member of the *I-L1, I-L7, I-L10,* and *I-M1* group of inhibitor genes which behave as a unit.

Although these data indicate a single inhibitor gene affecting five avirulence genes or a tightly linked complex of inhibitor genes, the lack of recombinants gives no information about the structure of such an inhibitor gene or gene complex. For the remainder of this paper the *I-L1, I-L7, I-L8, I-L10,* and *I-M1* inhibitor genes of rust CH5 will be referred to collectively as *I-1*.

Inhibitor gene testcross 2. A segregation of 29 progeny virulent to *L1, L7, L10,* and *M1* (i.e., inhibited by *I-1*) : 36 virulent to *M1* only (i.e., inhibited by the *I-M1* gene from rust I) was obtained, fitting a 1:1 ratio ($\chi^2_1 = 0.75, p = 0.3-0.5$), indicating that the *I-M1* genes of rusts CH5 and I are allelic (Table 3).

No progeny recombined for the *I-L1, I-L7,* or *I-L10* components of *I-1*, giving 0% recombination with an upper limit of 5.52% at

TABLE 2. Inhibitor gene testcross 1. Testcross progeny were obtained by crossing rusts CH5 and H.9.1, and their pathogenicity was tested on flax cultivars carrying *L1, L7, L10,* or *M1*. The cross is shown with the inhibitor genes linked, as indicated by the results of the cross

Rust CH5					×	Rust H.9.1				
A-L1 *a-L1*	*A-L7* *a-L7*	*a-L8* *a-L8*	*a-L10* *a-L10*	*A-M1* *a-M1*		*A-L1* *A-L1*	*A-L7* *A-L7*	*A-L8* *A-L8*	*A-L10* *A-L10*	*A-M1* *A-M1*
I-L1 *i-L1*	*I-L7* *i-L7*	*I-L8* *i-L8*	*I-L10* *i-L10*	*I-M1* *i-M1*		*i-L1* *i-L1*	*i-L7* *i-L7*	*i-L8* *i-L8*	*i-L10* *i-L10*	*i-M1* *i-M1*

Testcross progeny

background of avirulence	*A-L1* -	*A-L7* -	*A-L8* *a-L8*	*A-L10* *a-L10*	*A-M1* -	reaction[a] *L1, L7, L10, M1*	expected ratio	observed numbers
parentals	*I-L1* *i-L1*	*I-L7* *i-L7*	*I-L8* *i-L8*	*I-L10* *i-L10*	*I-M1* *i-M1*	+	1	79
	i-L1 *i-L1*	*i-L7* *i-L7*	*i-L8* *i-L8*	*i-L10* *i-L10*	*i-M1* *i-M1*	−	1	98
recombinants	new inhibitor gene combination							
	i-L1	*i-L7*	*i-L8*	*i-L10*	*i-M1*			0

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

TABLE 3. Inhibitor gene testcross 2. Testcross progeny were obtained by crossing rusts (CH5 × I)16 and H.9.1, and their pathogenicity was tested on flax cultivars carrying *L1, L7, L10,* or *M1*. The cross is shown with the *I-M1* genes of rusts CH5 and I as alleles, as indicated by the results of the cross

Rust (CH5 × I)16				×	Rust H.9.1			
- / -	*A-L7* / -	*A-L10* / *a-L10*	*A-M1* / -		*A-L1* / *A-L1*	*A-L7* / *A-L7*	*A-L10* / *A-L10*	*A-M1* / *A-M1*
I-L1 / *i-L1*	*I-L7* / *i-L7*	*I-L10* / *i-L10*	*I-M1* / *I-M1*		*i-L1* / *i-L1*	*i-L7* / *i-L7*	*i-L10* / *i-L10*	*i-M1* / *i-M1*

Testcross progeny

background of avirulence	*A-L1* / -	*A-L7* / -	*A-L10* / -	*A-M1* / -	reaction[a] *L1,L7,L10*	reaction[a] *M1*	expected ratio	observed numbers
parentals	*I-L1* / *i-L1*	*I-L7* / *i-L7*	*I-L10* / *i-L10*	*I-M1* / *i-M1*	+	+	1	29
parentals	*i-L1* / *i-L1*	*i-L7* / *i-L7*	*i-L10* / *i-L10*	*I-M1* / *i-M1*	−	+	1	36
recombinants	new inhibitor gene combination							
	i-L1	*i-L7*	*i-L10*	*i-M1*				0

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

$p = 0.05$. These data were combined with those of inhibitor gene testcross 1 to give 0% recombination for the *I-L1, I-L7,* and *I-L10* components of *I-1* with an upper limit of 1.51% at $p = 0.05$.

DISCUSSION

The inhibitors *I-L1, I-L7, I-L8, I-L10,* and *I-M1*, in rust CH5 behave as a discrete genetic unit, here designated *I-1*, and attempts to subdivide this unit, although limited, have failed. The data therefore suggest a single gene, but it is not possible to make a clear distinction between a cluster of closely linked genes and a single gene. If these inhibitor genes are separate genes, each affecting a different avirulence gene, and if inhibition can occur for all avirulence genes, then a tripartite gene-for-gene relationship exists. However, if there is a single inhibitor gene affecting a select number of different avirulence genes, then inhibition is not specific. This seems the more likely alternative.

The *I-M1* inhibitor gene in rust I, here designated *I-2*, appears to be allelic to *I-1*, indicating that *I-M1* can exist separately from *I-L1, I-L7, I-L8,* and *I-L10*. If, as seems likely, the latter can also exist separately from the former, then *I-1* is either two separate tightly linked genes or a single gene with two separate functional domains, one possessing *I-M1* activity and the other *I-L1, I-L7, I-L8,* and *I-L10* activity. This division suggests a pattern of inhibition according to the group specificity of the avirulence genes affected (i.e., L vs. M). An appropriate symbolism for these two inhibitor genes or inhibitor domains might be *I-L* and *I-M* where *I-L* = *I-L1 I-L7 I-L8 I-L10* and *I-M* = *I-M1*, so that *I-1* = *I-L I-M, I-2* = *i-L I-M*, and *i* = *i-L i-M*. This removes the assumption of individual specificity implicit in the inhibitor gene symbolism and indicates the group specificity of the avirulence genes affected but has the disadvantage of not indicating which members of each group are affected.

In addition to any possible group specificity, there appears to be some selectivity of inhibition because only some avirulence genes are affected. It is not clear whether this selectivity is real or only a sampling effect, but if it is real then the basis of the selectivity is obscure. However, the fact that several different avirulence genes are affected by what is possibly a single inhibitor gene would seem to indicate a relationship or similarity between these genes that has hitherto been unapparent.

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