Inheritance of Virulence of a Mutant Isolate of *Melampsora lini*

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ABSTRACT

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The inheritance of virulence was studied in a cross between two isolates of *Melampsora lini* with widely different virulence patterns. The F_1 of the cross (X82) was subjected to mutagenesis with nitrosoguanidine (MNNG). One mutant, isolate 33-27, had mutations to virulence on lines with host genes *L*8 and *N*2, and a mutation to avirulence on the line with *M*2 and was chosen to study the inheritance of virulence for a mutant isolate of *M. lini*. Although S_1 progenies from isolate 33-27 fit the same segregation ratios previous to MNNG treatment on many near-isogenic lines, differences in segregation occurred at several loci after MNNG treatment. These changes included lines on which X82 was homozygous avirulent and 33-27 S_1 progenies segregated 3:1, lines on which X82 progeny segregated 15:1 and 3:1 in the 33-27 S_1 progenies, and lines on which S_1 progenies from X82 segregated 3:1 and 13:3 in S_1 progenies from 33-27. S_1 progenies of isolate 33-27 segregated approximately 9:7, the same as the parental culture on lines with *L*8 and *N*2, even though this isolate was virulent to these loci after MNNG treatment. Isolate 33-27 had a mutation to avirulence at *AM*2. Progenies from selfing isolate X82 segregated approximately 3:1 on the line with *M*2, whereas S_1 progenies of isolate 33-27 segregated approximately 7:9. Segregation of S_1 progenies from X82 on several near-isogenic lines fit 3:1 ratios; however, 13:3 or 7:9 segregation ratios in progenies of 33-27 after treatment may suggest that expression of an additional recessive gene is affected by MNNG treatment.

The development of resistant cultivars has been one of the most successful methods of controlling rust diseases (8). However, *Melampsora lini* (Ehrenb.) Desmaz., the flax rust fungus, has the ability to change by mutation or sexual recombination and attack previously resistant flax (*Linum usitatissimum* L.) cultivars. This has happened several times throughout the history of flax cultivation (3,8). Thus, the development of resistant cultivars requires knowledge of mutation rates, virulence of the pathogen, and pathogen genetics.

Studies of mutations and inheritance of virulence provide knowledge of which genes for resistance would be most effective in a breeding program and how genes in *M. lini* are inherited (7,13–15). Most genetic studies of *M. lini* have indicated that virulence is inherited as single recessive genes, but digenic recessive combinations have also been reported (13,16). These single recessive genes for virulence have normally been inherited independently, but linkages have been reported (13,16). Lawrence et al (10) identified single pairs of allelic genes controlling virulence on 14 cultivars with avirulence dominant, but virulence to five cultivars involved both an avirulence-virulence locus and an inhibitor locus pair interacting to determine virulence.

There are several reports of mutations for virulence in the rust fungi (1,4–6,11,12,14,15). In 1956, Flor (4) used ultraviolet radiation to induce mutations to virulence in *M. lini*. In a later study, Flor (5) used X rays to induce mutations at both the same and different loci. Flor (4,5) determined that mutation rates differed at different loci. Statler (15) used *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) and induced mutations at loci different from those induced by Flor (4,5) and also found that mutation frequencies differed for different loci (14,15). This study was conducted to examine mutations affecting virulence in *M. lini* by analyzing changes in inheritance patterns after mutagen treatment of a culture known to segregate for virulence and avirulence on flax lines with many different resistance genes.

MATERIALS AND METHODS

Isolate X82 is an F_1 hybrid that resulted from a cross between two *M. lini* isolates with very different virulence patterns (16). To isolate X82, aeciospores were collected from an aecium by clipping a leaf with aeciospores into a 00 gelatin capsule. No other isolates were near the plant. Purity was evaluated by inoculating urediniospores of isolate X82 onto a set of 29 near-isogenic lines of flax. Contaminant pustules were not observed on the near-isogenic lines. This isolate was subjected to mutagenesis with MNNG (15) and screened for mutations to increased infection type (IT) on 22 near-isogenic host lines on which X82 was avirulent. An isolation was made from a pustule of high IT on one of 22 host lines that gave only low IT with X82 (15). The presumptive mutant to high IT was designated isolate 33-27. Isolate 33-27 was selfed by the method described below, and 88 progeny single-pustule isolates were recovered. The segregation of the 88 isolates was tested on near-isogenic lines of flax (Table 1).

ITs for isolates X82 and 33-27 are listed in Table 1. Isolate 33-27 was used to inoculate approximately 40 leaves on flax plants (cultivar Bison) that were approximately 15 cm tall. Leaves were sprayed with a suspension of 3 mg of urediniospores per milliliter of Soltrol 170 oil (Phillips Petroleum Co., Bartlesville, OK) and placed in a moist chamber at 18 ± 2 C and 100% RH for 24 h. The inoculated plants were then incubated in greenhouses at 21 ± 4 C. Sunlight was supplemented by sodium vapor lights for approximately 4 h at ~12,000 $\mu E \cdot s^{-1} \cdot m^{-2}$.

Teliospores of 33-27 were formed when the inoculated plants began to mature. Telia were conditioned to germinate by five alternate freeze-thaw, wet-dry cycles (15). The straw was frozen at about −5 C for 24 h, thawed at about 10 C for 24 h, dried at room temperature for 24 h, and then frozen. The wet straw was then suspended over seedlings of wet Bison flax for 24 h, dried at about 10 C for 24 h, and resuspended over seedlings until pycnia were observed. Pycnial infections on Bison plants were initiated by basidiospores released by the germinating teliospores.

Eighty-eight selfed isolates were produced by separately transferring spermatia from one pycnium to another by use of a sterile needle. Each pycnium was used only once to donate and/or receive

spermatia. Aeciospores resulting from fertilized pycnia were used to inoculate Bison plants. The 88 isolates were used to inoculate 29 near-isogenic lines of flax. The IT expressed on each line was classified on a scale of 0–4 (4) at 10–12 days after inoculation. ITs 0–2 were classified as avirulent and ITs 3 and 4 as virulent for genetic analyses. The chi-square method was used to test the hypothesis of independent segregation for various genetic ratios. The recombination values were calculated by the product method (9).

RESULTS

As reported previously (15), *M. lini* isolate 33-27 is virulent on lines with *L*8 and *N*2 after treatment with MNNG, whereas the parent isolate X82 is avirulent on these lines. The IT on the line with *M*2 changed from an intermediate type (2^+3^-) to a lower avirulent type (ITs 1 and 2) after MNNG treatment.

The S_1 progenies of the mutant isolate 33-27 segregated for about the same ratio as the S_1 progenies from isolate X82 on near-isogenic lines on which X82 was homozygous virulent (lines with *L*9, *M*1, *M*4, and *P*) and lines on which X82 was homozygous avirulent (lines with *M*6 and *N*), except for the line with *P*4 (Table 1). The S_1 progenies of isolate 33-27 also segregated about the same as the S_1 of X82 (3:1) on lines with *K, L*2, *L*5, *L*7, *L*11, *M, M*3, and *P*1 (Table 1). The S_1 of isolate 33-27 also segregated about the same as X82 on lines with *Ku* (gene from cultivar Kugine) and *L*3 (15:1). In addition, the S_1 of isolate 33-27 segregated 7:9, about the same as the F_2 of X82, on lines with *L*10 and 9:7 on lines with *L*4, *L*8, and *N*2 (Table 1).

Differences in segregation after MNNG treatment occurred at several loci. Isolate X82 was homozygous avirulent on the line with *P*4, but S_1 progenies of isolate 33-27 segregated approximately 3:1. S_1 progenies of isolate X82 segregated approximately 15:1 on the lines with *L* and *M*5, but S_1 progenies of isolate 33-27 segregated approximately 3:1 on these lines (Table 1). S_1 progenies of X82 segregated approximately 3:1 on lines with *L*6, *N*1, *P*2, and *P*3 but approximately 13:3 in progenies from 33-27 (Table 1). S_1 progenies of X82 segregated approximately 3:1 on the line with *M*2 but approximately 7:9 in S_1 progenies from 33:27.

The IT of isolate 33-27 was different from that of X82 on lines with *L*4, *L*8, and *N*2, yet segregation ratios of S_1 progenies from the two isolates were approximately the same. The IT of isolate X82 was 2^+ on the line with *L*4, but isolate 33-27 had an IT 0; on *L*4. The IT of isolate X82 was 0 to 0; on lines with *L*8 and *N*2, but isolate 33-27 was virulent (IT 3–4) on these lines.

The single recessive genes conditioning pathogenicity were apparently independently inherited, except for linkage indicated between *AL* and *AL*11; between *AM* and *AM*3; between *AL*5, *AL*6, and *AL*7; and between *AP*1, *AP*2, and *AP*3 (Table 2). Segregation for *AL*6 did not fit a 3:1 ratio, but chi-square tests for independence still indicated linkage between *AL*5, *AL*6, and *AL*7. Segregation on *AP*2, *AP*3, and *AM*2 did not fit a 3:1 ratio for single recessive genes, but linkage was indicated.

DISCUSSION

Mutagenic treatment may alter the expression of avirulence in the flax rust fungus, *M. lini,* without any apparent change in inheritance. This appears to have happened with virulence on flax lines with *L*8 and *N*2. Secondly, treatment with MNNG may cause simultaneous mutations at many avirulence or suppressor loci that can be detected by altered segregation ratios even when there is no change in IT induced. These results indicate that this happened at at least five and possibly at as many as nine loci in one rust isolate.

It was previously reported that isolate 33-27 had a mutation to virulence on near-isogenic lines with resistance genes *L*8 and

TABLE 1. Segregation for virulence among F_2 cultures of *Melampsora lini* derived from crossing races 1 and 400 (X82) and the S_1 progeny isolates of mutant culture 33-27

Gene loci on near-isogenic flax lines	IT[a] of X82	Segregation of X82[b]	IT of 33-27	S_1 isolates of isolate 33-17						Expected ratio	Chi-square	Probability
				Avirulent				Virulent				
				0–0;	0;1–0;2	1	2	3	4			
*M*6	0	HA	0;	70	9	6	1	0	0	HA	...	...
N	0	HA	0	84	2	0	0	0	0	HA	...	...
*L*9	4	HV	4	0	0	0	0	36	49	HV	...	...
*M*1	4	HV	4	0	0	0	0	31	56	HV	...	...
*M*4	4	HV	3	0	0	0	0	22	64	HV	...	...
P	4	HV	3	0	0	0	0	14	74	HV	...	...
K	0;2	3:1	0;	42	15	3	2	8	15	3:1	0.19	0.661
L	0	15:1	0	0	62	0	1	15	9	3:1	0.31	0.577
*L*2	0	3:1	0;	50	9	0	1	9	19	3:1	2.18	0.140
*L*5	0;	3:1	0	59	11	0	0	12	6	3:1	0.97	0.325
*L*7	0;	3:1	;1	43	23	2	3	9	6	3:1	2.62	0.106
*L*11	2	3:1	0;	53	5	2	4	14	10	3:1	0.24	0.622
M	0	3:1	0	53	6	0	0	7	22	3:1	2.97	0.085
*M*3	0	3:1	0	51	8	0	1	9	19	3:1	2.18	0.140
*M*5	2	15:1	;1	14	25	18	15	12	4	3:1	2.18	0.140
*P*1	0;	3:1	0;	54	17	0	2	10	4	3:1	3.68	0.055
*P*4	0	HA	0;	31	25	2	3	19	8	3:1	1.52	0.218
*L*6	0;	3:1	0	60	10	1	2	8	5	13:3	0.75	0.388
*N*1	0	3:1	0;	62	14	0	2	6	4	13:3	3.15	0.076
*P*2	0;	3:1	0;	67	6	1	1	8	5	13:3	0.91	0.339
*P*3	0;	3:1	;1	56	18	1	0	9	3	13:3	1.40	0.236
Ku[c]	0	15:1	0;	72	7	4	2	2	1	15:1	1.21	0.271
*L*3	12	15:1	;1	38	26	7	12	0	3	15:1	1.12	0.290
*L*10	3	7:9	3^-	7	21	5	10	30	15	7:9	0.94	0.334
*L*1	4	1:3	3^-	3	10	5	15	34	21	7:9	1.40	0.237
*M*2	2^+3^-	3:1	12	0	2	8	25	35	18	7:9	0.57	0.452
*L*4	2^+	9:7	0;	33	13	4	0	14	23	9:7	0.05	0.818
*L*8	0;	9:7	3	32	11	1	3	20	21	9:7	0.29	0.591
*N*2	0	9:7	4	32	12	1	1	16	25	9:7	0.40	0.526

[a] Infection type. 0–2 = avirulent, and 3–4 = virulent.
[b] HA = homozygous avirulent, and HV = homozygous virulent.
[c] Gene from cultivar Kugine.

*N*2 and a mutation to avirulence on near-isogenic lines with *M*2 (15). If there were a true mutation of a chromosomal gene, the mutant isolate, when selfed, should show a segregation pattern different from that of the parent isolate. Since the mutant and parent isolates show the same segregation ratios for virulence on both lines, it is possible that the change in virulence did not persist through meiosis.

The IT of isolate X82 on the line with *M*2 was reported to be susceptible (IT 4) in a previous study (15) but was later found to be intermediate (IT 2^+3^-) (16). In this study, the parent isolate X82 was determined to be intermediate (IT 2^+3^-) to the line with *M*2, so the mutation to avirulence on *M*2 was probably from intermediate to low IT rather than from virulence to avirulence. Segregation of isolate X82 changed from a 3:1 to a 7:9 ratio among the S_1 progenies of 33-27 on the line with *M*2 after MNNG treatment.

S_1 progenies of isolate X82 segregated 9:7 for virulence on three lines, those with *L*4, *L*8, and *N*2; and on all three lines the IT of 33-27 was different after treatment with MNNG. The IT on *L*4 changed from 2^+ to 0;. The IT on *L*8 changed from 0; to 3. The IT on *N*2 changed from 0 to 4. On none of these lines did the segregation ratio of S_1 progenies of 33-27 change from the 9:7 ratio among the S_1 progenies of X82. This suggests something other than a permanent genetic change.

It is hard to explain how a true mutation of a chromosomal gene could occur without affecting segregation in S_1 progenies of the mutant isolate. When the apparent mutant isolates were tested on the same lines from which they were selected in the previous study (15), most were no longer virulent. It could be argued that the apparent mutations to virulence to lines with *L*8 and *N*2 also were not completely stable and did not persist through meiosis. It could also be hypothesized that a chemical change in DNA could cause a temporary change in gene expression without altering segregation ratios. It could also be possible that mutations in gene expression are maintained through successive mitotic divisions but are finally repaired during meiosis to restore the original genotype.

It may be possible that the segregation of S_1 progenies from X82 on the line with *M*2 is 1:2:1 for avirulent:intermediate:virulent and that an induced mutation adds quantitative increases in aggressiveness to progenies that get one or two doses of the mutant allele. This would shift the intermediate reaction types toward the virulent end of the scale (isolate X82 would fit a 1:2:1 ratio [13:51:18; $P = 0.05$] on the line with *M*2). Conversely, when the low IT is 2, 2^+, or 2 and 3 and the high 3 and 2 or 3^-, it may be too difficult to consistently evaluate the segregation pattern.

The change from homozygous avirulent S_1 progenies of X82 on *P*4 to S_1 progenies of 33-27 segregating 3:1 after MNNG treatment could be explained by the mutation of one dominant allele for avirulence to a recessive allele for virulence in the homozygous avirulent X82. The change from 15:1 to 3:1 segregation for avirulence on *L* and *M*5 could be explained by the fact that isolate X82 was heterozygous at two loci where a dominant allele at either locus conditioned avirulence; the mutation changes the dominant allele to a recessive allele at one of the loci, making 33-27 homozygous virulent at that locus. The change from 1:3 to 7:9 segregation for avirulence on *L*1 could be explained by the fact that X82 is homozygous for dominant avirulence alleles at one locus but heterozygous at a second locus with a dominant allele that suppresses avirulence on *L*1 and a recessive allele that does not. The mutation at the avirulence locus makes 33-27 heterozygous at both loci.

Segregation changed from 3:1 to 13:3 after MNNG treatment on lines with *L*6, *N*1, *P*2, and *P*3. Virulence on *P*1 could be included in this group because the 73:14 ratio probably fit a 13:3 ratio better than a 3:1 ratio. One explanation is that these cases may represent some distortion in the normal 3:1 segregation rather than mutation. Perhaps there was some reduced fitness and lower survival of virulent progeny associated with the mutagen treatment. If the 13:3 ratio is real, expression of virulence must require a dominant allele at one locus and homozygous recessive alleles at the other.

Complementary dominant genes for virulence are uncommon in *M. lini* genetics but have been reported for the *AL*10 locus (16). However, it is unusual to have six cases of complementary genes in one study. The unusually high numbers of the 7:9 or 9:7 ratios could be the result of an additional recessive gene created by the MNNG treatment.

Although segregation for *AP*1, *AP*2, and *AP*3 did not fit 3:1 ratios ($P < 0.05$), chi-square tests for independence indicated that these loci are closely linked ($P < 0.001$) (Table 2). These genes were closely linked when Statler (16) studied the inheritance of virulence for isolate X82. Other studies also demonstrated linkage at the *AP*1, *AP*2, and *AP*3 loci (8,12). It is possible that genes for virulence *AP*1, *AP*2, and *AP*3 could be multiple alleles at the same locus, since few differences or no differences in segregation were observed.

Data from linkage analyses of *AL*5, *AL*6, and *AL*7 in this study and in the analysis of S_1 progenies from X82 (15) suggest that *AL*5 is located between *AL*6 and *AL*7 on the chromosome. The apparent linkage between *AL* and *AL*11 is interesting because it did not show up in the analysis of X82. The change from a 15:1 segregation for avirulence on line *L* in progenies of X82 to a 3:1 ratio in progenies of 33-27 obviously has something to do with this linkage.

The abnormal ratios reported here for the S_1 progenies from mutant isolate 33-27 are probably the result of treatment with MNNG, since logical Mendelian ratios were reported among the S_1 progenies of the parental isolate previous to treatment. One possible explanation for abnormal ratios might be that MNNG, a rather powerful mutagen, caused a chemical modification of DNA (2). This is plausible because MNNG is an alkylating agent that can cause guanine to ionize differently, which might result in pairing errors.

TABLE 2. Linkage analyses of avirulence gene loci of *Melampsora lini* developed from mutant culture 33-27 based on segregation on flax near-isogenic lines

Genes for virulence	*P* value	Recombination estimate[a] (%)	SE[b] (%)
AL vs. *AL*11	<0.005	4.9	2.5
*AL*5 vs. *AL*7	<0.001	5.1	0.1
*AL*5 vs. *AL*6	<0.001	14.2	4.3
*AL*6 vs. *AL*7	<0.001	18.9	4.9
AM vs. *AM*3	<0.005	4.9	2.5
AM vs. *AL*11	<0.005	40.5	7.3
*AP*1 vs. *AP*2	<0.001	1.3	5.3
*AP*1 vs. *AP*3	<0.001	1.3	5.3
*AP*2 vs. *AP*3	<0.001	0	5.3

[a]Based on formulas and tables developed by Immer (9).
[b]Standard error of recombination estimate.

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