Mutations to Virulence and Avirulence in *Melampsora lini*

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ABSTRACT

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A culture of race 1 of *Melampsora lini* was crossed with a culture of race 400 to produce a culture heterozygous at several loci. Flax plants inoculated with the heterozygous culture were treated with a solution of NTG (*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine) to induce mutations in *M. lini*. Isolations were made from compatible infection sites on near-isogenic lines resistant to the culture used. Seven mutant cultures were isolated that were virulent at the A_L^{11}, A_L^{8}, A_M^{5}, and/or A_N^{2} loci after NTG treatment. Three were virulent at A_L^{11}, three were virulent at A_L^{8}, A_M^{5}, and A_N^{2}, and one was virulent on A_L^{8} and A_N^{2} after NTG treatment. Nine mutant cultures were avirulent at the A_L^{1}, A_L^{10}, and/or A_M^{2} loci after NTG treatment. Three were avirulent at the A_L^{1} and A_L^{10} loci, one was avirulent at the A_L^{10} and A_M^{2} loci, two at A_L^{10}, and three at the A_M^{2} locus after NTG treatment.

Additional key words: *Linum*, flax rust, genetics.

Several reports of mutations for virulence in the rust fungi have been made (1,3–5,7,8). Many have been changes at a single locus. Watson (11) and Newton and Johnson (7) reported a single gene change affecting pathogenicity of *Puccinia graminis*. The mutations that attack only one additional gene can be explained on the basis of a point mutation. This could be a deletion or a structural alteration of the DNA comprising the gene.

The selection of induced mutations to virulence has been attempted in several different pathogenic fungi. Flor (3) first reported induced mutations for pathogenicity in *Melampsora lini* (Pers.) Lev. He used ultraviolet radiation as a mutagen with uredospores of *M. lini*. High frequencies of induced mutants were subsequently produced in *M. lini* by Flor (4) and by Schwinghamer (8) with X-rays. Gabriel et al (6) used a chemical mutagen, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG), to induce 29 mutations to increased virulence in *Erysiphe graminis*. They (6) reasoned that the loci appeared to function for avirulence and not for virulence since the rate of infection and the final infection types were indistinguishable from the wild type.

Melampsora lini is a binucleate dikaryon. A binucleate uredospore heterozygous for virulence at one locus carries a dominant allele for avirulence in one nucleus and a recessive allele for virulence in the other nucleus. Flor (3) reasoned that a uredospore heterozygous for virulence should be able to attack a resistant near-isogenic line following a single change by a mutagen to alter the dominant allele. It is also logical that a uredospore homozygous for virulence should become avirulent following alteration of one recessive allele by a mutagen. Day (1), however, indicated that methods were not available to select avirulent mutants. It is true that most avirulent mutants would be lost in a screening program. However, it may be possible to overcome this problem by screening treated cultures on several near-isogenic lines and searching for avirulent mutants. Mutations to avirulence will only be isolated from loci that were homozygous recessive for virulence prior to treatment with a mutagen.

The current study was undertaken to determine if mutations to virulence and avirulence could be induced by chemical mutagenesis in a culture of *M. lini* known to be heterozygous at several loci.

MATERIALS AND METHODS

Pure cultures of race 1 and race 400 of *M. lini* were established by two successive single-pustule isolations. Each culture was then used to inoculate flax (*Linum usitatissimum* L. 'Bison') plants that were approximately 15 cm tall. When teliospores formed, they were conditioned to germinate by several alternate freeze-thaw and wet-dry cycles (5). After several cycles, the telia-laden straw was suspended over Bison seedlings. Inoculated plants were held at approximately 100% relative humidity (RH) and 19 C for 48 hr prior to incubation in greenhouses at 20 ±4 C. Crossing was accomplished by separately transferring nectar and pycniospores from a pycnium of race 1 to pycnia of race 400. Aeciospores resulting from a single aecium of a cross were used to inoculate cultivar Bison plants. This F_1 culture (X82) was increased and purity was evaluated on near-isogenic lines. No virulent-type pustules were observed on the incompatible isogenic lines. Leaves of cultivar Bison were inoculated with a suspension of 3 mg of the

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pure F_1 culture, X82, in each milliliter of Soltrol 170 (Phillips Petroleum Company, Bartlesville, OK) and incubated in approximately 100 RH for 16 hr at 18.5 ± 2 C. Approximately 5 cm of the stem tip with leaves was placed in 10-cm-diameter petri dishes containing 2 ml aqueous benzimidazole (100 μg ml) and 2 ml of aqueous NTG (125 μg ml). The method of Gabriel et al (6) was used for the NTG treatment. Petri dishes were placed in a growth cabinet at 20 ±2 C with a 12-hr light period. Sunlight was supplemented by sodium vapor lights (~12,000 $\mu E \cdot s^{-1} \cdot m^{-2}$) to provide a 12-hr photoperiod. Uredospores were collected and inoculated onto cultivar Bison flax plants after 12 days of NTG treatment. Uredospores collected from Bison were used to inoculate a tester set of near-isogenic lines on which X82 was avirulent. The cultures obtained from NTG treatment were tested on near-isogenic flax lines with genes K, L, L^1, L^2, L^3, L^4, L^5, L^6, L^7, L^8, L^9, L^{10}, L^{11}, M, M^1, M^2, M^3, M^4, M^5, M^6, N, N^1, N^2, P, P^1, P^2, P^3, and P^4. Isolations were made from compatible infection sites, increased, and evaluated on near-isogenic lines. The infection type expressed on each near-isogenic line was classified on a scale of 0–4 at 10–12 days after inoculation (10). Infection types (IT) 0 and 0; were classified as highly avirulent, ITs 1 and 2 as avirulent, and ITs 3 or 4 as virulent. If more than one IT was observed, the predominant type was listed first (e.g., IT 12, is mostly IT 1 with a few IT 2 uredia). Contamination was negated by working only with culture X82 during the experiment.

RESULTS

Forty-eight cultures were produced by the NTG treatment of inoculated plants. These were used to inoculate a set of 22 near-isogenic lines that were immune or resistant to the F_1 culture (X82). Seventy-five isolates from single pustules of a virulent infection type were taken from the near-isogenic lines. These 75 isolates were separately increased on cultivar Bison and each resulting culture was used to inoculate the near-isogenic line from which it was isolated. Only seven cultures were virulent on the near-isogenic lines from which they were collected. The seven isolates that were virulent on the near-isogenic line from which they were originally isolated and twenty other NTG-treated cultures were increased on cultivar Bison and used to inoculate a full set of near-isogenic lines. Three of these isolates (8-27, 32-27, and 47-27) were mutations to virulence at A_L^{11} (Table 1). Three isolates (7-20, 7-21, and 7-22) were virulent at A_L^8, A_M^5, and A_N^2 after NTG treatment. One culture (33-27), was virulent at the A_L^8 and A_N^2 loci after NTG treatment. Culture 70-20 was isolated from the line with gene L^8, 7-21 from the line with gene N^2, 7-22 from the line with gene M^5 and 8-27, 32-27, 33-27, and 47-27 from the line with gene L^{11}.

Nine avirulent mutant cultures were detected by evaluating the NTG-treated isolates on a full set of near-isogenic lines. Three (7-20, 33-19, and 58-19) were avirulent after NTG treatment at the A_L^1 and A_L^{10} loci (Table 2). One isolate (36-19) was avirulent at A_L^{10} and A_M^2, two (20-21 and 32-27) at A_L^{10}, and three (7-29, 23-28, and 33-27) at the A_M^2 locus after treatment.

Several changes within the avirulent category were observed. Culture X82 was highly avirulent (IT 0 or 0;) at the A_L^7, A_L^8, A_K, A_N^2 and A_P^4 loci. Three mutant cultures were moderately avirulent (IT 1 or 2) at A_L^7, two at A_K and A_P^4 and one at the A_L^8 and A_N^2 loci after NTG treatment. Culture X82 was avirulent (IT 1 or 2) at A_L^3, A_L^4, and A_L^{11}. There were seven mutant cultures highly avirulent at A_L^3 and A_L^{11} and eight highly avirulent at A_L^4 after NTG treatment.

DISCUSSION

Avirulence in *M. lini* has been shown to be conditioned by dominant genes (9). The production of a mutation to virulence would involve a single event in a heterozygous clone or simultaneous mutations in both nuclei of the dicaryon in a homozygous clone (12). A culture heterozygous at many loci was developed in this study by crossing race 1 of *M. lini* with race 400. The genotype for pathogenicity of the F_1 labeled X82 was postulated from selfing studies of the parent races and the pathogenicity of the F_2 cultures (*unpublished*). The F_1 culture was heterozygous at 20 loci, homozygous virulent at five loci, and homozygous avirulent at two loci. The alteration of the dominant allele was detected phenotypically by the expression of virulence on a previously immune host. Mutations to virulence were all isolated from heterozygous loci. Mutations to avirulence were all isolated from loci homozygous recessive for virulence.

Seven mutant cultures had increased virulence at the A_L^8, A_L^{11}, A_M^5, and/or A_N^2 loci after NTG treatment (Table 1). Three mutants had increased virulence at only one locus (A_L^{11}). One had increased virulence at two loci (A_L^8 and A_M^5) and three at three loci (A_L^8, A_M^5, and A_N^2). The mutants that attack only one additional line can be accounted for by the mutation of a single gene (3). This could occur by a structural alteration of a few nucleotides of the DNA comprising a gene (2). Mutants virulent on several additional near-isogenic lines could be the result of a major alteration of DNA or gross chromosomal alterations, but more probably are single mutations with pleiotropic effects.

TABLE 1. Infection type[a] produced by parents (race 1 and 400), F_1 (X82), and seven mutations to virulence in *Melampsora lini* on near-isogenic lines of flax with single genes for rust resistance

Near-isogenic line	Parental race		F_1	Cultures from NTG[b] treatment						
	1	400	X82	7-20	7-21	7-22	8-27	32-27	33-27	47-27
L^8	0	4	0;	3	3	3	0;	0;	4	0
L^{11}	0	4	2	0;	0;	0;	3–	4	02	3–
M^5	12	4	2	4	4	4	0;	02	12	0;
N^2	0;	4	0;	4	4	4	0	12	3	0;

[a] Infection type (IT) 0 or 0; = highly avirulent, 1 or 2 = avirulent, and 3 or 4 = virulent. The predominant IT is listed first when more than one occurred. A + indicates a slightly higher IT while a – indicates a slightly lower IT.
[b] *N*-methyl-*N*′nitro-*N*-nitrosoguanidine, a mutagen.

TABLE 2. Infection type[a] produced by parents (race 1 and 400), F_1 (X82), and nine mutations to avirulence in *Melampsora lini* on near-isogenic lines of flax with single genes for rust resistance

Near-isogenic line	Parental race		F_1	Cultures from NTG[b] treatment								
	R1	400	X82	7-20	7-29	20-21	23-28	32-27	33-19	33-27	36-19	58-19
L^1	4	4	4	12	3–	4	4	4	12	3–	4	2
L^{10}	3	4	3	;2	3–	12	3–	01	12	3–	12	;2
M^2	3–	3	4	3–	12	4	2	3	3–	12	12	3–

[a] Infection type (IT) 0 or 0; = highly avirulent, 1 or 2 = avirulent, and 3 or 4 = virulent. The predominant IT is listed first when more than one occurred. A + indicates a slightly higher IT while a – indicates a slightly lower IT.
[b] *N*-methyl-*N*′nitro-*N*-nitrosoguanidine, a mutagen.

In 1956, Flor (3) used ultraviolet radiation on a cross of race 1 × race 22 of *M. lini* to induce mutations to virulence on lines with genes L^5, L^7, M, M^3, and N^1. In a later study, Flor (4) used X-ray to induce mutations on lines with genes L^5, L^6, M, M^3, N^1, P, and P^3. He (3,4) determined that mutation rates were different for different loci in *M. lini*. The current study was conducted with a chemical mutagen NTG and a culture of a cross of race 1 × race 400 *M. lini*. Mutations were induced at the A_L^8, A_L^{11}, A_M^5, and A_N^2 loci in this study. These mutants were at different loci than those induced by Flor (3,4), but mutation frequencies were still different for different loci. The fact that these mutants were at different loci than those induced by Flor (3,4) could be explained by the fact that he used x-radiation, whereas I used NTG in this study. Mutations induced by radiation in *M. lini* have been attributed to deletions (4,5,8), whereas chemical mutagens such as NTG probably act by chemical modification of DNA (2). That mutations are induced at different loci by different mutagens probably suggests that it may be inappropriate to use induced mutations to estimate gene longevity.

I have no explanation for the rather unusual phenomenon that of the 75 apparently virulent isolates from near-isogenic lines resistant to X82, only seven cultures were virulent on the same near-isogenic line from which each was isolated. The remainder were all avirulent with no virulent infection types. One hypothesis could be that some mutations caused by NTG could be unstable and revert back to the original form or undergo repair between the first and second generation. This could be plausible since NTG is an alkylating agent that can cause guanine to ionize differently, which might result in pairing errors (2). The pairing errors may not be stable in all cases. It could be argued that some of the single-pustule isolates were isolated from off-type plants (admixture). However, if this were the case the off type plant would have had more pustules. Normally, the plants we isolated from had only one or two pustules. Another possibility could be that the parent culture used (X82) was contaminated. This explanation was ruled out because off-type pustules were never observed when isogenic lines were inoculated with X82. This indicated that culture X82 was genetically pure and that natural mutants were not observed in the limited number of tests that were conducted.

Mutations to virulence at the A_L^8 and A_M^5 and A_N^2 or A_L^8 and A_N^8 loci appear to behave as a unit. Virulence has been reported to be linked with A_M^5 at the A_L^8 and A_N^2 loci but not at the A_L^8 or A_N^2 loci (5,9,10). As previously suggested (4), these mutations may simply be simultaneous mutations.

Mutations to avirulence were found in this study by isolating mutants that were virulent at one locus and screening for changes at other loci on different near-isogenic flax lines. These changes to avirulence could have been the result of single-gene mutations since virulence is recessive and only a single change is necessary for avirulence to occur in a virulent homozygote. Mutations to avirulence were detected at the A_M^2, A_L^1, and A_L^{10} loci, but the line with A_L^{10} was previously reported to be temperature sensitive (10).

Changes within the avirulent category were detected after NTG treatment on lines with genes A_K, A_L^3, A_L^4, A_L^7, A_L^8, A_L^{11}, A_N^2, and A_P^4 loci. These were all determined to be heterozygous loci. These changes could be explained by the fact that most chemical mutagens probably act by direct chemical modification of DNA (2). The chemical mutagen NTG is an alkylating agent that can react with amino groups of DNA bases (2). So, the changes within the avirulent category could be due to a slight change in a few nucleotides of the DNA causing highly avirulent (IT 0 or 0;) cultures to be moderately avirulent (IT 1 or 2) after NTG treatment or avirulent cultures to be highly avirulent after NTG treatment. Changes in avirulent ITs could also be changes in modifiers or environment, but all conditions were about the same in this study.

Gabriel (6) reasoned that if genes function for specific avirulence, mutations to increased virulence against specific genes should be more frequent than mutations to decreased virulence. Mutations to virulence were no more frequent than mutations to avirulence, but three mutations were mutations to virulence at certain loci and mutations to avirulence at other loci.

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