Mutations Affecting Virulence in *Puccinia recondita*

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ABSTRACT

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Wheat (*Triticum aestivum*) leaves inoculated with *Puccinia recondita* were treated with a chemical mutagen *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (NTG) in an attempt to alter virulence. Leaves were incubated for 10–12 days in NTG. Subsequently, uredospores were collected and inoculated onto near-isogenic "tester" wheats on which culture X65 of *P. recondita* was avirulent. Isolations were made from pustules showing a virulent infection type, increased, and evaluated on near-isogenic lines. Nine cultures with mutations to virulence were developed by NTG treatment. Genes in *P. recondita* for virulence and avirulence on wheat have been designated *p* and *P*, respectively, and assigned a number corresponding to that of the *Lr* gene for resistance in the host. Mutations to virulence were induced at the *p*1, *p*3, *p*16, *p*17, *p*27, and *p*29 loci. Seven cultures with mutations to avirulence were recovered by evaluating mutations to virulence on a full set of near-isogenic lines. Mutations to avirulence were recovered at the *P*2a, *P*2c, *P*23, and *P*28 loci. Seven mutations to orange color were detected by treating the red-orange parent with NTG.

Mutation studies with pathogens are important in plant breeding when avirulence genes mutate at different frequencies. The resistance gene corresponding to the avirulence gene with the lowest frequency of mutation is likely to last the longest (1). Mutation studies can provide knowledge of mutation rates and virulence changes in the natural population (5).

Mutations have been suggested to be one of the mechanisms operative in the asexual development of new pathogenic combinations in rust fungi (6,13). Several reports of mutations to virulence have been made (4,5,8). Mutations, or some mechanism other than the sexual cycle, probably account for much of the variability exhibited by *Puccinia recondita* Rob. ex. Desm f. sp. *tritici* since infection of the alternate host is not frequently observed in nature (9). Therefore, mutation studies could provide evidence about virulence changes and gene stability.

Genes conditioning avirulence in *P. recondita* have usually been dominant (10,12). A uredospore heterozygous for avirulence carries a dominant gene for avirulence in one nucleus and a recessive gene in the other. There is a greater probability of detecting a mutation to virulence in a uredospore heterozygous for avirulence than in one homozygous for avirulence because a mutation can be detected after a single change that alters the dominant allele (5). The same logic can be used to suggest that a uredospore homozygous for virulence should be avirulent on a line with the corresponding gene after a single change that altered its recessive gene. However, the avirulent mutant either does not sporulate or has reduced sporulation on its host and is usually lost (1). It may be possible to circumvent the problem by screening induced mutants on many isogenic lines.

The current study was undertaken to determine if the chemical mutagen NTG applied to inoculated wheat (*Triticum aestivum* L.) leaf tissue could alter virulence in *P. recondita* and if mutants to avirulence could be isolated.

MATERIALS AND METHODS

Culture X65 of *P. recondita* previously used in a study of inheritance of virulence and found to be heterozygous for virulence at several loci, was used in this study (12). The purity of the parent culture, X65, was evaluated on 24 near-isogenic lines. Plants were inoculated by spraying seedlings with a suspension of uredospores in Soltrol 170 oil (Phillips Petroleum Company, Bartlesville, OK) (3 mg/ml Soltrol 170). Inoculated plants were held at 18.5±2 C and approximately 100% relative humidity for 24 hr. Plants were then returned to the greenhouse at 20 ±4 C for the duration of the incubation period.

Leaves of leaf rust-susceptible Little Club wheat were inoculated with culture X65 of *P. recondita*. After 16 hr of incubation at 100% RH, the first leaves of 10 plants were placed in test tubes containing 1 ml of benzimidazole solution (100 μg/ml) and 1 ml of a solution of *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (NTG) (125 μg/ml). The chemical mutagen NTG was used in an attempt to alter virulence of *P. recondita* as reported for *Erysiphe graminis* by Gabriel et al (8). Leaves were incubated in NTG at 21 ±4 C with a 12-hr light period for 10–12 days. Subsequently, uredospores were collected and inoculated onto near-isogenic "tester" wheat lines on which X65 was either heterozygous or homozygous avirulent. A total of 105 cultures were developed from the NTG treatment and used to inoculate the set of tester wheats.

A total of 162 isolations were made from compatible infection sites on testers to which X65 was avirulent. These isolates were increased and inoculated onto the near-isogenic line from which they were isolated. Any culture that proved to be virulent to the near-isogenic line from which it was isolated was inoculated onto a full set of near-isogenic lines. The near-isogenic lines tested contained genes *Lr*1, *Lr*2a, *Lr*2c, *Lr*3, *Lr*3ka, *Lr*9, *Lr*10, *Lr*11, *Lr*15, *Lr*16, *Lr*17, *Lr*18, *Lr*19, *Lr*20, *Lr*21, *Lr*23, *Lr*24, *Lr*25, *Lr*27, *Lr*28, *Lr*29, *Lr*30, and *Lr*31. The infection type (IT) expressed on each near-isogenic line was classified with a scale ranging 0–4 at 10–12 days after inoculation. Infection types 0, 0;, 1, or 2 were considered to indicate an avirulent IT and types 3 and 4 a virulent IT (11). Normal precautions were followed to reduce contamination. The mutation study was conducted in a greenhouse in which no other cultures of *P. recondita* were sporulating. Plants for the study were grown in a disease-free greenhouse and other inoculations were not conducted on the same day.

Mutation rates were calculated by dividing the total number of pustules that would have been produced on susceptible wheat by the number of mutants detected on each near-isogenic line. Uredial color was visually compared to a Grumbacher color wheel (catalogue No. B420; Grumbacher Inc., New York, NY) and recorded as red red-orange, red-orange, orange-red, or orange.

RESULTS

The results of studies of the inheritance of virulence in *P. recondita* have provided evidence for gene-for-gene relationships in

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P. recondita with avirulence normally dominant (10,12). Therefore, the gene for virulence will be designated by "p" and the gene for avirulence by "P" in this study. The number following the gene designation corresponds to that of the *Lr* gene for resistance in wheat whose effect the *p* allele overcomes.

Nine mutant cultures virulent at different loci than the parent culture were developed by NTG treatment. One was virulent at *p*29 (mutant #5); two at *p*3 (#3 and #4); one at *p*1 and *p*3 1(#1); one at *p*27 and *p*29 (#2); one at *p*1, *p*17, and *p*29 (#9); two at *p*1, *p*3, *p*16, and *p*29 (#6 and #7); and one at *p*1, *p*3, *p*16, *p*27, and *p*29 (#8) (Table 1). The number of mutants at each locus were five at *p*1, six at *p*3, three at *p*16, one at *p*17, and six at *p*29.

Seven cultures with mutations to avirulence were recovered from the cultures treated with NTG. Five cultures were avirulent at *P*2a and *P*2c (#3, 4, 6, 7, and 8) (Table 1). All five of these mutations to avirulence were also mutations to virulence at *p*3. One mutation to avirulence was detected at *P*28 (#3) and two at *P*23 (#5 and 9). However, the interaction of X65 at *p*23 was intermediate (IT 32) and #5 differs only slightly from X65.

Some changes within avirulent categories were detected after NTG treatment. The parent culture X65 was moderately avirulent (IT 12 or 2) at the *P*17, *P*24, and *P*30 loci. Five isolates from the NTG treatment were highly avirulent (IT0 or 0;) at *P*17, ten isolates were less virulent at *P*24 than was X65, and seven isolates were less virulent at *P*30. Culture X65 was highly avirulent at *P*3, *P*9, and *P*25. Three mutant cultures were moderately avirulent at *P*3, one at *P*9, and four at *P*25.

Because mutation rates are affected by several usually uncontrolled variables, calculations of them are only estimates. The rates were one in 110,000 for *p*1, one in 91,000 for *p*3 and p29, one in 275,000 for *p*27, one in 183,000 for *p*16, and one in 550,000 for *p*17. Mutations were not detected for *p*3ka, *p*9, *p*11, *p*18, *p*19, *p*21, *p*24, *p*25, *p*16, *p*30, or *p*31.

The spore color in cultures of *P. recondita* X65 was red-orange. Seventy-seven cultures treated with NTG were observed for color. Thirty-eight were red-orange, 10 were red red-orange (darker red than the parent), 22 were orange-red, and seven were orange.

DISCUSSION

The chemical mutagen, NTG, effectively induced mutations in *P. recondita* X65. Several mutations to virulence were recovered. Mutations to avirulence were also detected by evaluating cultures treated with NTG on 24 near-isogenic wheat lines.

Flor (7) argued that a mutation to virulence in a homozygous dominant uredospore could be detected only if the corresponding gene in each dikaryotic nucleus mutated, and he reasoned that a culture heterozygous at several loci would be best for mutation studies. The culture of *P. recondita* used in this study was heterozygous at several loci; therefore, a mutation to virulence could be detected after a single change that altered the dominant allele. Mutations to virulence were isolated at the *p*1, *p*3, *p*16, *p*17, *p*27, and *p*29 loci in this study. Culture X65 was heterozygous for avirulence at *P*1, *P*3, and *P*17 but homozygous avirulent at *P*16 (12). Segregation of X65 was not tested at *P*27 or *P*29. A major change or several minor changes must have occurred in the DNA to cause virulence at *P*16 since *P*16 was homozygous avirulent and mutant cultures virulent at *p*16 were also virulent at *p*1 and *p*3.

A few mutants were virulent at only one additional locus than the parent culture in this study but some were virulent at several additional loci. Most chemical mutagens act by direct chemical modification of DNA (2,3). It is reasonable to assume that mutants that were virulent at only one additional locus were the result of DNA alteration that affected only one or a few nucleotides, eg, a point mutation (1,2). Several cultures had mutated at several virulence loci. These loci are not linked (10,12), so these multiple-mutant cultures may have been the result of several point mutations rather than a gross alteration of a large segment of DNA.

Gabriel et al (8) reasoned that if genes function for specific avirulence, then mutations to increased virulence against specific genes should be more frequent than mutations to decreased virulence. This was apparently true in this study based on the number of single-gene mutations. Nine cultures with mutations to virulence were recovered resulting in a total of 23 single-gene mutations at six loci. Seven cultures with mutations to avirulence were detected and affected four genes for a total of 13 single-gene mutations. However, the mutations to avirulence were the same cultures as those to virulence so any comparison may not be valid.

There is very little indication that any mutagen acts preferentially on one gene rather than another (2). However, this study suggests that mutation rates are different for different genes and that some are more susceptible to mutation by NTG. If mutations were random then more than a few of the genes should have been affected. Most mutations to virulence were detected for *p*3 and *p*29 followed by *p*1 and *p*16, but only a few at *p*27 and *p*17. One might conclude that the host genes *Lr*3, *Lr*29, *Lr*1 and *Lr*16 corresponding to the highly mutable pathogen genes *p*3, *p*29, *p*1, and *p*16 would not be as useful in a breeding program as those for which mutations were not reported. However, these mutations were induced by a specific chemical agent and may differ from natural mutations or those induced by X-rays or other mutagens.

Seven cultures with mutations to avirulence were detected by evaluating the isolates from the NTG treatment on a complete set of near-isogenic lines. Five mutations to avirulence were detected at *P*2a and *P*2c in this study. All five were also mutations to virulence at *p*3. Samborski and Dyck(10) reported that interactions at the *Lr*2 and *Lr*3 loci, each with several alleles, are unusual in that the action of a recessive gene for virulence is modified or inhibited by a second gene. This may explain the apparent disassociation of virulence of *p*2 and *p*3 in the mutant cultures in this study. All mutant cultures virulent at *p*3 were avirulent at *P*2a and *P*2c except for mutant #1.

Several small, but detectable, changes were found in the avirulent category of cultures treated with NTG. Some cultures were highly avirulent (IT 0;) before treatment but only moderately avirulent (IT 2) after treatment. Others were moderately avirulent

TABLE 1. Infection types[a] of mutant cultures of *Puccinia recondita*

Mutation to	Near-isogenic line	Parent X65	Mutant culture number 1	2	3	4	5	6	7	8	9
Virulence	*Lr*1	0	4	;	;	;	;	4	4	4	4
	*Lr*3	;1	3	1	4	4	1	4	4	4	;1
	*Lr*16	2	12	2	2	2^+	12	3^-	3^-	3^-	12
	*Lr*17	12	01	01	2	1	01	01	12	12	3
	*Lr*27	;2	2	4	01	1^+	;2	1	2^+	3	1^+
	*Lr*29	1	12	4	;	12	3	3	3	3^-	4
Avirulence	*Lr*2a	4	4	4	;	;2	4	;	;2	;2	4
	*Lr*2c	4	4	4	;	;2	4	;1	;2	;2	4
	*Lr*23	32	3^-	3^-	3	3^-	2	4	3	4	12
	*Lr*28	4	4	4	;	31	3	3	3	4	4

[a] Infection types 0, ; (0;), 1, and 2 indicate avirulence (*P*), and types 3 and 4 indicate virulence (*p*). A superscripted + or − indicates variance within categories 0–4 that indicate, respectively, slightly more or less compatibility than the designated category.

before treatment and highly avirulent after treatment. These small changes could have been due to slight changes in DNA by the chemical mutagen. This could be an example of intragenic recombination if these changes were at different sites within a gene (1,2). Variation in the intensity of the resistant interaction was previously reported by Samborski and Dyck (10) in genetic studies. They reasoned that a resistant interaction resulted from the interaction of a gene for resistance with a gene for avirulence but that the action of both genes could be influenced by modifiers in their own genetic background or by the environment. The influence of modifiers could explain the changes in the avirulent category in this study but the genetic background of the host should not be a factor since it was constant.

Culture X65 was previously shown to be heterozygous for color (12). The color of the mutated cultures ranged from red red-orange to orange. The red red-orange and orange-red mutants may have been only a variation of red-orange, but the seven orange mutants were definitely different from the parent and therefore were color mutants. None of the orange mutants were mutants to virulence or avirulence, so color was not associated with virulence.

Only a few of 162 isolates from compatible infection sites on near-isogenic lines resistant to X65 were virulent on the same near-isogenic line from which they were isolated. If these isolates were contaminants rather than mutants, then they should have produced compatible infection sites on the line from which they were isolated, but all of them were avirulent except for the mutant cultures. I have no interpretation for this but it does indicate a lack of contamination or admixture. One explanation may be that some NTG mutations are inconstant due to the alkylating effect of NTG causing a base to ionize differently resulting in pairing errors which may not be stable (3).

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