Genes for Pathogenicity in Cochliobolus carbonum

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ABSTRACT

The pathogenicity of 485 ascospore isolates from six different crosses of *Cochliobolus carbonum* (*Helminthosporium carbonum*) isolates to seven differential gramineous species was determined. A min of nine previously unreported genes for pathogenicity to seven grass species was identified. Segregation ratios and comparisons of reactions of paired differential hosts demonstrated that pathogenicity to five of the seven gramineous hosts is controlled by five different genes. Pathogenicity to two species is conditioned by two different sets of two genes each. Analyses of frequencies of parental and recombinant progeny indicated that seven of the nine identified genes for pathogenicity are independently inherited. Linkage was detected between the remaining two genes. Linkage was detected also between the gene for pathogenicity to *Cynodon dactylon* and the mating type locus in one cross. Additional segregation data identified linkage relationships between genes for pathogenicity reported herein and those reported previously. Phytopathology 60:1335-1337.

Previous studies (2, 3) presented evidence of the genetic basis for pathogenicity of *Helminthosporium* isolates to gramineous species. These isolates comprised one morphological group representative of *Cochliobolus carbonum* Nelson (*Helminthosporium carbonum* Ullstrup). Twenty-six genes for pathogenicity to 20 different species of the Gramineae were identified. The present paper presents evidence of additional genes for pathogenicity in *C. carbonum* to seven grass species. Linkage relationships between newly identified genes for pathogenicity and between newly and previously reported genes for pathogenicity are identified.

MATERIALS AND METHODS.—A total of 485 ascospore isolates obtained from six different crosses was used in the present studies. The source host and geographic origin of the parental isolates used in the crosses are identified in Table 1. Ascospore progeny from each cross were evaluated for pathogenicity in greenhouse infection studies on from one to six of seven differential hosts. Differential hosts are those species to which one parental isolate is pathogenic and the other parental isolate is nonpathogenic.

Additional segregations for pathogenicity and nonpathogenicity were evaluated on 14 differential hosts to which genes for pathogenicity had been reported previously (2, 3). The purpose of using the additional differentials was to obtain other pairs of hosts to determine possible linkage relationships between genes for pathogenicity identified in the present and previous studies. Two of the 14 previously used differential hosts will be discussed in context to linkage relationships later in this paper.

Techniques used to mate compatible parental isolates and procedures used to isolate ascospore progeny have been previously described in detail (1). The mating type *A* or *a* of each ascospore isolate was determined by pairing with both parental isolates or with the standard *A* and *a* tester isolates having vigorous mating capacities.

Test plants were grown in a 2:1 loam:sand mixture in 3- or 4-inch pots in greenhouses maintained at approximately 24 and 29 C, depending on the temp determined to be most suitable for each host species. There were considerable differences among host species in numbers of plants per pot and height and age of plants when inoculated, but numbers per pot, height, and age of plants of any given species were comparable in each infection trial.

Inoculum consisted of a water suspension of mycelia and spores prepared by scraping the surfaces of 7- to 14-day-old cultures grown on potato-dextrose agar (1.0% dextrose) in petri dishes at 24 C. Inoculum was sprayed onto the leaves with a small hand sprayer at a constant pressure of 10 psi. Inoculated plants were incubated at approximately 24 C for 48 hr in a moisture chamber in which a mist system operated for 30 sec every 3 hr. Plants were then returned to a controlled-temp greenhouse maintained at 24 C.

Plants were evaluated for disease reactions 7 days after inoculation. The criterion for pathogenicity of isolates was the presence of leaf lesions. Isolates were rated as nonpathogenic if they failed to incite a visible host response. The reactions of all plant species to each ascospore isolate were confirmed at least twice. No attempt was made to evaluate the observed differences among isolates in the number or size of lesions produced. The sole objective was to determine whether a specific isolate could induce some kind of lesion on a given test host under the conditions imposed upon the study.

RESULTS.—*Additional genes for pathogenicity*.—Data on segregation for pathogenicity and nonpathogenicity on seven differential hosts are summarized in Table 1 for 485 ascospore progeny from six crosses. Based upon approximately 1:1 segregations, the results indicate that pathogenicity to five of the seven differential hosts is conditioned by a single gene or by a gene system controlled by a single major gene. Pathogenicity to the remaining two host species is governed by two genes, as indicated by the 3:1 segregation for pathogenicity:nonpathogenicity on *Pennisetum glaucum* (L.) R.Br. and a 1:3 segregation on *Triticum aestivum* L.

Pathogenicity to each of the seven differential hosts is conditioned by different genes or gene systems. This

TABLE 1. Pathogenicity of parental isolates of *Cochliobolus carbonum* and 485 ascospore isolates derived from crosses on a group of gramineous host species

		Host species and isolate characterization						
Parental Isolates		*Avena sativa*	*Cynodon dactylon*	*Eleusine indica*	*Oryza sativa*	*Pennisetum glaucum*	*Triticum aestivum*	*Zea mays*
No.	Source							
1	(ex. *Agropyron*, Scotland)	+[a]	+	+	+	+	—	+
2	(ex. *Zea*, Brazil)	—	+	+	+	+	—	+
3	(ex. *Avena*, La.)	+	—	—	—	—	—	—
4	(ex. *Hordeum*, Minn.)	—	+	+	+	+	+	+
5	(ex. *Zea*, Mo.)	—	—	+	—	—	+	+
6	(ex. *Zea*, N.C.)	—	+	—	+	+	+	+
7	(ex. *Ixophorus*, Mex.)	+	—	+	—	—	+	—
8	(ex. *Digitaria*, Mex.)	+	+	—	+	+	—	+
9	(ex. *Cynodon*, Mex.)	—	+	—	+	+	+	+
10	(ex. *Zea*, Texas)	—	—	—	+	+	+	+
Crosses								
No.	Isolates							
1	1 × 2	27:28[b]						
2	3 × 4	[c]	44:46	42:48	48:42	69:21	22:68	46:44
3	5 × 6		44:40		45:39	62:22		
4	4 × 7	40:35	38:37					39:36
5	4 × 8	42:39						
6	9 × 10		52:48					
Min no. genes controlling pathogenicity		1	1	1	1	2	2	1

[a] + = Pathogenic; — = nonpathogenic.
[b] Ratios are given as pathogenic:nonpathogenic.
[c] Where no segregation data are given, the grass species either were not differential hosts for the crosses studied or were differential hosts that were not used.

conclusion was reached in part by comparing the reactions of all possible 15 combinations of two differential hosts to ascospore progeny from cross 2. All ascospore progeny were not similarly pathogenic or nonpathogenic to two differential hosts. In all paired combinations of hosts, several ascospore isolates were pathogenic to one host and nonpathogenic to the other. Such data from cross 2 indicate that pathogenicity to six hosts is conditioned by different genes. Since the parental isolate in cross 2 from *Hordeum vulgare* L. was pathogenic to all six differentials and was nonpathogenic to *Avena sativa* L., the gene for pathogenicity to *A. sativa* identified in crosses 1, 4, and 5 must be different from the other six genes.

The data indicate that a min of nine different genes for pathogenicity have been identified. The data cannot be used to conclude that the max is nine. Genes for pathogenicity in the different parental isolates to common hosts may be different; i.e., at different loci.

Linkage relationships.—Possible linkage relationships of genes for pathogenicity reported for the first time herein were evaluated by a total of 21 chi-square (X^2) analyses of the reactions of all possible pairs of differential hosts to ascospore progeny from a specific cross. For example, in comparing two differential hosts to which one parental isolate is pathogenic and the other is nonpathogenic, parental-type ascospore progeny would be pathogenic or nonpathogenic to both hosts, and recombinant progeny would be pathogenic to one of the two hosts. An additional 15 X^2 analyses were made between segregations for pathogenicity and mating type (*A-a*). For $P = .01$, a X^2 of 11.345 was needed. In 20 of 21 X^2 analyses for pathogenicity to paired differential hosts, the frequencies of parental and recombinant progeny were approximately 1:1, a gametic ratio indicating that the two genes for pathogenicity were inherited independently.

In the remaining analyses of paired hosts, the distribution of parental and recombinant types could not be explained by independent assortment. In cross 2, 76 of 90 progeny were parental types in their pathogenicity or nonpathogenicity to *Eleusine indica* (L.) Gaertn. and *Oryza sativa* L. ($X^2 = 43.4$). The results suggest that the distributions of parental and recombinant types are consistent with the assumption of linkage. The number of ascospore progeny used in this cross and other crosses is considered by the author to be insufficient to estimate cross-over units.

The segregation in one case is consistent with the assumption of linkage in cross 6 between the gene for pathogenicity to *Cynodon dactylon* (L.) Pers. and the mating type locus (*A-a*). Parental types were *A*-nonpathogenic and *a*-pathogenic, and the resulting segregation of progeny was 85 parental type: 15 nonparental ($X^2 = 50.16$). This association appears to be significant in that, while *C. dactylon* served as a differential host in crosses 2, 3, 4, and 6, linkage between pathogenicity and mating type was detected only among progeny of cross 6. Although the numbers of progeny were not large in any of the crosses, the consistent 1:1 segregation for pathogenicity and a 1:1 segregation for mating type in each cross suggest that we were not dealing with biased data accumulated as a result of unusual segregations. The linkage relationship in cross 6 suggests two possibilities; (i) the gene for pathogenicity of the pathogenic isolate in cross 6 is a

different gene than that possessed by pathogenic parental isolates in crosses 2, 3, and 4; or (ii) the mating type gene is not at the same locus in the parental isolates involved in the four crosses.

Additional linkage analyses were made possible as a result of including *Axonupus affinis* Chase as a differential host in crosses 2 and 3 and *Festuca elatior* L. as a differential host in cross 4. A single but different gene for pathogenicity to each of the two hosts was identified previously (2). Although other previously studied differential hosts also were used to evaluate segregations within the present six crosses, only the use of *A. affinis* and *F. elatior* resulted in the detection of previously unknown linkages. Segregation ratios on the previously studied differential hosts are not included, since their only value would be to conclude the number of genes conditioning pathogenicity. These conclusions were made previously, and similar conclusions herein would not constitute original findings. In crosses 2 and 3, linkage relationships were detected between genes for pathogenicity to *A. affinis* and *Oryza sativa*. Progeny from cross 2 segregated 72 parental:18 nonparental ($X^2 = 34.18$), and progeny from cross 3 segregated 66 parental:18 nonparental ($X^2 = 28.66$). Since X^2 analyses indicated linkage between genes for pathogenicity to *E. indica* and *O. sativa* in cross 2, it is concluded that genes for pathogenicity to *O. sativa*, *A. affinis*, and *E. indica* are on the same chromosome, and that genes for the latter two hosts are sufficiently separated to show independent assortment.

In cross 4, linkage was detected between genes for pathogenicity to *C. dactylon* and *F. elatior* (56 parental: 19 nonparental [$X^2 = 19.44$]). This is of interest, since a previous report showed that genes for pathogenicity to *Poa pratensis*, *Lolium multiflorum*, and *F. elatior* were linked when hybrid progeny from a cross of an isolate of *C. carbonum* × a *C. victoriae* isolate were studied on these hosts (2). In the present study, the parental isolate in cross 4 which is pathogenic to *F. elatior* was isolated from *H. vulgare* in Minnesota. In the previous study, the parental isolate pathogenic to *F. elatior* was isolated from *Zea mays* from Texas. If the gene for pathogenicity to *F. elatior* is the same in the two isolates, it could be concluded that genes for pathogenicity to *C. dactylon*, *F. elatior*, *L. multiflorum*, and *P. pratensis* are all on the same chromosome.

Data on certain crosses (Table 1) were concerned in part with segregations on *A. sativa* and *Z. mays*. Parental isolates in crosses 1, 4, and 5, pathogenic to *A. sativa*, incite a well-defined leaf spot on that host but do not induce a wilting or blighting syndrome associated with isolates of *Cochliobolus victoriae* Nelson (*Helminthosporium victoriae* Meehan & Murphy) producing a toxin specific to Victoria derivative oats. Since *C. victoriae* is included in the morphological group represented by *C. carbonum* on the basis of similar conidial morphology and cross-compatibility (3), two different genes for pathogenicity to *A. sativa* are identified among isolates of *C. carbonum*. Similarly, the common parent in crosses 2 and 4, pathogenic to *Z. mays*, has a gene for pathogenicity different than the gene associated with race 1 isolates of *C. carbonum* that produce a toxin and are pathogenic to certain inbred lines of corn. The isolate used in the present study is pathogenic to corn hybrids to which race 1 isolates of *C. carbonum* are nonpathogenic, and does not produce the toxin associated with race 1 isolates of *C. carbonum*. Thus, two different genes for pathogenicity to *Z. mays* have been identified.

DISCUSSION.—Genes for pathogenicity in *C. carbonum* to 25 gramineous species are now identified from the present and previous studies. These series of studies have utilized only 19 isolates from nearly 200 available within the morphological complex classified as *C. carbonum*. As additional isolates are studied and as additional grass species are evaluated, it is likely that many additional genes or gene systems will be identified.

The genes identified to date clearly demonstrate the complexity of the genetic systems for pathogenicity in *C. carbonum*. And yet, these studies have merely defined qualitative abilities and have not examined the systems conditioning differences in virulence among isolates, which have been clearly evident in all of the studies. Nor have these reports clarified the genetic control of pathogenicity of ascospore progeny to grass species resistant to both parental isolates from which these progeny were obtained (2).

The complexity of systems for pathogenicity and virulence within *C. carbonum* and the fact that many of the genes are inherited independently and are unique to isolates from diverse host sources and geographic areas suggest that the evolution of these genetic systems has been influenced by many factors.

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