Variation in Pathogenicity and Cultural Characteristics of Sorghum Isolates of *Colletotrichum graminicola* in India

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ABSTRACT

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The pathogenicity of nine sorghum isolates of *Colletotrichum graminicola* from different locations in India was tested on 30 sorghum genotypes in the greenhouse. Based on reaction class and disease severity, six genotypes were identified as susceptible and seven as resistant to all nine isolates of the pathogen. The remaining 17 genotypes exhibited differential responses to the nine isolates. The isolates also varied in their cultural characteristics. These results indicated that the nine isolates were distinct physiologic races.

Additional keywords: leaf anthracnose, *Sorghum bicolor*

Anthracnose of sorghum (*Sorghum bicolor* (L.) Moench) incited by the fungus *Colletotrichum graminicola* (Ces.) G. W. Wils. (= *C. sublineola* Henn. in Kab. & Bubák) was first reported from Togo, West Africa, in 1902 (20). The disease has since been reported in most sorghum-growing regions of the world (6), however, it is more prevalent and economically important where sorghum grows under warm and humid environmental conditions (4,17,21). *C. graminicola* infects all aboveground parts of the sorghum plant—stem, leaf, peduncle, inflorescence, and grain (11,21).

Leaf anthracnose, the most common form of the disease, is characterized by circular to elliptical spots with few or numerous fruiting bodies (acervuli) of the fungus on the leaf lesions (21). Differences in leaf symptoms are common and may be caused by variations in the pathogen, host reaction, or the physiological status of the host (8,17). The leaf phase of anthracnose can reduce yield of sorghum grain and fodder by as much as 50% or more in susceptible cultivars under severe epidemics (11,12,18).

Harris and Johnson (10) suggested the existence of physiological races of *C. graminicola* in the United States. Results of the 1976 International Sorghum Anthracnose Virulence Nursery (ISAVN) suggested the presence of different races of *C. graminicola* in the United States and Nigeria (13). Similarly, all 13 entries of the 1981 ISAVN were susceptible to anthracnose at Pantnagar, North India, suggesting that the Indian population of the pathogen was different from that in the United States (3). Seven physiological races of *C. graminicola* were identified in Brazil based on the reaction of 12 sorghum cultivars to seven isolates of the pathogen (7). Ali and Warren (2) identified three races among nine isolates of *C. graminicola* from the United States and Puerto Rico based on their pathogenicity on six sorghum genotypes. Recently, Cardwell et al (5) characterized eight pathotypes out of 12 isolates of *C. graminicola* on eight sorghum cultivars. Two new forma specialis, *C. g.* var. *zonatum* Rajasab & Ramalingam and *C. g.* var. *isolatum*, have been proposed from India (19) and Nigeria (1), based on the morphological characteristics and pathogenicity of the isolates. This paper reports the results of a detailed study of the variation in pathogenicity and cultural characteristics of nine sorghum isolates of *C. graminicola* from different locations in India.

MATERIALS AND METHODS

Pathogen isolates and production of inoculum. Diseased leaf samples were collected from research stations or farmers' fields in eight sorghum-growing regions of India. Symptoms as observed at the time of collection were recorded, and geographic location was used to designate the isolates (Table 1).

Specimens of diseased leaves were cut into small pieces, surface-sterilized in mercuric chloride solution (1:1,000) for 20–30 s, and washed three times in sterile distilled water before plating on Difco potato-dextrose agar (PDA). Plates were incubated under 24 hr of constant cool-white fluorescent light (40 W) at 26 ± 1 C to induce sporulation. Conidia from 8-day-old cultures on PDA plates were harvested by flooding each plate with 5–10 ml of sterile distilled water, serially diluted to 1:1,000, and streaked on 2% water agar in petri dishes to test germination. Fifteen single conidia per isolate were transferred to individual PDA plates and incubated for 7 days. The fastest growing culture from each of these 15 monoconidial cultures for each isolate

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was selected and used in these studies. All cultures were stored in a refrigerator at 4 C. Under these conditions, isolates were stable and retained their pathogenicity.

Conidial inoculum of the isolates for pathogenicity experiments was produced on sorghum leaf medium (SLM) on which conidial production was more abundant than on PDA. SLM was made as follows: 15 g of green leaves of the sorghum cultivar IS18442, at the eight- to 10-leaf growth stage, were washed in water and cut into 2 × 3 cm pieces. The pieces were then moistened, used to fill 250-ml Erlenmeyer flasks, and autoclaved at 121 C for 25 min. Each cooled flask was seeded with two mycelial plugs (1 cm^2) cut from the margins of single conidial colonies. Ten milliliters of 2% sucrose solution prepared in sterile distilled water was added to each flask at the time of inoculation as an additional carbon source. Inoculated flasks were incubated, as described earlier, for 10 days for conidial production. After incubation, cultures were flooded with 150 ml of sterile distilled water, stirred for 2–3 min on a magnetic stirrer, and the conidial suspension was passed through four layers of cheesecloth to remove mycelial and leaf fragments. The filtered suspension was adjusted to 4×10^5 conidia ml^{-1}. Two drops of Tween 20 was added to 100 ml of conidial suspension as a wetting agent before inoculation.

Pathogenicity tests. Thirty of 244 sorghum genotypes (Table 2) were selected for pathogenicity tests because of their differential reactions in preliminary inoculations in the greenhouse. They were grown in the greenhouse from disease-free, surface-sterilized seeds in 30.5-cm-diameter plastic pots filled with an autoclaved mixture of Vertisol and sand (1:1). The soil mix was autoclaved at 121 C for 2 hr, and three plants were grown in each pot. Two pots of 30-day-old plants at eight- to 10-leaf growth stage of each genotype were transferred to each of 10 compartments measuring 3 × 3 × 3 m inside a greenhouse. The side walls of the compartments were made of cheesecloth, and the roofs were covered with polythene sheets. One compartment was used for each isolate of *C. graminicola*; the 10th compartment was used for the uninoculated control plants. Experiments were arranged in a randomized complete block design.

Plants were inoculated on both leaf surfaces with the conidial suspension of an isolate with a hand sprayer attached to an air pump (103.5 KPa) until runoff. Control plants were sprayed with sterile distilled water with Tween 20. One hour after inoculation, humidifiers in each compartment were run continuously for 18 hr, and during this period 100% relative humidity (RH) was recorded. During the next 6 days, humidifiers were run for 8 hr a day to promote disease development. Mean maximum and minimum temperatures in each compartment were 28 ± 2 C and 22 ± 2 C, and mean natural light intensity during the day (12-hr photoperiod) ranged between 18 and 54 $\mu E \cdot m^{-2} \cdot s^{-1}$. In general, a hot and humid environment with low light intensity was maintained throughout the period of the experiment. All treatments were replicated twice and one pot of each genotype was a replicate. The experiment was repeated five times to verify results.

Disease rating. The top two inoculated leaves of each plant were evaluated for both symptom type and disease severity 8 days after inoculation. Disease severity scores were averaged for each pot (replication). Symptom types were scored on a 0–6 scale where 0 = no symptoms (inoculated leaves remained healthy), 1 = chlorotic flecks, 2 = reddening or red spots on the leaf lamina, 3 = necrotic lesions or patches but no acervuli formed, 4 = necrotic lesions with few pinpoint size acervuli restricted to the gray centers of the lesions, 5 = scattered lesions with distinct acervuli in the gray centers of the lesions, and 6 = necrotic lesions coalescing and abundant acervuli formed. Disease severity (percentage of leaf area covered by lesions) was estimated on a 0–5 rating scale where 0 = no visible lesions, 1 = ≤1, 2 = 1–5, 3 = 6–20, 4 = 21–40, and 5 = ≥40% leaf area covered with lesions.

For final tabulation of the data, symptom types were categorized into three broad reaction classes: R = resistant or no symptom development; HR = hypersensitively resistant and included 1, 2, and 3 types of symptoms; and S = susceptible comprising 4, 5, and 6 symptom

Table 1. Location, source, and symptoms elicited by nine Indian isolates of *Colletotrichum graminicola* on leaves of sorghum genotypes from which they were collected

Isolate	Location	Source[z]	Sorghum genotype	Leaf anthracnose symptoms
AK-1-83	Akola, Maharashtra (lat. 20°42′N, long. 77°2′E)	F	Local landrace	Elliptical to elongate scattered spots with yellow-red halos and acervuli in the ash-colored centers of the spots
DH-1-83	Dharwad, Karnataka (lat. 15°2′N, long. 95°E)	R	IS643	Circular to elliptical spots in patches and acervuli present in the centers of necrotic spots
HB-1-83	Hubli, Karnataka (lat. 15°22′N, long. 75°15′E)	F	Local landrace	Circular to elliptical spots in patches, a few spots with yellow halos, and acervuli abundant on necrotic spots
PAT-1-85	Patancheru, Andhra Pradesh (lat. 18°N, long. 78°2′E)	R	IS18442	Elliptical to elongated spots, leaf uniformly colonized, characteristic yellow halo around spots, and profuse acervuli production in the ash-colored centers of the spots
PAT-2-85	Patancheru, Andhra Pradesh (lat. 18°N, long. 78°2′E)	R	IS17803	Circular to elliptical scattered spots, yellow halo absent, and acervuli abundant on necrotic areas
ID-1-83	Indore, Madhya Pradesh (lat. 22°42′N, long. 75°54′E)	R	No. 654	Circular to elliptical scattered spots, yellow halo with distinct red rings, and acervuli in the centers of the spots
PAN-1-85.	Pantnagar, Uttar Pradesh (lat. 15°2′N, long. 75°E)	R	IS18442	Elliptical to elongated scattered spots, distinct yellow halos around leaf spots, and acervuli present in the centers of the spots
SR-1-85	Surat, Gujarat (lat. 21°12′N, long. 72°55′E)	R	Gundri	Elliptical to elongated diffuse spots large in size with yellow halos and acervuli
UD-1-83	Udaipur, Rajasthan (lat. 24°36′N, long. 73°44′E)	R	Local landrace	Circular to elliptical scattered spots, no typical yellow halo, and acervuli abundant in necrotic patches

[z] F = Farmer's field, R = research station.

types. Data were analyzed with the GENSTAT statistical program for randomized complete block design. Because there were no significant interactions for environment (run) ($P = 0.05$) either in symptom type or in disease severity across the five repetitions, data of each parameter were averaged. Analysis of variance was performed separately for symptom type and disease severity (Table 3).

Assessment of virulence and aggressiveness. Differences in virulence among isolates was examined by comparing the number of genotypes on which isolates produced susceptible symptoms. These genotypes were from a set of 30 sorghum lines used in this study.

Aggressiveness among the isolates was measured by comparing the variation in the degree of pathogenicity (mean disease severity) on six sorghum genotypes (IS914, IS1022, IS3089, IS17804, IS18442, and IS18531), which were susceptible to all nine pathogen isolates. The Waller-Duncan Bayesian *k*-ratio LSD rule ($P = 0.05$) was used to compare every possible pair of treatment means (14) and explain the relative aggressiveness among the isolates.

Morphology and cultural characteristics of isolates. Nine monoconidial isolates were selected and examined for morphological and cultural characteristics. The dimensions of 100 conidia of each isolate grown on SLM were measured. To measure growth of the colonies, 3-mm-diameter disks of the isolates were cut with a sterile cork borer, plated onto PDA plates, and incubated as mentioned previously. Radial growths were measured on alternate days up to 7 days after inoculation. Three replicate plates were used for each isolate and colony diameters were averaged. The experiment was arranged in a randomized block design inside the incubator considering each shelf as a block. This experiment was repeated three times. Analysis of variance was performed separately for conidial size and radial growth of the test isolates for each repetition. Because the results were similar for all three tests, only the analysis of variance (Tables 4 and 5) and results from the first test are presented. Means were compared with Waller-Duncan Bayesian *k*-ratio LSD rule ($P = 0.05$). Morphological characteristics of the cultures were recorded 7 days after inoculation.

Table 2. Race and origin of 30 sorghum genotypes used in the pathogenicity tests with isolates of *Colletotrichum graminicola*

Sorghum genotype	Race	Origin
IS643	Durra	United States
IS854	Kafir caudatum	United States
IS914	Caudatum bicolor	Mexico
IS1006	Kafir bicolor	United States
IS1022	Durra bicolor	India
IS2058	Kafir	United States
IS2596	Bicolor	India
IS3089	Caudatum bicolor	United States
IS3589	Caudatum	United States
IS5511	Durra	India
IS6958	Durra caudatum	Sudan
IS7142	Caudatum bicolor	Uganda
IS7775	Caudatum	Nigeria
IS8024	Caudatum	Japan
IS8283	Caudatum	Uganda
IS9600	Caudatum bicolor	Niger
IS12467	Caudatum	Sudan
IS12664C	NA[z]	United States
IS17141	Durra caudatum	Nigeria
IS17804	Durra	India
IS18433	Caudatum bicolor	India
IS18442	Guinea durra	India
IS18521	Caudatum	United States
IS18531	Durra	Egypt
IS18615	Durra caudatum	Nigeria
IS18680	Guinea caudatum	United States
IS18688	Durra	United States
IS18758	Guinea caudatum	Ethiopia
IS18760	Durra kafir	United States
UChV-2	NA	India

[z] NA = data not available.

Table 3. Analysis of variance for symptom type and disease severity ratings on 30 sorghum genotypes inoculated with nine Indian isolates of *Colletotrichum graminicola* in the greenhouse

Source	df	Mean squares: Symptom type[x]	Mean squares: Disease severity[y]
Replications	9	0.026	3.6059
Isolates	8	0.7151***[z]	64.2467***
Sorghum genotypes	29	0.0294***	83.5911***
Isolates × sorghum genotypes	232	9.9060***	5.5831***
Error	2,421	0.003	0.363
Total	2,690	3.543	1.900

[x] Symptom types based on a scale of 0–6 where 0 = no symptoms (inoculated leaves remained healthy), 1 = chlorotic flecks, 2 = reddening or red spots, 3 = necrotic lesions or patches but no acervulus formed, 4 = necrotic lesions with few pinpoint-sized acervuli restricted to the gray centers of the lesions, 5 = scattered lesions with distinct acervuli in the gray centers of the lesions, and 6 = necrotic lesions coalescing and abundant acervuli formed. Symptom types were categorized into three broad reaction classes: R = resistant (0 or no symptoms); HR = hypersensitively resistant and includes 1, 2 and 3 types of symptoms; and S = susceptible comprising 4, 5, and 6 symptom types.

[y] Disease severity rating based on a 0–5 scale where 0 = no visible lesions, 1 = <1% leaf area covered by lesions, and 5 = lesions covering >40% of leaf area.

[z] *** = Significant at the $P = 0.001$ level.

RESULTS

Variation in pathogenicity among isolates. Symptoms on inoculated leaves ranged from resistant (asymptomatic) to circular or elliptical lesions up to 3–7 mm in length (Fig. 1A and B) on both upper and lower leaf surfaces. Depending on

Table 4. Analysis of variance for conidial size of nine Indian isolates of *Colletotrichum graminicola* grown on sorghum leaf medium

Source	df	Mean squares: Length (μm)	Mean squares: Width (μm)
Isolates	8	356.549[z]	0.015
Error	891	7.387	0.032

[z] Significant at the $P = 0.001$ level.

Table 5. Analysis of variance (mean squares) for radial growth of nine Indian isolates of *Colletotrichum graminicola* grown on potato-dextrose agar

Source	df	Colony diameter (mm) after incubating for: 3 days	5 days	7 days
Replications	2	9.148	9.148	18.9259
Isolates	8	47.426***[z]	191.509***	346.870***
Error	16	0.898	1.273	1.01

[z] *** = Significant at the $P = < 0.001$ level.

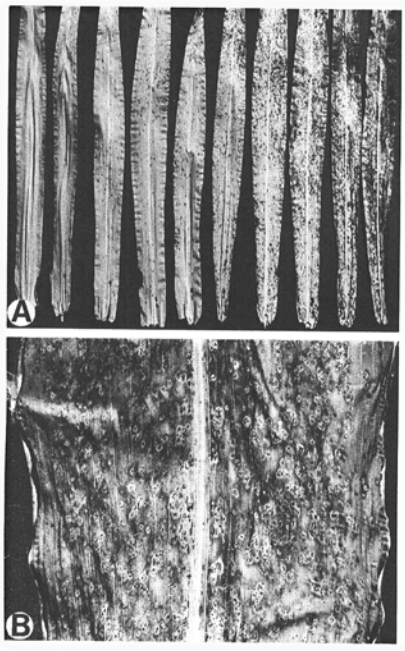

Fig. 1. Symptoms of anthracnose on sorghum leaves artificially inoculated with a conidial suspension of *Colletotrichum graminicola* in the greenhouse. **(A)** Variation in the hypersensitive resistance type of reaction (left = no symptoms, right = severe symptoms); **(B)** elliptical to elongated lesions with acervulli (black in photo) in the ash-colored centers of the lesions.

the sorghum genotype, lesion color varied from tan to cherry red to dark red. Some genotypes expressed necrosis but did not exhibit any fruiting structures (acervuli) of the fungus. Among the HR type symptoms, chlorotic flecks did not enlarge and few disappeared by 7 days after inoculation. Attempted isolations on PDA plates from HR symptoms did not give rise to any fungal colonies. Anthracnose symptoms did not develop in uninoculated plants.

Six sorghum genotypes out of the 30 tested were susceptible to all nine isolates of *C. graminicola*. However, the severity of susceptible symptoms varied among isolates. Similar variation in the severity of HR symptoms was recorded on seven

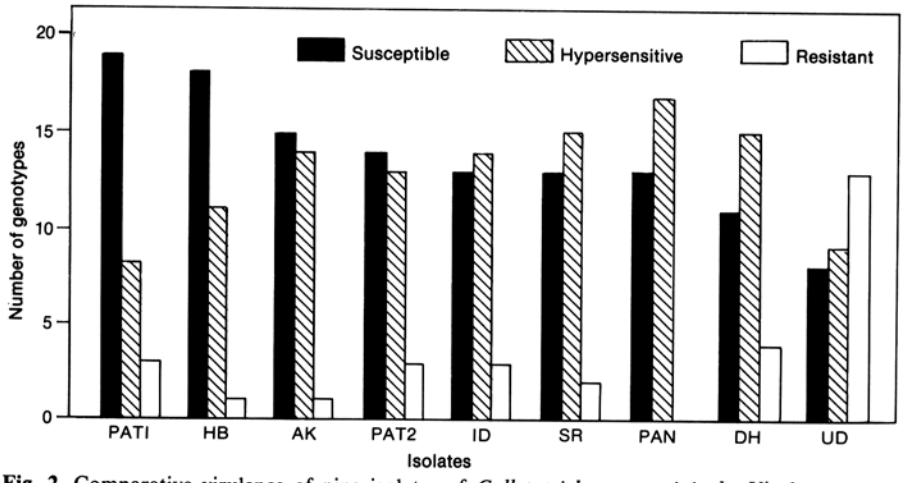

Fig. 2. Comparative virulence of nine isolates of *Colletotrichum graminicola*. Virulence was indicated by the number of susceptible sorghum genotypes to each isolate out of 30 test genotypes. Isolates: PAT1 = PAT-1-85, HB = HB-1-83, AK = AK-1-83, PAT2 = PAT-2-85, ID = ID-1-83, SR = SR-1-85, PAN = PAN-1-85, DH = DH-1-83, and UD = UD-1-83.

Table 6. Reaction class (RC)[x] and disease severity ratings (DS)[y] on 30 sorghum genotypes inoculated with nine Indian isolates of *Colletotrichum graminicola* in the greenhouse

Sorghum genotype	Isolates of *C. graminicola*																	
	AK-1-83		DH-1-83		HB-1-83		PAT-1-85		PAT-2-85		ID-1-83		PAN-1-85		SR-1-85		UD-1-85	
	RC	DS	RC	DS	RC	DS	RC	DS	RC	DS	RC	DS	RC	DS	RC	DS	RC	DS
IS914	S	3.0[z]	S	3.4	S	4.6	S	5.0	S	5.0	S	3.4	S	3.0	S	3.8	S	3.4
IS1022	S	3.0	S	3.8	S	4.4	S	4.6	S	4.2	S	3.4	S	3.8	S	4.0	S	3.4
IS3089	S	2.0	S	4.4	S	3.8	S	3.0	S	5.0	S	5.0	S	3.2	S	4.0	S	3.4
IS17804	S	3.6	S	5.0	S	2.0	S	3.2	S	4.8	S	5.0	S	2.2	S	3.4	S	2.6
IS18442	S	2.6	S	3.0	S	2.8	S	5.0	S	3.4	S	5.0	S	5.0	S	5.0	S	3.4
IS18531	S	3.6	S	2.8	S	1.4	S	4.2	S	3.2	S	5.0	S	4.0	S	4.8	S	2.2
IS643	S	2.2	S	3.4	S	2.8	S	3.2	HR	1.8	R	0.0	S	1.4	HR	2.8	HR	1.8
IS854	S	2.6	HR	1.4	HR	1.8	S	2.0	HR	1.4	HR	2.2	HR	1.4	HR	2.4	R	0.0
IS1006	HR	1.8	HR	2.2	HR	2.6	HR	1.2	S	2.4	HR	1.8	HR	1.6	HR	2.0	R	0.0
IS2596	S	2.4	HR	1.2	S	2.8	S	3.4	S	3.4	S	3.2	HR	2.6	S	2.6	S	1.0
IS3589	S	2.8	HR	1.8	S	2.0	S	2.8	S	3.4	S	3.0	HR	2.2	HR	2.4	HR	2.6
IS5511	S	3.0	S	2.4	S	4.2	S	3.6	S	4.0	S	2.6	S	3.2	S	2.4	HR	2.2
IS6958	R	0.0	R	0.0	S	2.6	R	0.0	HR	2.6	R	0.0	HR	1.2	HR	1.8	R	0.0
IS7142	HR	2.2	HR	1.8	S	2.6	S	2.4	HR	2.4	HR	2.4	S	1.8	S	1.8	HR	1.8
IS12467	HR	2.0	HR	1.4	S	3.0	S	3.0	HR	2.2	HR	1.4	HR	1.2	HR	1.6	R	0.0
IS17141	HR	1.4	HR	1.2	HR	2.0	S	3.6	HR	2.0	HR	2.2	HR	1.6	HR	2.2	R	0.0
IS18521	HR	1.4	HR	1.6	HR	1.6	S	2.2	HR	1.4	HR	1.8	HR	1.6	HR	2.8	HR	1.0
IS18615	S	3.2	HR	1.0	S	3.2	S	3.0	HR	2.0	S	3.0	S	2.2	S	3.0	HR	1.6
IS18680	S	2.8	S	3.2	HR	2.0	S	3.8	S	3.0	S	3.4	S	3.8	S	3.8	S	3.0
IS18758	R	0.0	HR	1.8	S	2.6	HR	2.4	HR	2.2	HR	1.8	HR	1.4	HR	2.4	R	0.0
IS18760	S	2.4	S	2.8	S	3.2	S	2.8	S	3.4	HR	2.4	HR	2.2	HR	2.6	R	0.0
IS18433	S	2.2	S	3.0	S	3.0	HR	2.4	S	2.8	S	3.0	S	2.4	S	3.4	HR	2.2
UchV-2	R	0.0	R	0.0	S	2.6	S	2.4	S	2.2	S	2.8	S	2.2	S	3.0	HR	1.4
IS2058	R	0.0	R	0.0	HR	2.6	R	0.0	R	0.0	HR	1.2	HR	1.2	R	0.0	R	0.0
IS7775	HR	1.6	HR	2.4	HR	2.4	HR	1.8	HR	3.2	HR	2.4	HR	2.6	HR	1.8	R	0.0
IS8024	R	0.0	HR	1.8	HR	1.8	HR	1.4	HR	2.6	HR	2.0	HR	1.8	R	0.0	R	0.0
IS8283	R	0.0	HR	1.8	HR	1.6	HR	1.8	HR	2.2	R	0.0	HR	1.4	HR	2.4	R	0.0
IS9600	HR	1.2	R	0.0	R	0.0	R	0.0	HR	1.0	HR	1.2	HR	1.4	HR	1.4	R	0.0
IS12664C	HR	1.4	HR	1.2	HR	1.2	HR	1.6	HR	2.0	HR	2.2	HR	1.4	HR	2.0	HR	1.8
IS18688	HR	1.4	HR	1.0	HR	1.6	HR	1.2	HR	2.8	HR	2.0	HR	2.4	HR	2.6	R	0.0

[x] R = resistant (no symptoms), HR = hypersensitive type resistance (flecking, reddening or red spots, necrosis), and S = susceptible lesions with acervuli.

[y] Based on a scale of 0–5 where 0 = no visible lesions, 1 = <1% leaf area covered by lesions, and 5 = lesions covering >40% of leaf area.

[z] $LSD_{0.05}$ = 0.53 used for pairwise (genotype × isolate) comparison of disease severity both across and within columns. Each value represents the mean of 10 replications (two replications per repetition) per isolate-genotype combination.

genotypes belonging to HR and R groups. Seventeen host genotypes clearly demonstrated the differential reaction with respect to all pathogen isolates tested, and no two isolates could be grouped into a race. This indicated that all nine isolates were distinct from each other, and were most probably different physiologic races of *C. graminicola* (Table 6).

Table 7. Relative aggressiveness as measured by disease severity of nine Indian isolates of *Colletotrichum graminicola* on six susceptible sorghum genotypes in the greenhouse

	Sorghum genotype[z]						
Isolate	**IS914**	**IS1022**	**IS3089**	**IS17804**	**IS18442**	**IS18531**	**All**
ID-1-83	3.4 bc	3.4 ab	5.0 a	5.0 a	5.0 a	5.0 a	4.46 a
SR-1-85	3.8 a–c	4.0 ab	4.0 bc	4.4 b	5.0 a	4.8 ab	4.32 a
PAT-2-85	5.0 a	4.2 ab	5.0 a	4.8 ab	3.4 b	3.2 de	4.28 a
PAT-1-85	5.0 a	4.6 a	3.0 e	3.2 e	5.0 a	4.2 bc	4.18 a
DH-1-83	3.4 bc	3.8 ab	4.4 ab	5.0 a	3.0 c	2.8 ef	3.76 b
PAN-1-85	3.0 c	3.8 ab	3.2 ed	2.2 de	5.0 a	4.0 c	3.54 b
HB-1-83	4.6 ab	4.4 a	3.8 b–d	2.0 e	2.8 cd	1.4 g	3.18 c
UD-1-83	3.4 bc	3.4 ab	3.4 c–e	2.6 d	3.4 b	2.2 f	3.06 c
AK-1-83	3.0 c	3.0 b	2.0 f	3.6 c	2.6 d	3.6 cd	2.96 c

[z] Disease severity rating based on susceptible reaction type on a 0–5 scale where 0 = no visible lesions, 1 = <1% leaf area covered by lesions, and 5 = lesions covering >40% of leaf area. Each value represents the mean of 10 replications (two replications per repetition) per isolate-genotype combination. Means followed by the same letter within the subgroup for each sorghum genotype are not significantly different at $P = 0.05$ according to the Waller-Duncan Bayesian k-ratio LSD rule.

Table 8. Conidial size of the nine Indian isolates of *Colletotrichum graminicola*

	Length (μm)		Width (μm)[y]
Isolate	**Mean**	**Range**	**Mean**
AK-1-83	25.56 c[z]	20–30	4.91 a[z]
DH-1-83	27.16 b	20–30	4.96 a
HB-1-83	24.86 cd	20–35	4.94 a
PAT-1-85	25.26 c	20–30	4.95 a
PAT-2-85	24.40 d	20–30	4.93 a
ID-1-83	25.40 c	20–30	4.93 a
PAN-1-85	29.56 a	25–35	4.94 a
SR-1-85	24.20 d	20–30	4.95 a
UD-1-83	23.06 e	20–30	4.90 a

[y] Width measured only at the center of the conidium.
[z] Mean of 100 conidia of each isolate. Means within each column followed by the same letter are not significantly different at $P = 0.05$ according to the Waller-Duncan Bayesian k-ratio LSD rule.

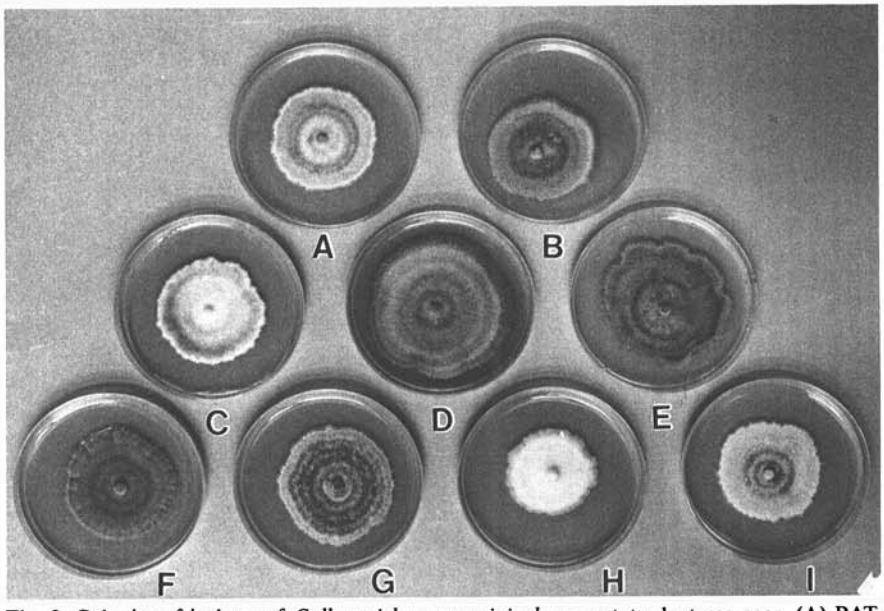

Fig. 3. Colonies of isolates of *Colletotrichum graminicola* on potato-dextrose agar. **(A)** PAT-1-85, **(B)** PAT-2-85, **(C)** AK-1-83, **(D)** DH-1-83, **(E)** HB-1-83, **(F)** PAN-1-85, **(G)** SR-1-85, **(H)** UD-1-83, and **(I)** ID-1-83.

Virulence and aggressiveness among isolates. On the 30 genotypes, isolates PAT-1-85 and UD-1-83 produced susceptible type lesions on 19 and eight genotypes, respectively. The remaining seven isolates were pathogenic to a varying range of genotypes (Fig. 2). Based on the number of genotypes susceptible to an isolate, isolates PAT-1-85 and UD-1-83 were identified as the most and least virulent, respectively, to the 30 host genotypes tested.

The nine isolates of *C. graminicola* could be subdivided into three groups based on the mean disease severity on six susceptible genotypes. Isolates ID-1-83, SR-1-85, PAT-2-85, and PAT-1-85, as a group, were most aggressive. Isolates DH-1-83 and PAN-1-85 were intermediate, whereas the remaining three isolates (HB-1-83, UD-1-83, and AK-1-83) were least aggressive. These three groups were significantly different ($P = 0.05$) from each other in disease severity. Isolates within a group were similar in aggressiveness (Table 7).

Morphology and cultural characteristics of the isolates. Conidia were sparsely produced by all isolates on PDA but were abundant on SLM. Conidia were falcate or spindle-shaped, hyaline, aseptate, and had a mean length of 23.06–29.56 μm. Significant differences were not observed in the width of conidia among the isolates, and the width of the conidia of all isolates ranged between 4.90 and 4.96 μm (Table 8). Significant differences were obtained in radial diameters of the test isolates. Radial diameters of colonies of each isolate are presented in Table 9. Mean radial diameters 7 days after incubation ranged from 33.0 to 72.33 mm. Isolate HB-1-83 produced the largest colony and UD-1-83 had the smallest colony.

Colonies of isolates on PDA ranged from white to a gray to dark brown, with light tan being most common. Colonies of isolates PAT-1-85, PAT-2-85, HB-1-83, and ID-1-83 were felty to woolly with well-defined zonation, and those of isolates AK-1-83, DH-1-83, and PAN-1-85 were tufted with faint zonation. The colony of isolate SR-1-85 had distinct concentric rings, whereas that of isolate UD-1-83 was compact with no zonation (Fig. 3). The bottom of colonies ranged from greenish gray to black. It was not possible from these observations to relate cultural characteristics of the nine isolates with any specific groups based on virulence or aggressiveness.

DISCUSSION

The nine Indian isolates of *C. graminicola* showed considerable variation in pathogenicity, virulence, and aggressiveness on 30 sorghum genotypes, and no two isolates elicited similar symptoms on 17 genotypes. The isolates also differed in cultural characteristics. Our data suggest that the nine isolates were distinct

Table 9. Radial growth of nine Indian isolates of *Colletotrichum graminicola* on potato-dextrose agar

Isolates	Colony diameter (mm) after incubating for					
	3 days		5 days		7 days	
	Mean	Range	Mean	Range	Mean	Range
AK-1-83	21.33 a[z]	21–22	33.33 c	32–35	46.33 c	44–48
DH-1-83	22.33 a	21–24	37.33 b	37–38	52.00 b	50–54
HB-1-83	15.00 c	14–16	46.33 a	44–48	72.33 a	71–74
PAT-1-85	13.33 d	11–15	22.66 f	22–23	46.33 c	44–48
PAT-2-85	13.00 c	12–14	26.33 e	24–28	43.00 d	42–43
ID-1-83	9.66 e	9–10	20.00 g	19–21	40.66 e	40–41
PAN-1-85	15.33 bc	14–17	30.00 d	28–32	50.66 b	48–54
SR-1-85	15.66 bc	15–16	31.66 cd	31–32	47.33 c	47–48
UD-1-85	16.66 b	15–19	26.66 e	25–28	33.00 f	32–34

[z] Mean of three replications of each isolate. Means within each column followed by the same letter are not significantly different at $P = < 0.05$ according to the Waller-Duncan Bayesian k-ratio LSD rule.

physiologic races, supporting previous reports of the variability of *C. graminicola* pathogenic to sorghum (1,2,5, 7,10,13).

Virulence of a pathogen may be determined by comparing the number of host genotypes on which isolates produce susceptible symptoms. In this study, isolate PAT-1-85 was the most virulent—it elicited susceptible lesions on 19 of 30 sorghum genotypes. However, the severity of lesions (aggressiveness) varied on these genotypes. This differential relationship between virulence and aggressiveness or degree of pathogenicity is ascribed to isolate-host genotypic interaction. The nine isolates could be grouped into three distinct aggressiveness categories based on the reaction of the six susceptible genotypes.

The observed differences in the cultural characteristics of the nine isolates of *C. graminicola* were similar to those reported by others (1,15,19). Alawode et al (1) used these differences to differentiate races as well as forma specialis in the populations of *C. graminicola* on sorghum in Nigeria. They proposed the variety name *C. g. isolatum* for an isolate that caused pinpoint leaf lesions. Similarly, zonate anthracnose was reported as a new disease of sorghum caused by *C. g. zonatum* in India (19). Both isolates produced concentric rings on PDA. Symptoms of the two new diseases (1,19) are similar to the foliar anthracnose symptoms produced by isolates of *C. graminicola* in our study and on several sorghum and maize lines (2,8–10,16, 18,22). Furthermore, isolates PAT-1-85, PAT-2-85, HB-1-83, and ID-1-83 produced colonies with well-defined zonations and that of SR-1-85 had distinct concentric rings. These isolates produced a range of symptoms on the host genotypes used as differentials in the pathogenicity tests. Thus, the different symptoms produced on sorghum genotypes and differences in cultural characteristics appeared to be attributable to physiologic variation within populations of *C. graminicola* and not to the occurrence of a new pathogen and disease.

The relationship of races of *C. graminicola* in India and those identified elsewhere (1,2,5,7,10,13) is unknown. Research at one location with pathogen isolates from different geographic areas and a wide range of sorghum genotypes would provide data necessary for the designation of races on a global basis and identification of differential sorghum genotypes for determining their occurrence. Such information would be valuable in disease resistance breeding programs.

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LITERATURE CITED

1. Alawode, D. A., Manzo, S. K., and Sundram, N. V. 1983. Anthracnose of sorghum in northern Nigeria caused by *Colletotrichum graminicola*. Ahmadu Bello Univ. Sorghum Pathol. Rep. 18 pp.
2. Ali, M. E. K., and Warren, H. L. 1987. Physiological races of *Colletotrichum graminicola* on sorghum. Plant Dis. 71:402-404.
3. Anonymous. 1984. International Sorghum Anthracnose Virulence Nursery (ISAVN). Page 27 in: Annual Report 1983. ICRISAT, Patancheru, A. P., India.
4. Bergquist, R. R. 1973. *Colletotrichum graminicola* on *Sorghum bicolor* in Hawaii. Plant Dis. Rep. 57:272-275.
5. Cardwell, K. F., Hepperly, P. R., and Frederiksen, R. A. 1989. Pathotypes of *Colletotrichum graminicola* and seed transmission of sorghum anthracnose. Plant Dis. 73:255-257.
6. Commonwealth Agricultural Bureau International. 1988. Distribution Map No. 586 in: Maps of Plant Diseases. 1st ed. Commonw. Mycol. Inst./Assoc. Appl. Biol, Kew, Surrey, England. 2 pp.
7. Ferreira, A. D. S., Frederiksen, R. A., Warren, H., and De Castillo, K. C. 1985. Identification of races of *Colletotrichum graminicola* in Brazil. Sorghum Newsl. 28:80-83.
8. Ferreira, A. S., and Warren, H. L. 1982. Resistance of sorghum to *Colletotrichum graminicola*. Plant Dis. 66:773-775.
9. Forgey, W. M., Blanco, M. H., and Loegering, W. Q. 1978. Differences in pathological capabilities and host specificity of *Colletotrichum graminicola* on *Zea mays*. Plant Dis. Rep. 62:573-576.
10. Harris, H. B., and Johnson, B. J. 1967. Sorghum anthracnose—symptoms, importance, and resistance. Proc. Bienn. Grain Sorghum Res. Util. Conf. 5:48-52.
11. Harris, H. B., Johnson, B. J., Dobson, J. W., Jr., and Luttrell, E. S. 1964. Evaluation of anthracnose on grain sorghum. Crop Sci. 4:460-462.
12. Harris, H. B., and Sowell, G., Jr. 1970. Incidence of *Colletotrichum graminicola* on *Sorghum bicolor* introductions. Plant Dis. Rep. 54:60-62.
13. King, S. B., and Frederiksen, R. A. 1976. Report on the International Sorghum Anthracnose Virulence Nursery. Sorghum Newsl. 19:105-106.
14. Madden, L. V., Knoke, J. K., and Louie, R. 1982. Considerations for the use of multiple comparison procedures in phytopathological investigations. Phytopathology 72:1015-1017.
15. Mordue, J. E. M. 1967. *Colletotrichum graminicola*. No. 132 in: Descriptions of Pathogenic Fungi and Bacteria. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England. 2 pp.
16. Nicholson, R. L., and Warren, H. L. 1981. The issue of races of *Colletotrichum graminicola* pathogenic to corn. Plant Dis. 65:143-145.
17. Pastor-Corrales, M. A., and Frederiksen, R. A. 1980. Sorghum anthracnose. Pages 289-294 in: Sorghum Diseases—a World Review. R. J. Williams, R. A. Frederiksen, L. K. Mughogho, and G. D. Bengtson, eds. ICRISAT, Patancheru, A. P., India.
18. Powell, P., Ellis, M., Alameda, M., and Sotomayor, A. 1977. Effect of natural anthracnose epiphytotic on yield, grain quality, seed health and seed-borne fungi in *Sorghum bicolor*. Sorghum Newsl. 20:77-78.
19. Rajasab, A. H., and Ramalingam, A. 1981. Zonate anthracnose, a new disease of sorghum caused by *Colletotrichum graminicola* var. *zonatum* var. nov. Current Sci. 50:34-36.
20. Sutton, B. C. 1980. The Coelomycetes. Fungi imperfecti with pycnidia, acervuli and stroma. Commonw. Mycol. Inst., Kew, Surrey, England. 696 pp.
21. Tarr, S. A. J. 1962. Diseases of Sorghum, Sudan Grass and Broom Corn. Commonw. Mycol. Inst., Kew, Surrey, England. 380 pp.
22. Wheeler, H., Politis, D. J., and Poneleit, C. G. 1974. Pathogenicity, host range, and distribution of *Colletotrichum graminicola* on corn. Phytopathology 64:293-296.