P. R. Verma M.Sc. Student Award

Pathogenic and Molecular Variability Among Twelve Isolates of *Colletotrichum graminicola* from Sorghum

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Abstract

Variability in 12 foliar and grain isolates of sorghum anthracnose pathogen *Colletotrichum graminicola* was studied through virulence and Random Amplified Polymorphic DNA (RAPD) assay. For virulence analysis, pot grown 21-day-old plants of six differential sorghum lines were spray inoculated with conidia (1 x 10^5 conidia ml^{-1}). The isolates showed significant ($P< 0.05$) variations in latent period, aggressiveness and virulence. Overall pathogenic potential was determined as Virulence Index. Hierarchical clustering using euclidean test based on "Virulence Index" grouped the 12 isolates into three groups, while that based on RAPDs using primer OPA8 grouped these into four groups. Monoconidial isolate Cg 226-3 had 85% genetic and pathogenic similarity like its parent, while Cg 227-3 showed virulence variation and 65% genetic similarity with its parent, indicating heterogeneity in Cg 227.

Key words: *Sorghum bicolor, Colletotrichum graminicola,* virulence, pathotypes, DNA polymorphism

ज्वार एन्थ्रेक्नोज रोगाणु *कोलीटोट्राइकम ग्रेमीनीकोला* के 12 पत्रीय और अनाज पृथक्कृतों में अग्रता और रेन्डम एम्प्लीफाइड पोलीमोरफिक डी एन ए आमापन द्वारा परिवर्तिता का अध्ययन किया गया। उग्रता विश्लेषणार्थ ज्वार की छः विभेद लाइन्स के 21-दिवसीय पौधों की कोनिडिया ($1x10^5$ कोनिडिया प्रति मि ली) से फुहार-संरोपण किया गया था। पृथक्कृतों ने अव्यक्त काल, आक्रमणशीलता और उग्रता में सार्थक ($P < 0.05$) परिवर्तन दर्शाया। सार्व रोगजनक विभव उग्रता सूचक द्वारा ज्ञात की गई। उग्रता सूचक पर आधारित यूक्लीडियन परीक्षण द्वारा हाइरारकिकल क्लस्टरिंग करने पर 12 पृथक्कृतों को तीन समूहों में रखा गया, जबकि OPA8 प्राइमर से RAPDs विश्लेषण से इनके चार समूह पाये गये। एकल कोनिडीयल पृथक्कृत Cg 226-3 ने अपने जनक से 85% आनुवंशिक और रोग जनकता में समानता दर्शायी जबकि Cg 227-3 ने उग्रता में परिवर्तन और अपने जनक से 65% आनुवंशिक समानता दर्शायी, जिससे Cg 227 में विषमता का संकेत मिलता है।

Sorghum anthracnose (also known as red leaf blight and seedling blight) is one of the major yield reducing factors under hot, humid conditions in most tropical and subtemparate regions of the world. Although leaf blight phase is the most common, grain anthracnose also becomes severe at times, reducing germinability and quality of seeds (Saifula and Rangnathiah, 1989). Sorghum being a marginal crop, control measures other than use of host resistance may not be economically feasible. For effective development and deployment of host resistance, information on the distribution of races / pathotypes and the extent of variability is desirable. The pathogen *Colletotrichum graminicola* (Ces.) Wils. (= *C. sublineolum* Henn. Kabat. et Bub.) is highly variable and its several races have been reported from different regions (Ferreira and Casela, 1986; Ali and Warren, 1987;Cardwell *et al.,* 1987;

Thakur, 1995). In India, existence of its races in different states as well as within states and locations have been reported on the basis of cultural characteristics, morphology, and pathogenicity (Pande *et al*., 1991; Mathur *et al.,* 1998; Rao *et al*., 1998). Random Amplified Polymorphic DNA analysis has also been found useful in elucidating variability in isolates of *C. graminicola* in different areas (Gutherie *et al.,* 1992; Thakur *et al.,* 1999). In the present investigations, extent of pathogenic and molecular diversity, and possible heterogeneity was studied among 12 foliar and grain isolates of C. *graminicola* collected from four states of India.

Materials and Methods

Inoculum and inoculation. Cultures of *C. graminicola* were raised from dried infected leaf and grain samples collected from different areas of four states (Table 1) and stored at ICRISAT-Patancheru at 4°C after blotter-drying. Leaf pieces with single-lesion were surface-sterilized with 0.1 % $HgCl_2$ for 2 min, rinsed twice with sterilized distilled water and placed on 2% oatmeal agar (OMA) in petri plates and incubated at 25° C under continuous fluorescent light. Colonies of C. *graminicola* growing from these single-lesions were transferred on fresh OMA plates. Infected seeds washed and surface sterilized, as above, were incubated in petridishes lined with moist blotting paper, and spores developing on these were streaked onto fresh OMA plates. The cultures were maintained on OMA slants at 4°C.

Table 1. Details of *Colletotrichum graminicola* isolates collected from four states of India

S.No.	Isolate designation	Location / State
1.	Cg 20	Koppal / Karnataka
2.	Cg 75	Peddapur / A.P.
3.	Cg 94	Thalval / Maharashtra
4.	Cg 118	Mariwad / Karnataka
5.	Cg 130	Jedcharla / A.P.
6.	Cg 138	Patancheru / A.P.
7.	Cg 150	Pudur / T. N.
S.	Cg 158	Thamaraikulum / T. N.
9.	Cg 226 (grain)	Patancheru / A.P.
10.	Cg 226-3 (monoconidial derivative *of* Cg 226)	
11.	Cg 227	Patancheru/ A.P.
12.	Cg 227-3 (monoconidial derivative of Cg 227)	Patancheru/ A.P.

Pathogenic variability

Pathogenicity. Pathogenic variability of the isolates was evaluated on differential lines (Mathur *et al*.. 1997). Surface sterilized seeds of six differentials (A 2267-2. IRAT 204, IS 3758, IS 8354, IS 3089, IS 18442) were sown in sterilized black soil: sand: farmyard manure mix (3:2:2 by volume) in 18cm square plastic pots in a glasshouse (25±2°C, rh <90%). The isolates were grown in 2% oatmeal broth for 96 h at 25°C in an incubator shaker at 125 rpm with 12 h photoperiod for 10 days. Conidia were separated and concentration of I x 10^5 conidia ml^{-1} was prepared using a haemocylomeler, and two drops of Tween-20 were added to each suspension. Plants were spray-inoculated at the 5-6 leaf stage (21-day old) with each of the 12 isolates. The inoculated plants were air-dried and incubated at 25°C and >95% rh for 24 h. The plants were then moved to glasshouse benches at 25±2°C.

The experiment was conducted in a completely randomized design involving 12 isolates x 6 host lines with two replications and 10 plants per replication, and was repeated once to confirm the results.

Observations for latent period were recorded every day for each isolate-sorghum line combination, beginning two days after inoculation. Plants were scored for disease reaction and disease severity 14 days after inoculation. Disease reaction was recorded as R = resistant (no symptoms or presence of chlorotic flecks); MR = moderately resistant (hypersensitive, necrotic lesions without sporulation) and S = susceptible (necrotic lesions with sporulation). Disease severity was recorded on 1-9 scale on fourth and fifth inoculated leaves, where 1 = no lesions; 2 = 1-5%; 3 = 6-10%; 4 = 10-20%; 5 = 21-30%; 6 = 31-40%; 7 = 41-50%; 8 = 51-75%= and 9 > 75% leaf area covered with lesions.

Host-pathogen interaction model. Host-pathogen interaction model was determined on the basis of virulence - the potential of an isolate to infect a host line, measured qualitatively as disease reaction types (R, MR, S) and expressed quantitatively as R = 1; MR = 2 and S = 3, and aggressiveness - the amount

of damage caused by a virulent isolate to the host line, measured quantitatively on a disease severity scale of 1-9. For designating virulence, isolates showing disease reaction of R/MR or MR on a sorghum line were considered as avirulent. Those showing R/MR/S or MR/S or S were considered as virulent. 'Virulence Index' (VI) was calculated as:

VI = [virulence (V) x aggressiveness (A)] x latent period $(L)^{-1}$ or $VI = (VA)L^{-1}$

DNA analysis

DNA Isolation. The isolates were multiplied by growing in 0.1% oatmeal broth at 25°C in a rotary shaker (125 rpm) under continuous fluorescent light for five days, and mycelium separated by filtering through Mira cloth (Calbiochem, USA), washed three times with 100 ml of sterile distilled water, and wiped with soft tissue paper to remove most of the moisture. DNA was extracted from the mycelia using the procedure of Murray and Thompson (1980) with few modifications. About 5 g of wet mycelia were ground in a prcchilled mortar using liquid nitrogen and the powder was transferred to a 5 ml polypropylene tube containing 20 ml of extraction buffer (2% CTAB, 0.2 M Tris-HCl, pH 8.0, 1.4 M NaCl, and 0.02 M EDTA). The contents of the tube were gently mixed by inversion and incubated in a waterbath at 65°C for 1 h. After cooling to room temperature an equal volume of chloroform : isoamylalcohol (24:1 v/v) was added and the contents were gently mixed for 5 min by inversion and the layers were separated by centrifugation at 6000 rpm for 15 min. The aqueous layer was removed and the above step was repeated. DNA was precipitated by the addition of an equal volume of isopropanol and was hooked out with a bent Pasteur pippett. DNA was washed twice with 70% ethanol and dissolved in TE (0.05M Tris-HCl, pH 8.0, 0.01 MEDTA). Ribonuclease was added to a final concentration of 50 µg/ml and incubated at 37°C for Ih. An equal volume of phenol : chlloroform (1:1 v/v) was added and mixed for 5 min by inversion. The aqueous layer was removed after centrifugation and an equal volume of chloroform : isoamyl alcohol (24:1) was added and extracted as before. DNA was precipitated by the addition of 0.3 M sodium acetate and an equal volume of chilled isopropanol. The DNA pellet was washed twice with 70% ethanol! and redissolved in TE (0.01 M Tris-HCl, pH 8.0,0.001 MEDTA).

Polymerase chain reaction. RAPD reactions were performed as described by Williams *et al.* (1990). The reactions were performed in a 25 µl volume containing 10 x Taq buffer (Promega), 100µM dNTPs (NEB), 15ng primer (OPA8, base sequence 5'GTGACGTAGG3' J, 50ng of fungal DNA and one unit Taq DNA polymerase (Promega). Amplification was carried out in a MJ Research DNA engine (PTC 200) thermal cycler programmed for 2 min at 94°C followed by 35 cycles *of* 30 *sec* at 94°C, 30 sec at 36°C and 1 min at 72°C. A final extension was done for4 min at 72°C. Amplified fragments were resolved by electrophoresis on 1.4% agarose gel containing 0.5 ug/ml ethidium bromide and the bands were visualized on a UV transilluminator. Presence or absence of bands was scored as 1 or 0, respectively.

Statistical analysis

Analysis of variance was done using GENSTAT (1986) to determine the significant interactions of isolate and lines in two experimental runs separately. The error MS of two experimental runs was subjected to F-test. The error variances were considered homogenous as the highest error MS was not three-fold larger than the smallest error MS (Gomez and Gomez, 1984). Therefore, the data from the two experimental runs were pooled and means were estimated through analyses of variance. Similarities of 12 isolates basead on virulence index was determined through hierarchical clustering procedure using Euclidean test of average linkage cluster method (GENSTAT, 1986). Dendrogram for the 12 isolates was produced to determine the variation in virulence.. For RAPD analysis, the data were subjected to cluster analysis, and dendrogram was developed with GENSTAT program.

Results and Discussion

Pathogenic variations

Latent period. The latent period of the isolates varied from 2.7 to 4.6 days (Table 2) across six sorghum lines. The isolate Cg 226 had the shortest mean latent period of 3.6 days, and Cg 130 had the longest (4.4 days). Among sorghum lines, shortest latent period was on IS 3758 (3.4 days) and the longest on A 2267-2 (4.4 days). Highly significant ($P<0.001$) differences were observed for isolates, sorghum lines and isolate x sorghum lines for latent period (Table 5).

Table 2. Latent period (days)* of 12 isolates of *Colletotrichum graminicola* on six sorghum lines in the glasshouse

Isolate designation	Sorghum lines						
	A 2267-2	IRAT 204	IS 3758	IS 8354	IS 3089	IS 18442	Mean
Cg 20	4.3	4.3	3.6	3.5	3.7	3.3	3.8[ab]
Cg 75	4.5	4.3	3.5	2.9	3.5	3.8	3.8[ab]
Cg 94	-**	-	3.9	3.4	4.4	4.0	3.9[b]
Cg 118	-	-	4.1	3.5	4.5	4.0	4.0"
Cg 130	4.5	4.3	3.8	3.6	4.3	4.1	4.1[b]
Cg 138	-	-	3.9	3.7	3.8	3.6	3.7[ab]
Cg 150	4.4	4.3	3.8	3.6	3.9	3.5	3.9[b]
Cg 158	-	4.3	3.1	3.3	3.6	3.9	3.7[ab]
Cg 226	4.1	4.2	3.5	2.7	3.5	3.4	3.6a
Cg 226-3	-	-	3.8	3.1	3.8	4.0	3.7[a]
Cg 227	-	4.1	4.1	3.1	3.9	3.6	3.8[ab]
Cg 227-3	-	4.6	4.0	3.8	3.6	3.9	4.0[b]
Mean***	4.4[c]	4.3[c]	3.8[b]	3.8[b]	3.9[b]	3.8[b]	

LSD (P<0.05) For Isolates = 0.24; Sorghum Lines = 0.17; Isolates x sorghum lines = 0.58.

* Mean of two experimental runs; ** No flecks or lesions; *** Means with same letter are not significantly different within a column, according to Duncan's multiple range test (P<0.05).

Table 3. Virulence (disease reaction) and aggressiveness (disease severity on a 1-9 ascale) of 12 *Colletotrichum graminicola* isolates on six sorghum lines in the glasshouse

Isolate designation	Sorghum lines						
	A 2267-2	IRAT 204	IS 8354	IS 3758	IS 3089	IS 18442	Mean
Cg 20	R /1.6	R/1.9	S/2.6	MR/ 3.6	S/ 3.1	S/3.4	2.7[b]
Cg 75	R/1.6	R/1.7	S/4.0	MR/ 3.1	S/3.2	S/3.2	2.8[b]
Cg 94	R /1.0	R/1.0	S/3.0	MR/ 3.9	S/4.0	S/4.2	2.9[b]
Cg 118	R/1.0	R/1.0	MR/2.6	MR/ 3.1	S/3.1	S/2.7	2.2[a]
Cg 130	R/1.5	R/1.7	S/2.4	MR/3.9	S/3.1	S/4.0	2.8[b]
Cg 138	R/1.0	R/1.0	MR/2.6	MR/3.7	S/5.2	S/2.5	2.7[b]
Cg 150	R/1.5	R/1.5	S/3.0	MR/ 3.3	S/2.7	S/3.5	2.6[ab]
Cg 158	R/1.0	R/1.1	S/3.7	S/3.2	S/ 4.7	S/3.4	2.9[b]
Cg 226	R/1.6	R/1.8	S/4.0	*S*/ 5.1	S/ 3.6	S/6.1	3.7[c]
Cg 226-3	R/1.0	R/1.0	S/3.5	S/ 3.2	S/ 3.0	S/4.8	2.9[b]
Cg 227	R/1.0	R/1.7	S/2.6	S/3.5	S/3.9	S/4.6	2.7[b]
Cg 227-3	R/1.0	R/1.8	S/4.5	MR/ 4.5	S/2.3	S/3.4	2.9
Mean***	1.2[a]	1.4[a]	3.2[b]	3.7[C]	3.4[bc]	3.8[C]	

LSD (P<0.05) For Isolates = 0.36; Sorghum Lines = 0.26; Isolates x sorghum lines = 0.88.

* Mean of two experimental runs; ** No flecks or lesions; *** Means with same letter are not significantly different within a column, according to Duncan's multiple range test (P<0.05).

Table 4. Virulence Index* of 12 isolates of *Colletotrichum graminicola* isolates on six sorghum lines in the glasshouse

Isolate designation	Sorghum lines						
	A 2267-2	IRAT 204	IS 3758	IS 8354	IS 3089	IS 18442	Mean
Cg 20	0.4	0.4	2.4	2.1	2.4	3.0	1.8[ab]
Cg 75	0.3	0.4	3.5	2.4	2.6	2.7	2.0[ab]
Cg 94	-**	-	2.3	2.3	2.7	3.2	2.7[c]
Cg 118	-		1.3	1.8	1.8	2.0	1.7[a]
Cg 130	0.3	0.4	1.9	2.2	2.0	3.0	1.6[a]
Cg 138	-	-	1.6	2.1	4.5	2.0	2.6[c]
Cg 150	0.3	0.4	2.5	1.9	1.9	3.1	1.7[a]
Cg 158	-	0.4	4.2	2.8	3.7	2.8	2.[cd]
Cg 226	0.4	0.4	3.5	5.0	2.9	5.8	3.0[d]
Cg 226-3	-	-	2.7	2.5	2.2	3.6	2.8[cd]
Cg 227	-	0.4	1.9	2.3	2.3	4.3	2.3[bc]
Cg 227-3		0.4	3.4	2.4	1.8	2.7	2.1[b]
Mean***	0.4'	0.4'	2.6"	2.5[b]	2.6"	3.2[c]	

LSD (P<0.05) For Isolates = 0.37; Sorghum Lines = 0.27; Isolates x sorghum lines = 0.92.

* Mean of two experimental runs; ** No flecks or lesions; *** Means with same letter are not significantly different within a column, according to Duncan's multiple range test (P<0.05).

Table 5. Analysis of variance for latent period (LP), virulence index (VI) and aggressiveness (AG)

Source of variation	Mean square			Mean square	
	df	LP	VI	df	AG
Experiment (E)	1	10.42***	10.40***	1	48.7***
Isolates (I)	1 1	0.6.V	5.94***	11	2.67***
Sorghum lines (G)	5	4.68***	45.19***	5	4.59***
E x I	11	0.37"	2.68***	11	0.89***
E x G	5	1.07***	9.98—	5	9.30***
1 xG	44	0.23***	1.71***	5 5	1.67***
Ex I x G	43	0.26**	0.81***	5 5	0.93***
Error	120	0.14	0.30	144	0.20
Total	240				

** significant at P< 0.01.
*** significant at P<0.001.

Virulence. All the 12 isolates were avirulent on A 2267-2, and IRAT 204, but virulent on IS 3089 and IS 18442. Other two lines IS 8354 and IS 3758 produced differential reactions with these isolates. Two isolates- Cg 118 and Cg 138 produced moderately resistant (MR) reaction on IS 8354, while others produced susceptible (S) reaction. On IS 3758, four isolates- Cg 158, Cg 226, Cg 226-3 and Cg 227 produced S reaction and others produced MR reaction. On the basis of viruelnce, the isolates could be divided into three groups: i). Cg 118 and Cg 138; ii). Cg 158, Cg 226, Cg 226-3 and Cg 227 and iii).Cg 20, Cg 75, Cg 94, Cg 130, Cg 150 and Cg 227-3 (Table 3).

Aggressiveness. Among the 12 isolates, Cg 226 was the most aggressive whereas Cg 118 was the least. Disease severity was highest on IS 18442, and lowest on A 2267-2. Monoconnidial derivative 226-3 varied significantly in aggressiveness from its parent isolate, but 227-3 didnot (Table 3). Highly significant (P<0.001) effects of isolates, sorghum lines and isolate and sorghum lines were observed for aggressiveness (Table 5).

Virulence Index. Highest mean virulence index was of isolate Cg 226 (3.0) and lowest in Cg 130 (1.6). Among sorghum lines, highest VI was on IS 18442 and lowest on A 2267-2 (Table 4). Dendrogram based on mean virulence index (Fig. 1) divided the 12 isolates into four groups: i) Cg 226; ii). Cg 20, Cg 118, Cg 150 and Cg 130; iii). Cg 75, Cg 227-3 , Cg 227 and iv). Cg 138, Cg 94, Cg 226-3 and Cg 158.

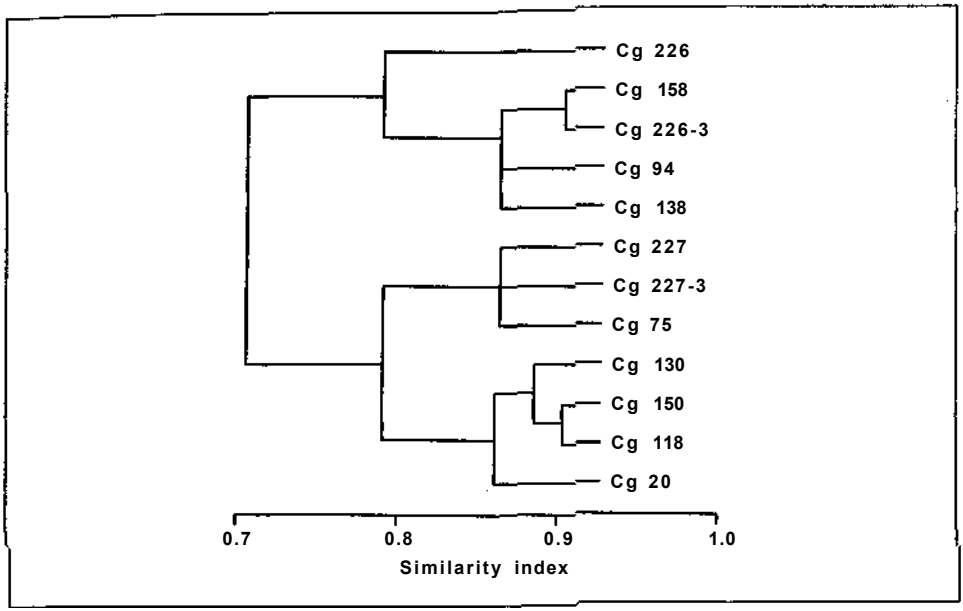

Figure1. Dendogram based on virulence index of 12 isolates of *Colletotrichum graminicola* on six sorghum lines.

Correlation matrix . Latent period was negatively correlated (r^2) with virulence (-0.37), aggressiveness (-0.47) and virulence index (- 0.67). Aggressiveness was positively correlated with virulence (r = 0.70), and virulence index was positively and strongly correlated with virulence (0.70) and aggressiveness (0.90) of the isolates.

Genetic variation

Pattern of bands on the electrophoretic gel indicated DNA polymorphism among the isolates, which varied in length of the fragment amplified, and number of fragments. The primer OPA8 produced bands in 15 different positions with sizes ranging between 0.2 to 2.0 kb (Plate 1). Similarity index revealed that 57.5% of the amplified bands were common to all isolates, while 42.5% were polymorphic. On this basis, the isolates could be grouped into four groups (Fig. 2) - i). Cg 138; ii). Cg 150, Cg 130, Cg 75, Cg 94, Cg 118; iii). Cg 20, Cg 227-3, Cg 130; and iv). Cg 226, Cg 226-3 and Cg 227. The isolate Cg 227 and its monoconidial derivative Cg 227-3 clustered into separate groups having 65% similarity, while Cg 226 and its monoconidial derivative Cg 226-3 were in the same group with 85% similarity.

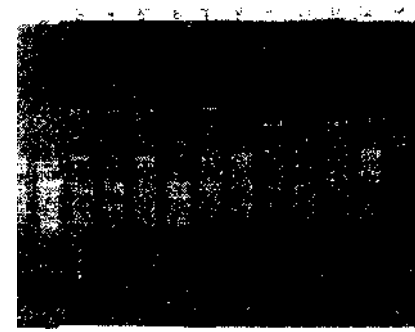

Plate 1. Random amplified Polymorphic DNA(RAPD) analysis of 12 isolates of *Colletotrichum graminicola* using OPA 8 primer (base sequence 5 'GTGACGTAGG3') [1-Cg20; 2-Cg75;3-Cg94;4-Cg118;5-Cgl30;6-Cgl38; 7-Cgl50;8,Cgl58;9-Cg226;10-Cg226-3;ll-Cg227; 12-Cg227-3 M-Marker].

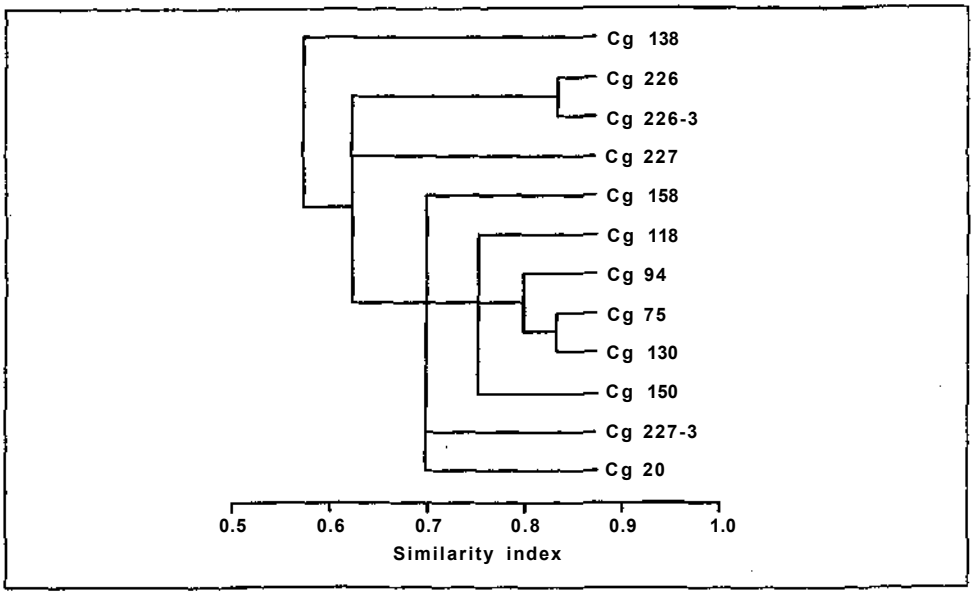

Figure 2. Dendrogram based on RAPD analysis of 12 isolates of *Colletotrichum graminicola* using OPA8 primer.

Pathogenic variability in *C. graminicola* has been demonstrated in isolates collected from different states of India (Pande *etal.,* 1991), and also within states (Rao *et al,* 1998). In the present study, 12 isolates from four states were found to belong to three pathogenic and four genetic groups. DNA analysis based on RAPDs showed more variability than that observed in their virulence. The cluster composition varied for virulence and RAPDs. This is quite expected because gene (s) controlling a particular character most likely present a minute fraction of cellular DNA (nuclear or mitochondrial), whereas RAPD banding pattern obtained from total DNA reflects total DNA divergence (Andebrhan and Furtek, 1994). The association of molecular markers with virulence pattern for plant pathogens has been classified into three categories - perfect, partial and no association (Leung *et al.,* 1993). Close association of molecular markers and pathogenicity has been reported for a few fungal pathogens only, like *Leptosphaeria maculans* (Koch *et al.,* 1991; *Goodwin and Annis, 1991*), *Fusarium solani f.sp. cucurbitae* (Crowhurst *et al.,* 1991), *Magnaporthe grisea* (Levy *et al.,* 1991), and low association in others like *Puccinia striiformis* (Chen *et al.,* 1993). Intra-population variability at molecular level was also observed in *C. graminicola* and cluster composition for RAPDs and virulence index were independent to each other (Mathur *et al.,* 1998). In our study also, of the two monoconidial derivatives from two isolates, one showed significant pathogenic and genetic diversity from its parent isolate. This shows variation in heterogeneity among the isolates. Intra-population variations may be attributed to heterothallism (Anderbrhan and Furtek., 1994). So far, perfect state of sorghum isolates of *C. graminicola* has not been observed, and the variations in asexual spore progenies may be due to parasexual DNA exchange as observed in rice blast pathogen *Magnaporthe grisea*(Zeigler *et al.,* 1997). These results also indicate to the potential of development of more aggressive isolates, which may have implications in epidemiology and resistance stability in sorghum.

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References

Ali, M.E.K. and H.L. Warren.) 987. Physiological races of *Colletotrichum graminicola* on sorghum. *Plant Dis.* 71:402-404.

Anderbrhan, T. and D.R. Furtek. 1994. Random amplified polymorphic DNA (RAPD)analysis of *Crimipcllis perniciosa* isolates from different hosts. *Plant Pathol.* 43: 1020-1027.

Cardwell. K.F., R.R.Duncan and R.A. Frederiksen. 1987. Failure of vertical resistance of selected differential sorghum cultivars due to variations in the populations of *Colletotrichum graminicola* and the relation to rainfall incidence over seven years. *Sorghum Newslett.* 30: 82.

Chen, X.,R.F. Line, and H. Leung. 1993. Relationship between virulence variation and DNA polymorphism in *Puccinia striiformis. Phytopathology* 83:11489-1497.

Crowhursl, R.N., B.T. Hawthorne, E.H.A. Rikkerink and M.D. Templeton. 1991. Differentiation of *Fusaritun solani* f. sp. *cucurbitae* race 1 and 2 by random amplification of polymorphic DNA. *Current Genet.* 20:391-396.

Ferreira, A.S. and C.R. Casela. 1986. Races pathogenicas de *Colletotrichum graminicola* agente causal de anthracnose em sorgo *(Sorghum bicolor* (L.) Moench). *Fitopatol. Brazil.* 11:833-87.

GENSTAT. 1986. A general statistical program. 1986.*Rothamsted Experiment Station, Harpenden, Herts* Al 52 JQ, U.K.

Goodwin, P.H. and S.L. Annis. 1991. RAPD identification of genetic variation and pathotypes of *Leptosphaeria maculans* by random amplified polymorphic DNA assay. *Appl. Environ. Microbiol.* 57: 1482-1486.

Gomez, K.A. and A. A. Gomez. 1984. *Statistical procedures for Agricultural Research.* 2nd edition. John Wiley and Sons, NY. pp 316-356.

Guthrie, P.A.I., C.W. Magill, R.A. Frederiksen and G.N.Odvody. 1992. Random amplified polymorphic DNA markers: a system for identifying and differentiating isolates of *Colleiotrichum graminicola. Phytopathology* 82: 832-835.

Koch, E., K. Song, T.C. Osborn and P.H. Williams. 1991. Relationship between pathogenicity and phyllogeny based *on* restricted fragment length polymorphism in *Leptosphaeria maculans. Mol. Plant-Microb Interact.* 4: 341-349.

Leung, H. , R. J.Nelson and J. E. Leach. 1993. Population structure of plant pathogenic fungi, and bacteria. *Adv. Plant Pathol.* 10: 157-205.

Levy, M., J. Romao, M. A. Marchetti and J. E. Hamer. 1991. DNA fingerprinting with a dispersed repeated sequence resolves pathogenic diversity in the rice blast fungus. *Plant Cell* 3: 95-102.

Mathur, K., V. P. Rao and R. P.Thakur. 1997. Intra-population variability in *Colletotricum sublineolum* infecting sorghum. *J. Mycol. Pl. Pathol.* 27: 302-310.

Mathur, K., V. P. Rao, R. P. Thakur and S. Sivaramakrishnan. 1998. RAPDs and virulence analysis for characterizing pathogenic variability in *Colletotrichum graminicola* infecting sorghum. *ICRISAT* (International Crops Research Institute for the Semi-arid Tropics) 1998. A research report on pathogenic variability in sorghum anthracnose by Kusum Mathur and R.P. Thakur. Patancheru 502 324, Andhra Pradesh, India. 172 pp.

Murray, M.G. and W.E. Thompson. 1980. Rapid isolation of high molecular weight plant DNA. *Nucl. Acids Res.* 8:4321-4325.

Pande, S., L.K.Mughogho, R. Bandhyopadhyay and R.I. Karunakar. 1991. Variation in pathogenicity and cultural characterictics of sorghum isolates of *Colletotrichum graminicola* in India. *Plant Dis.* 75: 778-783.

Rao, V.P., R.P. Thakur and K. Mathur. 1998. Morphological and pathogenic variability among grain sorghum isolates of *Colletotrichum graminicola* in India. *Indian Phytopath.* 51: 164-174.

Saifula, M and K.G. Rangnathiah. 1989. Seed health testing of sorghum with special reference to *Colletotrichum graminicola. Indian Phytopath.* 42: 73-78.

Thakur, R.P. 1995. Status of International Sorghum anthracnose and Pearlmillet Downy Mildew Virulence Nurseries. In: J.F. Leslie and R.A. Frederiksen (eds.) *Disease Analysis through Genetics and Biotechnology-Interdisciplinary Bridges to Improve Sorghum and Millet Crops.* Iowa State University Press, Iowa, Ames. pp 75-92.

Thakur, R.P., K. Mathur, V. P. Rao, S. Chandra, S. Shivramakrishnan, S.Kannan, R.V.Hiremath, H.C. Tailor, US. Kushwaha, R. R. Dwivedi and S. Indira. 1999. Pathogenic and genetic characterization of six Indian populations of *Colletotrichum sublineolum,* the causal agent of sorghum anthracnose. *Indian Phylopath.* 51:321-323.

Williams, J.K.G., A. R. Kubelik, K. J. Livak, J.A. Rafalski and S.V.Tingey. 1990. DNA polymorphism amplified by arbitary primers are useful as genetic markers. *Nucl. Acids Res.*18: 6531-6535.

Zeigler, R.S., R.P. Scott, H.Leung, A.A. Bordeos, J. Kumar and R. J. Nelson. 1997. Evidence of parasexual exchange of DNA in the rice blast fungus challanges its exclusive clonality. *Phytopathology* 87:284-294.