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Pathogenic and genetic characterization of six Indian populations of *Colletotrichum sublineolum,* the causal agent of sorghum anthracnose

R.P. THAKUR[a]*, K. MATHUR[a], V.P RAO, S. CHANDRA[a], S. SHIVRAMKRISHNAN[a], S. KANNAN[a], R.V. HIREMATH[b], H.C. TAILOR[c], U.S. KUSHWAHA[d], R.R. DWIVEDI[e] and S. INDIRA[f]

[a]*Genetic Enhancement and Biotechnology Program, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, 502 324*

[b]*Department of Plant Pathology, University of Agricultural Sciences, Dharwad 580 005*

[c]*Sorghum Research Station, Gujarat Agricultural University, Athwa Farm, Sural 395 007*

[d]*Department of Plant Pathology, JNKVV, Indore 451001*

[e]*Department of Plant Pathology, G.B.Pant University of Agriculture and Technology, Pantnagar 263 145*

[f]*National Research Center for Sorghum, Rajendranagar, Hyderabad 500 030*

ABSTRACT : Pathogenic and genetic characterization of populations of *Colletotrichum sublineolum,* the causal agent of anthracnose of sorghum, was done from six Indian locations. Multi-location field evaluation and greenhouse tests were done of 16 sorghum lines that comprised the International Sorghum Anthracnose Virulence Nursery (ISAVN). The lines were tested in a field trial for 4-5 years (1992-1996) at six Indian locations: Indore, Surat, Patancheru, Dharwad, Udaipur and Pantnagar. Plants were scored for disease reaction (R/MR/S) and for disease severity (on a 1-9 scale where 1= no lesion and 9 >75% leaf area covered with lesions) at the soft-dough stage in the field and at the seedling stage in the greenhouse. Significant (P<0.001) differences were observed for virulence (disease reaction) and aggressiveness (disease severity) across locations (isolates) and sorghum lines both in field and greenhouse tests. In both tests isolate x sorghum fine interactions were highly significant (P<0.001) suggesting that populations of *C. sublineolum* at these six locations were different. A random amplified polymorphic DNA (RAPD) analysis exhibited genetic dissimilarities among the isolates and these were classified in to six groups,

Key words : Sorghum, anthracnose, virulence, aggressiveness, *Colletotrichum sublineolum*

During the last two and half decades, systematic efforts have been made for developing high yielding sorghum (*Sorghum bicolor* (L.) Moench) cultivars for increased grain production. Incorporation of disease resistance into high yielding cultivars is of particular importance to provide stability of production. The success of disease resistance breeding program dependson the information and knowledge of the existence and distribution of races in the pathogen populations. Monitoring resistance stability of improved cultivars and virulence change in pathogen populations is important to planning of resistance utilization and resistance deployment. This is usually done through virulence survey and using host differential lines carrying different resistance genes.

Anthracnose of sorghum, caused by *Colletotrichum sublineolum* (Henn. Kabat *et* Bub.) is widely prevalent in areas with 500-1500 mm annual rainfall and temperature ranges of 26-32°C. The pathogen is known to be highly variable (Frederiksen and Franklin, 1980; Ferreira and Warren, 1982; Casela *et al,* 1992) and 44 races/pathotypes have been identified in different countries: Brazil (Nakamura, 1982; Ferreira and Casela, 1986; Casela and Ferreira, 1995), northern Nigeria (Ozolua *et al.,* 1986), US and Puerto-Rico (Ali and Warren, 1987; Cardwell *et al,* 1989), India (Pande *et al.,* 1991), and western Africa (Thomas, 1995). Molecular tools like RAPD have been found useful in differentiating isolates of *Colletotrichum sublineolum* isolates (Guthrie *et al.,* 1992). Most of these studies were carried out under varying field and glasshouse condi-

The second author is a Visiting Scientist at ICRISAT from Department of Plant Pathology, Rajasthan Agricultural University, R.C.A., Udaipur-313 001, Rajasthan.

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*Corresponding author, Fax: 241 239.
E-mail: r.thakur@cgiar.org

tions, using different sets of host differentials, and different disease rating scales. These studies are, therefore, not comparable and cannot provide a clear picture of the race composition in *C. sublineolum* populations (Thakur, 1995). In an initial attempt to assess pathogenic variability on a regional basis, an International Sorghum Anthracnose Virulence Nursery (ISAVN), comprising of a set of differential lines, was evaluated at key locations in North, Central, and South America, the Caribbean region, Asia and Africa and the results indicated existence of uniquely different populations of the pathogen (King and Frederiksen, 1976; ICRISAT, 1983). Further studies (Pastor-Corrales and Frederiksen, 1980) also suggested that *C. sublineolum* is a dynamic pathogen, and its virulence is affected by differential selection pressure by host resistance genes. To better understand the variability in the pathogen populations at the global level, a modified ISAVN, consisting of a new set of sorghum differential lines assembled from different countries and a standard disease rating scale (for disease reaction and disease severity) was established in 1992. The nursery has been evaluated at 20-28 locations annually in 12-14 countries of Asia, Africa and America (Thakur, 1995).

In this paper, the results of pathogenic and genetic characterization of C. *sublineolum* populations from six Indian locations only based on field, greenhouse and molecular tests, and discuss implications in effective utilization and deployment of host resistance against sorghum anthracnose have been reported.

MATERIALS AND METHODS

Multi-location field evaluations

Locations

The International Sorghum Anthracnose Nursery (ISAVN) is a part of the collaborative projects between ICRISAT and the Indian Council of Agricultural Research (ICAR-ICRISAT Collaborative Project). The centers of All India Coordinated Sorghum Improvement Project (AICSIP) where anthracnose has been prevalent were chosen as test locations. These centers are situated between latitudes 15°18' N to 29°12' N, and longitude 30°2'E to 79° 18' E. The locations and other details of these centers are presented in Table 1. The nursery was conducted for five years (1992-96) except at Dharwad and Pantnagar, where it was conducted only for four years.

Table 1. Locations of International Sorghum Anthracnose Virulence Nursery (ISAVN) in India, and collection of *Colletotrichum sublineolum* isolates from sorghum cultivars

Location, state	Source Cultivar Isolate	Isolate designate
Pantnagar, Uttar Pradesh	Local landrace	Cs 079
Udaipur, Rajasthan	Local landrace	Cs 029
Surat, Gujarat	C10-2	Cs 031
Indore, Madhya Pradesh	Local landrace	Cs 047
Patancheru, Andhra Pradesh	IS 18442	Cs 042
Dharwad, Kamataka	D 340	Cs080

Host differentials

A set of 15 sorghum lines (Table 2) was selected on the basis of their earlier performance in field test (ICRISAT, 1983) and greenhouse test (Pande *et al.,* 1991). Of the 15 lines, seven originated from USA, two from Sudan, three from India, and one each from Burkina Faso, Ethiopia and Nigeria. Each location included one local resistant and one local susceptible check, making the total number of lines 17. Seed stocks of these 15 differential lines were multiplied and maintained at ICRISAT, Patancheru and supplied from the same stocks each year to the collaborators.

Table 2. Description of sorghum lines tested as host differentials in International Sorghum Anthracnose Virulence Nursery

Designation	Race	Origin
IS 8354	Caudatum bicolor	USA
IS 3758	Caudatum bicolor	USA
IS 3552	Caudatum	USA
IS 2508	Kafir caudatum	USA
IRAT 204	Durra caudatum	Burkina Faso
IS 6928	Kafir caudatum	India
IS 854	Kafir caudatum	USA
IS 1006	Kafir bicolor	USA
IS 6958	Durra caudatum	Sudan
IS 12467	Caudatum	Sudan
IS 17141	Durra caudatum	Nigeria
IS 18758	Guinea caudatum	Ethiopia
IS 18760	Durra kafir	USA
ICSV 247	Caudatum	India
IS 18442	Guinea durra	India
A 2267-2[0]	Caudatum	India

[a]Used in greenhouse only

Nursery management

The nursery at each location was conducted in the rainy seasons to provide congenial conditions for anthracnose development. The nursery was laid out in a randomized complete block design in two replications. Each plot comprised two rows, each of 4m length. Ten days after emergence, plants were thinned to maintain 20-25 plants per row. NPK fertilizers were applied at the rate of 80, 40 and 40 kg ha^{-1}, respectively, and carbofuran granules were applied in furrows at 3g per row, at the time of sowing to control soil insects. Whorl application of carbofuran granules at 3g per row was made 30 days after sowing to control insect pests.

Inoculation

At some locations evaluations were done under natural anthracnose infection conditions, while at other locations inoculation was done either by placing the infected-sorghum grains in the whorls, or by spraying conidial suspension (1 x 10^5 conidia ml^{-1}) on 25 to 30-days old plants. In the absence of adequate rainfall, high relative humidity (>90%) was provided through sprinkler irrigation from inoculation to disease development and its subsequent spread, up to the soft-dough stage.

Data recording

At the soft-dough stage, 10 plants from each of 2 rows were randomly scored for disease reaction [R (resistant) = no symptoms or presence of chlorotic flecking; MR (moderately resistant) = hypersensitive reaction , presence of necrotic spots without acervuli, or S (susceptible) = necrotic spots with acervuli] and disease severity on a 1-9 scale (where, 1 = no lesions, 2 = 1-5%, 3 = 6-10%, 4 - 11-20%, 5 = 21-30%, 6 = 31-40%, 7 = 41-50%, 8 = 51-75%, and 9 >75% leaf area covered with lesions). The mean values of these on per plot basis were used for analysis.

Greenhouse evaluation

Host differentials

Greenhouse evaluation was done to compare seedling versus adult plant reaction to *C. sublineolum* isolates. Sorghum lines used as host differentials in ISAVN (Table 2), were used along with one resistant check, A 2267-2. To eliminate seedborne inoculum seeds were surface-sterilized by soaking in 0.1% $HgCl_2$ for 4-5 min, washed thoroughly with sterile distilled water, dried at room temperature (25°C) and sown in 18-cm square plastic pots filled with autoclaved potting mixture (black soil:sand:farmyard manure in a ratio of 3:2:2 v/v). The experiment was conducted in a completely randomized design. There were five plants per pot, and two pots as two replications for each sorghum line and for each isolate.

Inoculum and inoculation

Cultures of *C. sublineolum* were raised from dried infected leaf-samples collected from the six ISAVN locations (Table 1) and stored at ICRISAT-Patancheru at 4°C after blotter-drying. Leaf pieces with single-lesion from these were surface-sterilized with 0.1% $HgCl_2$ for 2 minutes, rinsed twice with sterile distilled water and placed on 2% oatmeal agar in petri plates and incubated at 25° C, under continuous fluorescent light. Colonies of C. *sublineolum* growing from these single-lesions were allowed to sporulate, and the cultures were maintained on oatmeal agar slants at 4°C.

For inoculation, the cultures were grown at 25°C in *2%* oat meal broth in Erlenmeyer flasks for 5 days on a shaker (125 rpm) with continuous fluorescent light, and the conidia separated by filtering through a double-layered muslin cloth. Twenty-one-day old plants (at 5-6 leaf stage) of each sorghum line were spray-inoculated with a conidial suspension (1x10^5 ml^{-1}) containing Tween-20 (1 ml /liter) of each of the six isolates, using an atomizer. Polyethylene sheets were used to separate plants when inoculating with different isolates, to avoid the inoculum drift. Following inoculation the plants were air dried and then kept in a humidity chamber (>95% RH) for 24 h. The plants were then transferred to a greenhouse at 25 ± 2°C and arranged on benches in a randomized complete block design, keeping isolates as blocks.

Disease scoring

Data were recorded for latent period (time in days from inoculation to appearance of first necrotic lesion in case of MR and sporulating lesion in case of S reaction) from two days after inoculation. Fourteen days after inoculation the plants were scored for disease reaction and disease severity, following the ISAVN system described earlier. The experiment was repeated once.

To determine pathogenic variability among isolates, disease reaction types were equated to virulence, where S= virulent, and MR/R = avirulent; and disease severity to aggressiveness. The relative quantitative virulence of the isolates termed as "virulence index" (Mathur *et al.,* 1997) was determined from the greenhouse data on latent period, virulence and aggressiveness and was calculated thus: Virulence index (VI)= [1 + (VA) LP^{-1}.], where V = virulence, A = aggressiveness, and LP = latent period. Virulence was quantified

to denote R = 1; MR = 2 and S = 3, and 1 was added to the equation to provide adjustment for reaction type R with no visible symptom which had no latent period and no severity.

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) and washed three times with 100ml of sterile distilled water. The mycelia were wiped with soft tissue paper to remove most of the moisture. DNA was extracted from the mycelia using the procedure of Murray and Thomson (1980) with a few modifications. About 5 g of wet mycelia was ground in a prechilled 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. The aqueous layer was removed and the above step was repeated one more time. DNA was precipitated by the addition of an equal volume of isopropanol and was hooked out with a bent Pasteur pipet. DNA was washed twice with 70% ethanol and dissolved in TE (0.05M Tris-HCl pH 8.0, 0.01 M EDTA). Ribonuclease was added to a final concentration of 50 µg/ml and incubated at 37°C for 1h. An equal volume of phenol: chloroform (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 cold isopropanol. The DNA pellet was washed twice with 70% ethanol and redissolved in TE (0.01M Tris-HCl, pH 8.0, 0.001 M EDTA).

Polymerase chain reaction

RAPD reactions were performed as described by Williams *et al.* (1990). The reactions were performed in a 25 µl volume containing 10x Taq buffer (Promega), 100µM dNTPs (NEB), 15ng primer (10-mer Operon), 50ng of fungal DNA and one unit Taq DNA polymerase (Promega). Amplification was carried out in an 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 for 4 min at 72° C. Amplified fragments were resolved by electrophoresis on a 1.4% agarose gel containing 0.5 µg/ll ethidium bromide and the bands were visualized on a UV transilluminator. Of 60 oligonucleotide primers (10-mer) tested, only A-9 and A-18 provided useful results.

Statistical analysis

Analysis was done using GENSTAT (Rothamsted Experiment Station, Herpenden, Herts, U.K.). Analysis of variance was used to determine significance of differences in isolates, sorghum lines and isolate x line interactions for virulence and aggressiveness.

For multi-location field trials the data were subjected to a pooled analysis of variance using the following linear additive fixed-effects model

$$y_{ijkl} = i + S_i + L_j + SL_{ij} + B_{ijk} + G_l + SG_{il} + LG_{jl} + SLG_{ijl} + a_{ijkl} \quad (1)$$

i=1,..., 5; j=1,..,,6; k=1,2;1=1, ,15

where y_{ijkl} is the observation on genotype 1 in block k at location j in year i; S_i, L_j, and G_i are respectively the effects of year i, location j and genotype 1; a_{ijkl} is the residual associated with the observation y_{ijkl} in ijkl-th plot and assumed to be normally and independently distributed with mean zero and a constant variance $6.^2$; the remaining terms in model (1) represent the interactions among various factors. It was decided not to transform the data as the conclusions from both the transformed and the untransformed analyses were found to be similar. The basic plot data used for analysis being an average of 10 randomly selected plants in effect reasonably followed a normal distribution due to the central limit theorem.

The pooled analysis of variance indicated the presence of highly significant GxE interactions. Analysis of variance, however, does not help to reveal the patterns of these interactions. Pattern analysis (PA) (Williams, 1976; DeLacy *et al.,* 1996) was employed to study the patterns, if any, in these interactions. PA is the joint and complementary application of classification and ordination techniques. The objective is to classify the genotypes and/or environments into relatively homogeneous groups with the genotypes/environments within any class/cluster having similar patterns for the characteristic under study. Ordination is an attempt to order the genotypes and the environments to study the interrelationships among genotypes and environments.

The mean values of genotypes across replications and years in different locations were used as the basic

data for PA. Due to strong interaction of genotypes with years at any location for disease severity the genotype performance over years in principle should not be averaged over years. However, this was done as the location classification based on disease severity matched very closely to that based on disease reaction. This gave rise to a data matrix $Y_{6x15} = \{y_{pq}\}$, p=1, ,6 and q=1, ,15, with 6 rows corresponding to 6 locations and 15 columns corresponding to 15 genotypes. The elements y of matrix Y were standardized (Fox and Rosielle 1982) to get the standardized data matrix Z = $\{z_{pq}\}$, with $z_{pe}=(y_{pq}-y_{.q})/s_q$ where $y_{.q}$ is the mean of genotype q over the 6 locations and s_q^2 is the phenotypic variance of the 6 location mean values for genotype q. The matrix Z was subjected to PA using an agglomerative hierarchical classification procedure with incremental sum of squares grouping strategy and squared Euclidean distance dissimilarity measure. The GEBEI package (Watson *et al.,* 1996) was used to implement the PA. The data of the greenhouse evaluations were subjected to analysis of variance as per the randomized complete block design, separately for both the runs, and the pooled analysis of variance was done over two experimental runs since there was no significant difference in their error mean sum of squares.

RESULTS

Multi-location field evaluations at soft-dough stage

Virulence

The 15 sorghum lines developed varying reaction types over 4-5 years of evaluation at the six locations. At Patancheru 14 lines recorded S reaction and one MR, while at Dharwad only 2 lines recorded S reaction

Table 3. Mean disease reaction/severity scores of 15 sorghum lines induced by *Colletotrichum sublineolum* at six field locations during 1992-1996

	Locations (isolate designation)						
Sorghum lines	Indore (5)[a] (Cs 047)	Surat (5) (Cs 031)	Dharward (4) (Cs 080)	Patancheru (5) (Cs 042)	Pantnagar (4) (Cs 079)	Udaipur (5) (Cs 029)	Mean severity
IS 8354	R/1.0	S/4.3	MR/2.3	S/5.3	S/6.5	MR/2.5	3.7-
IS 3738	S/1.3	S/4.4	MR/3.0	S/3.6	S/6.7	MR/2.4	3.6
IS 3552	S/2.3	S/4.6	MR/2.3	S/3.7	S/6.0	S/4.6	3.9
IS 2508	MR/1.8	S/5.1	MR/2.3	S/2.7	S/4.7	MR/3.0	3.3
IRAT 204	R/1.1	MR/2.2	MR/3.4	S/2.I	MR/6.6	R/2.2	2.9
IS 6928	S/1.0	MR/2.3	MR/2.1	S/I.8	S/3.9	S/8.5	3.3
IS 854	R/1.7	S/4.5	MR/2.2	S/5.3	S/5.7	S/3.6	3.8
IS 1006	R/1.0	S/5.2	S/3.0	S/3.8	MR/5.2	MR/3.3	4.2
IS 6958	S/1.4	MR/2.8	S/2.0	S/1.8	S/2.9	MR/2.3	2.2
IS 12467	MR/1.1	S/3.5	MR/2.4	S/3.4	S/6.2	MR/2.9	3.2
IS 17141	S/1.4	S/5.5	MR/2.4	S/3.1	MR/5.0	MR/2.8	3.4
IS 18758	S/3.0	MR/2.2	MR/3.5	S/2.0	S/3.8	R/1.9	2.7
IS 18760	S/1.3	S/4.5	MR/2.0	MR/3.9	S/5.0	S/3.8	3.4
ICSV 247	R/1.0	MR/2.2	MR/3.0	S/2.1	MR/3.9	MR/1.8	2.3
IS 18442	S/5.8	S/5.7	MR/5.1	S/6.7	S/8.7	S/8.0	6.7
Mean	1.5	3.9	2.8	5.2	3.5	3.6	
Local succ.	7.6	4.4	5.7	6.0	6.9	7.3	6.3
Local rest.	1.8	2.0	2.4	2.1	2.1	1.7	2.0
Number of lines with S reaction	8	10	2	14	11	5	

SE for disease severity Location (isolate) x sorghum line = ± 0.15

R = Resistant reaction, no symptoms or presence of chlorotic flecks; MR = Moderately resistant, red or necrotic spots without acervuli; and S = Susceptible reaction, spots with acervuli.

[a]Number of years of data from the location.

and the remaining recorded MR reaction. Based on the number of lines that developed S reaction, Patancheru isolate (Cs 042) was the most virulent, followed by that from Pantnagar (Cs 079), Surat (Cs 031), Indore (Cs 047) and Udaipur (Cs 029), and the Dharwad isolate (Cs 080) was the least virulent (Table 3).

Aggressiveness

The disease severity was high (5.1-8.7) on susceptible check IS 18442, and on the 14 other differential lines low to high (1.1-8.5). The highest mean disease severity across the 15 sorghum lines was at Patancheru (5.2); followed by that at Surat (3.9), Udaipur (3.6), Pantnagar (3.5); Dharwad (2.8) and the lowest (1.5) was at Indore (Table 3).

To explore the nature and structure of variations and *to* quantify GxE interactions, hierarchical classification based on mean disease severity over years at each location was done and presented as dendrogram (Fig. 1). The point of maximum dissimilarity (E) divides the six locations into two major groups: i) Indore and Dharwad, and ii) Surat, Patancheru, Udaipur and Pantnagar. Further division occurred in the second group, making four distinct groups of the six locations.

Greenhouse evaluations at seedling stage

Virulence

In the greenhouse evaluations made on 21-day-old seedlings, the isolate from Dharwad (Cs 080) induced MR reaction on all the 16 lines, while those from Indore (Cg 047) and Patancheru (Cs 042) infected 15 lines each based on the number of lines showing S reaction. Indore isolate (Cs 047) was the most virulent, and Udaipur isolate (Cs 029) was the least virulent (Table 4). Th& differences in virulence across isolates, sorghum lines and isolates x sorghum lines were highly significant (P<0.001).

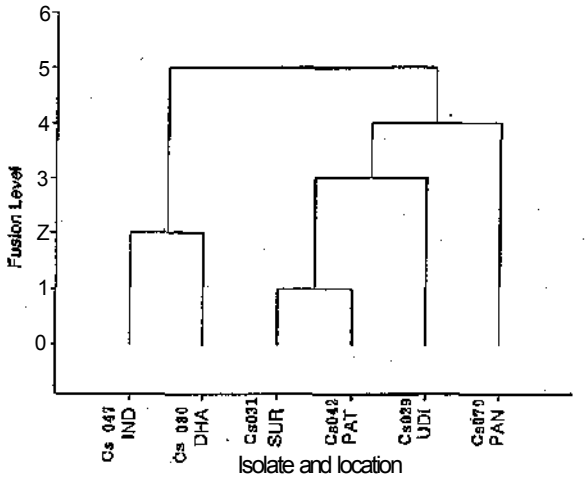

Fig. 1. Dendrogram based on aggressiveness (mean disease severity) of six populations of *Colletotrichum sublineolum* across 15 sorghum lines (soft-dough stage) from field evaluation at different locations for 4-5 years; locations (isolates) classified into four major groups: i) Indore and Dharwad; ii) Surat and Patancheru; iii) Udaipur and iv) Pantnagar

Aggressiveness

Disease severity varied across the isolates and sorghum lines. Six isolates developed high disease severity (score 5.2-7.3) on susceptible line IS 18442, and low to moderate (score 1.1-4.4) on the other 14 differential sorghum lines (Table 4). The mean disease severity induced across the sixteen sorghum lines was highest by Dharwad isolate (Cs 080), and lowest by Udaipur isolate (Cs 029). Indore isolate (Cs 047), and Patancheru isolate (Cs 042) had mean severity score 2.4 across the 16 lines. Dharwad isolate produced highest disease severity on IS 3552; Surat, Indore and Pantnagar isolates on IS 854; and Patancheru isolate on IS 2508.

Sorghum lines also differed significantly in disease severity across the six isolates. The highest mean severity developed on IS 18442, followed by IS 854, IS 3552, and the least on A 2267-2 and IS 6928. The differences in disease severity across the isolates and sorghum lines and for isolate x sorghum line interactions were highly significant (P <0.001).

Latent period

Latent period of the six isolates across the 16 sorghum lines varied from 3 to 6 days (Table 5). The shortest mean latent period (3.7 days) was of Cs 029 and longest (4.7 days) of Cs 042. The mean latent period ranged from 3.2-4.0 days on seven lines (IS 3758, IS 3552, IS 854, IS 1006, IS 12467, IS 17141, and IS 18442) while it was 4.2 to 5.7 days on the remaining- eight lines (IS 8354, IS 2508, IRAT 204, IS 6928, IS 6958, IS 18758, IS 18760, and ICSV 247). The differences in latent period for the isolates, sorghum lines and isolates x sorghum lines were highly significant (P<0.001).

Virulence index

Significant differences were observed in virulence index of the isolates across the sorghum lines. The nignest mean virulence index was of Indore isolate (Cs 047), followed by that of Surat (Cs 031) and Dharwad (Cs 080), and the lowest of Udaipur isolate (Cs 029). Low virulence index below 2.0 was observed on lines

Table 4. Mean disease reaction[a]/severity induced by six *Colletotrichum sublineolum* isolates when inoculated on 16 sorghum lines in greenhouse

Sorghum lines	Locations (isolate designation)						
	Indore (Cs 047)	Surat (Cs 031)	Dharwad (Cs 080)	Patancheru (Cs 042)	Pantnagar (Cs 079)	Udaipur (Cs 029)	Mean Severity
IS 8354	S/2.6	MR/2.8	MR/2.5	MR/2.3	MR/2.9	MR/2.1	2.5
IS 3758	S/2.8	MR/2.0	Mr/3.8	S/2.8	MR/2.8	MR/2.1	.2.7
IS 3552	MR/2.3	MR/3.1	MR/4.3	MR/2.5	MR/2.5	MR/2.7	2.9
IS 2508	MR/2.2	MR/3.3	MR/2.4	MR/3.1	MR/1.8	MR/1.6	2.4
IRAT 204	MR/2.4	R/1.0	MR/2.0	MR/1.8	R/1.2	R/1.0	3.0
IS 6928	MR/1.5	R/1.0	MR/2.0	MR/1.4	R/1.0	MR/1.3	1.4
IS 854	S/3.1	MR/4.4	MR/2.5	MR/2.7	MR/3.4	MR/2.3	3.2
IS 1006	MR/2.4	MR/3.0	MR/3.9	MR/2.2	MR/2.6	MR/2.6	2.3
IS 6958	MR/1.6	MR/3.2	MR/2.1	MR/2.0	MR/2.4	R/1.0	2.0
IS 12467	S/2.3	MR/2.1	MR/2.0	MR/2.4	MR/2.3	MR/2.0	2.2
IS 17141	MR/2.0	MR/2.3	MR/2.9	MR/2.0	MR/2.1	MR/2.5	2.3
IS 18758	MR/2.6	MR/1.5	MR/3.6	MR/2.4	MR/1.7	R/1.0	2.2
IS 18760	MR/2.2	MR/2.0	MR/2.0	MR/2.0	R/2.4	MR/2.3	2.1
ICSV 247	S/2.5	MR/1.1	MR/2.9	MR/2.4	MR/2.6	MR/2.2	2.9
IS 18442	S/6.3	S/5.3	MR/5.7	S/5.6	S/5.2	S/7.3	3.6
A 2267-2	R/1.0	R/1.0	MR/2.0	R/1.0	R/1.0	R/1.0	1.1
Mean sev	2.8	2.7	2.8	2.5	2.4	2.0	

[D]Based on 2 experiments

R=Resistant reaction, no symptoms or presence of chlorotic flecks; MR=Moderately resistant, red or necrotic lesions without acervuli; and S=Susceptible reaction, lesions with acervuli

A 2267-2, IRAT 204, IS 6958, IS 18758 and ICSV 247 (Table 6). The differences in virulence index for isolate x sorghum line were also highly (P<0.001) significant.

Hierarchical classification based on mean virulence index across the 16 lines is presented as dendrogram (Fig. 2). The point of maximum dissimilarity divides the six isolates into two major groups: i). Indore and Patancheru, and ii). Surat, Pantnagar, Udaipur and Dharwad. Further classification occurred in the second group, making four groups of the isolates.

Polymorphism among isolates using RAPDs

Of the 60 oligonucleotide primers (10-mer) tested only two showed polymorphism among the six isolates. The bands amplified were in the range of 1-3kb in size. The A-18 primer was able to classify the six isolates into four groups (Fig. 3 a). The isolate Cs 031 from Surat (Fig. 3a lane 2), Cs 080 from Dharwad (Fig. 3a lane 3) and Cs 029 from Udaipur (Fig. 3a lane 5) formed separate groups whereas Cs 047 (Fig. 3 a lane 1), Cs 042 (Fig. 3a lane 4) and Cs 079 (Fig. 3a lane 6) from

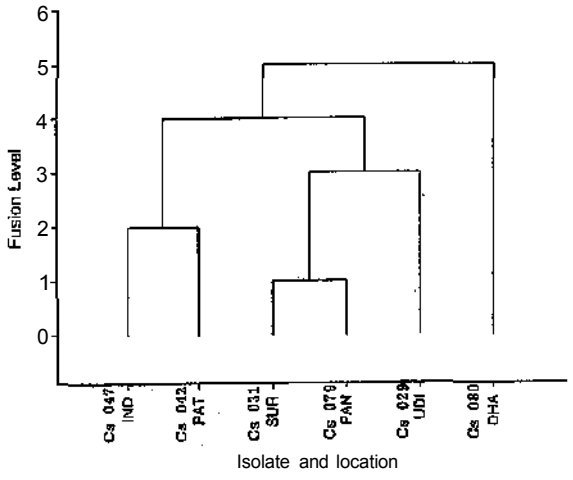

Fig. 2. Dendrogram based on virulence index of six populations of *Colletotrichum sublineolum* across 16 sorghum lines (seedling stage) from greenhouse-evaluation; isolates classified into four major groups: i) Cs 047 and Cs 042, ii) Cs 031 and Cs 079, iii) Cs 029 and iv) Cs 080.

Table 5. Mean latent period[a] (days) of six *Colletotrichum sublineolum* isolates inoculated on 16 sorghum lines in a greenhouse

Sorghum lines	Locations (isolate designation)						
	Indore (Cs 047)	Surat (Cs 031)	Dharwad (Cs 080)	Patancheru (Cs 042)	Pantnagar (Cs 079)	Udaipur (Cs 029)	Mean
IS 8354	5.0	5:0	4.0	6.0	5.0	6.0	5.2
IS 3758	,3.0	3.0	3.0	5.0	4.0	3.0	3.5
IS 3552	3.0	3.0.	3.0	4.0	3.0	3.0	3.2
IS 2508	5.0	4.0	5.0	5.0	5.0	4.0	4.7
IRAT 204	6.0	-	5.0	6.0	6.0	-	5.7
IS 6928	6.0	-	5.0	6.0	-	6.0	5.7
IS 854	3.0	3.0	6.0	3.0	3.0	4.0	3.7
IS 1006	5.0	3.0.	4.0	6.0	3.0	3.0	4.0
IS 6958	6.0	6.0	5.0	6.0	5.0	-	5.5
IS 12467	3.0	3.0	4.0	4.0	4.0	3.0	3.5
IS 17141	5.0	3.7	3.0	4.0	5.0	3.0	4.0
IS 18758	5.0	4.0	5.0	3.0	5.0	-	4.4'
IS 18760	3.0	4.0	5.0	4.0	5.0	4.0	4.2
ICSV 247	*5.0*	5.0	5.0	*5.0*	*5.0*	-	4.9
IS 18442	3.0	4.0	3.0	4.0	5.0	3.0	3.7
A 2267-2	-[b]	-	4.0	-	-	-	4.0
Mean	4.3	3.9	4.3	4.7	4.4	3.7	

SE : For Isolates x Sorghum lines = ± 0.04

[a]Means of two experimental runs.

[b]No infection (resistant reaction).

Indore, Patancheru and Pantnagar, respectively formed a single group. On the other hand the A-9 primer only formed three distinct groups (Fig. 3b), but it was able to distinguish Cs 042 from other isolates.

DISCUSSION

In this study, the sorghum lines exhibited differential disease reaction and variable disease severity across the six locations as well as across the respective iso-

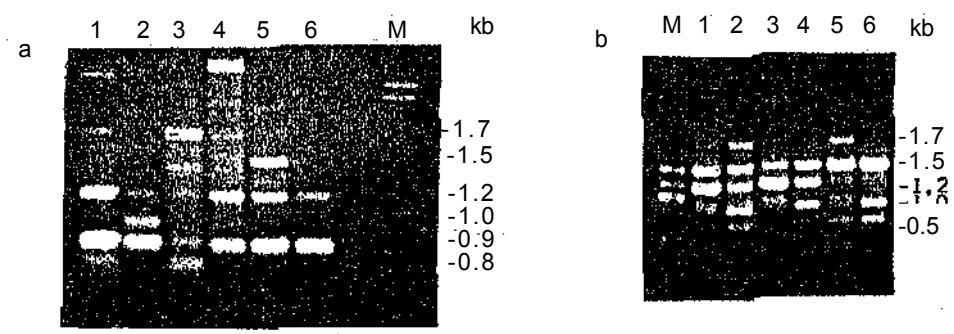

Fig 3. RAPD patterns of six *Colletotrichum sublineolum* isolates a) primer A-18, b) primer A-9. Lane 1-Cs 047(Indore); lane 2-Cs 031 (Surat); lane 3- Cs 080 (Dharwad); lane 4-Cs 042 (Patancheru); lane 5- Cs 029 (Udaipur); lane 6- Cs 079 (Pantnagar); and lane 7- M (marker). Primer A-9 identifies three groups: i) lanes 1,3 and 4; ii) lanes 2 and 5; and iii) lane 6 while primer A-18 identifies four groups: lanes 1,4 and 6; ii) lane 2; iii) lane 3; and iv) lane 5.

Table 6. Virulence index[a] (VI) of six *Colletotrichum sublineolum* isolates inoculated on 16 sorghum lines in a greenhouse

	Locations (isolate designation)						
Sorghum lines	Indore (Cs 047)	Surat (Cs 031)	Dharwad (Cs 080)	Patancheru (Cs 042)	Udaipur (Cs 029)	Pantnagar (Cs 079)	Mean
IS 8354	2.1	2.1	2.2	1.6	1.7	2.2	2.0
IS 3758	3.4	2.2	3.6	2.5	2.3	2.9	2.8
IS 3552	2.5	3.1	3.9	2.1	2.8	2.5	2.8
IS 2508	1.9	2.7	2.0	2.3	1.5	1.6	2.0
IRAT 204	1.6	1.0	2.2	1.8	1.0	1.1	1.4
IS 6928	1.2	1.0	1.6	1.2	1.2	1.0	1.2
IS 854	3.4	3.9	1.8	2.8	2.3	3.3	2.9
IS 1006	2.0	2.8	2.9	1.7	2.8	2.8	2.5
IS 6958	1.3	2.1	1.8	1.5	1.0	2.0	1.6
IS 12467	2.6	2.4	2.0	2.0	2.3	2.2	2.3
IS 17141	1.8	2.0	2.9	1.9	2.3	2.2	2.2
IS 18758	2.0	1.5	2.4	2.5	1.0	1.5	1.8
IS 18760	2.5	2.0	1.8	1.8	2.2	2.0	2.1
ICSV 247	2.4	1.8	2.1	2.0	1.2	2.0	1.9
IS 18442	6.7	6.5	3.3	5.7	6.3	4.1	5.5
A 2267-2	1.0	1.0	2.0	1.0	1.0	1.0	1.2
Mean	2.4	2.4	2.3	2.2	2.1	2.1	

SE : Isolates x sorghum lines = ± 0.16.

[a]Mean of two experimental runs, VI = [1 + (Virulence x Aggressiveness) x Latent period^{-1}]

lates from these locations in greenhouse testing. No line was scored uniformly R, MR or S across all the locations over five years. Highly significant (P<0.001) effects of environment (locations- pathogen population), sorghum lines, and environment x sorghum lines were observed. In this study we also included differential lines used by earlier workers (Casela *et al.,* 1992; Cardwell *et al.,* 1989; Pande *et al.,* 1991) to permit comparison of published race classification and to establish the usefulness of these as potential differentials. The pathogen populations from Indian locations seem to be different from those reported from other countries (Thakur, 1995).

On the basis of virulence analysis on sorghum lines both under field and greenhouse conditions, the six isolates could be differentiated into four major groups and six putative pathotypes (Table 7). The cluster analysis of virulence index, severity, and DNA polymorphism from RAPD analysis classified the six isolates into four distinct groups, although these were not similar. In greenhouse-evaluation-line-A2267-2-was-resistant-to-five isolates and moderately resistant to one isolate. Of the 15 sorghum lines, 11 exhibited moderate resistance to all the six isolates. In sorghum, anthracnose resistance is reported to be governed by a single dominant gene (Frederiksen and Rosenow, 1971), but no information is available on the number of genes imparting partial resistance. Further work is needed to establish the gene-for-gene relationship for designating these pathotypes as distinct races. Although six lines, IS 8354, IS 3758, IRAT 204, IS 854, IS 6928 and IS 18760, were found effective differentials, use of larger number of differentials is desirable to discern finer level of variability, and identify cultivar-specific virulence (Kelemu *et al.,* 1996). Such information would also be useful for assigning lineage amongst the diverse population, and identifying resistance genes and their allelism in the resistant sorghum lines. Line IS 18442, included as a susceptible check, developed high disease at all the locations and exhibited S reaction at five locations and MR at Dharwad and so it also acted as a differential line.

Disease reaction and disease severity scores of seedlings in greenhouse were different from those recorded at the soft-dough stage in the fields. Most lines scored MR reactions in greenhouse and S in field on

Table 7. Putative pathotyping of six *Colletotrichum sublineolum* isolates on six sorghum lines

Isolate		Sorghum lines						Putative pathotype
		IS 8354	IS 3758	IRAT204	IS 6928	IS 854	IS 18760	
Cs 042	F[1]	S	S	S	S	S	MR	P1
(Patancheru)	GH[2]	MR	S	MR	MR	MR	MR	
Cs 079	F	S	S	MR	S	S	S	P2
(Pantnagar)	GH	MR	MR	R	R	MR	R	
Cs 031	F	S	S	MR	MR	S.	S	P3
(Surat)	GH	MR	MR	R	R	MR	MR	
Cs 029	F	MR	MR	MR	S	S	S	P4
(Udaipur)	GH	MR	MR	R	MR	MR	MR	
Cs 047	F	R	S	R	S	R	S	P5
(Indore)	GH	S	S	MR	MR	S	MR	
Cs 080	F	MR	MR	MR	MR	MR	MR	P6
(Dharwad)	GH	MR	MR	MR	MR	MR	MR	

[1]Field evaluation for 4-5 year at 6 locations

[2]Greenhouse evaluation

adult plants. Adult sorghum plants are, generally more susceptible to anthracnose than seedlings (Pastor-Corrales and Frederiksen, 1980). The differences between field and greenhouse reactions may also be due to late appearance of disease in the field (at soft-dough stage) on certain lines which show delayed or slow disease development, called dilatory resistance (Casela and Frederiksen, 1995). Delayed symptom appearance in the field could also be due to prevalent of congenial conditions and high inoculum build up at post flowering stage of the crop.

Recent studies on other Indian isolates have revealed that some populations are heterogeneous, and significant morphological and pathogenic diversity was reported in progenies of mono-conidial isolates (Mathur *et al.,* 1997). Relatively higher genetic heterogeneity of Indian populations of *C. sublineolum* have also been observed through DNA analysis (Guthrie *et al.,* 1992). In our further studies at ICRISAT, Patancheru conducted under controlled conditions in the greenhouse, virulence variations have also been observed among C. *sublineolum* populations collected from farmers' fields within these individual states (Rao *et al.,* 1998). Inclusion of more locations within states for evaluation of this nursery is desirable, for more information on pathogenic variability in the populations of *C. sublineolum* prevalent therein. Analysis of the impact of prevalent weather conditions on disease expressions would also help-in-better understanding the response of the host lines.

Virulence index has been used to indicate the relative pathogenic potential of individual isolates by correlating the three independent parameters, latent period, virulence and aggressiveness. Analysis of variance showed that virulence index was more attributed to sorghum lines than to isolates or isolate x sorghum line interactions, and it was positively and significantly (P<0.001) correlated with disease severity.

Most of the sorghum lines used in this study developed MR reaction with variable aggressiveness, and six lines were potential differentials for discerning putative pathotypes. Some more lines with diverse anthracnose resistance are being evaluated for inclusion in ISAVN.

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