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Development of screening methods and identification of st resistance to anthracnose in sorghum [1]

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Abstract

Effective greenhouse- and field-screening techniques were developed to identify resistance to anthracnose (*Co raminicola*) in grain sorghum (*Sorghum bicolor*). In greenhouse screening, sorghum plants were spray-inoculated eaf stage with a conidial suspension (4×10^5 conidia ml^{-1}) of *C. graminicola*. Inoculated plants were incubate umidity chamber ($\geqslant$90% RH) for 24 h at 25–28°C and relocated to a greenhouse at 25 ± 2°C. Anthracnose develo cored 7–8 days after inoculation. In the field-screening technique, in every fifth row, a highly anthracnose-susceptib ine was sown as an infector row. Ten days later, test lines were sown between infector rows, and infector row noculated at the 6–8 leaf stage with either spore suspension or by dropping infected sorghum grains into the leaf umidity was provided by frequent overhead sprinkler or furrow irrigation. Test lines were scored for anthracnose t the hard-dough stage. Significant positive correlation ($r=0.88$, $P<0.001$) was found for anthracnose severi eedling screening in greenhouse and adult plant screening in the field. The field-screening technique was successfully o several locations in Africa and India. Thirty lines were selected from more than 13 000 sorghum germplasm ac dvanced breeding lines screened for anthracnose resistance, using the field-screening technique at Pantnagar (N between 1982 and 1991. They were evaluated in multilocational tests at hot spots in Burkina Faso, India, Nigeria, Z Zimbabwe for 1–10 years. Eleven lines (A 2267-2, IS 3547, IS 8283, IS 9146, IS 9249, IS 18758, SPV 386, PB 18601-3, PM 20873-1-3, and M 35610) showed stable resistance across these locations over the years. Some of lines are being converted into male-sterile lines through backcrossing with different sources of cytoplasmic male

Keywords: *Colletotrichum graminicola*; Screening technique; Sorghum; Stable resistance

1. Introduction

Anthracnose, caused by *Colletotrichum graminicola* (Ces.) G.W. Wills. (syn. *C. sublineola* Henn. in Kab. and Bubak.), is recognized as an important disease of sorghum (*Sorghum bicolor* (L.) Moench) in Africa, Asia and the Americas (Sutton, 1980; Alawode et al., 1983; CABI, 1988). The disease is more pr economically important under warm and hu ronments (Tarr, 1962; Pastor-Corrales and sen, 1980; CABI, 1988). Anthracnose app blight, stalk rot and head blight, and limit production in most sorghum-growing regi world (Luttrell, 1950; Harris et al., 1964; R al., 1968; Harris and Sowell, 1970; Bergu Leaf anthracnose is the most common and

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of the disease and can reduce yield of sorghum grain and fodder by 50% or more in susceptible cultivars during severe epidemics (Harris et al., 1964; Harris and Sowell, 1970; Powell et al., 1977; ICRISAT, 1984; Ali et al., 1987). A number of sorghum lines have been screened for resistance to anthracnose in the greenhouse and in the field (Harris et al., 1964; Harris and Johnson, 1965; Harris and Sowell, 1970; Ferreira and Warren, 1982) with variable results. Variations in results of these studies were attributed to factors such as inoculum concentration, inoculation method, plant age at inoculation, and environmental conditions. Studies of various factors influencing anthracnose infection and development are important to develop effective greenhouse- and field-screening techniques. Since 1982, efforts have been made at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India, to develop effective and simple greenhouse- and field-screening techniques for the identification of sources of resistance to anthracnose in sorghum. This paper describes studies leading to the development of screening techniques, and their use in identifying stable resistance to anthracnose in sorghum.

2. Materials and methods

2.1. Pathogen isolates and inoculum production

Single conidial isolates of *C. graminicola* obtained from the naturally infected sorghum leaves at ICRISAT Center (IC) farm, Patancheru, in southern India, and from the G.B. Pant University of Agriculture and Technology, Pantnagar, in northern India were used in greenhouse and field studies. A single isolate from among the 50 fast-growing single conidial isolates on oat-meal agar plates, each from IC and from Pantnagar, were selected for use in the various studies reported here.

2.2. Culture media and sporulation

Five culture media – sorghum leaf medium (SLM), oat meal agar (OMA), V-8 juice agar (VJA), sorghum grain medium (SGM) and potato-dextrose agar (Difco PDA) – were evaluated to identify the most suitable medium supporting adequate sporulation of *C. graminicola*. Sorghum grain medium was made by autoclaving 100 g sorghum seeds that had been soaked in water for 24 h at 121°C for 25 min. The flasks (250 ml) were then seeded with two mycelial plugs (1 cm^2) cut from the margins of the young, single conidial colonies. Similarly, the flasks containing SLM were inoculated. Petri dishes containing OMA, VJA and PDA were inoculated by placing 3-mm disks of the IC isolate of *C. graminicola*. Inoculated plates and flasks were incubated at 28°C with a 12-h photoperiod. Three replicates were used in each case. Sporulation on each medium was measured by suspending 1 g of culture with substrate from a 10-day-old culture in 10 ml sterilized distilled water. The suspensions were stirred with a magnetic stirrer and passed through four layers of cheese cloth to remove mycelial and media fragments. The concentrations of the resulting conidial suspensions were measured using a hemacytometer.

2.3. Inoculum concentration and anthracnose development

Five inoculum concentrations (4×10^3 to 4×10^7 conidia ml^{-1}) in sterile distilled water were made from a 10-day-old culture of the IC isolate on SLM. The suspensions were used to spray inoculate the top four leaves of the anthracnose-susceptible line IS 18442 at the 8–9 leaf stage in a greenhouse. Three pots containing three plants per pot were inoculated with each spore concentration. Inoculum suspensions containing Tween-20 (2 drops per 100 ml) were sprayed on plants with a hand sprayer until run-off. A randomized complete block design with six concentrations (including water spray as control) and two replications (2 pots per replication) was used. The inoculum was allowed to dry on the leaf surface before shifting the plants to the high humidity (>90% RH) chambers in a greenhouse. High RH was maintained for the first 18 h and later, only about 8 h per day for the next six days. The mean temperatures were between 28 ± 2°C and 22 ± 2°C, and the mean natural photosynthetically active radiation during the day (12-h photoperiod) ranged between 18 and 54 $\mu E\ m^{-2}\ s^{-1}$. In general, a warm and humid environment with diffused light was maintained throughout the experiment. Anthracnose severity was assessed 8–10 days after inoculation.

2.4. *Disease assessment*

For convenience, anthracnose severity was assessed either as percentage of leaf area covered with lesions or on a 1–5 rating scale, where 1 (highly resistant) = no visible lesions, 2 (resistant) = ≤5% leaf area with lesions, 3 (moderately resistant) = 6–20%, 4 (susceptible) = 21–40%, and 5 (highly susceptible) = >40% leaf area with lesions. In greenhouse experiments, only inoculated leaves were evaluated, while in the field experiments, the top four leaves were evaluated for disease severity.

2.5. *Plant growth stage at inoculation*

Seven sorghum lines whose resistance/susceptibility to *C. graminicola* was known from previous results, were grown in 15-cm plastic pots in the greenhouse. They included two highly resistant (A 2267-2 and IS 8283), two resistant (ICSV 173 and ICSV 247), two susceptible (ICSV 700 and ICSB 13) and one highly susceptible (IS 18442) lines. These lines were sown at 10 different dates at 7-day intervals to obtain 10 growth stages (2-leaf to boot-leaf stage) for inoculation at the same time. Plants were inoculated with conidial suspensions (4×10^5 conidia ml^{-1}) and incubated for 24 h in the high humidity chambers as described previously. A split-plot design with growth stages as main plots and sorghum lines as sub-plots was used. Plants (3 plants/pot) were placed randomly on a greenhouse bench at 25 ± 2°C, and disease severity (percentage leaf area infected) was recorded weekly for 2 weeks. The experiment was replicated three times. The average of the two scores were used for analysis.

2.6. *Temperature and disease development*

Pot-grown plants of three lines (A 2267-2, IS 8283 and IS 18442) were acclimatized at six different temperatures (10, 15, 20, 25, 30 and 35°C) for 24 h in the incubators. They were then spray-inoculated at the 8–9 leaf stage with an aqueous conidial suspension (4×10^5 conidia ml^{-1}) of the IC isolate, as described earlier. Plants were covered with wet polyethylene bags for 24 h after inoculation to provide high humidity, and incubated at the above temperatures for 14 days with a 12-h photoperiod. A randomized complete block design with three replications was used. Anthracnose severity (%) was scored 14 days after incubation.

2.7. *Leaf wetness duration and disease development*

Pot-grown plants of the above three lines were inoculated with *C. graminicola* IC isolate as described before and covered with wet polyethylene bags to provide leaf wetness for 2, 4, 6, 8, 24, and 48 h. Plants were shifted to a greenhouse at 25 ± 2°C after the required exposure periods. The experiment was in a factorial randomized complete block design with three replications. Disease scoring was done 14 days after inoculation.

2.8. *Spread of anthracnose and infector row technique*

Anthracnose is known to spread by rain-splashed conidia of *C. graminicola* (Nicholson and Warren, 1981). To estimate the distance and direction of spread from an infection focus, during the rainy season 1985, two plots of 33 × 33 m each were grown with a susceptible line ICSV 700. The plots were separated by growing six rows of a resistant sorghum line ICSV 173. In one plot anthracnose infection focus was established by inoculating plants in the center of the plot (4 × 4 m), and the other plot was maintained as a control. Severe anthracnose developed in the focus of inoculated plants 7 days after inoculation. Overhead sprinkler irrigation was provided in both plots for 1 h each in the morning and evening after disease symptoms appeared in the focus.

Plants were tagged for evaluation at 0.75-m increments, up to 10 m from the periphery of the focus at compass readings of 22.5°, 45°, 67.5° and 90°. Thus, there were five plant samples per plot for each direction and distance, and a total of 65 plants in each of the four sectors. Tagged plants were scored (weekly) for anthracnose severity up to physiological maturity. Results were used to determine dissemination and development of disease in different combinations of infector and test rows at Pantnagar.

Eight combinations of infector and test rows (1:1 to 1:8) were planted in a randomized complete block design with three replications. Plots were separated from each other by sowing a 4-m band of the anthracnose-resistant line, IS 3547, 20 days before the infector

rows. An anthracnose-susceptible line, IS 18442, was sown as infector rows 10 days before the test rows of a sorghum line, Bulk Y. Plants in the infector rows were inoculated by placing the anthracnose-infected grains in the whorl at the 6–8 leaf stage, and the disease severity in test rows was assessed at the hard-dough growth stage.

2.9. *Field screening techniques and their use*

Based on our results, an effective field screening technique was developed, which involved growing infector and test rows in the ratio of 1:4 as described before, during the rainy season (normally high RH > 80% and 25–35°C), and increasing humidity through irrigation when required. Using this technique, more than 13 000 sorghum germplasm accessions from various countries and breeding materials from IC were evaluated for anthracnose resistance in the field between 1982 and 1991 at Pantnagar. Lines found resistant (< 3 mean score) in 3–5 years of testing at Pantnagar were screened multilocationally for 1–2 years at Farako Ba (Burkina Faso), Bagauda (Nigeria), Golden Valley and Mansa (Zambia), and Henderson and Panmure (Zimbabwe), and IC.

2.10. *Greenhouse screening technique*

Sorghum lines found resistant in the multilocational screening were evaluated in the greenhouse at IC, where potted plants of test lines were spray inoculated with conidial suspension (4×10^5 conidia ml^{-1}) at the 8–9 leaf growth stage. Inoculated plants were incubated in the humid chambers as described earlier, and disease severity was scored 7–8 days after inoculation.

2.11. *Data analysis*

Depending on the experimental designs, analysis of variance was performed, using GENSTAT statistical package.

3. Results

3.1. *Effect of culture media on sporulation*

All five culture media supported conidial production of *C. graminicola*. SLM and OMA supported significantly higher spore production (5.8×10^7 and 6.4×10^6 conidia, respectively) per gram of medium than others. Potato-dextrose agar supported the least conidial production (4.1×10^3) (Fig. 1).

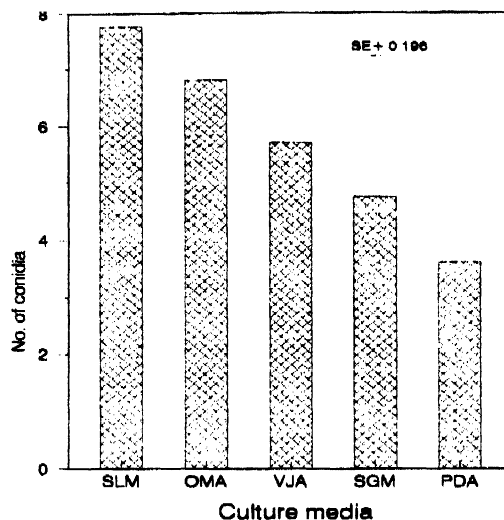

Fig. 1. Effect of different culture media, sorghum leaf medium (SLM), oat meal agar (OMA), V-8 juice agar (VJA), sorghum grain medium (SGM), and potato-dextrose agar (PDA) on conidial production (number g^{-1} substrate) of *Colletotrichum graminicola* at 28°C with a 12-h photoperiod.

3.2. *Effect of inoculum concentrations*

Anthracnose severity in IS 18442 increased from 2.3 to 4.8 (on a 1–5 scale) with increasing levels of inoculum concentration from 4×10^3 to 4×10^7 conidia ml^{-1} (Table 1). Concentrations of 4×10^5 conidia ml^{-1} and above produced significantly higher disease severity than the lower concentrations. No significant differences were observed in disease severity between 4×10^5 conidia ml^{-1} and the two higher concentrations.

3.3. *Effect of plant growth stage at inoculation*

Sorghum lines inoculated in the greenhouse at 10 different growth stages exhibited different anthracnose severity levels (Table 2). The 5- to 8-leaf stages were generally more susceptible, particularly in the three susceptible lines where anthracnose severity ranged from 19.57% in ICSV 700 at the 5-leaf stage to 51.41% in IS 18442 at the 8-leaf stage. Anthracnose severity in

esistant lines at 5- to 9-leaf stages varied from 0.11% n A 2267-2 to 2.9% in ICSV 173. In general, the esistance/susceptibility levels of lines did not change when inoculated at different growth stages (Fig. 2). Highly significant non-linear relationships between plant growth stage and infection severity were found or the susceptible ICSV 700 (r^2=0.84) and highly usceptible IS 18442 (r^2=0.91) lines. Effects of growth stage, lines, and line × growth stage interaction were highly significant ($P \leqslant 0.001$) for anthracnose everity. The 6–8 leaf stage which occurred in most ines 30–35 days after emergence was most sensitive o infection.

.4. *Effect of temperature*

Maximum anthracnose (⩾60% severity) developed n the highly susceptible line (IS 18442) at 25°C (Fig.). Temperatures below 15°C and above 30°C were letrimental. Highly resistant (A 2267-2) and resistant IS 8283) lines changed very little between 25 and 0°C, while in the susceptible line (IS 18442) ratings were 63% at 25°C to 45% at 30°C.

.5. *Effect of wetness duration*

The leaf wetness duration of 24–48 h produced significantly higher anthracnose than the duration of 2 to h in all three lines. Disease severity generally

able 1
ffect of different inoculum concentrations of *Colletotrichum graminicola* on anthracnose development in the sorghum line IS 18442

Inoculum concentration (conidia ml^{-1})[a]	Anthracnose severity (1–5)[b]
Control[c]	1.0
4×10^3	2.3
4×10^4	3.0
4×10^5	4.5
4×10^6	4.6
4×10^7	4.8
SE	±0.29

ained as water dilution from a 10-day-old growth on an autocla- sorghum leaf medium at 26±2°C.
erity scale of 1–5, where 1 = no visible lesions; 2 = <5% leaf with lesions; 3 = 6–20% leaf area with lesions; 4 = 21–40% leaf with lesions; and 5 = >40% leaf area with lesions. Each value e mean of three replications.
nts sprayed with sterilized distilled water.

Table 2
Effect of different plant-growth stages at inoculation on anthracnose on seven sorghum lines

Plant-growth stage	Anthracnose severity (%)[a]						
	A 2267-2	IS 8283	ICSV 173	ICSV 247	ICSV 700	ICSB 13	IS 18442
2-leaf	0.17	1.16	0.51	3.22	10.73	13.69	24.69
3-leaf	0.09	1.09	0.83	1.49	10.60	14.55	24.40
4-leaf	0.37	1.45	7.31	3.58	16.52	17.37	38.30
5-leaf	0.42	1.37	2.90	1.16	19.57	19.79	38.30
6-leaf	0.11	1.21	2.22	4.88	24.51	23.42	48.37
7-leaf	0.26	1.22	2.02	6.88	23.58	24.57	49.83
8-leaf	0.53	0.95	1.55	3.36	18.87	19.82	51.41
9-leaf	0.34	0.88	1.05	2.69	18.69	21.57	44.93
10-leaf	0.26	1.03	1.44	3.10	17.47	16.89	37.48
Boot leaf	0.25	0.78	1.34	2.57	12.84	17.68	34.45
LSD (P<0.05) = 4.02							

[a]Percentage leaf area with anthracnose lesions, mean of three plants from each of three replications.

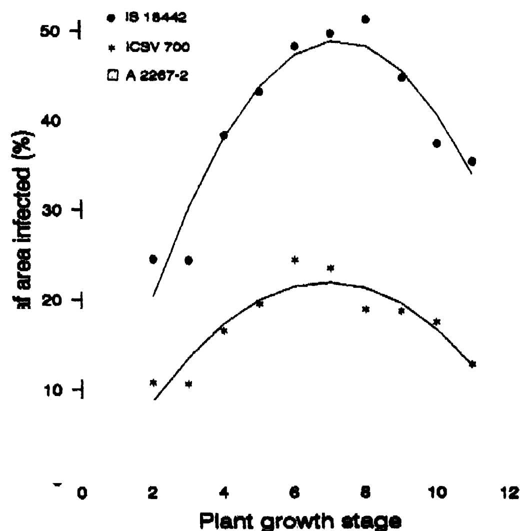

Fig. 2. Effect of plant growth (2- to 10-leaf) stages at inoculation on anthracnose development in sorghum lines highly susceptible (IS 18442), resistant (ICSV 700), and highly resistant (A 2267-2) to *Colletotrichum graminicola*. Regression equations for anthracnose development for IS 18442: $y = -5.6 + 15.12x - 1.05x^2$ (r^2 = 0.91**); for ICSV 700: $y = -4.33 + 7.61x - 0.55x^2$ (r^2 = 0.84**); and for A 2267-2: $y = -0.045 + 0.103x - 0.006x^2$ (r^2 = 0.23), where y = percentage leaf area infected and x = plant growth stage.

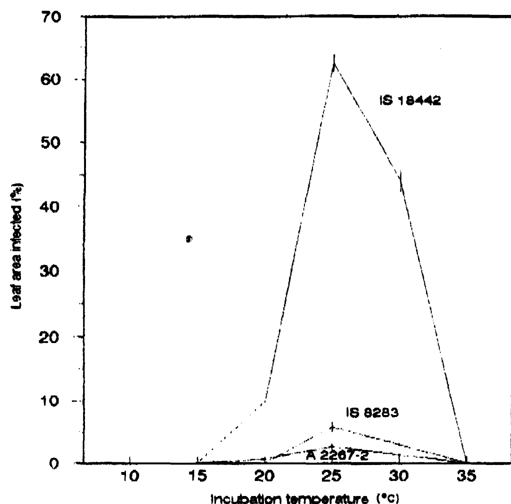

Fig. 3. Effect of post-inoculation incubation temperature on anthracnose development in three sorghum lines: highly susceptible (IS 18442), resistant (IS 8283), and highly resistant (A 2267-2) to *Colletotrichum graminicola*. Each data point represents the mean of three replications.

increased with the increasing periods of leaf wetness up to 24 h. There was no significant increase in disease severity beyond 24 h of wetness and plant growth was adversely affected. A linear relationship was found between wetness duration and anthracnose severity for the highly susceptible IS 18442 ($r^2=0.96, P<0.001$), resistant IS 8283 ($r^2=0.95, P<0.001$) and highly resistant lines ($r^2=0.93, P<0.001$) (Fig. 4). The rate of disease development, however, was much slower in the highly resistant and resistant lines than in the susceptible line.

3.6. Spread of anthracnose from an infection focus

Within 7 days of disease symptoms in the infection focus, anthracnose symptoms in ICSV 700 were observed downwind from the focus up to 3.25 m at the 13–14 leaf stage and up to 9.75 m at physiological maturity. Disease did not develop in the control plot.

3.7. Infector row technique

Anthracnose severity was higher ($\geqslant 4$ on a 1–5 rating scale) in the test rows nearest to the infector rows (1:1 to 1:4) than in those away from the infector rows. The 1:4 infector and test row arrangement was found most convenient and economical for effective field screening of sorghum for anthracnose.

3.8. Effectiveness of the field screening technique

Of more than 4000 sorghum germplasm accessions and 9000 breeding lines screened, 30 lines (12 germplasm accessions and 18 breeding lines) remained consistently resistant in 10 years of field screening at Pantnagar and 1 year at IC (Table 3). These entries, however, showed variable anthracnose severity in 1–2 years of testing at six locations in western and southern Africa. Two germplasm accessions (IS 3547 and IS 8283) and one breeding line (A 2267-2) showed <2 mean severity. Several entries showed differential reactions across locations/pathogen populations. For example, IS 7775, IS 12664-C and ICSV 173 were highly susceptible at Farako Ba and Bagauda (western Africa), but were resistant at Henderson and Panmure (southern Africa), and Pantnagar and Patancheru (India), while IS 18688 was resistant at Pantnagar but susceptible at Patancheru and Bagauda (Nigeria).

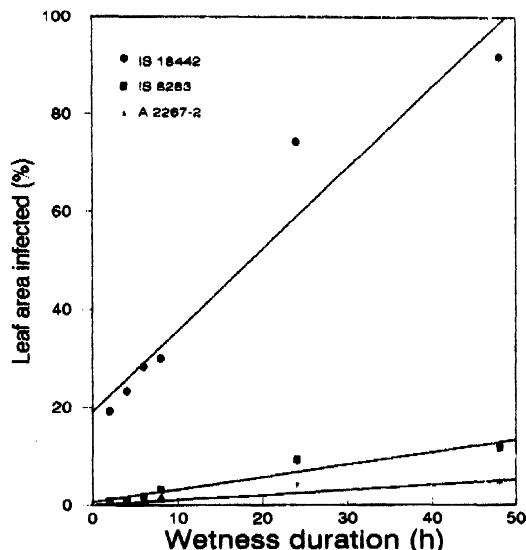

Fig. 4. Effect of post-inoculation wetness duration on anthracnose development in three sorghum lines: highly susceptible (IS 18442), resistant (IS 8283), and highly resistant (A 2267-2) to *Colletotrichum graminicola*. Regression equations for anthracnose development for IS 18442: $y=19.04+1.66x$ ($r^2=0.96^{**}$); for IS 8283: $y=0.64+0.25x$ ($r^2=0.95^{**}$); and for A 2267-2: $y=0.34+0.10x$ ($r^2=0.93^{**}$), where y = percentage leaf area infected and x = wetness duration in hours.

ble 3
nose severity[a] on sorghum lines in multilocational testing during 1982–1991

ghum line[b]	India		Western Africa		Southern Africa				Mean
	Pantnagar (10)[c]	Patancheru (1)	Farako-Ba (2)	Bagauda (1)	Golden Valley (2)	Mansa (2)	Henderson (2)	Panmure (2)	
2058	2.2	2.0	3.0	3.0	2.3	2.5	1.5	1.5	2.2
3413	1.8	4.0	3.3	3.3	2.5	2.7	1.8	1.3	2.6
3547	1.9	1.0	1.0	1.0	1.2	1.2	1.5	1.2	1.2
7775	2.3	2.7	4.8	4.0	3.8	4.0	1.3	1.2	3.0
8283	1.8	1.0	1.7	1.3	1.0	1.0	1.5	2.0	1.4
9146	1.8	2.0	1.2	1.0	2.0	1.8	2.3	2.2	1.8
9249	2.1	2.7	1.2	1.3	1.7	1.8	2.3	2.2	1.9
12662-C	2.2	3.0	5.0	5.0	3.7	4.3	1.2	1.2	3.2
14390	1.8	2.0	3.0	3.0	3.0	2.7	2.8	2.3	2.6
17141	2.4	1.7	3.3	3.7	2.0	1.8	2.0	1.8	2.3
18688	1.9	5.0	3.8	4.3	2.7	3.0	2.7	2.8	3.3
18758	2.5	2.0	2.0	2.0	2.5	2.7	1.8	1.8	2.2
SV 173	2.6	1.0	5.0	5.0	2.0	1.3	2.2	2.3	2.7
chV 2	2.6	2.7	4.0	4.3	3.5	4.5	3.3	3.2	3.5
RL 74/C-57	1.4	2.0	4.0	5.0	1.8	1.5	1.8	2.7	2.5
PV 386	2.6	2.0	1.8	2.0	2.8	2.2	2.3	2.3	2.2
YT-2ENo.1	2.0	2.7	3.8	3.7	2.7	4.2	2.3	2.5	3.0
YT-2ENo.6	2.0	2.7	3.0	3.7	2.7	3.7	2.5	2.5	2.8
YT-2ENo.77	2.2	2.3	3.3	3.3	2.8	2.2	2.7	2.7	2.7
B 8892-24	2.2	2.6	1.0	1.7	2.3	1.8	2.0	2.0	1.9
S 18601-3	2.2	2.0	1.7	2.0	2.5	2.2	2.3	2.3	2.1
M 2911	2.3	2.0	4.0	3.7	2.0	1.7	1.7	1.8	2.4
M 20873-1-3	2.1	2.3	1.2	2.7	1.0	1.0	1.3	1.7	1.7
I 36203	2.3	2.7	2.0	2.0	2.0	3.0	2.7	2.7	2.4
I 36248	2.3	2.3	2.0	2.3	3.0	3.0	3.3	3.3	2.7
I 35610	2.4	2.0	2.0	2.3	2.7	2.0	2.3	2.5	2.3
I 36172	2.3	2.3	3.2	3.0	3.7	3.7	3.7	3.7	3.2
I 36266	2.4	2.0	4.5	4.3	4.0	3.7	3.7	3.7	3.5
I 60328	2.1	2.3	4.2	4.0	2.3	4.8	2.0	2.0	3.0
2267-2	1.6	1.0	1.0	1.7	1.0	1.0	1.0	1.0	1.2
18442 Susceptible ontrol)	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
E	±0.454	±0.37	±0.35	±0.43	±0.45	±0.53	±0.49	±0.47	

On a 1–5 rating scale (as in Table 1). Infector and test rows were grown in a 1:4 ratio.
S = International sorghums; ICSV = ICRISAT sorghum variety; SPV = Sorghum preliminary variety; UchV = University Cholum (sorghum Tamil) Variety; PYT = Preliminary yield trial; PB = Pest-resistant breeding (borer) progenies; PM = Pest-resistant breeding (midge) progenies; PS = Pest-resistant breeding (shootfly) progenies; M = Grain mold-resistant breeding progenies; and A = Progenies from population reeding project.
Number of years of testing.

leven of these entries showed mean anthracnose ratngs of <3 across eight locations in India, Burkina aso, Nigeria, Zambia, and Zimbabwe (Table 4). hese entries represent diverse origins, plant characeristics and agronomic traits, and are thus potential ources of stable resistance to anthracnose.

3.9. Comparison of field and greenhouse screening techniques

Anthracnose severity ratings were generally higher in the greenhouse than in the field (Table 5). The disease severity ratings of 14 lines ranged from 1.0 to

Table 4
Plant characteristics and agronomic traits of 11 sorghum lines with stable resistance to anthracnose

Line	Origin	Race[a]	Plant type[b]	Grain color[c]	Days to 50% flowering	Plant height (m)	Anthracnose severity[d]
IS 3547	Sudan	C	P	RB	81	210	1.2
IS 8283	Uganda	C	P	RB	95	200	1.4
IS 9146	USA	C	P	W	71	220	1.8
IS 9249	Uganda	C	P	W	72	220	1.9
IS 18758	Ethiopia	GC	P	S	78	145	2.2
SPV 386	ICRISAT	–[e]	T	W	71	270	2.2
PB 8894-2	ICRISAT	–	T	W	76	255	1.9
PS 18601-3	ICRISAT	–	T	W	84	265	2.1
PM 20873-1-3	ICRISAT	–	T	W	70	190	1.7
M 35610	ICRISAT	–	T	W	71	245	2.3
A 2267-2	ICRISAT	–	T	W	72	200	1.1
IS 18442[f]	India	GD	P	Y	67	260	5.0

[a]C = Caudatum; GC = Guinea caudatum; GD = Guinea durra.
[b]P = Pigmented; T = Tan.
[c]RB = Red brown; W = White; S = Straw; Y = Yellow.
[d]On a 1–5 rating scale (from Table 4).
[e]Most of the ICRISAT Center bred sorghum used in this study were based on caudatum.
[f]Anthracnose-susceptible check.

3.3 in greenhouse screening and 1 to 2.7 in field screening. The susceptible control line IS 18442 scored 5.0 in both the greenhouse and field. The correlation coefficient for sorghum cultivars evaluated for leaf anthracnose in the greenhouse and field was highly significant ($r = 0.88$, $P = 0.01\%$).

Table 5
Anthracnose ratings (1–5)[a] of 15 sorghum lines in greenhouse and field screenings

Sorghum lines	Greenhouse	Field
IS 2058	2.0	1.7
IS 3547	1.3	1.0
IS 8283	1.0	1.0
IS 9246	2.3	2.0
IS 9249	2.7	2.3
IS 14390	2.3	2.0
IS 18758	2.7	2.0
SPV 386	2.7	2.0
PB 8892-2	2.7	2.3
PS 18601-3	2.3	2.0
PM 10873-1-3	2.7	2.3
M 36203	2.7	2.3
M 35610	3.3	2.7
A 2267-2	1.3	1.0
IS 18442 (susceptible control)	5.0	5.0
Paired t test value	0.04[ns]	

[a]A 1–5 rating scale (as in Table 1).
[ns]Nonsignificant (at $P < 0.05$).

4. Discussion

The components important for developing a disease screening technique are identification and determination of a suitable medium for mass multiplication of the pathogen, an optimum spore concentration for inoculation, optimal plant-growth stage at inoculation, and knowledge of the influence of environmental factors, particularly temperature and wetness duration, on disease development and spread. These studies addressed all these components leading to the development of effective field and greenhouse screening techniques for anthracnose in sorghum. Significant changes in any of these components might reduce the efficiency of the screening technique, including the failure of disease development.

Inoculum of the sorghum anthracnose pathogen can be easily maintained on sorghum leaves and artificial growth media, and the same substrates can also be used to increase the inoculum for inoculation (Harris et al., 1964; Harris and Sowell, 1970; Ferreira and Warren, 1982). However, SLM (Pande et al., 1991) is inexpensive and less cumbersome to prepare, and thus, the most suitable for mass multiplication of *C. graminicola* inoculum for a large-scale field screening.

High RH or leaf wetness from inoculation to disease development is critical for anthracnose development

though maximum anthracnose developed with 48 h wetness at 25°C, plants were etiolated and remained ak throughout the test. A leaf wetness period of 24 25°C was found most favorable for severe anthrac- development without detrimental effects on plant wth and a linear relationship was found between tness and anthracnose severity. In general, our ults agree with those of others who have reported nperatures between 25–30°C and a wetness duration 24–48 h favorable for anthracnose disease in maize eonard and Thompson, 1976), tomato (Dillard, 89) and barley (Skoropad, 1967). Our results sug- st that reliable field screening for sorghum anthrac- se can be done during the rainy season at locations here warm humid weather prevails throughout the wing season. However, more critical experiments needed to better understand the interaction of wet- ss and temperature for anthracnose infection and velopment to further refine the screening technique.

Anthracnose development in the early plant growth ges (2–3 leaf) was comparatively less than other owth stages, although we failed to observe any dif- ences in disease reactions in early growth stages hich was reported by Ferreira and Warren (1982). s plants were highly susceptible between 5- and 9- af stage, these growth stages can be conveniently used screen for anthracnose resistance in the greenhouse nd/or field.

The significant and high positive correlation between reenhouse and field screenings suggests that resis- ance to *C. graminicola* can be more easily and pre- isely determined under greenhouse conditions. Large cale screening at the seedling stage of segregating reeding populations for anthracnose resistance could e more economical and rapid in the greenhouse than the field. A significant contribution was the success- ul use of the infector-row based field screening tech- ique at several locations in Africa and India. The oculation techniques and infector-row arrangement an be modified, depending upon the need of the local nvironment.

Control of plant diseases through host-plant resis- ance mandates identifying stable resistance sources nd utilization of this resistance. A number of sorghum nes have shown stable resistance across diverse loca- ions in India, western, and southern Africa, although he number of years and test environments have been ew, particularly in Africa. Some of the best resistance sources (A 2267-2, IS 8283 and IS 3547) are already being utilized in ICRISAT and in Indian breeding programs. The line TRL 74/C-57, a maintainer of all four male-sterile cytoplasms (A_1, A_2, A_3, and A_4-Maladandi), and A 2267-2, a maintainer of A_3 and A_4 cytoplasms, are being converted into male-sterile lines through backcross breeding (ICRISAT, 1993). Test crossing and conversion of selected single plants with male-sterility maintaining ability have been undertaken and the progenies are in various stages of backcross generations.

Differential susceptibility of lines between locations can be attributed to variation in environmental parameters, particularly temperature and wetness, and also to the possible existence of variable pathotypes or physiological races of *C. graminicola* (Ali and Warren, 1987; Pande et al., 1991). Identification of lines with different resistance genes would be useful in designing a set of host differentials for use in pathogenic variability.

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