Evaluation of Field Screening Techniques for Resistance to Sorghum Grain Molds

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ABSTRACT

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Three methods of promoting grain mold development (overhead sprinkler irrigation on rain-free days, inoculation of panicles with mold-causing fungi, and bagging of panicles) for field screening of sorghum lines for resistance to grain molds were evaluated for two seasons (1982 and 1983) using genotypes susceptible and resistant to grain mold. Threshed grain mold ratings (TGMRs) of these genotypes in overhead sprinkled plots were not significantly different from ratings in unsprinkled plots when rainfall was abundant and frequent from flowering through grain maturity to harvest. In a season of low and infrequent rainfall, however, TGMRs were higher in sprinkled plots. Grain germination was significantly lower in sprinkled than in unsprinkled plots in the season of frequent rainfall. There were no significant differences in TGMRs between inoculated and/or bagged treatments and noninoculated and nonbagged treatment for susceptible genotypes in years of either frequent or infrequent rainfall. Inoculation and bagging increased moldiness in resistant genotypes but not to the threshold level of susceptibility. Screening nursery results (1983–1985) showed that mold resistance screening without inoculation and bagging of panicles appears feasible if overhead sprinkler irrigation is used from flowering to harvest.

Grain mold is an important disease of grain sorghum (*Sorghum bicolor* (L.) Moench) where it matures under warm and wet conditions (7). This disease is caused by fungal species belonging to several genera, notably *Fusarium, Curvularia, Phoma, Alternaria*, and *Cladosporium* (1,2,5,7). Most of these fungi are unspecialized or facultative parasites, and the predominant species vary with location, year, and season (7). Infection by mold-causing fungi may begin at anthesis (1), but in low frequency (2), and continues beyond maturity until harvest (1,2). The ideal method of controlling grain molds is to sow the crop so that it matures in dry weather after the end of the rainy season or humid period. This is not always practical, however, because of the need to maximize yields by early sowing and because of the unpredictability of rainfall, particularly in the semiarid tropics where most of the crop is grown. Chemical control of grain mold is not economical. The use of resistant cultivars offers the most practical and effective means of control.

Breeding of resistant cultivars requires the availability of screening methods to identify sources of resistance. Three methods have commonly been used (1,5): overhead sprinkler irrigation to wet panicles, inoculation to ensure pathogen presence, and bagging to enhance humidity in the panicles. The efficacy of each of these methods in the screening technique has not been well documented. The objectives of this study were to compare each of these methods with appropriate controls and to assess their usefulness in screening for grain mold resistance.

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MATERIALS AND METHODS

Evaluation of overhead sprinkler irrigation and panicle inoculation and bagging, 1982 and 1983. Experiments were conducted during the rainy seasons at ICRISAT Center, near Hyderabad, India. Seeds were sown in dry soil on 4-m-long ridges 0.75 m apart 3–6 days before the expected onset of the southwest monsoon. Seedlings emerged by 16 June in 1982 and 19 June in 1983 after the first rains on 12 June and 15 June, respectively, and were thinned 14 days later to 25–30 plants per row.

Seed was sown in a split-split plot design with five replications. Main plots were assigned to an irrigation treatment with two levels, i.e., with and without overhead sprinkler irrigation. Subplots were assigned to six sorghum genotypes, two susceptible to grain mold (CSH 1 and 2077B) and four resistant to grain mold (IS 8763, IS 9484, IS 9498, and IS 11227). Sub-subplots were assigned to pathogen inoculation and bagging of the panicle. In 1982, inoculation and bagging treatments were: 1) panicles inoculated and bagged, 2) panicles not inoculated but bagged, and 3) panicles neither inoculated nor bagged. In 1983, a fourth sub-subplot treatment was added: panicles inoculated but not bagged. Each sub-subplot was a single 4-m row with 25–30 plants.

Overhead sprinkling was provided from onset of flowering through grain maturity, i.e., black layer formation (3), to harvest about 2 wk later. The plots were sprinkled for 1 hr in the morning if it did not rain the previous night and same morning, and for an additional hour in the evening if it did not rain throughout the day. Sprinklers were arranged in a square grid pattern, the shortest distance between any two sprinklers being 12 m.

Inocula of the predominant mold-causing fungi at ICRISAT Center—*Fusarium moniliforme* Sheld., *F. pallidoroseum* (Cooke) Sacc., and *Curvularia lunata* (Wakker) Boedijn—were produced separately on autoclaved sorghum grain in 250-ml Erlenmeyer flasks incubated for 7–10 days at 30 C. Grains showing profuse sporulation of the pathogens were removed from the flasks, washed in a bucket of distilled water, and strained through two layers of cheesecloth. Spores and mycelial fragments in the resulting suspension were counted with a hemacytometer and appropriately diluted with distilled water to make nearly 15 L of inoculum suspension containing 3×10^6 spores and mycelial fragments per milliliter. Suspensions of the three fungi were made separately. The inoculum for inoculation treatments was formed by mixing equal quantities of each of the three fungal suspensions. Thus, the mixed fungal suspension had 1×10^6 spores and mycelial fragments of each fungus per milliliter. This inoculum was sprayed with a hand sprayer onto panicles when approximately 50% of the spikelets had flowered. Spraying was done carefully to wet the whole panicle to runoff. The noninoculated panicles were not sprayed with either the inoculum suspension or water. In the bagging treatment, paper bags were placed over panicles immediately after inoculation and removed 7 days later (1,5).

Flowering dates were recorded when anthesis progressed halfway down in nearly 50% of panicles in a plot. In each plot, 10 panicles that flowered on the same day were tagged for the various treatments described above. Grain was harvested 14 days after physiological maturity and the extent to which it was molded determined. Each panicle was hand-threshed separately, care being taken to minimize damage to the grain surface. A sample of about 35 g of

threshed grain was spread evenly in a 9-cm-diameter petri dish and scored visually for mold severity on a scale where 1 = no mold visible on grain surface, 2 = scant superficial mold growth and up to 10% of grain surface covered by mold, 3 = moderate mold growth and 11–25% of grain surface molded, 4 = considerable mold growth and 26–50% of grain surface molded, and 5 = extensive mold growth and more than 50% of grain surface molded. Three samples, separate for white and colored grain, with 10, 25, and 50% of the grain surface area molded were used as standards in assessing threshed grain mold ratings (TGMRs).

Grain germination was determined by incubating 100 grains from each sub-subplot in 9-cm-diameter petri dishes (10 grains per dish) lined with wet filter paper for 4 days at 30 C.

Data were analyzed using the GENSTAT statistical analysis program for split-split plot design. Since there were no significant differences within genotypes of susceptible and resistant groups, data of all genotypes in a group were averaged and analysis of variance performed separately for each group of genotypes. Standard errors were calculated and differences attributed at $P < 0.05$.

Evaluation of panicle inoculation and bagging in resistance screening nurseries, 1983–1985. The effect of inoculation and bagging and of overhead sprinkling on the severity of grain mold was also evaluated for three rainy seasons during 1983–1985 in a nursery consisting of 25 sorghum lines resistant to grain mold and three lines susceptible to grain mold. These lines were of early (less than 66 days to 50% flowering) and medium (66–80 days to 50% flowering) maturity. The nursery design was a split plot with two replications, and all genotypes in each maturity group were grown in a separate block. Entries were assigned to main plots and inoculation and bagging treatments were assigned to subplots. Each plot comprised two rows 4 m long. Ten panicles of one row were tagged, inoculated, and bagged, and 10 panicles of the other row were tagged but not inoculated or bagged. Other treatment details, observations, and grain mold assessment were as described above.

RESULTS

The weather data for the period from 50% flowering to grain harvest of the genotypes are given in Table 1. Rainfall, rainy days, and relative humidity were higher and therefore more favorable for grain mold development in 1983 than in 1982.

Effect of overhead sprinkler irrigation and panicle inoculation and bagging. In the relatively drier 1982 rainy season, TGMRs of susceptible and resistant genotypes were significantly greater, and germination of susceptible genotypes lower, in plots with sprinkler irrigation than in plots receiving only rainfall (Table 2). In 1983, TGMRs and germination were similar in both sprinkled and unsprinkled treatments for susceptible and resistant genotypes.

Under sprinkler irrigation, susceptible genotypes showed equally high mold ratings in all the inoculation and bagging treatments (Tables 3 and 4). In 1982, in the absence of sprinkler irrigation, inoculated and bagged panicles of susceptible genotypes had higher mold ratings than noninoculated and non-bagged ones. This difference was not observed in 1983, however. Whether sprinkler irrigation was used or not, resistant genotypes had higher mold scores when inoculated and bagged than when not inoculated and bagged (Table 3). The differences were not large, however, and the TGMRs of resistant genotypes did not exceed 3.0, the threshold level of resistance. Inoculation without bagging and bagging without inoculation produced as much grain mold as no inoculation and no bagging.

Evaluation of inoculation and bagging in resistance screening trials. In both inoculated and bagged and noninoculated and nonbagged treatments, the TGMRs of resistant lines were not above 3.0, the threshold level of resistance, whereas the TGMRs of susceptible lines ranged from 3.1 to 5.0 (Table 4). Although most of the resistant lines had higher TGMRs in the inoculated and bagged treatment than in the noninoculated and nonbagged treatment, TGMRs never exceeded 3.0, and the differences were mostly not significant. The exception was IS 14332, which had a rating of less than 3.0 in 1983 but of more than 3.0 in 1984 and 1985.

DISCUSSION

The availability of simple, reliable, and large-scale field screening techniques is necessary for progress in any resistance breeding program. For sorghum grain molds, resistance screening has been done under natural conditions by adjusting planting dates such that periods of grain development and

Table 1. Days to 50% flowering of mold-susceptible and mold-resistant sorghum genotypes and relative humidity, rainfall, and rainy days from flowering to grain harvest in 1982 and 1983

Genotype[a]	Days to 50% flowering		Relative humidity[b] (%)				Rainfall[c] (mm)		Rainy days[d] (no.)	
			Minimum		Maximum					
	1982	1983	1982	1983	1982	1983	1982	1983	1982	1983
CSH 1	56	58	63	72	89	94	212	558	27	38
2077 B	68	71	57	66	89	93	182	444	20	29
IS 8763	55	55	63	72	89	94	212	558	27	38
IS 9484	62	62	59	70	89	94	197	492	24	34
IS 9498	62	62	59	70	89	94	197	492	24	34
IS 11227	58	59	62	72	89	94	199	554	26	37

[a] CSH 1 and 2077 B, susceptible; IS 8763, IS 9484, IS 9498, and IS 11227, resistant.
[b] Mean of days from flowering to harvest at 14 days after physiological maturity (black layer formation).
[c] Total rainfall from flowering to harvest at 14 days after physiological maturity (black layer formation).
[d] Out of the 54 days from 50% flowering to harvest at 14 days after physiological maturity (black layer formation).

Table 2. Threshed grain mold rating (TGMR)[a] and percent germination (Germ) of grain of mold-susceptible and mold-resistant sorghum genotypes in two sprinkler irrigation treatments in 1982 and 1983

Sprinkler irrigation		Susceptible genotypes[b]		Resistant genotypes[c]	
		TGMR	Germ (%)	TGMR	Germ (%)
			1982		
Present		4.9[d]	23	1.6[e]	84
Absent		4.6	35	1.4	84
	SE	±0.03	±5.1	±0.06	±2.4
			1983		
Present		5.0	17	2.4	77
Absent		5.0	22	2.3	80
	SE	±0.00	±2.3	±0.04	±1.0

[a] Based on a scale where 1 = no mold visible on grain surface, 2 = scant superficial mold growth and up to 10% of grain surface covered by mold, 3 = moderate mold growth and 11–25% of grain surface molded, 4 = considerable mold growth and 26–50% of grain surface molded, and 5 = extensive mold growth and more than 50% of grain surface molded. Data recorded 14 days after physiological maturity.
[b] CSH 1 and 2077 B.
[c] IS 8763, IS 9484, IS 9498, and IS 11227.
[d] Mean of the two susceptible genotypes.
[e] Mean of the four resistant genotypes.

Table 3. Threshed grain mold rating (TGMR[a]) and percent germination (Germ) of mold-susceptible and mold-resistant sorghum genotypes in sprinkler irrigation and inoculation and bagging treatments in the 1982 and 1983 rainy seasons

Sprinkler irrigation	Inoculation	Bagging	Susceptible genotypes[b]				Resistant genotypes[c]			
			1982		1983		1982		1983	
			TGMR	Germ (%)	TGMR	Germ (%)	TGMR	Germ (%)	TGMR	Germ (%)
Present	Yes	Yes	5.0[d]	19	5.0	9	1.9[e]	78	2.8	68
	Yes	No	...[f]	...	5.0	16	...	...	2.2	81
	No	Yes	4.9	18	5.0	16	1.6	85	2.4	77
	No	No	5.0	31	5.0	28	1.5	88	2.2	83
Absent	Yes	Yes	4.9	30	5.0	9	1.8	83	2.7	73
	Yes	No	...	...	5.0	22	...	...	2.2	79
	No	Yes	4.4	33	5.0	23	1.3	81	2.3	81
	No	No	4.5	42	5.0	33	1.3	89	2.1	86
SE to compare two levels of:										
Sprinkler at same inoculation and bagging level			±0.06	±5.7	±0.0	±3.0	±0.12	±2.9	±0.07	±1.6
Inoculation and bagging at same sprinkler level			±0.06	±3.0	±0.0	±2.2	±0.10	±1.8	±0.07	±1.5

[a] Based on a scale where 1 = no mold visible on grain surface, 2 = scant superficial mold growth and up to 10% of grain surface covered by mold, 3 = moderate mold growth and 11–25% of grain surface molded, 4 = considerable mold growth and 26–50% of grain surface molded, and 5 = extensive mold growth and more than 50% of grain surface molded. Data recorded 14 days after physiological maturity.
[b] CSH 1 and 2077 B.
[c] IS 8763, IS 9484, IS 9498, and IS 11227.
[d] Mean of the two susceptible genotypes.
[e] Mean of the four resistant genotypes.
[f] Data not available (treatment not included in 1982).

Table 4. Days to 50% flowering (DTF), frequency of rainy days from flowering to harvest (% RF),[a] and threshed grain mold rating (TGMR)[b] of 28 genotypes in inoculated and bagged (IB) and noninoculated and nonbagged (NINB) treatments in resistance screening trials during 1983–1985 rainy seasons

Entry	1983				1984				1985			
			TGMR				TGMR				TGMR	
	DTF	% RF	IB	NINB	DTF	%RF	IB	NINB	DTF	% RF	IB	NINB
IS no.												
625	56	70	2.0	1.9	54	26	2.6	2.2	55	33	2.0	2.0
2821	55	70	2.1	1.9	54	26	2.2	2.0	56	33	2.0	2.0
2825	58	70	2.2	2.0	54	26	2.1	2.0	53	35	1.8	1.5
2867	56	70	2.0	1.9	56	22	2.0	2.0	54	33	2.0	1.6
3547	74	50	3.0	2.3	69	28	2.3	2.2	74	37	2.3	2.2
8545	59	69	2.0	2.0	55	24	2.4	2.0	57	33	2.0	2.0
8614	58	70	2.0	2.1	53	28	2.8	2.3	55	33	1.9	1.8
8763	58	70	2.0	1.9	56	22	2.1	2.0	53	35	2.0	1.8
8848	60	67	2.1	1.9	57	22	1.5	1.4	55	33	1.2	1.2
9353	61	65	2.0	1.8	64	28	2.1	2.2	62	37	2.0	2.0
9487	60	67	2.4	2.0	59	22	2.0	2.0	58	33	1.9	2.0
9498	67	61	2.6	2.1	66	28	2.4	2.0	66	33	2.0	1.4
10301	60	67	2.3	1.8	57	22	2.3	2.1	58	33	1.8	2.7
10892	63	65	2.0	2.0	63	26	2.0	2.0	58	33	1.9	1.8
11227	60	67	2.4	2.1	63	26	2.5	2.4	58	33	2.0	1.7
14332	71	54	2.6	2.9	69	28	3.4	3.4	69	33	3.3	3.2
14375	74	50	2.4	2.0	69	28	2.8	2.1	69	33	2.2	2.1
14380	72	52	2.1	2.0	69	28	2.9	2.2	68	31	2.6	2.4
14384	72	52	2.2	2.0	69	28	2.8	2.1	69	33	2.1	2.3
14387	71	54	2.0	1.8	68	28	2.1	2.0	68	31	2.0	1.9
14388	60	67	2.0	1.8	60	22	2.3	2.0	55	33	1.8	1.8
17141	71	54	2.7	2.3	69	28	2.3	2.2	70	35	2.1	2.3
18759	50	69	2.0	2.0	57	22	2.0	1.8	54	33	2.0	1.7
20620	59	69	2.0	1.8	57	22	2.1	2.3	52	33	2.0	2.0
21454	57	70	2.0	2.0	59	22	2.7	2.1	53	35	2.0	2.0
Susceptible controls												
CSH 1	57	70	5.0	5.0	59	22	4.9	4.7	56	33	4.4	4.4
SPV 104	67	61	5.0	5.0	64	28	5.0	5.0	60	35	5.0	5.0
E 35-1	78	46	3.7	3.5	79	26	4.8	4.5	79	31	5.0	5.0
	SE[c]		±0.3				±0.14				±0.20	

[a] Percentage of rainy days of total days from 50% flowering through maturity to harvest.
[b] Based on a scale where 1 = no mold visible on grain surface, 2 = scant superficial mold growth and up to 10% of grain surface covered by mold, 3 = moderate mold growth and 11–25% of grain surface molded, 4 = considerable mold growth and 26–50% of grain surface molded, and 5 = extensive mold growth and more than 50% of grain surface molded. Data recorded 14 days after physiological maturity.
[c] To compare two levels of treatments at the same level of genotype.

maturation coincided with expected periods of frequent rainfall (2,7). In years of frequent rains during the grain development stages, this technique succeeds because wet and humid conditions created by the rains promote sufficient grain mold pressure for screening. In the semiarid tropics, however, the length of the rainy season varies (6) and resistance screening may be unsatisfactory in years when rains are erratic or cease early. Provision of overhead sprinkler irrigation on rain-free days from flowering through grain maturity to harvest would overcome the problem of infrequent rains. For example, in 1982, a year characterized by low and infrequent rainfall, overhead sprinklers significantly increased mold severity. When ambient relative humidity is low, however, sprinkler irrigation may also be ineffective in maintaining sufficient humidity for grain mold development. Our experience at ICRISAT Center shows that sorghums that flower after the first week of September are difficult to screen despite our using sprinklers, because grain development occurs after the rains have ceased and humidity and temperature are too low for mold development.

Our data agree with an earlier work (1) that inoculating and bagging panicles can be used to screen for grain mold resistance in the absence of overhead sprinkler irrigation. Moreover, our data clearly show that if overhead sprinkler irrigation is used, both the inoculated and bagged treatment and the non-inoculated and nonbagged treatment were equally consistent in separating resistant genotypes from susceptible ones. Therefore, contrary to an earlier report (5), inoculation and bagging are not required for screening if overhead sprinkler irrigation is used. This is because conditions favorable for mold development are created during the susceptible panicle development stages by the presence of abundant naturally occurring inoculum (4) and the mold-conducive wet and humid environment produced by overhead sprinkler irrigation. Thus, the use of overhead sprinkler irrigation alone to wet the panicles from flowering to harvest is a simple, effective, and epidemiologically meaningful method to screen for mold resistance in the rainy season, and the laborious and time-consuming method of inoculation and bagging can be avoided.

We observed two problems with the use of inoculation and bagging, particularly in conjunction with the use of overhead sprinkler irrigation. First, the inoculum load during the early grain development stages increased tremendously inside the moist environment of the bags, as fungi rapidly multiplied on decaying anthers (Fig. 1A) that would have been blown away if the panicle had not been bagged. Inoculation and bagging imposed an abnormally harsh selection pressure rarely encountered in nature (Fig. 1B). Second, bagging created an excellent microenvironment for panicle-feeding insects, such as head bugs and earhead caterpillars (Fig. 1C), which are favored by excessive humidity. Insect excreta also served as good substrate for mold growth (Fig. 1D), thereby increasing inoculum. Grains damaged by insects showed more severe mold invasion than undamaged grains (R. Bandyopadhyay and H. C. Sharma, *unpublished*). Thus, insect damage and subsequent excessive mold growth significantly affect the TGMR. Bags were retained over the panicles for 7 days after inoculation (1,5). We did not attempt to determine the effect of the duration of bagging on mold severity and insect activity.

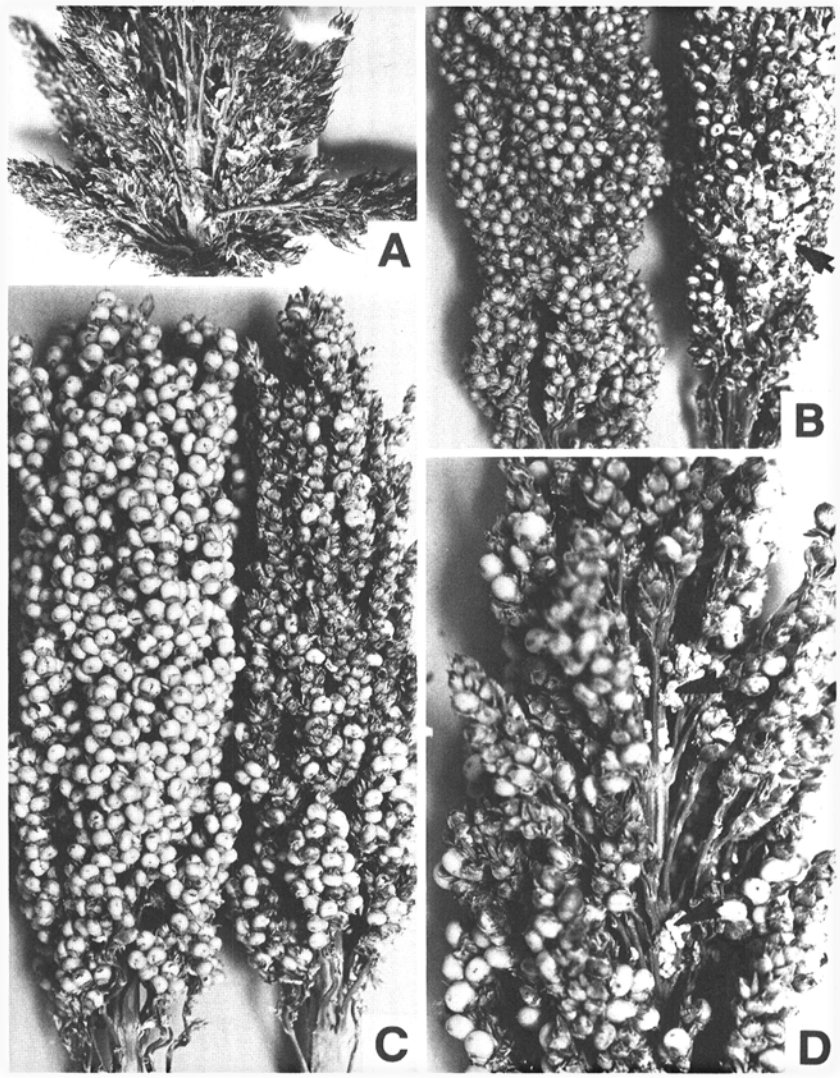

Fig. 1. Effect of inoculation and bagging on mold development: **(A)** Excessive mold growth on decaying anthers of an inoculated and bagged panicle causing rotting of developing grains. **(B)** (Right) Fungal mat (arrow) on an inoculated and bagged panicle; (left) noninoculated and nonbagged panicle. **(C)** (Right) Grains damaged by earhead caterpillars on an inoculated and bagged panicle at the hard dough growth stage; (left) noninoculated and nonbagged panicle. **(D)** Fungal growth enveloping insect excreta (arrow) in an inoculated and bagged panicle.

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