Table 4. Weather variables at four locations in India in the Sorghum Grain Mold Variability Nursery-2002 during rainy season 2002.

	No. of	Temperature <sup>1</sup> (°C)		Relative humidity <sup>1</sup> (%)		No. of rainy	Total rainfall
Location	days <sup>2</sup>	Minimum	Maximium	Minimum	Maximium	days	(mm)
Akola	48	18-24	25-39	21-98	53-98	12	166
Parbhani	42	17-33	29-37	29-76	68-100	5	22
Palem	59	12-22	26-36	26-85	66-95	6	92
Patancheru	34	14-27	26-38	25-85	79-99	6	69
1. Range.							

2. From flowering to PPM; sprinkler irrigation was provided at all locations except Palem during this period.

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# Sorghum Grain Mold: Resistance Stability in Advanced B-lines

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## Introduction

Grain mold resistance breeding in sorghum *(Sorghum bicolor)* at ICRISAT and in Indian national programs has focused on developing varieties, restorer lines, and hybrid seed parents utilizing resistance from germplasm

lines of diverse geographical origin. During the past few years, ICRISAT has developed a large number of highyielding, grain mold resistant B-lines using pedigree breeding with single- and three-way crosses and selecting the progenies under high disease pressure in field screenings (Reddy et al. 2000). Resistance stability of some selected elite B-lines was tested through a collaborative Sorghum Grain Mold Resistance Stability Nursery (SGMRSN) established in 2002. The results of trials conducted at diverse locations in India are presented.

## **Materials and Methods**

The nursery and its management. The SGMRSN is a collaborative nursery between ICRISAT and the AII India Coordinated Sorghum Improvement Project (AICSIP) of National Research Centre for Sorghum (NRCS) under the Indian Council of Agricultural Research (ICAR), coordinated by ICRISAT. The nursery was established at Akola, Parbhani, Palem and Patancheru in India. It included 43  $F_6$  to  $F_8$  male-sterility maintainer progenies from 17 crosses involving 20 B-lines, 8 inbred lines and one B-line population that had shown desirable agronomic traits and grain quality, moderate grain yield potential and moderate to high level of grain mold resistance at Patancheru, and two resistant and two susceptible check lines.

Each entry was grown in 2 rows, 4 m long in 2 replications. The recommended agronomic and cultural practices were followed at each location. Sprinkler irrigation was provided on dry days for 30 min per day in the evening during the flowering to post-physiological maturity (PPM) stages to maintain high relative humidity (RH) (>95%). No artificial inoculation was done with any mold fungi. Damage by insect pests, particularly by shoot fly, stem borer and head bug was minimized by timely application of pesticides.

#### **Data Recording**

**Plant traits.** Data were recorded for quantitative traits such as days to 50% flowering (DTF) and plant height at each location, and for other traits such as glume coverage, panicle compactness and grain color only at Patancheru as the latter traits are least influenced by weather factors.

Field grain mold severity. Five plants with uniform flowering in each row of the 2-row plot (10 plants  $plot^{-1}$ ) were tagged and the overall grain mold severity scores were taken on a progressive 1 to 5 scale (1 = no mold, 2 = 1-10%, 3 = 11-25%, 4 = 26-50% and 5 = >50% grains molded on a panicle) at PPM, 10 days after physiological maturity (PM).

Threshed grain mold severity. Threshed grain (20 g) from the bulk of 10 panicles per plot, spread in a petri dish was scored for mold severity on the above 1 to 5 scale, using a magnifying lens under proper lighting.

**Grain hardness.** From each plot 50 grains were subjected to grain hardness tester (Kiya Seisakusho Ltd., Tokyo, Japan) after the grain samples were dried to 7% grain moisture level.

### **Results and Discussion**

Variation in plant traits. Significant (P < 0.05) variations occurred for DTF and plant height between sorghum lines within and across locations; and also for glumes coverage, panicle compactness and grain color between sorghum lines. The mean and range of each trait for the 43 test lines and for resistant and susceptible check lines are given in Table 1. The mean DTF for 43 test lines varied from 68 (at Palem) to 77 (at Akola), and the mean plant height from 175 cm (at Parbhani) to 198 cm (at Patancheru) compared with 122-182 cm for susceptible checks and 233-281 cm for the resistant checks across locations.

The mean glumes coverage for test lines was 42% compared with 31% for susceptible check and 50% for

 Table 1. Plant traits and grain mold severity of 47 entries in the Sorghum (train Mold Resistance Stability Nursery during rainy season 2002 at four locations in India.

		A	kola	Pa	rbhani	Pa	lem	Patan	icheru
Parameter	Lines <sup>1</sup>	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Days to 50% flowering	Test lines	77	65-88	74	60-77	68	63- 73	73	60-85
	S lines	72	60-84	70	61-78	61	56-66	61	49-73
	R lines	85	81-88	77	76-77	67	61-72	75	72-78
	LSD (P<0.05)	3.1	-	1.0		2.1		3.4	-
Plant height (cm)	Test lines	190	126-261	175	117-238	177	148-245	198	133-245
	S lines	122	119-125	128	127-129	182	178-185	130	120-140
	R lines	273	190-355	281	207-338	233	208- 258	253	190-315
	LSD (P<0.05)	25.4		9.8	-	14.1	-	23.3	-
Grain hardness <sup>2</sup> (kg seed ')	Test lines	6.6	4.9-8.0	5.4	2.2-8.2	6.8	4.1-11.4	2.9	0.9-6.4
	S lines	5.8	4.9-6.6	4.5	4.1-4.9	6.3	4.6-8.0	2.0	1.6 2.0
	R lines	7.8	7.8	10.0	9.0-10.9	9.7	8.5-10.8	7.2	6.2-8.1
	LSD ( <i>P</i> <0.05)	1.04	-	0.74	-	3.1	-	2.2	
Glumes coverage <sup>2</sup> (%)	Test lines							42	25-75
	S lines							31	25-38
	R lines							50	25-75
	LSD ( <i>P</i> <0.05)							25.7	
Grain mold severity at PPM	Test lines	2.6	1.8-3.3	3.9	2.8-4.8	1.8	1.2-2.7	2.6	1.2-3.8
	S lines	4.4	4.0-4.7	4.4	4.1-4.8	3.7	3.6-3.8	4.8	4.6-4.9
	R lines	1.5	1.3-1.7	3.4	3.3-3.6	1.7	1.0-2.4	2.1	1.0-3.2
	LSD ( <i>P</i> <0.05)	0.54	-	0.45	-	0.20	-	0.34	
Grain mold severity on threshed grain	Test lines	2.5	2.0-3.5	4.4	3.4-5.0	1.8	1.1-2.9	2.8	1.3-3.7
	S lines	4.0	4.0	5.0	5.0	3.9	3.8-3.9	5.0	5.0
	R line	1.5	1.0-2.0	3.3	3.0-3.5	1.8	1.1-2.4	1.7	1.0-2.3
	LSD (P <0.05)	0.70		0.20	-	0.16	-	0.37	-

1. Test lines 43. S (susceptible) lines 2. R (resistant) lines 2.

2. Observations of samples from the four locations were recorded at Patancheru.

Table 2. Agroi	nomic traits and grain mold severity of selected sorghu	m B-lines aç	ross four lo	cations in I	adle dariag	rainy seas	on 2002'.			
		Days to	Plant		Glumes	Panick	100-grațin	Grain	Mold se (1-5 s	verity <sup>2</sup> cale)
Entry	Pedigree	50 <del>4</del> Nowering	height (cm)	Grain color	coverage (Ŧ)	campact- ness'	mass (g)	bardness (kg seed")	PPM PPM	Threshed grain
SGMR 14	(3CSB 392 × SP 1792-11-3-1-1-1-1-1	76	192	Red	50	sc	2.17	5.8	2.2	2.4
SGMR 19	(()S 13817 × ICSB 270) × ICSB 392:Red)-2-1-1-1-1	75	162	Red	50	U	2.58	6.1	2.1	2.4
SGMR 20	((IS_13817 × JCSB_270) × JCSB_392:Red)-2-1-1-2-1	76	148	Brown	38	ç	1.75	6.5	2.2	2.4
SGMR 21	((IS 13817 × JCSB 270) × ICSB 392:Red)-2-1-1-4-1	76	175	Brown	50	SC	2.20	5.2	2.3	2.5
SGMR 23	(IS 86]4 × ICSB 293)-2-1-1-1-4-1	<b>7</b> 6	161	Brown	75	S	1.59	9.E	2.4	2.4
ICSB 383	(ICSB 17 × IS 10646)-5-1-2	74	221	Вточи	38	SC	1.86	4.4	1.8	2.1
ICSB 392	(ICSB 37 × IS 10475B)-2-2-1-3-1	73	183	Red	50	SC	2.51	5.9	2.3	2.5
ICSB 403	(ICSB 42 × IS 23585)-1-7-1-2-2	5	176	Brown	38	S	2.28	4.4	2.3	2.4
Bulk Y'		57	138	White	36	L	3.02	4.9	4.4	4.4
IS 14384'		76	317	Red	75	ر	1.68	8.4	1.7	1.5
Mean		5	186	ı	42	ų	2.33	5.5	2.6	2.9
LSD (P <0.05	()	<del>د</del> ا	Ð. 11	I	15.7	٩	0.38	1.69	16'0	0.97
1. Mean of four	r locations except grain color, glumes coverage and 100-grain mi	us that were r	ecurded only -	at Patancheru	. Grain bardn	ess of the su	nples from th	te foar location	i osht saw si	recorded at

Patancheru.

1 = No mold, 2 = 1-10% mold. 3 = 11-25% mold. 4 = 25-50% mold. and 5 = >50% grains molded on panicle; PPM = post-physiological maturity.
 3. SC e semi-compact; C = compact; L = koose.
 4. Susceptible check.
 5. Resistant check.

the resistant check. Thirty-eight of the 43 lines had semicompact panicle, 3 had compact, and 2 had loose panicles, compared with loose to semi-compact panicles of the resistant and susceptible check lines. Grain color varied from white (17 lines), red (17 lines) and brown (9 lines). The susceptible check lines had white grain while one resistant check line had white grain and the other red grain. These plant traits have been shown to be associated with grain mold resistance in several studies (Esele et al. 1993, Audilakshmi et al. 1999, Rooney and Klein 2000).

**Variation in grain hardness.** Significant variations were recorded for grain hardness between entries within and across locations (Table 1). The mean grain hardness of the 43 test lines varied from 2.9 kg seed<sup>-1</sup> (at Patancheru) to 6.8 kg seed<sup>-1</sup> (at Palem) while it was 7.2-10.0 kg seed<sup>-1</sup> in the resistant check lines and 2.0-6.3 kg seed<sup>-1</sup> in susceptible checks across locations. Grain hardness is considered to be one of the most desirable traits often positively correlated with mold resistance (Audilakshmi et al. 1999). Most white- and bold-grain sorghums have soft endosperm and thus they are more susceptible to mold fungi than the red-grain or brown-grain sorghums that have smaller grain size (Reddy et al. 2000).

**Grain mold severity scores.** Grain mold severity of the 43 test lines differed significantly between and within locations. The mean grain mold severity at PPM varied from 1.8 (at Palem) to 3.9 (at Parbhani), and that of threshed grains from 1.8 (at Palem) to 4.4 (at Parbhani), compared with 1.5 to 3.4 in resistant checks and 3.7 to 4.8 in the susceptible checks across locations (Table 1). Generally, mold severity scores of sorghum lines at PPM and of threshed grains were similar at a location. Pooled ANOVA for threshed grain mold severity indicated highly significant (P < 0.001) effects of location, entry, and location x entry interaction; of these, location effect was more pronounced than the other two.

**Resistance stability.** Of the 43 test lines, 8 (SGMRs 14, 19,20,21, 23, ICSBs 383, 392 and 403) recorded 1 to 2.5 mold severity across locations compared with 4.4 on the susceptible check Bulk Y and 1.6 on the resistant check IS 14384 (Table 2). These eight lines are derivatives from six crosses involving 12 B-lines and inbreds. The different traits in these lines varied: DTF from 70 to 76 days; plant height from 148 to 221 cm; grain color from red to brown; glumes coverage from 38 to 75%; panicle compactness from compact to semi-compact; 100-grain mass from 1.59 to 2.58 g; and grain hardness from 3.8 to 6.5 kg seed<sup>-1</sup>. These B-lines that have stable resistance and desirable agronomic traits could be converted into A-lines for utilization in hybrid programs.

Relationships between agronomic traits and mold severity. Significant negative correlations were found between DTF and threshed grain mold severity, and between plant height and threshed grain mold severity. In late-maturing tall lines, grain mold severity tends to decrease probably because of relatively dry period prevailing towards the end of the crop season resulting in less humid microclimate than that of shorter plants which are usually shaded by taller plants. Rooney and Klein (2000) reported strong negative correlation between plant height and grain mold incidence, and found association between quantitative trait loci (QTLs) for plant height and grain mold incidence on linkage groups D and E. In this study, however, a negative and weak correlation was found between grain hardness and threshed grain mold severity, which was unexpected. This could be partly due to the equipment and method used to determine grain hardness.

Grain mold severity scores at PM (not reported here) were usually different from those at PPM and that of threshed grain, but those at PPM and of threshed grain were similar. We, therefore, suggest that mold scoring should be done twice; first, at PM in the field and second, of threshed grain in the laboratory. However, it is important to determine the right PM stage (black-layer formation) for each sorghum line in the field nursery to record the data at the right time. We suggest that when more than 50% grains in the middle portion of a panicle of most plants in a line show black-layer formation, this should be considered as PM. The nursery will be conducted again during 2003 crop season to confirm the results.

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## Reduction of Sorghum Seedling Vigor by Inoculation with *Fusarium thapsinum* and *Curvularia lunata* at Anthesis

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The two most common fungi associated with molded seed of sorghum *(Sorghum bicolor)* are *Fusarium thapsinum* (FT) *(F. moniliforme* mating group F) and *Curvularia lunata* (CL) (Bandyopadhyay et al. 2002, Prom et al. 2003). These fungi have proven quite aggressive in the developing kernel. These fungi typically cause discoloration, molding on the grain surface, endosperm degradation, reduced grain-filling ('small-seed syndrome' in the case of *Fusarium* spp) and reduced germination (Bandyopadhyay et al. 2002, Prom et al. 2003). Reduced seedling vigor, as measured by seedling height, however, has not been reported in association with grain molded seed.

Earlier reports have indicated that important levels of infection occur at or near anthesis. Nene (1975) inoculated sorghum panicles with *Fusarium* and *Curvularia* spp at varying times after panicle emergence and found the greatest mold occurring when panicles were inoculated 3-7 days after emergence from the boot. Rao and Williams (1977) conducted a similar study and found that the greatest reduction in kernel viability occurred when panicles were inoculated at anthesis compared with later inoculations.

Four sorghum cultivars were tested in a recent study to determine the effect of early inoculations on vigor of surviving kernels. Sureno is a white-tan, zerazera variety that is resistant to grain mold. RTx2911 is highly grain mold resistant, derived from a SC719-11E\*SC650 cross and is a bright-red *kafir-caudatum* plant type. SC170 is a white-grained, white-pigmented *caudatum* line which is moderately susceptible to grain mold and moderately resistant to grain weathering (personal observation). RTx430 is a highly susceptible variety derived from SC170 and RTx2536.

Inoculations of sorghum panicles were made precisely at anthesis with FT or CL at 1 x  $10^6$  conidia ml<sup>-1</sup> suspended in a 0.5% gelatin solution and measured using a hemacytometer. Mock-inoculations (control) were

Table 1. Seedling height (mm) of four sorghum lines inoculated with *Fusarium thapsinum* (FT), *Curvularia lunata* (CL), or mock-inoculated with water (control) at anthesis.

Genotype	Treatment	14 DDP <sup>1</sup>	% Control <sup>2</sup>	17 DDP <sup>1</sup>	% Control'
Sureno	Control	232.5	-	377.6	-
	FT	230.1	98.9 ab	352.7	93.4 a
	CL	221.8	95.4 b	344.2	91.1 a
SC 170	Control	244.8	-	361.2	-
	FT	155.6	63.6 d	288.5	79.9 b
	CL	85.3	34.8 f	160.6	44.5 c
RT x 2911	Control	182.2	-	275.6	-
	FT	185.7	101.9 a	255.1	92.6 a
	CL	158.8	87.2 c	220.5	80.0 b
RTx430	Control	212.7	-	298.5	-
	FT	100.5	47.2 e	146.2	49.0 c
	CL	63.4	29.8 g	150.8	50.5 c

1. A total of 30 seedlings were harvested and measured in each genotype x treatment category at 14 and 17 days post-planting (DPP) with the exception of RTx2911 where only 20 seedlings were harvested.

Seedlings were measured (in mm) from the crown to the tip of the first leaf as an estimation of seedling vigor.

2. Overall % Control values for 14 DPP are not significantly different if followed by the same letter (P < 0.05, Bonferroni's test).

3. Overall % Control values for 17 DPP are not significantly different if followed by the same letter (P < 0.05. Bonferroni's test).