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Variation in occurrence and severity of major sorghum grain mold pathogens in India

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ABSTRACT: Variation in occurrence and severity of pathogenic grain mold fungi were studied through a collaborative Sorghum Grain Mold Variability Nursery (SGMVN), consisting of 12 sorghum genotypes, that was established at five locations (Akola, Parbhani, Palem, Patancheru and Surat) during the three rainy seasons 2002-2004. Grain mold infection severity by the major pathogens was recorded at physiological maturity and on threshed grain, and grain colonization was measured using the blotter method. Among the fungal species, *Fusarium* spp., *Curvularia lunata, Alternaria alternata* and *Phomasorghina,* in receding order, were predominant across locations and genotypes. Analysis of variance indicated highly significant effects of location, year, genotype and their interactions on grain mold seventy and grain colonization by the four fungi. Grain colonization was highest by *Fusarium* spp. at Parbhani (54%), by *C. lunata* at Surat (45%), by *A. aiternata* both at Parbhani (25%) and Patancheru (23%), and by *P. sorghina* at Patancheru (17%). Four of the sorghum genotypes (ICSV 96101, ICSV 95001, SPV 351/ICSV 1, and ICSV 91008) showed tolerance to mold infection (≤3.0 severity) and these could serve as sources of grain mold resistance.

Key words: Sorghum, grain mold pathogens, host resistance

Grain moid of sorghum (*Sorghum bicoior* (L.) Moench), caused by a complex of pathogenic and saprophytic fungi, is the greatest constraint for optimum grain yield and quality (Williams and Rao, 1981; Bandyopadhyay *et al.,* 2000). Early-maturing, high yielding hybrids and improved varieties that are grown during the rainy season are particularly more vulnerable. Grain molding and grain weathering are two successive events occurring on sorghum caryopsis. Grain molding occurs during anthesis to physiological maturity while grain weathering succeeds the grain molding in which case saprophytic fungi continue to grow on matured grains (Waniska, 2000). Both these events are greatly favored by the prevailing high humidity and panicle wetness during the rainy season resulting in grain discoloration and considerable reduction in grain quality and seed viability (Waniska, 2000). Such grains are not suitable for food and feed and thus get poor market price.

Major fungi that are associated with early infection events are species of *Fusarium, Curvularia lunata, Alternaria aiternata* and *Phoma sorghina* (Indira *et al.,* 1991; Bandyopadhyay *et al.,* 2000; Thakur *et al*., 2003). Girish *et al.* (2004b) detected the seedborne nature of the above fungi with infection appearing in seed coat, endosperm and embryo. Damage resulting from these early infection events includes reduced kernel development, discoloration of grain, colonization and degradation of endosperm and germ, decreased grain density, germination, seedling vigor, and possible mycotoxin contamination particularly with *Fusarium* species (Leslie *et al.,* 2005; Nayi. *et al*., 2005). Knowledge of variation in occurrence of the major pathogenic fungi in a given sorghum-growing area is critical to study host-pathogen interaction and identify genetic resistance

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to individual and multiple pathogens involved with sorghum grain mold complex. This study was conducted to determine the major pathogenic fungi involved in the mold complex, and their variation and severity of occurrence in major sorghum growing areas of India.

MATERIALS AND METHODS

Sorghum genotypes and test locations

Ten sorghum genotypes that had shown moderate to high level of resistance to grain mold at ICRISAT, Patancheru, India and possessed desirable agronomic traits, and the two check genotypes a resistant and a susceptible (Table 1) were evaluated through a collaborative ICRISAT-ICAR (AICSIP) Sorghum Grain Mold Variability Nursery (SGMVN). The SGMVN was established at five grain mold hot spots - Akola and Parbhani (Maharashtra state), Paiem and Patancheru (Andhra Pradesh state) and Surat (Gujarat state) in India during the three rainy seasons 2002 -2004, except Surat where it was conducted only for two seasons, thus making a total of 14 environments. The details of geographical locations and variation in weather factors (temperature, relative humidity and rainfall) over three crop seasons are presented in Tabie 2.

Each genotype was grown in two rows, 4m long with two replications in a randomized block design. Local crop production and protection (except for grain mold) practices with protective irrigation were followed to raise a good crop. Sprinkler irrigation was provided on dry days during 5 to 6 PM to maintain high relative humidity (>95%) from flowering to physiological maturity (PM) at Patancheru and Parbhani; water was sprayed on the panicles at Akola, and panicles were covered with pre-wetted polythene bags at Surat for grain mold development. Five plants with uniform flowering in each row of a 2-row plot (10 plants per plot of each genotype) were tagged for recording the grain mold severity. All the tagged panicles were harvested, threshed, and their grain mold severity was recorded.

Grain mold scoring

Two grain mold severity scores were taken, first at physiological maturity - the panicle grain mold rating (PGMR) on a progressive scale of 1 to 5 (where, 1 = no mold, 2 =1-10%, 3 =11-25%, 4 = 26-50% and 5 >50% grains molded on a panicle) on the tagged panicles in the field and second on the bulked threshed grain - the threshed grain mold rating (TGMR) of each genotype after harvesting, threshing and drying the grain. For this purpose, threshed grain (20g) from the bulk of 10 panicles per plot spread in the Petridish was scored for mold severity using the above 1 to 5 scale, with the help of a magnifying lens under proper lighting.

Table 1. Genotypes used in Sorghum Grain Mold Variability Nursery, 2001-2003

Genotype	Known reactions[a]	Origin	Remarks
IS 18758C-618-2	R	Ethiopia	Zera zera conversion
IS 18522	MR	U S A	Germplasm genotype
ICSV 96101	MR	India	ICRISAT designated variety
CS 3541	MR	India	NRCS designated R-genotype
Sepon 78-1/SPV 350	MR	India	ICRISAT designated variety
ICSV 95001	MR	India	ICRISAT designated variety
IS 30469C-140	R	Ethiopia	Zera zera conversion
SPV 351/ICSV 1	S	India	ICRISAT designated variety
ICSV 91008	MR	India	ICRISAT designated variety
CSH 9	S	India	NRCS designated hybrid
SPV 104	S	India	NRCS designated variety
IS 8545	R	Ethiopia	Germplasm accession

R = resistant; MR = moderately resistant and S = susceptible
NRCS = National Research Centre for Sorghum

Table 2. Geographical and climatic features during the three crop seasons (2001-2003) of the Sorghum Grain Mold Variability Nursery locations in India

Location	Dates of planting	Latitude (N)	Longitude (E)	No of days[a]	Mean temperature (°C)		Mean RH (%)		Rainfall (mm)	
					Min.	Max.	Min.	Max.	No of days	Average
Akola	22 June-24 July	20°42'	77°03'	61	20±0.5	32±0.4	46±2.5	79±1.6	12 (9-16)[b]	144
Parbhani	18 June-6 July	19°16'	76°47'	54	21±0.4	32±0.2	53±2.0	81±1.2	10 (5-13)	49
Palem	26 June-18 July	16°35'	78°16'	56	19±0.3	30±0.3	58±1.7	86±1.1	14 (6-23)	155
Patancheru	13 June-7 July	17°32'	78°17'	49	20±0.3	31±0.4	49±1.8	91±1.3	16 (6-24)	161
Surat	10-22 August	20°12'	72°52'	52	18±0.3	33±0.3	35±0.9	69±1.2	-[c]	-

[a]Period from flowering to physiological maturity across early to late maturing lines.
[b]Range of rainy days.
[c]Data not available.

Frequency of major mold fungi

During the two seasons (2003 and 2004), mold infection percentage by individual fungi were also recorded on the panicle at the PM stage. This was done based on the typical symptoms produced by each fungus on the grain (white to pinkish white by *Fusarium* spp.; shiny velvety black by *C. lunata,* grayish black by A *altemata,* and pin-head black pycnidia by *P. sorghina*). In addition, threshed grain samples of each genotype from the tagged panicles obtained from all the five locations were surface sterilized and plated on moist blotter paper in glass Petri plates, 50 grains (25 per plate) in each of two replications. After 4 days of incubation at 28 °C with 12 hr photoperiod, they were examined under stereomicroscope to record the frequency (%) cf grain colonization by specific fungi based on their growth characteristics and spore morphology.

Data analysis

Analysis of variance was performed on pooled data of 3 years from five locations on grain mold severity scores and grain colonization using the GenStat 8.1, Lawes Agricultural Trust (Rothamsted Experimental Station, UK) to find the effects of genotypes, locations, seasons and their interactions on the development of mold fungi, and to find the means of individual factors and the significant differences among the factors.

RESULTS AND DISCUSSION

Incidence and frequency of mold fungi

Among various fungi colonizing sorghum grains in the field, species of *Fusarium, Alternaria, Curvularia,* and *Phoma* were more prominent than others at PM. Analysis of variance indicated highly significant effects *(P*> 0.001) of year, location, genotypes and their interactions on frequency of these fungi (Table 3). Similarly, the grain colonization incidence in the blotter test by the above fungi was significantly affected by individual variables and their interactions (Table 4).

Among the fungal species, *Fusarium* species (comprising of *F. proliferatum, F. thapsinum, F. verticillioides, F. nygamai* and *F. andiyazi*) (32%), *A. alternata* (19%), *C. lunata* (25%) and P. *sorghina* (10%) were more pronounced than others across

Table 3. Analysis of variance for grain infection caused by *Fusarium* spp. (*F.* spp.), *Curvularia lunata* (Cl), *Alternaria alternata* (Al) and *Phoma sorghina* (Ps) at physiological maturity in sorghum.

Source of variation	df	MS[a]			
		F. spp.	Cl	Al	Ps
Replications	1	1.43	0.33	3.69	3.43
Year (Y)	1	5907.10*	9987.43*	258.27*	4539.85*
Location (L)	4	2872.13*	8159.64*	3836.56*	2149.44*
Genotype (G)	11	1202.83*	3421,06*	1380.87*	107.11*
Y x L	2 (2)	64.38*	371.54*	1817.30*	1253.70*
Y x G	11	259.53*	663.88*	116.42*	309.69*
L x G	43 (1)	316.55*	1097.15*	489.72*	191.19*
Y x Lx G	22 (22)	96.79*	293.68*	115.13*	167.39*
Residual	1703 (576)	3.77	24.72*	10.11	2.60

*Significant at P<0.001

[a]Based on 2 seasons' data on grain infection by individual fungi.

Table 4. Analysis of variance for sorghum grain colonization caused by *Fusarium* spp. (F. spp.), *Curvularia lunata* (Cl), *Alternaria alternata* (Al) and *Phoma sorghina* (Ps) using blotter method.

Source of variation	df	MS			
		F. spp.	Cl	Al	Ps
Replications	1	38.00	27.41	804.68	50.64
Year(Y)	2	5017.3*	579.44*	18927.28*	3640.22*
Location (L)	4	43913.50*	18281.72*	4910.42*	3766.82*
Genotype (G)	11	3128.9*	3430.10*	913.63*	207.48*
Y x L	7 (1)	4305.3*	1085.44*	459.68*	2696.05*
Y x G	22	619.1*	388.29*	646.27*	173.91*
L x G	44	893.5*	517.28*	218.41*	266.08*
Y x Lx G	76 (12)	718.4*	308.64*	354.44*	215.05*
Residual	498 (41)	100.8	69.18	54.41	41.42

*Significant at P<0.001

locations, years and genotypes (Table 5). However, the frequencies of occurrence of fungi across genotypes were quite variable. The frequency of occurrence of *Fusarium* spp. was highest (54%) at Parbahni; of *C. lunata* (45%) at Surat; of A *alternata* (25%) at Parbhani; and of *P. sorghina* (17%) at Patancheru (Fig. 1). Grain colonization by *Fusarium* spp. was more (41%) on IS 18522 and least (13%) on a resistant check IS 8545. Colonization by A *alternata* was more (23%) on ICSV 95001 and SPV 351, *C. lunata* was more (34%) on a susceptible check SPV 104 and least (9%) on IS 8545, whereas *P. sorghina* was more (12%) on IS 30469C-140 and ICSV 91008 and least (7%) on IS 8545. Species of *Fusarium* have been found to be major component of sorghum grain mold complex in the United States (Prom *et al.,* 2003) and in Mexico (Montes-Belmont *et al,* 2003) as well. Among the *Fusarium* species involved, *F. thapsinum* seems to be dominant over others at most locations. According to Leslie et al., (2005), F. *thapsinum* produces more of moniliformin and traces of fumonisins, while *F. verticillioides* and *F. nygamai* produce more of furnonisins and traces of moniliformin. These findings have implication with this study as we should determine the prevalence of toxin producing *Fusarium* strains in the grain mold complex in India.

In blotter and potato-carrot agar platings, the incidence of mold fungi on grains collected from

Table 5. Frequency of four major mold fungi on sorghum genotypes across 15 environments

Genotype	Grain colonization (%)[a] by				Mean
	Fusarium spp.	*Alternaria alternata*	*Curvularia lunata*	*Phoma sorghina*	
IS 18758C-618-2	36	17	30	13	24
IS 18522	41	12	22	9	21
ICSV 96101	36	17	21	10	21
CS 3541	37	15	28	10	23
Sepon/78-1	36	17	20	8	20
ICSV 95001	30	23	29	11	23
IS 30469C-140	36	17	25	12	23
SPV 351/ICSV1	25	23	23	9	20
ICSV 91008	29	18	19	12	20
CSH 9	31	22	32	9	24
SPV 104 (SC)	35	22	34	8	25
IS 8545 (RC)	13	20	9	7	12
Mean	32	19	25	10	22
SE (m)±	4.6	3.5	4.5	2.6	3.8
LSD (P<0.05)	13.4	10.0	13.1	7.6	11.0

[a]Mean across 15 environments, 5 locations x 3 seasons (Akola, Parbhani, Palem, Surat and Patancheru during 2001-2003 rainy seasons) based on blotter method using 100 seeds/location/season at 28^{S}C.

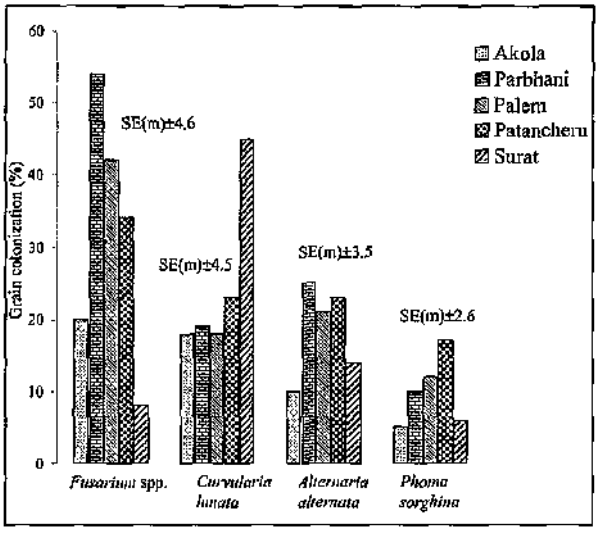

Fig.1. Frequency of major sorghum grain mold pathogens at five locations across genotypes and seasons

molded sorghum genotypes at ICRISAT-Patancheru varied from 24 to 95%, and *A. alternata* appeared most prominent followed by *C. lunata, Fusarium* spp., *Bipolaris* sp., *Exserohilum sp.and P. sorghina* (Girish *et al*., 2004a). These results indicate the variation in frequency of fungi in different environments.

Variation in severity of grain colonization on sorghum genptypes

Among the sorghum genotypes, IS 8545 (resistant check) recorded the lowest mean colonization (10%) and SPV 104 (susceptible check) the highest (27%). Of the 10 sorghum genotypes evaluated, five (ICSV 91008, Sepon 78-1, IS 18522, SPV 351, and ICSV 96101) had overall colonization of 20 to 21% that was significantly less than in CSH 9 and the susceptible check (Table 5).

The overall PGMR scores across the genotypes, locations and seasons ranged from 2.4 to 2.9 compared to 1.7 on resistant check and. 3.6 on the susceptible check (Table 6). Similarly, the TGMR scores of test genotypes across locations and years varied from 2.8 to 3.3 compared to 1.8 on resistant check and 4.0 on the susceptible check.

Four of the 10 test genotypes (ICSV 96101, ICSV 95001, SPV 351 and ICSV 91008) recorded lower overall mean PGMR (<2.5) and TGMR (<3.0) than others and thus could be considered as good sources of re sistarice. The germplasm accession IS 8545, the resistant check remained highly resistant with mean PGMR of 1.7 and TGMR of 1.8. In order to breed for grain mold resistance in

Table 6. Grain mold infection on 12 genotypes of Sorghum Grain Mold Variability Nursery at physiological maturity (PGMR) and on threshed grain (TGMR) across five locations

Genotype	PGMR[a]						TGMR[3]					
	Akl	Par[b]	Pal	Pat	Sur[b]	Mean	Akl	Par	Pal	Pat	Sur[b]	Mean
IS 18758C-618-2	2.4	3.0	2.9	4.0	2.2	2.9	2.6	3.1	3.7	4.9	2.4	3.3
IS 18522	2.6	3.0	2.5	3.0	2.0	2.6	2.8	3.0	3.5	4.1	1.9	3.1
ICSV 96101	2.5	3.0	2.1	2.9	2.1	2.5	2.4	2.8	3.0	3.7	2.0	2.8
CS 3541	2.7	2.8	2.6	3.0	2.4	2.7	2.8	2.6	3.7	3.8	2.3	3.1
Sepon 78-1/SPV 350	2.7	2.8	2.2	2.8	2.6	2.6	2.7	2.7	3.1	4.2	2.8	3.1
ICSV 95001	2.0	2.8	1.7	3.7	1.7	2.4	2.0	2.7	2.9	4.5	1.7	2.8
IS 30469C-140	2.5	4.3	2.9	3.1	1.5	2.9	2.7	2.7	3.9	4.1	1.5	3.0
SPV 351/ICSV 1	2.5	2.5	2.2	2.2	2.6	2.4	2.5	2.6	3.5	3.3	2.9	3.0
ICSV 91008	2.5	2.5	2.5	2.4	2.1	2.4	2.2	2.6	3.7	3.5	2.0	2.8
CSH 9	3.0	3.2	2.9	3.8	1.8	2.9	2.8	2.9	4.1	4.7	1.8	3.3
SPV 104-Sus check	3.0	3.5	3.5	4.3	3.7	3.6	3.1	3.3	4.5	5.0	4.1	4.0
IS 8545-Res check	2.1	2.4	1.1	1.6	1.3	1.7	2.1	2.4	1.5	1.9	1.2	1.8
Mean	2.5	3.0	2.4	3.1	2.1	2.6	2.5	2.8	3.5	3.9	2.2	3.0
SE (m)±	0.1	0.3	0.2	0.1	0.1	0.2	0.1	0.1	0.3	0.1	0.1	0.1
LSD (P<0.05)	0.3	1.0	0.4	0.3	0.4	0.5	0.3	0.3	0.7	0.4	0.4	0.4

[a]Mean of 3 seasons, 2 replications/season, 10 plants/rep/genotype, based on mold infection scale: 1= no mold; 2= 1-10%; 3=11-25%; 4=26-50%; and 5 = >50% mold infection.

[b]Mean of 2 years. Akl = Akola; Par = Parbhani; Pal = Palem; Pat = Patancheru; and Sur = Surat.

sorghum, it is imperative to identify genetic resistance to individual grain mold pathogens and then introgress the resistance genes by gene-pyramiding.

Variation in grain mold severity among locations

The average PGMR was highest at Patancheru (3.1) and lowest at Surat (2.1), and that of TGMR, it was highest at Patancheru (3.9) and lowest at Surat (2.2) (Table 6). This variation could be attributed to the prevailing weather factors especially temperature and relative humidity (RH) during the crop season. Patancheru had the maximum RH (91%) and moderate maximum temperature (31 °C) compared to Surat that had 69% RH and temperature of 33 °C (Table 2). More detailed studies are needed to understand the critical role of weather variables in grain infection and colonization by individual pathogens.

Relationships between PGMR, TGMR and grain colonization

Highly significant (P<0.01) positive correlations were obtained between PGMR and TGMR (r = 0.766); between PGMR and grain colonization (r = 0.461); and between TGMR and grain colonization (r = 0. 592). These results suggest that grain mold severity scores at physiological maturity in the field is more reliable than on threshed grain, and thus for all grain mold screening the PGMR scores alone should be adequate to discern lines into different resistance and susceptibility classes. This is more precise, both time and cost effective, and would thus enhance the efficiency and effectiveness of grain mold resistance breeding. Grain colonization data are important to identify the association of individual fungi with grain molding and would be helpful in identifying sorghum genotypes with tolerance/resistance to individual and multiple pathogens.

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