

## Evaluation of advanced sorghum breeding lines for grain mold resistance

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**ABSTRACT:** Grain mold, caused by a fungal complex, is a major production constraint of early-maturing high-yielding rainy season sorghum (*Sorghum bicolor*) hybrids. A total of 34 selected elite B-lines bred for grain mold resistance at ICRISAT were evaluated for their resistance stability through a collaborative Sorghum Grain Mold Resistance Stability Nursery under natural infection at five locations (Akola, Parbhani, Palem, Patancheru, and Surat) in India during three rainy seasons, 2002 to 2004. Grain mold severity scores were recorded at two stages, first at physiological maturity- panicle grain mold rating (PGMR) in the field nursery and second after harvest on threshed grain- threshed grain mold rating (TGMR), using a progressive 1–5 scale. Results indicated significant differences among the genotypes (G), locations (L), years (Y) and their interactions for both PGMR and TGMR scores. A strong positive correlation ( $r = 0.89$ ) between PGMR and TGMR indicated the adequacy of grain mold severity recording, preferably PGMR. Relatively, larger variance due to G than to G  $\times$  L and G  $\times$  Y interaction components justified breeding sorghum hybrid for grain mold resistance for wider adaptation in India.

**Key words:** Sorghum, grain mold, stable resistance, agronomic traits

Grain mold of sorghum (*Sorghum bicolor* (L.) Moench), caused by a complex of pathogenic and saprophytic fungi (Forbes *et al.*; 1992; Navi *et al.*, 1999), is the greatest constraint for optimum grain yield and quality in the semi-arid tropics of India, Africa, and the Americas (Williams and McDonald, 1983). Early-maturing, high grain yielding hybrids 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 and physiological maturity - characterized by the formation of the black layer on the grain hilum, while grain weathering succeeds grain molding in which fungi colonize physiologically matured grain prior to harvest (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 seed viability. In different sorghum growing areas of India, the major pathogens associated with grain mold across variable weather conditions (humidity and temperature) during the rainy season are *Fusarium* spp., *Curvularia lunata*, *Alternaria alternata* and *Phoma sorghina* (Indira *et al.*, 1991; Thakur *et al.*, 2003a, 2006a, 2006b). Damage resulting from these early infection events includes reduced kernel development, discoloration of grain, colonization and degradation of endosperm and germ, decreased grain density, decreased germination, decreased seedling vigor and possible mycotoxin contamination particularly with *Fusarium* species (Waniska, 2000; Leslie *et al.*, 2005; Navi *et al.*, 2005).

Among several approaches to manage the sorghum grain mold, host-plant resistance is the

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most effective and economical option (Thakur *et al.*, 1997, 2003b; Reddy *et al.*, 2000). Studies on biology and epidemiology of sorghum grain mold (Bandyopadhyay *et al.*, 2000) led to the development of an effective field screening technique (Bandyopadhyay and Mughogho, 1988) and number of resistant lines were identified by screening a large number of germplasm accessions at ICRISAT (Bandyopadhyay *et al.*, 1988). Considering that grain mold infection and development are greatly influenced by weather variables, such as temperature, relative humidity and rainfall quantity and distribution (Das *et al.*, 2003; Navi *et al.*, 2005) it was important to assess the responses of these lines for grain mold resistance in the target production areas. Therefore, this study was planned to evaluate some of the new advanced maintainer (B) lines and established B-lines bred at ICRISAT through multi-location testing in the major sorghum growing areas of India, and identify lines with high levels of resistance for use in developing grain mold resistant hybrids.

## MATERIALS AND METHODS

### Nursery and its locations

Thirty-four selected elite B-lines, including 17 newly bred B-lines (not converted into A- lines) and 17 established B-lines, bred at ICRISAT, Patancheru with two resistant and two susceptible checks were used in this study (Table 1). These B-lines were evaluated for their resistance to grain mold through a collaborative Sorghum Grain Mold Resistance Stability Nursery (SGMRSN), that was established at five grain mold hot spots in three states -Akola and Parbhani (Maharashtra), Palem and Patancheru (Andhra Pradesh) and Surat (Gujarat) during the three rainy seasons 2002 – 2004 (only two seasons at Surat), thus making a total of 14 environments. Each line was grown in two rows, 4m long with two replications in a randomized block design. Local crop production and crop protection (except for grain mold) practices were followed to raise good crop. Sprinkler irrigation was provided on dry days to maintain high relative humidity (>95% RH) from flowering to physiological maturity. In each line 10 plants with uniform flowering were tagged for recording the grain mold severity. All the tagged panicles were harvested and threshed, and their grain mold severity recorded (Thakur *et al.*, 2006b).

### Grain mold scoring

Grain mold severity scores were taken twice, first at physiological maturity – the panicle grain mold rating (PGMR) on a progressive 1 to 5 scale (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 nursery, and second on the bulked threshed grain – the threshed grain mold rating (TGMR) of each line after harvesting and drying. For this purpose threshed grain (20g) from the bulk of 10 panicles per plot, were spread in the Petridish and scored for mold severity following the same 1-5 scale using a magnifying lens under proper lighting (Thakur *et al.*, 2006b).

### Agronomic traits

At each location, data were recorded for agronomic traits, such as, days to 50% flowering, plant height and grain hardness following the standard methods. Grain hardness was recorded for 50 grains from each plot by subjecting individual grains to grain hardness tester (Kiya Seisakusho Ltd., Tokyo, Japan) after the grain samples were uniformly dried to 7% grain moisture level. The tester records the pressure (kg seed<sup>-1</sup>) applied manually to crack the grain. In addition, environment-insensitive qualitative traits, such as, per cent glumes coverage of the grains, panicle compactness (compact, semi-compact, or loose based on the arrangement of the primary and secondary rachises on the panicle), and grain color (white, brown, or red) were recorded only at Patancheru.

### Data analysis

The grain mold severity scores recorded on the ordinal 1-5 scale were expressed as the midpoint of the range of proportions of molded grain (e.g. 1 = 0, 2 = 0.055, 3 = 0.18, 4 = 0.38, and 5 = 0.76). This was essential to bring homogeneity in dataset before subjecting it to statistical analysis. The combined analysis of variance served the basis to estimate components of variance attributable to lines (genotype), location and year/environment and their interactions for agronomic traits and grain mold severity scores. For partitioning of total variation into different sources, mixed model was followed, where genotype (G) effects were considered as fixed, while location (L) and years (Y) effects as

**Table 1.** Pedigrees, agronomic traits and grain mold scores of 34 advanced sorghum B- lines and four checks evaluated for grain mold resistance at five locations in India during 2002-2004

Line	Pedigree	Agronomic traits <sup>a</sup>				Grain mold PGMR <sup>b</sup>
		Panicle type	Glumes colour	Glumes coverage (%)	Grain colour	
SGMR 01	(ICSB 300 × IS 25060)-1-1-2-1-1-1	SC	White	50	White	0.23
SGMR 03	(ICSB 306 × GM 973303)-27-1-1-4-1-1-1	SC	Red	75	White	0.15
SGMR 04	(ICSB 403 × ICSB 11)-1-1-3-1-2-1	SC	Red	50	White	0.20
SGMR 05	(ICSB 403 × ICSB 11)-1-1-3-1-4-1-1	SC	Red	50	White	0.21
SGMR 06	(ICSB 403 × ICSB 11)-1-1-3-1-5-1	SC	Red	50	White	0.19
SGMR 07	(ICSB 403 × ICSB 11)-1-1-3-1-6-1-1	SC	Red	50	White	0.16
SGMR 08	(PKV 400 × ICSB 101)-2-1-1-1-1-1-1	SC	White	50	White	0.17
SGMR 09	(PKV 400 × ICSB 101)-2-1-1-1-2-1-1	SC	White	50	White	0.15
SGMR 10	(ICSB 383 × ICSB 101)-2-1-1-1-1-1-1	SC	Red	25	White	0.14
SGMR 11	(ICSB 383 × ICSB 101)-2-1-1-1-2-1-1	SC	Red	50	Red	0.15
SGMR 12	ICSP-B-Population -5-1-1-1-1-1-1	SC	White	25	Red	0.13
SGMR 13	ICSP-B-Population -5-1-1-1-2-1	SC	Red	50	Red	0.10
SGMR 14	(ICSB 392 × SP 1792-1)-3-1-1-1-1-1	SC	White	50	Red	0.12
SGMR 17	(ICSB 392 × SP 1792-1)-4-1-1-1-1-1	SC	Red	25	Red	0.17
SGMR 21	((IS 13817 × ICSB 270) × ICSB 392:Red) -2-1-1-4-1-1	SC	Red	50	Red	0.14
SGMR 23	(IS 8614 × ICSB 293)-2-1-1-1-4-1-1	SC	Red	50	Brown	0.17
SGMR 24	(IS 8614 × ICSB 293)-2-1-1-1-5-1-1	SC	White	25	Brown	0.15
ICSB 352	(ICSB 11 × IS 2815)-2-1-2-1-1	SC	Red	25	White	0.13
ICSB 355	(ICSB 11 × IS 2815)-12-1-1	SC	Brown	50	Brown	0.10
ICSB 362	(ICSB 11 × IS 2815)-25-1-2-1	SC	Red	25	White	0.17
ICSB 363	(ICSB 11 × IS 2815)-25-1-3-1-2	SC	Red	50	White	0.15
ICSB 368	(ICSB 11 × IS 2815)-30-1-2-3	SC	Red	25	White	0.20
ICSB 370	(ICSB 11 × IS 2815)-30-1-3-3-2	SC	Brown	50	Red	0.09
ICSB 380	(ICSB 11 × IS 2815)-61-2-1-2-1-1	SC	Red	50	White	0.15
ICSB 382	(ICSB 11 × IS 2815)-62-4-3-1	SC	Red	50	White	0.20
ICSB 383	(ICSB 17 × IS 10646)-5-1-2	SC	Red	25	Brown	0.06
ICSB 385	(ICSB 17 × IS 21599)-14-1-2-3-2	SC	Brown	50	Red	0.12
ICSB 391	(ICSB 37 × IS 10475B)-2-2-1-2-1-1	SC	Red	50	Red	0.08
ICSB 393	(ICSB 37 × IS 10475B)-4-3-2-1-2	SC	Red	50	Red	0.06
ICSB 394	(ICSB 37 × IS 10475B)-6-1-1-1-1	SC	Brown	50	White	0.17
ICSB 401	(ICSB 42 × IS 23585)-1-2-2-2-2	SC	Red	50	Brown	0.14
ICSB 402	(ICSB 42 × IS 23585)-1-7-1-1-1	SC	Brown	50	Brown	0.13
ICSB 403	(ICSB 42 × IS 23585)-1-7-1-2-2	SC	Brown	50	Brown	0.12
ICSB 407	(IS 3436 × PQ 35B)-5-1-1-2-1-1	SC	Red	50	Red	0.13
	Bulk Y – Susc check	SC	Brown	25	White	0.54
	IS 14384 – Res check	SC	Brown	90	Red	0.03
	296B – Susc check	SC	White	25	White	0.45
	PVK 801 – Moderately res check	SC	Red	50	White	0.10
	SE (m)±					0.04
	LSD (P<0.05)					0.10

<sup>a</sup>Recorded only at Patancheru<sup>b</sup>Mean of 14 environments across 5 locations based on the mid values of 1-5 scale

random. The year factor was nested within the location with a total of 14 environments (5 locations  $\times$  3 years; only 2 years at Surat). Pearson correlation coefficients were estimated to assess the relationships between PGMR and TGMR scores of B-lines. In view of a very high positive correlation between PGMR and TGMR scores and the fact that PGMR scores provide more realistic score under field conditions than TGMR score (as discussed below), only PGMR scores were considered to assess the resistance levels of the test lines. All the statistical analyses were carried out using GENSTAT package (GenStat Release 9.1, Lawes Agricultural Trust, UK).

## RESULTS

Rainfall pattern, the total amount of rainfall and their distribution, and relative humidity varied considerably across locations and across years. However, the use of sprinkler irrigation and/or mist spray with water on the panicles during the critical periods of flowering and grain development provided near favourable conditions for mold development at each location. This facilitated fair assessment of genotypic responses to grain mold infection and development.

### Variance components

Analysis of variance indicated significant mean squares due to genotypes/lines (G), locations (L), years (Y), and interactions of genotype with locations and years for grain mold severity (Table

2). The mean squares due to year and G  $\times$  Y interaction components were lower than those due to location and G  $\times$  L components for both PGMR and TGMR. The mean squares due to G  $\times$  L  $\times$  Y interaction component was higher than those due to G  $\times$  Y or G  $\times$  L interaction.

### Grain mold resistance at individual locations

Mean grain mold severity scores (PGMR) of the 34 B-lines across 14 environments varied from 0.06 to 0.23 compared to 0.03 on the resistant check IS 14384, and 0.54 on the susceptible check Bulk Y (Table 1). Based on the mean mold scores of 0.12 (2.5 on a 1-5 scale) as cut-off point for resistance, 24 lines (SGMR 08 to -14, -17, -24, ICSB 352, -355, -362, -363, -368, -370, -380, -383, -385, -391, -393, -401-403, and -407) were resistant at Akola; 4 (SGMR 06, -14, ICSB 363 and -383) at Parbhani; 12 (SGMR 12, -13, -23, ICSB 355, -370, -383, -385, -391, -393, -401, -402 and -403) at Patancheru; 24 (SGMR 08 to -14, -17, -24, ICSB 352, -355, -362, -363, -368, -370, -380, -383, -385, -391, -393, -401, -403 and -407) at Palem; and 25 (SGMR 03, -05 to -14, -17, -21, ICSB 352, -355, -362, -363, -368, -370, -380, -382, -383, -385, -391, -393, -403 and -407) at Surat. These lines will be useful for location-specific grain mold resistance breeding.

### Grain mold resistance across environments

Eight of the 34 B-lines showed high grain mold resistance across 14 environments with mean PGMR

**Table 2.** Mean squares due to genotypes, locations, years, and their interactions for sorghum grain mold severity and agronomic traits

Source of Variation	df	Days to 50% flowering	Plant height (m)	Grain hardness (kg seed <sup>-1</sup> )	PGMR	TGMR
Replications	1	12.50	0.01	1.48	0.0065	0.0179
Genotypes (G)	37	884.52**	1.66**	43.27**	0.2065***	0.2371***
Locations (L)	4	1156.02**	8.24**	291.81**	0.9432***	5.4510***
Year (Y)	2	438.17**	4.19**	138.50**	0.1439***	2.7782***
G $\times$ L	148	75.68**	0.09**	4.20**	0.0328***	0.0576***
G $\times$ Y	74	49.61**	0.10**	4.08**	0.0319***	0.0533***
L $\times$ Y	7	1635.56**	0.85**	139.20**	0.9902***	2.0259***
G $\times$ L $\times$ Y	252	23.14**	0.05**	2.42**	0.0233***	0.0373***
Residual	517	3.08	0.02	0.66	0.0098	0.0051

\*\*Significant at  $P < 0.01$ ; \*\*\*Significant at  $P < 0.001$

PGMR = Panicle grain mold rating; TGMR = Threshed grain mold rating

**Table 3.** Agronomic traits and mean grain mold severity scores of eight sorghum B-lines identified as stable grain mold resistance across 14 environments in India

Sorghum B-lines	Agronomic traits <sup>a</sup>			PGMR (mid values of 1-5 scale) <sup>b</sup>					Mean
	DTF	Plant height (m)	Grain hardness (kg seed <sup>-1</sup> )	PAT (3) <sup>c</sup>	PAL (3)	AKL (3)	PAR (3)	SUR (2)	
SGMR 13 <sup>d</sup>	71	1.9	7.1	0.11	0.08	0.08	0.18	0.06	0.10
SGMR 14 <sup>d</sup>	79	1.8	5.6	0.25	0.07	0.07	0.11	0.11	0.12
ICSB 355	76	1.7	4.9	0.05	0.11	0.11	0.18	0.05	0.10
ICSB 370	67	1.4	5.8	0.06	0.07	0.07	0.19	0.04	0.09
ICSB 383	67	1.6	5.8	0.04	0.07	0.07	0.12	0.01	0.06
ICSB 385	72	1.7	6.9	0.12	0.09	0.09	0.25	0.06	0.12
ICSB 391	66	1.5	6.9	0.11	0.07	0.07	0.15	0.02	0.08
ICSB 393	74	1.9	9.0	0.05	0.04	0.04	0.17	0.02	0.06
IS 14384 Res check	73	2.6	8.7	0.00	0.02	0.02	0.11	0.00	0.03
296B Susc check	76	1.3	4.9	0.63	0.31	0.31	0.79	0.20	0.45
Trial mean	73	1.7	6.0	0.22	0.13	0.13	0.22	0.10	0.16
LSD ( $P < 0.05$ )	1.6	5.4	1.6	0.13	0.06	0.06	0.19	0.09	0.10

<sup>a</sup>Recorded only at Patancheru; DTF = Days to 50% flowering

<sup>b</sup>Mean severity of 14 environments (5 locations × 3 years) based on the mid values of 1-5 scale where 1= no mold; 2 = 1-10% mold; 3 = 11-25% mold; 4 = 26-50% mold and 5 = >50% molded grain on a panicle

<sup>c</sup>Number of years of testing at each location; PAT = Patancheru, PAL = Palem, AKL = Akola, PAR = Parbhani and SUR = Surat

<sup>d</sup>Newly developed B-lines

of 0.06 to 0.12 compared to 0.03 in resistant check and 0.45 in susceptible check (Table 3). The PGMR scores of these lines were consistent at all the locations except Parbhani where the scores were relatively higher than at other locations. Of these, two (SGMR 13 and SGMR 14) are new advanced B-lines, and the remaining six (ICSB 355, -370, -383, -385, -391 and -393) are established B-lines that have already converted into A-lines for utilization in hybrid development. These lines have desired days to 50% flowering (66 to 79 days) and soft to hard grains (grain hardness = 4.9 to 9.0 kg seed<sup>-1</sup>). Besides, most of the lines have colored glumes with 25% to 50% glumes coverage and most of the lines have semi-compact panicles and white grains (Table 1).

#### Relationship between plant traits and grain mold resistance

Significant negative correlations were found between grain mold severity scores and glumes coverage and between grain mold scores and plant height (Table 4). A significant strong positive

correlation ( $r = 0.89$ ) was found between PGMR and TGMR scores (Table 4, Fig. 1).

#### Agronomic traits and grain mold severity

Agronomic traits, such as days to 50% flowering and plant height of test lines varied depending on the variation in weather variables across locations and years (data not shown). However, the general pattern remained consistent. The fixed quality traits, such as panicle type varied from compact (3 lines) to semi-compact (31 lines); glumes color red (22 lines), brown (6 lines) and white (6 lines); glumes coverage 75% (one line), 50% (25 lines), and 25% (8 lines); and grain color varied from red (11 lines), brown (7 lines) to white (16 lines) (Table 1).

#### DISCUSSION

The progress in breeding for grain mold resistant hybrids has been rather slow due to the complex nature of the grain mold disease, interactions between mold fungi and weather variables,

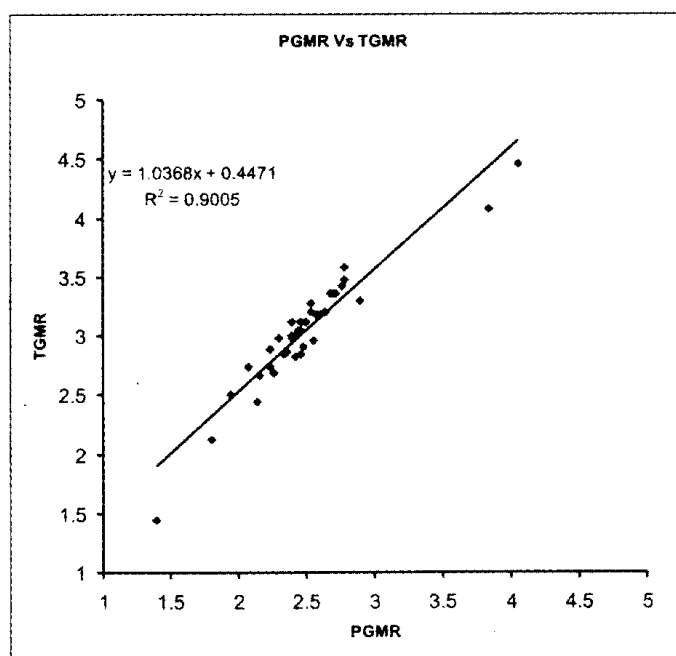
**Table 4.** Correlation coefficients between agronomic traits and grain mold reactions of 38 sorghum lines

	DTF	Glumes coverage	Grain hardness	PGMR	Plant height	TGMR
DTF	1.000					
Glumes coverage	0.267	1.000				
Grain hardness	0.012	0.409**	1.000			
PGMR	-0.185	-0.268	-0.233	1.000		
Plant height	0.261	0.521**	0.327*	-0.465**	1.000	
TGMR	-0.251	-0.244	-0.264	0.894**	-0.326*	1.000

\*Significant at  $P < 0.05$  and \*\* Significant at  $P < 0.01$

DTF=Days to 50% flowering; PGMR = Panicle grain mold rating at physiological maturity;

TGMR= Threshed grain mold rating of threshed grain



**Fig. 1.** Relationship of mold severity scores of 38 sorghum lines recorded at physiological maturity (PGMR) and on threshed grain (TGMR)

association of plant traits with resistance, varied adaptation in different sorghum growing areas, and the lack of a simple disease severity scoring method. Because of these factors, identification of reliable and stable resistance has been difficult. Grain mold development is greatly influenced by weather conditions (Das *et al.*, 2003; Ratnadass *et al.*, 2003) and this was apparent in variances due to various component interactions in this study. Variation in grain mold severity could also be due to plant qualitative traits, such as panicle structure type, glumes coverage and grain hardness and grain colors (Table 1).

The grain mold resistant B-lines identified in this study could be used as potential seed parents for the development of hybrids having stable grain mold resistance for the target sorghum areas of India. These lines have desirable agronomic traits, such as early to medium flowering period, shorter to medium plant height, and soft to hard grains, colored glumes, semi-compact panicles and white grains that are preferred by growers in India and these can provide dual-purpose (grain and dry fodder after harvest of grain) hybrid cultivars with easy threshability. However, despite higher grain mold resistance, the use of either parent with colored grain results in colored-grain hybrids (as grain color is dominant trait), which are not preferred in India for food use. Nevertheless, such colored grain hybrids with high grain mold resistance would be preferred for poultry feed, which is a tremendously growing industry in India (Seshaiah, 2000).

In this study, the significant mean squares due to  $G \times L$  interaction supports breeding for specific adaptation (characterized by predictable variability), while those due to  $G \times Y$  interaction suggests breeding for stability over temporal (unpredictable) variability. In the present study, both  $G \times L$  and  $G \times Y$  interaction mean square components are although significant for grain mold scores, their magnitudes are much lower than that of genotypic variance. Therefore, it could be concluded that these lines are fairly adapted to the test locations and justify their utilization in grain mold resistance breeding for a wider adaptation.

A significant positive correlation ( $r = 0.89$ ) between PGMR and TGMR scores suggested that recording grain mold severity at one of the two

stages, preferably at physiological maturity could be adequate. The PGMR scores provide more realistic reaction under field conditions at the right stage of the grain development (physiological maturity) than the TGMR scores, which are recorded about a week later (after harvesting, drying and threshing) until which time some saprophytic growth occurs on the grain. This is a significant step towards simplifying the screening process in terms of improving precision of disease scoring and economizing on time and resources. This procedure would also increase the pace of screening breeding materials and hence improves the efficiency of the grain mold resistance breeding programs. Significant negative correlations between glumes coverage and grain mold severity scores (both PGMR and TGMR) supported the earlier results (Audilakshmi *et al.*, 1999; 2000; Reddy *et al.*, 2000) that glumes coverage of grain was associated with grain mold resistance. However, in this study, grain hardness did not contribute directly to resistance as indicated by low and non-significant correlation between grain hardness and grain mold severity scores, which needs further confirmation. One possible reason for this lack of correlation could be the method of measuring grain hardness, which is tedious and highly subjective because the manual pressure applied to crack individual grains could be highly variable. The significant negative correlation between grain mold scores and plant height indicates that tall and late maturing plants might escape mold infection due to less favorable microclimatic conditions. The weak and non-significant correlation between days to flowering and grain mold severity scores, suggests that grain mold resistance could be combined with desired maturity groups to develop cultivars for specific adaptation (Ibrahim *et al.*, 1985). However, grain mold resistant lines identified in this study have medium maturity, medium height and not very hard grains and thus offer the possibility of breeding grain mold resistant hybrids with desirable agronomic traits. Future research will focus on better understanding the genetics of the traits associated with grain mold resistance and mechanisms of resistance for further improving the parental lines for developing grain mold resistant hybrids.

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