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Research Article
Grain Biodeterioration of Sorghum Converted Lines Inoculated with a Mixture of *Fusarium thapsinum* and *Curvularia lunata*

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Abstract

Background and Objective: Globally, grain mold is a major hurdle affecting sorghum productivity and quality. This disease is caused by complex fungal pathogens, among them *Fusarium thapsinum* and *Curvularia lunata* are the major fungi prevalent in many sorghum growing regions. This study examined the effect of inoculating a mixture of *F. thapsinum* and *C. lunata* on 60 sorghum converted lines with five adapted inbred lines as checks. **Materials and Methods:** Sorghum lines and checks were evaluated in field trials at the Texas AgriLife Research Station. Plants were inoculated with a mixture of *F. thapsinum* and *C. lunata* at 50% bloom. **Results:** The overall result showed that SC 725 (PI 534101), SC 218 (PI 534127), SC 691 (PI 534050), SC 91 (PI 534145) and Sureno exhibited grain mold severity of 2.3 or less. This level of grain mold infection was lower than the scores exhibited by the two resistant checks RTx 2911 (2.8) and SC 719-11E (2.5). Significant negative correlation (r = -0.385, p = 0.002) between grain mold and germination indicated the impact of these two fungi infection on germination rates. The significant negative correlation detected between germination and daily maximum temperature during the evaluation period shows planting of sorghum cultivars/hybrids that mature during periods of dry moderate weather will avoid problem of grain mold infection. **Conclusion:** The identified four converted lines for grain mold resistance in this study is recommended to use in breeding program to introgress grain mold resistance genes into other adapted sorghum inbred lines to increase the yield and seed quality traits.

Key words: *Sorghum bicolor*, grain mold, *Fusarium thapsinum*, *Curvularia lunata*

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Grain mold is a major biotic constraint to sorghum [*Sorghum bicolor* (L.) Moench] production worldwide[1,2], particularly in areas where sorghum is grown during the rainy season under warm and humid conditions[3-5]. Grain mold is a disease complex associated with various fungal species, of these *Fusarium thapsinum* Klittich, Leslie, Nelson et Marasas and *Curvularia lunata* (Wakker) Boedijn are prevalent in most sorghum production regions[6-8]. *Fusarium* infection may lead to mycotoxins production during the grain development or post-harvest during storage[8-12]. *Curvularia lunata* infects the developing caryopses prior to maturity leading to pre-mature black layer deposition in susceptible sorghum[13]. Generally, grain mold symptoms may be manifested as seed discoloration and smaller seed size[3,13,14]. In some cases, yield losses due to grain mold may be severe and on highly susceptible sorghum lines can reach 100%[1]. The management strategies aimed at reducing the impact of grain mold may include planting of sorghum cultivars that mature during periods of dry weather, the use of cultivars with colored grain high in tannins and planting resistant cultivars[3,15,16]. The development of genetically resistant sorghum lines to grain mold is the most practical method of reducing the impact of the disease[3,14-16]. Differences in grain mold disease levels among different sorghum varieties grown in the same environment imply that host genes play a role in controlling disease severity[17]. However, the complexity of the disease requires continuous evaluation to identify new sources of resistance to grain mold for successful management. In this study, the reaction of sorghum converted lines developed from exotic germplasms were used and inoculated with a mixture of *F. thapsinum* and *C. lunata* to assess the resistance reaction of these fungi causing grain mold disease and to identify resistance sources for further breeding programs.

MATERIALS AND METHODS

Field trial: A total of 60 sorghum converted originally from different exotic sources of diversified origin (Ethiopia, Sudan, Nigeria, India, USA, Uganda, Zimbabwe, Mali, Japan, Burkina Faso and Uganda) with five checks from the USA were evaluated (Table 1). Sorghum germplasm used in this study were converted from photoperiod sensitive exotic accessions to photoperiod insensitive lines by introgressing many important traits into BT$\times$406 with early maturity and dwarfing traits background using a minimum of four backcrosses and readily usable in the USA and other temperate-zone areas of the world. These lines contain sources of desirable novel traits such as diversity, insect resistance, drought resistance and improved grain quality[18]. Accessions were evaluated in two years (2008 and 2009 growing seasons) in a randomized complete block design at the AgriLife Research Farm, Burleson County, Texas. Each accession was replicated thrice in both experiments. The RTx 2911, SC 719-11E and Sureno as resistant checks and RTx 2536 and RTx 430 as susceptible checks were included. Fertilizer application and other agronomic managements were followed as recommended.

Screening for grain mold: Plants (three panicles/line/replicate) were inoculated with a mixture of *F. thapsinum* and *C. lunata* at 50% bloom following the method by Prom *et al.*[19]. The conidial suspensions used in this study were filtered through four layers of sterile cheesecloth into two separate beakers and diluted with sterile water to a final concentration of 1×10^6 conidia mL^{-1}. All flasks were thoroughly agitated before inoculation. Due to differences in developmental stage, panicles were inoculated at different dates. Seed germination evaluation has been described by Prom *et al.*[19] and Prom[20]. Inoculated panicles were assessed for grain mold severity in the field using a 1-5 scale, where 1 = Seed bright with no mold and no discoloration due to weathering, 2 = Seed is not as bright and has little or no mold but has some discoloration due to weathering, 3 = Seed is not bright, there is some mold and some discoloration, 4 = Seed is almost entirely covered in mold and is deteriorating and 5 = Seed is covered entirely with mold, is deteriorated and looks dead[21]. At maturity, plant height was measured from the soil to the top of the plant in centimeters and panicle length was measured from the first branch with racemes to the top of the panicle. Seed weight was based on weight in grams of 100 randomly selected seeds from each replication. Germination rate was based on the number of seeds that germinated after 7 days from 300 seeds (100 seeds per replication) planted in flats containing Metro Mix 200 potting medium (Scotts-Sierra Horticultural Products Company, Maryland, OH)[19]. In addition to seed germination rate, weather parameters during the growing seasons also were measured to determine the effect on grain mold severity. Maximum and minimum temperature and daily precipitation measurements were averaged every 2 days through the months of May-July for 2008 and 2009.

Statistical analysis: Data for grain mold severity, seed weight, percent germination rate, plant height and panicle length were subjected to the analysis of variance using the command PROC GLM (SAS version 9.4, SAS Institute, Cary, NC)

Table 1: Reaction of Sorghum Converted (SC) lines inoculated with a mixture of *Fusarium thapsinum* and *Curvularia lunata* fungi causing grain mold disease

SC line[#]	Accession	Country	PGMR[##]	Seed weight (g)	Plant height (cm)	Panicle length (cm)	Germination rate (%)
SU 623	PI 571184	Sudan	4.8^{a*}	2.6^{b-m}	250.5^{bc}	39.2^{ab}	80.4^{abc}
SC 370	PI 533776	Unknown	4.4^{a}	4.7^{a}	104.6^{f-j}	31.1^{abc}	45.5^{bc}
SC 382	PI 534088	Nigeria	4.3^{a}	3.1^{a-k}	88.3^{hij}	24.9^{b-h}	37.0^{bc}
SC 55	PI 533755	Unknown	4.0^{a}	3.3^{a-h}	58.2^{j}	14.0^{h}	33.5^{bc}
SC 345	PI 534073	Nigeria	4.0^{a}	3.6^{abc}	96.4^{g-j}	31.9^{abc}	66.2^{abc}
SC 4	PI 534119	Ethiopia	4.0^{a}	1.1^{m}	79.0^{hij}	13.7^{h}	26.9^{bc}
RTx 2536	Inbred	USA	3.8^{a}	3.1^{a-j}	86.4^{hij}	29.1^{b-e}	23.2^{bc}
SU 805	PI 571342	Sudan	3.8^{a}	3.5^{a-e}	185.3^{cd}	14.3^{gh}	68.7^{abc}
SC 863	PI 576360	India	3.8^{a}	2.5^{b-m}	96.1^{g-j}	15.3^{gh}	68.7^{abc}
SC 1325C	PI 597970	USA	3.8^{a}	2.9^{b-m}	99.3^{f-j}	15.6^{fgh}	54.3^{abc}
SU 186	PI 570841	Sudan	3.7^{ab}	[**]	-	-	-
SC 460	PI 534017	India	3.7^{ab}	3.6^{a-d}	76.0^{hij}	20.7^{c-h}	39.0^{bc}
SC 352	PI 534042	Sudan	3.7^{ab}	3.9^{ab}	103.9^{f-j}	24.0^{b-h}	48.8^{bc}
SC 929	PI 595699	USA	3.7^{ab}	2.5^{b-m}	68.5^{hij}	21.9^{c-h}	47.8^{bc}
SC 389	PI 533894	Nigeria	3.7^{ab}	3.3^{a-l}	102.7^{f-j}	40.0^{a}	34.0^{bc}
SC 94	PI 533950	Sudan	3.5^{ab}	3.2^{a-j}	150.5^{d-g}	39.3^{a}	71.2^{abc}
SC 256	PI 533818	Nigeria	3.5^{ab}	2.6^{b-m}	153.6^{def}	35.8^{ab}	69.3^{abc}
SC 568	PI 576343	Nigeria	3.5^{ab}	3.6^{a-e}	110.9^{e-i}	28.2^{b-e}	46.2^{bc}
SC 530	PI 533892	Nigeria	3.5^{ab}	2.6^{b-m}	95.4^{hij}	30.4^{a-d}	59.1^{abc}
SU 1715	PI 569853	Sudan	3.4^{ab}	3.2^{a-j}	131.7^{d-h}	18.0^{d-h}	80.7^{ab}
SC 296	PI 533885	Nigeria	3.3^{ab}	1.9^{j-m}	91.1^{hij}	31.6^{abc}	61.8^{abc}
SC 969	PI 534164	Uganda	3.3^{ab}	1.5^{m}	94.1^{hij}	16.0^{fgh}	90.2^{ab}
SC 349	PI 595712	USA	3.3^{ab}	3.5^{a-e}	99.2^{g-j}	24.1^{b-h}	74.0^{abc}
SC 186	PI 533802	Unknown	3.3^{ab}	3.2^{a-j}	117.4^{e-i}	22.3^{c-h}	69.2^{abc}
SC 155	PI 534155	Ethiopia	3.3^{ab}	2.6^{b-m}	85.7^{hij}	27.5^{b-e}	67.3^{abc}
SC 58	PI 533911	Sudan	3.3^{ab}	2.4^{b-m}	80.8^{hij}	24.8^{b-h}	79.8^{abc}
SC 748	PI 533991	Sudan	3.3^{ab}	2.7^{b-m}	109.8^{e-j}	22.7^{c-h}	62.3^{abc}
SC 451	PI 533934	India	3.2^{ab}	1.9^{i-m}	61.0^{ij}	14.7^{gh}	73.5^{abc}
RTx 430	Inbred	USA	3.2^{ab}	-	79.3^{hij}	30.6^{a-d}	13.0^{c}
SC 30	PI 534131	Ethiopia	3.2^{ab}	-	58.7^{ij}	26.3^{b-g}	42.7^{bc}
SC 37	PI 534134	Ethiopia	3.2^{ab}	-	61.0^{ij}	25.5^{b-h}	50.7^{abc}
SC 50	PI 533787	Unknown	3.2^{ab}	2.8^{b-m}	108.8^{f-j}	26.2^{b-g}	66.7^{abc}
SC 118	PI 533759	Unknown	3.2^{ab}	2.6^{b-m}	68.1^{hij}	23.1^{c-h}	78.7^{abc}
SC 1339	PI 597977	USA	3.2^{ab}	2.6^{b-m}	132.7^{d-h}	25.9^{b-g}	81.7^{ab}
SC 737	PI 533983	Sudan	3.2^{ab}	2.4^{b-m}	69.7^{hij}	19.6^{c-h}	64.5^{abc}
SC 564	PI 534053	Uganda	3.2^{ab}	3.5^{a-f}	63.4^{ij}	20.2^{c-h}	55.7^{abc}
SC 183	PI 534160	Unknown	3.2^{ab}	1.8^{j-m}	99.4^{f-j}	26.9^{b-f}	72.0^{abc}
SC 185	PI 534161	Nigeria	3.0^{ab}	2.3^{b-m}	104.1^{f-j}	17.8^{e-h}	45.1^{bc}
SC 17	PI 533903	Ethiopia	3.0^{ab}	1.7^{j-m}	78.6^{hij}	31.4^{abc}	83.1^{ab}
SC 403	PI 533898	Nigeria	3.0^{ab}	3.1^{a-k}	96.4^{g-j}	30.4^{a-d}	23.5^{bc}
SC 475	PI 534022	India	3.0^{ab}	2.3^{c-m}	64.5^{ij}	20.0^{c-h}	59.7^{abc}
SC 218	PI 534162	India	3.0^{ab}	3.1^{a-j}	92.2^{hij}	15.7^{fgh}	54.8^{abc}
SC 654	PI 533975	Rhodesia	2.8^{ab}	2.2^{e-m}	90.5^{hij}	27.2^{b-e}	69.8^{abc}
RTx 2911	Inbred	USA	2.8^{ab}	2.6^{b-m}	125.2^{e-h}	30.8^{abc}	98.3^{a}
SC 987	PI 534116	Ethiopia	2.8^{ab}	1.5^{m}	105.3^{f-j}	35.3^{ab}	46.7^{bc}
SC 207	PI 533815	India	2.8^{ab}	2.1^{f-m}	68.4^{hij}	15.5^{gh}	71.0^{abc}
SC 599	PI 534163	USA	2.8^{ab}	1.7^{klm}	76.9^{hij}	20.0^{c-h}	71.8^{abc}
SC 1109	PI 576405	India	2.8^{ab}	1.9^{i-m}	73.8^{hij}	17.7^{fgh}	38.0^{bc}
BTx 623	PI 564163	Sudan	2.8^{ab}	2.1^{f-m}	118.3^{e-i}	30.7^{a-d}	70.7^{abc}
SC 1111	PI 576409	Sudan	2.8^{ab}	2.0^{h-m}	65.8^{hij}	19.5^{c-h}	40.7^{bc}
SU 5	PI 571012	Sudan	2.8^{ab}	2.9^{a-m}	276.4^{ab}	19.2^{c-h}	90.2^{ab}
SC 328	PI 534112	India	2.7^{ab}	2.2^{d-m}	118.7^{e-h}	19.6^{c-h}	66.2^{abc}
SC 311	PI 533753	Unknown	2.7^{ab}	2.0^{h-m}	92.9^{hij}	25.4^{b-h}	91.5^{a}
SC 724	PI 533993	Sudan	2.7^{ab}	2.8^{b-m}	80.1^{hij}	22.8^{c-h}	59.8^{abc}
SC 1328	PI 597971	USA	2.7^{ab}	3.0^{a-l}	83.2^{hij}	21.7^{c-h}	81.5^{ab}
SC 680	PI 576374	India	2.7^{ab}	2.4^{b-m}	91.0^{hij}	15.3^{gh}	92.0^{a}
SC 605	PI 534096	Mali	2.6^{ab}	-	101.0^{f-j}	29.5^{a-e}	72.0^{abc}
SC 625	PI 534097	Japan	2.5^{ab}	1.6^{lm}	94.6^{hij}	20.4^{c-h}	60.3^{abc}
SC 1124	PI 576418	Nigeria	2.5^{ab}	3.4^{a-g}	117.2^{e-i}	36.7^{ab}	44.0^{bc}
SC 719-11E	PI 534047	Sudan	2.5^{ab}	3.7^{ab}	113.4^{e-i}	25.6^{b-g}	81.7^{ab}

Table 1: Continue

SC line[#]	Accession	Country	PGMR[##]	Seed weight (g)	Plant height (cm)	Panicle length (cm)	Germination rate (%)
SC 725	PI 534101	Japan	2.3[ab]	2.8[b-m]	90.3[hij]	23.1[c-h]	80.0[abc]
SC 218	PI 534127	Unknown	2.2[ab]	2.8[b-m]	330.8[a]	20.7[c-h]	98.3[a]
SC 691	PI 534050	Burkina Faso	2.2[b]	2.8[b-m]	108.6[f-j]	20.3[c-h]	93.7[a]
SC 91	PI 534145	Rhodesia	2.0[b]	2.6[b-m]	249.9[bc]	19.8[c-h]	87.7[ab]
Sureno	Inbred	-	2.0[b]	2.0[g-m]	165.1[de]	19.6[c-h]	83.8[ab]

[#]Sorghum lines were planted at the AgriLife Research Farm, near College Station, Texas during the 2008 and 2009 growing seasons. Seed weight in gram was based on weight of 100 randomly selected seeds from each replication. Germination rate was based on the No. of seeds that germinated after 7 days from 300 seeds (100 seeds per replication) planted in flats containing potting soil, [##]PGMR: Inoculated panicles rated in the field using a scale 1-5, where 1 = Seed bright with no mold and no discoloration due to weathering, 2 = Seed is not as bright and has little or no mold, but has some discoloration due to weathering, 3 = Seed is not bright, there is some mold and some discoloration, 4 = Seed is almost entirely covered in mold and is deteriorating, 5 = Seed is covered entirely with mold, is deteriorated and looks dead[21]. At maturity, plant height was measured from the soil to the top of the plant in centimeters and panicle length (in centimeters) was measured from the first branch with racemes to the top of the panicle, [*]Means within a column followed by the same letter(s) are not significantly different at the 5% probability level based on Tukey-Kramer, [**]Missing data

to determine the effect of the accessions selected. Mean comparisons were conducted using Tukey-Kramer at the 5% probability level. Pearson correlation coefficient was calculated among the measured components.

RESULTS AND DISCUSSION

The complexity of grain mold on sorghum requires continuous evaluation of germplasm either exotic or converted to identify new sources of resistance. In this study, 60 converted sorghum lines along with five checks (resistant: RTx 2911, Sureno and SC 719-11E, susceptible: RTx 430 and RTx 2536) were inoculated with a mixture of *Fusarium thapsinum* and *Curvularia lunata* in the field. SC 91 (PI 534145) and Sureno had the lowest grain mold severity (2.0) while SU 623 (PI 571184) had the highest grain mold severity (4.8) (Table 1). During the evaluations, parameters such as seed weight, plant height, panicle length and germination rate were measured. Kernels weight and seed germination are important traits in determining the reaction of sorghum germplasm to grain mold[4,22]. The SC 370 (PI 533776; 4.7 g/100 kernels) recorded the highest seed weight followed by SC 352 (PI 534042, 3.9 g) and SC 719-11E (3.7 g), whereas, SC 4 (PI 534119, 1.1 g) recorded the lowest. The RTx 2911 recorded the highest germination rate (98.3%) while RTx 430 (13%) exhibited the lowest rate. Sorghum line SC 218 (PI 534127) was the tallest plant recording a mean height of 330 cm while lines SC 30 (PI 534131) and SC 55 (PI 533755) were the shortest, 58.2 and 58.7 cm, respectively. The SC 389 (PI 533894) recorded the longest panicle length of 40.0 cm while SC 4 (PI 534119) had the shortest (13.7 cm).

There was a highly significant negative correlation between grain mold severity and germination ($r = -0.385$, $p = 0.002$), indicating that germination rate was adversely affected when the sorghum lines were inoculated with a mixture of *Fusarium thapsinum* and *Curvularia lunata* (Table 2). Similar negative correlation betweeen grain mold severity and germination rate was recorded by Castor[13], Hepperly *et al.*[23], Garud *et al.*[24] and Prom *et al.*[19] reported that fungal treatments markedly reduced seed germination rates when compared to water-sprayed controls. *Curvularia lunata* tended to have a greater negative impact on germination in the drier season, whereas *F. thapsinum* in mold favorable environments. Hepperly *et al.*[23] observed significant negative correlations between seed germination with the incidence of *C. lunata* and *F. moniliforme* for mold infected sorghum seed from Mayaguez, Puerto Rico. Castor[13] noted significant negative correlations between germination and *Fusarium* spp. Prom[20] reported significant negative correlations between seed germination with the incidence of *F. thapsinum* and *C. lunata* isolated from sorghum panicles inoculated with grain mold.

Significant negative correlation was detected between germination rate and daily maximum temperature during the evaluation period. Most sorghum breeding programs have developed photoperiod-insensitive, short to medium duration lines with broad adaptability and mature before the end of rains but are frequently infected by grain mold. Despite mold susceptibility, photoperiod-insensitive lines are chosen by most farmers in the tropics due to higher yield than that of the photoperiod-sensitive cultivars[7].

Furthermore, results from this study showed that SC 725 (PI 534101), SC 218 (PI 534127), SC 691 (PI 534050) and SC 91 (PI 534145) exhibited grain mold severity of 2.3 or less with good seed weight (≥2.6 g), germination rate (≥80%) and panicle length (≥19.8 cm). The SC 91 (PI 534145) and SC 218 (PI 534127) are tall with 250.0 and 331.0 cm plant height, respectively. The SC 725 (PI 534101) and SC 691 (PI 534050) are short with 90.0 and 109.0 cm in plant height. The level of grain mold infection in the aforesaid

Table 2: Inter-relationship between grain mold severity and weather parameters across two growing seasons (2008 and 2009)

	Germ		T_{max}		T_{min}		Precip	
Parameters	r	p	r	p	r	p	r	p
GMS	-0.385	0.002***	0.194	0.131	-0.198	0.122	0.165	0.200
Germ			-0.553	0.0001***	0.030	0.815	-0.031	0.813
T_{max}					0.120	0.352	-0.228	0.074
T_{min}							-0.035	0.787

GMS: Grain mold severity across the sorghum converted lines, germ: Germination was based on the No. of seeds that germinated after 7 days out of 300 seeds per line planted in flats with potting soil, T_{max}: Maximum daily temperature during the evaluation period, T_{min}: Minimum daily temperature during the evaluation period, precip: Daily precipitation during the evaluation period, ***Significant at 1% probability level

four converted lines was lower than the scores exhibited by two resistant checks RTx 2911 (2.8) and SC 719-11E (2.5). Several other accessions SU 1873 (PI 570011), SU 1884 (PI 570022), SU 1854 (PI 569992), SU 1744 (PI 569882), SU 766 (PI 571312), SU 424 (PI 570759) and SU 109 (PI 267548) from Sudan were shown to possess high levels of resistance to grain mold[16]. Four red-seeded lines IS 14375, IS 14387, IS 18144 and IS 18528 and white-seeded lines IS 21443, IS 24495 and IS 25017 exhibited grain mold resistance when sorghum lines were evaluated under sprinkler irrigation in India[25]. Kumar *et al.*[26] identified 9 hybrids including ICSA 101×PVK 801, ICSA 382×GD 65055 and ICSA 400×GD 65028 that exhibited resistance to grain mold. Challenged with *F. thapsinum*, accessions PI525954, PI276841 and PI276840 exhibited lower grain mold severities and higher germination rates when compared to the resistant controls[27] Sureno and SC719.

CONCLUSION

Grain mold is a major obstacle to sorghum productivity and seed quality. The use of resistant sources is the best means to control the disease complex. Based on this study, the four lines SC 725 (PI534101), SC 218 (PI 534127), SC 691 (PI 534050) and SC 91 (PI 534145) identified may possess grain mold resistance genes and these lines could be used in grain mold resistance breeding programs.

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