Short Communication

Evaluation of sorghum accessions from Ethiopia and Mali against *Fusarium thapsinum*

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Abstract

Thirty-eight sorghum accessions from Ethiopia and Mali along with resistant (Sureno and SC719) and susceptible (RTx430 and RTx2536) controls were evaluated in replicated plots for resistance against *Fusarium thapsinum* at Isabela, Puerto Rico. Environmental conditions such as the temperature, relative humidity (>83%), and rainfall during this study, especially at and after physiological grain maturity were optimal for grain mould development. Highly significant negative correlations were observed between grain mould severity ratings in the field and on threshed grains with germination rate, indicating that germination rate was adversely affected when challenged with *F. thapsinum*. Of the 38 accessions tested, 18 accessions were either highly susceptible or susceptible to the pathogen. Accessions PI525954, PI276841, and PI276840 exhibited lower mean grain mould severities and higher germination rates when compared with the resistant controls Sureno and SC719. The aforementioned accessions may possess grain mould resistant genes and further studies are underway to determine the resistance mechanisms.

Keywords: Sorghum, Exotic accessions, Grain mould resistance, *Fusarium thapsinum*.

Grain sorghum (*Sorghum bicolor* (L.) Moench) is grown in a wide range of different environments, due to its natural ability to tolerate drought and heat, without compromising grain production. This crop is a major source of food in the drier tropics of Africa, Asia, and some parts of Central and South America (Singh and Bandyopadhyay, 2000). In the U.S. and Australia, the crop is used mainly as feed (Singh and Bandyopadhyay, 2000; Mukuru, 1992; Garud et al., 2000b). Grain mould, a complex fungal disease, is one of the principal constraints to sorghum productivity and grain quality, especially in areas with moist conditions later in the growing season (Bandyopadhyay and Chandrashekar, 2000; Garud et al., 2000a). Fungi of several genera, including *Fusarium thapsinum* Klittick, Leslie, Nelson et al., Manasas; *Fusarium semitectum* Berk. & Ravenel; *Curvularia lunata* (Wakk.) Boedijn; *Colletotrichum graminicola* (Ces.) G. W. Wilson; *Alternaria alternata* (Fr.: Fr.) Keissl.; and *Phoma sorghina* (Sacc.) Boerema, Dorenbosch, & Van Kesteren have been reported to be associated with the grain mould disease complex (Singh and Bandyopadhyay, 2000; Bandyopadhyay and Chandrashekar, 2000; Esele et al., 1995). Worldwide, *F. thapsinum*, *F. nygamy*, and *C. lunata* are considered to be the most important grain moulding fungi (Singh and Bandyopadhyay, 2000; Forbes et al., 1992; Bandyopadhyay and Chandrashekar, 2000). Management strategies for controlling grain mould disease complex include planting photosensitive cultivars that mature during periods of dry weather and chemical treatment to enhance seed germination and vigour. However, the

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use of resistant cultivars offers the most effective method for controlling the disease complex (Forbes et al., 1992; Singh and Bandyopadhyay, 2000). Efforts to improve sorghum grain mould resistance have been only partially successful mainly due to differences in environmental conditions and genotype x isolate interaction (Prom et al., 2003). Hence, continuous evaluation and identification of grain mould resistant sources, particularly from the exotic germplasm is one of the major breeding objectives for the development of new cultivars/ hybrids with stable resistance. Ethiopia and Mali are important sources of genetic diversity for sorghum. Thus, subsets of sorghum accessions from Ethiopia and Mali collections were evaluated for resistance against the grain moulding fungus *F. thapsinum*.

Thirty-eight accessions from Ethiopia (18) and Mali (20) collections maintained by the United States Department of Agriculture, Agricultural Research Service, National Plant Germplasm System (USDA-ARS, NPGS) that showed tolerance to grain weathering (GRIN, 2006) were selected for the field evaluation in 2006. Seed samples were obtained from the USDA-ARS Genetic Resources Conservation Unit, Griffin, Georgia. RTx430 and RTx2536 as susceptible control genotypes along with Sureño and SC719 exotic as resistant controls were included in the experiment. The study was conducted at the USDA-ARS Tropical Agriculture Research Station in Isabela, Puerto Rico, during the rainy season. The sorghum accessions were planted in a randomized complete block design with three replications. Seeds were planted in 1.8 m rows with 0.9 m row spacing. To control fire ants, Lorsban 15G (Chlorpyrifos) granular insecticide (Dow AgroSciences, Indianapolis, IN) at 8 kg ha^{-1} was applied at planting. Fertilizer (15-5-10 NPK) at 560 kg/ha was applied at planting and 30 days after planting to the field plots. Weeds were controlled by using both mechanical tillage and hand hoeing. Overhead irrigation was applied three times with the third irrigation conducted 16 days after planting and no irrigation applied after panicles were inoculated with the pathogen. To prevent bird damage, the study was conducted in a mesh screen covered structure.

F. thapsinum was isolated from grain mould infected sorghum seeds collected from research fields in Isabela, Puerto Rico, (Erpelding and Prom, 2006) and cultured on ½ potato-dextrose agar (PDA) media at room temperature for 7 days. Inoculum preparation and inoculation protocol have been previously reported (Prom and Erpelding, 2009). Briefly, inoculum was prepared by culturing the fungus on sorghum seeds. The sorghum seeds were soaked in water overnight, rinsed, transferred to media bottles, autoclaved, and inoculated with the 7 day old fungal cultures grown on ½ PDA media. The inoculated seeds were incubated at room temperature for approximately 5 days until the seeds were completely colonized by the fungus. Fungal spores and mycelium fragments were removed from the seeds by adding water to the media bottles and vigorously mixing. The suspension was filtered through cotton cloth into a backpack sprayer, approximately 5 mL tween 20 was added as a sticking agent, and the suspension was diluted to approximately 12 L with tap water to achieve a spore concentration of 1 x 10^6 conidia ml^{-1}. Field inoculations were conducted when the panicles within a row were at 50% bloom. All panicles within the row were inoculated with the spore suspension until runoff. Inoculations were repeated at weekly intervals until flowering was completed and seed development was observed in the row. Due to differences in developmental stage, accessions were inoculated at different dates. Panicles were harvested at maturity for grain mould assessment.

Ten panicles per replication were evaluated for grain mould severity in the field (PGMR) and for the threshed grains (TGMR) from the same panicles in the laboratory using a 1 to 5 rating scale, where 1 = no mould (highly resistant); 2 = 1 – 10% moulded kernels (resistant); 3 = 11 – 25% moulded kernels (moderately resistant); 4 = 26 – 50% moulded kernels (susceptible); and 5 = >50% moulded kernels (highly susceptible) (Prom and Erpelding, 2009; Thakur et al., 2007). Seed weight was based on weight in grams of 100 randomly selected seeds per replication, and germination rates were based on the number of seeds that germinated in 7 days out of 300 seeds per accession planted in flats containing Metro Mix 200 potting medium (Scotts-Sierra Horticultural Products Company, Maryland, OH) (Prom et al., 2003).

Data for PGMR, TGMR, seed weight, and percent germination rate were analyzed using the command PROC GLIMMIX (SAS version 9.2, SAS Institute, Cary, NC) to determine the effect of the accessions. Mean comparisons among the sorghum accessions for the different measured parameters were based on Tukey-Kramer grouping at the 5% probability level. The Pearson correlation coefficient was calculated among PGMR, TGMR, seed weight, percent germination rate, daily precipitation, daily maximum and minimum temperatures, and relative humidity.

One of the principal fungal pathogens that incite sorghum grain mould in the United States is *Fusarium thapsinum* (Esele et al., 1993; Bandyopadhyay et al., 2002). Because of the complexity, little is known about the genetic control of grain mould resistance in sorghum. Several crop improvement programmes have been conducted to face this problem, but the results were only partially successful. In this study, the mean values of the 38 accessions for the recorded variables are shown in Table 1 and the phenotypic correlations between the traits are shown in Table 2. Differences in grain mould reactions among the selected accessions when challenged with *F. thapsinum* were noted as indicated by the main effect being highly significant ($P<0.0001$). Accessions PI525954 and PI276840 were resistant to moderately resistant, whereas PI585697, PI585754, PI585814, PI585744, PI585743, and PI585752 were moderately resistant both at PGMR and TGMR levels (Table 1). Thakur et al., (2007) reported that IS 2815, IS 21599, IS 10288, IS 3436, IS 10646, IS 10475, and IS 23585 were resistant to grain mould and are currently been used to develop restorer lines, varieties and hybrids parents for breeding programmes. Prom and Erpelding (2009) recorded several accessions PI570011, PI570022, PI569992, PI569882, PI571312, PI570759, and PI267548 from Sudan that possess high levels of resistance to grain mould. Four coloured-seeded lines IS 14375, IS 14387, IS 18144, and IS 18528 and white-seeded lines IS 21443, IS 24495, and IS 25017 exhibited grain mould resistance when grown under sprinkler irrigation in India (Audilakshmi et al., 1999). Also, 9 hybrids, including ICSA 101 x PVK 801, ICSA 382 x GD 65055, and ICSA 400 x GD 65028 exhibited resistance to grain mould (Kumar et al., 2008). PI276841 and PI585823 were resistant under field conditions but showed susceptibility when threshed grains were evaluated. Eleven accessions exhibited moderately resistant reaction at PGMR but were susceptible at the TGMR level. Eighteen accessions out of the 38 accessions were either highly susceptible or susceptible. Accession PI276841 had the highest average seed weight (3.08g/100seeds) while PI585827 had the lowest seed weight (1.11g/100 seeds). All others had an average hundred seed weight ranging from 1.17g (PI585822) to 3.05g (PI276840) Accession PI525954 (56.7%) was recorded with the highest germination rate followed by PI276841 (36.3%) and PI585647 (27.6%), while the susceptible check RTx430 recorded zero germination rate. All other accessions exhibited germination rate ranging from 0.67% (PI455719) to 26.7% (PI276840). Due to high level of disease development, poor germination was recorded in most of the accessions.

There was a strong positive correlation ($r = 0.72$, $P < 0.0001$) between PGMR and TGMR in this study, indicating that either field or laboratory scoring techniques are equally effective for grain mould evaluation (Table 2). Significant negative correlations for PGMR and TGMR with germination rate ($r = -0.67$, $P < 0.0001$, and r = -

Table 1. Reaction of subsets of sorghum accessions from Ethiopia, Mali, and checks against the grain moulding fungus *Fusarium thapsinum*[1]

Accession	PGMR[2]	TGMR[3]	Seed weight[4]	Germination[5]
PI456635	5.0a[6]	4.9ab	1.83bcdefgh	5.01bcd
PI454308	5.0a	5.0a	2.46abc	3.27bcd
PI455181	5.0a	5.0a	2.03bcdefgh	5.73bcd
PI455187	5.0a	5.0a	1.78cdefgh	1.60cd
PI455719	5.0a	4.0bcde	1.63cdefgh	0.67cd
PI452650	5.0a	5.0a	1.88bcdefgh	1.43cd
PI453578	5.0a	5.0a	2.27abcdef	1.10cd
RTx430	5.0a	5.0a	1.61cdefgh	0.00d
PI456320	5.0a	5.0a	2.44abcd	8.00bcd
PI452944	4.9ab	4.5abcd	1.21fgh	17.21bcd
RTx2536	4.8ab	4.3abcd	1.53cdefgh	1.17cd
PI456024	4.7ab	4.0bcde	2.13abcdefg	23.00abcd
PI456605	4.7ab	5.0a	2.24abcdef	4.77bcd
PI452721	4.0abc	4.0abcde	1.31efgh	12.28bcd
PI452641	4.0abc	4.0abcde	2.78ab	17.30bcd
PI457838	4.0abc	5.0a	1.35efgh	9.33bcd
PI267647	4.0abc	5.0a	1.48defgh	2.00bcd
PI585822	4.0abc	4.7abc	1.17gh	8.03bcd
PI585755	3.7bcd	4.0abcde	1.81bcdefgh	12.87bcd
PI585827	3.7bcd	4.0abcde	1.11h	21.43bcd
PI585697	3.3cde	3.0e	1.64cdefgh	17.37bcd
Sureno	3.0cdef	3.0e	2.20abcdef	11.93bcd
PI585754	3.0cdef	3.0e	1.91bcdefgh	24.33abcd
PI585814	3.0cdef	3.3de	2.14abcdefg	33.30abc
PI585647	3.0cdef	3.7cde	1.57cdefgh	27.60abcd
PI276843	3.0cdef	4.0abcde	2.34abcde	25.20abcd
PI585744	3.0cdef	3.0e	1.73cdefgh	16.73bcd
PI585825	2.7def	4.7abc	1.93bcdefgh	6.43bcd
PI525506	2.7def	4.0abcde	1.30fgh	2.03bcd
PI585828	2.7def	3.7cde	1.86bcdefgh	23.83abcd
PI585739	2.7def	3.7cde	1.95bcdefgh	29.40abcd
PI585817	2.7def	4.0abcde	1.75cdefgh	10.70bcd
PI585805	2.7def	4.0abcde	1.50cdefgh	17.33bcd
PI585743	2.7def	3.0e	1.61cdefgh	31.67abc
PI585752	2.7def	3.0e	2.05bcdefgh	27.00abcd
SC719 Exotic	2.6ef	4.0abcde	2.33abcde	6.18bcd
PI585824	2.3ef	4.0abcde	1.95bcdefgh	18.83bcd
PI585816	2.3ef	4.0abcde	1.91bcdefgh	21.53bcd
PI276840	2.0f	3.0e	3.05a	26.70abcd
PI276841	2.0f	3.7cde	3.08a	36.33ab
PI525954	2.0f	3.0e	1.70cdefgh	56.70a
PI585823	2.0f	4.0abcde	1.96bcdefgh	18.20bcd

[1]Sorghum accessions were selected based on preliminary screening showing them as having grain weathering tolerance. Accessions and checks were planted in replicated plots in Isabela, Puerto Rico under mesh screen. Grain mould severity ratings conducted in the field and in the laboratory on thrashed seeds were based on a scale of 1 to 5 (Prom and Erpelding, 2009; Thakur et al. 2007).
[2]PGMR=non destructive evaluation of the tagged sorghum panicle grain mould rating in the field.
[3]TGMR=evaluation in the laboratory of the threshed seed grain mould rating from the same tagged sorghum panicles.
[4]Seed weight=seed weight in grams of 100 seeds per replicate per accession.
[5]Germination= the percentage of germinated seeds out of 300 seeds per accession.
[6]Means within a column followed by the same letter(s) are not significantly different (P=0.05) based on Tukey-Kramer adjustment for multiple comparisons.

Table 2. Correlation coefficients among grain mould severity scored from the field (PGMR), threshed seeds (TGMR), seed weight (SW), germination rate (Germ), daily precipitation (PRE), maximum daily temperature (Tmax), minimum daily temperature (Tmin), and percent relative humidity (RH)[1]

	TGMR		SW[2]		Germ[3]		PRE		Tmax		Tmin		RH	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
PGMR	0.71723	0.0001***[4]	-0.16424	0.2986	-0.66981	0.0001***	-0.04343	0.7848	-0.30023	0.0534*	-0.22013	0.1613	0.08057	0.6120
TGMR			-0.11684	0.4612	-0.72429	0.0001***	0.05102	0.7483	-0.13355	0.3992	-0.30137	0.0524*	0.01491	0.9254
SW					0.18651	0.2369	-0.10399	0.5122	0.25748	0.0997*	0.19999	0.2041	0.03803	0.8110
Germ							0.13306	0.4009	0.41465	0.0063***	0.08174	0.6068	-0.09001	0.5708
PRE									0.04598	0.7725	0.13760	0.3848	0.08133	0.6086
Tmax											-0.08570	0.5895	0.04947	0.7557
Tmin													0.03330	0.8341

[1]Experiment was conducted at Isabela, Puerto Rico during the rainy season when conditions are optimal for grain mould infection. Grain mould severity was based on a scale of 1 to 5 (Prom and Erpelding, 2009: Thakur et al. (2007). Eighteen accessions from Ethiopia, 20 accessions from Mali, and four controls were planted in replicated plots (three replicates per accession). PGMF was evaluated on tagged sorghum panicles on the field, and TGMR scores based on grain mould severity of threshed seeds (same tagged panicles) in the laboratory. Precipitation, maximum and minimum temperatures were recorded daily 8 days prior to the first inoculation until harvest.
[2]Seed weight=seed weight in grams of 100 seeds per replicate per accession.
[3]Germination= the percentage of germinated seeds out of 300 seeds per accession.
[4]*, or *** denotes significant at 10, or 1% probability level.

0.72, $P < 0.0001$) were recorded, indicating that germination rate was adversely affected when challenged with *F. thapsinum*. Similar negative correlation between grain mould severity and seed germination was reported earlier by Castor (1981), Hepperly et al. (1982), Garud et al. (2000a), and Prom et al. (2003). Thakur et al. (2007) noted that high humidity (>90%) and temperature range of 25-35°C are quite favorable for infection and mould development. The environmental conditions such as temperature, relative humidity (>83%), and rainfall during this study, especially at and after physiological maturity were optimal for grain mould development, making the screening for the identification of grain mould resistance sources in this year's evaluation at Isabela, Puerto Rico, more effective. Though frequent precipitations and high relative humidity were noted during the experiment period, we failed to detect any significant association between grain mould severity and these two variables (Table 2). However, studies have shown that the intensity of grain mould severity varies with rainfalls during grain development to maturity (Shinde et al., 2003). In conclusion, this study has shown that sorghum accessions PI525954, PI276840, PI585697, PI585754, PI585814, PI585744, PI585743, and PI585752 may possess grain mould resistant genes and might be useful in crop improvement programs. Studies are underway to determine the resistance mechanisms within these sorghum accessions.

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