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NEW SOURCES OF GRAIN MOLD RESISTANCE AMONG SORGHUM ACCESSIONS FROM SUDAN

[NUEVAS FUENTES DE RESISTENCIA AL MOHO DEL GRANO ENTRE ACCESIONES DE SORGO DEL SUDAN]

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SUMMARY

Fifty-nine sorghum accessions from Sudan were evaluated in replicated plots at Isabela, Puerto Rico, for resistance against *Fusarium thapsinum*, one of the causal agents of grain mold. The environmental conditions such as temperature, relative humidity, and rainfall during this study, especially at and after physiological maturity were optimal for grain mold development. Highly significant negative correlations between grain mold severity ratings in the field and on threshed grains with germination rate and seed weight were recorded, indicating that germination and seed weight were adversely affected when challenged with *F. thapsinum*. Temperature showed a significant negative correlation with grain mold severity and a significant positive correlation with germination rate. However, no significant correlation was observed between rainfall and grain mold severity or germination rate. Accessions PI570011, PI570027, PI569992, PI569882, PI571312, PI570759, and PI267548 exhibited the lowest grain mold severities and among the highest germination rates, indicating that these accessions may possess genetic resistance to grain mold and might be useful in sorghum enhancement programs. Four of these accessions had significantly higher germination rates than the resistant control genotypes with PI267548 having the highest germination rate. PI267548 was the only white seeded accessions showing significantly better grain mold resistance than the control genotypes.

Key words: Sorghum; exotic lines; grain mold resistance; *Fusarium thapsinum.*

RESUMEN

Cincuenta y nueve lineas de sorgo provenientes del Sudan fueron evaluadas en parcelas repetidas en Isabela, Puerto Rico para resistencia a *Fusarium thapsinum*, uno de los agentes causales de mohos del grano. Las condiciones ambientales como temperatura, humedad relativa y lluvia durante el estudio, especialmente durante y después de la madurez fisiológica fueron las óptimas para el desarrollo de los mohos del grano. Se registraron correlaciones negativas altamente significativas entre el moho del grano en el campo y los granos trillados, la tasa de germinación y el peso de los granos, indicando que la germinación y el peso del grano fueron adversamente afectados cuando se enfrentaron a *Fusarium thapsinum*. La temperatura mostró una correlación negativa significativa con la severidad del moho del grano y una correlación positiva significativa con la tasa de germinación. Sin embargo, no se observó una correlación significativa entre la precipitación y la severidad del moho del grano o con la tasa de germinación. Las accesiones PI570011, PI570027, PI569992, PI569882, PI571312, PI570759 y PI267548 mostraron los valores más bajos de severidad de moho del grano y las más altas tasas de germinación, indicando que estas accesiones poseen resistencia genética al moho del grano y pueden ser útiles en los programas de mejoramiento de sorgo. Cuatro de estas accesiones presentaron las más altas tasas de germinación que el testigo resistente, teniendo PI267548 la más alta tasa de germinación. PI267548 fue la única accesión de grano blanco que mostró significativamente la mejor resistencia al moho del grano que el genotipo testigo.

Palabras clave: Sorgo; líneas exóticas; resistencia a mohos del grano; *Fusarium thapsinum.*

INTRODUCTION

Sorghum grain mold is a complex disease caused by fungi from several genera, including the most common species *Fusarium thapsinum* Klittick, Leslie, Nelson et al., Marasas and *Curvularia lunata* (Wakk.) Boedijn (Singh and Bandyopadhyay, 2000; Bandyopadhyay and Chandrashekar, 2000; Esele et al., 1995). Annually, losses in market value of the sorghum crop due to grain mold are estimated at $130 million U.S. dollars worldwide (Bandyopadhyay et al., 2002). Although, management strategies such as planting cultivars that mature during periods of dry weather can reduce the impact of sorghum grain mold, nevertheless, the use of resistant cultivars offers the most effective method for controlling the disease (Forbes et al., 1992; Singh and Bandyopadhyay, 2000). However, grain mold resistance in sorghum is complex, and it involves several mechanisms (Castor, 1981; Forbes et al., 1992; Waniska et al., 2001). Studies have shown that resistance to grain mold can be enhanced by certain characteristics, such as kernel hardness, kernels with red pericarp, endosperm texture, kernels high in tannins with a pigmented testa, high concentrations of flavan-4-ol, and plants with tan plant color and the pericarp intensifier (*I*) gene (Mukuru, 1992; Esele et al., 1995; Waniska et al., 2001). Recent studies have indicated that antifungal proteins such as sormatin, chitinases, glucanases, and ribosome-inhibiting protein may play a role in grain mold resistance (Seetharaman et al., 1996; Rodriguez-Herrera et al., 1999; Bueso et al., 2000). Worldwide, few sources of grain mold resistance are available for sorghum improvement, and evaluation of germplasm collections will be essential to identify new sources of resistance. Sudan is an important source of genetic diversity for sorghum, and this study was undertaken to identify potential sources of grain mold resistance among 59 germplasm lines with different agronomic traits from the Sudan sorghum collection.

MATERIAL AND METHODS

Field trial

Fifty-nine accessions from the Sudan collection maintained by the United States Department of Agriculture, Agricultural Research Service, National Plant Germplasm System (USDA-ARS, NPGS) were selected for the grain mold field evaluation. Germplasm lines that showed tolerance to grain weathering (GRIN, 2006) were selected and included 39 white seeded lines and 20 red seeded lines that have a pigmented testa. Seed samples were obtained from the USDA-ARS Genetic Resources Conservation Unit, Griffin, Georgia. RTx430 (NSL92562) and RTx2536 were included as susceptible control genotypes with Sureño (PI561472) and NSL51941 included as resistant controls. Sureño is a white seeded cultivar developed in Honduras (Meckenstock et al., 1993) and shows a high level of resistance to grain mold (Prom et al., 2003). NSL51941 is the exotic parent used in the development of SC719 (Stephens et al., 1967) and is a red seeded line with a pigmented testa that was collected from Sudan. The evaluation 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 on 24 January 2006. Seeds were planted in 1.8 m length rows with 0.9 m row spacing. Lorsban 15G (Chlorpyrifos) granular insecticide (Dow AgroSciences, Indianapolis, IN) at 8 kg/ha was applied at planting for fire ant control. Fertilizer at 560 kg/ha (15-5-10 NPK) was applied at planting and 30 days after planting to the field plots. Weeds were controlled by mechanical tillage and hand hoeing. Overhead irrigation was applied three times with the third irrigation conducted 16 days before flowering and no irrigation applied after inoculation. To prevent bird damage, the evaluation was enclosed in a mesh screen covered structure.

Grain mold inoculation

Fusarium thapsinum was isolated from grain mold infected sorghum seed collected from research fields in Isabela, Puerto Rico (Erpelding and Prom, 2006) and cultured on ½ strength potato-dextrose agar (PDA) media at room temperature for 7 days. Inoculum was prepared by culturing the fungus on sorghum seed. The sorghum seed was 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 seed was incubated at room temperature for approximately 5 days until the seed was completely colonized by the fungus. Fungal spores and mycelial fragments were removed from the seed by adding water to the media bottles and mixing vigorously. The suspension was filtered through cotton cloth into a backpack sprayer, the seed was rinsed three times with water and the rinse was added to the sprayer, approximately 5 mL of 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 approximately $1x10^6$ conidia/mL. Field inoculations were conducted when in a row the first panicles to flower were at 50% bloom, and 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 for panicles in the row. Due to differences in the flowering pattern, accessions were inoculated at different dates. Panicles were harvested approximately 60 days after flowering for grain mold assessment.

Parameters measured

Ten to 15 panicles per replication were evaluated for percentage grain mold severity rating in the field (PGMSR) and for percentage moldy threshed grains rating (PMTGR) from the same panicles in the laboratory using a 1 to 5 rating scale, where 1 = no mold (highly resistant), 2 = 1 – 10% molded kernels (resistant), 3 = 11 – 25% molded kernels (moderately resistant), 4 = 26 – 50% molded kernels (susceptible), and 5 = >50% molded kernels (highly susceptible) (Thakur et al., 2007). Seed weight was based on weight in grams of 100 randomly selected seeds per replication, and germination percentage were based on the number of seeds that germinated in 7 days out of 300 seeds per line planted in flats containing Metro Mix 200 potting medium (Scotts-Sierra Horticultural Products Company, Maryland, OH) (Prom et al., 2003).

Statistical analysis

Data for PGMSR, PMTGR, seed weight, and percent germination rate were subjected to the analysis of variance using the command PROC GLIMMIX (SAS version 9.2, SAS Institute, Cary, NC) to determine the effect of disease on the accessions selected. 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 PGMSR, PMTGR, seed weight, percent germination, daily precipitation, and daily maximum and minimum temperatures.

RESULTS

Grain mold reaction measured by PGMSR and PMTGR with other components seed weight and germination of 59 sorghum exotic accessions collected from Sudan inoculated with *F. thapsinum* were presented in Table 1. Accessions PI570011 and PI570027 exhibited the lowest grain mold severities both at PGMSR and PMTGR levels when compared with Sureño and NSL51941. PI569992 was highly resistant under field evaluation but moderately resistant for the PMTGR, whereas accessions PI569882, PI571312, PI570759, and PI267548 were resistant for the PGMSR and were moderately resistant for the PMTGR. Forty-two out of the 59 accessions tested were either highly susceptible or susceptible to grain mold. Germination rate ranged from 0 to 81.33% (Table 1). The highest germination was observed for PI267548 followed by PI570011 (45.1%) and PI570027 (44.0%). Only ten accessions had higher germination percentage than Sureño. The susceptible controls, RTx2536 and RTx430, had germination percentages less than 0.5% and germination was 0% for six accessions with germination less than 10% for 43 accessions. Accession PI571244 recorded the highest average seed weight of 3.28 g/100 seeds, which was significantly higher than the average seed weight of 22 of the 59 accessions tested.

Table 1. Grain mold reaction of 59 sorghum accessions from Sudan inoculated with *F. thapsinum*[1]

Accession	PGMSR[2]	PMTGR[3]	Seed weight[4]	Germination[5]
PI569919	5.0a[6]	5.0a	2.09abcdefghi	0.67h
PI562932	5.0a	5.0a	2.49abcdefgh	1.37gh
PI217826	5.0a	5.0a	1.47efghi	0.37h
PI562286	5.0a	5.0a	1.60defghi	1.00h
PI562188	5.0a	5.0a	1.84bcdefghi	2.67fgh
PI562189	5.0a	5.0a	1.85bcdefghi	1.42gh
PI569896	5.0a	5.0a	1.78bcdefghi	6.75efgh
PI562274	5.0a	4.9a	1.04i	3.06efgh
PI562275	5.0a	4.9a	1.97abcdefghi	2.47fgh
PI562288	5.0a	5.0a	1.84bcdefghi	0.00h
PI562281	5.0a	5.0a	1.90bcdefghi	4.68efgh
PI569899	5.0a	5.0a	2.17abcdefghi	2.35gh
PI562287	5.0a	5.0a	1.71cdefghi	0.00h
PI570578	5.0a	5.0a	2.46abcdefhi	5.00efgh
PI570915	5.0a	5.0a	1.39ghi	0.00h
PI570298	5.0a	4.0abcd	2.46abcdefgh	25.33bcdef
PI569961	5.0a	4.67ab	2.59abcdef	0.00h
PI569104	5.0a	5.0a	1.38hi	1.46gh
PI563379	5.0a	5.0a	1.80bcdefghi	1.01h
PI570842	5.0a	5.0a	2.01abcdefghi	2.03gh
PI570801	5.0a	4.7ab	2.19abcdefghi	3.07efgh

Accession	PGMSR[2]	PMTGR[3]	Seed weight[4]	Germination[5]
PI562927	5.0a	4.33abc	2.00abcdefghi	3.01fgh
RTx2536	5.0a	5.0a	1.41ghi	0.11h
RTx430	5.0a	5.0a	2.00abcdefghi	0.34h
PI568543	5.0a	5.0a	1.84bcdefghi	0.33h
PI569189	5.0a	5.0a	1.71bcdefghi	0.00h
PI569105	5.0a	5.0a	1.12i	0.68h
PI571191	4.7ab	5.0a	2.51abcdefgh	7.00efgh
PI571117	4.7ab	5.0a	1.99abcdefghi	0.00h
PI571019	4.7ab	5.0a	1.95bcdefghi	0.00h
PI562223	4.7ab	5.0a	1.68cdefghi	2.00gh
PI571000	4.7ab	4.3abc	2.08abcdefghi	11.33defgh
PI217680	4.7ab	5.0a	1.71cdefghi	2.67fgh
PI569969	4.3abc	4.0abcd	2.73abcd	14.67defgh
PI571054	4.3abc	5.0a	1.43fghi	0.33h
PI562278	4.3abc	4.7ab	2.71abcd	4.33efgh
PI571024	4.3abc	4.3abc	2.27abcdefghi	4.31efgh
PI563237	4.0abcd	4.0abcd	2.82abc	8.67efgh
PI562920	4.0abcd	4.3abc	2.28abcdefghi	7.35efgh
PI570686	4.0abcd	4.3abc	2.70abcd	2.00gh
PI570683	4.0abcd	4.0abcd	2.63abcde	13.00defgh
PI570756	4.0abcd	4.0abcd	2.51abcdefgh	9.00efgh
PI571259	4.0abcd	4.7ab	2.39abcdefgh	0.33h
PI570929	3.7bcde	4.7ab	2.02abcdefghi	22.33cdefg
PI569903	3.3cdef	3.0def	2.79abcd	15.33defgh
PI570685	3.3cdef	3.3cde	2.32abcdefghi	39.13bc
PI570748	3.3cdef	4.0abcd	2.00abcdefghi	26.40bcde
PI571244	3.0defg	3.7bcd	3.28a	7.37efgh
PI571126	3.0defg	4.0abcd	2.75abcd	7.37efgh
PI571297	3.0defg	3.7bcd	1.65cdefghi	4.03efgh
Sureno	3.0defg	3.7bcd	1.96bcdefghi	15.36defgh
NSL51941	2.8efg	3.8bcd	1.91bcdefghi	14.27defgh
PI570888	2.7efg	3.7bcd	2.40abcdefgh	8.07efgh
PI570878	2.7efg	3.3cde	2.59abcdefg	6.67efgh
PI570751	2.7efg	4.0abcd	2.34abcdefghi	12.67defgh
PI267548	2.3fgh	3.0def	2.18abcdefghi	81.33a
PI152580	2.3fgh	4.0abcd	2.91ab	14.33defgh
PI570759	2.3fgh	3.0def	2.16abcdefghi	19.00cdefgh
PI571312	2.0gh	3.0def	2.20abcdefghi	10.40efgh
PI569882	2.0gh	3.0def	2.61abcdef	22.33cdefg
PI570011	1.3h	2.0f	2.72abcd	45.10b
PI570027	1.3h	2.3ef	1.64cdefghi	44.00b
PI569992	1.3h	3.0def	1.69cdefghi	32.57bcd

[1]Plant introduction numbers for the accessions included in the grain mold evaluation. Sorghum accessions selected were rated as tolerant to grain weathering (GRIN, 2006). Accessions and control genotypes (RTx430, RTx2536, Sureño, and NSL51941) were planted in replicated plots in Isabela, Puerto Rico in 2006 during the rainy season under mesh screen. Grain mold severity ratings conducted in the field and in the laboratory on threshed seeds were based on a scale of 1 to 5 (Thakur et al., 2007).

[2]PGMSR=non destructive evaluation of the tagged sorghum panicle grain mold rating in the field.

[3]PMTGR=evaluation in the laboratory of the threshed seed grain mold rating from the same tagged sorghum panicles.

[4]Seed weight=mean seed weight in grams of 100 seeds per replicate per line.

[5]Germination=percentage of germinated seeds out of 300 seeds per line.

[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.

Correlation coefficients among grain mold severity scored from the field, threshed seeds, seed weight, germination and other weather parameters were presented in Table 2. A strong positive correlation (r =0.91, $P < 0.0001$) between PGMSR and PMTGR was observed. Whereas, negative correlations for PGMSR and PMTGR with germination (r = -0.68, $P < 0.0001$, and r = -0.74, P < 0.0001) and seed weight (r = -36, P < 0.0039, and r = -43, P < 0.0005) were recorded. Also, maximum and minimum temperature recorded significant negative correlation with PGMSR and PMTGR. But, precipitation in this study was not recorded any significant correlation with PGMSR and PMTGR.

DISCUSSION

Grain mold is one of the major constraints to sorghum productivity and grain quality, especially if frequent rains occur after physiological maturity (Garud et al., 2000). In addition, several of the grain molding fungi can produce mycotoxins in infected grain (Singh and Bandyopadhyay, 2000; Bandyopadhyay et al., 2002). The disease is caused by an array of fungal species with over 40 species identified from mold infected sorghum seed. *Fusarium semitectum*, *F. verticillioides*, *F. thapsinum* and *C. lunata* were the most frequently recovered fungal species from naturally infected grain produced in Isabela, Puerto Rico (Erpelding and Prom, 2006). Since numerous fungal species can contribute to grain mold, identification of new resistance sources is of paramount importance and hence screening of germplasm lines is a continuous process for sorghum crop improvement programs.

There were differences in grain mold reactions among the selected 59 sorghum accessions and the two resistant and two susceptible controls when challenged with *F. thapsinum* (P<0.0001). Accessions PI570011 and PI570027 may possess genes for grain mold resistance, as they exhibited the lowest grain mold severities both at PGMSR and PMTGR levels when compared with the resistant checks. Whereas, a maximum number of 42 out of 59 accessions tested were either highly susceptible or susceptible to grain mold indicating that there was conducive environment for grain mold infection. Although there were no significant differences between the overall means for grain mold severity among the white and red seeded accessions, the lowest grain mold severities for the PGMSR and PMTGR were recorded for the red seeded accessions with a pigmented testa, this suggests that tannin content of the kernel may affect grain mold development. The majority of the red seeded accessions showed grain mold severity and germination similar to that of the resistant controls. Three red seeded accessions were rated as highly susceptible and showed germination percentages less than 4%. Only one white seeded accession showed greater resistance than the resistant controls with five accessions showing higher germination percentages. However, for the development of grain sorghum hybrids in the United States, germplasm lines with low kernel tannin content are essential for sorghum improvement.

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

	PMTGR		SW[2]		Germ[3]		PRE		Tmax		Tmin	
	r[4]	*P*[5]	*R*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
PGMSR	0.9	0.01[c6]	-0.36	0.01[c]	-0.68	0.01[c]	0.01	0.98	-0.42	0.01[c]	-0.37	0.01[c]
PMTGR			-0.43	0.01[c]	-0.75	0.01[c]	-0.03	0.83	-0.42	0.01[c]	-0.28	0.03[b]
SW					0.21	0.09[a]	-0.06	0.65	0.09	0.45	0.20	0.12
Germ							0.02	0.90	0.32	0.01[b]	0.22	0.08[a]
PRE									-0.01	0.94	0.22	0.08[a]
Tmax											0.04	0.78

[1]Experiment was conducted at Isabela, Puerto Rico during the rainy season when conditions are optimal for grain mold infection. Grain mold severity was based on a scale of 1 to 5 (Thakur et al., 2007). Fifty-nine accessions from Sudan and four control genotypes were planted in replicated plots (three replicates per line). PGMSR was evaluated on tagged sorghum panicles in the field, and PMTGR scores based on grain mold 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=mean seed weight in grams of 100 seeds per replicate per line.

[3]Germination=percentage of germinated seeds out of 300 seeds per line.

[4]*r*=correlation coefficient.

[5]*P*=probability value (p-value).

[6a], [b], or [c] denotes significant at 10, 5, or 1% probability level.

A strong positive correlation between PGMSR and PMTGR indicates that either field or laboratory scoring techniques are equally effective for grain mold evaluation. However, Thakur et al. (2007) suggested using only the PGMSR since it represents a more realistic evaluation of the line/accession reaction to grain mold in the field, as opposed to using PMTGR which in most cases is evaluated several days after harvest. Due to high levels of grain mold infection, most of the accessions including the resistant control genotypes recorded very poor germination. However, significant negative correlations for PGMSR and PMTGR with germination and seed weight observed in this study indicates that germination and seed weight were adversely affected when challenged with *F. thapsinum*. Similar negative correlations between grain mold severity and seed germination was reported earlier by Castor (1981), Hepperly et al. (1982) Garud et al. (2000), and Prom et al. (2003).

High humidity (>90%) and temperatures ranging from 25 to 35°C are quite favorable for infection and mold development. Panicle wetness during flowering and grain development due to frequent rain showers contributes to greater mold development (Thakur et al., 2007). The environmental conditions during this study, especially at and after physiological maturity were highly conducive for grain mold development resulting in high grain mold severities being recorded for the majority of the accessions in the evaluation and poor germination rates for nearly all accessions including the resistant controls. Although the study was conducted for only one year, resistant sources were identified and a single year of data could be effectively used to eliminate susceptible germplasm from further evaluation.

Though frequent precipitations were noted during the course of this study, we failed to detect any significant association between grain mold severity and daily precipitation (Table 2). However, earlier studies have shown that the intensity of grain mold severity varies with rainfall during grain development to maturity (Shinde et al., 2003). In this study, rainfall occurred on approximately 60% of the days from flowering to harvesting with over 467 mm of rainfall received during this period. This excessive rainfall could have effected the influence of precipitation on grain mold severity, as the majority of the accessions were rated as susceptible or highly susceptible.

Since a significant strong positive correlation was noticed between PGMSR and PMTGR in this study, and the exotic sorghum accessions PI570011, PI570027, PI569992, PI569882, PI571312, PI570759, and PI267548 from Sudan showed the lowest grain mold severities both at PGMSR and PMTGR levels, these accessions may possess genes for grain mold resistance and might be useful in sorghum enhancement programs. Grain mold resistance was frequently associated with red seeded accessions. PI267548 showed the highest germination rate and was the only white seeded accession rated as resistant.

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CONCLUSION

With optimal environmental conditions in 2006 at Isabela, Puerto Rico, fifty-nine exotic sorghum accessions collected from Sudan were evaluated for resistance against the grain molding fungus, *Fusarium thapsinum*. Seven accessions PI570011, PI570027, PI569992, PI569882, PI571312, PI570759, and PI267548 were selected based on low disease severity and other related components and can be used for further resistance breeding program.

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