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Research Article
Mycoflora Analysis and Other Measured Parameters of Sorghum Seeds Collected from Puerto Rico and Mexico

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Abstract

Background and Objective: Grain mold is one of the major biotic constraints to sorghum production worldwide. This disease complex is associated with many genera of fungi, including mycotoxigenic *Fusarium* species. Yield losses can be high, especially when susceptible lines are planted in areas with wet conditions later in the growing season. The aim of this study was to determine the frequency of fungal genera/species contaminating sorghum seeds, grain mold severity, seed weight and germination rate. **Materials and Methods:** During the 2016 and 2017 growing seasons, 62 sorghum lines were collected from Puerto Rico and Mexico. Panicles from these locations were threshed and the seed samples were put in separate paper bags and stored at 7°C in a refrigerator in the laboratory. Seed samples were evaluated for grain mold severity, seed mycoflora, seed weight and germination rate. **Results:** In Isabela, Puerto Rico, *Fusarium thapsinum* was the dominant fungal species isolated from sorghum grain, followed by *Aspergillus* spp. and *F. semitectum* in 2016 and 2017, *F. semitectum* was the most frequently isolated fungal species. In Guayanilla, Puerto Rico and Puerto Vallarta, Mexico, FIESC (*Fusarium incarnatum*, *F. acuminatum*, *F. equiseti* and *F. semitectum* Complex) were the dominant species isolated from sorghum seed samples. Among the sorghum lines evaluated for grain mold severity, KS-963 collected from Guayanilla, Puerto Rico, exhibited a moderate resistance response to grain mold, while the other sorghum lines were either moderately susceptible or susceptible. Across locations, KS-835 exhibited the highest seed weight (4.7 g) while PI534152 exhibited the lowest seed weight (1.0 g). Germination rates ranged from 100-0% among the sorghum lines surveyed. **Conclusion:** While *F. thapsinum*, *F. nygamy* and *C. lunata* are considered the most common grain molding species, the frequency of recovery of these three fungal species in some sorghum growing regions, including locations surveyed in this study, may be low. Thus, in grain mold resistance studies, selecting the most dominant fungal species in a sorghum growing region and using them as inocula in either the field or greenhouse is more practical and beneficial.

Key words: *Sorghum bicolor*, grain mold, fungal species, sorghum seed mycoflora

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Competing Interest: The authors have declared that no competing interest exists.

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

INTRODUCTION

Sorghum is the 5th most important cereal crop and provides the caloric needs of millions of people, especially in the drier tropics[1]. However, the crop is hampered by a number of biotic stresses, including grain mold, a complex fungal disease considered to be the most important disease worldwide. Grain mold is associated with fungi in several genera, including *Fusarium thapsinum* Klittick, Leslie, Manassas; *Fusarium semitectum* Berk. and Ravenel; *Curvularia lunata* (Wakk.) Boedijn; *Colletotrichum graminicola* (Ces.) G. W. Wilson; *Alternaria alternata* (Fr.: Fr.) Keissl; and *Phoma sorghina* (Sacc.) Boerema, Dorenbosch and Van Kesteren[2-4]. Some of the fungal genera, in particular *Fusarium* species, associated with this disease complex are mycotoxigenic either during the grain development or post-harvest during storage[5-9]. The disease is most severe in areas where wet conditions occur later in the growing season and if mature grains are not harvested on time[3,10]. Generally, grain mold symptoms may range from seed discoloration to smaller seed size[4,11,12]. Yield losses on highly susceptible sorghum lines can reach[13] 100%. *Fusarium thapsinum*, *F. nygamy* and *C. lunata* are considered the most important grain mold fungi[3,4,14]. Thus, this communication reports the analysis of sorghum seed samples collected from Puerto Rico and Mexico for the frequency of fungal genera/species contaminating the seeds, grain mold severity, seed weight and germination rate.

MATERIALS AND METHODS

A total of 23 and 19 sorghum lines were collected from experimental plots at the USDA-ARS Tropical Agriculture Research Station, Isabela, Puerto Rico, between June-July, 2016 and 2017, respectively. Also, seeds from 10 sorghum lines each from Guayanilla, Puerto Rico and Puerto Vallarta, Mexico, were collected from experimental fields between June-July, 2017. Panicles from these locations were threshed and the seed samples were put in separate paper bags and stored at 7°C in a refrigerator in the laboratory. Seed samples were evaluated for grain mold severity based on a scale of 1-5 where, 1: No mold observed on the seeds, 2: 1-9%, 3: 10-24%, 4: 25-49% and 5: 50% or more of the seeds molded[10,15]. The samples also were assessed for mycoflora, germination rate and seed weight.

Mycoflora analysis was determined by placing 50 seeds per sample in vials. The vials were put in a small beaker containing 10% NaCl for 1 min, then rinsed three times with sterilized water and dried under a laminar flow hood. Ten surface-disinfested seeds were plated on each Petri dish containing a half-strength potato dextrose agar and incubated at 25±2°C for 5-7 days[16,17]. Using a microscope (Olympus America, Inc., Melville, N.Y.) and according to descriptions provided by Booth[18], Nelson *et al.*[19] and Barnett and Hunter[20], the fungal species were identified based on the conidia, conidiophores, colony morphology and color. The FIESC (i.e., *Fusarium incarnatum*, *F. acuminatum*, *F. equiseti* and *F. semitectum* Complex) was identified by using TEF-1-alpha primers and checking on NCBI for confirmation.

Seed weight recorded in grams was based on the weight of 100 randomly selected seeds per sample and germination rate (%) was based on the number of seeds that germinated in 7 days out of 100 seeds per sample placed on Anchor seed germination paper (Anchor Paper CO, St. Paul, MN).

Statistical analysis: Data for the mycoflora, grain mold severity, seed weight, percent germination rates were analyzed using the commands PROC GLM (SAS Institute, SAS version 9.4, Cary, NC). Differences in means for fungal isolate, grain mold severity, seed weight, germination rates (%) among lines were determined at the 5% probability level based on pair wise comparisons of least-square means with *t*-tests.

RESULTS

Mycoflora analysis: In this study, the fungal community of sorghum seeds collected from several locations in Puerto Rico and Mexico in 2016 and 2017 was examined. The main effect of isolate in each location was significant ($p<0.05$), indicating that the frequency of recovery of the different fungal genera and/or species was not the same. In Isabela, Puerto Rico, *F. thapsinum* (27%) was the dominant fungal species isolated from sorghum grain, followed by *Aspergillus* spp. (17%) and *F. semitectum* (15%) in 2016 (Fig. 1). Other fungi isolated from seed samples were *F. proliferatum*, *C. lunata* and *Penicillium* spp. During the 2017 survey in Isabela, *F. semitectum* (41%) was the most frequently isolated fungal species, followed by FIESC (*Fusarium incarnatum*, *F. acuminatum*, *F. equiseti* and *F. semitectum* Complex) (17%), *C. lunata* (14%) and *F. verticillioides* (6%). *Fusarium thapsinum*, *Aspergillus* spp., *Alternaria* spp. and *Bipolaris* spp. were isolated in trace amounts in 2017 (Fig. 1). In Guayanilla, Puerto Rico and Puerto Vallarta, Mexico, FIESC was the dominant species isolated from sorghum seed samples, accounting for 55 and 20%, respectively (Fig. 2, 3). Other fungal species recovered from

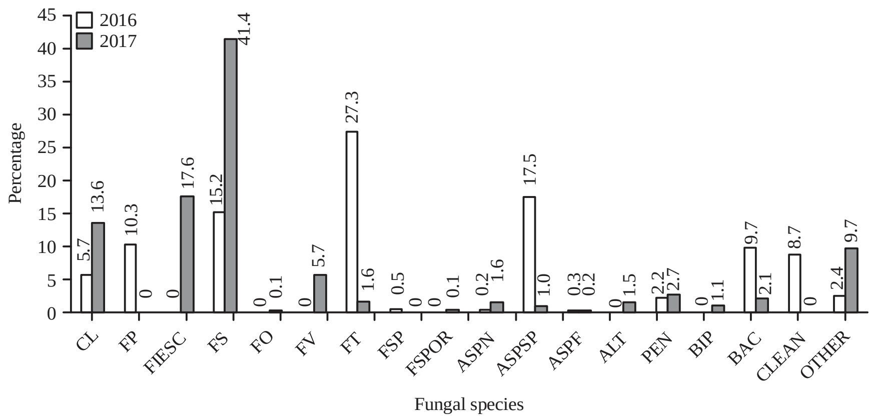

Fig. 1: Frequency of isolation (%) of fungal species from sorghum seeds collected in Isabela, Puerto Rico, 2016 and 2017

CL: *Curvularia lunata*, FP: *Fusarium proliferatum*, FIESC: *Fusarium incarnatum*, *F. acuminatum*, *F. equiseti* and *F. semitectum* Complex, FS: *F. semitectum*, FO: *F. oxysporum*, FV: *F. verticillioides*, FT: *F. thapsinum*, FSP: *Fusarium* species, FSPOR: *F. sporotrichioides*, ASPN: *Aspergillus niger*, ASPSP: *Aspergillus* spp., ASPF: *Aspergillus flavus*, ALT: *Alternaria* spp., PEN: *Penicillium* spp., BIP: *Bipolaris* spp., BAC: Bacteria, CLEAN: Clean seeds i.e., not contaminated or infected and OTHER: Fungal spp. i.e., not identified

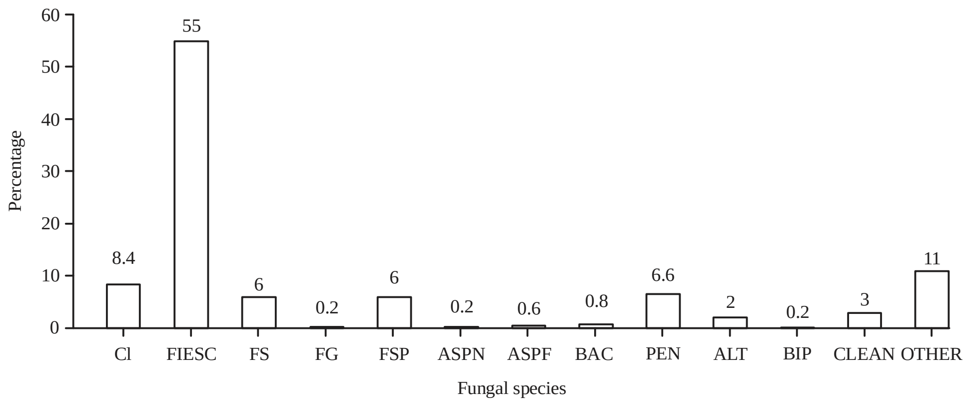

Fig. 2: Frequency of isolation (%) of fungal species from sorghum seeds collected in guayanilla, Puerto Rico, 2017

CL: *Curvularia lunata*, FIESC: *Fusarium incarnatum*, *F. acuminatum*, *F. equiseti* and *F. semitectum* Complex, FS: *F. semitectum*, FG: *F. graminearum*, FSP: *Fusarium* species, ASPN: *Aspergillus niger*, ASPF: *Aspergillus flavus*, BAC: Bacteria, PEN: *Penicillium* species, ALT: *Alternaria* species, BIP: *Bipolaris* species, CLEAN: Clean seeds i.e., not contaminated or infected, and OTHER: Fungal species i.e., not identified

seed samples collected from Guayanilla, Puerto Rico were *C. lunata*, *Penicillium* spp. and *F. semitectum*. *F. graminearum*, *A. niger*, *A. flavus* and *Bipolaris* spp. were isolated in trace amounts (Fig. 2). In Mexico (Fig. 3), other fungi isolated from sorghum seed samples were *C. lunata* (16%), *Alternaria* spp. (12%) and *F. proliferatum* (11%).

Grain mold severity, seed weight and germination rate: Grain mold severity was significant across all locations ($p<0.05$) indicating that sorghum lines responded different. In Isabela, grain mold severity (GMS) for the sorghum lines collected during the 2016 and 2017 surveys exhibited scores of 3 and above, indicating a moderately susceptible to susceptible response (Table 1). LAF-5 (4.0 g) exhibited the highest seed weight, while LAF-77 (1.3 g) the lowest in 2016 in Isabela (Table 1). The seed weight of LAF-5 was significantly higher than the seed weights of 90% of the lines sampled. Similarly, PI563482 recorded the highest seed weight (3.7 g) and this was significantly higher than the seed weights of 90% of the lines surveyed in Isabela during the 2017 growing season. While, PI534152 exhibited the lowest seed weight (1.0 g). Seeds collected from Isabela in 2016 showed poor germination rate; whereas in 2017, the germination rate ranged from 89.1 (PI514601)-5.0% (PI533799) (Table 1).

In Guayanilla, Puerto Rico, KS-963 (2.3 GMS) exhibited a moderate resistance response to grain mold, while the other sorghum lines were moderately susceptible with GMS scores ranging from 3.3-3.0 (Table 2). KS-835 exhibited the highest

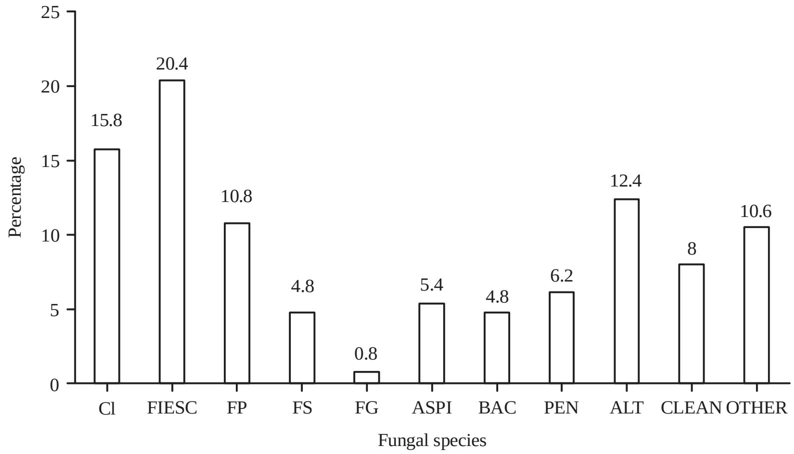

Fig. 3: Frequency of isolation (%) of fungal species from sorghum seeds collected in Puerto Vallarta, Mexico, 2017

CL: *Curvularia lunata*, FIESC: *Fusarium incarnatum*, *F. acuminatum*, *F. equiseti* and *F. semitectum* Complex, FP: *F. proliferatum*, FS: *F. semitectum*, FG: *F. graminearum*, ASPF: *Aspergillus flavus*, BAC: Bacteria, PEN: *Penicillium* species, ALT: *Alternaria* species, CLEAN: Clean seeds i.e., not contaminated or infected and OTHER: Fungal species i.e., not identified

Table 1: Measured parameters of sorghum seeds collected from Isabela, Puerto Rico, in 2016 and 2017

		2016				2017	
Line	GM*	Seed WT**	Germ$^{\#}$	Line	GM	Seed WT	Germ
LAF-32	4.3^{a4}	3.3^{bcd}	0.0^{f}	PI533799	5.0^{a}	1.6^{ghij}	5.0^{f}
LAF-81	4.0^{ab}	2.7^{efg}	8.7^{cd}	PI669639	5.0^{a}	2.2^{efg}	43.5^{d}
LAF-102	3.7^{bc}	1.6^{klm}	3.7^{def}	PI534152	4.7^{a}	1.0^{k}	19.3^{ef}
LAF-36	3.7^{bc}	3.2^{cde}	1.3^{ef}	PI33.819	4.0^{b}	1.7^{ghij}	18.8^{ef}
LAF-107	3.7^{bc}	2.4^{gh}	0.0^{f}	PI197462	4.0^{b}	3.3^{a}	60.8^{c}
LAF-82	3.3^{cd}	1.4^{lm}	0.0^{f}	PI514605	3.7^{bc}	2.4^{bcd}	43.8^{d}
LAF-38	3.3^{cd}	2.5^{fgh}	0.0^{f}	PI533903	3.3^{cd}	1.3^{kj}	50.1^{cd}
LAF-28	3.3^{cd}	2.9^{def}	31.3^{a}	PI669795	3.3^{cd}	1.9^{fgh}	44.8^{d}
LAF-85	3.3^{cd}	2.3^{ghi}	4.0^{def}	PI514601	3.3^{cd}	2.1^{defg}	89.1^{a}
LAF-50	3.3^{cd}	2.1^{hij}	0.3^{f}	PI534131	3.0^{d}	1.7^{ghij}	61.8^{c}
LAF-140	3.0^{d}	3.4^{bc}	0.0^{f}	PI669698	3.0^{d}	2.0^{defg}	79.5^{a}
LAF-64	3.0^{d}	2.2^{hij}	21.7^{b}	PI267588	3.0^{d}	2.4^{bcd}	23.1^{e}
LAF-56	3.0^{d}	2.1^{hij}	0.0^{f}	PI660637	3.0^{d}	2.8^{b}	78.8^{ab}
LAF-45	3.0^{d}	3.8^{ab}	0.0^{f}	PI668717	3.0^{d}	1.4^{ijk}	11.1^{ef}
LAF-47	3.0^{d}	3.1^{cde}	13.0^{c}	PI669702	3.0^{d}	1.8^{ghi}	78.1^{ab}
LAF-77	3.0^{d}	1.3^{m}	0.3^{ef}	PI534001	3.0^{d}	2.8^{bc}	64.1^{bc}
LAF-70	3.0^{d}	2.5^{fgh}	0.0^{f}	PI534116	3.0^{d}	1.2^{k}	85.5^{a}
LAF-5	3.0^{d}	4.0^{a}	7.0^{cdef}	PI576377	3.0^{d}	1.6^{hij}	52.8^{cd}
LAF-86	3.0^{d}	1.8^{jklm}	0.0^{f}	PI563482	3.0^{d}	3.7^{a}	58.1^{cd}
LAF-13	3.0^{d}	2.1^{hij}	10.0^{cd}				
LAF-4	3.0^{d}	1.9^{ijkl}	0.0^{f}				
LAF-78	3.0^{d}	2.8^{efg}	0.7^{f}				
LAF-2	3.0^{d}	2.1^{hij}	8.3^{cde}				

*GM: Grain mold severity based on a scale of 1-5 where, 1: No mold observed on the seeds, 2: 1-9%, 3: 10-24%, 4: 25-49% and 5: 50% or more of the panicle molded[10,15]. Grain mold severity was assessed on naturally-infected threshed seeds. **Seed WT: Seed weight (100 seeds in grams). Main effect of seed weight for 2016 was non-significant. $^{\#}$Germ: Germination rates (%) based on the number seeds that germinated after 1 week on a blotter paper. Means within a column followed by the same letter(s) are not significantly different at the 5% probability level based on pairwise comparisons of least-square means with t-tests

seed weight (4.7 g) and KS-573 (1.9 g) had the lowest score. The highest and lowest germination rates were 97.3% (KS-967) and 66% (KS-835), respectively. All other lines surveyed had germination rate ranging from 96% (KS-815) to 68.3% (KS-320). Seed samples obtained from MX-900 collected from Puerto Vallarta had GMS score of 2.3, indicating a moderately resistant response (Table 3). All other lines had GMS scores ranging from 2.7 (MX-MA) to 3.3 (MX-392). MX-535 had mean seed weight of 4.3 g and this score was significantly higher than the scores of all other sorghum lines surveyed in Puerto Vallarta. Table 3 showed that the germination rate for sorghum lines MX-HKZ and MX-MA was 100% and all other lines had rates ranging from 98.7% (MX-KO) to 66.7% (MX-849).

Table 2: Measured parameters of sorghum seeds collected from Guayanilla, Puerto Rico, 2017

Line	GM*	Seed WT#	GermΦ
KS-835	3.3^{a4}	4.7^{a}	66.0^{d}
KS-596	3.0^{a}	3.1^{e}	80.7^{bc}
KS-573	3.0^{a}	1.9^{h}	94.0^{a}
KS-841	3.0^{a}	3.4^{d}	89.3^{ab}
KS-967	3.0^{a}	2.5^{g}	97.3^{a}
KS-815	3.0^{a}	2.8^{f}	96.0^{a}
KS-629	3.0^{a}	2.7^{fg}	92.0^{ab}
KS-169	3.0^{a}	3.7^{c}	75.3^{cd}
KS-320	3.0^{a}	4.1^{b}	68.3^{d}
KS-963	2.3^{b}	2.0^{h}	92.3^{ab}

*GM: Grain mold severity was assessed on naturally-infected threshed seeds and based on a scale of 1 to 5 where, 1: No mold observed on the seeds, 2: 1-9%, 3: 10-24%, 4: 25-49% and 5: 50% or more of the panicle molded[10,15]. #Seed WT: Seed weight (100 seeds in grams). ΦGerm: Germination rates (%) based on the number seeds that germinated after one week on a blotter paper. [4]Means within a column followed by the same letter(s) are not significantly different at the 5% probability level based on pairwise comparisons of least-square means with t-tests

Table 3: Measured parameters of sorghum seeds collected from Puerto Vallarta, Mexico in 2017

Line	GM*	Seed WT#	GermΦ
MX-392	3.3a^{4}	4.0^{b}	70.0^{ed}
MX-HKZ	3.0^{ab}	3.3^{f}	100.0^{a}
MX-849	3.0^{ab}	3.6^{cd}	66.7^{e}
MX-529	3.0^{ab}	3.8^{bc}	83.3^{bcd}
MX-535	3.0^{ab}	4.3^{a}	73.3^{cde}
MX-546	3.0^{ab}	3.6^{de}	90.0^{ab}
MX-978	3.0^{ab}	3.4^{ef}	80.0^{bcde}
MX-KO	3.0^{ab}	1.9^{h}	98.7^{a}
MX-MA	2.7^{bc}	2.8^{g}	100.0^{a}
MX-900	2.3^{c}	2.9^{g}	87.3^{abc}

*GM: Grain mold severity was assessed on naturally-infected threshed seeds and based on a scale of 1 to 5 where, 1: No mold observed on the seeds, 2: 1-9%, 3: 10-24%, 4: 25-49% and 5: 50% or more of the panicle molded[10,15]. #Seed WT: Seed weight (100 seeds in grams). ΦGerm: Germination rates (%) based on the number seeds that germinated after one week on a blotter paper. [4]Means within a column followed by the same letter(s) are not significantly different at the 5% probability level based on pairwise comparisons of least-square means with t-tests

In this study, non-significant negative correlation between GMS and germination rate was noted in locations tested, except in 2017 survey conducted in Isabela, Puerto Rico, where the association between the two parameters was significant (r = -0.53882; p = 0.0173).

DISCUSSION

Sorghum grain mold is one of the most complex host-pathosystems. The disease is associated with fungi in several genera; *F. thapsinum*, *F. nygamy* and *C. lunata* are considered the most common grain mold species[3,4,14]. However, the frequency of recovery of these three fungal species in many sorghum growing regions may be low[21-27]. In this current research, *F. thapsinum*, *F. semitectum* and FIESC (*Fusarium incarnatum*, *F. acuminatum*, *F. equiseti* and *F. semitectum* Complex) were the dominant species isolated from sorghum seed samples collected in Puerto Rico and Mexico. It is not surprising that *F. thapsinum* was isolated in high level since it was one of the fungi used to inoculate the panicles in Isabela, PR, 2016. Other fungal species isolated in various frequencies included *C. lunata*, *F. verticillioides*, *F. graminearum*, *Aspergillus* spp., *A. niger*, *A. flavus*, *Alternaria* spp., *Penicillium*s pp and *Bipolaris*s pp. Nagaraja *et al.*[25] noted that among the fusarium isolates infecting or contaminating sorghum seeds in Karnataka, India, *F. verticillioides* followed by *F. graminearum* and *F. proliferatum* were the most recovered species. Fungal community of sorghum seed samples collected from 6 regions in Egypt, showed *A. niger*, followed by *A. flavus*, *Alternaria* spp. and *Fusarium* spp. as the most frequently isolated fungi[27].

Mycoflora analysis of sorghum seeds collected from northwestern Sierra Leone, West Africa, showed that *Phoma sorghina* was the most frequently isolated fungus, followed by *F. verticillioides* and *Bipolaris bicolor*, while *C. lunata* was among the lowest recovered fungal species[26]. However, *Alternaria alternata* was the most recovered fungal species on sorghum seeds collected from different locations in Turkey[22]. Naqvi *et al.*[23] also isolated *Alternaria* spp. on all sorghum seed samples collected from 14 locations in Eritrea, North East Africa.

In this study, all the lines evaluated, except for KS-963 (moderate resistance response) were moderately susceptible to grain mold. However, studies in Puerto Rico and other locations have identified a number of sorghum lines with high level of grain mold resistance[12,28-30]. There was significant negative correlation between GMS and germination rate for samples collected in 2017 survey in Isabela, Puerto Rico. Erpelding and Prom[21] also noted a significant negative association between germination rate with *F. semitectum*, *C. lunata* and *Bipolaris* spp.

CONCLUSION

The work showed that fungal species such as *F. semitectum* and FIESC (*Fusarium incarnatum*, *F. acuminatum*, *F. equiseti* and *F. semitectum* Complex) are the most dominant species infecting or contaminating mature grain in some sorghum growing regions of Puerto Rico and Mexico. Therefore, in grain mold resistance studies, selecting the most dominant fungal species in a sorghum growing region and using them as inocula in either the field or greenhouse is more practical and beneficial. Among the sorghum lines evaluated for GMS across locations, KS-963 (Guayanilla, Puerto Rico) and MX-900 (Puerto Vallarta, Mexico), exhibited a moderate resistance response to grain mold, while the other sorghum lines were either moderately susceptible or susceptible.

SIGNIFICANCE STATEMENTS

This study shows that different fungal species may be contaminating or infecting sorghum seeds instead of the three species, *F. thapsinum*, *F. nygamy* and *C. lunata*, which are considered the most important grain mold fungi. Our study also showed that the frequency of isolation of grain molding fungi may differ within similar regions and years. Thus, in grain mold resistance evaluations, the use of either the dominant fungal species or a mixture of species in such regions will be most beneficial. The lack of highly resistant grain mold line in the current study also warrants further surveys of sorghum fields in these locations to identify resistant sources.

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REFERENCES

1. Frederiksen, R.A. and G.N. Odvody, 2000. Compendium of Sorghum Diseases. The American Phytopathological Society, St. Paul, MN, USA.
2. Esele, J.P., R.A. Frederiksen and F.R. Miller, 1995. Importance of plant colour and modifier genes in grain mould resistance in sorghum. J. East Afr. Agric. For., 61: 31-37.
3. Bandyopadhyay, R. and A. Chandrashekar, 2000. Biology and management of sorghum grain mold. Proceedings of Consultative Group Meeting on Technical and Institutional Options for Sorghum Grain Mold Management, May 18-19, ICRISAT, Patancheru, India, pp: 2.
4. Singh, S.D. and R. Bandyopadhyay, 2000. Grain Mold. In: Compendium of Sorghum Diseases, Frederiksen, R.A. and G.N. Odvody (Eds.). The American Phytopathological Society, St. Paul, MN, USA., pp: 38-40.
5. Sashidhar, R.B., Y. Ramakrishna and R.V. Bhat, 1992. Moulds and mycotoxins in sorghum stored in traditional containers in India. J. Stored Prod. Res., 28: 257-260.
6. Leslie, J.F., K.A. Zeller, S.C. Lamprecht, J.P. Rheeder and W.F.O. Marasas, 2005. Toxicity, pathogenicity and genetic differentiation of five species of *Fusarium* from sorghum and millet. Phytopathology, 95: 275-283.
7. .Funnell-Harris, D.L., L.K. Prom, S.E. Sattler and J.F. Pedersen, 2013. Response of near-isogenic sorghum lines, differing at the P locus for plant colour, to grain mould and head smut fungi. Ann. Applied Biol., 163: 91-101.
8. Isakeit, T., L.K. Prom, M. Wheeler, L.S. Puckhaber and J. Liu, 2008. Mycotoxigenic potential of ten *Fusarium* species grown on sorghum and *in vitro*. Plant Pathol. J., 7: 183-186.
9. Little, C.R., R. Perumal, T. Tesso, L.K. Prom, G.N. Odvody and C.W. Magill, 2012. Sorghum pathology and biotechnology-a fungal disease perspective: Part I. Grain mold, head smut and ergot. Eur. J. Plant Sci. Biotechnol., 6: 10-30.
10. Thakur, R.P., V.P. Rao, B.V.S. Reddy and S.P. Reddy, 2007. Grain Mold. In: Screening Techniques for Sorghum Diseases, Thakur, R.P., B.V.S. Reddy, K. Mathur (Eds.). ICRISAT, Patancheru, India, pp: 5-14.
11. Castor, L.L., 1981. Grain mold histopathology, damage assessment and resistance screening within *Sorghum bicolor* (L.) Moench lines. Ph.D. Thesis, Department of Plant Pathology and Microbiology, Texas A and M University, College Station, TX., USA.
12. Prom, L.K., R. Perumal, N. Cisse and C. Little, 2014. Evaluation of selected sorghum lines and hybrids for resistance to grain mold and long smut fungi in Senegal, West Africa. Plant Health Progr., 15: 74-77.
13. Navi, S.S., R. Bandyopadhyay, R.K. Reddy, R.P. Thakur and X.B. Yang, 2005. Effects of wetness duration and grain development stages on sorghum grain mold infection. Plant Dis., 89: 872-878.
14. Forbes, G.A., R. Bandyopadhyay and G. Garcia, 1992. A Review of Sorghum Grain Mold. In: Sorghum and Millets Diseases: A Second World Review. De Milliano, W.A.J., R.A. Frederiksen and G.D. Bengston (Eds.). ICRISAT, Patancheru, India, pp: 253-264.
15. Isakeit, T., S.D. Collins, W.L. Rooney and L.K. Prom, 2008. Reaction of sorghum hybrids to anthracnose, grain mold and grain weathering in Burleson County, Texas, 2007. Plant Disease Management Reports, 2, FC003.
16. Prom, L.K., R.D. Waniska, A.I. Kollo and W.L. Rooney, 2003. Response of eight sorghum cultivars inoculated with *Fusarium thapsinum, Curvularia lunata* and a mixture of the two fungi. Crop Protect., 22: 623-628.
17. Prom, L.K., 2004. The effects of *Fusarium thapsinum*, *Curvularia lunata* and their combination on sorghum germination and seed mycoflora. J. New Seeds, 6: 39-49.
18. Booth, C., 1971. The Genus *Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, England, Pages: 237.
19. Nelson, P.E., T.A. Toussoun and W.F.O. Marasas, 1983. *Fusarium* species: An Illustrated Manual for Identification. Pennsylvania State University Press, USA., ISBN: 0271003499, Pages: 193.
20. Barnett, H.L. and B.B. Hunter, 1998. Illustrated Genera of Imperfect Fungi. 4th Edn., APS Press, St. Paul. Minnesota.

21. Erpelding, J.E. and L.K. Prom, 2006. Seed mycoflora for grain mold from natural infection in sorghum germplasm grown at Isabela, Puerto Rico and their association with kernel weight and germination. Plant Pathol. J., 5: 106-112.
22. Turgay, E.B. and F. Unal, 2009. Detection of seed borne mycoflora of sorghum in Turkey. J. Turk. Phytopath., 38: 9-20.
23. Naqvi, S.D.Y., T. Shiden, W. Merhawi and S. Mehret, 2013. Identification of seed borne fungi on farmer saved sorghum (*Sorghum bicolour* L.), pearl millet (*Pennisetum glaucum* L.) and groundnut (*Arachis hypogaea* L.) seeds. Agric. Sci. Res. J., 3: 107-114.
24. Prom, L.K., R. Perumal, Z. Jin, G. Radwan, T. Isakeit and C. Magill, 2015. Mycoflora analysis of hybrid sorghum grain collected from different locations in South Texas. Am. J. Exp. Agric., 6: 1-6.
25. Nagaraja, H., G. Chennappa, K.P.C. Rao, G.M. Prasad and M.Y. Sreenivasa, 2016. Diversity of toxic and phytopathogenic *Fusarium* species occurring on cereals grown in Karnataka state, India. 3 Biotech, Vol. 6, No. 1. 10.1007/s13205-016-0399-5.
26. Taylor, D.R. and A.S. Ngaujah, 2016. Seed borne fungi of rice, maize, sorghum, fundi, cowpea, groundnut, pigeon pea and pepper cultivated in the Kambia District of Sierra Leone. Int. J. Agric. Res. Rev., 4: 569-578.
27. Osman, M.A., A. Salama, K.H.M. Naguib, M.A. Abdel-Wahhab and S.R. Sherif, 2017. Fungi and mycotoxins associated with Egyptian sorghum grains. MOJ. Toxicol., 3: 51-56.
28. Prom, L.K. and J.E. Erpelding, 2009. New sources of grain mold resistance among sorghum accessions from Sudan. Trop. Subtrop. Agroecosyst., 10: 457-463.
29. Sharma, R.,V.P. Rao, H.D. Upadhyaya, V.G. Reddy and R.P. Thakur, 2010. Resistance to grain mold and downy mildew in a mini-core collection of sorghum germplasm. Plant Dis., 94: 439-444.
30. Cuevas, H.E., L.K. Prom, T. Isakeit and G. Radwan, 2016. Assessment of sorghum germplasm from Burkina Faso and South Africa to identify new sources of resistance to grain mold and anthracnose. Crop Protect., 79: 43-50.