Sorghum Seed Fungal Community and Their Association with Grain Mold Severity, Seed Weight, and Germination Rate

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Abstract

Grain mold, considered the most important disease of sorghum, is associated with several fungal genera. The disease reduces both yield and quality. In this study, over 300 sorghum seed samples collected from Texas, Florida, and Georgia were evaluated for grain mold severity, seed weight, germination rate, and seed fungal community. Grain mold severity of the seed samples, except for those collected from Cameron, Texas, were rated 3 or higher, indicating that these sorghum lines were moderately susceptible under naturally-infected field conditions during the 2016 and 2017 growing seasons. Seed weight across surveyed locations ranged from 1.1 g to 4.0 g for samples collected in Texas during the same period. Percent germination rates for samples collected in Texas ranged from 59.6% to 86.7%. Sorghum samples collected from Florida and Georgia exhibited moderately susceptible response to grain mold infection. Mean seed weight was 1.9 g for samples collected from Florida, while in Georgia, mean seed weight was 2.3 g. Germination rate was low for samples collected from Florida and Georgia. Mycological analysis of sorghum seed samples collected from farmers’ fields in Central and South Texas during the 2016 and 2017 growing seasons showed Alternaria species as the most frequently isolated fungal genus, accounting for 40% and 42% in 2016 and 2017, followed by Fusarium incarnatum, F. acuminatum, F. equiseti, & F. semitectum Complex. In Florida and Georgia, Fusarium incarnatum, F. acuminatum, F. equiseti, & F. semitectum Complex was the most frequently recovered fungal species, accounting for 77% and 72% of the total. genera/species isolated from seed samples. Other fungal species, including Curvularia lunata, Bipolaris sp., Colletotrichum sublineola, F. verticilloides, Penicillium sp., Aspergillus flavus, F. thapsinum, F. oxysporum, F. sporotrichioides, F. graminearum, F. proliferatum, and Aspergillus niger were also isolated from sorghum seeds in various frequencies. In conclusion, the presence of large number of fungal genera associated with grain deterioration and their effect on other traits, makes management of this disease complex challenging. To identify grain mold resistant sources in a region, using the most dominant species in that region to screen the sorghum germplasm is recommended.

Keywords: Sorghum bicolor; Grain mold; Fungi; Mycoflora; Sorghum seed; Fungi.

1. Introduction

Sorghum is considered a vital cereal crop that provides the calorie intake needs of millions of people, especially in the drier tropics [1]. The production and profitability of the crop are hampered by both abiotic and biotic stresses. Among the biotic constraints on sorghum production is grain mold, a complex fungal disease considered to be the most important worldwide. Sorghum grain mold is associated with fungi in several genera, including Fusarium thapsinum Klittick, Leslie, Nelson et al., Manasas; Fusarium semitectum Berk. & Ravenel; Curvularia lunata (Wak.) Boedijn; Colletotrichum sublineola Henn ex Sacc & Trotter; Alternaria alternata (Fr.: Fr.) Keissl.; and Phoma sorgihna (Sacc.) Boerema, Dorenbosch, & Van Kesteren [2-4]. Some of the fungal genera, in particular Fusarium species, are mycotoxigenic either during the grain development or post-harvest during storage [5-9]. Severity of this pathosystem is most pronounced in areas where wet conditions occur later in the growing season and if mature grains are not harvested on time [3, 10]. Manifestation of the disease may range from seed discoloration to smaller seed size [4, 11, 12]. Moreover, losses in grain yield on highly susceptible sorghum lines can reach 100% [13]. Fusarium thapsinum, F. nygamé and C. lunata are considered the most important contributors to grain molding
fungi [3, 4, 14]. However, the frequency and recovery of the grain molding fungi vary from location to location, and in some sorghum production areas, the frequency of isolation of these fungal species is either low or non-existent [15-18]. Thus, this study reports the grain mold severity, seed weight, germination rate, and seed fungal community of sorghum samples collected from Texas, Georgia, and Florida.

2. Materials and Methods

A total of 165 and 109 hybrid sorghum seed samples were collected from farmers’ fields in Central and South Texas during the 2016 and 2017 growing seasons, respectively. These samples were obtained from 6 counties in Georgia were analyzed using the command PROC MEANS and PROC GLM (SAS Institute, SAS version 9.4, Cary, NC). Differences in means for grain mold severity, seed weight, and percent germination rates among counties were determined at the 5% probability level based on pairwise comparisons of least-square means with t-tests. Grain mold severity, seed weight, percent germination rates of the samples collected from Florida and Georgia were analyzed using the command PROC UNIVARIATE.

2.2. Statistical Analysis

Data for the mycoflora, grain mold severity, seed weight, percent germination rates were analyzed using the command PROC MEANS and PROC GLM (SAS Institute, SAS version 9.4, Cary, NC). Differences in means for grain mold severity, seed weight, and percent germination rates among counties were determined at the 5% probability level based on pairwise comparisons of least-square means with t-tests. Grain mold severity, seed weight, percent germination rates of the samples collected from Florida and Georgia were analyzed using the command PROC UNIVARIATE.

3. Results

3.1. Mycoflora Analysis

Mycological analysis was performed on 165 and 109 sorghum seed samples collected from farmers’ fields in Central and South Texas during the 2016 and 2017 growing seasons. Alternaria species was the most frequently isolated fungal genus from the samples, accounting for 40% and 42 % in 2016 and 2017, respectively (Fig. 1). In 2016, Alternaria spp., followed by Fusarium incarnatum, F. acuminatum, F. equiseti, & F. semitectum Complex (FIESC) (27.8%) and C. lunata (13.7%) were the most recovered species. Other fungal genera and species recovered from seed samples included Bipolaris sp., C. sublineola, F. verticillioides, Penicillium sp., and Aspergillus flavus. Fusarium thapsinum, F. oxysporum, F. sporotrichioides, and A. niger were also isolated but in trace amounts. During the 2017 survey, FIESC (32.9%) also was the second most frequently recovered fungal species, followed by Bipolaris sp. (13.2%), other unidentified fungal species (4.7%) and C. lunata (4.6%). Other fungi isolated from seed samples in trace amounts were F. graminearum, F. thapsinum, F. sporotrichioides, F. verticillioides, Aspergillus sp., and A. niger. However, Colletotrichum sublineola, F. proliferatum, Penicillium sp., Aspergillus flavus, and F. oxysporum were not detected in sorghum seeds collected in 2017. In both years, trace amounts of bacteria were detected.

In Florida and Georgia, FIESC was the most frequently recovered fungal species, accounting for 77% and 72.4% of the total genera/species isolated from seed samples (Fig. 2). In both states, C. lunata (7.2% and 8.4%) was the second most isolated species. Other fungal species isolated were F. oxysporum, Alternaria sp., and Bipolaris sp. F. thapsinum, F. verticillioides, F. sporotrichioides, and A. niger. Trace amounts of bacteria were also isolated on seed samples from Florida and Georgia, while fungi recovered on 6.1% of the seed samples from Florida and 3.4% from Georgia were not classified.
Figure 1. CS= Colletotrichum sublineola; CL=Curvularia lunata; FIESC=Fusarium incarnatum, F. acuminatum, F. equiseti, and F. semitectum Complex; FO=F. oxysporum; FP=F. proliferatum; FG=F. graminearum; FV=F. verticillioides; FT=F. thapsinum; FSPOR= F. sporotrichioides; FSP=Fusarium species; ASPN=Aspergillus niger; ASPFL=A. flavus; ASPSP=Aspergillus species; ALT=Alternaria species; BIP=Bipolaris species; PEN=Penicillium species; and OTHER=unknown fungal species i.e., not identified.

3.2. Disease Assessment, Seed Weight, Germination Rate

The main effect of Texas county for grain mold severity ($P=0.0107$) was highly significant and significant for seed weight ($P=0.0313$) and germination rate ($P=0.0376$) in 2016. In 2017, grain mold severity and seed weight were highly significant ($P=0.0001$) and significant for percent seed germination rate ($P=0.0318$), indicating differences in response among the counties for the measured traits (Table 1). In 2016, Willacy and Burleson Counties recorded the lowest mean grain mold severities 3.0 and 3.1, respectively. Hidalgo County recorded the highest mean grain mold severity of 3.8 (Table 1). Mean seed weight was highest on samples collected from farmers’ fields in Nueces County (4.0 g) and this was significantly higher than the level recorded from Willacy County. Seed samples from Willacy County exhibited the highest germination rate (82.3%), followed by samples from Burleson and Kleberg Counties. In 2017, mean grain mold severity of samples from Cameron County (2.4) was significantly lower than mean levels obtained from the rest of the counties surveyed (Table 1). Similarly, samples from Cameron County recorded the highest mean seed weight (3.2 g); whereas, samples from Jim Wells County exhibited the lowest (2.2 g). Mean percent seed germination rate was highest on samples collected from Jim Wells County (86.7%) and this amount was significantly higher than samples collected from Hidalgo County (59.6%) which was the lowest rate among the counties surveyed in 2017.
Table-1. Mean grain mold severity, seed weight, and germination rate of sorghum samples collected from farmers’ fields location in a number of Counties in Central and South Texas, during the 2016 and 2017 growing seasons

| County    | 2016       | 2017       |
|-----------|------------|------------|
|           | GM¹        | Seed WT²   | Germ²      | GM  | Seed WT | Germ    |
| Burleson  | 3.1b⁴      | 3.6ab      | 78.0a      | 3.6bc | 2.8ab   | 76.9ab  |
| Cameron   | -³         | -          | -          | 2.4d | 3.2a    | 78.8a   |
| Hidalgo   | 3.8a       | 2.2bc      | 72.3ab     | 3.4bc | 2.4cd   | 59.6b   |
| Jim Wells | -          | -          | -          | 3.2c | 2.2d    | 86.7a   |
| Kleberg   | 3.6ab      | 2.2bc      | 76.2ab     | 3.4bc | 2.8ab   | 79.8a   |
| Nueces    | 3.3b       | 4.0a       | 71.4b      | 3.2c  | 2.8ab   | 77.5a   |
| Refugio   | 3.7ab      | 2.2abc     | 63.7b      | 4.7a  | 2.8ab   | 76.9ab  |
| San Patricio | -          | -          | -          | 3.4bc | 3.1ab   | 78.5a   |
| Willacy   | 3.0b       | 1.1c       | 82.3a      | 4.0ab | 2.6bcd  | 74.7ab  |

¹GM=grain mold severity based on a scale of 1 to 5 where, 1 = no mold observed on the panicle; 2 = 1 to 9 %, 3 = 10 to 24%, 4 = 25 to 49% and 5 = 50% or more of the panicle mold.  
²Seed WT=seed weight (100 seeds in grams). Main effect of seed weight for 2016 was non-significant.  
³Germ=percent germination rates based on the number seeds that germinated after one week on a blotter paper.  
⁴Means within a column with the same letter(s) are not significantly different at the 5% probability level based on pairwise comparisons of least-square means with t-tests.  
⁵Counties not surveyed in 2016.

Table 2 shows the descriptive statistics of the data collected from samples obtained in Florida and Georgia. Mean grain mold severity of 3.4 was recorded for the seed samples collected from both locations. The percent seed germination rate was low, 30.7% and 38% from Florida and Georgia, respectively. While mean seed weight was 1.9 g for samples collected from Florida, and 2.3 g for those from Georgia.

| County | Florida | Georgia |
|--------|---------|---------|
| N      | Mean    | SD      | N      | Mean    | SD      |
| GM¹    | 19      | 3.4     | 0.68   | 10      | 3.4     | 0.70    |
| SeedWT² | 19      | 1.9     | 0.64   | 10      | 2.3     | 0.50    |
| Germ²  | 19      | 30.7    | 26.2   | 10      | 38.8    | 20.6    |

¹GM=grain mold severity based on a scale of 1 to 5 where, 1 = no mold observed on the panicle; 2 = 1 to 9 %, 3 = 10 to 24%, 4 = 25 to 49% and 5 = 50% or more of the panicle mold.  
²Seed WT=seed weight (100 seeds in grams). Main effect of seed weight for 2016 was non-significant.  
³Germ=percent germination rates based on the number seeds that germinated after one week on a blotter paper.  

4. Discussion

Globally, grain mold is considered the most important sorghum disease [18]. The fact that large number of fungal genera are associated with this pathosystem makes management challenging [3, 4, 11, 18, 21]. In this study, sorghum samples collected from Texas, Georgia, and Florida were evaluated for disease severity, seed weight, germination rate, and seed fungal community. Grain mold severity of the seed samples, except for those collected from Cameron, Texas were rated 3 or higher, indicating that the sorghum lines being grown were moderately susceptible under naturally-infected field conditions. Over the years, grain mold resistance studies conducted either under naturally-infected fields or inoculated with individual fungal species or in combination have yielded sorghum lines with high levels of resistance [10, 25-29]. Seed weight across surveyed locations ranged from 1.1 g to 4.0 g for samples collected in Willacy and Nueces Counties, Texas, while germination rate ranged from 59.6% to 86.7%. The mean seed weight and germination rate of samples from Texas were higher than those from Florida and Georgia. Seed weight and germination rate are factors to consider when evaluating sorghum germplasm response to grain mold. Mycological analysis of sorghum seed samples collected from farmers’ fields in Central and South Texas, showed Alternaria species as the most frequently isolated fungal genus. Similarly, Prom, et al. [18], noted that Alternaria spp. was the dominant fungal species isolated from sorghum seeds across several counties in South Texas. Also, Alternaria spp. and F. semitectum were the most frequently recovered fungi on naturally infected sorghum seeds collected from Burleson County, Texas [21]. Turgay and Ünal [30], also found that Alternaria alternata was the most frequently recovered fungal species on sorghum seeds collected from different locations in Turkey. Also, Naqvi, et al. [31], isolated Alternaria spp. on all sorghum seed samples collected from 14 locations in Erıtrea, North East Africa. Unlike the Texas samples in this study, F. incarnatum, F. acuminatum, F. equiseti, & F. semitectum Complex (FIESC) was the most frequently recovered fungal species from Florida and Georgia seeds. Erpelding and Prom [32], also noted that F. semitectum was the dominant fungal species infecting or contaminating sorghum seeds collected from Isabela, Puerto Rico, during the 2002 and 2003 growing seasons. In the present study other fungal species, including C. lunata, Bipolaris sp., C. sublineola, F. verticillioides, Penicillium sp., Aspergillus flavus, F. thapsinum, F. oxyssporum, F. sporotrichioides, and A. niger were also isolated. Fungal community of sorghum seeds collected from Northwestern Sierra Leone, West Africa, revealed Phoma sorghina as the most frequently isolated fungus, followed by F. verticillioides, and Bipolaris bicolor, but C. lunata was among the least recovered fungal species [16]. However, sorghum samples collected from six regions in Egypt, showed Aspergillus
niger, followed by *A. flavus*, *Alternaria* spp., and *Fusarium* spp. as the most frequently isolated fungi infecting or contaminating the seed [17].

Overall, similar fungal communities on sorghum seeds collected from Texas, Florida, and Georgia were noted in the study. The climatic classification of the collection sites in these states is similar according to Köppen Classification Cfa i.e., warm temperate, fully humid, and hot summer [33, 34].

5. Conclusion

In conclusion, the presence of a large number of fungi associated with grain deterioration and their effect on other traits makes management of this disease complex very challenging. Future research in grain mold studies would require planting sorghum lines in multiple geographic locations to identify stable resistant lines. However, in specific regions, exposing the lines to a mixture of the predominant fungal species will be more practical and beneficial in identifying the most stable grain mold resistant sources. Recommendation is when screening sorghum germplasm for resistance to grain mold in a particular region, one should use the dominant fungal species either contaminating or infecting sorghum grain in that region.

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